CHEMICAL TRANSFORMATIONS OF SESQUITERPENE LACTONES BY CONVENTIONAL AND NON-CONVENTIONAL METHODOLOGIES AND THEIR EVALUATION AS AGROCHEMICALS

Dissertation

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY
in
CHEMISTRY
(Minor Subject: Botany)

By

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LUDHIANA – 141 004

2006

CERTIFICATE I

This is to certify that the dissertation entitled, "**Chemical transformations of sesquiterpene lactones by conventional and non-conventional methodologies and their evaluation as agrochemicals**" submitted for the degree of **Ph. D.**, in the subject of **Chemistry** (Minor subject: **Botany**) of Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Rajat Rekha (L-2003-BS-46-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

8.11.06

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CERTIFICATE II

This is to certify that the dissertation entitled, "**Chemical transformations of sesquiterpene lactones by conventional and non-conventional methodologies and their evaluation as agrochemicals**" submitted by Rajat Rekha (L-2003-BS-46-D) to Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph. D.**, in the subject of **Chemistry** (Minor subject: **Botany**) has been approved by the Student's Advisory Committee after an oral examination of the same.

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(Rajat Rekhia)

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ABSTRACT

Sesquiterpene lactones found in plants are remarkably diverse in terms of their structure, properties and proposed functions. Sesquiterpene lactones contain a common functional structure, an α-methylene-γ-lactone group and this important chemical characteristic means that the thiol-reactivity of sesquiterpene lactones is an underlying mechanism responsible for their bioactivities. Sesquiterpenoids isolated from various natural sources include dehydrocostus lactone from *Saussurea lappa*, alantolactone and isoalantolactone from *Inula racemosa*, parthenin from *Parthenium hysterophorus* and lactucin, 8-deoxylactucin and lactucopicrin from *Cichorium intybus*. Four new alantolides *viz.* 4,10 β-dimethyl-5,6α-epoxy-7αH,8αH-eudesman-11-ene-8,12-olide, 4,10β-dimethyl-7αH,8αH-eudesman-5,11-diene-8,12-olide, 4,10β-dimethyl-7αH,8αH-eudesman-11-ene-8,12-olide and 4,15α-epoxy-10β-methyl-7αH,8αH-eudesman-5,11-diene-8,12-olide have been isolated from *Inula racemosa*. Research has been directed towards extensive use of microwave radiations for various reactions including double bond migration reaction in case of dehydrocostus lactone and isoalantolactone and a few monoterpenes, decomposition of pyrazoline derivatives of the sesquiterpenes, Hoffmann elimination of some quaternary ammonium salts and the decomposition of Mannich bases to their respective arylidenes. All the reactions afforded the products in high yields with substantial decrease in reaction times as expected. Other reactions included epoxidation of the olefinic bonds using sodium perborate and allylic oxidations with selenium dioxide and *tert.*-butyl hydroperoxide. Parthenin was employed as the substrate for reduction with various reagents like sodium borohydride, zinc borohydride, sodium cyanoborohydride, lithium tri-*tert.*-butoxy aluminohydride and magnesium in methanol. All the natural compounds and their derivatives were evaluated for their plant growth regulatory effects with the aim to establish and further clarify the structure activity relationships. The biological testing studies included the screening of seed germination and seedling growth behaviour of pea (*Pisum sativum*) and two weed species *viz. Avena fatua* (wild oats) and *Phlaris minor*. Parthenin and its derivatives showed promising potential as weedicides.

Key words: Microwave energy, double bond isomerisation, α-methylene-γ-lactone, reductions, weedicides.

8.11.06

Signature of Major Advisor

Signature of Student

CONTENTS

ABBREVIATIONS

C	Celsius	sec	seconds
CC	Column Chromatography	d	doublet
cm	centimeter	t	triplet
g	grams	brs	broad singlet
kg	kilograms	mp	melting point
µg	micrograms	IR	Infrared
l	litres	dec. pt.	decomposition point
ml	millilitres	ppm	parts per million
mg	milligrams		
µW	microwave		
h	hours		
min	minutes		
s	singlet		
rt	room temperature		
TLC	Thin Layer Chromatography		
PTLC	Preparative Thin Layer Chromatography		
^{1}H NMR	Proton Nuclear Magnetic Resonance		
^{13}C NMR	Carbon Nuclear Magnetic Resonance		
THF	Tetrahydrofuran		
PBA	Perbenzoic acid		
SPB	Sodium perborate		
TBHP	*Tert*- butyl hydroperoxide		
GA	Gibberelic acid		
ABA	Abscisic acid		
δ	delta		

CHAPTER I

INTRODUCTION

Sesquiterpene lactones found in plants are remarkably diverse in terms of their structure, properties and proposed functions. Among the Asteraceae, over 500 different members of the sesquiterpene lactone family have been described including not only constitutively produced secondary metabolites but also phytoalexins, which are only synthesized following microbial challenge. The growing interest in secondary metabolites of the plants has directed attention to study the role of sesquiterpene lactones in various developmental and metabolic processes and thus explore their potential as regulators of plant growth and development.

Sesquiterpene lactones are widely distributed natural products exhibiting a broad bioactivity profile. They are active constituents of a variety of medicinal plants used in traditional medicine for the treatment of inflammatory diseases. In recent years several biological activities related to sesquiterpene lactones have attracted a great deal of interest some of the which include anticancer potential (Zhang *et al* 2005), leishmanicidal activity (Wilson *et al* 2004), antibacterial properties (Melliou *et al* 2005), cytotoxicity (Tian *et al* 2006, Yang *et al* 2003), allelopathic effects (Bajwa *et al* 2004), as enzyme inhibitors and novel pharmacophores (Rahman and Choudhary 2001) and hydroxyl radical scavenging activity (Jung *et al* 2004) to mention a few.

Based on the aforementioned bioregulatory effects of these compounds, extensive studies have been directed towards the characterization of these properties, the related molecular mechanisms and the potential chemotherapeutic applications of sesquiterpene lactones. All the sesquiterpene lactones contain a common functional structure, an α-methylene-γ-lactone group and this important chemical characteristic means

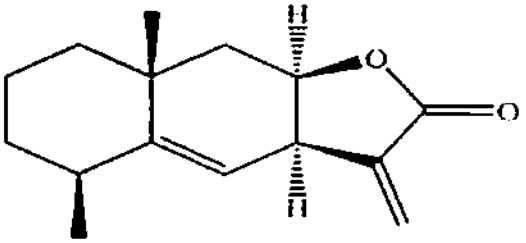
that the thiol-reactivity of sesquiterpene lactones is an underlying mechanism responsible for their bioactivities.

Nowadays, microwave rapid heating techniques are widely used in organic synthesis to accelerate organic reactions and substantially reduce reaction times. The microwave region of the electromagnetic spectrum lies between infrared radiation and radio frequencies and corresponds to wavelengths ranging between 1 cm to 1 m. In order to avoid interferences with RADAR and telecommunication applications, domestic and industrial microwave reactors for chemical use operate at 2.54 GHz. In contrast to the conventional heating which involves conduction and convection, microwave energy transfer occurs by dielectric loss.

Chemical selectivity in microwave assisted reactions is an expression of changes in the preferred reaction pathway or mechanism under these conditions. Consequently, the manner in which microwave radiation interacts with reaction systems can be probed by examining the change in selectivity that it induces. Microwave radiations have thus, been applied to carry out a wide range of chemical reactions.

Based on these facts regarding the structure bioactivity inter relationship and the utility of microwave radiations in organic synthesis, it was intended to modify the structure of the lactones by various chemical, stereochemical and microwave assisted transformations that could have marked effects on their growth regulating potential.

Alantolides, guaianolides and pseudoguaianolides are widely distributed in the family Compositae. Alantolactone (1) and isoalantolactone (2) are the major sesquiterpene lactones from *Inula racemosa;* dehydrocostus lactone (3) and costunolide (4) present in chicory and costus roots whereas parthenin (5) is the major constituent of *Parthenium hysterophorus.*



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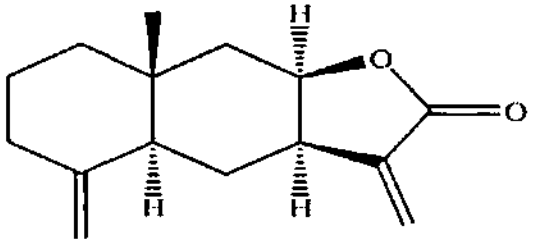

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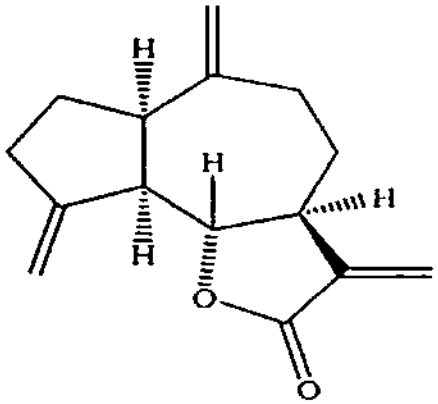

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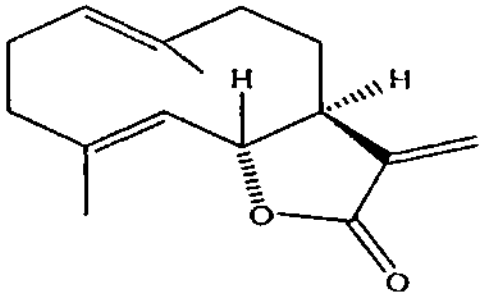

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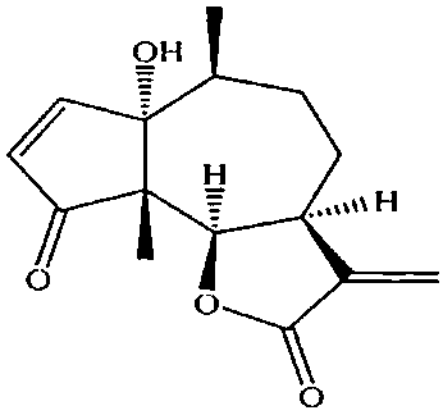
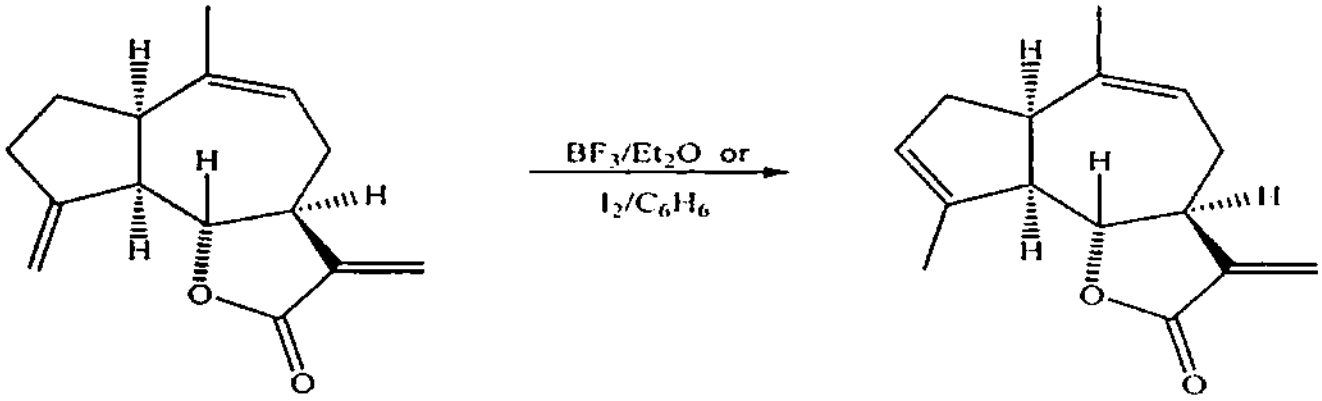


[5]

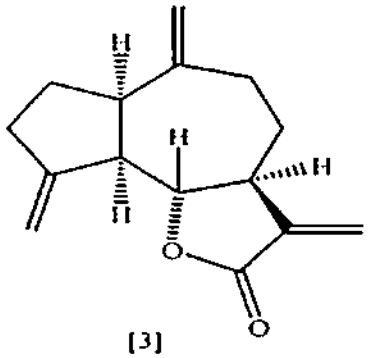
Double bond migration that was observed initially as an additional modification accompanying several organic reactions is now a reaction of great chemical significance.

The double bond migration from *exo* to *endo* position in eremanthine was carried out by Macaira *et al* (1977) using BF_3/etherate. Iodine in dry benzene is also known to cause the isomerisation reaction.



[6] [7]

Acid catalysed isomerisation of dehydrocostus lactone has also been reported in dichloromethane along with which addition of a methoxy group takes place at C_4 exomethylene position (Singh *et al* 1992).



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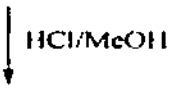
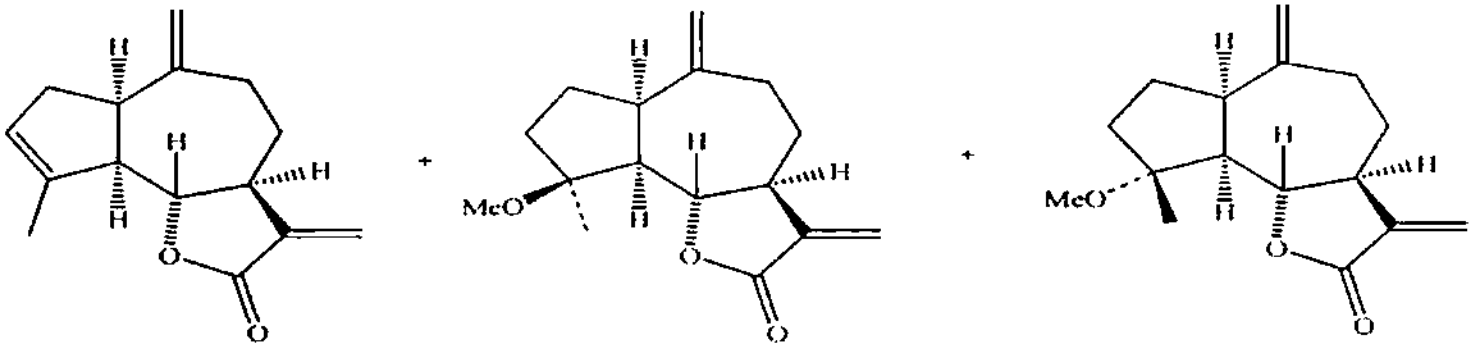




[8] [9] [10]

Three-membered heterocyclic ring of the epoxide offers an uncommon combination of reactivity, synthetic flexibility and atom economy. Emphasis in this field has been given to novel synthetic methods and new insights into existing methodologies for the selective construction and controlled reaction of the title compounds reported in the past few years. Epoxidation of olefins is one of the important reactions in organic synthesis as epoxy compounds are widely used as intermediates in laboratory and for chemical manufacturing (Smith 1984). Research in the area of epoxidation has been carried out for several reasons.

- Oxidation reactions are always challenging in industry in order to achieve satisfactory yields, use of cheapest reagents as well as catalysts with simple and efficient procedures.
- Extensive application in areas ranging from the fine laboratory synthesis to cost effective industrial approach.
- Epoxides form the backbone of many industrially important molecules.

Allylic oxidations also hold sustained interest owning to their practical and theoretical importance and a reference to literature reveals the use of wide range of catalysts in concern with several stoichiometric oxidizing agents (Serni and Acar, 2002).

Reductions are another set of chemical reactions that are highly significant in synthetic organic chemistry. Several reducing agents are available that can reduce a vast variety of functional groups and at the same time exhibit selectivity in their action.

Literature reveals growth regulating properties of many naturally occurring plant constituents. In some cases, the results obtained in the bioassays with synthetic analogues of active substances have been used as a basis for proposing relationship between stereostructure and biological activity. In case of the sesquiterpene lactones, it is the α-methylene-γ-butyrolactone moiety with which their biological activity is associated.

The importance of the above mentioned classes of chemical reactions and the structural significance that they impart to the variously modified

moieties both from synthetic and bioregulation points of view, inspired us to direct the research with the aim to introduce novel structural variations in some naturally occurring sesquiterpenoids. The reactions were carried out both by conventional methods and using microwave radiations to obtain chemically transformed products that were evaluated for their plant growth regulatory effects.

Chemical transformations included the double bond migration reaction in case of dehydrocostus lactone and isoalantolactone and a few monoterpenes under microwave irradiated conditions. In order to further prove the utility of microwave irradiation in chemically modifiying various functional groups, several microwave-mediated reactions were carried out which included the decomposition of pyrazoline derivatives of the sesquiterpenes, Hoffmann elimination of some quaternary ammonium salts and the decomposition of Mannich bases to their respective arylidenes.

All these reactions were achieved successfully under microwave-irradiated conditions with a significant enhancement in reaction rates coupled with amelioration in yields of the products.

Other chemical transformations included epoxidation of the olefinic bonds using sodium perborate in various solvent systems in comparison with the conventional epoxidation reagent, perbenzoic acid; allylic oxidations of the sesquiterpene lactones were carried out using selenium dioxide and *tert.*-butyl hydroperoxide.

Parthenin obtained from the extract of congress grass was employed as the substrate for reduction with various reagents like sodium borohydride, zinc borohydride, sodium cyanoborohydride and lithium tri-*tert.*-butoxy aluminohydride with the aim to compare the selectivity of the reducing agents. Parthenin and anhydroparthenin were also subjected to reaction with magnesium in methanol.

All the chemically modified products obtained from various reactions were evaluated for their plant growth regulatory effects in comparison with the naturally occurring sesquiterpenoids in order to study the influence of

structural modifications on the growth regulating properties with the aim to establish and further clarify the structure activity relationships.

The biological testing studies included the screening of seed germination and seedling growth behaviour of pea (*Pisum sativum*) and two weed species *viz. Avena fatua* (wild oats) and *Phlaris minor*. Data pertaining to per cent germination, length of root and shoot, fresh and dry weight of seedlings and leaf chlorophyll content were recorded.

The dissertation has been divided into five chapters. Chapter I comprises introduction. Chapter II presents a review of literature regarding the chemistry of various sesquiterpene lactones reported from different plant sources and their biological activity. Chapter III deals with the material and methods employed for research. Chapter IV describes the results obtained during the present investigation and their discussion. Chapter V summarizes all the work presented in the dissertation. The numbers ascribed to the structures are concordant for chapters III and IV. The references cited in the text are alphabetically arranged at the end of the dissertation.

CHAPTER II

REVIEW OF LITERATURE

Natural products represent a rich source of biologically active compounds and are an example of molecular diversity, with recognized potential in drug discovery and development. In recent years, a nearly exponentially increasing number of these natural secondary metabolites being employed in research, has led to the development of a new perspective of combinatorial chemistry in which natural product chemistry complements on a synergistic perspective, since nature continues to be the most diverse and active compound library known.

Secondary metabolites are chemical compounds derived from living organisms. The study of natural products involves isolation in a pure form of these compounds and investigation of their structure, formation, use and purpose in the organisms. Secondary metabolites appear to function primarily in defense against predators and pathogens, and in providing reproductive advantage as intra-specific and inter-specific attractants (Seigler 1998).

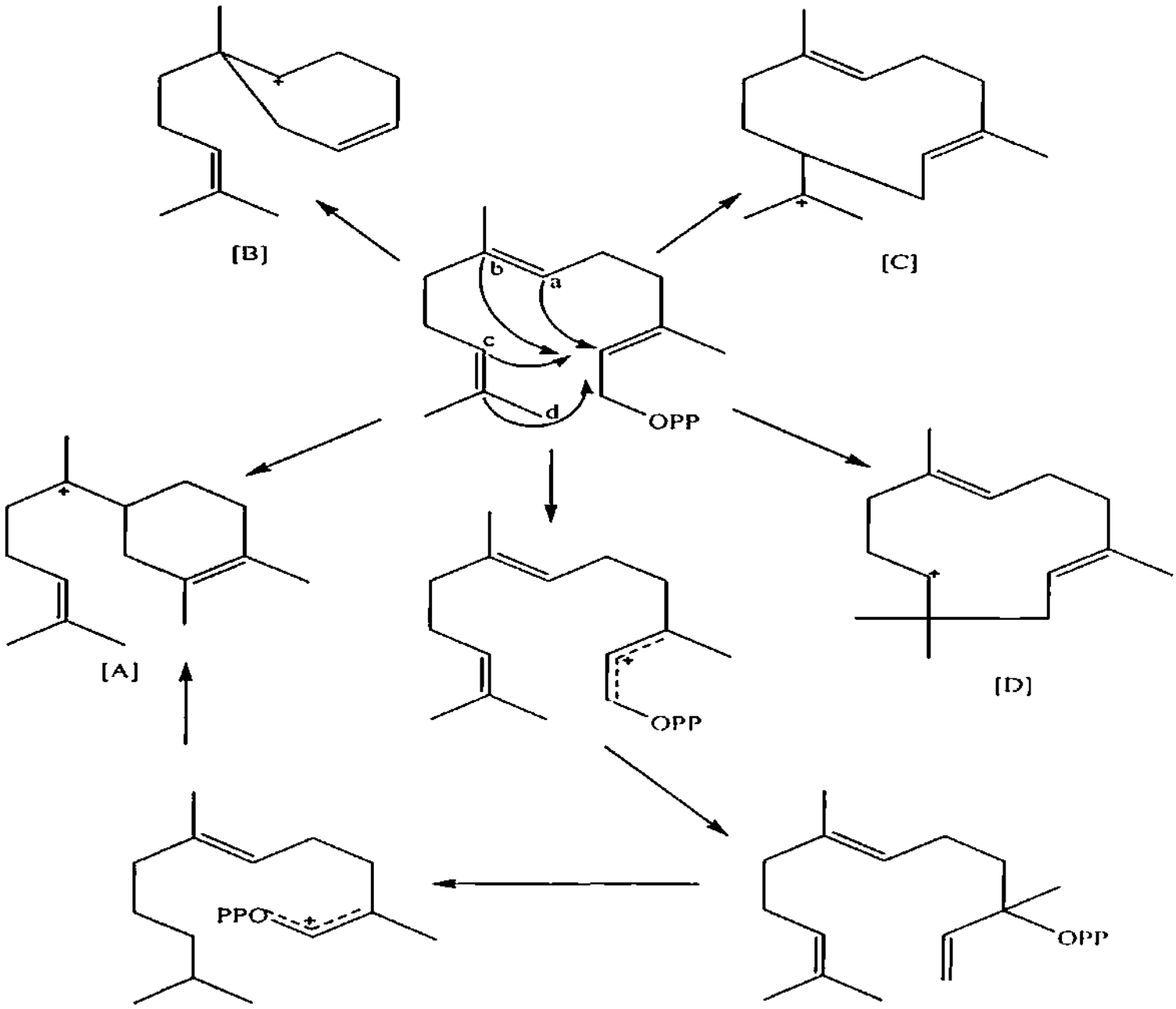
Most natural products can be classified into a few groups: acetogenins as well as propanogenins, terpenoids, derivatives of amino acids, and aromatic compounds. Many plant terpenoids are toxins and feeding deterrents to herbivores or are attractants and many posses pharmacological activity. Throughout evolution, plants have developed defenses against herbivory and microbial attack and produced other natural products to foster competitiveness. The study of natural products has had a number of rewards. It has led to the discovery of a variety of useful drugs for the treatment of diverse ailments and contributed to the development of separation science and technology, spectroscopic methods of structure elucidation and synthetic methodologies that now make up the basis of analytical organic chemistry.

The theory that provided the first conceptual framework for a common structural relationship among the terpenoids was formulated by Wallach in 1887 after carrying out structural investigations of several terpenes. His

theory stated that terpenoids can be viewed as made up of two or more isoprene (2-methyl-1,3-diene) units joined together in a head to tail manner. Wallach's idea was further refined in the 1950 by Ruzicka's formulations of the biogenetic isoprene rule, emphasizing the mechanistic considerations of terpenoid synthesis in terms of electrophilic elongations, cyclizations and rearrangements.

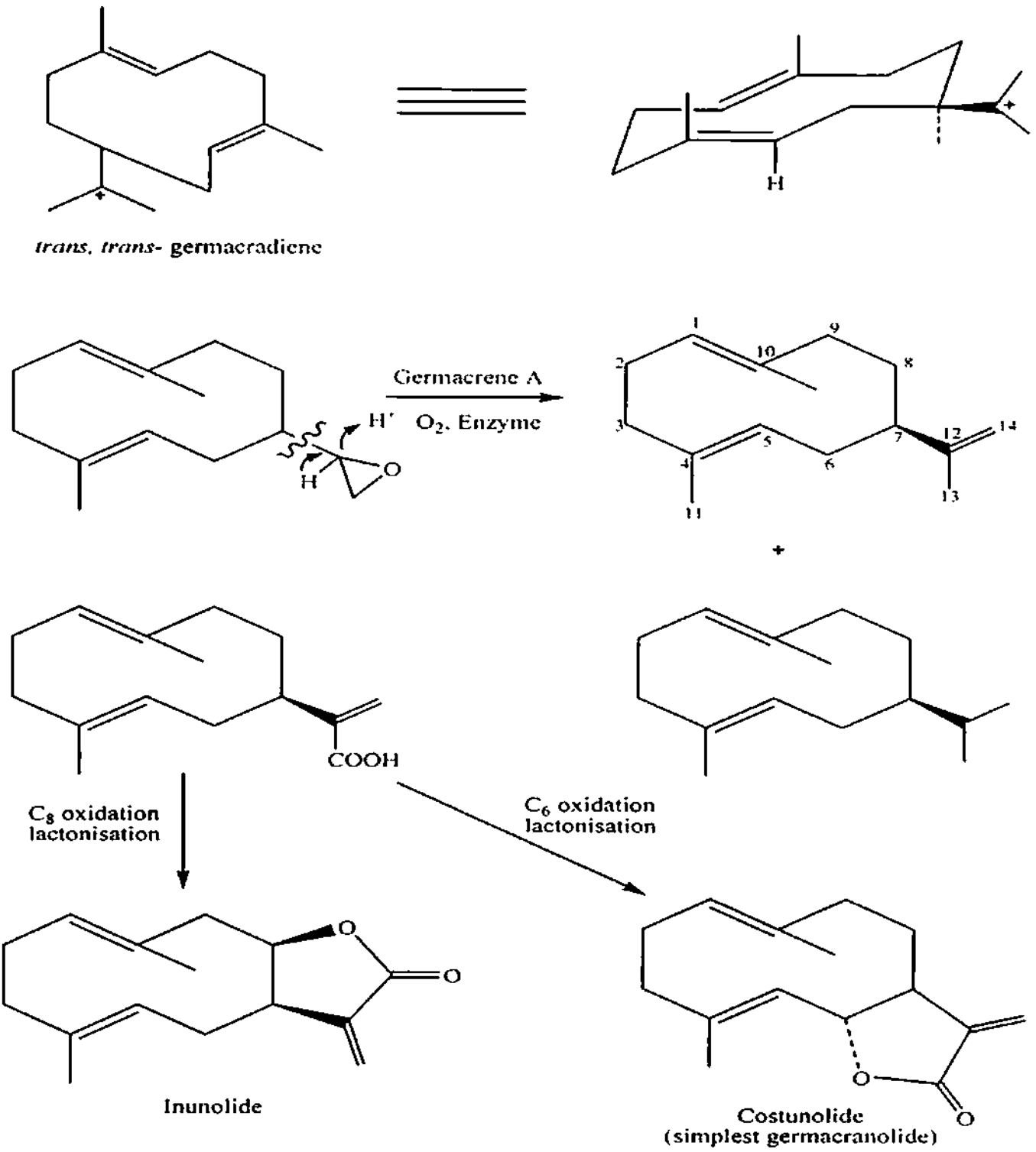
Among the terpenoids, sesquiterpene lactones are a major class of plant secondary metabolites that are mainly found in the family Asteraceae but also occur infrequently in other high plant families and lower plants. Sesquiterpene lactones are formed from head-tail condensation of three isoprene units and subsequent cyclization and oxidative transformation to produce a *cis*- or *trans*- fused lactone. These secondary compounds are primarily classified on the basis of their carbocyclic skeletons into germacranolides, guaianolides, eudesmanolides, pseudoguaianolides and xanthonolides. The suffix 'olide' refers to the lactone function and is based on costunolide, a germanocranolide which is related to the ten-membered carbocyclic sesquiterpene, germacrane.

According to the accepted hypothesis as originally formulated by Ruzicka and subsequently elaborated by several groups (Richard and Hendrickson 1964, West 1981, Parker *et al* 1967), the formulation of all sesquiterpenes can be accounted through A, B, C or D intermediates (Scheme I).



SCHEME I

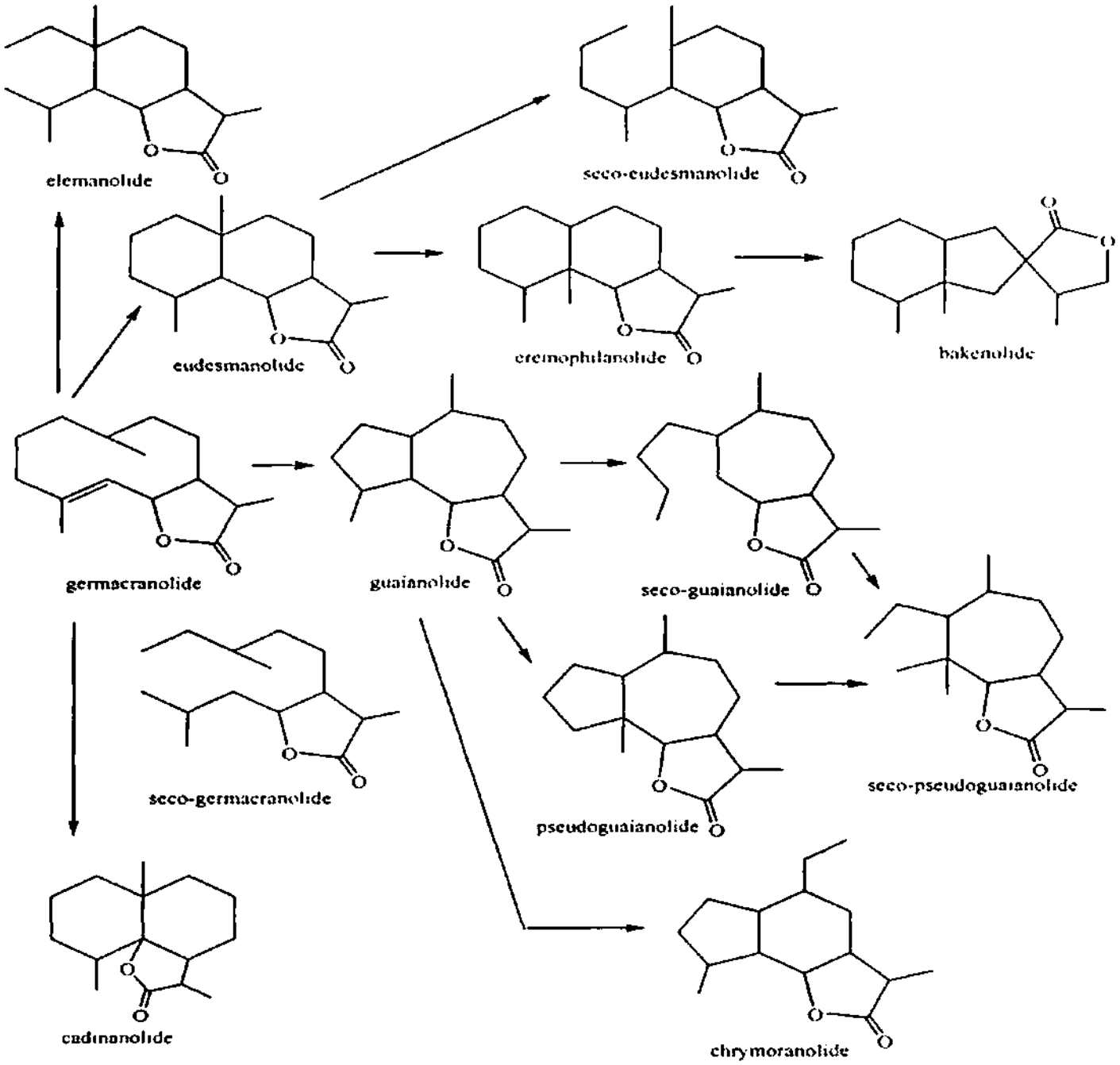
Trans-trans germacranolide intermediate (C) by enzymatic oxidation provides the germacranolides (Scheme II).



SCHEME II

Majority of sesquiterpene lactones representing an α-methylene-γ-lactone moiety or biomodified functionality derived from this, can be

considered by biogenetic derivatives of the largest class, the germacranolides. The presently known structural classes with their carbocyclic ring systems as presented as follows:



SCHEME III

An individual plant species generally produces one skeletal type of sesquiterpene lactone concentrated primarily in the leaves and flower heads. The percentage of sesquiterpene lactone per dry weight may vary from 0.01% to 8.0%.

Sesquiterpene lactones have a distinctive functional group like α,β-unsaturated lactone moiety, which represents a reactive receptor site for biological nucleophiles, in particular, thiol and amino groups. Consequently, a wide spectrum of biological activities involving such lactones has been reported. Some of these biological roles are as anticancer (Douglas 2000), anti-inflammatory (Cho and Baik 2000, Schinella *et al* 1998), anti-mycobacterial (Cantrell *et al* 1998), antifungal (Tan *et al* 1998), antitumor (Cho and Park 1998), cytotoxic (Robles *et al* 1997, Lee *et al* 2005), antimicrobial (Wayuono *et al* 1992), antimitotic and anti-histosomal (Ando *et al* 1987), nematicidal (Mahajan *et al* 1986), molluskicidal agents (Fronczek *et al* 1984) and as plant growth regulators (Chhabra *et al* 1998).

Chemotaxonomic importance of the natural sesquiterpene lactones, as well as their prominent and diverse physiological activity, is the major reason for continuous interest regarding these compounds. As stated earlier, this activity is most likely associated with the α-methylene-γ-lactone part of a molecule. Nevertheless, in addition to structural demands, it is well known that geometry of the entire molecule is important for the expression of biological activity. Accordingly, elucidation of conformational properties of these compounds could be interesting. During the last few decades, an appreciable number of sesquiterpene lactones has been isolated from the plant sources and chemically transformed with the aim of relating variable biological properties with the variation in functional moieties associated with the molecule.

The Costus Plant

Botanical name : *Saussurea lappa*

Plant family : Asteraceae

Common names : Costus, Kushtha, Qusht Shirin

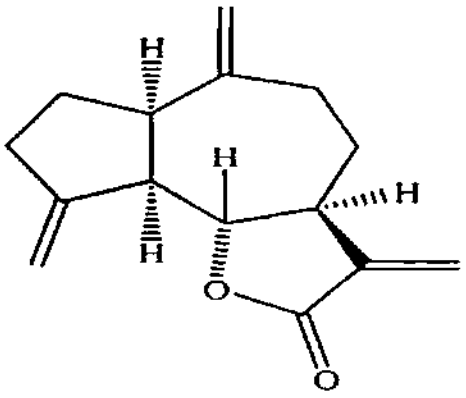
Origin: *S. lappa* is a high altitude plant growing on the moist slopes of the Himalayas at altitudes of 8,000-12,000 feet in Kashmir, Himachal Pradesh, Lahul-Spiti etc.

Phytochemical constituents: The roots of the plant contain an essential oil, alkaloid saussurine and a bitter resin. The resinoid on distillation with superheated steam under reduced pressure yields an essential oil. The chemical constituents of the plant include heptadecatetraene, 22-dihydrostigmasterol, 3-isopropylpentanoic acid, 3-methylbutyric acid, 4-ethyloctanoic acid, 7-octenoic acid, acetic acid and alkaloids. The major sesquiterpene lactones present in the plant are costunolide, costus acid, costus-lactone, dehydrocostus lactone, dihydro-dehydrocostus lactone, dihydrocostunolide, dihydrocostus lactone, isozaluzanin and isoalantolactone.

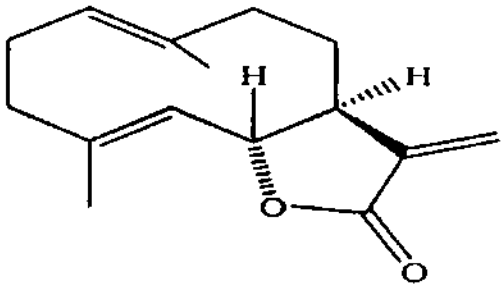
Medicinal parts used: Roots

Medicinal uses: *Saussurea* is beneficial in the treatment of respiratory disorders like bronchitis, asthma and cough. The combined action of the essential oil and the alkaloid in the root restricts the paroxysms. The alkaloid saussurine has a depressant action on the vagus center in the medulla. The root has a pungent taste and a peculiar fragrance. It strengthens the functioning of the stomach and promotes its action. It is also helpful in arresting secretion or bleeding.

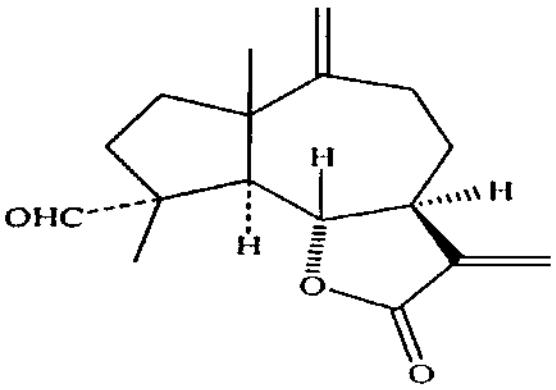
The essential oil has antiseptic and disinfectant properties. It releases the involuntary muscle tissues and serves as a cardiac stimulant. The reflex inhibition effect of the essential oil is produced due to its stimulatory properties.



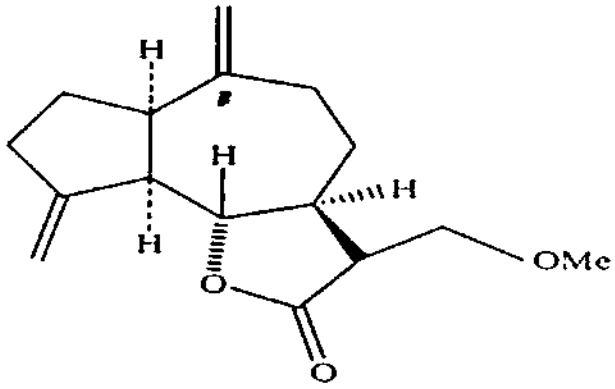
Dehydrocostus lactone [1]



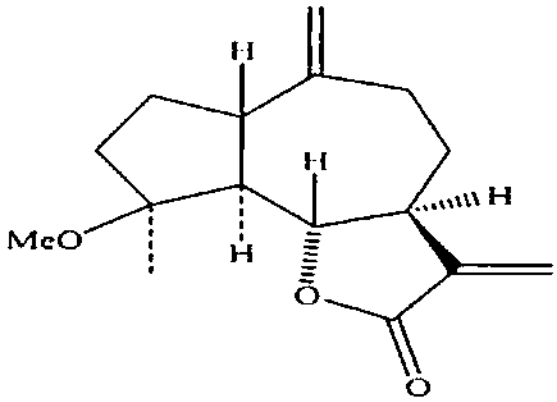
Costunolide [2]



Saussureal [3]



13-methoxydihydrodehydrocostus lactone [4]



4-β-methoxydehydrocostus lactone [5]

Pushkar

Botanical name : *Inula racemosa*

Plant family : Asteraceae

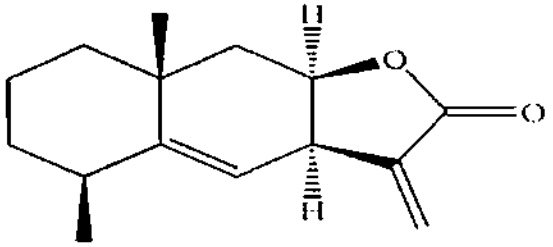
Common names : Pushkar, pushkarmoola

Origin: The plant grows wild in Temperate and Alpine Western Himalayas and commonly found in Kashmir.

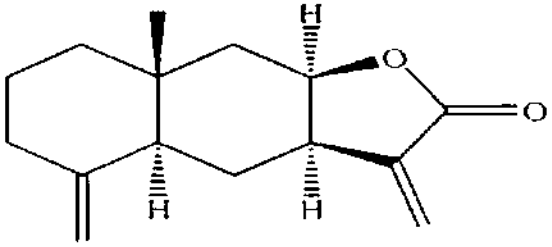
Phytochemical constituents: The plant is predominantly constituted by the eudesmanolide group of sesquiterpene lactones. These include alantolactone, isoalantolactone, inunal and isoalloalantolactone. The roots also contain an essential oil, α-ionone, phellandrene, resinoids, taraxasterol, cedrene, cedrol etc.

Medicinal parts used: Roots

Medicinal uses: The plant is an effective antispasmodic, beta-blocker, an antianginal and hypotensive agent. It is thus useful in cardiovascular conditions of angina pectoris and other heart diseases. The use of *Inula* has been reported to decrease the total cholesterol by 39%, triglycerides by 51% and total blood lipids by 32%. *Inula racemosa* especially combined with *Commiphora mukul* is useful in ischemic heart disease and has been shown to be superior to nitroglycerine in human trials (Tripathi *et al* 1984).



Alantolactone [6]



Isoalantolactone [7]

Congress Grass

Botanical name : *Parthenium hysterophorus*

Plant family : Asteraceae

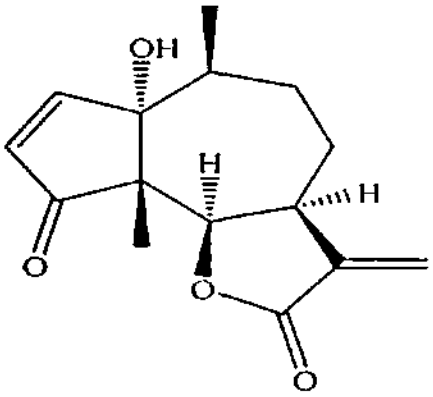
Common names : Congress weed, stenweed, fever few, white top, chatak chandani, bitter seed, carrot weed.

Origin: It is believed to have entered India accidentally in the mid 1950s and is now considered as one of the most feared noxious weed species (Rao, 1956).

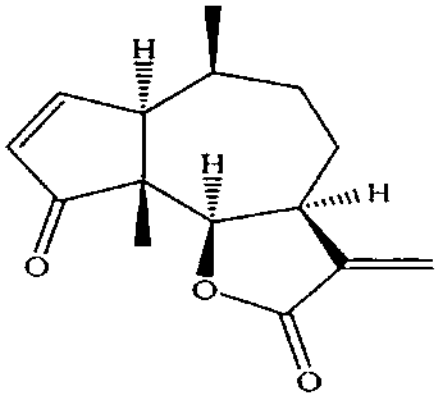
Phytochemical constituents: The major toxin component of the plant is parthenin. The other sesquiterpenoids include coronopilin, dihydroparthenin, hysterin, ambrosin, hymenin, dihydroisoparthenin. A rare lignan, (+)-syringaresinol has also been reported (Venkataiah *et al* 1999). Some phenolic acids are also present (Mahadenappa 1997). These include cafferic acid, vanillic acid, anisic acid, *p*-anisic acid, chlorogenic acid and *p*-hydroxy benzoic acid.

Medicinal parts used: Roots

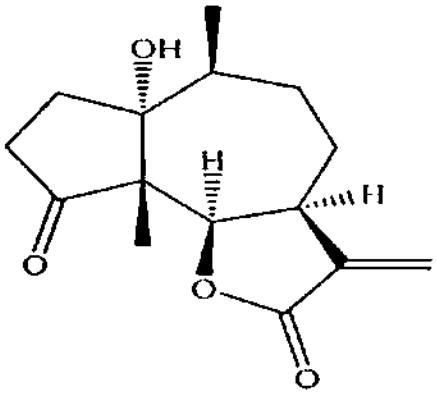
Medicinal uses: Root decoction is useful in dysentery (Singh *et al* 1996). Sublethal doses of parthenin exhibit antitumor activity in mice (Mew *et al* 1982). *Parthenium* is also reported as promising remedy against hepatic amoebiasis (Sharma and Bhutani 1988). It is used as fold remedy in the Caribbean and Central America. It is applied extensively on skin disorders and decoction of the plant is often taken internally as a remedy for wide variety of ailments (Dominguez and Sierra 1970). In Jamaica, the decoction is used as a flea repellant for dogs and other animals (Morton 1981).



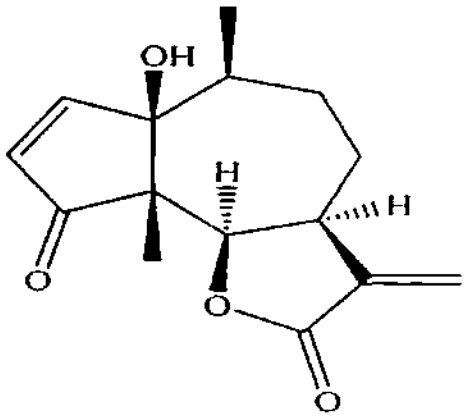
Parthenin [8]



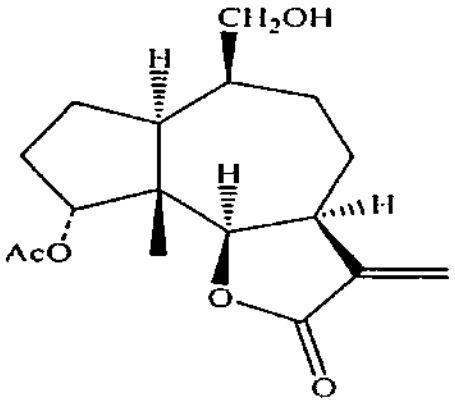
Ambrosin [9]



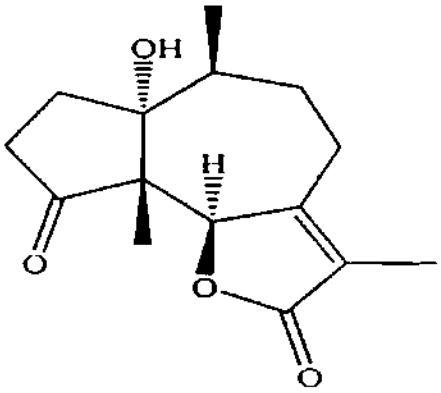
Coronopilin [10]



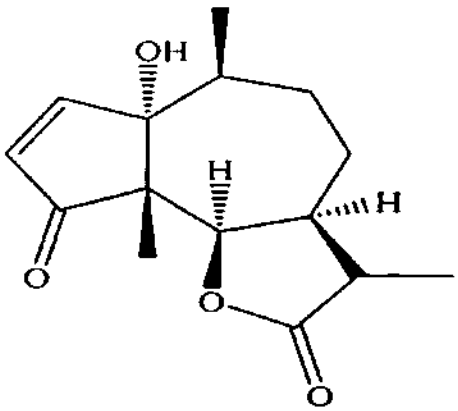
Hymenin [11]



[12]



Dihydroisoparthenin [13]



Dihydroparthenin [14]

Chicory

Botanical name : *Cichorium intybus*

Plant family : Asteraceae

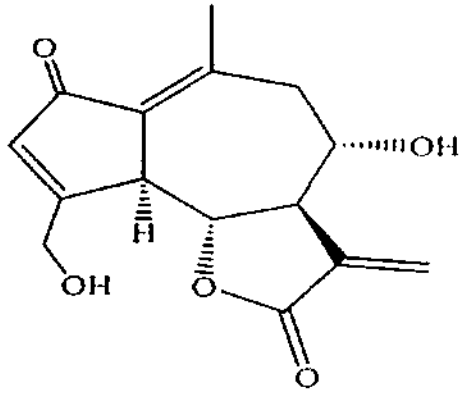
Common names : Chicory, succory, wild succory, hendibeh

Origin: Chicory is a prolific plant that can grow in all types of soils. Some consider it a weedy nuisance, while others appreciate its stately beauty and culinary virtue. It is more common on gravel as chalk, especially on the downs of the south-east coast.

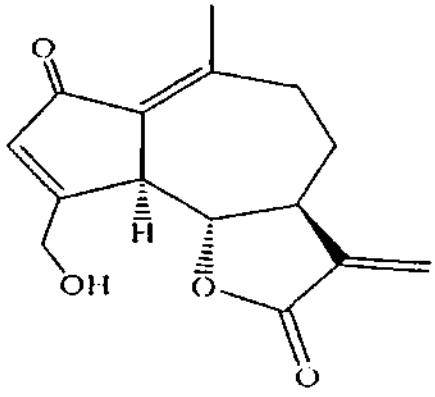
Phytochemical constituents: The major sesquiterpene lactones are lactucin, 8-deoxylactucin and lactucopicrin. Sesquiterpene lactone glycosides include crepidiaside B, sonchuside A, ixerioside D, cichorioside B, dichorioside C (Malarz *et al* 2002). Chicory roots also contain inulin, tartaric acid, hyperoside and some hydroxycoumarins.

Medicinal parts used: Roots

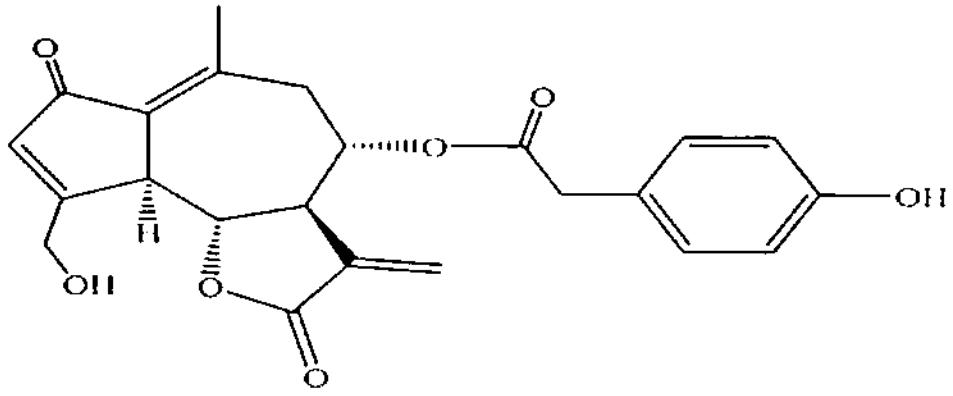
Medicinal uses: Chicory is used to treat skin disorders, gout, jaundice and to reduce an enlarged liver. As a poultice, chicory is thought to improve inflammations, swellings, bruises and eye disorders. In India, the plant is often used by herbalists to treat dyspepsia, vomiting, diarrhoea, headache and skin allergies. Animal studies have revealed that chicory preparations can lower pulse rate and cholesterol level in rat liver and plasma (Bischoff *et al* 2004).



Lactucin [15]



8-deoxylactucin [16]



Lactucopicrin [17]

Microwave chemistry

Microwave chemistry involves the use of microwave radiations to conduct chemical reactions and essentially pertains to chemical analysis and chemical synthesis. Microwaves lie in the electromagnetic spectrum between infrared waves and radio waves. They have wavelengths between 0.01 and 1.0 metre, and operate in a frequency range between 0.3 and 300 GHz. However, for their use in laboratory reactions, a frequency of 2.45 GHz is preferred, since this frequency has the right penetration depth for laboratory reaction conditions.

Thermally driven organic transformations take place by either of two ways: conventional heating or microwave-accelerated heating. In the first way, reactants are slowly activated by a conventional external heat source. Heat is driven into the substance, passing first through the walls of the vessel in order to reach the solvent and reactants. This is a slow and inefficient system. In the second way, microwaves couple directly with the molecules of the entire reaction mixture, leading to a rapid rise in temperature. Since the process is not limited by the thermal conductivity of the vessel, the result is an instantaneous localized superheating of any substance that will respond to either dipole rotation or ionic conduction – the two fundamental mechanisms for transferring energy from microwaves to the substance(s) being heated (Hayes 2002).

The rate of reaction is determined by the Arrhenius equation

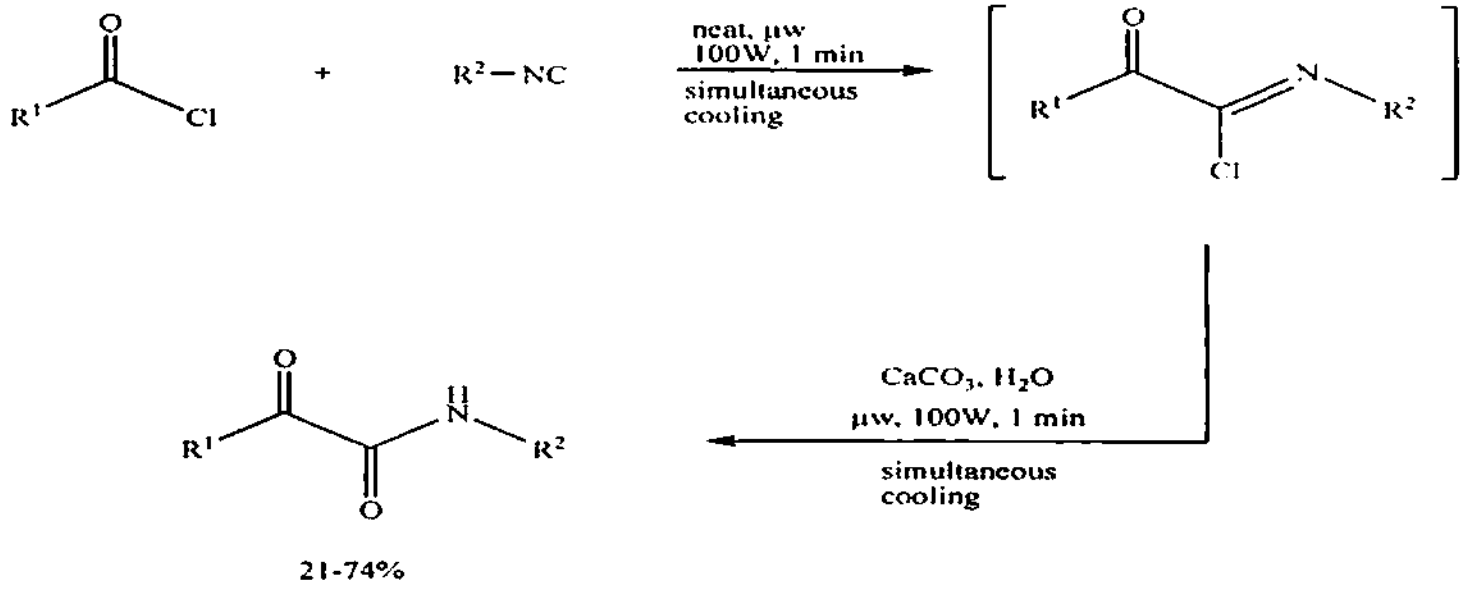
$$k = Ae^{-Ea/RT}$$

where T is the absolute temperature that controls the kinetics of the reaction. In conventionally heating reactions, this temperature is a bulk temperature (T_B). Microwave-assisted reactions are different. Microwave irradiation directly activates most molecules that possess a dipole or are ionic. Since energy transfer occurs in less than a nanosecond (10^{-9}s), the molecules are unable to completely relax (~10^{-5}s) or reach equilibrium. This creates a state of non equilibrium that results in a high instantaneous temperature (T_i) of the molecules and is a function of microwave power input. The instantaneous temperature is not directly measurable, but it is much greater than the measured T_B ($T_i >> T_B$). Thus, the greater the intensity of microwave power being administered to a chemical reaction, the higher and more consistent T_i will be.

Based on experimental data from numerous studies that have been performed over the last ten years, chemists have found that microwave-enhanced chemical reaction rates can be faster than those of conventional heating methods by as much as 1000-fold.

Recently, an alternative method for performing microwave-assisted organic synthesis, termed "Enhanced Microwave Synthesis" (EMS), has been examined (Hayes and Collins 2004). By externally cooling the reaction vessel with compressed air, while simultaneously administering microwave irradiation, more energy can be directly applied to the reaction mixture. Simultaneous cooling thus enables a greater amount of microwave energy to be introduced into a reaction, while keeping the reaction temperature low. This results in significantly greater yields and cleaner chemistries.

EMS was employed in the synthesis of a variety of α -keto amides to support a protease inhibitor discovery project. Acyl chlorides were coupled with various isonitriles and α -keto imidoyl chloride intermediates were formed. These were then hydrolysed to afford α -keto amides (Scheme IV, Chen and Deshpande, 2003).

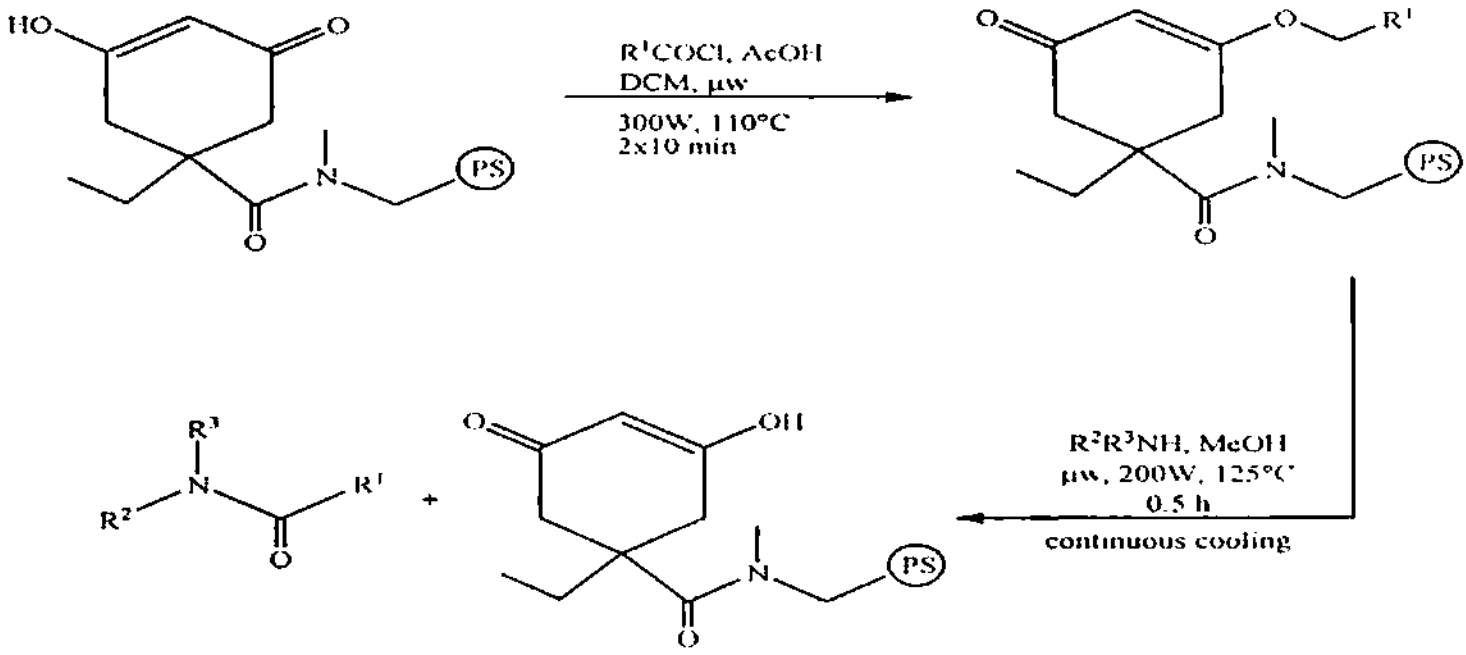


21-74%

R^1= Ph, phenethyl, 4-anisyl, 1-naphthyl
R^2= *n*-Bu, Bn, Cy

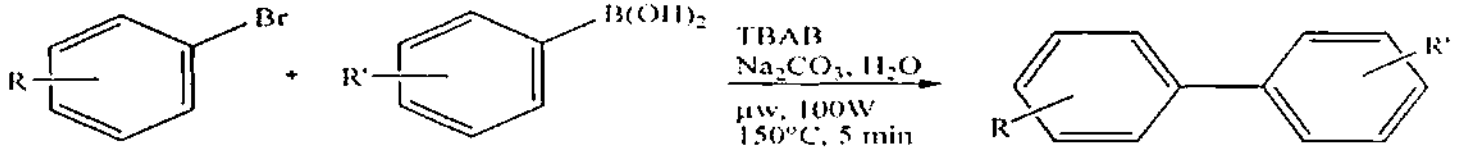
SCHEME IV

EMS has also been beneficial in producing higher release levels of the desired amides from the solid-phase resin, as compared with microwave heating alone (Scheme V, Humphrey *et al* 2003).



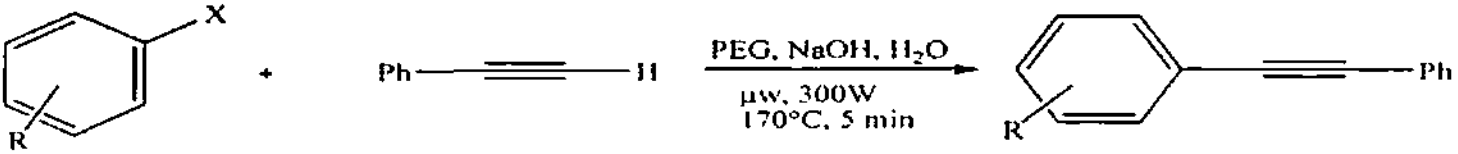
SCHEME V

In organometallic chemistry, two of the most phenomenal recent discoveries are transition-metal free Suzuki and Sonogashira couplings. Leadbeater and coworkers (2003) have shown that reacting an activated aryl bromide with an aryl boronic acid in water, using tetrabutyl ammonium bromide (TBAB) as a phase-transfer catalyst, results in a successfully coupled biaryl Suzuki product without the aid of palladium catalyst.



R= H, 2-Me, 4-Me, 2-OMe, 4- OMe, 4- Ac,
2- Pyr, 2,6- Me_2
R'= H, 4- Me, 4- Ac

In addition, a transition metal free Sonogashira reaction between an aryl bromide or iodide and phenylacetylene results in respectable yields (Leadbeater and Marco 2003).



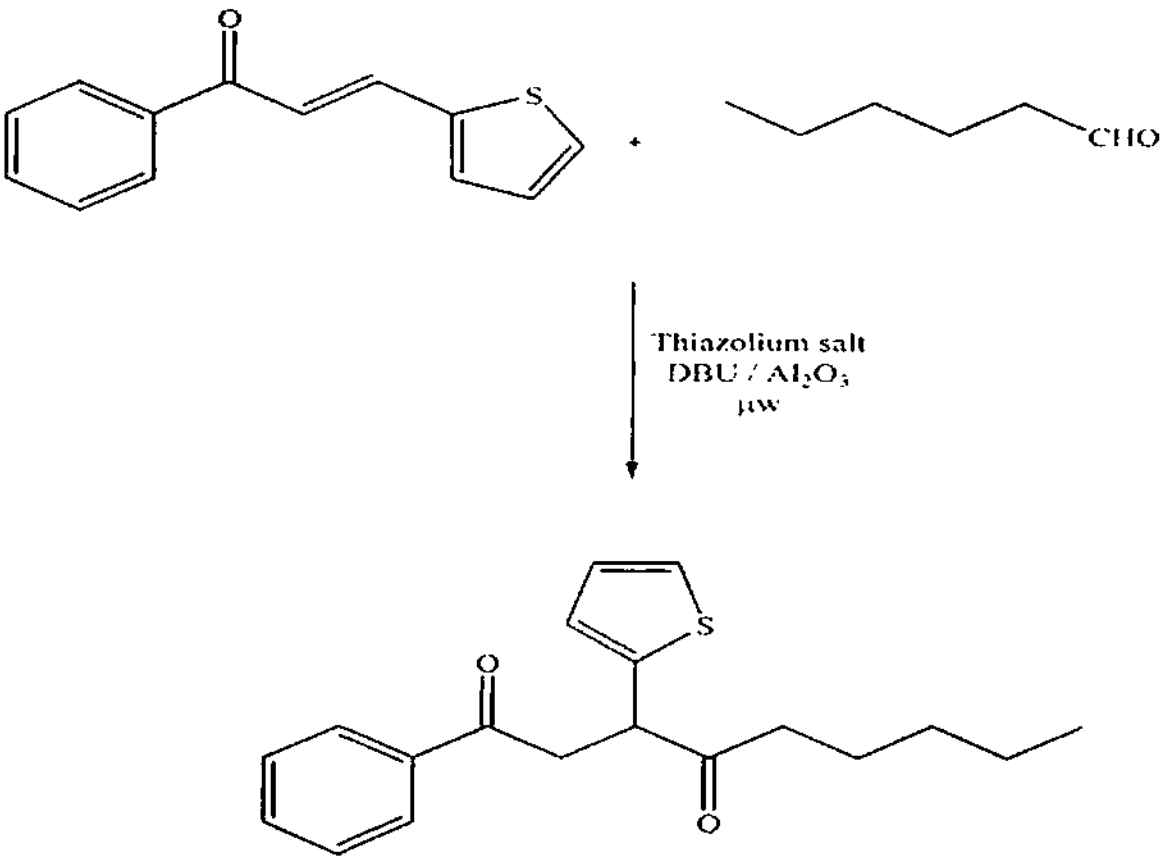
X= Br, I

R= H, 4-Me, 4- Ac, 4- MeO

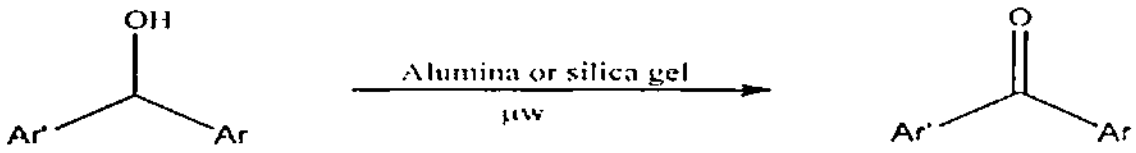
9-92% yields

10-100% conversion

Microwave irradiation has also been reported to accelerate the conjugate addition of aldehydes to α, β- unsaturated ketones using a solid supported reagent system, thiazolium salt-DBU-Al_2O_3 (Yadav et al 2003).



Pesyan and Dabbagh (2005) reported the successful use of microwaves for the oxidation of benzoins to benzils in the presence of alumina and silica oxides as catalysts.



The use of microwave irradiation greatly enhanced the reaction rate and percent yield.

Chemical selectivity in microwave-assisted reactions is an expression of changes in the preferred reaction pathway or mechanism under these conditions. Consequently, the manner in which microwave radiation interacts with reaction systems can be probed by examining the change in selectivity that it induces. Microwave radiations have been successfully employed in the

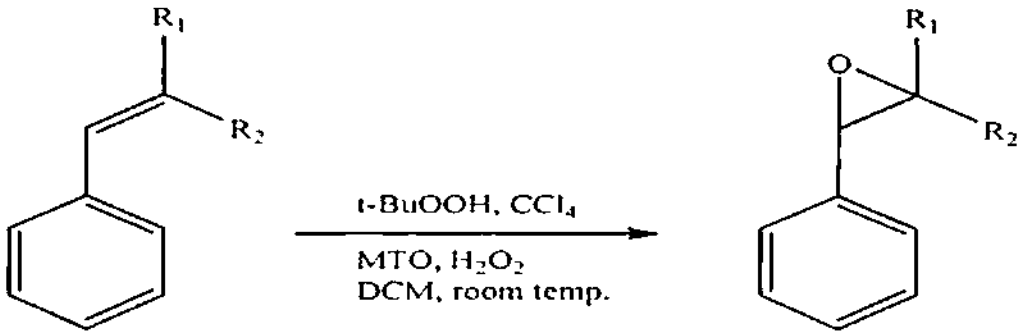
decomposition of pyrazolines of sesquiterpene lactones (Chhabra and Jain 2002).

Microwave radiations have also been employed in combination with UV light to facilitate some important reactions. One such reaction is the combined μw-UV irradiation on 2-*tert*-butylphenol (2TBP) transformation, which was carried out in the absence of sensitizers with different value of singlet and triplet energy and in the presence of solvents with different polarity (Vladimir *et al* 2004).

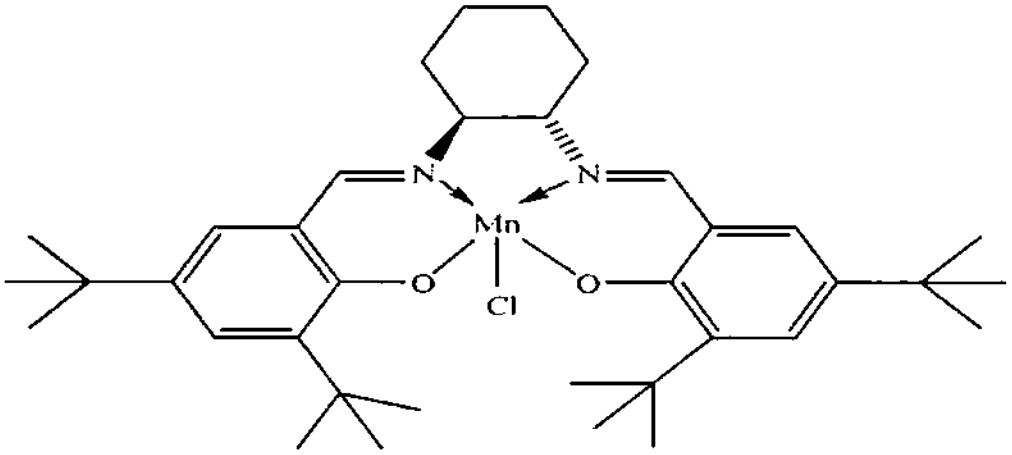
Chemistry of sesquiterpene lactones

Epoxidations

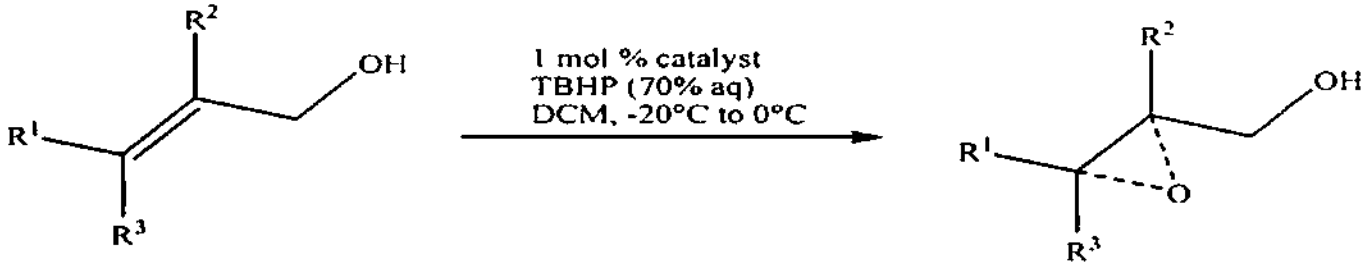
During the last decades, substantial progress has been made in the development of efficient synthetic methods to obtain optically active compounds. Enantioselective alkene epoxidation is an important reaction for synthesis of optically active organic compounds (Rao, 1991). Particularly, the titanium tartarate catalyzed enantioselective epoxidation of allylic alcohols constitutes one of the most widely applied reactions in organic synthesis (Katsuki and Sharpless, 1980). Also, molybdenum peroxo complexes have been reported to catalyse epoxidation of olefins with 50 per cent chemical yield and 40 per cent enantiomeric excess (Chaumette *et al* 1983). Transition metal (W, Mo and Re)- peroxo complexes have also been reported to bring about enantioselective epoxidation of styrene derivatives (Park *et al* 2000).



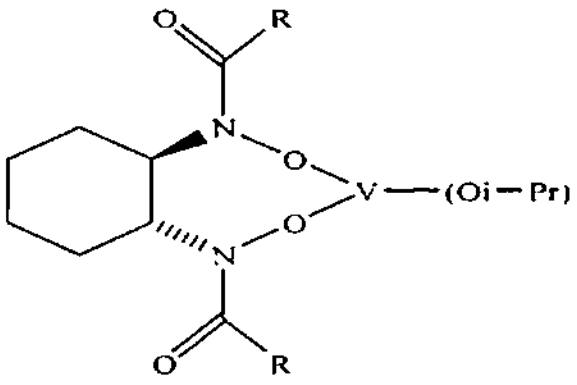
Secondary allylic alcohols have recently been reported to be epoxidised in high *threo* diastereoselectivity by an achiral Mn^{III} (salen) complex with iodosyl benzene as oxygen source (Adam *et al* 2001).



Zhang *et al* (2005) have reported the vanadium-catalysed epoxidation of allylic alcohols in moderate to excellent yields. This catalytic process features robust conditions for easy operation and requires relatively low catalyst loading without the exclusion of moisture and air.



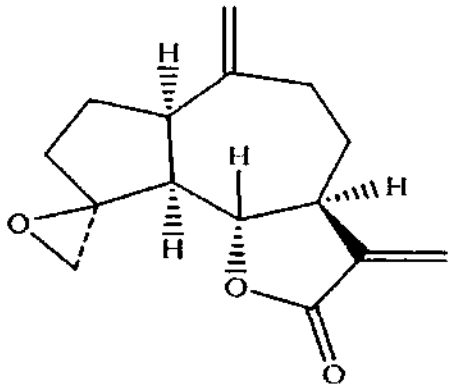
R^1, R^2, R^3= H, Alk, Ar



R= $CHPh_2$, CH_2CPh_3

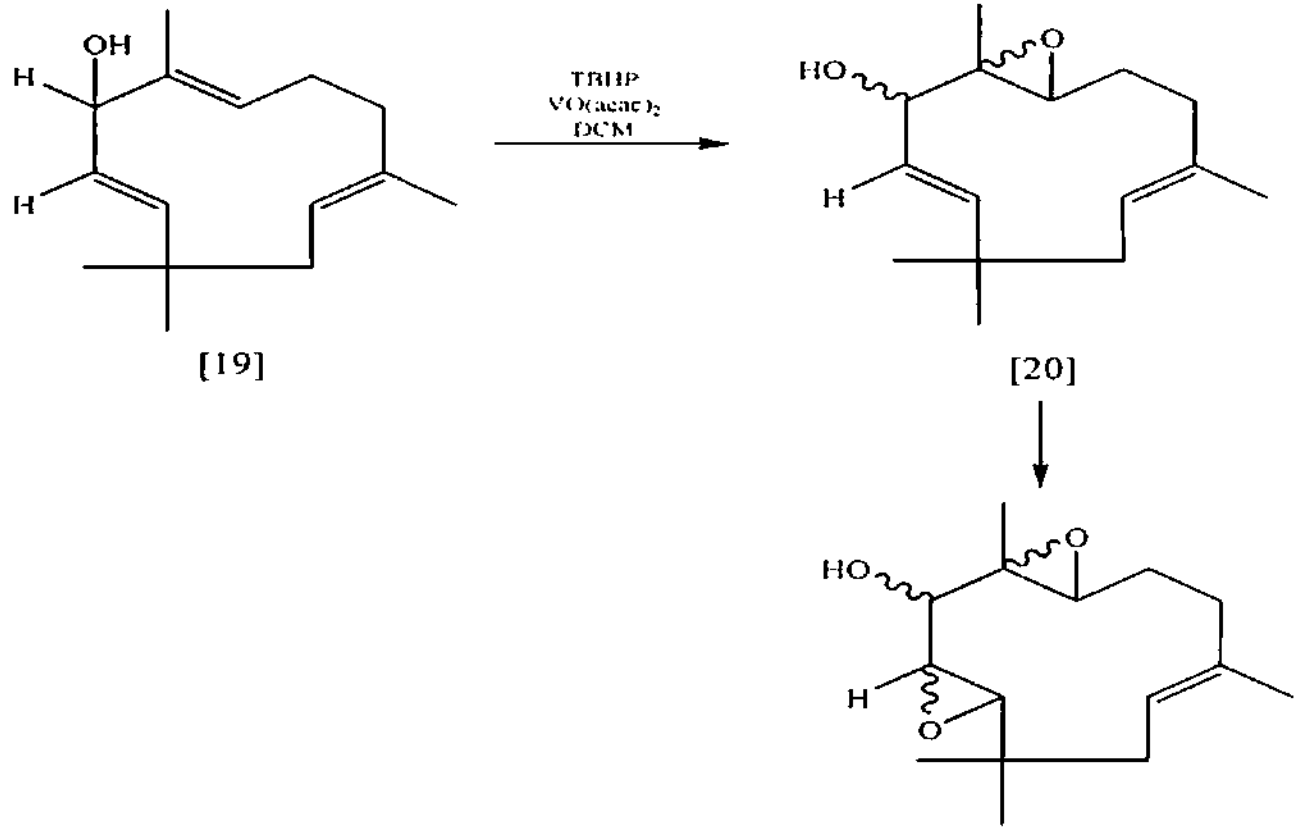
Chiral vanadium catalyst

Perbenzoic acid (PBA) has also been reported to cause epoxidation of olefins. PBA brings about the epoxidation of isolated double bond at different positions in a terpenoid, with difference in their stereochemistry when a functional group is present as is evident in the formation of (18) on treatment of dehydrocostus lactone with one mole of PBA (Kalsi *et al* 1981).

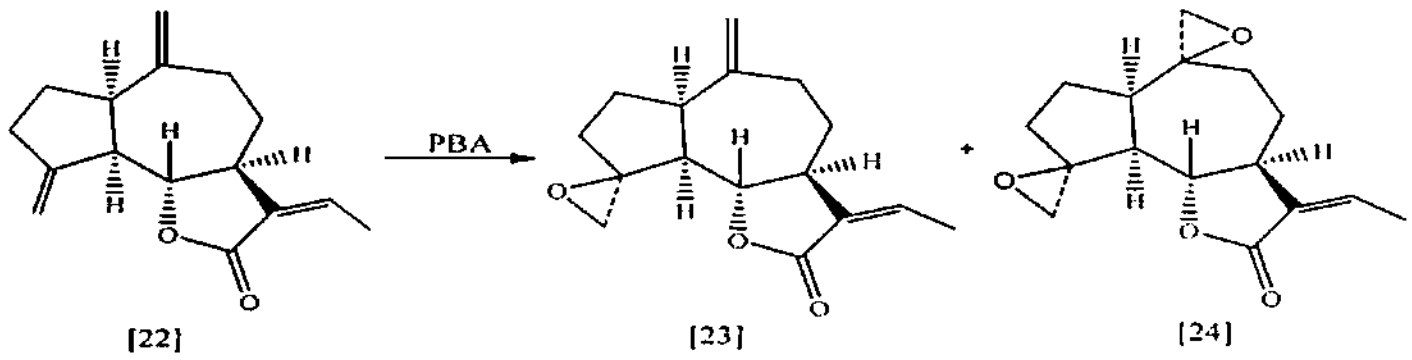


[18]

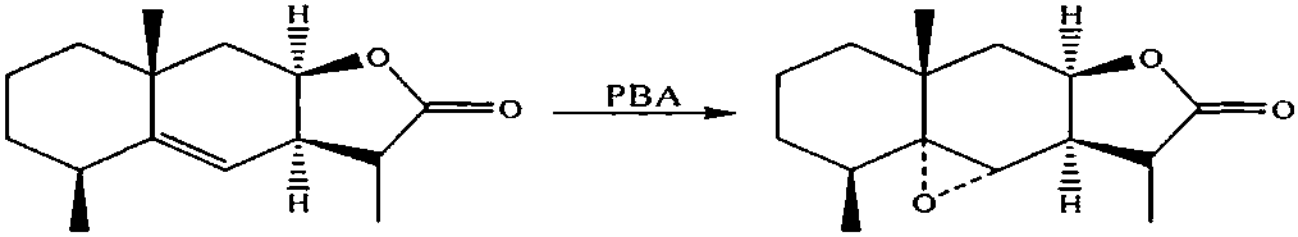
Zerumbol (19) when treated with one mole of TBHP in the presence of catalytic amount of $VO(acac)_2$ in CH_2Cl_2 gave (20) and when (20) was treated with two moles of TBHP under similar conditions, it afforded (21) (Chhabra *et al* 2001).



13-Methyl dehydrocostus lactone which is a pyrolysed product of the pyrazoline of dehydrocostus lactone, on epoxidation with PBA afforded two compounds (23 and 24) (Kalsi *et al* 1981).

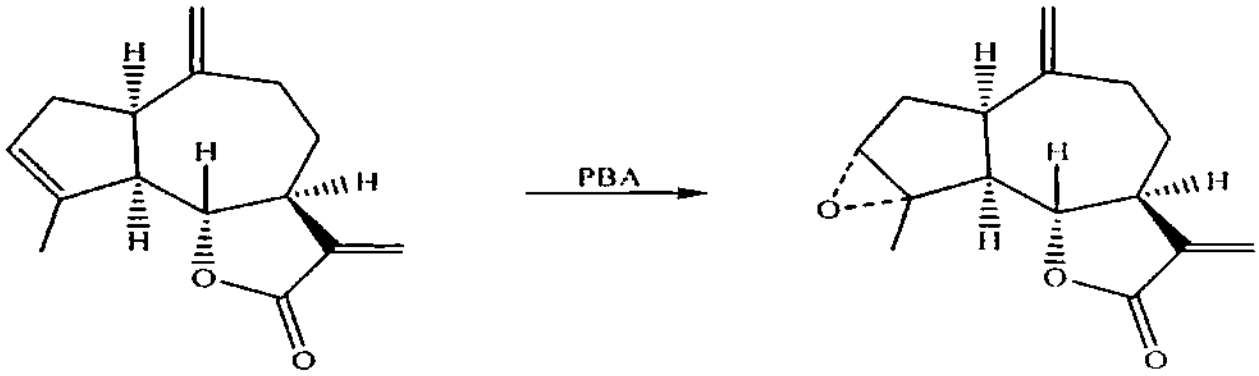


Epoxidation of dihydroalantolactone (25) with PBA results in the formation of 5,6-epoxydihydroalantolactone (26) (Srivastava *et al* 1971).



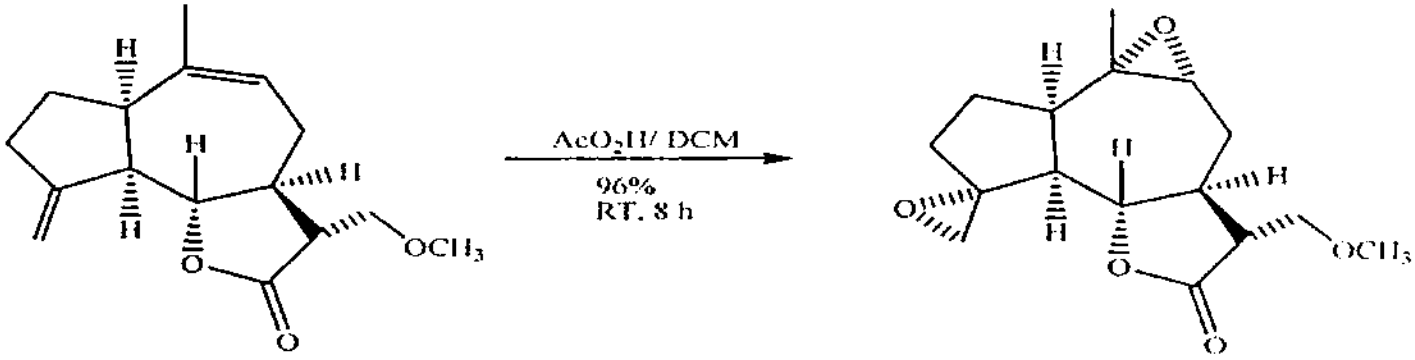
[25] [26]

Estafiatin (28) is formed on epoxidation of isodehydrocostus lactone (27) with PBA (Kalsi *et al* 1983).



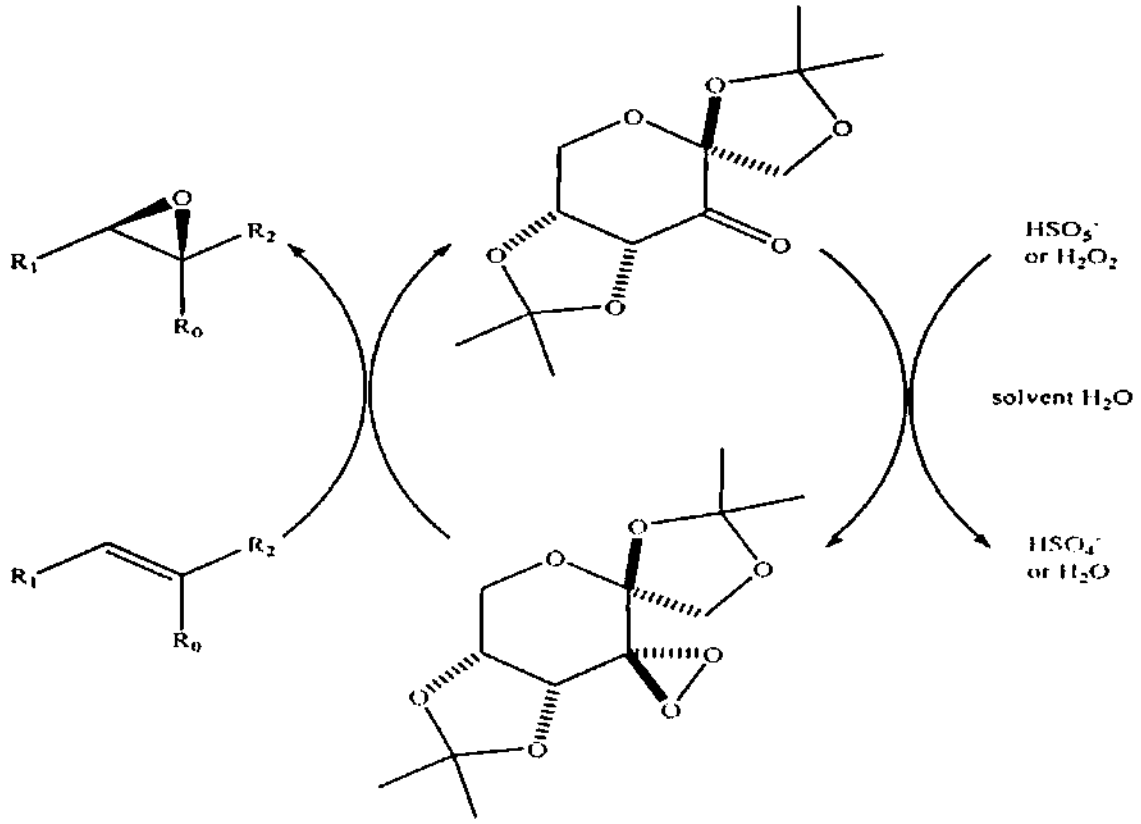
[27] [28]

The methoxy derivative of eremanthine (29) has been successfully transformed into its diepoxide using excess of peracetic acid in dichloromethane (Alves and Fantini 2005).



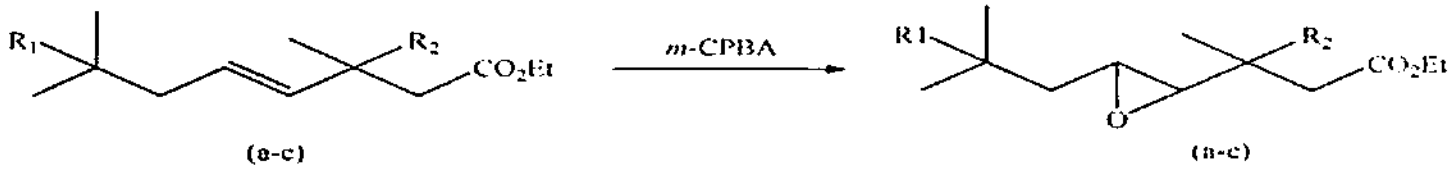
[29] [30]

Recently, a chiral ketone catalyst has been employed to give high enantioselectivities for the epoxidation of *cis*-olefins (Scheme VI, Lopp *et al* 2001).



SCHEME VI

Olejniczak *et al* (2000) have shown the usefulness of *m*-chloroperbenzoic acid in the epoxidation of some racemic esters.



(a-c) (a-c)

a: R_1 = H, R_2 = H
b: R_1 = CH_3; R_2 = H
c: R_1 = H; R_2 = CH_3

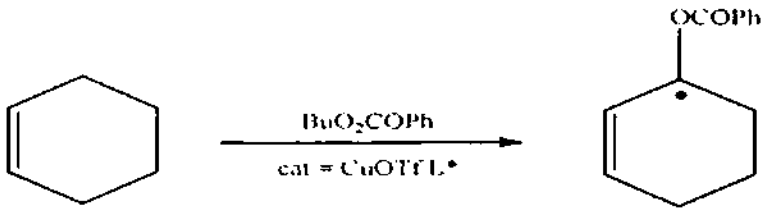
A biological route towards chemical transformations is revealed from the effective use of certain fungi for bringing about modifications in various moieties of sesquiterpenoids. Incubation of the fungi *Cunninghamella echinulata* and *Rhizopus oryzae* with sesquiterpene lactones *viz*. costunolide,

dehydrocostus lactone, salonitenolide and eremantholide has afforded epoxidation products (Barrero *et al* 2000).

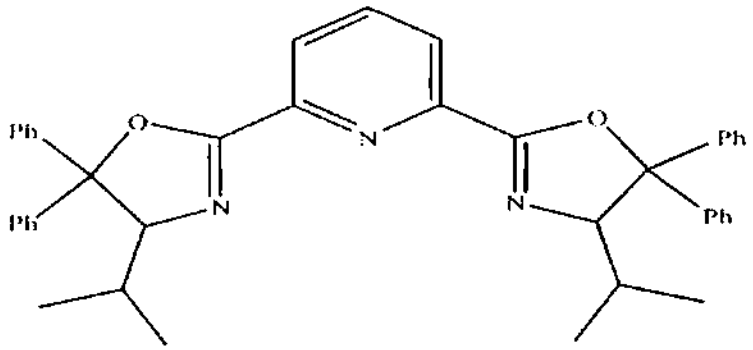
Allylic oxidations

Allylic alcohols are of high importance in chemical industry. Oxidation of alkenes to epoxides, diols and allylic alcohols thus, forms a major part of the modern synthetic methods (Stork and Kahn 1983). The aim therefore, remains to synthesize α, β-unsaturated alcohols by allylic oxidation of olefins in the presence of non-toxic oxidative agents. Oxidizing agents attack either at carbon-carbon double bond or at α-position to the double bond. In the former case, diols, α-ketols or cleavage products are formed while in the latter case, α, β-unsaturated ketones or alcohol derivatives are formed.

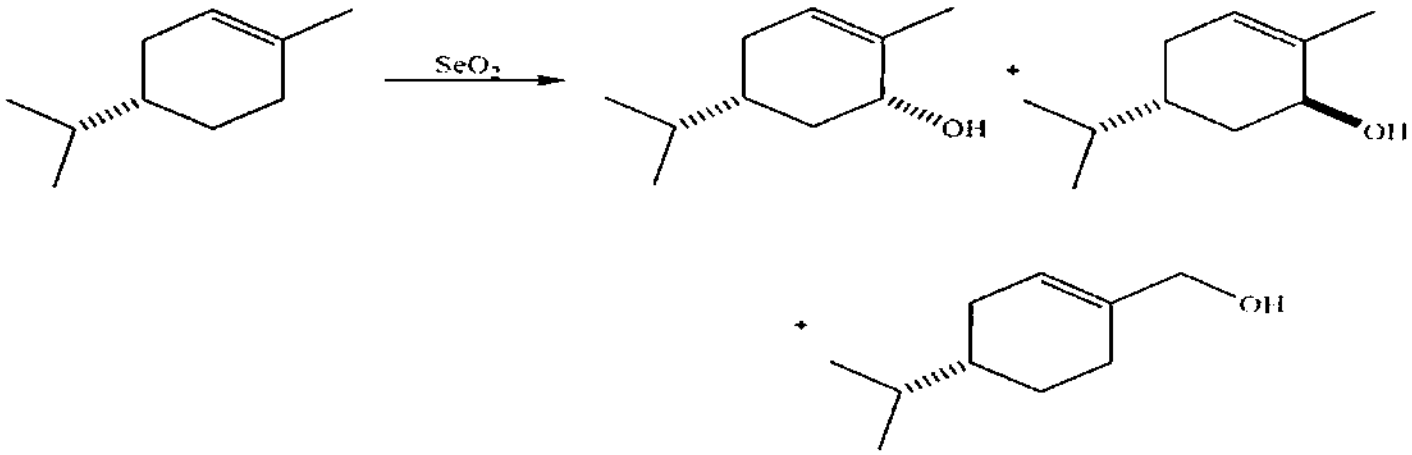
Osmium tetroxide, rhenium tetroxide, permanganate, sodium chromate etc. are some of the various oxidising reagents that react with the carbon-carbon double bond. More recently, the use of chiral catalysts has led to the formation of chiral allylic alcohols as revealed by some studies carried out using cyclic olefins. In this pretext, the most commonly used enantioselectivity inducers are the chiral oxazolines that react in the presence of *tert.*-butyl perbenzoate (Datta Gupta and Singh 1996).



L*

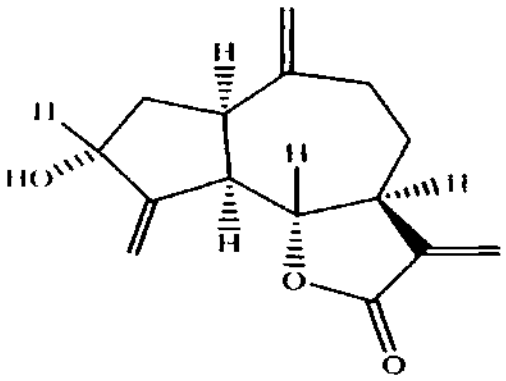


However, selenium dioxide is the most effective reagent for allylic oxidation. SeO_2 can perform several common types of oxidations such as alcohol to ketones and aldehydes as well as dehydrogenation of ketones to enones. Use of SeO_2 for the oxidation of reactive methylene groups to carbonyl groups was introduced by Riley *et al* 1932. This reagent has a great advantage of inserting oxygen directly into an allylic C–H bond. Due to its toxicity and sometimes malodorous Se-containing byproducts formed, SeO_2 is used only where it competes well with other methods, or provides unique reactivity. One of these is the allylic oxidation of alkenes to allylic alcohols with regiocontrol (without migration of the double bond), and a second is the α - oxidation of ketones to α- diketones which is effective only if there are no β-hydrogens.



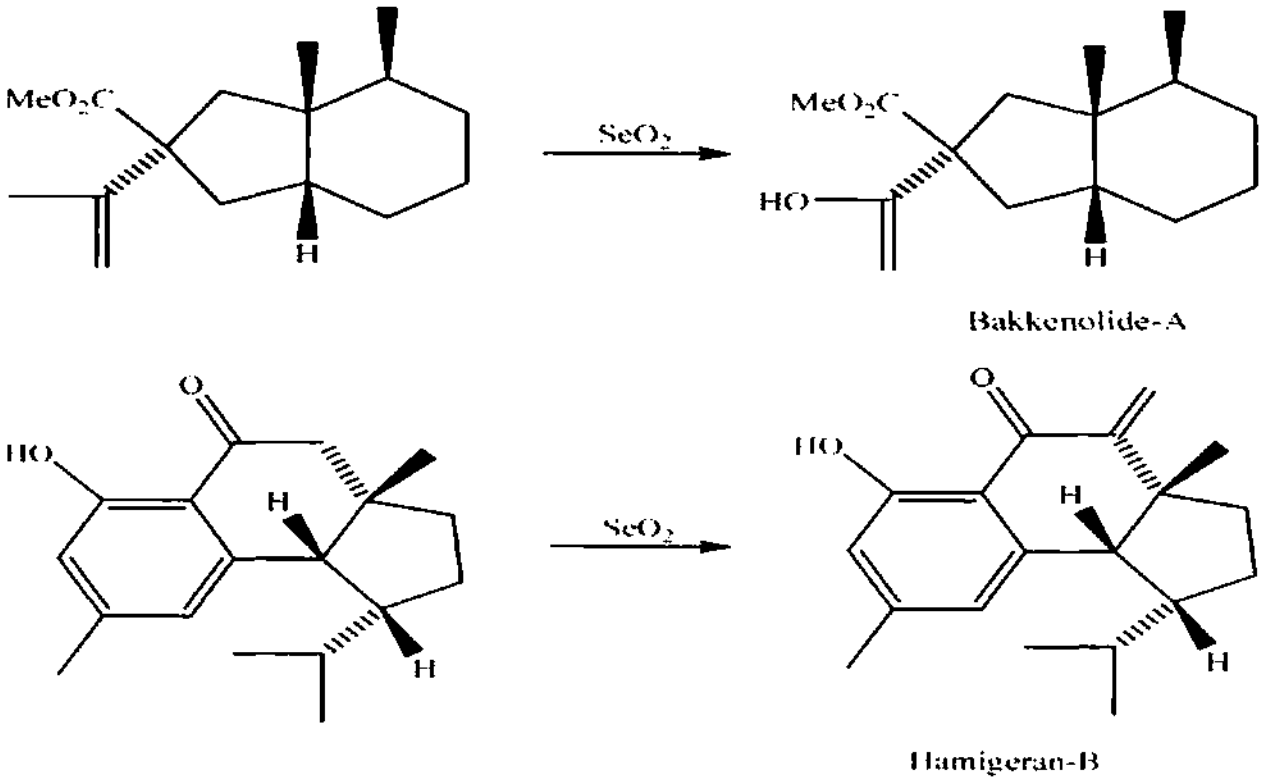
An alternative to overcome the serious complication of production of reduced Se lies in the usage of an oxidant in combination with SeO_2. This oxidant may be hydrogen peroxide, peracetic acid or *tert.*-butyl hydroperoxide (TBHP). It was observed that the oxidation of olefins with TBHP in the presence of catalytic amount of SeO_2 afforded excellent results (Umbreit and Sharpless 1977). Products of allylic oxidation with SeO_2 in combination with TBHP are formed in higher yields than those obtained with stoichiometric SeO_2 alone.

Allylic oxidation of dehydrocostus lactone (1) with SeO_2 in the presence of TBHP afforded several new compounds out of which isozaluzanin-C (31) was found to be biologically active (Kalsi *et al* 1979).



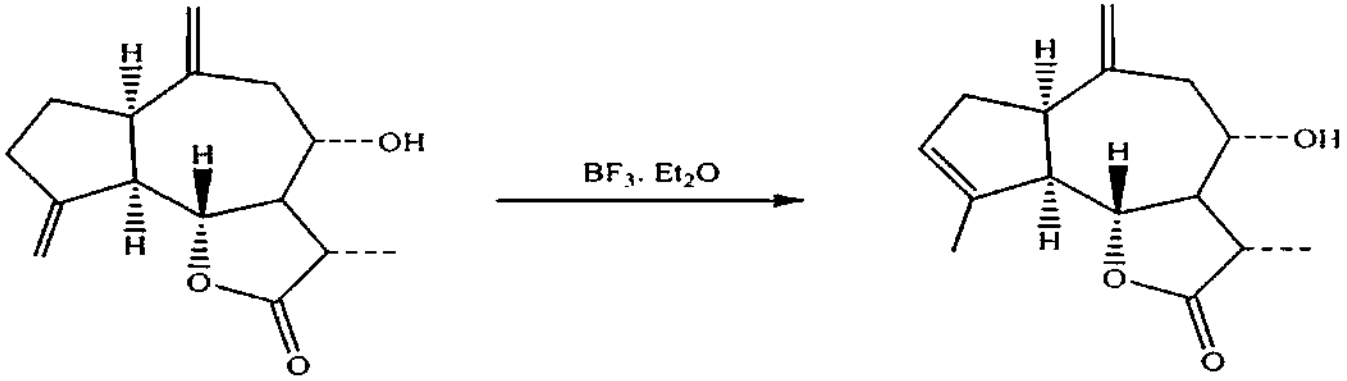
[31]

Another application of SeO_2 in oxidation is the formation of Bakkenolide-A (Reddy 2004) and Hamigeran B (Trost *et al* 2004).



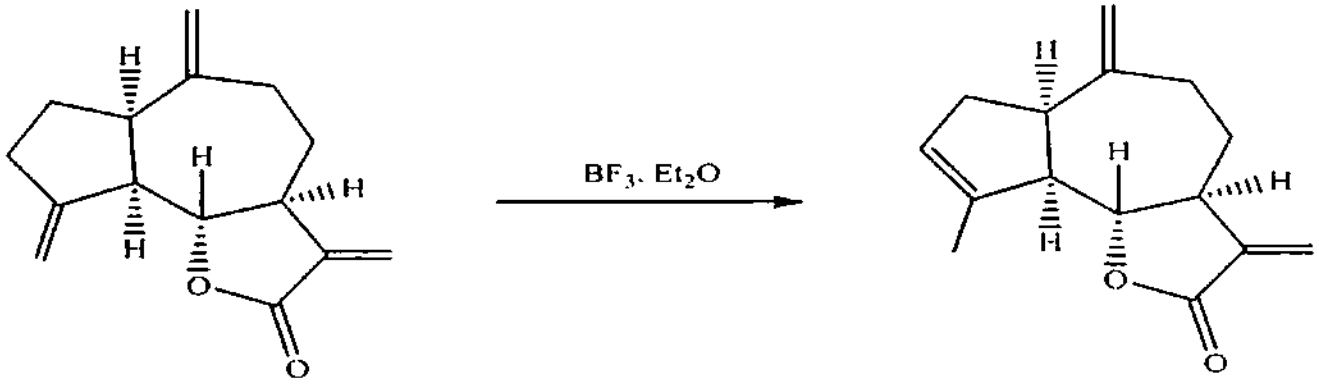
Double bond migrations

Double bond migration is a well studied reaction under catalyst-induced acidic and basic conditions. Double bond isomerisation was initially studied as an additional modification that accompanied several chemical transformations. For instance, boron triflouride-etherate which is known to cause the conversion of epoxides to respective ketones, brings about double bond isomerisation as well. Dihydrodecyl subexpinnatin (32) undergoes double bond migration to give (33) (Gonzalez *et al* 1982).



[32] [33]

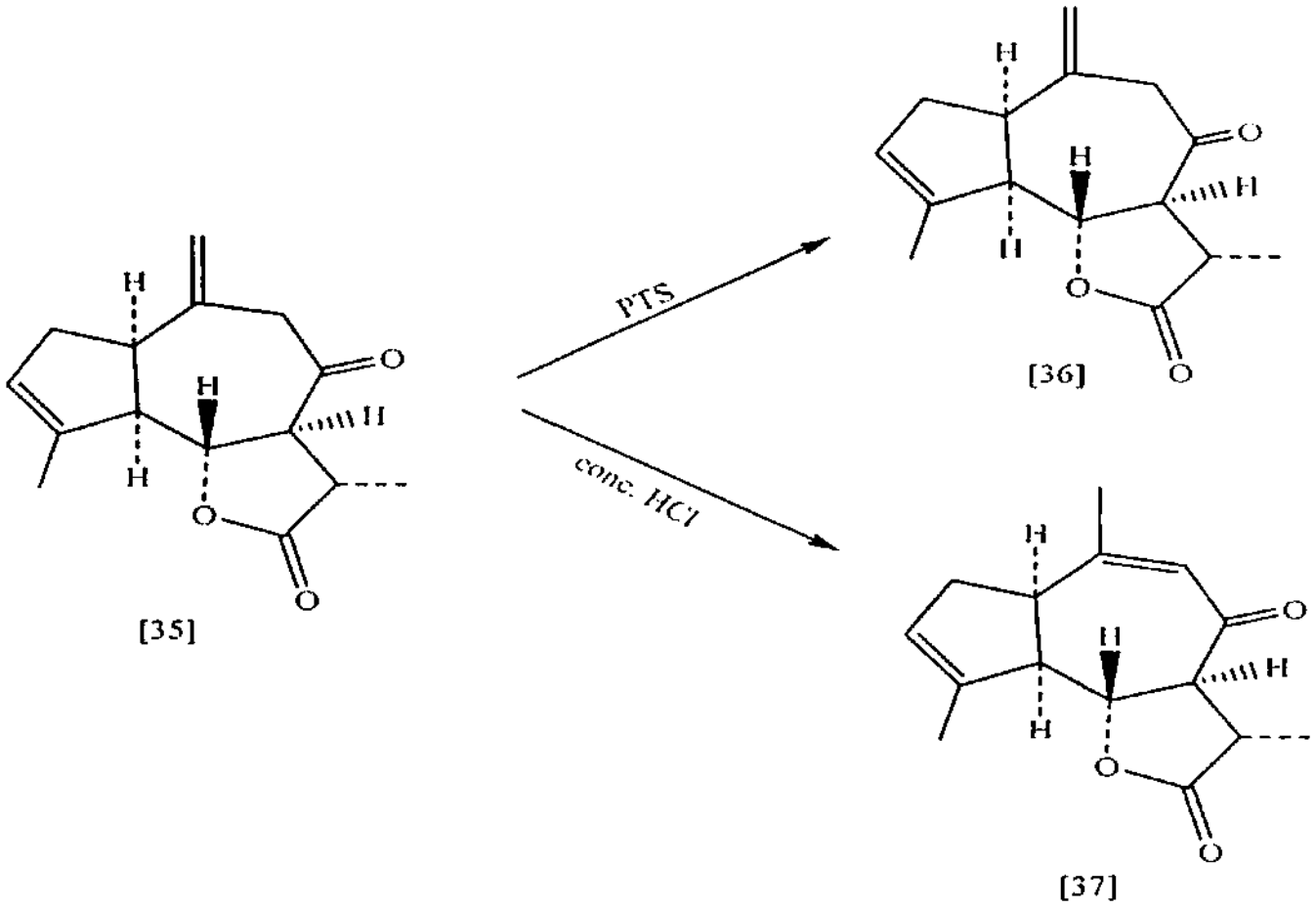
Dehydrocostus lactone also undergoes this reaction (Macaira *et al* 1977).



[1] [34]

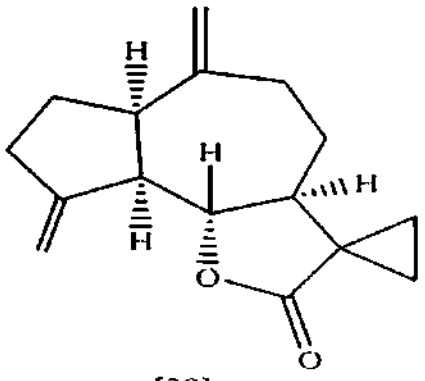
This migration of double bond in dehydrocostus lactone has also been effected by iodine / hydrobromic acid (Golden *et al* 1964). Iodine in benzene was found to be equally efficient for this reaction (Kalsi *et al* 1984). Mechanism of this reaction has been investigated to follow a free radical pathway (Abell 1966).

Double bond migration has been observed in certain other acid-catalysed reactions as well. Dehydrodihydro-ligustein (35) on mild acid treatment affords (36) by the isomerisation of C-10 exocyclic double bond to endocyclic conjugation while a stronger acid forms an unconjugated ketone (37) (Romo *et al* 1968).

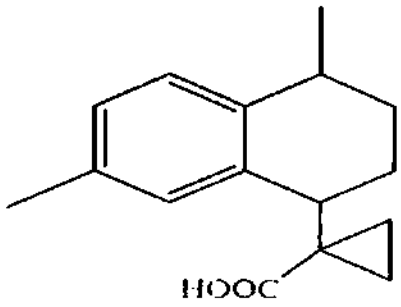


[35] [36] [37]

Hydrochloric acid when used in combination with dioxane also affects double bond migration as in case of 11-spirocyclopropyl dehydrocostus lactone (38). However, in this case an accompanying molecular rearrangement of guaiane skeleton to a cadinane skeleton in (39) occurs.

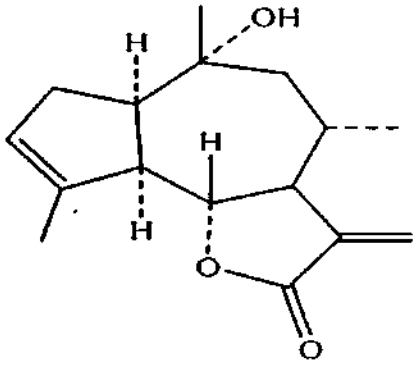


[38]

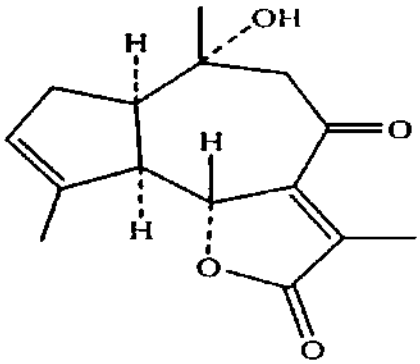


[39]

Chromium trioxide oxidation is usually accompanied by migration of double bonds as in case of cumambrine-B (40). In this case, $\Delta^{11(13)}$ bond migrates to $\Delta^{7(11)}$ i.e. in endocyclic conjugation with the ketone group giving (41) (Irwin and Geissman 1969).

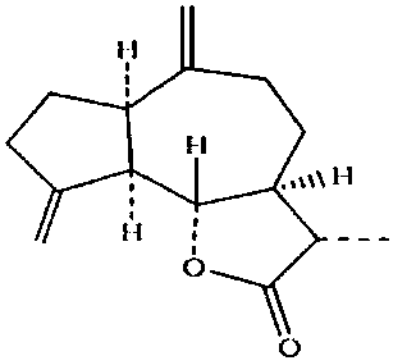


[40]

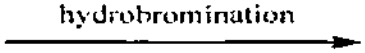


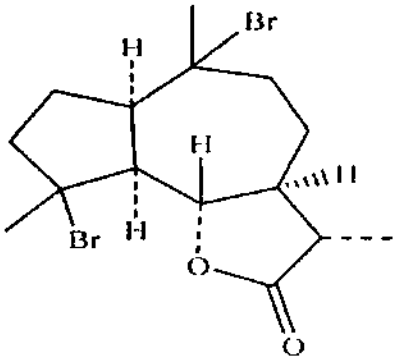
[41]

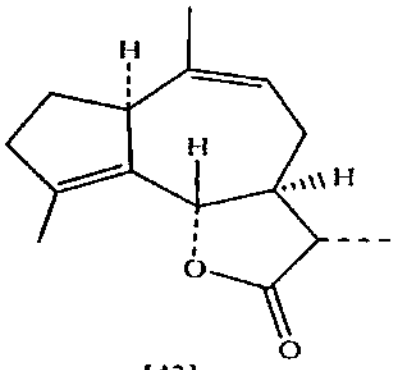
Dihydrodehydrocostus lactone (42) on hydrobromination–dehydrobromination afforded dienic lactone (43) with both the double bonds migrated (Mathur *et al* 1965).



[42]

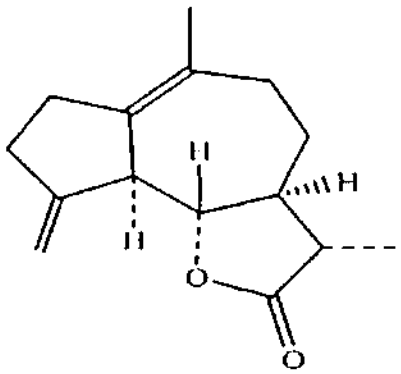






[43]

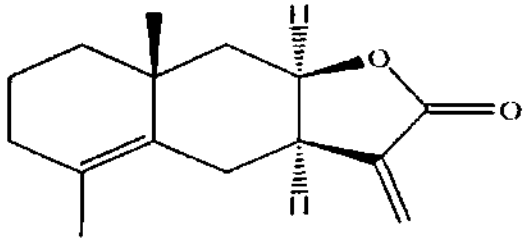
Compound (42) is found to give an isomerised product (44) with one exocyclic double bond migrated to endocyclic position when hydrogenated in ethanol over Pd/C.



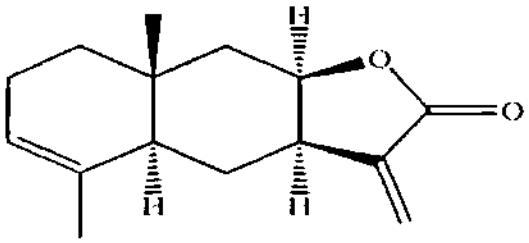
[44]

Such isomerisations have also been reported in germacranolides under alkaline conditions (Ognjanov *et al* 1958; Suchy *et al* 1961).

Bhattacharyya and Govindan (1978) have reported the formation of two double bond isomers (45 and 46) by treating isoalantolactone (7) with formic acid.

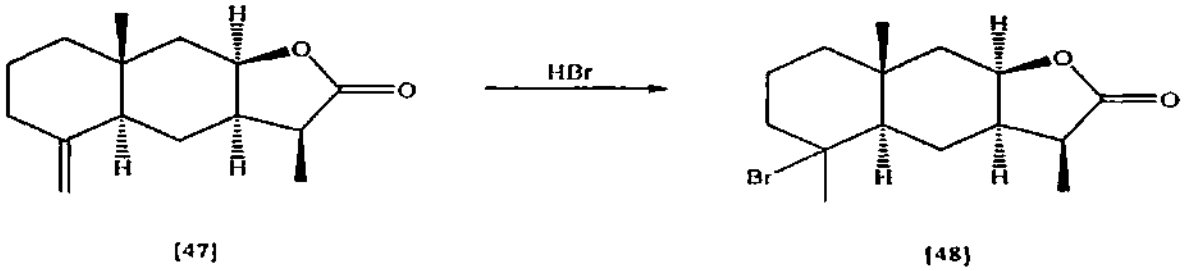


[45]

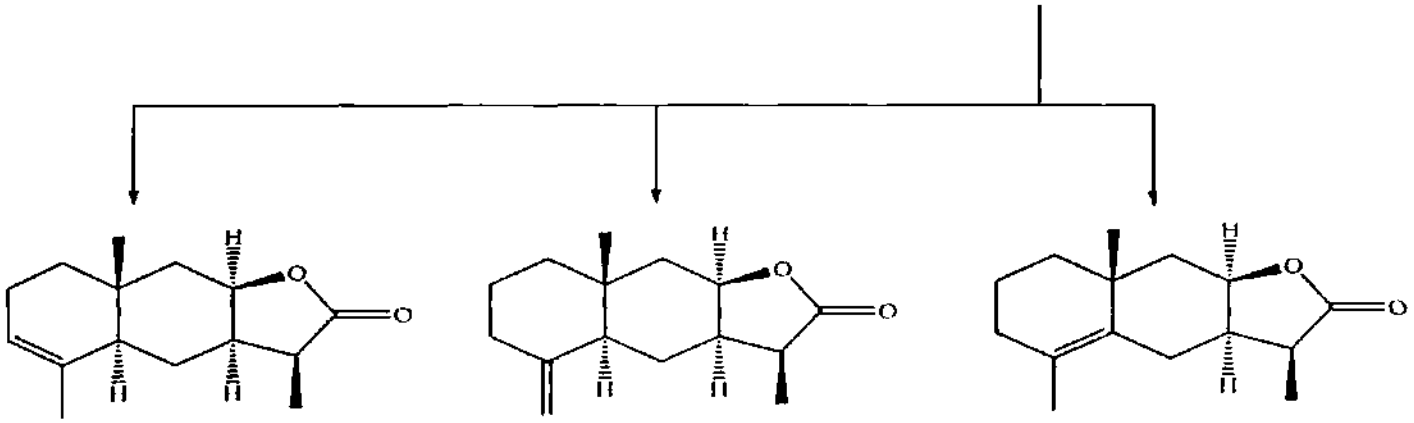


[46]

Nakazawa (1960) also prepared two isomers (49 and 50) of dihydroisoalantolactone (47). Reaction of (47) with HBr afforded 4-bromo-tetrahydroalantolactone (48), which on dehydrobromination with γ-collidine afforded three dehydrobrominated products (49-51).

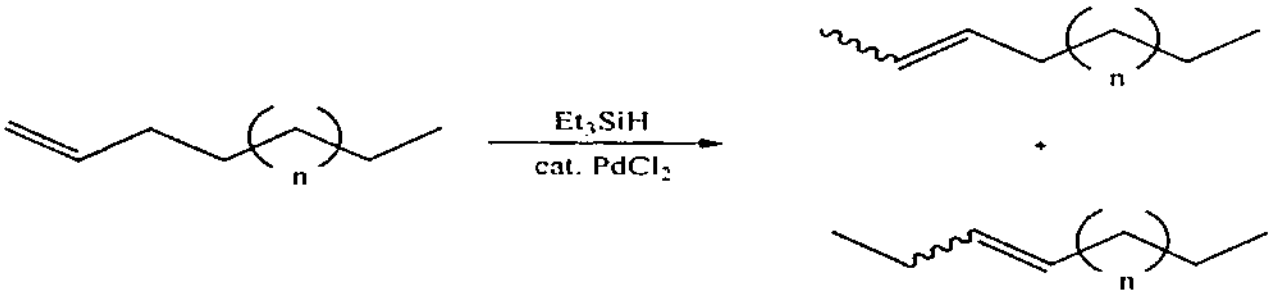


[47] [48]



[49] [50] [51]

A novel and efficient method for double bond isomerisation has been proposed by Aghayan *et al* (2003) in which isomerisation of α-alkenes is carried out in the presence of catalytic amount of palladium (II) chloride and triethyl silane at room temperature. This novel and efficient method affords high yield in the absence of solvent for double bond isomerisation of alkenes.



Double bond isomerisation reaction finds application in triggering mechanism for enediyne activation via alkene from bridgehead *endo* position to an *exo* position (Semmelhack *et al* 2002).

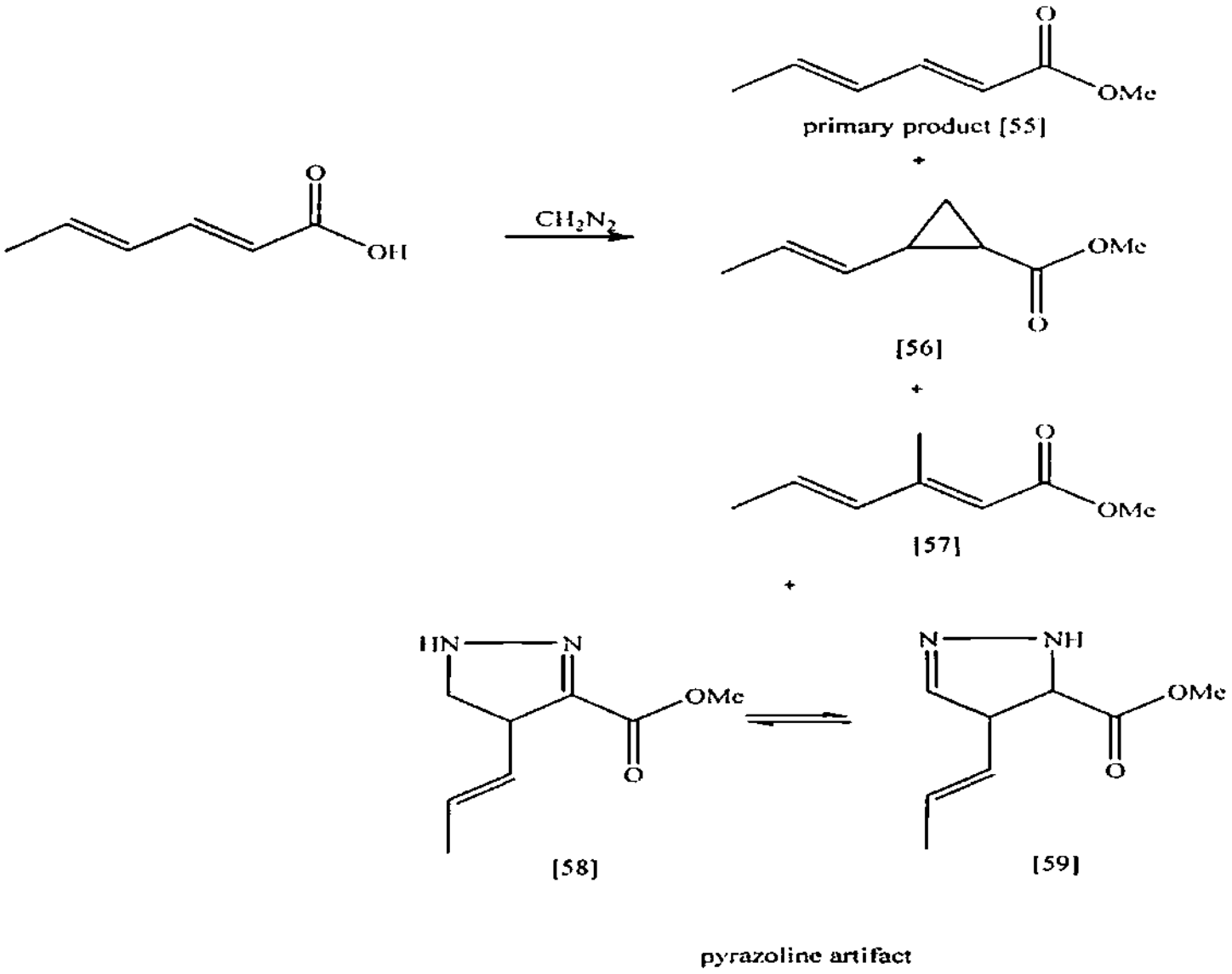
[52] [53]

Olefinic hydrocarbons have also been transformed at double bond position using zinc aluminate (Welch 1986).

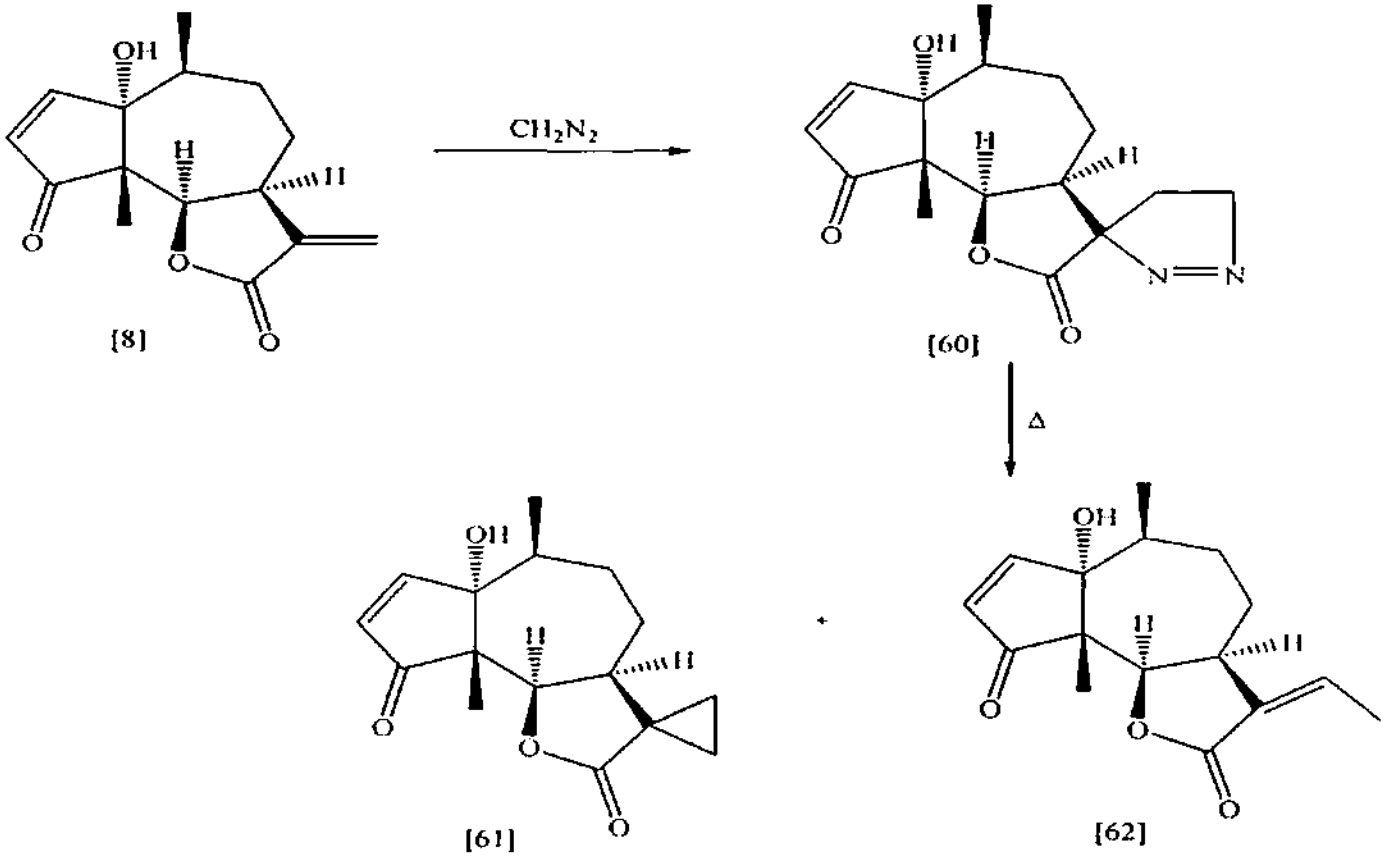
Pyrazoline adducts

Addition of nitrogen to the α-carbon in an olefinic moiety of sesquiterpenoids has been reported to greatly alter the biological activity. Diazomethane has been added to a large number of olefins and the reaction leads to the formation of pyrazolines, cyclopropane a C-methyl artifacts e.g.

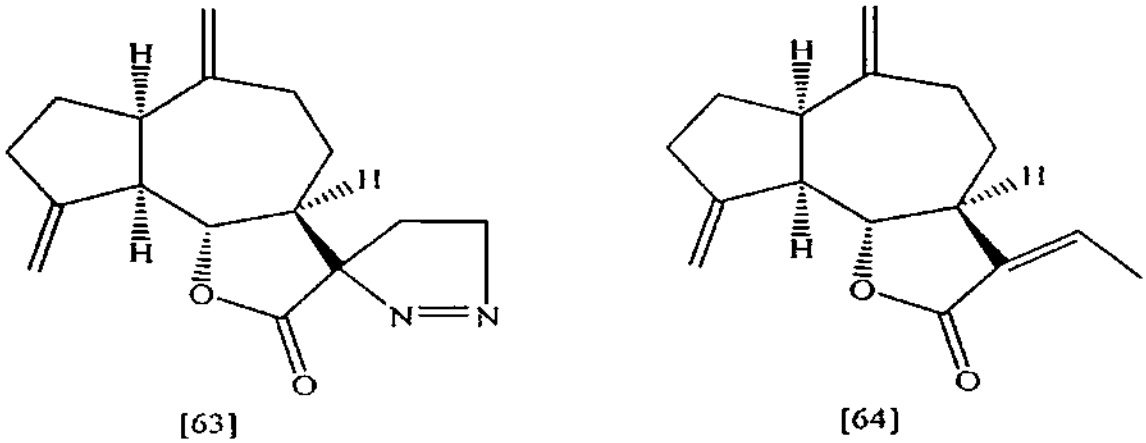
Sorbic acid (54) dissolved in DMF and reacted with diazomethane for one minute gave the following artifacts (58 and 59) at a concentration between 50-100 ppm in addition to expected products (55-57).

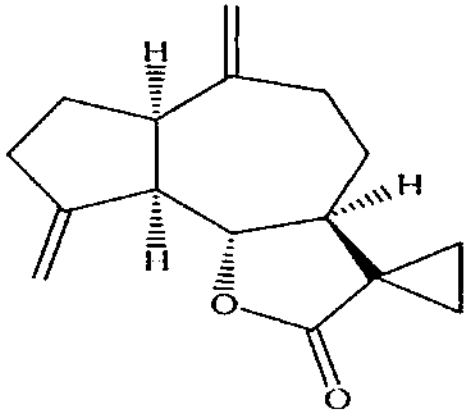


In order to study structure activity relationship for allelopathic effects of parthenin (8), it was transformed into its pyrazoline adduct (60) which on pyrolysis afforded two products (61 and 62). Of these, the cyclopropyl derivative was found to show significant bioregulatory properties (Saxena *et al* 1991).



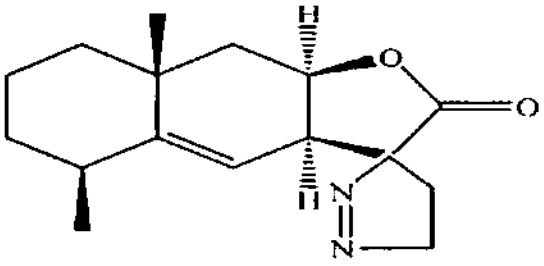
Dehydrocostus lactone (1) on reaction with diazomethane afforded a crystalline pyrazoline derivative (63) that on pyrolysis, gave two compounds i.e. 13-methyl dehydrocostus lactone (64) and 11-spirocyclopropyl derivative (65) (Kalsi *et al* 1979).



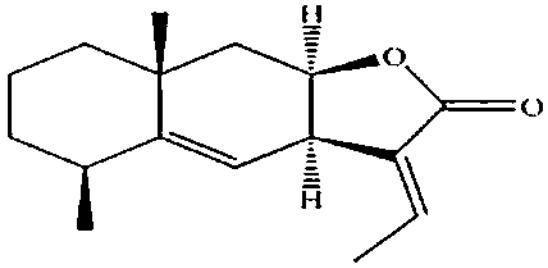


[65]

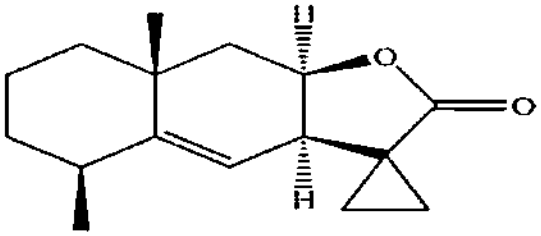
A similar reaction has also been reported for isoalantolactone (7) to yield compounds (66-68) (Kalsi *et al* 1984).



[66]

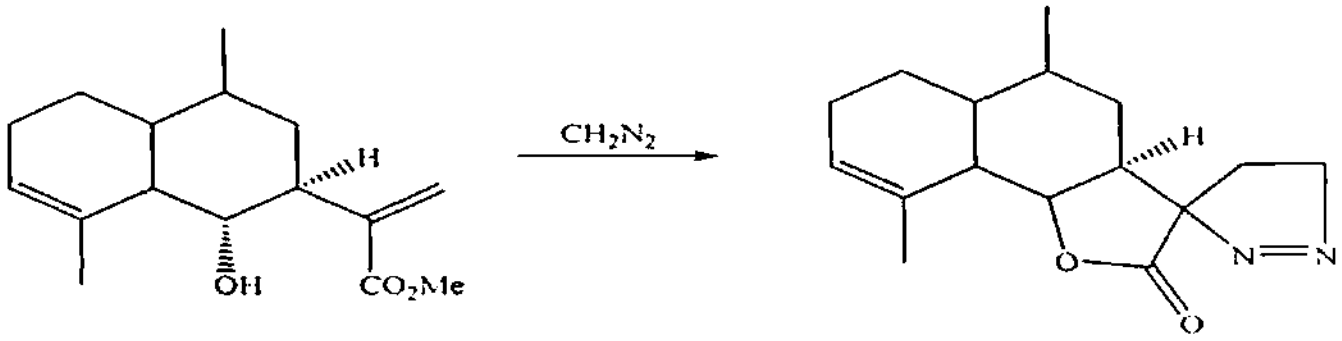


[67]



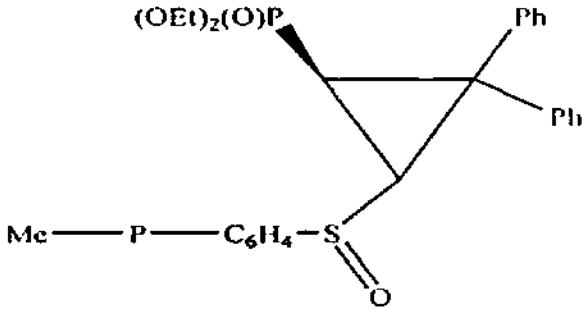
[68]

γ-Hydroxy ester (69) derived from cyclocostunolide on reaction with diazomethane yielded pyrazoline (70) (Singh and Kalsi 1992).

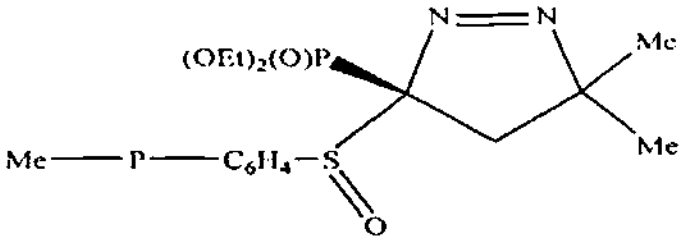


[69] [70]

Cycloaddition of diazomethane and ethyl diazoacetate to α- (diethoxyphosphoryl) vinyl *p*-tolyl sulfoxide and its β-substituted analogues (Me, Ph) gave 3-phosphoryl pyrazoles in high yield (Midura *et al* 1999).

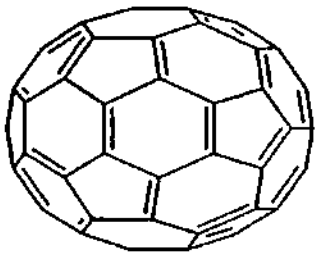


[71]



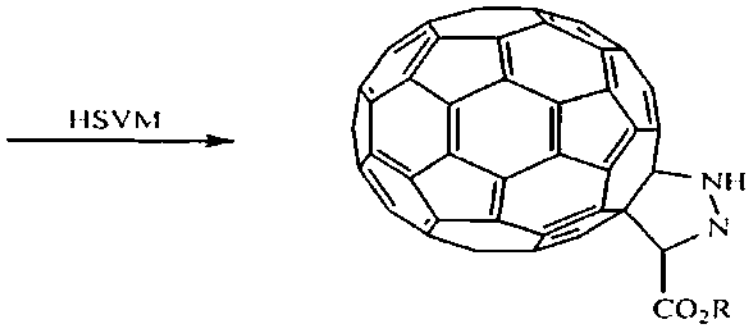
[72]

The addition of diazo compounds to [60] fullerene (C_{60}) is one of the first investigated reactions in fullerene chemistry. Wang *et al* (2004) reported the preparation of fullerene-fused pyrazolines under the solid state high speed vibration milling (HSVM) conditions as well as in the liquid phase solution.



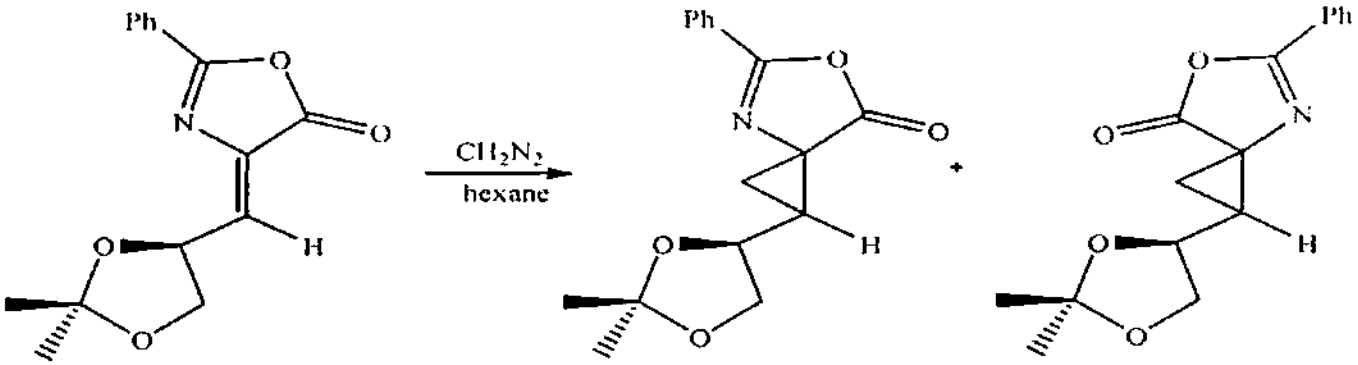
+ $NH_2CH_2CO_2R$. HCl + $NaNO_2$

[73]



[74] R= Et
[75] R= $(CH_2)_7$ CH_3

Azlactones (76) derived from glyceraldehydes have been demonstrated to undergo 1,3-dipolar cycloadditions of diazomethane. These compounds serve as the synthetic precursors of cyclopropyl amino acids (Cativiela *et al* 2006).

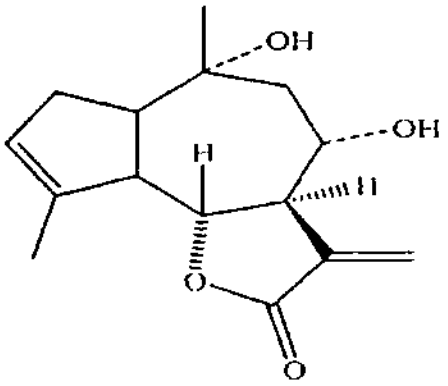


[76] [77] [78]

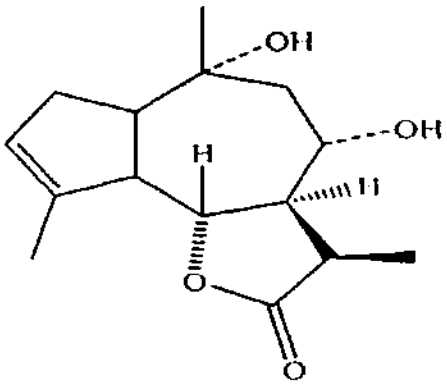
Diazomethane has also been employed for the preparation of some carbohydrate pyrazolines by addition to nitroalkenic sugars (Baer and Gilron 1987).

Reduction reactions

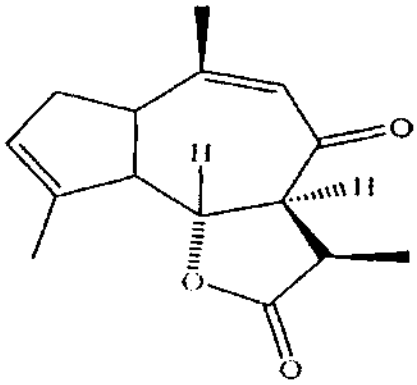
Reduction is one of the frequently used reactions in organic synthesis and a vast variety of reducing agents have been introduced for this achievement. Among the powerful and mild reducing agents which have been developed for the reduction of functional groups, lithium aluminium hydride (LAH) and sodium borohydride are the commonly used reagents in the synthetic organic laboratories. A wide variety of reduction reactions have been reported on sesquiterpene lactones. Romo *et al* (1968) undertook the reduction of cumbaunbring (79) with $NaBH_4$, which yielded a dihydroderivative (80). This derivative upon oxidation followed by dehydration gave a conjugated ketone (81).



[79]

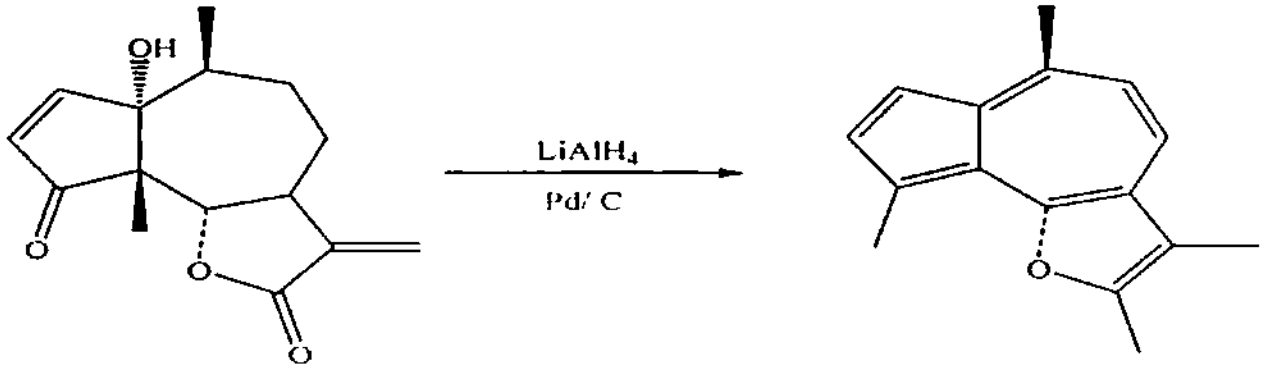


[80]



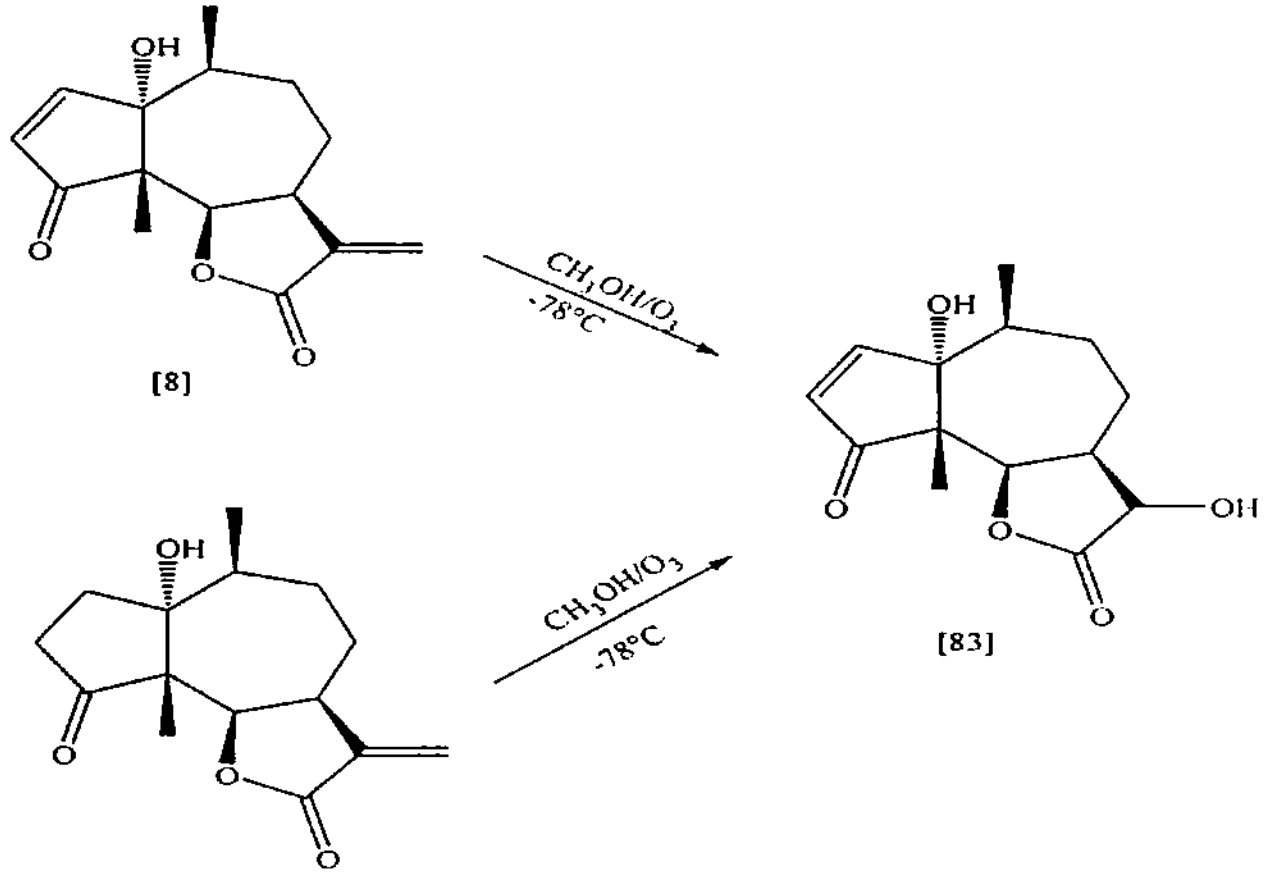
[81]

Parthenin (8), on reduction with LAH followed by dehydrogenation with Pd-C afforded atremazulene (82) (Herz and Watanabe 1959).

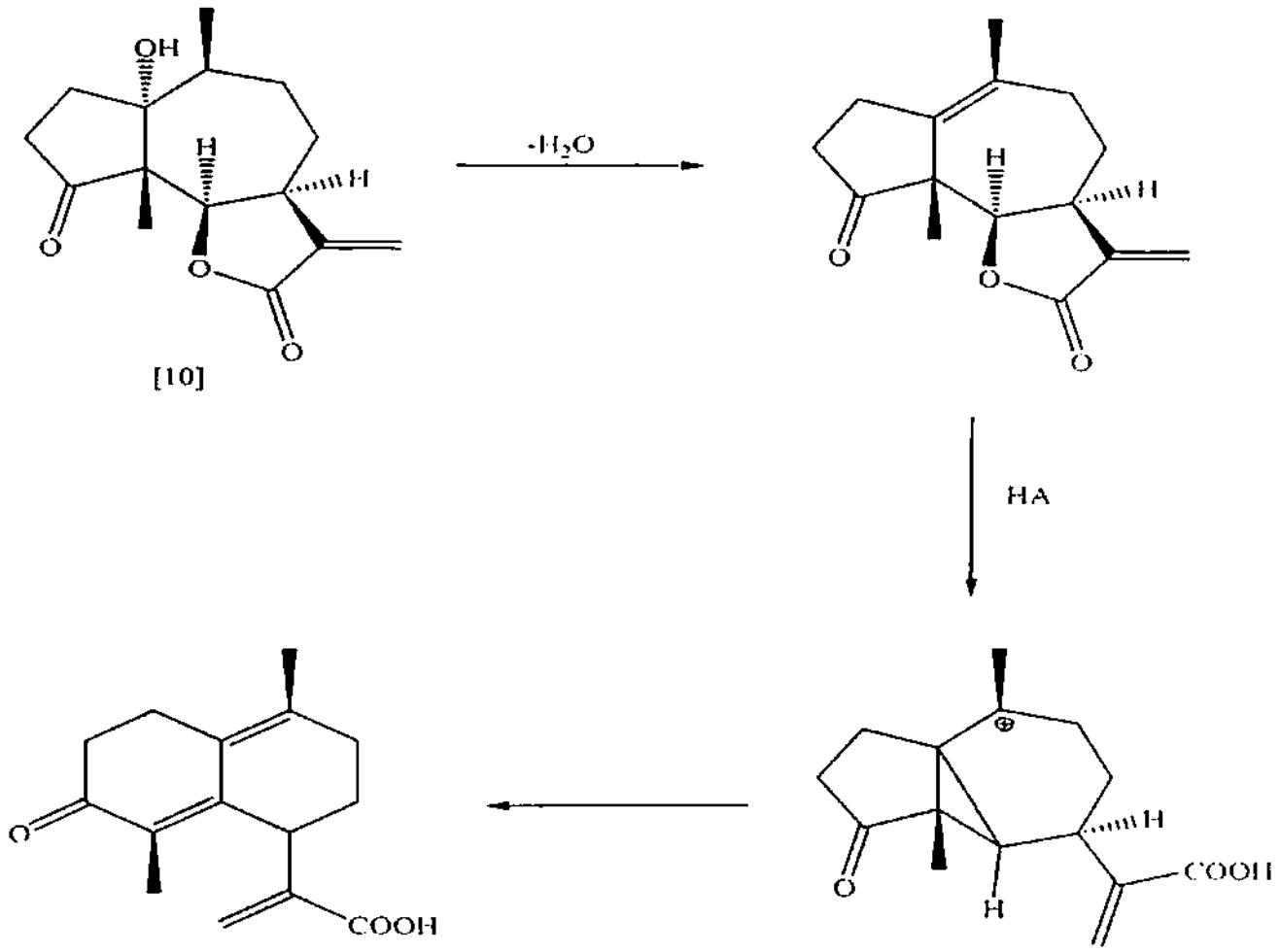


[8] [82]

Parthenin (8) and coronopilin (10) were reported to form norparthenone on ozonolysis in methanol at –78°C (Herz and Hogenauer 1961).



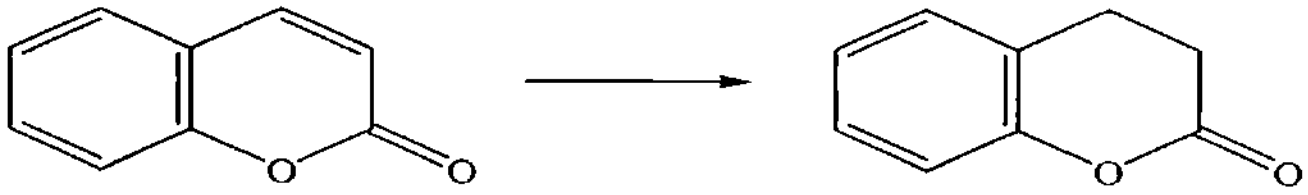
An attempt to dehydrate coronoplin however, failed as a carboxylic acid (84) was formed after treatment of coronopilin with acetic acid and sulfuric acid (Geissmann and Turkey 1964).



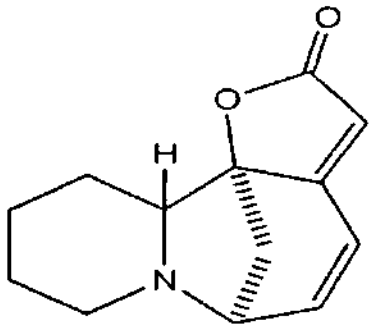
[10]

[84]

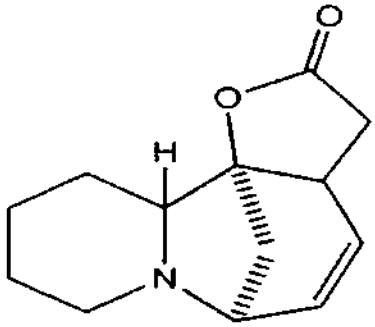
Sodium hydrogen telluride has been shown to serve as a convenient reagent for the hydrogenation of carbon-carbon double bonds. The reaction operates smoothly, the conditions are mild and tellurium metal can be recovered almost quantitatively (Geethamalika *et al* 2004).



A new isolate of *Aspergillus* spp. has been reported to hydrogenate the double bond of securinine (85) to give 14,15-dihydrosecurinine (86) at over 98% (Hong *et al* 2005).

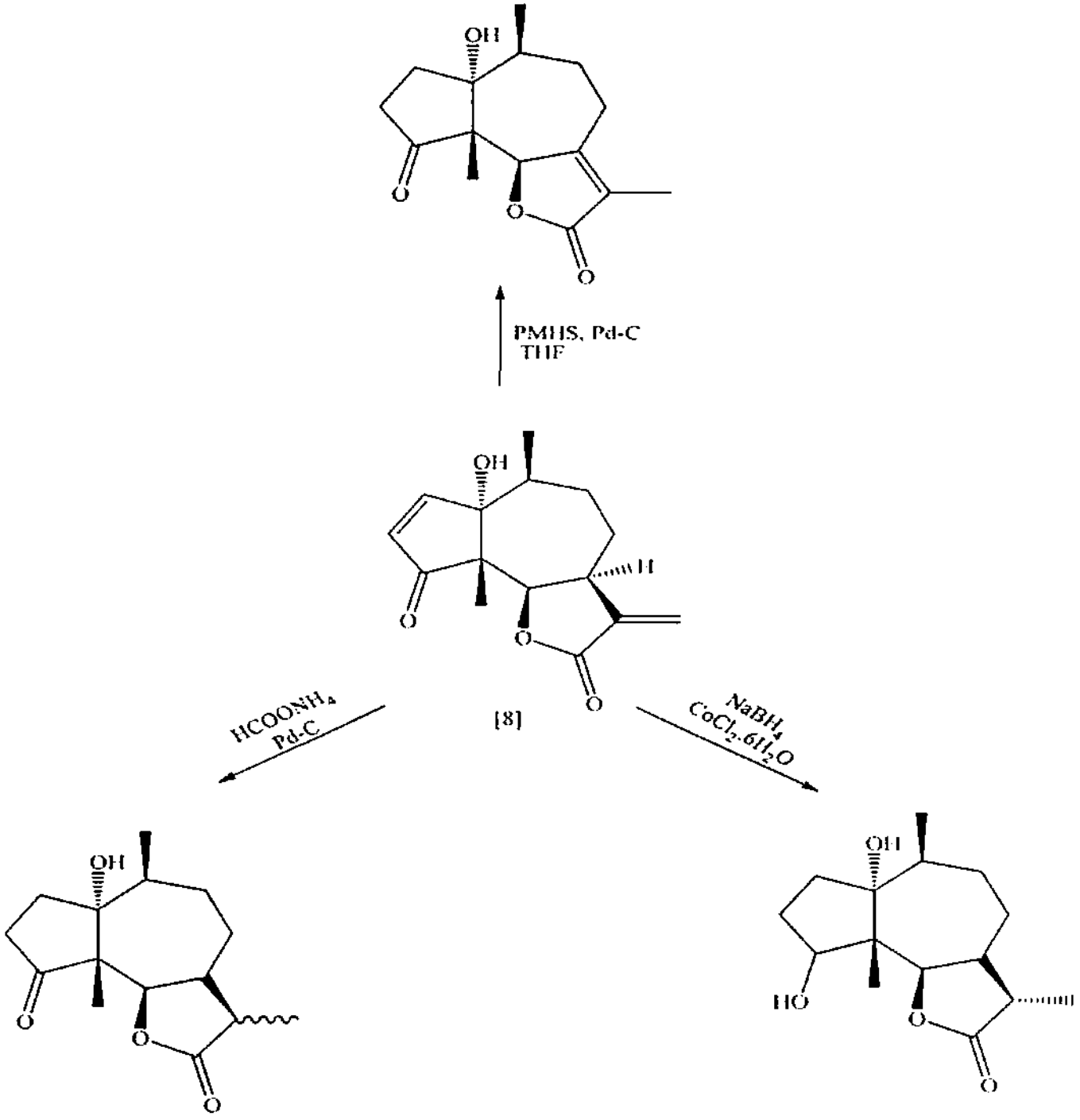


[85]



[86]

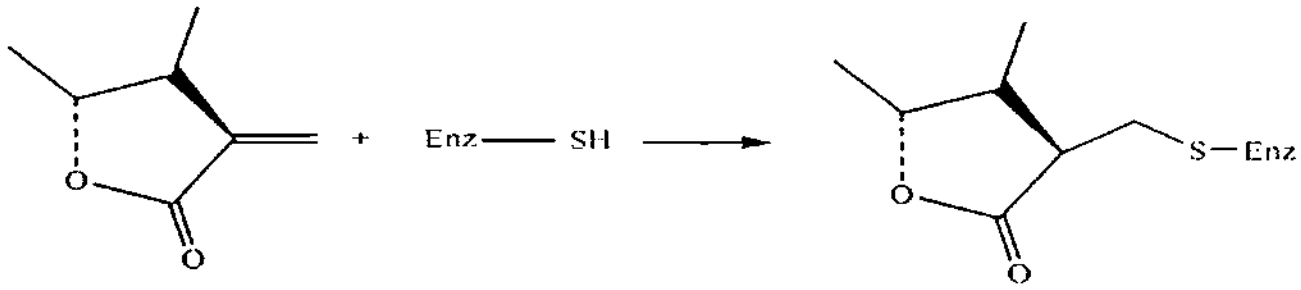
Several other reagents have also been employed for carrying out the reduction reactions of parthenin. These include polymethylhydrosiloxane (PMHS) and Pd-C in THF that afforded dihydroisoparthenin; $HCOONH_4$ and Pd-C which forms dihydroisoparthenin and dihydrocoronopilin and sodium borohydride in the presence of $CoCl_2.6H_2O$ which leads to the formation of a hexahydro derivative (Ramesh *et al* 2005).



Biological activity

Use of synthetic chemicals has no doubt improved crop yields quantitatively as well as qualitatively but at the same time, has created undesirable side effects in the form of health hazards, deterioration of environment quality and development of resistance and cross resistance. In

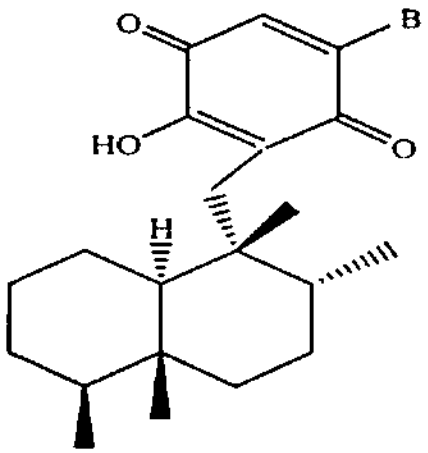
order to circumvent these problems, efforts are being made to find alternatives to these chemicals. Among the various alternatives, use of natural plant products offers a novel approach for management of obnoxious weeds and pests in a sustainable manner (Macias *et al* 2001). Sesquiterpene lactones are one of the very important groups of natural products known for their biological activities. A number of structural moieties present in these compounds have been reported to contribute to their biological properties. Sesquiterpene lactones possess the unsaturated double bonds conjugated with a carbonyl in the α-exomethylene-γ-lactone and cyclopentenone moieties that are also able to form an adduct with glutathione or L-cysteine following a Michael addition reaction (Lee *et al* 1977).



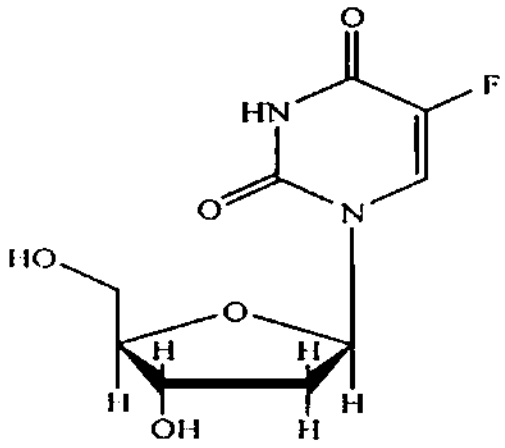
The diverse biological activities of these compounds are supported by several references in literature. Parthenolide and its photochemical derivatives have been studied for structure-activity relationship using human leucocyte chemotaxis (Neukirch *et al* 2003). Sesquiterpene lactones of the pseudoguaianolide skeleton *viz.* helenalin and dihydrohelenalin have been reported to show activities against asexual blood forms of *Plasmodium falciparum in vitro* (Francois and Passreiter 2004).

Sunflower sesquiterpene lactone models which share structural features of the lactone rings of strigol and its synthetic analogues, with different conformational flexibilities have been tested as *Orobanche cumana* germination stimulants. Among these, parthenolide and 3,5-dihydroxy dehydrocostus lactone significantly increased germination of *O. cumana* seeds (Luque *et al* 2000).

Marine sesquiterpenoids from *Spongia* spp. have been reported to inhibit the lyase activity of DNA polymerase β (Cao *et al* 2004).

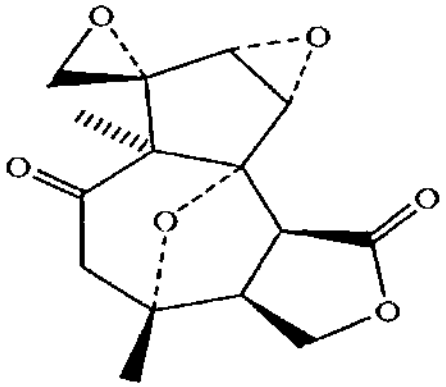


Ilimaquinone



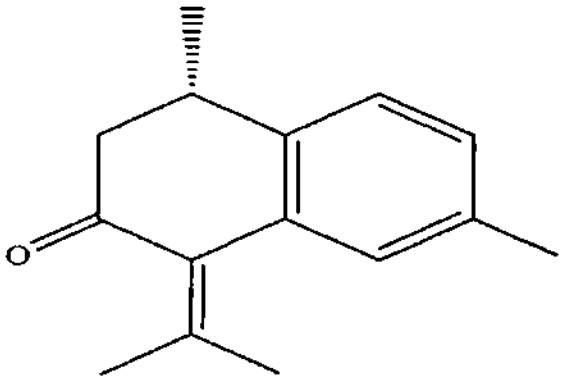
Deoxyuridine

Some melampolides and *cis, cis*-germacranolides on cluster analysis have shown potential phytotoxic activity (Macias *et al* 2004).



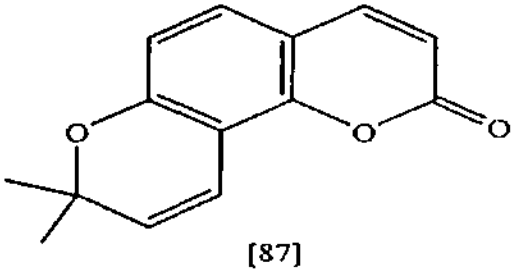
Corianlactone

β-Turmerone and ar-turmerone, sesquiterpenoids isolated from the rhizome of *Curcuma zedoaria*, inhibited lipopolysaccharide (LPS) – induced prostaglandin E2 production in cultured mouse macrophage cells. In addition, these compounds exhibited inhibitory effects on LPS-induced nitric oxide production in the cell system (Hong *et al* 2002).

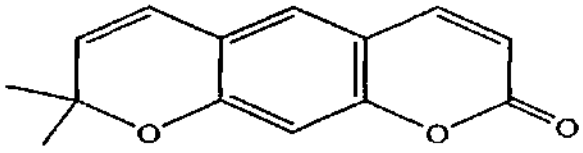


ar- Turmerone

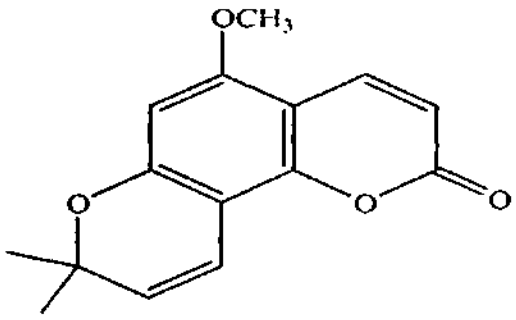
Natural and synthetic pyranocoumarins isolated from *Citrus grandis* have been reported to possess antibacterial activity against *S. aureus, S. epidermidis, E. coli* and *Presteus mirabilis* (Melliou *et al* 2005).



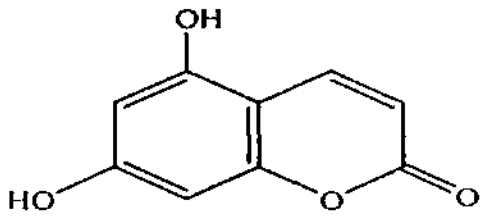
[87]



[88]



[89]

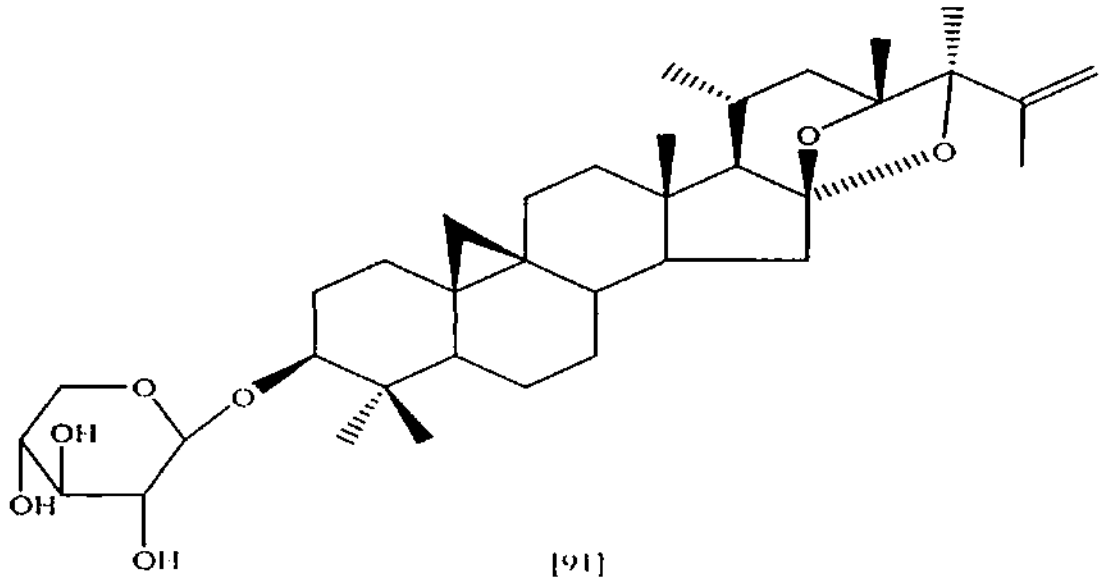


[90]

In recent years, sesquiterpene lactones have been in limelight due to their anti-cancer properties that have attracted a great deal of interest. Extensive research work has been carried out to characterise the anticancer activity, the molecular mechanisms and the potential chemopreventive and chemo-therapeutic application of sesquiterpene lactones (Zhang *et al* 2005).

The interactions of the three sesquiterpene lactones dihydrohelenalin acetate, dihydrohelenalin methacrylate and helenalin isobutyrate from *Arnica montana* and of parthenolide from *Tanacetum parthenium* have been investigated on human blood, plasma and human serum albumin solution (Wagner *et al* 2004).

Cyclo-artane triterpenoids from *Cimicifuga foetida* have been reported to show cytotoxic effect against R-HepG2 cells and its drug resistant sub line R-HepG2 (Tian *et al* 2006).

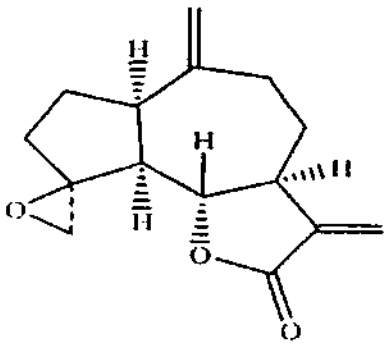


[91]

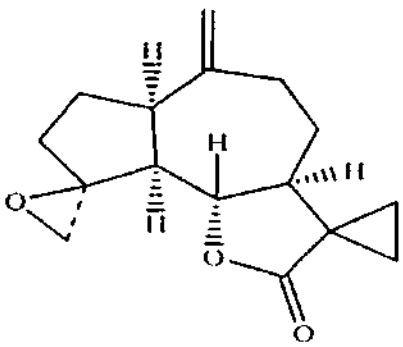
Leaf extracts from *Sida cordifolia* have been reported to possess some cardiovascular effects on rats (Medeiros *et al* 2006).

Pyrazoline derivatives of some sesquiterpene lactones have antinociceptive effects in mice. They have been reported to impair the motor coordination and increase immobility (Tabarelli *et al* 2004).

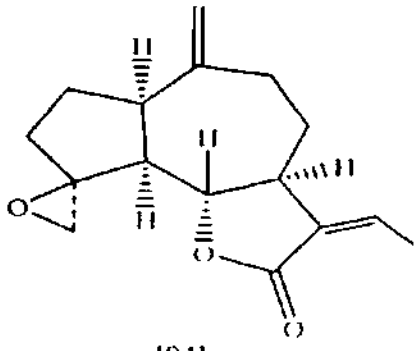
Kalsi *et al* (1981) reported the potential of epoxy derivatives of some sesquiterpene lactones in initiation of roots in *Phaseolus aureus* seeds.



[92]

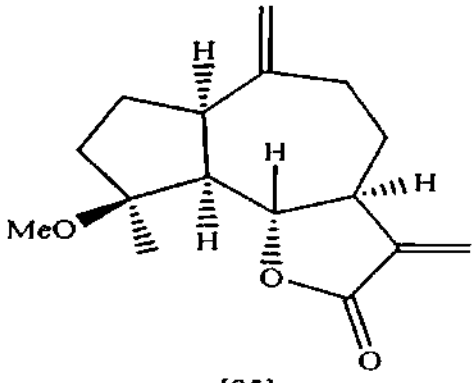


[93]

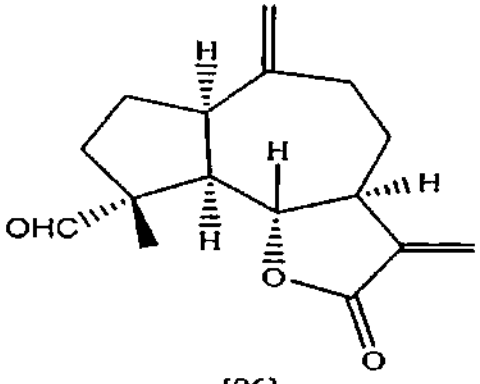


[94]

4-β-Methoxy dehydrocostus lactone (Singh *et al* 1992) and saussureal (Talwar *et al* 1992) isolated from costus roots, displayed significant biological activity as plant growth regulators.

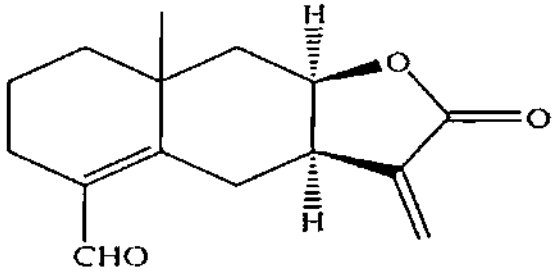


[95]



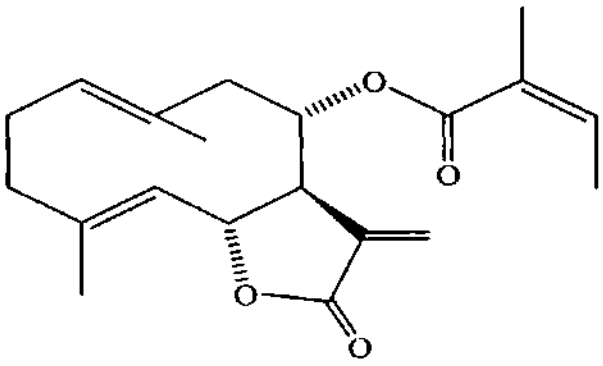
[96]

Epoxy-alantolide inunal, a new potent plant growth regulator has been isolated from *Inula racemosa* (Kalsi *et al* 1988).



[97]

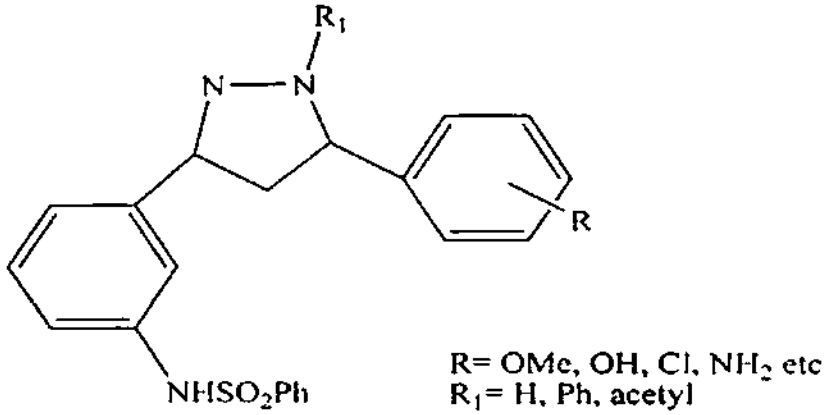
8 –Angeloyloxy costunolide has shown antifeedant activity against neonate larvae of *Spodoptera lettoralis* (Goren *et al* 1994).



[98]

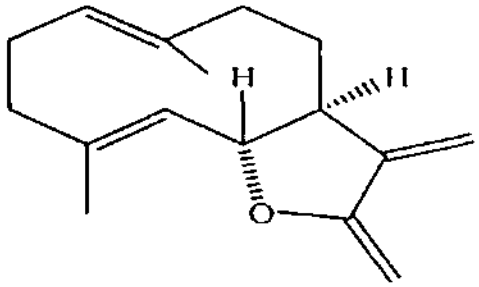
Sesquiterpene lactones can cause allergic contact dermatitis. The principle immunochemical requirement is the presence of α-methylene γ-lactone moiety that binds to the skin via a Michael reaction, thereby forming an antigen that causes sensitization (Elakowich 1987).

3-(3-Phenylsulfonamidophenyl)-5-aryl pyrazolines were reported to exhibit fungicidal activity (Fernandes and Parekh 1997).



[98]

Takasugi *et al* (1985) for the first time isolated two antifungal sesquiterpenes namely costunolide and lettucenin A (99).



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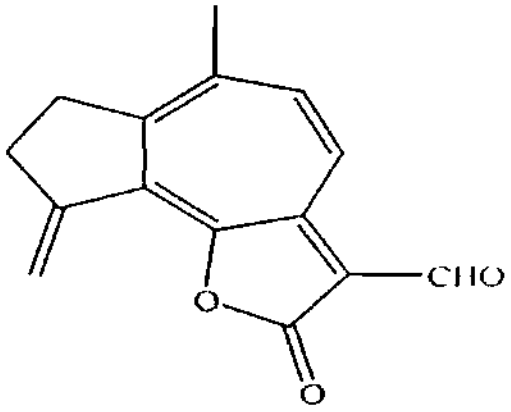
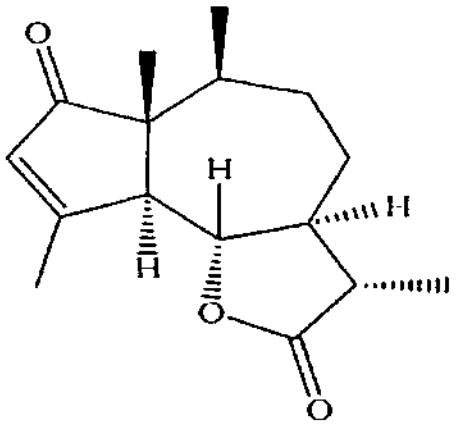


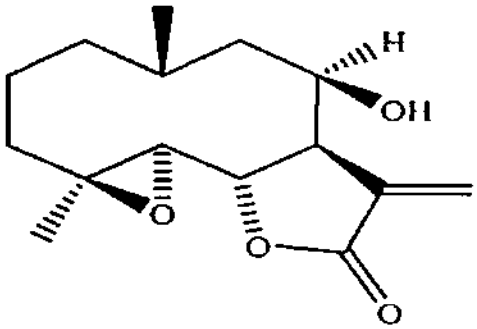
[99]

Cichoralexin (100) isolated from *Cichorium intybus* has been shown to exhibit antifungal activity (Mondi *et al* 1990).

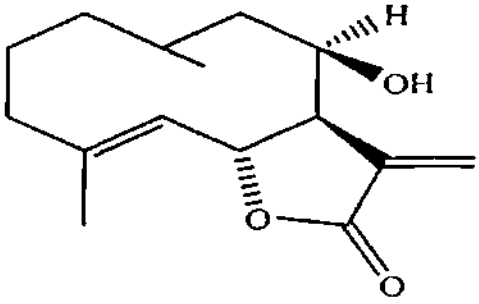


[100]

Sesquiterpene lactones salsolin (101) and eupatolide (102) isolated from *Inula salsoides* exhibited cytotoxic activity against KB and P-388 cell lines (Zhou *et al* 1994).

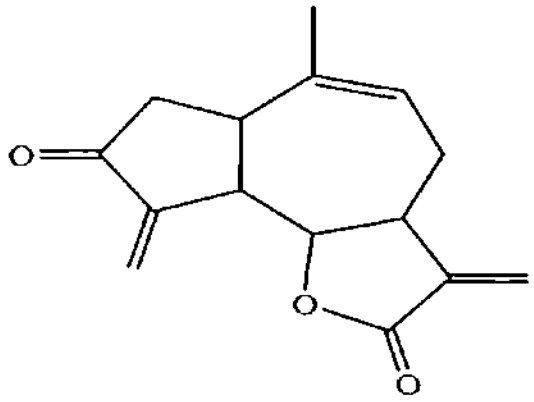


[101]



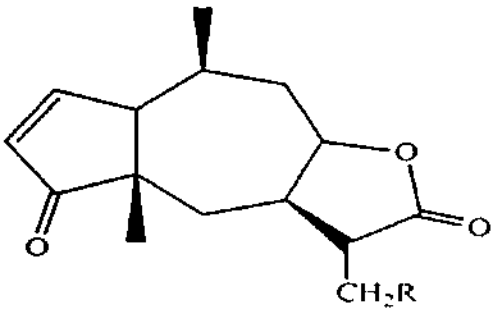
[102]

Eremantholide (103) with α-methylene-γ-lactone moiety provides the plant with a defence mechanism against predatory attack (Vichnewski and Gilbert 1972).

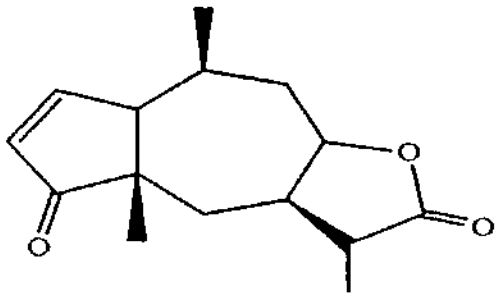


[103]

Lee *et al* (1977) conducted studies on compounds like helenalin and plenalin to correlate structure and activity in sesquiterpenes and proved that α-methylene-γ-lactone moiety is essential for terpenoids to exhibit cytotoxic and antitumor activities. Modification of this moiety by either hydrogenation or Michael type amine addition yields compounds which are about 5 to 10 times less active than the parent molecule helenalin. This compound inhibits DNA synthesis and DNA polymerase activity (Lee *et al* 1972). It also inhibits incorporation of leucine into proteins.



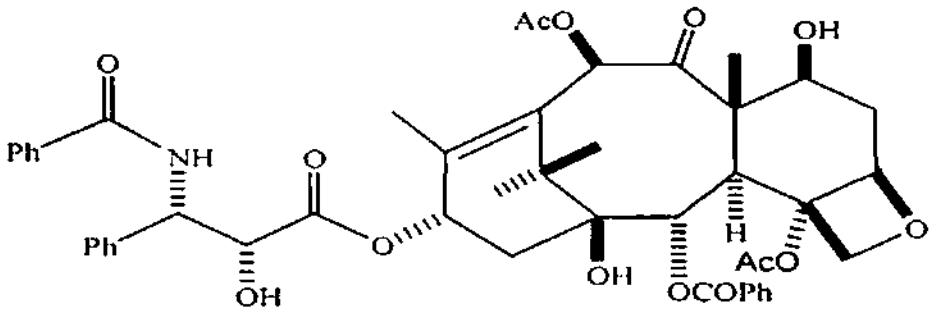
[104]



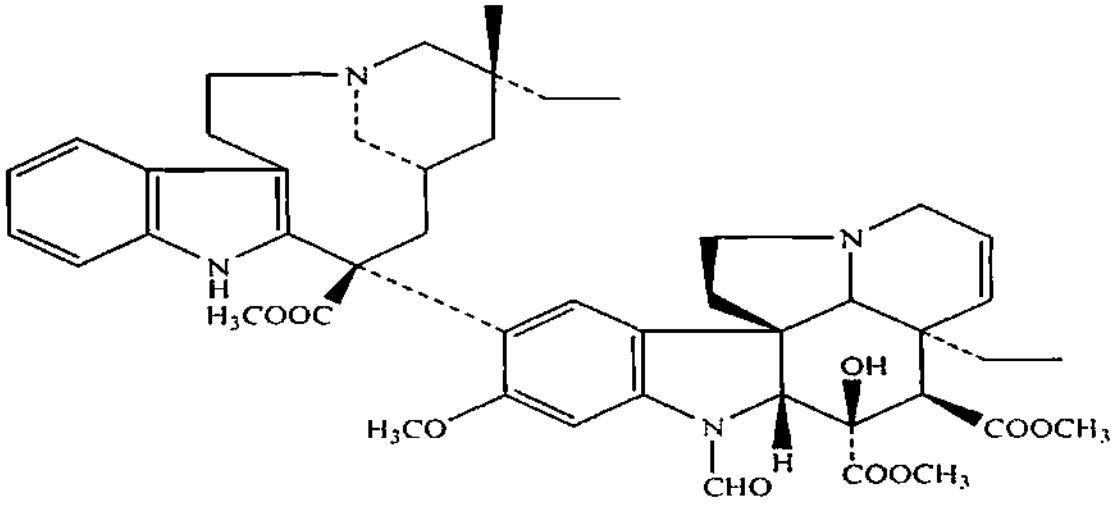
[105]

A recent survey showed that of the 87 approved anticancer drugs over the past ten years, 62% are of natural origin or are modelled on natural product parents. Among those clinically useful drugs include paclitaxel, vincristine (Oncovinâ), 9-podophyllotoxin (a natural product recursor) and

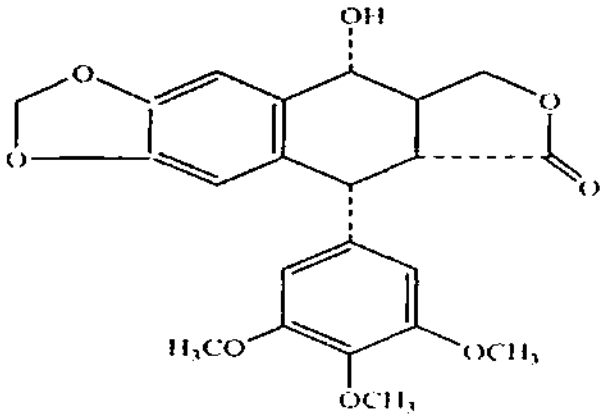
camptothecin (a natural product precursor for water-soluble derivatives). These substances embrace some of the most exciting new chemotherapeutic agents currently available for use in a clinical setting.



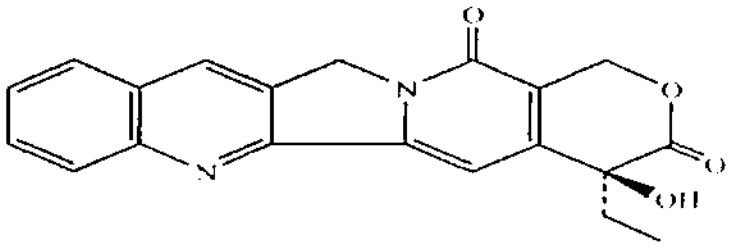
Paclitaxel



Vincristine



Podophyllotoxin

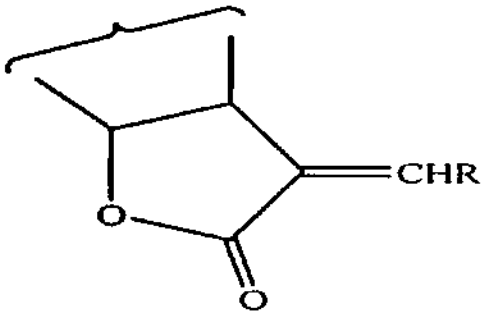


Camptothecin

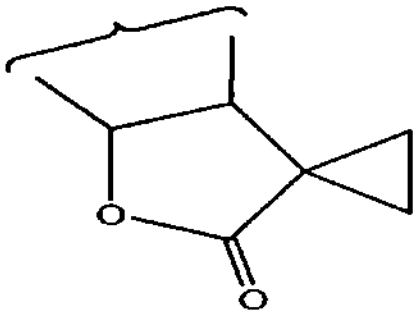
Parthenin, isolated from *Parthenium hysterophorus*, also plays a vital role in the defense of plants against insects. It inhibits sporangial germination and zoospore mobility in *Sclerospora graminicola* (Char and Shankarabhat 1975). The weed has also been reported to have allergic effects and inhibits cholinesterase activity (Kumar *et al* 1990). At high concentrations, parthenin inhibited germination and seedling growth of rye, while at lower concentrations, it enhanced the inhibitory effects caused by 2,4-D (Singh *et al* 1990).

The sesquiterpene lactones may act by inhibiting the respiratory chain of mitochondria and the main target of their activity appears to be NADH isoenzyme Q_{10} or succinate dehydrogenase coenzyme (Van *et al* 1982).

Various activities of sesquiterpene lactones suggest their evolutionary significance in plants as deterrents against herbivores and anti-fungal and anti-bacterial allelopathic agents. It has been confirmed that the plant growth regulatory activity of the terpenoid lactones depends upon the presence of an α-methylene group in conjugation with the lactone carbonyl (Kalsi *et al* 1977). Studies have established that the biological activity associated with α-methylene-γ-lactone moiety (107) in a terpenoid is further enhanced when a cyclopropane ring (108) is placed in the α,β-position to the lactone carbonyl. This activity is increased when a trisubstituted (*Z*)-double bond (109) is in the position of conjugation (Kalsi *et al* 1983).



[106] R= H
[108] R= Me



[107]

CHAPTER III

MATERIALS AND METHODS

IR spectra were measured in $CHCl_3$ solution or mentioned otherwise on Perkin Elmer, Model RX-1 FT-IR spectrometer. ^1H NMR spectra were recorded with Bruker AC 300 F (300 MHz) as solutions (in $CDCl_3$) using TMS as internal reference. The chemical shifts were expressed in δ (ppm) values and the abbreviations 's', 'brs', 'd', 't' and 'm' stand for singlet, broad singlet, doublet, triplet and multiplet respectively. The spectroscopic analysis were obtained from Department of Applied Biological Chemistry, Faculty of Agriculture, Shizouoka University, Shizouoka, Japan and Central Instrumentation Laboratories (CIL), Panjab University, Chandigarh. Microwave-induced reactions were done in a LG domestic oven MS-194 W. All the melting points were determined in open capillaries on a Buchi B-545 melting point apparatus and are uncorrected.

An overview of the analytical methods

Chromatographic methods

Chromatography is the method of choice in handling the problem of isolation of a compound of interest from a complex natural mixture. Therefore, the chromatographic methods used during the present work are briefly described.

Thin layer chromatography (TLC)

TLC involves the use of a particulate sorbant spread on an inert sheet of glass, plastic or metal as a stationary phase. The mobile phase is allowed to travel up the plate carrying the sample that was initially spotted on the sorbant just above the solvent. Depending on the nature of the stationary phase, the separation can be either partition or adsorption chromatography. The advantage of TLC is that the samples do not have to undergo the extensive cleanup steps, and the ability to detect a wide range of compounds, using reactive spray reagents.

Plates required for TLC were prepared by spreading a uniform layer of silica gel–G in the form of aqueous slurry (10 g silica gel/ 100 ml water). The plates were dried at room temperature followed by activation at 120°C for 45 minutes.

Column chromatography (CC)

CC consists of a column of particulate material such as silica or alumina that has a solvent passed through it at atmospheric pressure. The separation can be liquid/solid (adsorption) or liquid/liquid (partition). The columns are usually glass or plastic with sinter frits to hold the packing. The sample is dissolved in solvent and applied to the front of the column (neck packing), or alternatively adsorbed on coarse silica gel (dry packing). The solvent elutes the sample through the column, allowing the compounds to separate. Normally, the solvent is non-polar and the surface polar. The solvent is changed stepwise, and fractions are collected according to the separation required, with the eluting products being monitored by TLC.

Chromatographic solvent "polarity"

There are four major intermolecular interactions between sample and solvent molecules in liquid chromatography, dispersion, dipole, hydrogen bonding and dielectric. The total interaction of the solvent and sample is the sum of the four interactions. The total interaction for a sample or solvent molecule in all four ways is known as the "polarity" of the molecule. Polar solvents dissolve polar molecules and for normal phase partition chromatography, solvent strength increases with solvent polarity.

Spray reagents

TLC plates were developed in suitable solvent systems like chloroform: acetone (6:1) or benzene: ethyl acetate (95:5) and visualization of spots was done by spraying the plates with vanillin spray reagent or the methanol sulfuric acid spray reagent.

Vanillin spray reagent: Ethanol (95%) 9.0 ml, concentrate H_2SO_4 0.5 ml, glacial acetic acid 3 drops and vanillin 0.5 g.

Methanol sulfuric acid reagent: 95 ml methanol and 5 ml concentrated H_2SO_4

Extraction techniques

Solvent extraction

Solvent extraction is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Some extraction techniques involve partition between two immiscible liquids, others involve either continuous extractions or batch extractions. During the present work, dried and pulverized plant materials were subjected to solvent extraction in a Soxhlet apparatus using chloroform or hexane. The Soxhlet apparatus was run for 24 hrs in batches and the solvent was distilled off to obtain the crude extracts of different plant materials.

Spectroscopic techniques

Spectroscopy is the study of the interaction of electromagnetic radiation (EMR) with matter. The spectroscopic techniques employed for the present study include IR, ^{1}H NMR and ^{13}C NMR analysis.

Plant materials

Four different plant species from the family *Asteraceae* have been included in the present investigation. The roots of *Saussurea lappa* and *Inula racemosa* were taken from our laboratory stock. Leaves of *Parthenium hysterophorus* were collected from PAU campus and identified by Dr (Mrs) N P Kaur, Professor, Department of Botany. Dried and roasted root powder of *Cichorium intybus* was procured from Mohini Coffee Beans, Mumbai. For biological testing of the compounds, seeds of *Pisum sativum*, *Avena fatua* and *Phlaris minor* were procured from Department of Plant Breeding, Genetics and Biotechnology, PAU, Ludhiana.

Preparation of reagents

Diazomethane

Preparation of nitrosomethyl urea

A solution of methyl ammonium chloride (1.5 moles, 100 g), urea (5.0 moles, 300 g) in water (500 ml) was refluxed for 6 hrs. After cooling the solution, sodium nitrite (1.5 mole, 110 g) was added to it and the mixture was cooled to 0°C. This solution was added slowly to an ice cold mixture of

sulfuric acid (60 ml) in ice (300 g) slowly, not allowing the temperature of mixture to rise above –5°C. Nitrosomethyl urea rose to the surface as a foam and was filtered through suction pump till almost dry.

Generation of diazomethane

Diazomethane was prepared whenever required by decomposing the required quantity of nitrosomethyl urea with solid potassium hydroxide in sufficient quantity of mixture of ice and diethyl ether, when a yellow colored gas got trapped in ether and was used as such.

Diazoethane

A solution of ethylamine (100 ml, 70%) in water (250 ml), was treated with concentrated hydrochloric acid (95 ml) with continuous cooling under water until it became acidic. To this was added urea (150 g) and refluxed for 6 hrs. After cooling the above solution, sodium nitrite (55 g) was added to it and the contents were cooled to 0°C. This solution was added slowly to an ice cold mixture of sulfuric acid (60 ml) in ice (300 g) slowly, not allowing the temperature of mixture to rise above –5°C. Nitrosoethyl urea rose to the surface as a foam and was filtered through suction pump till almost dry. Diazoethane was prepared when required by following a procedure similar to that used for generation of diazomethane.

Diazopropane

A solution of n-propyl amine (45 ml, 98%) in water (45 ml), was prepared in a conical flask. To this was added concentrated hydrochloric acid (45 ml) dropwise with continuous cooling under water until it became just acidic. To this was added urea (80 g) and refluxed for 8 hrs. After cooling the above solution, sodium nitrite (30 g) was added to it and the contents were cooled to 0°C. This solution was added slowly to an ice cold mixture of sulfuric acid (30 ml) in ice (300 g) slowly, not allowing the temperature of mixture to rise above –5°C. Nitrosopropyl urea rose to the surface as a slightly yellow foam and was filtered through suction pump till almost dry. Diazopropane was prepared when required by following a procedure similar to that used for generation of diazomethane.

Perbenzoic acid

Sodium (5.2 g) was added to methanol (100 ml) in a round-bottomed flask fitted with a condenser and the solution was cooled to 0°C in a mixture of crushed ice and salt. A solution of benzoyl peroxide (50.0 g) in chloroform (200 ml) was added to it with constant shaking and cooling after regular intervals so as to keep the temperature below 0°C. The mixture was kept in ice-salt bath for 5 minutes. As it turned milky, it was transferred to a separatory funnel. Sodium perbenzoate was extracted with water (3 x 150 ml) containing sufficient amount of ice. To remove methyl benzoate, the aqueous layer was extracted with chloroform (2 x 100 ml). The water layer was then acidified with ice cold sulfuric acid (1 N, 250 ml), perbenzoic acid was extracted out of the acidified aqueous layer with chloroform (3 x 150 ml) and dried (sodium sulfate) and stored in refrigerator in an amber colored bottle.

Dry methanol

Methanol AR (250.0 ml) was mixed with sodium metal (5.0 g) in a round-bottomed flask. It was distilled under dry conditions. After rejection of initial distillate (20.0 ml), the collected methanol (dry) was used as such immediately.

Activation of magnesium turnings

Mg turnings (2.5 g) were washed with dilute sulfuric acid in a suction funnel. The turnings were then washed with water to remove any traces of acid. Finally, Mg turnings were rinsed with acetone and kept in oven overnight for activation.

Silver nitrate impregnated silica gel

To a solution of $AgNO_3$ in acetonitrile, silica gel (500 g) was added in small portions and mixed thoroughly with occasional shaking. Acetonitrite was distilled and the last traces of acetonitrile were removed *in vacuo*. The silica gel was activated in the oven until the adsorbent was obtained in the form of light grey powder. It was stored in amber colored jar in the dark.

Zinc borohydride

A mixture of reagent grade anhydrous zinc chloride (4.0 g, 0.029 moles) with 50 ml diethyl ether (distilled from lithium aluminium hydride) was

boiled until most of the solid had dissolved. The mixture was allowed to stand and the supernatant liquid was carefully decanted from 0.4 g of the insoluble material. The ethereal zinc chloride solution was added drop wise to the stirred suspension of 2.7 g of 96% sodium borohydride (0.059 moles) in 150 ml of absolute ether. Stirring was continued overnight. The solids were allowed to settle and the liquid was removed by decantation and if necessary, clarified by centrifugation. The ethereal solution of zinc borohydride, thus obtained, was stored in a stoppered bottle at 5°C.

Hoagland solution

Hoagland solution was used in the biological activity experiments as the control medium for the germination of various seeds. The composition of Hoagland solution is as follows:

Macronutrients (g/l)

Potassium nitrate (KNO_3)	1.020
Calcium nitrate [$Ca(NO_3)_2$]	0.492
Ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$)	0.230
Magnesium sulfate ($MgSO_4.7H_2O$)	0.490
Ferrous sulfate ($FeSO_4.7H_2O$)	0.005

Micronutrients (mg/l)

Boric acid (H_3BO_4)	2.86
Manganese chloride ($MnCl_2.4H_2O$)	1.181
Copper sulfate ($CuSO_4.5H_2O$)	0.08
Zinc sulfate ($ZnSO_4$)	0.22
Molybdic acid ($H_2MoO_4.4H_2O$)	0.09

To 1 l of macronutrient solution, 0.1 ml of micronutrient solution was added and then the solution was diluted to make it to half strength.

Experimental

Isolation of dehydrocostus lactone (1)

The dried costus roots were crushed to a fine powder. Extraction of 250 g of powdered plant material at a time was done using 1.0 l hexane in a Soxhlet apparatus for 24 hrs. The distillation of solvent afforded a crude extract which was stored in a deep freezer to obtain a solid. The solid mixture (10 g) was subjected to CC over silica gel (600 g). The details of chromatography are presented in Table 1.

Table 1

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (10 x 100)	-	-
2	Hexane: dichloromethane 5% (5 x 100)	1.2	Mixture
3	Hexane:dichloromethane 10% (20 x 100)	7.8	Pure compound (1) mp 58-60°C
4	Hexane: dichloromethane 20% (5 x 100)	0.8	Mixture

Fraction 3 (Table 1) was identified as dehydrocostus lactone (1) on the basis of its spectroscopic analysis and comparison of mp with an authentic sample (Li *et al* 2005).

^{1}H NMR signals (400 MHz, $CDCl_3$), δ at:

6.22 (1H, d, J = 3.2 Hz C_{13}-H),

5.50 (1H, d, J = 3.2 Hz, C_{13}-H),

5.27 (1H, brs, H-15a),

5.07 (1H, brs, H-15b),

4.82 (1H, brs, H-14b),

3.97 (1H, t, J = 9.2 Hz, H-5).

^{13}C NMR (400 MHz, $CHCl_3$), δ:

45.04 (C_1-d), 30.24 (C_2-t), 36.24 (C_3-t), 149.17 (C_4-s), 51.95 (C_5-d), 85.22 (C_6-d), 47.52 (C_7-d), 30.88 (C_8-t), 32.54 (C_9-t), 30.24 (C_{10}-s),

151.22 (C_{11}-s); 170.24 (C_{12}-s), 120.18 (C_{13}-t), 109.53 (C_{14}-t), 112.55 (C_{15}-t).

Isolation of alantolactone and isoalantolactone

The roots of *Inula racemosa* were crushed to a fine powder, 250 g of the plant material was packed in the Soxhlet apparatus and extracted with chloroform (1.0 l) for 18 hrs. The chloroform extract was concentrated to yield a yellow oil (5.5 g). Extract of 4 batches (20.0 g) was collected and chromatographed over silver nitrate impregnated silica gel (1.2 kg). The details of CC are as follows:

Table 2

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 100)	-	-
2	Hexane (10 x 100)	6.7	Pure compound (2) mp 76-78°C
3	Hexane: dichloromethane 5% (5x 100)	7.5	Pure compound (1) mp 105-106°C
4	Hexane: dichloromethane 10% (5 x 100)	3.2	4 compound mixture
5	Hexane: dichloromethane 5% (8 x 100)	2.2	4 compound mixture
6	Hexane:dichloromethane 20% (5 x 100)	-	-

Fraction 2 (Table 2) was identified as alantolactone (2) by comparison of its melting point, IR and ^{1}H NMR spectra with that of an authentic sample.

Fraction 3 (Table 2) was identified as isoalantolactone (3) by comparison of its mp and spectral data with an authentic sample.

Fractions 4 and 5 (Table 2) were clubbed. The compounds of the mixture were separated by preparation thin layer chromatography.

Preparative thin layer chromatography of the alantolide mixture

The mixture obtained from the CC of plant extract of *Inula racemosa* was resolved by preparative TLC. For this, the mixture (1.0 g) was dissolved

in 5.0 ml of benzene and 1.0 ml of the mixture was charged to the plates by the spot method. Development of the plates with benzene : ethyl acetate (95:5) solvent mixture was carried out by the ascending method and led to the formation of four separate zones. The four zones were separated by scraping the silica gel from each zone of the developed plates. Compounds obtained from all the four zones (in descending order of Rf values) were analyzed spectrometrically and the data for the same is as follows:

Zone 1 (compound 4) showed following spectral data:

IR bands ν_{max} /cm^{-1} at: 1760,1660, 1460, 1375, 1140, 1060. 980, 890, 810.

^{1}H NMR ($CDCl_3$, 300 MHz) δ at:

1.11 (3H, d, J 7.83Hz, C_4-Me),

2.97 (1H, d, J 7.5Hz, C_6-H),

3.06 (1H, m, C_7-H),

4.88 (1H, m, C_8-H),

5.48, 6.23 (1H each, br s, C_{13}-H),

1.22 (3H, s, C_{10}-Me).

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

36.8 (C_1-t), 20.7 (C_2-t), 29.9 (C_3-t), 30.9 (C_4-d), 74.1 (C_5-s), 59.8 (C_6-d), 47.1 (C_7-d), 72.8 (C_8-d), 40.4 (C_9-t), 27.5 (C_{10}-s), 138.5 (C_{11}-s), 169.8 (C_{12}-s), 124.0 (C_{13}-t), 18.6 (C_{14}-q), 15.2 (C_{15}-q).

Zone 2, compound 5 revealed the following data on spectroscopic analysis:

IR bands ($CHCl_3$) ν_{max} /cm^{-1} at: 1760, 1669, 1650.

^{1}H NMR ($CDCl_3$, 300 MHz) δ at:

3.25 (1H, m, C_7-H),

4.2 (1H, m, C_8-H),

5.45, 6.20 (1H each, br s, C_{13}-H),

1.08 (3H, s, C_{14}-H),

4.81 (1H, d, J 2.3Hz, C_{15}-H),

4.86 (1H, d, J 2.4Hz, C'_{15}-H)

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

38.4 (C_1-t), 23.8 (C_2-t), 38.8 (C_3-t), 145.4 (C_4-s), 142.5 (C_5-s), 122.5 (C_6-s), 39.5 (C_7-d), 76.5 (C_8-d), 41.5 (C_9-d), 27.9 (C_{10}-s), 139.8 (C_{11}-s), 169.8 (C_{12}-s), 124.0 (C_{13}-t), 25.8 (C_{14}-q), 109.5 (C_{15}-t).

Zone 3, compound 6 showed the following spectrometric data:

IR bands ($CHCl_3$) ν_{max} /cm^{-1} at: 1760, 1645, 1475, 1370.

^1H NMR ($CDCl_3$, 300 MHz) δ at:

1.58 (1H, d, J= 7.2 Hz, C_4-H),

2.9 (1H, m, C_7-H),

4.1 (1H, m, C_8-H),

5.40, 6.25 (1H each, br s, C_{13}-H),

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

40.8 (C_1-t), 21.3 (C_2-t), 34.5 (C_3-t), 33.2 (C_4-d), 60.0 (C_5-d), 28.8 (C_6-d), 44.2 (C_7-d), 76.5 (C_8-d), 44.8 (C_9-t), 31.5 (C_{10}-s), 138.5 (C_{11}-s), 169.8 (C_{12}-s), 124.1 (C_{13}-t), 22.8 (C_{14}-q), 19.3 (C_{15}-q).

Zone 4, compound 7 on spectroscopic analysis showed the following data:

IR bands ($CHCl_3$) ν_{max} /cm^{-1} at: 1765, 1645, 1470, 1370, 1130, 860, 810.

^1H NMR ($CDCl_3$, 300 MHz) δ at:

3.25 (1H, m, C_7-H),

4.24 (1H, m, C_8-H),

5.5 (1H, br s, C_{13}-H),

6.1 (1H, br s, C'_{13}-H),

2.55 (1H, d, J 4.0Hz, C_{15}-H),

2.65 (1H, d, J 4.0 Hz, C'_{15}-H)

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

38.1 (C_1-t), 15.5 (C_2-t), 32.7 (C_3-t), 63.4 (C_4-s), 150.1 (C_5-s), 122.8 (C_6-d), 39.1 (C_7-d), 76.3 (C_8-d), 41.1 (C_9-t), 26.8 (C_{10}-s), 139.7 (C_{11}-s), 169.9 (C_{12}-s), 124.0 (C_{13}-t), 24.9 (C_{14}-q), 55.5 (C_{15}-t).

Isolation of parthenin

Parthenium hysterophorus leaves collected from PAU campus during the months of May and June, were air dried and shredded. They were then extracted in a Soxhlet apparatus using 250.0 g of powdered plant material

and 1.0 l of chloroform at a time. The extraction was done in a 24 hr batch. The chloroform extract so obtained was distilled to yield thick syrup (2.5 g). Extract of 4 batches (10.0 g) was collected and to this was added methanol (150.0 ml), water (150.0 ml), lead acetate (5.0 g) and acetic acid (1.0 ml) and kept overnight. The clarified solution, yellow in color, was filtered and the filter was concentrated to minimum volume by distilling off methanol on a heating mantle. The residue was diluted with water in 1:1 ratio and thoroughly extracted with chloroform (4 x 50 ml). The organic layer was dried (sodium sulfate) and chloroform was distilled to afford crude extract (8.0 g).

The extract was dissolved in minimum quantity of chloroform and chromatographed over silica gel (500.0 g). The details of chromatography was given in Table 3.

Table 3

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (10 x 100)	2.0	Mixture
2	Chloroform (2 x 100)	-	-
3	Chloroform: acetone 5% (10 x 100)	0.7	Mixture
4	Chloroform: acetone 5% (2 x 100)	-	-
5	Chloroform: acetone 8% (10 x 100)	1.5	Mixture Major coronopilin
6	Chloroform: acetone 10% (20 x 100)	2.5	Crystalline solid Major parthenin (8)
7	Chloroform: acetone 10% (2 x 100)	-	-
8	Chloroform: acetone 15% (10 x 100)	1.2	Highly polar liquid

Fraction 5 and 6 (Table 3, 4.0 g) were mixed and further subjected to CC over silica gel (400.0 g) and the details are given in Table 4.

Table 4

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (5x 100)	Traces	-
2	Chloroform: acetone 2% (10 x 100)	1.3	Mixture solid (major coronopilin)
3	Chloroform: acetone 5% (2 x 100)	-	-
4	Chloroform: acetone 5% (10 x 100)	2.5	Pure parthenin mp 160-162°C
5	Chloroform: acetone 10% (5 x 100)	-	-

Fraction 4 (Table 4) was identified as parthenin mp 160-162°C lit 163°C (Herz *et al* 1962). It showed the following spectroscopic data:

IR ($CHCl_3$) ν_{max} /cm^{-1} at: 3600, 3418, 3070, 1760, 1720, 1645 and 880.

^1H NMR signals ($CDCl_3$, 300 MHz) δ at:

1.11 (d, 3H, J= 7.56 Hz, C_{10}-CH_3),

1.23 (s, 3H, C_5-CH_3),

3.54 (m, 1H, exchangeable),

5.03 (d, 1H, J= 7.83 Hz, C_6-H),

5.62 (d, 1H, J= 2.70 Hz, C_{13}-H_a),

6.26 (d, 1H, J= 2.70 Hz, C_{13}-H_b),

6.15 (d, 1H, J= 6.00 Hz, C_3-H),

7.62 (d, 1H, J= 5.99 Hz, C_2-H)

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

17.43 (C_{15}-q), 18.35 (C_{14}-q), 28.40 (C_9-t), 29.81 (C_8-t), 40.47 (C_7-d)[a], 44.19 (C_{10}-d)[b], 59.05 (C_5-s), 79.06 (C_6-d), 84.28 (C_1-s), 121.87 (C_{13}-d), 131.43 (C_3-d), 140.47 (C_{11}-s), 163.77 (C_2-d), 171.19 (C_{12}-s), 211.12 (C_4-s), (a) and (b) are interchangeable.

Isolation of guaianolides from *Cichorium intybus*

Dried and roasted root powder was obtained from Mohini Coffee Beans, Bombay. The root powder (300.0 g) was extracted in a Soxhlet

extractor using chloroform as the solvent. The extract thus obtained was concentrated (8.5 g) to a minimum volume by evaporation of solvent and was then chromatographed over silica gel (500.0 g). The details of CC are as follows:

Table 5

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (5x 100)	-	-
2	Chloroform: acetone 5% (10 x 100)	0.8	Pure compound (9)
3	Chloroform: acetone 7% (3 x 100)	0.2	Mixture
4	Chloroform: acetone 10% (10 x 100)	1.5	Pure compound (10)
5	Chloroform: acetone 10% (5 x 100)	0.5	Mixture
6	Chloroform: acetone 15% (5 x 100)	2.3	Pure compound (11)
7	Chloroform: acetone 20% (5 x 100)	0.8	Mixture
8	Chloroform: acetone 25% (5 x 100)	1.8	Pure compound (12)
9	Chloroform: acetone 30% (5 x 100)	0.2	Mixture

Fraction 2 (Table 5) was identified as lactucin (9) on the basis of following spectral data:

^{1}H NMR signals (d_6-Me_2SO, 400 MHz) δ at:

6.60 (1H, br s, C_3-H),

3.53 (1H, m, C_7-H),

3.97 (1H, m, C_6-H),

3.28 (1H, m C_8-H),

2.85 (1H, dd, J= 13.0, 12.0 Hz, C_9-H),

2.34 (1H, dd, J= 13.0, 2.0, C_9-H),

6.04 (1H, d, J= 3.0 Hz, C_{13}-H),

5.67 (1H, d, J= 3.0 Hz, C_{13}-H),

2.13 (3H, br s, C_{14}-H),

4.66 (2H, dd, J= 10.0, 1.5 Hz, C_{15}-H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

132.7 (C_1-s), 194.1 (C_2-s), 133.4 (C_3-d), 169.4 (C_4-s), 47.6 (C_5-d), 80.2 (C_6-d), 52.8 (C_7-d), 43.5 (C_9-t), 145.0 (C_{10}-s), 136.5 (C_{11}-s), 168.1 (C_{12}-s), 120.9 (C_{13}-t), 20.8 (C_{14}-q), 67.8 (C_{15}-t).

Fraction 4 (Table 5), compound 10 was identified as 8-deoxy-lactucin on the basis of following spectral data:

^{1}H NMR signals (d_6-Me_2SO, 400 MHz) δ at:

6.60 (1H, br s, C_3-H),

3.53 (1H, m, C_7-H),

3.97 (1H, m, C_6-H),

6.04 (1H, d, J= 3.0 Hz, C_{13}-H),

5.67 (1H, d, J= 3.0 Hz, C_{13}-H),

2.13 (3H, br s, C_{14}-H),

4.66 (2H, dd, J= 10.0, 1.5 Hz, C_{15}-H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

132.7 (C_1-s), 194.1 (C_2-s), 133.4 (C_3-d), 169.4 (C_4-s), 47.6 (C_5-d), 80.2 (C_6-d), 52.8 (C_7-d), 31.2 (C_8-t), 33.5 (C_9-t), 145.0 (C_{10}-s), 136.5 (C_{11}-s), 168.1 (C_{12}-s), 120.9 (C_{13}-t), 20.8 (C_{14}-q), 67.8 (C_{15}-t).

Fraction 6 (Table 5), compound 11 was identified as lactucopicrin on the basis of following spectral data:

^{1}H NMR signals (d_6-Me_2SO, 400 MHz) δ at:

6.31 (1H, br s, C_3-H),

4.03 (1H, br d, J= 10 Hz, C_5-H),

3.90 (1H, t, J= 10 Hz, C_6-H),

3.52 (1H, dt, J= 10.0, 3.0 Hz, C_7-H),

4.85 (1H, dt, J= 10.0, 3.0 Hz, C_8-H),

2.84 (1H, dd, J= 13.0, 10.0 Hz, C_9-H),

2.27 (1H, dd, J= 13.0, 1.5 Hz, C_9-H),

5.37 (1H, d, J= 3 Hz, C_{13}-H_a),

5.89 (1H, d, J= 3 Hz, C_{13}-H_b),

2.32 (3H, s, C_{14}-Hs),

4.24, 4.66 (2d, J= 18.0 Hz, C_{15}-Hs),

3.62, 3.66 (2d, J= 18 Hz, CH_2(bz)),

6.72, 7.04 (2d, J= 8Hz, Ar-H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

133.0 (C_1-s), 194.1 (C_2-s), 132.0 (C_3-d), 174.9 (C_4-s), 47.6 (C_5-d), 80.3 (C_6-d), 52.9 (C_7-s), 69.2 (C_8-d), 43.3 (C_9-t), 144.6 (C_{10}-s), 138.5 (C_{11}-s), 168.1 (C_{12}-s), 120.9 (C_{13}-t), 20.6 (C_{14}-q), 61.2 (C_{15}-t), 39.6 (CH_2(bz)-t), 123.8 (Ph-C_1-s), 130.4 (Ph-C_2, C_6-d), 115.2 (Ph-C_3, C_5-d), 156.4 (Ph-C_4-s).

Fraction 8 (Table 5), compound 12 was identified as isodehydrocostus lactone on the basis of comparison of its spectroscopic data with an authentic sample.

Reaction of dehydrocostus lactone (1) under microwave irradiation

TLC-spotting technique

Six TLC plates spotted with pure sample of (1) and irradiated with microwaves. After every one minute, one TLC plate was removed and developed using standard solution of ethyl acetate: benzene (5: 95). Successive TLC plates showed the extent of completion of the reaction. Thus, the conditions of the reaction were standardized and the reaction was scaled up.

Scaling up of reaction of (1) under microwave irradiation

Dehydrocostus lactone (1.0 g) was dissolved in dichloromethane (10.0 ml). The solution was thoroughly mixed with silica gel (30 g) in a 100 ml beaker and the solvent was removed under reduced pressure. The silica gel was then exposed to microwave radiations at an output of 680 W for 6 min 40 sec. After cooling to room temperature, the irradiated silica gel was eluted with dichloromethane (20 ml). Evaporation of the solvent *in vacuo* afforded pure compound (12, 0.9 g) which showed the following spectroscopic data.

^{1}H NMR signals (400 MHz, $CDCl_3$), δ at:

6.22 (1H, d, J = 3.2 Hz, C_{13}-H_a),

5.50 (14, d, J = 3.2 Hz, C_{13}-H_b),

4.90 (1H, brs, C_{14}-H_a),

4.82 (1H, brs, C_{14}-H_b),

5.3 (1H, m, C_3-H),

1.93 (3H, s, C_{15}-Me),

4.15 (1H, t, J = 8 Hz, C_6-H).

^{13}C NMR (400 MHz, $CDCl_3$), δ at:

45.04 (C_1), 30.24 (C_2), 125.0 (C_3-d), 145.3 (C_4-5), 53.0 (C_5-d), 84.5 (C_6-d), 51.0 (C_7-d), 36.0 (C_9-t), 148.5 (C_{10}-s), 32.5 (C_{11}-s), 171.2 (C_{12}-s), 120.5 (C_{13}-t), 109.2 (C_{14}-t), 54.8 (C_{15}-q).

Reaction of dehydrocostus lactone (1) with iodine in benzene

To the solution of dehydrocostus lactone (1, 2.0 g) in benzene (25 ml), was added iodine (catalytic) and refluxed for 12 hrs. The reaction mixture was diluted with water and extracted with ether. Last traces of iodine in the organic layer were removed by washing thoroughly with sodium thiosulfate and finally drying over sodium sulfate. Evaporation of the solvent afforded a thick brown liquid compound. The spectroscopic data of this compound was identical to that obtained by the microwave method.

Reaction of isoalantolactone (3) under microwave irradiation

The solution isoalantolactone (3, 1.0 g) in dichloromethane (10.0 ml), was adsorbed onto silica gel (30.0 g) in a beaker. Evaporation of the solvent furnished free flowing silica gel adsorbed with the compound. This was irradiated under the microwave for 2 minutes followed by a time lapse of 1 min before the next irradiation. The reaction reached completion in 9 min 30 sec after which the silica gel was cooled to room temperature. It was then eluted through a column with dichloromethane (20.0 ml). The solvent was distilled off and the compound (13) obtained was analyzed spectrometrically. It showed the following data:

^{1}H NMR signals (400 MHz, $CDCl_3$), δ at:

2.95 (1H, *m*, C_7-H),

4.1 (1H, *m*, C_8-H),

5.40, 6.25 (1H each, *br s*, C_{13}-H),

1.08 (3H, *s*, C_{14}-H),

1.71 (3H, bs, C_4-Me),

5.37 (1H, s, C_3-Me)

^{13}C NMR (400 MHz, $CDCl_3$), δ at:

36.2 (C_1-t), 22.1 (C_2-t), 123.1 (C_3-d), 135.2 (C_4-s), 52.9 (C_5-d), 24.8 (C_6-t), 59.1 (C_7-d), 76.5 (C_8-d), 46.8 (C_9-t), 31.5 (C_{10}-s), 138.5 (C_{11}-s), 169.8 (C_{12}-s), 124.1 (C_{13}-t), 22.8 (C_{14}-q), 21.5 (C_{15}-q).

Reaction of isoalantolactone with iodine in benzene

Isoalantolactone (3, 6.0 g) was refluxed in dry benzene (50.0 ml) with iodine (catalytic) for 12 hrs, the progress of the reaction being monitored by TLC. Usual work up followed by evaporation of the solvent afforded a pure compound (13) which was identical to that obtained by the microwave method.

Reactions of limonene (14) and *p*-menthene (17) under microwave irradiation

Limonene (14) was dissolved in dichloromethane (10.0 ml) and mixed thoroughly with silica gel, the solvent was evaporated and the silica gel was irradiated with microwaves. The reaction reached completion in 8 minutes. Silica gel was cooled to room temperature and eluted with dichloromethane (50 ml). Distillation of the solvent yielded pure compound (16) which was identified by comparison with product obtained from reaction of limonene with iodine dry benzene.

A similar process for *p*-menthene (17) afforded its isomerized product (18) with a reaction time of 5 min 30 sec.

Reaction of dehydrocostus lactone (1) with diazomethane

A solution of dehydrocostus lactone (1, 3.0 g) in ether containing 2-3 drops of triethylamine was reacted with an excess of diazomethane. After keeping overnight, the evaporation of the solvent afforded a crystalline compound (3.1 g) which was purified by crystallization. This was identified as

the pyrazoline (19) of dehydrocostus lactone on the basis of comparison of its mp and mmp and NMR studies (mp 92°C).

IR ν_{max} /cm^{-1} at: 1770, 1640, 1550 and 850.

^{1}H NMR ($CDCl_3$, 300 MHz) δ at:

4.6 (1H, t, J= 9Hz, C_6-H),

4.72 (2H, br s, C_{14}-H),

4.88 (2 H, t, J=9 Hz, C_{16}-Hs),

5.48 and 5.60 (1H each, br s, C_{15}- Hs)

^{13} C NMR ($CDCl_3$, 67.8 MHz) δ at:

47.26 (C_1 –t), 32.25 (C_2 –t), 36.44 (C_3 –t), 52.49 (C_5 –d), 84.72 (C_6 –d), 52.05 (C_7 –d), 30.09 (C_8 –t), 30.60 (C_9 –t), 149.00 (C_{10} –s), 100.57 (C_{11}–s), 151.03 (C_{12} –s), 25.84 (C_{13}-t), 112.65 (C_{14} –t), 109.66 (C_{15} –t), 86.63 (C_{16} –d).

Pyrolysis of pyrazoline derivative (19)

The pyrazoline derivative (19, 2.4 g) of dehydrocostus lactone was dissolved in dichloromethane and adsorbed over silica gel (50.0 g). The solvent was evaporated in order to obtain free flowing silica gel. This was irradiated in microwave oven at 800 W power for 6 minutes, with a relaxation time interval of 2 minutes between every two consecutive irradiations of 1 minute each. The silica gel was then cooled to room temperature followed by elution through a column using dichloromethane as the solvent. Evaporation of the solvent afforded a mixture (2.2 g) that was resolved by CC. The details of the chromatographic procedure was given in Table 6.

Table 6

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (7 x 100)	-	Mixture
2	Hexane: diethyl ether 5% (10 x 100)	1.2	Pure compound (20) mp 74°C
3	Hexane: diethyl ether 5% (5 x 100)	0.1	Mixture

4	Hexane: diethyl ether 10% (11 x 100)	1.0	Pure compound (21) mp 70°C
5	Hexane: diethyl ether 10% (4 x 100)	-	-

Fraction 2 (Table 6), compound (20, mp 74°C) was identified as 13-methyl dehydrocostus lactone by comparison of its IR spectrum, mp and mmp with that of an authentic sample.

Fraction 4 (Table 6), compound (21, mp 70°C) was identified as 11-spirocyclopropyl derivative of dehydrocostus lactone by comparison of its IR, NMR, mp and mmp with that of authentic sample (Kalsi *et al* 1979).

Reaction of dehydrocostus lactone (1) with diazethane

Dehydrocostus lactone (2, 2.0 g) was treated with an excess of diazoethane in ether medium containing 2-3 drops of triethylamine. After keeping overnight, white crystalline product (22, 2.4 g) was obtained with dec. pt. 87-90°C.

EI-MS m/z: 258 [M^+-28] corresponding to molecular formula $C_{17}H_{22}O_2$.

IR bands (KBr) ν_{max}/cm^{-1} at: 3080, 1770, 1600, 1550, 1150 and 850.

^{1}H NMR ($CDCl_3$, 270 MHz) δ at:

1.54 (3H, d, J = 7.29 Hz, C_{17}-H)

2.91 (1H, brs, C_7-H)

4.71-4.90 (2H, m, C_6-H and C_{16}-H)

4.83 (2H, bs, C_{14}-H)

4.87 (1H, d, J = 4 Hz, C_{15}-H)

5.29 (1H, d, J = 4 Hz, C_{15}-H)

^{13}C NMR signals at δ :

37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4-s), 109.1 (C_{14}-t), 57.1 (C_5-d), 50.8(C_1-d), 148.5 (C_{10}-s), 109.0 (C_{15}-t), 36.2 (C_9-t), 25.0 (C_8-t), 35.2 (C_7-d), 80.2 (C_6-d), 106.1 (C_{11}-8), 48.7 (C_{13}-t), 52.4 (C_{16}-d), 19.9 (C_{17}-q), 172.0 (C_{12}-s).

Pyrolysis of pyrazoline derivative (22)

Pyrolysis of pyrazoline derivative (22, 2.4g) was carried out under microwave irradiation using the previously described procedure.

A mixture of 3 compounds was obtained that were isolated by CC and details of the same are presented in Table 7.

Table 7

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 100)	-	-
2	Hexane: diethyl ether 5% (5 x 100)	0.1	Mixture
3	Hexane: diethyl ether 5% (10 x 100)	0.5	Pure compound (23)
4	Hexane: diethyl ether 5% (10 x 100)	0.6	Pure compound (24)
5	Hexane: diethyl ether 10% (10 x 100)	1.0	Pure compound (25)
6	Hexane: diethyl ether 10% (4 x 100)	0.1	Mixture

Fraction 3 (Table 7), compound (23) showed the following spectroscopic data:

^{1}H NMR signals at δ:

1.1 (3H, t, J = 7.5 Hz, C_{17}-H),

1.2 (3H, t, J = 8 Hz, C_6-H),

4.0 (3H, 5, J = 8 Hz, C_6-H),

4.78 (1H, s, C_{14}-H),

4.86 (1H, s, C_{14}-H),

5.24 (1H, s, C_{15}-H),

5.29 (1H, s, C_{15}-H),

6.74 (1H, dt, J = 3 Hz; 7.5 Hz, C_{13}-H)

^{13}C NMR signals at δ:

37.5 (C_2-t), 37.0 (C_3-t), 150.7 (C_4-3), 109.1 (C_{15}-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 85.0 (C_6-d), 41.9 (C_7-d), 31.5 (C_8-t), 36.5 (C_9-t), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 14.3 (C_{17}-q), 169.8 (C_{12}-s).

Fraction 4 (Table 7), compound (24) showed the following data on spectrometric analysis:

^1H NMR signals at δ:

1.03 (3H, t, J = 7.6 Hz, C_{17}-H),

3.86 (1H, t, J = 8 Hz, C_6-H),

4.76 (1H, s, C_{14}-H),

5.03 (1H, s, C_{15}-H),

5.28 (1H, s, C_{15}-H),

5.95 (1H, dt, J = 2 Hz; 7 Hz, C_{13}-H)

^{13}C NMR signals at δ:

37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4-s), 109.1 (C_{15}-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 57.0 (C_5-d), 35.8 (C_9-t), 32.0 (C_8-t), 48.5 (C_7-d), 85.0 (C_6-d), 169.8 (C_{12}-s), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 20.0 (C_{16}-t), 14.3 (C_{17}-q).

Fraction 5 (Table 7), compound (25) was identified as the spirocyclopropyl derivative on the basis of following spectroscopic data:

^1H NMR signals at δ:

0.81-0.92 (3H, m, C_{11}-cycloprophyl-H),

1.40 (3H, t, J = 7 Hz, C_{17}-H),

3.98 (1H, t, J = 7 Hz, C_6-H),

4.76 (1H, s, C_{14}-H),

5.05 (1H, s, C_{15}-H),

5.25 (1H, s, C_{15}-H),

^{13}C NMR signals at δ:

37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4-s), 109.1 (C_{15}-t), 37.0 (C_3-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 57.5 (C_5-d), 36.1 (C_9-t), 28.2 (C_8-d), 38.0 (C_7-d), 82.9 (C_6-d), 31.5 (C_{11}-s), 15.0 (C_{16}-d), 20.5 (C_{17}-q), 32.4 (C_{13}-t), 172.0 (C_{12}-s).

Reaction of dehydrocostus lactone (1) with diazopropane

A solution of dehydrocostus lactone (1, 3.0 g) in ether containing 2-3 drops of triethylamine was reacted with an excess of ethereal solution of diazopropane. The reaction was completed after keeping overnight (TLC). Evaporation of solvent afforded a crystalline solid compound. This was identified as pyrazoline (27) of dehydrocostus lactone by the following data.

^{1}H NMR signals δ at:

1.14 (3H, d, J = 7.29 Hz, C_{18}-H),

2.90 (1H, brs, C_7-H),

4.73-4.90 (3H, m, C_{14}-H and C_{16}-H),

5.12 (1H, brs, C_{15}-H),

5.29 (1H, brs, C_{15}-H).

^{13}C NMR signals δ (ppm) at:

37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4s), 109.1 (C_{15}-t), 50.8 (C_1-d), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 36.2 (C_9-t), 28.3 (C_{17}-t), 59.2 (C_{16}-d), 9.0 (C_{18}-q), 172.7 (C_{12}-s).

Pyrolysis of the pyrazoline derivative (27)

Pyrolysis of pyrazoline derivative (27, 3.2 g) of dehydrocosus lactone was carried out under microwave irradiated conditions by an aforestated procedure to afford a mixture of 3 compounds. The mixture was chromatographed over silica gel and the details of the same are presented in Table 8.

Table 8

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (7 x 100)	Traces	-
2	Hexane: diethyl ether 5% (10 x 100)	0.7	Pure compound (28)
3	Hexane: diethyl ether 5% (4 x 100)	0.2	Mixture
4	Hexane: diethyl ether 7% (5 x 100)	0.8	Pure compound (29)

5	Hexane: diethyl ether 10% (10 x 100)	1.0	Pure compound (30)
6	Hexane: diethyl ether 15% (5 x 100)	0.1	Mixture

Fraction 2 (Table 8), compound (28) showed that following spectroscopic data:

^{1}H NMR signals at δ:

0.97 (3H, t, J = 7.5 Hz, C_{18}-H)

3.99 (1H, t, J = 9 Hz, C_{6}-H)

4.7 (1H, s, C_{14}-H)

4.85 (1H, s, C_{14}H)

5.06 (1H, d, J = 1.2 Hz, C_{15}-H)

5.3 (1H, d, J = 1.2 Hz, C_{15}-H)

6.75 (1H, dt, J = 3Hz; 9 Hz, C_{13}-H)

^{13}C NMR signals at δ:

37.5 (C_{2}-t), 37.0 (C_{3}-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_{1}-d), 57.0 (C_{5}-d), 150.7 (C_{4}-s), 109.5 (C_{15}-t), 85.0 (C_{6}-d), 41.9 (C_{7}-d), 36.5 (C_{9}-t), 129.0 (C_{11}-s), 142.5 (C_{13}-d), 29.6 (C_{8}-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s), 22.1 (C_{16}-t).

Fraction 4 (Table 8), compound (29) showed spectral peaks at:

^{1}H NMR signals δ at:

0.93 (3H, t, J = 7.5 Hz, C_{18}-H)

3.90 (1H, t, J = 9 Hz, C_{6}-H)

4.76 (1H, s, C_{14}-H)

4.86 (1H, s, C_{14}-H)

5.06 (1H, d, J = 1.5 Hz, C_{15}-H)

5.25 (1H, d, J = 1.5 Hz, C_{15}-H)

6.02 (1H, dt, J = 2.7 Hz; 7.5 Hz, C_{13}-H)

^{13}C NMR signals at δ:

37.5 (C_{2}-t), 37.0 (C_{3}-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_{1}-d), 57.0 (C_{5}-d), 150.6 (C_{4}-s), 109.4 (C_{15}-t), 85.2 (C_{6}-d), 48.5 (C_{7}-d), 36.5 (C_{9}-t),

129.0 (C_1-s), 142.5 (C_{13}-d), 29.6 (C_8-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s), 30.4 (C_{16}-t).

Fraction 5 (Table 8), compound (30) was identified as the spirocyclopropyl derivative on the basis of following spectroscopic data:

^{1}H NMR signals δ (ppm) at:

0.86-0.90 (3H, m, C_{11}-cyclopropyl-H)

0.90 (3H, t, J = 7.5 Hz, C_{18}-H)

3.98 (1H, t, J = 9 Hz, C_6-H)

4.76 (1H, s, C_{14}-H)

4.86 (1H, s, C_{14}-H)

5.06 (1H, d, J = 1.5 Hz, C_{15}-H)

5.25 (1H, d, J = 1.5 Hz, C_{15}-H)

6.02 (1H, dt, J = 2.7 Hz; 7.5 Hz, C_{13}-H)

^{13}C NMR signals at δ (ppm) :

37.5 (C_2-t), 37.0 (C_3-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 150.7 (C_4-s), 109.5 (C_{15}-t), 85.0 (C_6-d), 38.5 (C_7-d), 27.8 (C_8-t), 36.5 (C_9-t), 29.0 (C_{11}-s), 22.5 (C_{13}-d), 28.5(C_8-t), 11.6 (C_{18}-q), 29.7 (C_{17}-t), 171.9 (C_{12}-s).

Reaction of isodehydrocostus lactone with diazomethane

A solution of isodehydrocostus lactone (12, 2.8 g) in ether containing 2-3 drops of triethyl amine was treated with an excess of diazomethane. Reaction mixture was kept overnight. Evaporation of solvent *in vacuo* afforded a crystalline compound (3.2 g) which was purified by crystallization. This was identified as the pyrazoline (31, 3.0 g) of isodehydrocostus lactone (mp 110°C).

^{1}H NMR signals (400 MHz, $CDCl_3$), δ at:

4.6 (1H, t, J= 9Hz, C_6-H),

4.72 (2H, br s, C_{14}-H),

4.88 (2 H, t, J=9 Hz, C_{16}-Hs),

5.48 and 5.60 (1H each, br s, C_{15}-Hs)

5.3 (3H, m, C_3-H),

4.15 (1H, t, J = 8 Hz, C_6-H).

^{13}C NMR (400 MHz, $CDCl_3$), δ at:

48.5 (C_1-d), 35.8 (C_2-t), 125.0 (C_3-d), 142.6 (C_4-s), 53.0 (C_5-d), 79.5 (C_6-d), 35.2 (C_7-d), 36.0 (C_9-t), 148.5 (C_{10}-s), 108.8 (C_{11}-s), 171.2 (C_{12}-s), 33.5 (C_{13}-t), 77.8 (C_{14}-t), 108.8 (C_{15}-t), 20.9 (C_{16}-q).

Pyrolysis of pyrazoline (31) of isodehydrocostus lactone

Microwave assisted pyrolysis of the pyrazoline (31, 3.2 g) was carried out by the typical procedure mentioned earlier. The reaction afforded a mixture (3.1 g) which was chromatographed over silica gel (180 g). Details of the chromatography are as follows:

Table 9

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 100)	-	-
2	Hexane: diethyl ether 5% (5 x 100)	0.1	Mixture
3	Hexane: diethyl ether 5% (10 x 100)	1.3	Pure compound (32) mp 81°C
4	Hexane: diethyl ether 5% (5 x 100)	-	-
5	Hexane: diethyl ether 10% (11 x 100)	1.4	Pure compound (33) mp 80°C
6	Hexane: diethyl ether 10% (2 x 100)	0.1	Mixture

Fraction 3 (Table 9), compound (32, mp 81°C) was identified as 13-methyl isodehydrocostus lactone on the basis of comparison of spectroscopic data with authentic sample.

Fraction 5 (Table 9), compound (33, mp 80°C) was proved to be 11-spirocyclopropyl isodehydrocostus lactone on the basis of its spectral analysis.

Reaction of isodehydrocostus lactone with diazoethane

Isodehydrocostus lactone (12, 2.0 g) was treated with an excess of ethereal diazoethane containing 2-3 drops of triethylamine. After keeping

overnight 50% reaction was completed. More diazoethane was added after 24 hours, products (34, 2.4 g) was obtained with mp 165°C which was identified to be C_{16}-methyl pyrazoline of isodehydrocostus lactone. It showed the followed spectroscopic data.

IR bands ($CHCl_3$) ν_{max} /cm^{-1} at: 3080, 1770, 1600, 1550, 1150 and 850.

^1H NMR signals ($CDCl_3$, 270 MHz) δ at

1.54 (3H, d, J = 7.2 Hz, C_{17}-H)

1.70 (3H, bs, C_{15}-H)

4.70 (2H, m, C_6-H and C_{16}-H)

5.20 (2H, bs, C_{14}-H and C'_{14}-H)

5.62 (1H, brs, C_3-H)

^{13}C NMR signals ($CDCl_3$, 67.8 MHz) δ at :

19.35 (C_{17}-q), 25.84 (C_{13}-t), 30.09 (C_8-t), 30.60 (C_9-t), 32.25 (C_2-t), 36.44 (C_3-t), 47.26 (C_7-d), 52.05 (C_1-d), 100.57 (C_{11}-s), 109.65 (C_{15}-q), 109.70 (C_3-t), 112.65 (C_{14}-t), 149.00 (C_{10}-s), 151.03 (C_4-s), 172.50 (C_{12}-s)

Pyrolysis of pyrazoline (34) of isodehydrocostus lactone

Microwave assisted pyrolysis of the pyrazoline (34, 3.2 g) was carried out by the typical procedure mentioned earlier. The reaction afforded a mixture (3.1 g) which was chromatographed over silica gel (180 g). Details of the chromatography are as follows:

Table 10

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 100)	-	-
2	Hexane: diethyl ether 5% (5 x 100)	0.1	Mixture
3	Hexane: diethyl ether 5% (10 x 100)	1.3	Pure compound (35)
4	Hexane: diethyl ether 7% (5 x 100)	-	Pure compound (36)
5	Hexane: diethyl ether 10% (11 x 100)	1.4	Pure compound (37)

6	Hexane: diethyl ether 10% (2 x 100)	0.1	Mixture

Fraction 3 (Table 10), compound (35) showed the following spectroscopic data:

^{1}H NMR signals at δ:

1.3 (3H, t, J = 7.5 Hz, C_{17}-H),

4.2 (3H, s, C_6-H),

1.5 (3H, bs, C_{15}-Hs),

4.78 (1H, s, C_{14}-H),

4.86 (1H, s, C_{14}-H),

5.24 (1H, bs, C_3-H),

6.84 (1H, dt, J = 3 Hz; 7.5 Hz, C_{13}-H)

^{13}C NMR signals at δ:

37.5 (C_2-t), 123.5 (C_3-t), 142.9 (C_4-s), 109.1 (C_{15}-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 85.0 (C_6-d), 41.9 (C_7-d), 31.5 (C_8-t), 36.5 (C_9-t), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 14.3 (C_{17}-q), 169.8 (C_{12}-s).

Fraction 4 (Table 10), compound (36) showed the following data on spectrometric analysis:

^{1}H NMR signals at δ:

1.03 (3H, t, J = 7.6 Hz, C_{17}-H),

1.6 (3H, bs, C_{15}-Hs)

3.86 (1H, t, J = 8 Hz, C_6-H),

4.76 (1H, s, C_{14}-H),

5.03 (1H, s, C_{14}-H),

5.28 (1H, bs, C_3-H),

5.95 (1H, dt, J = 2 Hz; 7 Hz, C_{13}-H)

^{13}C NMR signals at δ :

37.5 (C_2-t), 122.8 (C_3-t), 143.8 (C_4-s), 109.1 (C_{15}-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 35.8 (C_9-t), 32.0 (C_8-t), 48.5 (C_7-d), 85.0 (C_6-d), 169.8 (C_{12}-s), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 20.4 (C_{16}-t), 14.3 (C_{17}-q).

Fraction 5 (Table 10), compound (37) was identified as the spirocyclopropyl derivative on the basis of following spectroscopic data:

^{1}H NMR signals at δ:

0.81-0.92 (3H, m, C_{11}-cyclopropyl-H),

1.40 (3H, d, J = 7 Hz, C_{17}-H),

1.65 (3H, bs, C_{15}-H)

4.76 (1H, s, C_{14}-H),

5.05 (1H, s, C_{14}-H),

5.25 (1H, s, C_3-H),

^{13}C NMR signals at δ:

37.5 (C_2-t), 122.9 (C_3-t), 142.8 (C_4-s), 109.1 (C_{15}-t), 37.0 (C_3-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 36.1 (C_9-t), 28.2 (C_8-d), 38.0 (C_7-d), 31.5 (C_{11}-s), 15.0 (C_{16}-d), 20.5 (C_{17}-q), 32.4 (C_{13}-t), 172.0 (C_{12}-s).

Reaction of isodehydrocostus lactone (12) with diazopropane

A solution of isodehydrocostus lactone (12, 3.0 g) in ether containing 2-3 drops of triethylamine was reacted with an excess of diazopropane. The completion of the reaction was checked by TLC. Evaporation of solvent afforded a crystallization product which was identified as pyrazoline (38) of isodehydrocostus lactone.

^{1}H NMR signals δ at:

1.14 (3H, t, J = 7.29 Hz, C_{18}-H),

4.73-4.90 (3H, m, C_{14}-H and C_{16}-H),

1.53 (3H, brs, C_{15}-H),

5.29 (1H, brs, C_3-H).

^{13}C NMR signals δ at:

37.5 (C_2-t), 123.5 (C_3-d), 142.8 (C_4-s), 109.1 (C_{15}-t), 50.8 (C_1-d), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 36.2 (C_9-t), 28.3 (C_{17}-t), 59.2 (C_{16}-d), 9.0 (C_{18}-q), 172.7 (C_{12}-s).

Pyrolysis of the pyrazoline derivative (38)

Pyrolysis of pyrazoline derivative (38, 3.2 g) of isodehydrocostus lactone was carried out under microwave irradiated conditions by an aforestated procedure to afford a mixture of 3 compounds. The mixture was

chromatographed over silica gel and the details of the same are presented in Table 11.

Table 11

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (7 x 100)	Traces	-
2	Hexane: diethyl ether 5% (10 x 100)	0.7	Pure compound (39)
3	Hexane: diethyl ether 5% (4 x 100)	0.2	Mixture
4	Hexane: diethyl ether 7% (5 x 100)	0.8	Pure compound (40)
5	Hexane: diethyl ether 10% (10 x 100)	1.0	Pure compound (41)
6	Hexane: diethyl ether 15% (5 x 100)	0.1	Mixture

Fraction 2 (Table 11), compound (39) showed that following spectroscopic data:

^{1}H NMR signals at δ:

0.97 (3H, t, J = 7.5 Hz, C_{18}-H)

3.99 (1H, t, J = 9 Hz, C_{6}-H)

4.7 (1H, s, C_{14}-H)

4.85 (1H, s, C_{14}H)

1.56 (3H, bs, C_{15}-H)

5.3 (1H, d, J = 1.2 Hz, C_{15}-H)

6.75 (1H, dt, J = 3Hz; 9 Hz, C_{13}-H)

^{13}C NMR signals at δ:

37.5 (C_{2}-t), 123.5 (C_{3}-d), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_{1}-d), 57.0 (C_{5}-d), 142.7 (C_{4}-s), 109.5 (C_{15}-t), 85.0 (C_{6}-d), 41.9 (C_{7}-d), 36.5 (C_{9}-t), 129.0 (C_{11}-s), 142.5 (C_{13}-d), 29.6 (C_{8}-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s)

Fraction 4 (Table 11), compound (40) showed spectral peaks at:

^{1}H NMR signals δ at:

0.93 (3H, t, J = 7.5 Hz, C_{18}-H)

3.90 (1H, t, J = 9 Hz, C_6-H)

4.76 (1H, s, C_{14}-H)

4.86 (1H, s, C_{14}-H)

1.6 (3H, bs, C_{15}-H)

5.25 (1H, d, J = 1.5 Hz, C_3-H)

6.02 (1H, dt, J = 2.7 Hz; 7.5 Hz, C_{13}-H)

^{13}C NMR signals at δ:

37.5 (C_2-t), 122.8.0 (C_3-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 142.6 (C_4-s), 109.4 (C_{15}-t), 85.2 (C_6-d), 48.5 (C_7-d), 36.5 (C_9-t), 129.0 (C_1-s), 142.5 (C_{13}-d), 29.6 (C_8-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s).

Fraction 5 (Table 11), compound (41) was identified as the spirocyclopropyl derivative on the basis of following spectroscopic data:

^{1}H NMR signals δ at:

0.86-0.90 (3H, m, C_{11}-cyclopropyl-H)

0.90 (3H, t, J = 7.5 Hz, C_{18}-H)

3.98 (1H, t, J = 9 Hz, C_6-H)

4.76 (1H, s, C_{14}-H)

4.86 (1H, s, C_{14}-H)

1.6 (3H, bs, C_{15}-H)

5.25 (1H, d, J = 1.5 Hz, C_3-H)

^{13}C NMR signals at δ :

37.5 (C_2-t), 123.0 (C_3-d), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 142.7 (C_4-s), 109.5 (C_{15}-t), 85.0 (C_6-d), 38.5 (C_7-d), 27.8 (C_8-t), 36.5 (C_9-t), 29.0 (C_{11}-s), 22.5 (C_{13}-d), 28.5(C_8-t), 11.6 (C_{18}-q), 29.7 (C_{17}-t), 171.9 (C_{12}-s).

Reaction of alantolactone (2) with diazomethane

To a solution of alantolactone (2, 3.0 g) in ether containing 2-3 drops of triethylamine, was added an excess of ethereal solution of diazomethane. It was kept overnight. After completion of reaction TLC), the solvent was

evaporated which afforded crystalline compound (42, 3.4 g) identified as pyrazoline (mp 119°C), being identical in all respects to authentic sample (Kalsi *et al* 1984).

Pyrolysis of pyrazoline (42) of alantolactone

The pyrazoline derivative (42, 3.4 g) was pyrolysed under microwave radiations to afford a mixture (3.2 g) that was resolved by CC to furnish two products. The details of chromatography are depicted in Table 12.

Table 12

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (7 x 100)	Traces	-
2	Hexane: diethyl ether 5% (10 x 100)	1.2	Pure compound (44) mp 78°C
3	Hexane: diethyl ether 5% (5 x 100)	0.2	Mixture
4	Hexane: diethyl ether 10% (11 x 100)	1.6	Pure compound (45) mp 78°C
5	Hexane: diethyl ether 10% (3 x 100)	0.1	Mixture

Fraction 2 (Table 12), compound (44, mp 78°C) was identified as 13-methyl alantolactone by comparison of its IR spectrum, mp and mmp with that of an authentic sample.

Fraction 4 (Table 12), compound (45, mp 75°C) was identified as 11-spirocyclopropyl derivative by comparison of its IR, NMR, mp and mmp with that of an authentic sample.

Reaction of isoalantolactone (3) with diazomethane

A solution of isoalantolactone (3, 2.5 g) in ether containing 2-3 drops of triethylamine was reacted with an ethereal solution of diazomethane. After keeping the reaction mixture overnight, the evaporation of solvent afforded a single compound (2.9 g) which was purified by crystallization. This was identified as the pyrazoline (43) of isoalantolactone on the basis of

comparison of its mp, mmp and IR spectrum with that of an authentic sample (mp 165°C).

Pyrolysis of pyrazoline (43) of isoalantolactone

Microwave-assisted pyrolysis of the pyrazoline derivative (43, 3.0 g) was carried out by the previously described procedure. Elution of irradiated silica gel followed by evaporation of the solvent yielded a mixture, the CC of which furnished two compounds (Table 13).

Table 13

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (7 x 100)	Traces	-
2	Hexane: diethyl ether 5% (10 x 100)	1.0	Pure compound (46) mp 151°C
3	Hexane: diethyl ether 5% (4 x 100)	0.2	Mixture
4	Hexane: diethyl ether 10% (11 x 100)	1.2	Pure compound (47) mp 78°C
5	Hexane: diethyl ether 10% (3 x 100)	0.2	Mixture

Fraction 2 (Table 13), compound (46, mp 151°C) was identified as 13-methyl isoalantolactone by comparison of its IR spectrum, mp and mmp with that of an authentic sample.

Fraction 4 (Table 13), compound (47, mp 111°C) was identified as 11-spirocyclopropyl derivative of isoalantolactone by comparison of its IR, NMR, mp and mmp that of an authentic sample.

Reaction of alantolactone (2) with diazoethane

The solution of alantolactone (2, 2.0 g) in ether was treated with ethereal solution of diazoethane in the presence of 2-3 drops of triethyl amine. After completion of the reaction, solvent was evaporated to yield a white crystalline compound (48, dec. pt. 94°C), which showed the following spectral data:

IR bands ν_{max}/cm^{-1} at: 2929, 1754, 1455, 1373, 1197, 1126, 1037, 887.

^{1}H NMR signals (270 MHz), δ (ppm) at:

1.23 (3H, d, J = 7.76 Hz, C_{15}-H)

1.26 (3H, s, C_{14}-H)

1.57 (3H, d, J = 7.29 Hz, C_{17}-H)

4.78 (1H, m, C_8-H)

5.51 (1H, m, C_{16}-H)

Pyrolysis of compound (48)

Microwave-assisted pyrolysis of the pyrazoline derivative (48, 2.0 g) was carried out by the previously described procedure. Elution of irradiated silica gel followed by evaporation of the solvent yielded a mixture, the CC of which furnished three compounds (Table 14).

Table 14

S. No.	Eluent (ml)	Wt (g)	TLC based remarks
1	Pet. ether (2 x 50)	-	-
2	Pet. ether:diethyl ether (2%) (8 x 50)	0.2	Pure compound (49) mp 83°C
3	Pet. ether:diethyl ether (2%) (5 X 50)	0.1	Mixture
4	Pet. ether:diethyl ether (5%) (10 X 50)	0.7	Pure compound (50) mp 147°C
5	Pet. ether:diethyl ether (5%) (4 X 50)	0.1	Mixture
6	Pet. ether:diethyl ether (8%) (8 X 50)	0.4	Pure compound (51) liquid

Fraction 2 (Table 14), compound (49, mp 83°C)

IR bands ν_{max} /cm^{-1} at: 2927, 1739, 1668, 1440, 1371, 11199 970, 867

^{1}H NMR signals (270 MHz, δ) at

1.05 (3H, t, J = 7.56 Hz, C_{17}-H)

1.11 (3H, d, J = 7 Hz, C_{15}-H)

1.25 (3H, s, C_{14}-H)

3.47 (1H, brs, C_7-H)

4.77 (1H, m, C_8-H)

5.08 (1H, d, J = 3.8 Hz, C_6-H)

6.14 (1H, dt, J = 1.6 Hz; 6 Hz, C_{13}-H)

Fraction 4 (Table 14) compound (50, mp 147°C)

IR bands (cm^{-1}) at: 2929, 12743, 1326, 1247, 1132, 975, 902

^{1}H NMR signals (270 MHz, δ) at

0.98 (3H, m, C_{11}-cyclopropyl-H)

1.14 (3H, d, J = 7.56 Hz, C_{15}-H)

1.23 (3H, d, J = 5 Hz, C_{17}-H)

1.27 (3H, s, C_{14}-H)

4.9 (1H, m, C_8-H)

5.07 (1H, d, J = 3 Hz, C_6-H)

Fraction 6 (Table 14), compound (51, liquid)

IR bands (cm^{-1}) at: 2927, 1757, 1668, 1629, 1549, 1459, 1379, 1266, 1198, 1030, 977, 866

^{1}H NMR signals (300 MHz, δ) at

1.12 (3H, t, J = 7.3 Hz, C_{17}-H)

1.15 (3H, d, J = 6.6 Hz, C_{15}-H)

1.21 (3H, s, C_{14}-H)

3.47 (1H, brs, C_7-H)

4.87 (1H, m, C_8-H)

5.16 (1H, d, J = 3 Hz, C_6-H)

6.65 (1H, dt, J = 1.8 Hz; 7.8 Hz, C_{13}-H)

Reaction of isoalantolactone (3) with diazoethane

The solution of isoalantolactone (3, 2.0 g) in ether (5.0 ml) was treated with ethereal solution of diazoethane. Shining white crystalline product (52, 1.8 g, dec. pt. 100-102°C) was obtained. It showed the following data on spectroscopic analysis:

IR bands ν_{max}/cm^{-1} at: 2933, 1768, 1644, 1441, 1160, 993, 944, 890.

^{1}H NMR signals (270 MHz) δ (ppm) at:

0.85 (3H, s, C_{14}-H)

1.55 (3H, d, J = 7.29 Hz, C_{17}-H)

4.45 (1H, m, C_8-H)

4.74 – 4.85 (2H, m, C_{15}-H)

5.57 (1H, m, C_{16}-H)

Pyrolysis of compound (52)

The pyrazoline derivative (47, 2.0 g) was pyrolysed under microwave radiations to afford a mixture (3.2 g) that was resolved by CC to furnish three products. The details of chromatography are depicted in Table 15.

Table 15

S. No.	Eluent (ml)	Wt (g)	TLC based remarks
1	Pet. Ether (2 x 50)	-	-
2	Pet. Ether:diethyl ether (2%) (4 x 50)	0.2	Pure compound (53) mp 97°C
3	Pet. Ether:diethyl ether (2%) (3 X 50)	0.1	Mixture
4	Pet. Ether:diethyl ether (5%) (12 X 50)	0.4	Pure compound (54) mp 130°C
5	Pet. Ether:diethyl ether (10%) (4 X 50)	0.1	Mixture
6	Pet. Ether:diethyl ether (10%) (15 X 50)	0.4	Pure compound (55) mp 98°C

Fraction 2 (Table 15) compound (53, mp 97°C)

IR bands ν_{max}/cm^{-1} at: 2935, 1741, 1704, 1671, 1133, 1091, 991, 914, 883.

^{1}H NMR signals (270 MHz) δ at:

0.84 (3H, s, C_{14}-H)

1.06 (3H, t, J = 7.56 Hz, C_{17}-H)

2.62 (1H, m, C_7-H)

4.46 (1H, d, J = 1.6 Hz, C_{15}-H)

4.76 (1H, d, J = 1.1 Hz, C_{15}-H)

4.48 (1H, brs, C_8-H)

6.12 (1H, d, J = 8 Hz, C_{13}-H)

Fraction 4 (Table 15) compound (54, mp 130°C)

IR bands ν_{max}/cm^{-1} at: 2933, 1741, 1145, 954, 900.

^{1}H NMR signals (270 MHz) δ at:

0.86 (3H, s, C_{14}-H)

0.99-1.16 (3H, m, C_{11}-cyclopropyl-H)

1.19 (3H, d, J = 5.9 Hz, C_{17}-H)

4.49 (1H, s, C_{15}-H)

4.76 (1H, s, C_{15}-H)

4.67(1H, m, C_8-H)

Fraction 6 (Table 15), compound (55, mp 98°C)

IR bands ν_{max}/cm^{-1} at: 2927, 1747, 1683, 12216, 997, 960, 896, 773

^{1}H NMR signals (270 MHz) δ at:

0.83 (3H, s, C_{14}-H)

1.1 (3H, t, J = 7.6 Hz, C_{17}-H)

2.99-3.08 (2H, m, C_{16}-H)

2.98-3.05 (1H, m, C_7-H)

4.43 (1H, s, C_{15}-H)

4.48 (1H, m, C_8-H)

4.77 (1H, s, C_{15}-H)

6.63 (1H, dt, J = 1.9 and 8.0 Hz, C_{13}-H)

Reaction of parthenin (8) with diazomethane

To a suspension of parthenin (8, 2.0 g) in ether, was added an excess of ethereal solution of diazomethane. Evaporation of solvent after 24 hrs at 0°C yielded a powder (1.89g), which upon recrystallization from ethyl acetate gave white crystals of pure pyrazoline (56), mp 140°C. It showed the following spectral data:

IR bands ν_{max}/cm^{-1} at: 3600, 3390, 1770, 1730, 1340, 980.

^{1}H NMR signals (270 MHz) δ at:

1.2 (d, 3H, C_{10}-CH_3, J= 7.0 Hz),

1.43 (s, 3H, C_5-CH_3),

4.66 (m, 2H, -CH_2-N=N-),

5.67 (d, 1H, C_6-H, J= 7.0 Hz),

6.26 (d, 1H, C_3-H, J= 7.0 Hz),

7.7 (d, 1H, C_2-H, J= 7.0 Hz).

Pyrolysis of the pyrazoline derivatiove (56)

The pyrazoline (51, 3.0 g) was dissolved in dichloromethane (10.0 ml) and this was adsorbed on to silica gel (30 g). Free flowing silica gel was then irradiated under microwaves at 800W level for 5 minutes intermittently (30 second exposure and 1 minute interval). The silica gel was eluted with 10% ethanol in hexane and evaporation of solvent furnished a mixture that was resolved by chromatography. The details of CC are as follows:

Table 16

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (7 x 100)	-	Mixture
2	Hexane: diethyl ether 5% (10 x 100)	1.2	Pure compound (57) mp 130°C
3	Hexane: diethyl ether 5% (5 x 100)	0.1	Mixture
4	Hexane: diethyl ether 10% (11 x 100)	1.0	Pure compound (58)
5	Hexane: diethyl ether 10% (4 x 100)	-	-

Fraction 2 (Table 16), compound (52, mp 130°C) was identified as 13-methyl derivative of parthenin by comparison of its IR spectrum, mp and mmp with that of an authentic sample.

Fraction 4 (Table 16), compound (53, mp 70°C) was identified as 11-spirocyclopropyl derivative of parthenin by comparison of its IR, NMR, mp and mmp with that of authentic sample (Kalsi *et al* 1979).

Decomposition of Mannich bases under microwave irradiation

In a typical procedure, Mannich base (59, 1.0 g) was dissolved in minimum amount of dichloromethane and to this was added silica gel (50.0 g). The mixture was stirred and air-dried to obtain free flowing silica gel with adsorbed Mannich base. This was then subjected to microwave irradiation for 5 minutes at 600 W level, cooled and pure arylidene (60) was extracted with dichloromethane. It showed the following spectral data:

^{1}H NMR signals ($CDCl_3$) at δ:

1.12 (3H, s), 1.11 (3H, s), 1.22 (3H, s), 2.47 (4H, bs), 5.57 (1H, s), 7.22 – 7.28 (5H, m).

^{13}C NMR signals at δ:

194.3 (C_1-s), 52.8 (C_2-t), 30.3 (C_3-s), 52.8 (C_4-d), 194.4 (C_5-s), 250.9 (C_6-s), 146.0 (C_7-d), 135.3 (C_8-s), 126.5 (C_9-d), 128.5 (C_{10}-d), 128.1 (C_{11}-d), 128.5 (C_{12}-d), 126.3 (C_{13}-d), 26.5 (C_{14}-q), 26.6 (C_{15}-q)

Similarly, other Mannich bases (61-65) were decomposed on solid matrix using microwave radiations to furnish the respective arylidenes (66-70), that were identified on the basis of their spectral analysis.

Spectral data of compound 66

^{1}H NMR signals at δ:

1.12 (3H, s), 1.24 (3H, s), 2.32 – 2.48 (4H, m), 3.75, 3.83, 3.93 (3H each, s), 5.50 (1H, s), 6.34 (1H, s) and 6.63 (1H, s).

^{13}C NMR signals at:

194.3 (C_1-s), 52.8 (C_2-t), 30.3 (C_3-s), 52.8 (C_4-d), 194.4 (C_5-s), 250.9 (C_6- s), 146.0 (C_7-d), 135.3 (C_8-s), 126.5 (C_9-d), 136.5 (C_{10}-s), 135.1 (C_{11}-s), 136.5 (C_{12}-s), 123.3 (C_{13}-d), 26.5 (C_{14}-q), 26.6 (C_{15}-q), 22.5 (C_{16}-q), 18.8 (C_{17}-q), 22.1 (C_{18}-q).

Spectral data of compound 67

^{1}H NMR signals δ at:

1.09 (3H, s), 1.21 (3H, s), 2.32 – 2.46 (4H, m), 5.66 (1H, s), 7.02 (2H, d, J = 9 Hz) and 7.20 (2H, d, J = 9 Hz)

^{13}C NMR signals at δ:

194.3 (C_1-s), 52.8 (C_2-t), 30.5 (C_3-s), 52.7 (C_4-d), 194.3 (C_5-s), 140.8 (C_6-s), 146.1 (C_7-d), 132.9 (C_8-s), 127.8 (C_9-d), 128.6 (C_{10}-d), 133.2 (C_{11}-s), 128.5 (C_{12}-d), 127.5 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q)

Spectral data of compound 68

^{1}H NMR signals at δ :

1.09 (3H, s), 1.22 (3H, s), 2.32-2.41 (4H, s), 3.76 (3H, s), 5.48 (1H, s), 6.80 (2H, d, J = 9 Hz), 6.99 (2H, d, J = 9 Hz)

^{13}C NMR signals at δ :

194.4 (C_1-s), 52.9 (C_2-t), 30.4 (C_3-s), 52.9 (C_4-d), 194.38 (C_5-s), 140.8 (C_6-s), 145.9 (C_7-d), 127.6 (C_8-s), 127.4 (C_9-d), 114.5 (C_{10}-d), 159.9 (C_{11}-s), 113.9 (C_{12}-d), 127.5 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q)

Spectral data of compound 69

^{1}H NMR signals at δ:

1.13 (3H, s), 1.25 (3H, s), 2.0-2.21 (4H, m), 5.56 (1H, s) and 7.44-8.23 (4H, m)

^{13}C NMR signals at δ:

194.2 (C_1-s), 52.8 (C_2-t), 30.2 (C_3-s), 52.5 (C_4-d), 194.0 (C_5-s), 141.2 (C_6-s), 145.9 (C_7-d), 124.7 (C_8-s), 127.1 (C_9-d), 113.9 (C_{10}-d), 148.5 (C_{11}-s), 114.2 (C_{12}-d), 127.1 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q), 40.2 (q, N-CH_3), 40.3 (q, N-CH_3)

Spectral data of compound 70

^{1}H NMR signals at δ :

1.09 (6H, s), 2.37-2.56 (4H, m), 2.91 (3H, s), 3.01 (3H, s), 6.64 (24, d, J = 9 Hz), 6.26 (2H, d, J = 9 Hz), 6.02 (1H, s)

^{13}C NMR signals at δ :

194.2 (C_1-s), 52.8 (C_2-t), 30.2 (C_3-s), 52.7 (C_4-d), 194.4 (C_5-s), 140.5 (C_6-s), 145.2 (C_7-d), 135.8 (C_8-s), 132.2 (C_9-d), 129.8 (C_{10}-d), 122.8 (C_{11}-d), 147.2 (C_{12}-s), 119.8 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q)

Microwave-assisted Hoffmann elimination of quaternary ammonium salts

In a typical procedure, the morpholide adduct of dehydrocostus lactone (71), was dissolved in dichloromethane and then adsorbed on basic alumina which was subjected to microwave irradiation at 800 W level for 5 minutes. Elution of the irradiation silica gel with dichloromethane and further evaporation of the solvent resulted in the isolation of parent compound (1) in almost 90% yield.

Similarly, the morpholide adducts (72-77) were also subjected to microwave irradiation and the pure parent compounds (2, 3, 71, 72, 69, 8) were isolated, respectively.

Reaction of dehydrocostus lactone with sodium perborate

1. In acetic anhydride – dichloromethane

The reaction was attempted using different relative preparations of acetic anhydride and dichloromethane. Dehydrocostus lactone (1, 100 mg) was taken in 5 different flasks. To each added the solvent system (10.0 ml) with different ratios of acetic anhydride: dichloromethane (3:1, 1:1, 1:3, 9:1 and 1:9) followed by 88 mg of sodium perborate (1.1 eq). Stirred the reaction mixtures over a magnetic stirrer and checked the completion of reaction by TLC. The reaction employing the ratio 9:1 for acetic anhydride and dichloromethane reached proved to be best suited for the reaction. The reaction was then scaled up using 1 g of dehydrocostus lactone and 1.1 eq. of sodium perborate. The reaction mixture was washed with distilled water (3 x 20 ml) and extracted with dichloromethane (2 x 20 ml). Evaporation of solvent afforded a mixture that was subjected to CC, the details of which are presented in Table 17.

Table 17

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 50)	-	-

2	Hexane: dichloromethane 5% (10 x 50)	0.25	Pure compound (1) (unreacted)
3	Hexane: dichloromethane 5% (5 x 50)	Traces	Mixture
4	Hexane:dichloromethane 10% (5 x 50)	0.2	Pure compound (78)
5	Hexane:dichloromethane 15% (5 x 50)	0.055	mixture

Fraction (2) (Table 17) proved to be unreacted dehydrocostus lactone by comparison of its TLC with pure sample.

Fraction 4 (Table 17) compound (78) was identified as epoxy derivative of (1) by comparison of spectral datawith authentic sample.

^{1}H NMR signals (300 MHz, $CDCl_3$, δ:

6.07 and 5.37 (d, 1H each, J= 3.0 Hz, C_{13}-Hs),

3.89 (1H, dd, J= 9.0 and 10.0 Hz, C_6-H),

5.10 and 4.90 (brs, (1H each, C_{14}-Hs),

3.27 and 2.74 (d, 1H each, J=5 Hz, C_{15}-Hs)

^{13}C NMR signals (300 MHz, $CDCl_3$) δ (ppm):

43.2 (C_1-d), 25.5 (C_2-t), 34.5 (C_3-t), 61.7 (C_4-d), 78.2 (C_6-d), 40.5 (C_7-d), 27.8 (C_8-d), 36.0 (C_9-t), 148.5 (C_{10}-s), 171.2 (C_{12}-s), 15.3 (C_{13}-t), 109.2 (C_{14}-t), 52.2 (C_{15}-t), 15.1 (C_{16}-t).

The data proved fraction 2 (Table 17) to be the monoepoxide of (1).

2. In the presence of phase transfer catalyst

Dehydrocostus lactone (1, 2.0 g) was dissolved in dichloromethane (20.0 ml). In another flask, a solution of sodium perborate (1.86 g) was prepared in distilled water (10.0 ml). The solutions were mixed and stirred for 6 hours in the presence of catalytic amount of cetyl trimethyl ammonium bromide. Usual workup afforded a mixture (1.9 g) that was resolved by CC. Details of the same are as follows:

Table 18

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 50)	-	-
2	Hexane: diethyl ether 5% (10 x 50)	0.7	Pure compound (1) (unreacted)
3	Hexane: diethyl ether 5% (3 x 50)	0.2	Mixture
4	Hexane: diethyl ether 10% (8 x 50)	1.1	Pure compound (78)
5	Hexane: diethyl ether 10% (4 x 50)	Traces	Mixture

Fraction 2 (Table 18), compound (1) was unreacted dehydrocostus lactone that was identified by TLC with pure sample.

Fraction 4 (Table 18), compound (78, 1.1 g) was identified as the epoxy derivative of dehydrocostus lactone by comparison of its spectral data with an authentic sample.

3. In isopropanol-water

Dehydrocostus lactone (1, 1.0 g) was taken in a flask and to this was added the solvent system (10.0 ml) of isopropanol and water in the ratio 3:1, followed by 88 mg of sodium perborate (1.1 eq). Stirred the reaction mixtures over a magnetic stirrer and checked the completion of reaction by TLC. The reaction reached completion in 2 hrs. The reaction mixture was washed with distilled water (3 x 20 ml) and extracted with dichloromethane (2 x 20 ml). Evaporation of solvent afforded a pure compound that that proved to be epoxy-derivative of dehydrocostus lactone by spectral comparison with samples.

Reaction of dehydrocostus lactone (1) with perbenzoic acid

Dehydrocostus lactone (1, 1.0g) was dissolved in chloroform (10.0 ml) and perbenzoic acid (PBA) was added to it dropwise till there is excess of PBA (as indicated by liberation of iodine from potassium iodide in acetic acid by this reaction mixture). Reaction mixture was kept at 0°C for 12 hrs. Usual

workup with sodium bicarbonate/ether afforded a two component mixture which was chromatographed over silica gel (50 g). Details of chromatography are presented in Table 19.

Table 19

S. No.	Eluent (mL)	Weight (g)	TLC based remarks
1	Petroleum ether (3 x 100)	-	-
2	Petroleum ether : diethyl ether 5% (5 x 100)	0.4	Pure compound (79)
3	Petroleum ether : diethyl ether 10% (5 x 100)	Traces	Mixture

Fraction 2 (Table 19) was identified as the mono-epoxide of dehydrocostus lactone by comparison of its spectroscopic data with that of an authentic sample.

Reaction of isodehydrocostus lactone (12) with sodium perborate

Isodehydrocostus lactone (12, 1.0 g) was taken in a flask and to this was added the solvent system (10.0 ml) with acetic anhydride: dichloromethane (9:1) followed by 100 mg of sodium perborate (1.1 eq). The reaction mixture was stirred and the completion of reaction was checked by TLC. The reaction reached completion in 3 hrs. The reaction mixture was washed with distilled water (3 x 20 ml) and extracted with dichloromethane (2 x 20 ml). Evaporation of solvent afforded a mixture that was subjected to CC, the details of which are presented in Table 20.

. Table 20

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 50)	-	-
2	Hexane: diethyl ether 5% (10 x 50)	0.4	Pure compound (12) unreacted

3	Hexane: diethyl ether 5% (3 x 50)	traces	mixture
4	Hexane: diethyl ether 10% (8 x 50)	0.5	Pure compound (82)
5	Hexane: diethyl ether 10% (4 x 50)	traces	mixture

Fraction 2 (Table 20), compound (12) was the unreacted isodehydrocostus lactone (12).

Fraction 4 (Table 20), compound (82) showed the following spectroscopic data:

IR bands ν_{max} /cm^{-1} at 2995, 2800, 1790, 1470, 1380, 1260, 1150, 940, 880, 860.

^{1}H NMR signals (90 MHz, $CDCl_3$) (ppm) at:

1.25 (3H, s, C_{14}-H),

1.6 (3H, s, C_{15}-H),

3.1-3.5 (3H, m, C_3-H),

3.95 (1H, t, J= 9 Hz, C_6-H),

5.2 (1H, d, J= 2 Hz, C_{13}-H),

5.9 (1H, d, J= 2 Hz, C_{13}-H)

Reaction of isoalantolactone (3) with sodium perborate

Isoalantolactone (3, 2.0 g) was taken in a flask and to this was added the solvent system (10.0 ml) with acetic anhydride: dichloromethane (9:1) followed by 200 mg of sodium perborate (1.1 eq). The reaction mixture was stirred and the progress of reaction was checked by TLC. The reaction reached completion in 4 hrs and 30 minutes. For workup, the reaction mixture was washed with distilled water (3 x 20 ml) and extracted with dichloromethane (2 x 20 ml). Evaporation of solvent afforded a mixture (1.8 g) that was subjected to CC, the details of which are presented in Table 21.

Table 21

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 50)	-	-
2	Hexane: diethyl ether 5% (10 x 50)	0.6	Pure compound (3) (unreacted)
3	Hexane: diethyl ether 5% (3 x 50)	0.1	Mixture
4	Hexane: diethyl ether 10% (8 x 50)	1.0	Pure compound (83)
5	Hexane: diethyl ether 10% (4 x 50)	Traces	Mixture

Fraction 2 (Table 21), compound (3) was unreacted isoalantolactone that was identified by TLC with pure sample.

Fraction 4 (Table 21), compound (83) was identified as epoxy-derivative of isoalantolactone by spectroscopic analysis, the details of which are as follows:

IR bands ν_{max} /cm^{-1} at 1765, 1645, 1470, 1272, 942, 812.

^{1}H NMR ($CDCl_3$) δ (ppm) at:

1.18 (s, 3H, C_{10}-CH_3),

2.80 (d, 1H each, J= 3.0 Hz, C_{15}-Hs),

4.67 (m, 1H, C_8-H),

5.73 and 6.40 (brs, 2H, J= 3.2 Hz, C_{13}-Hs).

Reaction of alantolactone (2) with sodium perborate

Alantolactone (2, 1.0 g) was taken in a flask and to this was added the solvent system (10.0 ml) with acetic anhydride: dichloromethane (9:1) followed by 100 mg of sodium perborate (1.1 eq). The reaction mixture was stirred and the completion of reaction was checked by TLC. The reaction reached completion in 5 hrs. The reaction mixture was washed with distilled water (3 x 20 ml) and extracted with dichloromethane (2 x 20 ml). Evaporation of solvent afforded a mixture that was subjected to CC, the details of which are presented in Table 22.

Table 22

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 50)	-	-
2	Hexane: diethyl ether 5% (10 x 50)	0.7	Pure compound (2) unreacted
3	Hexane: diethyl ether 5% (3 x 50)	0.2	Mixture
4	Hexane: diethyl ether 10% (8 x 50)	1.1	Pure compound (84)
5	Hexane: diethyl ether 10% (4 x 50)	Traces	Mixture

Fraction 2 (Table 22), compound (2) was unreacted alantolactone that was identified by TLC with pure sample.

Fraction 4 (Table 22), compound was identified as epoxy-derivative of alantolactone by spectrometric screening, the details of which are as follows:

IR bands ν_{max}/cm^{-1} at 1760, 1660, 1460, 1375, 1140, 1060, 980, 890, 810.

^1H NMR ($CDCl_3$) δ (ppm) at:

1.04 (d, 3H, J= 7 Hz, C_4- CH_3),

1.10 (s, 3H, C_{10}-CH_3),

2.89 (d, 1H, J= 7.29 Hz, C_6-H),

4.52 (m, 1H, C_8-H),

5.50 and 6.23 (d, 1H, C_{13}-Hs).

Reaction of isotelekin (93) with sodium perborate

Isotelekin (93, 1.0 g) was dissolved in acetic anhydride: dichloromethane (9:1, 10.0 ml) and sodium perborate (0.3 g) was added to it. Reaction mixture was stirred for 12 hrs. Usual workup afforded a mixture that was resolved to afford two pure compounds (85 and 86). Compound (86) showed the following spectral data:

IR bands ν_{max}/cm^{-1} at 3490, 1750, 1660, 1450, 1370, 1130, 1050, 960, 810.

^1H NMR signals ($CDCl_3$) δ (ppm) at:

0.97 (s, 3H, C_{14}-Hs),

2.79 and 2.62 (d, 1H each, C_{15}-Hs),

3.40 (br s, 1H, C_3-H),

4.47 (m, 1H, C_8-H),

5.54 (br s, 1H, C_{13}-H),

6.09 (br s, 1H, C_{13}-H).

Reaction of isozaluzanin-C (89) with sodium perborate

Isozaluzanin-C (89, 1.0 g) was dissolved in acetic anhydride: dichloromethane (9:1, 10.0 ml) and sodium perborate (0.2 g) was added to it. Reaction mixture was stirred for 5 hrs. Usual workup afforded a mixture (87 and 88). Compound (87) was the major product, which showed the following spectral data:

IR bands ν_{max}/cm^{-1} at 3450, 1760, 1720, 1640, 1070.

^{1}H NMR signals ($CDCl_3$) δ (ppm) at:

2.80 and 3.00 (br s, 1H each, C_{15}-Hs),

4.01 (t, 1H, J= 9.2 Hz),

4.00 (t, 1H, J=8.0 Hz, C_6-H),

5.00 (br s, 2H, C_{14}-Hs),

5.45 and 6.20 (d, 1H each, J= 2.0 Hz, C_{13}-Hs).

Reaction of dehydrocostus lactone (1) with SeO_2/ TBHP

1. Conventional method

Selenium dioxide (5.0 mg) was added to *tert.*-butyl hydroperoxide (1.5 ml, 70%) and the mixture was stirred for half an hour. To this mixture, a solution of dehydrocostus lactone (2.0 g) in dichloromethane (25.0 ml) was added and the reaction mixture was stirred at room temperature, and the progress was monitored by TLC. The reaction was completed after 5 hrs. The reaction mixture was diluted with cold water and extracted with dichloromethane. The combined organic extracts were washed with water and dried over Na_2SO_4. Evaporation of solvent afforded a mixture of two components (TLC, 1.9 g) which was chromatographed over silica gel (200 g) and the details of same are given in Table 23.

Table 23

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (5 x 50)	traces	-
2	Hexane: diethyl ether 5% (10 x 50)	0.8	Pure compound (88) mp 143°C
3	Hexane: diethyl ether 5% (3 x 50)	0.5	Mixture
4	Hexane: diethyl ether 10% (8 x 50)	0.4	Pure compound (91) mp 160°C
5	Hexane: diethyl ether 10% (4 x 50)	traces	Mixture

Fraction 2 (Table 23) was characterized as isozaluzanin-C (88) mp 143°C. Similarly, fraction 4 (Table 23) mp 160°C was identified to be diol (91) being identical in all respects to authentic sample (Kalsi *et al* 1984).

2. Microwave method

Selenium dioxide (5.0 mg) was added to *tert.*-butyl hydroperoxide (1.5 ml, 70%) and the mixture was stirred. To this mixture, a solution of dehydrocostus lactone (2.0 g) in minimum quanity of dichloromethane was added. The mixture was adsorbed over silica gel (60 g). The excess solvent was evaporated to furnish a free flowing powder that was irradiated with microwaves at 640W for 7 minutes with a time lapse of 1 minute after every irradiation of 2 minutes. The irradiated silica gel was cooled to room temperature and eluted with dichloromethane (50.0 ml). Evaporation of solvent *in vacuo* afforded a mixture that was resolved by CC to yield the mono- and di-hydroxy derivatives of dehydrocostus lactone as obtained by the conventional method.

Reaction of isodehydrocostus lactone (12) with SeO_2/ TBHP

Selenium dioxide (10 mg) was added to *tert.*-butyl hydroperoxide (3.0 ml, 70%) and the mixture was stirred for half an hour. To this mixture, a solution of isomerised dehydrocostus lactone (12, 1.0 g) in dichloromethane (25 ml) was added and the reaction was completed after stirring for 5 hrs at

room temperature. The reaction mixture was diluted with cold water and extracted with dichloromethane. The combined organic extracts were washed with water and dried over Na_2SO_4. Evaporation of solvent afforded a single compound (92) which showed spectral data identical with an authentic sample.

^1H NMR signals (400 MHz, $CDCl_3$), δ (ppm) at:

6.22 (1H, d, J = 3.2 Hz, C_{13}-H_a),

5.50 (14, d, J = 3.2 Hz, C_{13}-H_b),

4.90 (1H, brs, C_{14}-H_a),

4.82 (1H, brs, C_{14}-H_b),

5.3 (3H, m, C_3-H),

1.93 (3H, s, C_{15}-H),

4.09 (1H, t, J = 8 Hz, C_6-H).

^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm) at:

40.04 (C_1), 30.24 (C_2), 125.0 (C_3-d), 145.3 (C_4-5), 90.0 (C_5-d), 87.5 (C_6-d), 42.0 (C_7-d), 36.0 (C_9-t), 148.5 (C_{10}-s), 32.5 (C_{11}-s), 171.2 (C_{12}-s), 120.5 (C_{13}-t), 109.2 (C_{14}-t), 14.8 (C_{15}-q).

Reaction of isoalantolactone (3) with SeO_2/ TBHP

Selenium dioxide (10 mg) was added to *tert.*-butyl hydroperoxide (3.0 ml, 70%) and the mixture was stirred for half an hour. To this mixture, a solution of isoalantolactone (3, 4.0 g) in dichloromethane (25.0 ml) was added and the reaction was completed after stirring for 4 hrs at room temperature. The reaction mixture was diluted with cold water and extracted with dichloromethane. The combined organic extracts were washed with water and dried over Na_2SO_4. Evaporation of solvent afforded a mixture of two components (TLC, 3.9 g) which was chromatographed over silica gel (400 g) and the details of same are given in Table 24.

Table 24

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (4 x 50)	-	-

2	Hexane: diethyl ether 5% (4 x 50)	0.4	mixture
3	Hexane: diethyl ether 5% (10 x 50)	1.0	Pure compound (93) mp 157°C
4	Hexane: diethyl ether 10% (3 x 50)	0.5	mixture
5	Hexane: diethyl ether 10% (8 x 50)	1.1	Pure compound (85) mp 144°C
6	Hexane: diethyl ether 20% (3 x 50)	0.3	mixture
7	Hexane: diethyl ether 20% (7 x 50)	0.5	Pure compound (94) mp 163°C
8	Hexane: diethyl ether 20% (3 x 50)	-	-

Fraction 3 (Table 24) was characterized as telekin (93) mp 157°C. Fraction 5 (Table 24) mp 144°C was identified to be isotelekin (85) and fraction 7 (Table 24) mp 163°C was identified to be diol (94) being identical in all respects to authentic sample (Kalsi *et al* 1985).

The same reaction of isoalantolactone (3, 2.0 g), when carried out under the influence of microwave radiations, reached completion in 8 minutes. Elution of irradiated silica gel with dichloromethane afforded a mixture that was resolved by CC (Table 25).

Table 25

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Hexane (4 x 50)	-	-
2	Hexane: diethyl ether 5% (4 x 50)	0.05	mixture
3	Hexane: diethyl ether 5% (10 x 50)	0.8	Pure compound (93) mp 157°C
4	Hexane: diethyl ether 10% (3 x 50)	0.1	mixture

5	Hexane: diethyl ether 10% (8 x 50)	0.9	Pure compound (85) mp 144°C
6	Hexane: diethyl ether 20% (3 x 50)	traces	mixture

Fractions 3 and 5 (Table 25) were identified to be similar to compounds (93 and 85) obtained by conventional method (Table 24).

Reaction of isomerised isoalantolactone (13) with SeO_2/ TBHP

Selenium dioxide (10 mg) was added to *tert.*-butyl hydroperoxide (3.0 ml, 70%) and the mixture was stirred for half an hour. To this mixture, a solution of isomerised isoalantolactone (13, 1.0 g) in dichloromethane (25.0 ml) was added and the reaction was completed after stirring for 4 hrs at room temperature. The reaction mixture was diluted with cold water and extracted with dichloromethane. The combined organic extracts were washed with water and dried over Na_2SO_4. Evaporation of solvent afforded a single compound (95) which showed the following spectral data:

^{1}H NMR signals (400 MHz, $CDCl_3$), δ (ppm) at:

2.95 (1H, *m*, C_7-H),

4.1 (1H, *m*, C_8-H),

5.40, 6.25 (1H each, *br s*, C_{13}-H),

1.08 (3H, *s*, C_{14}-H),

1.71 (3H, s, C_4-Me),

5.37 (3H, s, C_3-Me)

^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm) at:

30.2 (C_1-t), 22.1 (C_2-t), 123.1 (C_3-d), 135.2 (C_4-s), 72.9 (C_5-d), 36.8 (C_6-t), 39.1 (C_7-d), 76.5 (C_8-d), 46.8 (C_9-t), 36.5 (C_{10}-s), 138.5 (C_{11}-s), 169.8 (C_{12}-s), 124.1 (C_{13}-t), 16.8 (C_{14}-q), 15.5 (C_{15}-q).

Reaction of parthenin (8) under microwave irradiation

Parthenin (3.0 g) was dissolved in dichloromethane (15.0 ml) and the solution was adsorbed on to silica gel (100.0 g). The excess solvent was evaporated in order to get free flowing silica gel, which was then irradiated with microwaves at 640W level for 8 minutes intermittently with every

radiation of 1 minute following a relaxation time of 2 minutes. The silica gel was cooled to room temperature and the eluted through a column with 10% ethanolic hexane. Evaporation of the solvent in vacuo afforded a pure compound (8'), mp 120°C. The compound was identified as anhydroparthenin by comparison of spectral data with an authentic sample.

Reaction of parthenin (8) with sodium borohydride

Parthenin (1.0 g) was taken in a round bottomed flask and dissolved in methanol (20.0 ml). To this, sodium borohydride (1:2 molar ratio) was added. The reaction mixture was stirred for 15 minutes. The extent of completion of the reaction was monitored by TLC of the reaction mixture.

For work up, a few drops of acetic acid were added and the reaction mixture was extracted with water was then with chloroform (3 x 50 ml). The organic (100.0 ml) layer was dried (sodium sulfate) and the excess solvent was distilled off. The mixture (0.9 g) was chromatographed over silica gel. The details of the chromatography are discussed below:

Table 26

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (4 x 100)	0.55	Mixture
2	Chloroform (2 x 100)	-	-
3	Chloroform: Acetone (5%) (8 x 100)	0.28	Pure compound (98)
4	Chloroform: Acetone (5%) (5 x 100)	0.075	Mixture
5	Chloroform: Acetone (10%) (5 x 100)	0.25	Pure compound (99)
6	Chloroform: Acetone (5%) (4 x 100)	Traces	-

Fraction 3 (Table 26), compound (98) showed the following ^{1}H NMR data δ (ppm) at:

1.11 (d, 3H, J = 7.5 Hz, C_{10}-CH_3)

1.23 (s, 3H, C_5-CH_3)

3.54 (m, 1H, exchangeable)

5.03 (d, 1H, J = 7.83 H_2, C_6-H)

6.15 (d, 1H, J = 6 Hz, C_3-H)

7.62 (d, 1H, J = 5.99 Hz, C_2-H)

3.75 (d, J = 3.5 Hz)

Fraction 5 (Table 26), compound (99) showed that following spectroscopic data:

^{1}H NMR signals δ (ppm) at:

5.62 (d, 1H, J = 2.70 Hz, C_{13}-Ha)

6.26 (d, 1H, J = 2.70 Hz, C_{13}-Hb)

1.11 (d, 3H, J = 7.56 Hz, C_{10}-CH_3)

1.27 (s, 3H, C_5-CH_3)

5.03 (d, 1H, J = 7.83 Hz, C_6-H)

6.15 (d, 1H, J = 6.00 Hz, C_3-H)

7.62 (d, 1H, J = 5.99 Hz, C_2-H)

Reaction of parthenin (8) with zinc borohydride

Parthenin (0.5 g) was taken in a round bottomed flask and dissolved in tetrahydrofuran (THF). 50.0 ml of zinc borohydride solution was added to the parthenin – THF solution and the reaction mixture was then stirred for 2 hrs.

The progress of the reaction was monitored by TLC. The mixture (0.45 g) obtained after usual work up was subjected to CC over silica gel (50 g). The details of the chromatography are presented in Table 27.

Table 27

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (4 x 50)	0.075	Mixture
2	Chloroform: Acetone (2%) (2 x 50)	-	-
3	Chloroform: Acetone (5%) (5 x 50)	0.20	Pure compound (98)

4	Chloroform: Acetone (7%) (4 x 40)	Traces	Mixture

Fraction 3 (Table 27), compound (98) was proved to be identical to one of the products of sodium borohydride reaction by comparison of its spectral data.

Reaction of parthenin (8) with sodium cyanoborohydride

Parthenin (2.0 g) was dissolved in methanol (20.0 ml) and to this was added sodium cyanoborohydride (1.8g). The reaction mixture was stirred for 6 hours, the progress of reaction being screened by TLC. Usual work up afforded a mixture (1.8 g) that was chromatographed over silica gel (120 g). The details of the chromatographic procedure are as follows:

Table 28

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (4 x 100)	-	-
2	Chloroform: Acetone (5%) (6 x 100)	0.6	Pure compound (98)
3	Chloroform: Acetone (7%) (4 x 100)	0.1	Mixture
4	Chloroform: Acetone (10%) (8 x 100)	0.4	Pure compound (101)
5	Chloroform: Acetone (15%) (3 x 100)	Traces	Mixture
6	Chloroform: Acetone (15%) (6 x 100)	0.45	Pure compound (102)
7	Chloroform: Acetone (20%) (4 x 100)	-	Mixture

Fraction 2 (Table 28), compound (98) was identified to be similar to one of the products of sodium borohydride reaction.

Fraction 4 (Table 28), compound (101) was subjected to spectroscopic analysis and it showed the following data:

IR bands ν_{max} /cm^{-1} at 3500, 1760, 1740, 1110, 1090.

^{1}H NMR signals ($CDCl_3$, 270 MHz) δ at:

1.14 (3H, s, C_5-Me),

1.16 (3H, d, J= 8.37 Hz, C_{10}-Me),

3.34 (3H, s, -CH_2-O**C**H_3),

3.47 (3H, s, >CH-O**C**H_3),

3.62 (2H, d, J= 5.37 Hz, **C**H_2-OCH_3),

4.95 (1H, d, J= 8.10 Hz, C_6-H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

84.20 (C_1-s), 76.28 (C_2-d), 37.12 (C_3-t), 213.40 (C_4-s), 57.80 (C_5-s), 80.09 (C_6-d), 37.63 (C_7-t), 30.02 (C_8-t), 26.27 (C_9-t), 49.72 (C_{10}-d), 43.68 (C_{11}-d), 171.16 (C_{12}-s), 71.44 (C_{13}-t), 16.42 (C_{14}-q), 14.23 (C_{15}-q), 58.94 (C_{16}-q), 60.25 (C_{17}-q).

Fraction 4 (Table 28), compound (102) also showed the following spectral data:

IR bands ν_{max}/cm^{-1} at 1770, 1745, 1600, 1360, 1110, 980.

^{1}H NMR signals ($CDCl_3$, 270 MHz) δ at:

1.15 (3H, s, C_5-Me),

1.3 (3H, d, J= 7.0 Hz, C_{10}-Me),

6.15 (1H, d, C_3-H, J= 6.0 Hz),

7.62 (1H, C_2-H, J= 5.95 Hz),

3.5 (3H, s, -CH_2-O**C**H_3),

5.03 (1H, d, J= 7.83 Hz, C_6-H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ at:

84.20 (C_1-s), 155.8 (C_2-d), 132.12 (C_3-t), 213.40 (C_4-s), 68.80 (C_5-s), 80.09 (C_6-d), 41.63 (C_7-t), 30.02 (C_8-t), 26.27 (C_9-t), 40.72 (C_{10}-d), 47.68 (C_{11}-d), 177.16 (C_{12}-s), 74.44 (C_{13}-t), 13.42 (C_{14}-q), 7.23 (C_{15}-q), 74.94 (C_{16}-t), 60.25 (C_{17}-q).

Reaction of parthenin (8) with lithium tri-*tert*.- butoxy aluminohydride

Parthenin (2.0 g) was dissolved in dry THF (20.0 ml) and to this was added lithium tri-*tert*.-butoxy aluminohydride (1.0 g). The reaction mixture was stirred for 15 minutes, the progress of reaction being screened by TLC. Usual work up afforded a mixture (1.8 g) that was chromatographed over

silica gel (120 g). The details of the chromatographic procedure are as follows:

Table 29

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (3 x 50)	-	-
2	Chloroform: Acetone (2%) (4 x 50)	0.05	Mixture
3	Chloroform: Acetone (5%) (6 x 50)	1.1	Pure compound (98)
4	Chloroform: Acetone (5%) (3 x 50)	Traces	-
5	Chloroform: Acetone (10%) (5 x 100)	0.4	Pure compound (103)
6	Chloroform: Acetone (10%) (3 x 100)	0.08	Mixture

Fraction 3 (Table 29), compound (98) was identified to be similar to one of the products of sodium borohydride reaction.

Fraction 5 (Table 29), compound (103) was subjected to spectroscopic analysis and it showed the following data:

IR bands ν_{max}/cm^{-1} at 3400, 1745, 1700, 1660, 1590, 825.

^1H NMR signals ($CDCl_3$, 270 MHz) δ at:

7.50 (d, 1H, J= 5.9 Hz, C_2-H),

6.17 (d, 1H, J= 5.9 Hz, C_3-H),

5.01 (d, 2H, J= 7.90 Hz, C_6-H),

3.54 (m, 1H, C_7-H),

1.70 (s, 3H, C_5-Me),

1.13 (d, 3H, J= 7.54 Hz, C_{10}-Me).

Reaction of parthenin (8) with Mg/methanol

To dry methanol (50.0 ml), parthenin (0.5 g) and active Mg turnings (0.5 g) were added with constant stirring under dry conditions. A mild exothermic reaction was observed after 3 hours with the evolution of H_2 gas.

The progress of reaction was monitored by TLC. Untreated metal was then separated and the mixture was added to dilute HCl (150.0 ml) to get a clear solution. pH was adjusted around 8.0 – 9.0 by adding ammonia solution. The reaction mixture was then concentrated diluted with water and extracted with dichloromethane (4 x 20 ml). The organic layer was dried (sodium sulfate) and the solvent was evaporated *in vacuo* to afford a mixture (0.42 g) which was chromatographed over silica gel (30 g), the details of same are as follows:

Table 30

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1	Chloroform (3 x 50)	-	-
2	Chloroform: Acetone (2%) (4 x 50)	0.05	Mixture
3	Chloroform: Acetone (5%) (6 x 50)	0.12	Pure compound (101)
4	Chloroform: Acetone (5%) (3 x 50)	Traces	-
5	Chloroform: Acetone (10%) (5 x 100)	0.2	Pure compound (102)
6	Chloroform: Acetone (10%) (3 x 100)	0.08	Mixture

Fractions 3 and 4 (Table 30) were found to be same as compounds obtained by reaction of parthenin with sodium cyanoborohydride on comparison of their spectroscopic data (Table 28).

Reaction of anhydroparthenin with Mg/methanol

A similar procedure was followed to carry out the reaction of anhydroparthenin (0.5 g) with Mg (0.5 g) in dry methanol (50.0 ml). Usual work up afforded a yellow liquid (0.355 g) which showed a single spot. The compound (104) showed following spectroscopic data:

IR bands ν_{max} /cm^{-1} at 1750, 1650.

^{1}H NMR signals ($CDCl_3$, 270 MHz) δ at:

1.20 (d, 3H, J= 7.0 Hz, C_{10}-Me),

3.65 (d, 2H, J= 6.5 Hz, C_{13}-H),

4.5 (d, 1H, J= 8.0 Hz, C_6-H),

6.45 (bd, 1H, J= 6.3 Hz, C_3-H),

8.1 (bd, 1H, J= 6.0 Hz, C_2-H),

1.35 (s, 3H, C_5-CH_3),

3.3 (s, 3H, -OCH_3)

Biological activity

The biological testing studies included the screening of seed germination and seedling growth behaviour of pea (*Pisum sativum*) and two weed species *viz. Avena fatua* (wild oats) and *Phlaris minor.* Data pertaining to per cent germination, length of root and shoot, fresh and dry weight of seedlings and leaf chlorophyll content were recorded.

Seed germination and seedlind growth

Seeds of uniform size were surface sterlilized with 0.1% mercuric chloride solution for 1 min, followed by thorough washing with distilled water. The pea seeds were germinated in glass Petri dishes lined with double layer of filter paper moistened with the test solutions (50, 100, 300 and 500 10 and 20 µg ml^{-1}). Hoagland solution was used as control and ABA (10 and 20 µg ml^{-1}) and GA_3 (25 and 50 µg ml^{-1}) were used as standard compounds. The Petri dishes were kept in BOD incubator at 18±1°C for 10 days. Germination was recorded after 72 hrs and the data pertaining to length of root and shoot, fresh weight of seedlings and leaf chlorophyll content were recorded on the 10^{th} day. Dry weights of the seedlings were recorded after drying in oven at 70°C for 72 hrs.

In case of the weed seeds, the germination was carried out in small pots containing field soil moistened with different concentrations of the test solutions. The seeds were covered with soil upto 2 cm in thickness. The germination assay was carried out for 21 days in a growth chamber. Data pertaining to various parameters were recorded on the 21^{st} day.

Vigour index (V.I.) was calculated (Abdul-Baki and Anderson 1973) as follows:

V.I.= % germination X seedling dry weight

Chlorophyll content (Anderson and Boardman 1964)

Samples (100mg) were homogenized in 5 ml of 80% acetone in a pestle and mortar. The homogenate was centrifuged at 3000 rpm for 10 min. the supernatant was retained and the residue was again centrifuged. Both these extracts were pooled and final volume was adjusted with acetone. Absorbance was recorded at 645 and 663 nm. From the absorbance values the concentration of the total chlorophyll was calculated by using the following equation:

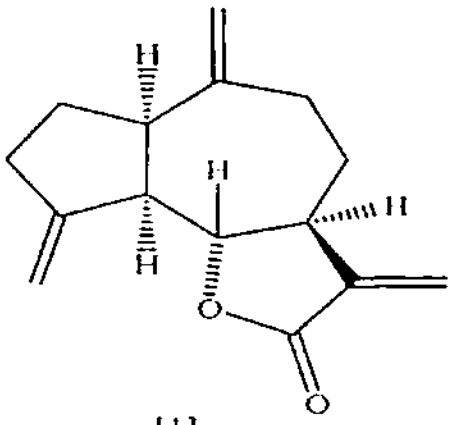
Total chlorophyll = [20.2 (A645) + 8.06 (A663)] x V/1000 x W

CHAPTER IV

RESULTS AND DISCUSSION

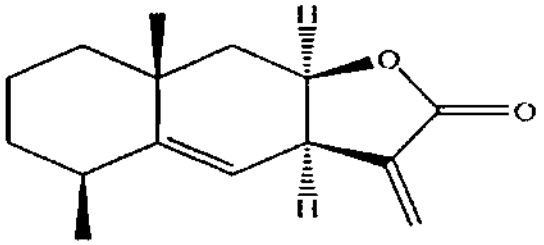
Sesquiterpene lactones with α-methylene-γ-lactone moiety fused on various skeletons are a rapidly expanding group of natural products that represent a rich source of biologically active compounds. These are an example of molecular diversity, with recognized potential in drug discovery (Abreu and Branco 2003), and a wide spectrum of biological activities like anticancer (Douglas 2000), anti-inflammatory (Cho and Baik 2000) and anti bacterial activities (Yoo *et al* 2005). They are particularly known for their potential as plant growth regulators (Chhabra *et al* 1998). Sesquiterpene lactones from the members of family Asteraceae are particularly significant as far as the aforementioned properties are concerned. The extracts from *Saussurea lappa*, *Inula racemosa*, *Parthenium hysterophorus* and *Cichorium intybus* have thus been chosen to isolate, purify and chemically modify the sesquiterpene lactones which can lead to the successful establishment and further clarification of the structure-activity relationship.

The oil obtained from the roots of *Saussurea lappa* is a rich source of sesquiterpenoids. It was obtained from the powdered costus roots by Soxhlet extraction in hexane followed by refrigeration. The solidified material was purified by column chromatography over silica gel to afford pure, crystalline dehydrocostus lactone (1, mp 58-60°C). The structure of the compound was established on the basis of ^{1}H NMR and ^{13}C NMR analyses, the data for which is presented in Table 1.

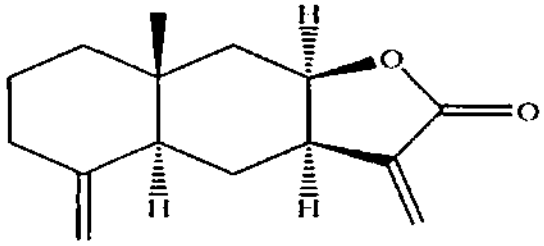


[1]

The extract from the dried and powdered roots of *Inula racemosa* was obtained following a similar extraction procedure using Soxhlet method. The extract was chromatographed over silica gel impregnated with silver nitrate to furnish two isomeric lactones, alantolactone and isoalantolactone which were identified by spectroscopic analyses (Table 1).



[2]



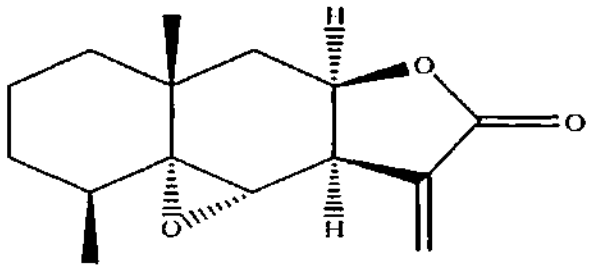
[3]

Column chromatography of the *Inula* extract also afforded a several component mixture that was identified by thin layer chromatography. The less quantities of the mixture, however, made the separation of its components to be a tedious job with chances of wastage of material fairly high. Thus, the mixture was resolved into its components by preparative thin layer chromatography. In the process, a few new alantolides have been isolated from the roots of *Inula racemosa*. These were characterized on the basis of spectroscopic analysis.

One of the compounds showed IR bands ($CHCl_3$) at 1760,1660 cm^{-1} for the intact lactone moiety and at 1460 and 890 cm^{-1} for the exomethylene group. Other peaks were obtained at 1375, 1140, 1060, 980 and 810 cm^{-1}.

Amongst the ^1H NMR signals ($CDCl_3$, 300 MHz) it showed a doublet due to 3 hydrogens at δ 1.11 with a coupling constant of 7.83 Hz showing the presence of a secondary methyl group, another doublet at δ 2.97 with J= 7.5 Hz due to C_6-H, a multiplet at δ 4.88 typical of C_8-H with an intact lactone in alantolides, a pair of broad singlets at δ 5.48 and 6.23 each may be due to the α–methylene of γ-lactone and a singlet δ 1.22 due to three C_{10}-Me hydrogens, a tertiary methyl. All these spectral features when compared to the prepared compounds were indicative of the presence of a natural epoxy derivative in the plant.

Based on this data, the compound was confirmed to be 4,10 β-dimethyl-5,6α-epoxy-7αH,8αH-eudesman-11-ene-8,12-olide (4) with the following structure:



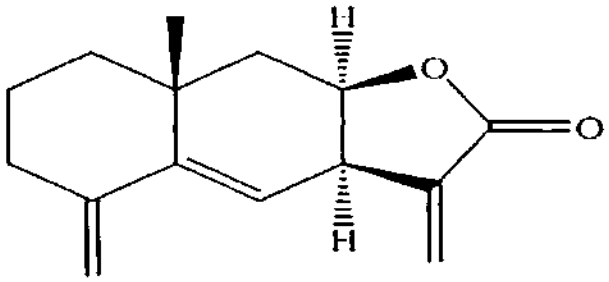
[4]

The structure was further supported by the ^{13}C NMR ($CDCl_3$, 300 MHz) data which showed signals at δ 36.8 (C_1-t), 20.7 (C_2-t), 29.9 (C_3-t), 30.9 (C_4-d), 74.1 (C_5-s), 59.8 (C_6-d), 47.1 (C_7-d), 72.8 (C_8-d), 40.4 (C_9-t), 27.5 (C_{10}-s), 138.5 (C_{11}-s), 169.8 (C_{12}-s), 124.0 (C_{13}-t), 18.6 (C_{14}-q), 15.2 (C_{15}-q).

Another compound procured by PTLC showed IR peaks ($CHCl_3$) at 1760, 1669 and 1650 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) spectrum of the compound comprised of a multiplet due to a C_7-H at δ 3.25, another multiplet at δ 4.2 due to C_8-H, both indicating the presence of tertiary hydrogens, two broad singlets at δ 5.45 and 6.20 due to the C_{13}-H and a singlet at δ 1.08 for the three hydrogens at C_{14} confirming the presence of tertiary methyl groups.

The signals for two C_{15} hydrogens appeared as doublets at δ 4.81 and 4.86 with coupling constant values being 2.3Hz and 2.4Hz respectively, which were affirmative of the presence of an exomethylene group.

^{13}C NMR ($CDCl_3$, 300 MHz) data with δ 38.4 (C_1-t), 23.8 (C_2-t), 38.8 (C_3-t), 145.4 (C_4-s), 142.5 (C_5-s), 122.5 (C_6-s), 39.5 (C_7-d), 76.5 (C_8-d), 41.5 (C_9-d), 27.9 (C_{10}-s), 139.8 (C_{11}-s), 169.8 (C_{12}-s), 124.0 (C_{13}-t), 25.8 (C_{14}-q), 109.5 (C_{15}-t) further ascertained the structure to be 4,10β-dimethyl-7αH,8αH-eudesman-5,11-diene-8,12-olide (5).



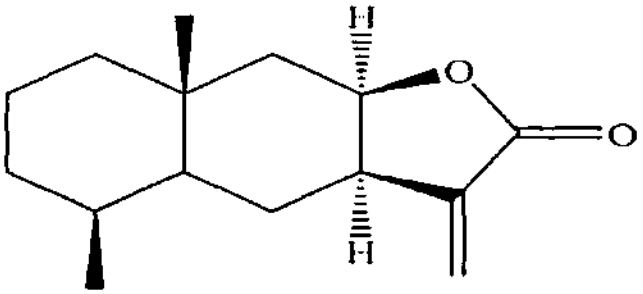
[5]

The third compound obtained by PTLC was 4,10β-dimethyl-7αH,8αH-eudesman-11-ene-8,12-olide (6). The structure was supported by IR bands ($CHCl_3$) at 1760, 1645, 1475, 1370 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) peaks were obtained at δ 1.58 (1H, *m*, C_4-H) showing the presence of a secondary methyl group, multiplets at δ 2.9 and 4.1 due to C_7 and C_8 hydrogens respectively representing an intact lactone moiety, a pair of doublets at δ 5.3 and δ 5.9 each due to one C_{11} hydrogen with the respective coupling constants for the two being 1.9Hz and 1.8Hz. Other signals were obtained at δ 5.40, 6.25 (1H each, *br s*, C_{13}-H), 4.81 (1H, *d*, *J* 2.4Hz, C_{15}-H), 4.86 (1H, *d*, *J* 2.4Hz, C'_{15}-H). From the spectroscopic data, it was clear that the compound was a reminiscent of alantolides except for the absence of secondary methyl at C_4 position. The position of the exomethylene group could be explained on the basis of the biogenesis of these compounds.

^{13}C NMR ($CDCl_3$, 300 MHz) data showed peaks at δ 40.8 (C_1-t), 21.3 (C_2-t), 34.5 (C_3-t), 33.2 (C_4-d), 60.0 (C_5-d), 28.8 (C_6-d), 44.2 (C_7-d), 76.5 (C_8-

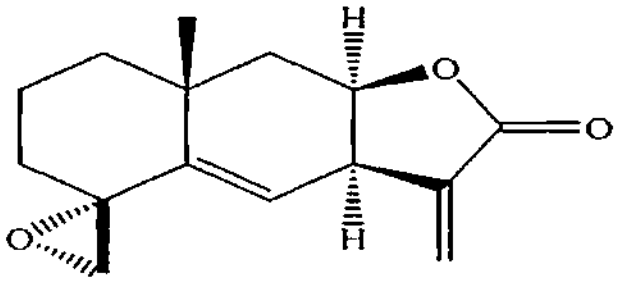
d), 44.8 (C_9-t), 31.5 (C_{10}-s), 138.5 (C_{11}-s), 169.8 (C_{12}-s), 124.1 (C_{13}-t), 22.8 (C_{14}-q), 19.3 (C_{15}-q).

Based on the spectrometric analysis, structure (6) is confirmed for the compound; 4,10 β-dimethyl-7αH,8αH-eudesman-11-ene-8,12-olide.



[6]

The fourth compound was 4,15α-epoxy-10β-methyl-7αH,8αH-eudesman-5,11-diene-8,12-olide (7).



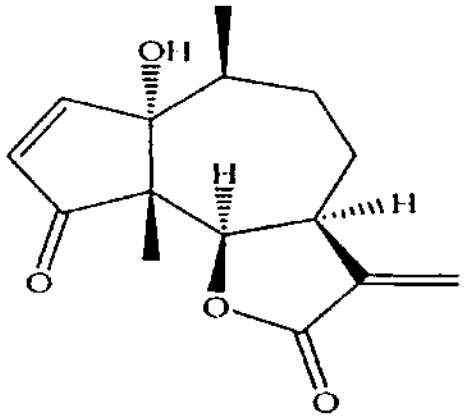
[7]

The structure was conclusively based on the spectroscopic information. The compound showed IR bands ($CHCl_3$) at 1765 and 1645 cm^{-1} for the lactone. Other signals included bands at 1470, 1370, 1130, 860 and 810 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) data showed peaks at δ 3.25, a multiplet due to C_7-H, another multiplet at δ 4.24 due to C_8-H which is typical of an intact lactone moiety, two broad singlets at δ 5.5 and 6.1 each due to the C_{13} hydrogen attributable to α–methylene of the γ–lactone and a pair of doublets at δ 2.55 and 2.65 corresponding to the C_{15} hydrogens with coupling constant of 4.0Hz in each case, confirming the presence of an epoxy ring.

The structure was further supported by ^{13}C NMR ($CDCl_3$, 300 MHz) signals at δ 38.1 (C_1-t), 15.5 (C_2-t), 32.7 (C_3-t), 63.4 (C_4-s), 150.1 (C_5-s),

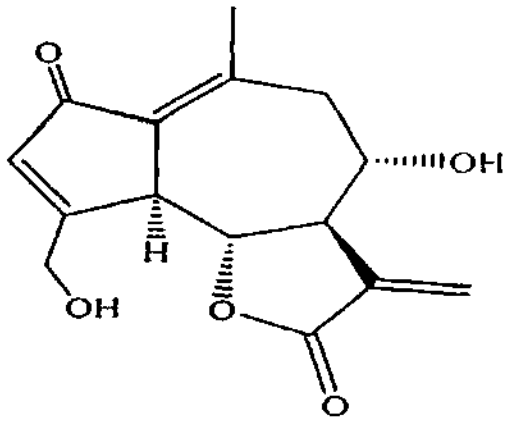
122.8 (C_6-d), 39.1 (C_7-d), 76.3 (C_8-d), 41.1 (C_9-t), 26.8 (C_{10}-s), 139.7 (C_{11}-s), 169.9 (C_{12}-s), 124.0 (C_{13}-t), 24.9 (C_{14}-q), 55.5 (C_{15}-t).

The extract of *P. hysterophorus* was obtained from the dried and powdered plant by Soxhlet extraction method using chloroform as the solvent. Pure parthenin (8) was isolated from the extract by column chromatography. The purity of the compound was checked by TLC and the structure was confirmed by spectroscopic analysis (Table 1).

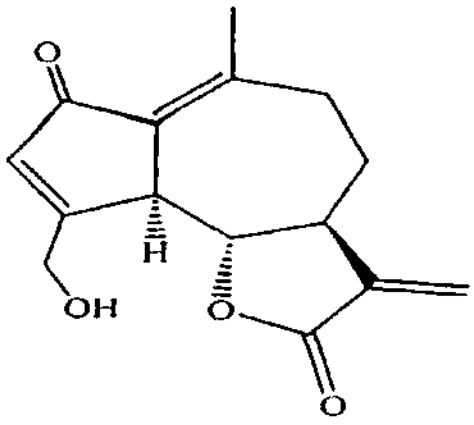


[8]

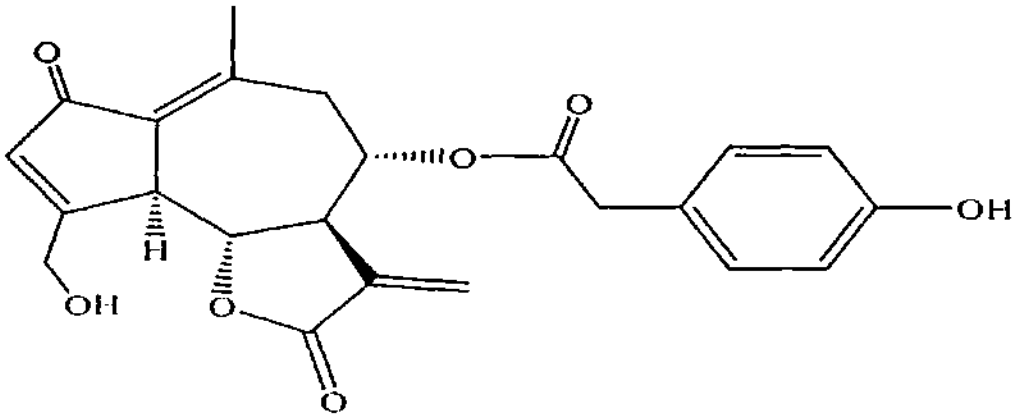
For extracts of *Cichorium intybus*, the commercially available roasted root powder was used. The root powder was extracted in chloroform in the Soxhlet apparatus. Evaporation of solvent *in vacuo* afforded a crude extract which was chromatographed over silica gel to yield four compounds. Compound (9) showed a molecular ion at m/z 277 is positive ion ES-MS and was identified as lactucin (molecular weight 276) by comparison of ^{1}H NMR and ^{13}C NMR data (Table 1) with published spectra. Another compound (10), 8-deoxylactucin was also identified on the basis of the spectral data. Similarly, compound (11) gave a molecular ion at m/z 411 and a daughter ion at m/z 259 (loss of 4-hydroxyphenyl acetic acid) in positive ion ES-MS and was identified as lactucopicrin (molecular weight 410) by comparison with published NMR data (Sessa *et al* 2000).



[9]

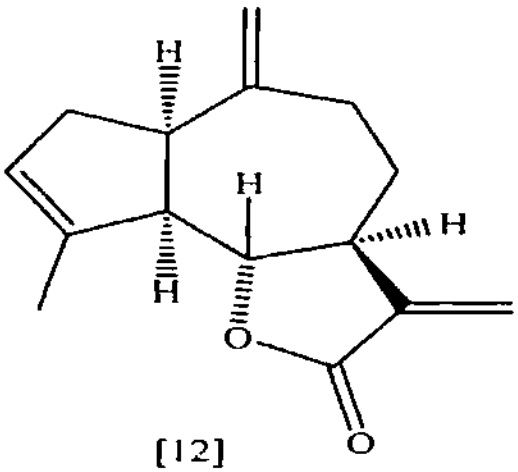


[10]



[11]

In addition to these reported compounds, another sesquiterpenoid has been isolated from the chicory extract. The screening of its ^{1}H NMR and ^{13}C NMR led to the following structure for the compound:



[12]

The structure is same as that for isodehydrocostus lactone leading to the inference that isodehydrocostus lactone is another sesquiterpene present in the roots of *Cichorium intybus*. However, the observation that isodehydrocostus lactone has been isolated from the chicory roots does not necessarily prove it to be natural component owing to the fact that roasted roots of the plant have been used for extraction. Roasting is a heat mediated process, so is the double bond migration reaction. Therefore, the presence of isodehydrocostus lactone in the plant extract might be a result of heat-assisted transformation of dehydrocostus lactone.

Table 1: Spectroscopic data of all the isolated natural compounds

Compound	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
1	6.22 (1H, d, J = 3.2 Hz H-13a), 5.50 (1H, d, J = 3.2 Hz, H-13b), 5.27 (1H, brs, H-15a), 5.07 (1H, brs, H-15b), 4.82 (1H, brs, H-14b), 3.97 (1H, t, J = 9.2 Hz, H-5).	45.04 (C_1-d), 30.24 (C_2-t), 36.24 (C_3-t), 149.17 (C_4-s), 51.95 (C_5-d), 85.22 (C_6-d), 47.52 (C_7-d), 30.88 (C_8-t), 32.54 (C_9-t), 30.24 (C_{10}-s), 151.22 (C_{11}-s); 170.24 (C_{12}-s), 120.18 (C_{13}-t), 109.53 (C_{14}-t), 112.55 (C_{15}-t).
2	1.18 (s, 3H, C_{10}-Me), 1.40 (d, 3H, J= 7.0 Hz, C_4-Me), 4.81 (m, 1H, C_8-H), 5.15 (d, 1H, J= 4.0 Hz, C_6-H), 5.59 and 6.17 (d, 1H each, J= 2.0 Hz, C_{15}-Hs).	38.8 (C_1-t), 21.3 (C_2-t), 36.5 (C_3-t), 35.2 (C_4-d), 144.0 (C_5-s), 122.8 (C_6-d), 39.2 (C_7-d), 76.5 (C_8-d), 41.8 (C_9-t), 31.5 (C_{10}-s), 170.5 (C_{11}-s), 139.8 (C_{12}-s), 124.1 (C_{13}-t), 24.8 (C_{14}-q), 19.3 (C_{15}-q).
3	0.82 (s, 3H, C_{10}-Me), 1.40 (d, 3H, J= 7.0 Hz, C_4-Me), 4.51 (m, 1H, C_8-H), 4.43 and 6.12 (d, 1H each, J= 1.5 Hz, C_{15}-Hs), 5.57 and 6.12 (bs, 1H each, C_{13}-H).	38.8 (C_1-t), 23.3 (C_2-t), 38.5 (C_3-t), 148.2 (C_4-s), 56.0 (C_5-d), 30.8 (C_6-d), 44.2 (C_7-d), 76.5 (C_8-d), 44.8 (C_9-t), 28.5 (C_{10}-s), 170.5 (C_{11}-s), 139.8 (C_{12}-s), 124.1 (C_{13}-t), 23.8 (C_{14}-q), 109.3 (C_{15}-t).
8	1.11 (d, 3H, J= 7.56 Hz, C_{10}-CH_3), 1.23 (s, 3H, C_5-CH_3), 3.54 (m, 1H, exchangeable), 5.03 (d, 1H, J= 7.83 Hz, C_6-H), 5.62 (d, 1H, J= 2.70 Hz, C_{13}-H_a), 6.26 (d, 1H, J= 2.70 Hz, C_{13}-H_b), 6.15 (d, 1H, J= 6.00 Hz, C_3-H), 7.62 (d, 1H, J= 5.99 Hz, C_2-H)	17.43 (C_{15}-q), 18.35 (C_{14}-q), 28.40 (C_9-t), 29.81 (C_8-t), 40.47 (C_7-d)[a], 44.19 (C_{10}-d)[b], 59.05 (C_5-s), 79.06 (C_6-d), 84.28 (C_1-s), 121.87 (C_{13}-d), 131.43 (C_3-d), 140.47 (C_{11}-s), 163.77 (C_2-d), 171.19 (C_{12}-s), 211.12 (C_4-s), (a) and (b) are interchangeable.

9	6.60 (1H, br s, C_3-H), 3.97 (1H, m, C_5-H), 3.53 (1H, m, C_7-H), 3.97 (1H, m, C_6-H), 3.28 (1H, m C_8-H), 2.85 (1H, dd, J= 13.0, 12.0 Hz, C_9-H^1), 2.34 (1H, dd, J= 13.0, 2.0, C_9-H^2), 6.04 (1H, d, J= 3.0 Hz, C_{13}-H), 5.67 (1H, d, J= 3.0 Hz, C_{13}-H), 2.13 (3H, br s, C_{14}-H), 4.66 (2H, dd, J= 10.0, 1.5 Hz, C_{15}-H).	132.7 (C_1-s), 194.1 (C_2-s), 133.4 (C_3-d), 169.4 (C_4-s), 47.6 (C_5-d), 80.2 (C_6-d), 52.8 (C_7-d), 43.5 (C_9-t), 145.0 (C_{10}-s), 136.5 (C_{11}-s), 168.1 (C_{12}-s), 120.9 (C13-t), 20.8 (C_{14}-q), 67.8 (C_{15}-t).
10	6.60 (1H, br s, C_3-H), 3.97 (1H, m, C_5-H), 3.53 (1H, m, C_7-H), 3.97 (1H, m, C_6-H), 6.04 (1H, d, J= 3.0 Hz, C_{13}-H), 5.67 (1H, d, J= 3.0 Hz, C_{13}-H), 2.13 (3H, br s, C_{14}-H), 4.66 (2H, dd, J= 10.0, 1.5 Hz, C_{15}-H).	132.7 (C_1-s), 194.1 (C_2-s), 133.4 (C_3-d), 169.4 (C_4-s), 47.6 (C_5-d), 80.2 (C_6-d), 52.8 (C_7-d), 31.2 (C_8-t), 33.5 (C_9-t), 145.0 (C_{10}-s), 136.5 (C_{11}-s), 168.1 (C_{12}-s), 120.9 (C_{13}-t), 20.8 (C_{14}-q), 67.8 (C_{15}-t).
11	6.31 (1H, br s, C_3-H), 4.03 (1H, br d, J= 10 Hz, C_5-H), 3.90 (1H, t, J= 10 Hz, C_6-H), 3.52 (1H, dt, J= 10.0, 3.0 Hz, C_7-H), 4.85 (1H, dt, J= 10.0, 3.0 Hz, C_8-H), 2.84 (1H, dd, J= 13.0, 10.0 Hz, C_9-H), 2.27 (1H, dd, J= 13.0, 1.5 Hz, C_9-H), 5.37 (1H, d, J= 3 Hz, C_{13}-H_a), 5.89 (1H, d, J= 3 Hz, C_{13}-H_b), 2.32 (3H, s, C_{14}-Hs), 4.24, 4.66 (2d, J= 18.0 Hz, C_{15}-Hs), 3.62, 3.66 (2d, J= 18 Hz, CH_2(bz)), 6.72, 7.04 (2d, J= 8Hz, Ar-H).	133.0 (C_1-s), 194.1 (C_2-s), 132.0 (C_3-d), 174.9 (C_4-s), 47.6 (C_5-d), 80.3 (C_6-d), 52.9 (C_7-s), 69.2 (C_8-d), 43.3 (C_9-t), 144.6 (C_{10}-s), 138.5 (C_{11}-s), 168.1 (C_{12}-s), 120.9 (C_{13}-t), 20.6 (C_{14}-q), 61.2 (C_{15}-t), 39.6 (CH_2(bz)-t), 123.8 (Ph-C_1-s), 130.4 (Ph-C_2, C_6-d), 115.2 (Ph-C_3, C_5-d), 156.4 (Ph-C_4-s).

Microwave induced organic transformations

Microwave technology is emerging as an alternative energy source powerful enough to accomplish chemical transformations in minutes, instead of hours. For this reason, microwave irradiation is presently seeing an exponential increase in acceptance as a technique for enhancing chemical synthesis. A growing number of investigators are adopting microwave-assisted synthesis as a means to increase their productivity.

In addition to the preparative interest in this method in terms of use, separation and economical aspects, safe and clean procedures, absorption of microwave radiation is now limited to the reactive species. Microwave heating effect results from material-wave interactions and due to the dipolar polarization phenomenon, the greater the polarity of a molecule (such as the solvent) the more pronounced the microwave effect when the rise in temperature is considered (Gedye *et al* 1998).

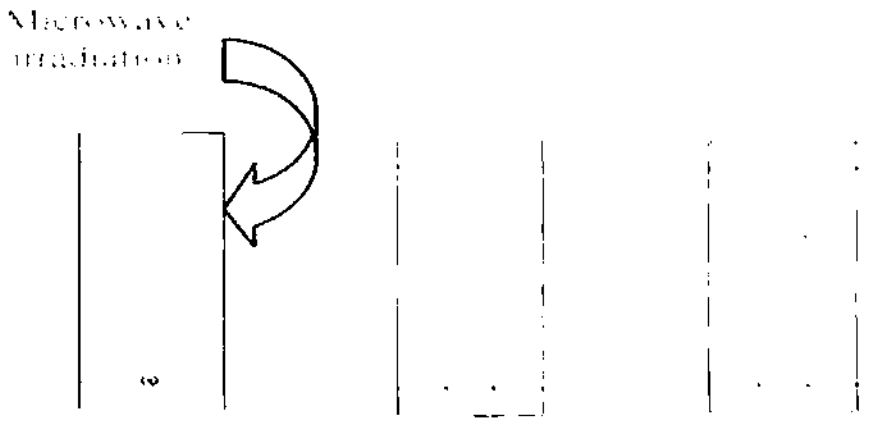
Clearly, microwave irradiation has emerged as a powerful tool for organic synthesis. In concert with a rapidly expanding applications base, microwave synthesis can be effectively applied to any type of chemistry, resulting in faster reaction times and improved product yields. In addition, microwave synthesis creates new possibilities in performing chemical reactions. Because microwaves can transfer energy directly to the reactive species, they can promote transformations that are currently not possible using conventional heat, creating a new realm in synthetic organic chemistry.

The double bond migration is a well studied reaction under catalyst - induced, acidic and basic conditions. Boron triflouride/ etherate (Macaira *et al* 1977) and iodine in dry benzene (Kalsi *et al* 1984) have been reported to bring about carbon-carbon double bond migrations in dehydrocostus lactone. The C=C migrations using catalytic amounts of palladium chloride have also been observed (Aghayan *et al* 2003). More recently, singlet oxygen induced double bond migrations have been reported during sulfide photooxidation (Clennan and Aebisher 2002). Despite the flurry of activity in this area, new fascinating aspects of these reactions are still being undertaken. Thus, attempting this reaction under microwave irradiation seemed to be a

fascinating new aspect towards chemical transformations of sesquiterpenoids.

The development of the use of microwave technology in organic chemistry started in mid 1980s but still many reactions lie undiscovered because of the scarce availability and the high cost of the starting materials. Keeping this view in mind, a method was devised to check the feasibility of reactions rapidly microwave assisted reaction in minimum possible time with the help of thin layer chromatography, that uses micro scale quantities of starting compounds. The TLC spotting technique involved the spotting of the reactant(s) onto a number of TLC plates, which were then irradiated under microwave for set intervals of time. After every one minute, one TLC plate was removed and developed using standard solvents to check the progress of the reaction while the remaining plates still being exposed to microwaves. Thus, using minutest quantity of the starting material(s), reactions could be carried out in microwave and the time period for the completion of reaction could be standardized. Once the reaction conditions are optimized, the reaction can be scaled up suitably.

To start with, using this TLC spotting technique, double bond migration of dehydrocostus lactone (1) was attempted under microwave irradiated conditions. The reaction reached completion in 6 min 40 sec.



Spot of the pure sample applied and the TLC plate irradiated with microwaves

Another spot of pure sample applied after irradiation

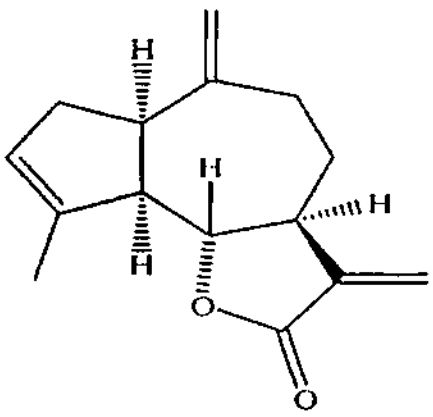
Developed spots before and after irradiation

TLC spotting technique

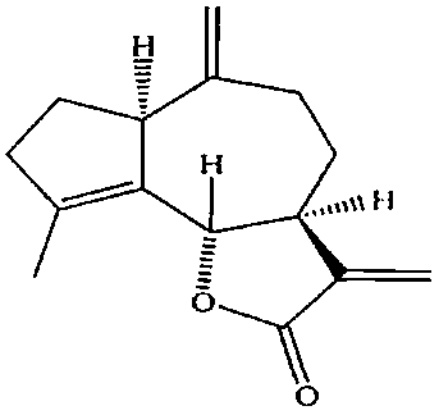
In order to confirm this observation, the reaction was scaled up to gram level by adsorbing the compound over silica gel and subsequently irradiating it with microwaves. Elution of the silica gel with dichloromethane afforded a product that was characterized by spectroscopic analysis. The compound showed ^{1}H NMR spectrum with the two characteristic low field doublets (δ 5.50 and 6.22, J= 3 Hz) corresponding to the protons of an exocyclic methylene conjugated with the lactone carbonyl function. Two broad singlets at δ 4.90 and 4.82 were attributed to the hydrogens of the exocyclic methylene group attached to C-10. A multiplet at δ 5.3 due to one hydrogen at the C-3 position along with a singlet at δ 1.93 attributable to the methyl hydrogens at C-15 position were indicative of the migration of the double bond from the *exo* to the *endo* position.

Taking into consideration the preferential abstraction of tertiary hydrogen, a tetrasubstituted position for the migrated bond was expected,

giving rise to a large number of possible structures for the isomerised product. But this possibility is in contradiction with the observed spectroscopic data wherein under the present reaction conditions, migration of only one of the exocyclic double bonds to endocyclic position has occurred and that too, with a trisubstituted nature. Based on these observations, two structures were proposed fro the isomerised product. It may be suggested that for a five membered ring in a guaianolide skeleton, an sp^3 hybridization for C-5 seemed to provide greater stability rather than sp^2 hybridization that would lead to increase in strain in the molecule.



[A]

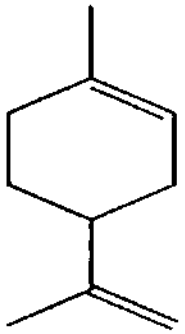


[B]

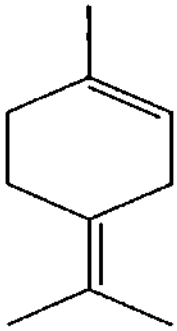
Based on these observations, structure (A) was confirmed for the isomerised product of dehydrocostus lactone. The observation was further strengthened by comparison of the structure of the microwave-assisted double bond migration product with that obtained by the conventional I_2 / benzene refluxing method. Moreover, the microwave-assisted reaction seemed to hold great advantage over the conventional method due to substantial decrease in reaction time from 12 h refluxing in case of I_2 / benzene reaction to 5 min 40 sec in the microwave method.

With the successful achievement of double bond migration under microwave irradiation for dehydrocostus lactone, several other sesquiterpenoids and a few monoterpenes were subjected to similar reactions and the reaction times for the conventional and non conventional

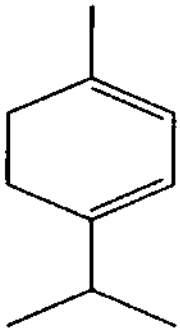
methods were compared. Details of the same are presented in Table 2. In all the cases, a decreased reaction time with the microwave method was observed.



[14]



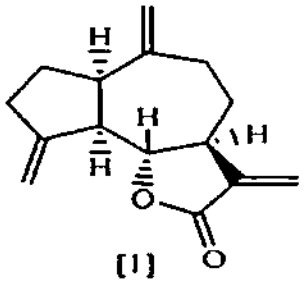
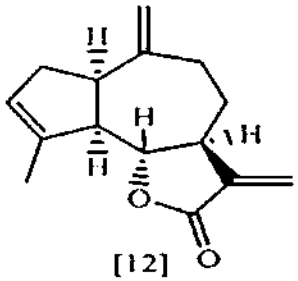
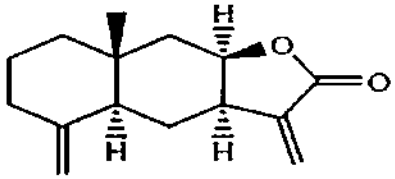
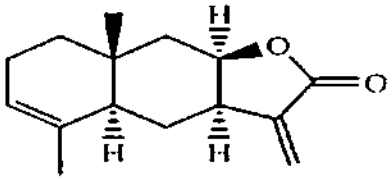
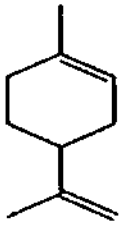
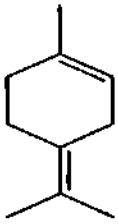
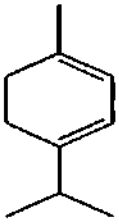
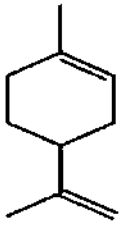
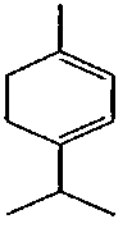
[15]

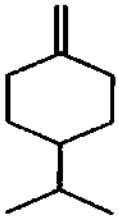
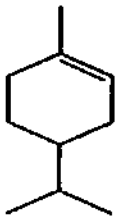


[16]

Interestingly, during the double bond migration in (14), the major product obtained was (16) with the irradiation time being 8 minutes. This could be explained by the fact that compound (16) might have been generated by the intermediacy of (15). In order to prove this, the same reaction was screened for lower time intervals it was observed that after 5 minutes of microwave irradiation when both (15) and (16) were obtained in the ratio (1:2) and the same reaction mixture after further irradiation of 2-3 minutes yielded sole product (16).

Table 2: Comparison of reaction times in some double bond migration reactions by conventional and microwave methods

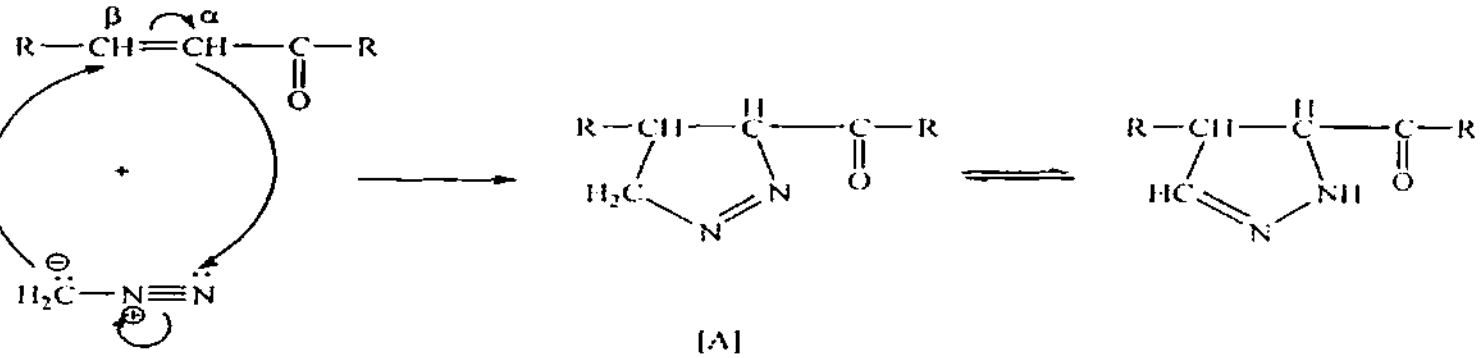
S.No.	Reactant	Product	Reaction time	
			Conven. Method	*μW Method*
1.	 [1]	 [12]	12 h	6 min 40 sec
2.	 [3]	 [13]	12 h	9 min 30 sec
3.	 [14]	 [15]  [16] (1:2)	-	5 min
4.	 [14]	 [16]	24 h	8 min

5.	 [17]	 [18]	8 h	5min 30 sec

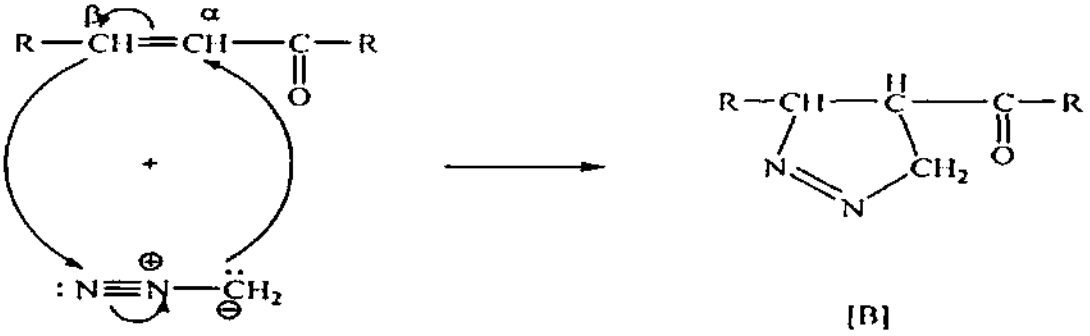
Microwave assisted decomposition of pyrazolines

Sesquiterpene lactones having α-methylene-γ-lactone moiety are known to undergo 1,3-dipolar addition to diazoalkanes resulting in the formation of pyrazolines. The pyrazoline addend formation is activated by electron attracting groups, of which α-methylene-γ-lactone is the promising site. Various substituted pyrazolines and their pyrolyzed products are important biological agents and a significant amount of research activity has been directed towards this class due to the fact that they are efficiently utilized as anti-bacterial, anti-parasitic, anti-tubercular, anti-viral (Husain and Shukla 1986), anti-microbial (Azarifar and Ghasemnejad 2003) and insecticidal agents. Pyrazolines function as useful synthons in organic chemistry and thus play a crucial role in development of heterocyclic chemistry (Abdelhamid *et al* 2000).

Diazomethane has been added to a large number of conjugated olefins, the reaction that leads to the formation of pyrazolines. It is a concerted reaction since the stereochemistry of the olefin is generally maintained in the resulting pyrazolines (Van Auken and Rinehart 1960). The addition to diazoalkanes can take place by two modes resulting in the formation of two types of pyrazolines. The path followed depends upon whether the carbon atom of diazoalkane attaches itself to the β-carbon or the α-carbon atom of the conjugated olefin. In the former case pyrazoline of type A also called normal pyrazoline is formed and the later one resulted with a pyrazoline of type B or the abnormal pyrazoline.



[A]



[B]

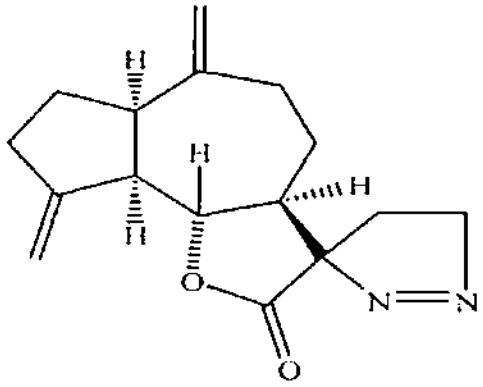
SCHEME I

The distinction between type A and B pyrazolines is best achieved by the IR spectroscopy. The normal pyrazoline (Type A) exhibits absorption band at 3340 cm^{-1} characteristic of the –NH group and it lacks absorption band near 1600 cm^{-1} which is characteristic of –N=N- group. Reverse is true in case of abnormal pyrazolines (Type B). This imparts a great extent of certainity in the placement of double bond in the structures of the two types of pyrazolines.

Literature revealed that sesquiterpene lactones having α-methylene-γ-lactone moiety form type B or the Δ' pyrazolines with diazomethane. Also, the pyrolysed derivatives obtained by the heat induced decomposition of these pyrazolines exhibit immense potential in regulating processes of plant growth and development. This reaction affords the olefin and cyclopropane derivatives and has been of great interest both from synthetic and mechanistic point of view. Chemical and biological significance of the reaction coupled with the immense utility that microwave radiations hold in

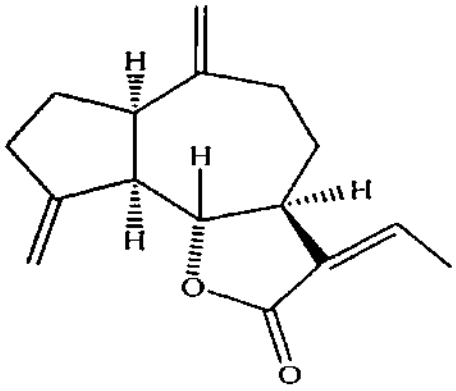
organic chemistry, led to an attempt to pyrolyse the pyrazoline addends of some sesquiterpene lactones under microwave irradiated conditions.

The pyrazoline of dehydrocostus lactone (19) was prepared by treating with ethereal solution of diazomethane. The product was identified by the IR, ^{1}HNMR and ^{13}CNMR data (Table 3).

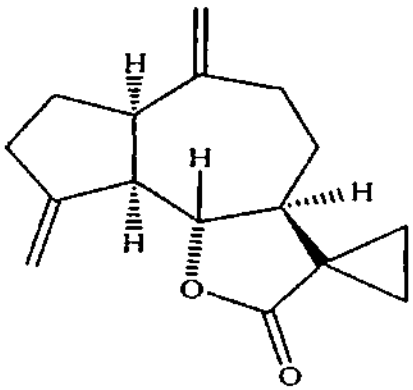


[19]

In a typical procedure, for pyrolysis of this compound, it was adsorbed onto silica gel and irradiated under microwaves for 6 minutes following a time lapse of 2 minutes between every two successive irradiations. Elution of the silica gel with dichloromethane and subsequent evaporation of the solvent afforded a mixture of two compounds. CC of the extract yielded the corresponding olefinic (20) and cyclopropyl (21) derivatives. Their structures were established in accordance with the spectroscopic data presented in Table 3.



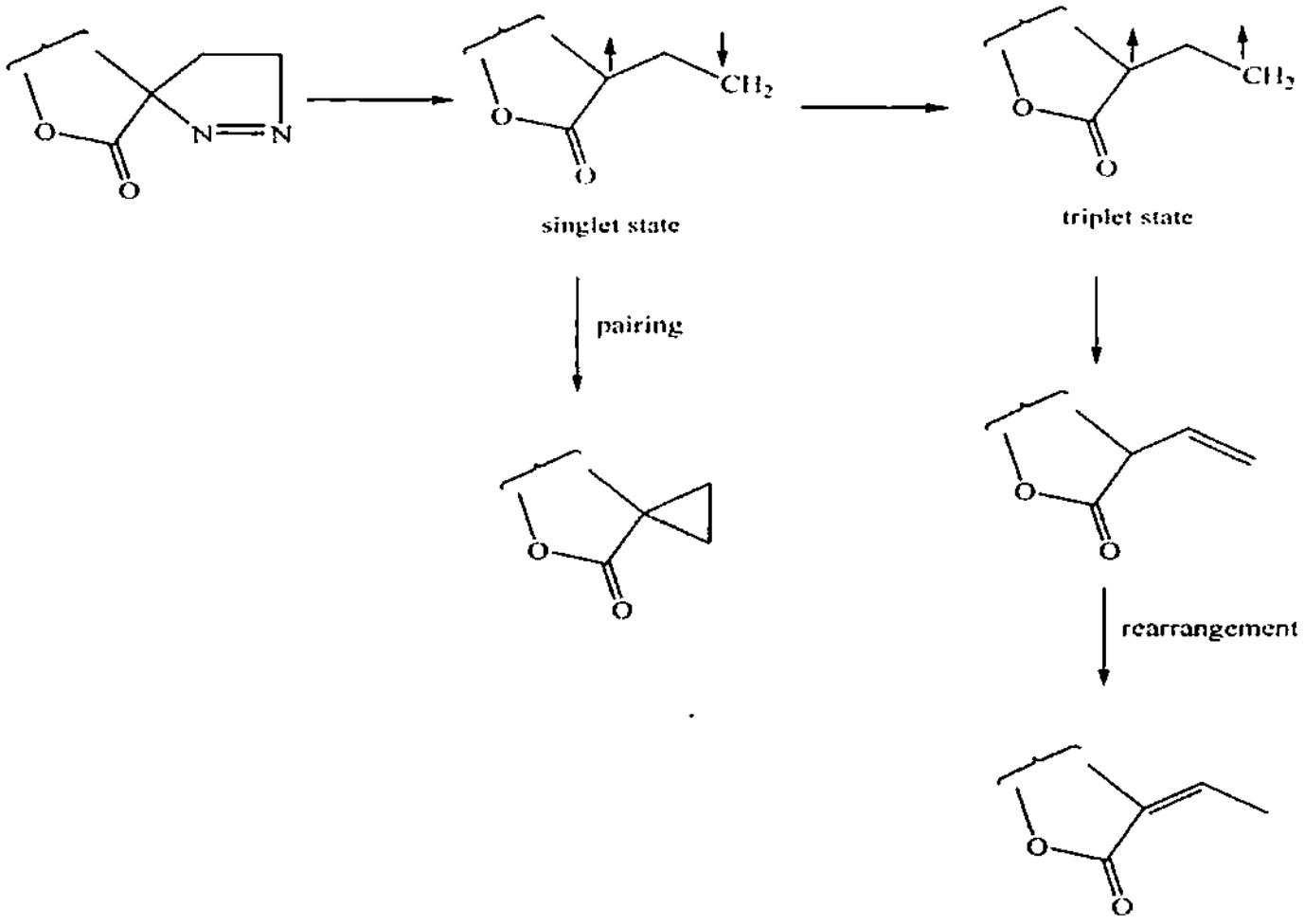
[20]



[21]

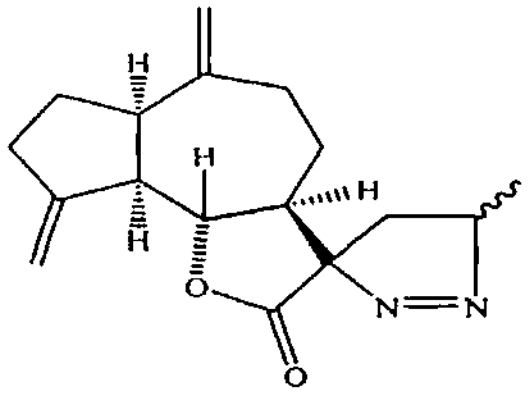
According to the suggested mechanism, the formation of two compounds from pyrazoline is explained via biradical mechanism. The

elimination of N_2 under heat yields biradical in singlet state which gives a cyclopropyl derivative or by losing energy is converted into a triplet state that yields propenyl derivative (Scheme II).



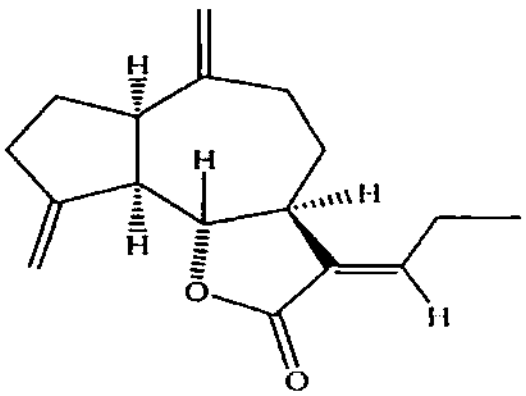
SCHEME II

In order to introduce methyl group at C_{16}, dehydrocostus lactone (1) was made to react with diazoethane to afford the corresponding pyrazoline. ^1H NMR showed all the spectral features for two exomethylene double bonds, a lactone moiety and a doublet for $-CH_3$ at δ 1.54. Taking into account the chemistry of the reaction, structure (22) has been suggested for the compound.

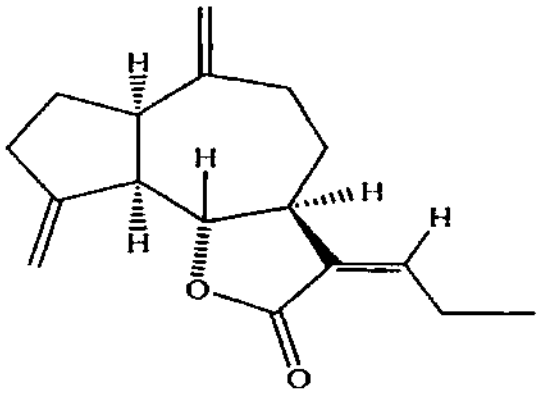


[22]

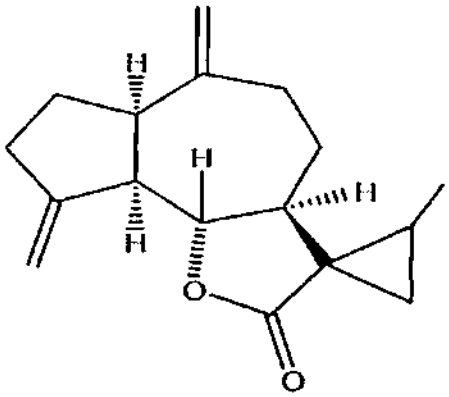
This pyrazoline was also subjected to pyrolysis in microwave with irradiation time of 5 min 20 sec following a 2 min relaxation time between two successive exposures of one minute each. The structures of pyrolysed products were established on the basis of their IR, ^{1}H NMR and ^{13}C NMR data (Table 3).



[23]



[24]



[25]

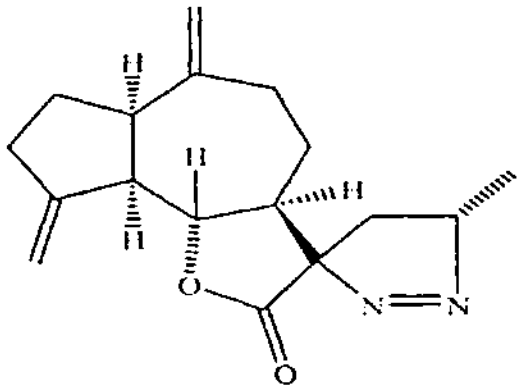
Table 3: Spectroscopic data of pyrazoline derivatives of dehydrocostus lactone (1)

Compound No.	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
19	4.6 (1H, t, J= 9Hz, C_6-H), 4.72 (2H, br s, C_{14}-H), 4.88 (2 H, t, J=9 Hz, C_{16}-Hs), 5.48 and 5.60 (1H each, br s, C_{15}- Hs)	47.26 (C_1 –t), 32.25 (C_2 –t), 6.44 (C_3 –t), 52.49 (C_5 –d), 84.72 (C_6 –d), 52.05 (C_7 –d), 30.09 (C_8 –t), 30.60 (C_9 –t), 49.00 (C_{10} –s), 100.57 (C_{11}–s), 151.03 (C_{12} –s), 25.84 (C_{13}-t), 112.65 (C_{14} –t), 109.66 (C_{15} – t), 86.63 (C_{16} –d).
22	1.54 (3H, d, J = 7.29 Hz, C_{17}-H), 2.91 (1H, brs, C_7-H), 4.71-4.90 (2H, m, C_6-H and C_{16}-H), 4.83 (1H, d, J = 4 Hz, C_{14}-H), 4.87 (1H, d, J = 4 Hz, C_{15}-H), 5.29 (1H, d, J = 4 Hz, C_{15}-H)	37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4-s), 109.1 (C_{14}-t), 57.1 (C_5-d), 50.8(C_1-d), 148.5 (C_{10}-s), 109.0 (C_{15}-t), 36.2 (C_9-t), 25.0 (C_8-t), 35.2 (C_7-d), 80.2 (C_6-d), 106.1 (C_{11}-8), 48.7 (C_{13}-t), 52.4 (C_{16}-d), 19.9 (C_{17}-q), 172.0 (C_{12}-s).
23	1.1 (3H, t, J = 7.5 Hz, C_{17}-H), 1.2 (3H, t, J = 8 Hz, C_6-H), 4.0 (3H, 5, J = 8 Hz, C_6-H), 4.78 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 5.24 (1H, s, C_{15}-H), 5.29 (1H, s, C_{15}-H), 6.74 (1H, dt, J = 3 Hz; 7.5 Hz, C_{13}-H)	37.5 (C_2-t), 37.0 (C_3-t), 150.7 (C_4-3), 109.1 (C_{15}-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 85.0 (C_6-d), 41.9 (C_7-d), 31.5 (C_8-t), 36.5 (C_9-t), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 14.3 (C_{17}-q), 169.8 (C_{12}-s), 20.0 (C_{16}-t).
24	1.03 (3H, t, J = 7.6 Hz, C_{17}-H), 3.86 (1H, t, J = 8 Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 5.03 (1H, s, C_{15}-H), 5.28 (1H, s, C_{15}-H), 5.95 (1H, dt, J = 2 Hz; 7 Hz, C_{13}-H)	37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4-s), 109.1 (C_{15}-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 57.0 (C_5-d), 35.8 (C_9-t), 32.0 (C_8-t), 48.5 (C_7-d), 85.0 (C_6-d), 169.8 (C_{12}-s), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 29.8 (C_{16}-t), 14.3 (C_{17}-q).
25	0.81-0.92 (3H, m, C_{11}-cycloprophyl-H), 1.40 (3H, t, J = 7 Hz, C_{17}-H), 3.98 (1H, t, J = 7 Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 5.05 (1H, s, C_{15}-H), 5.25 (1H, s, C_{15}-H).	37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4-s), 109.1 (C_{15}-t), 37.0 (C_3-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 57.5 (C_5-d), 36.1 (C_9-t), 28.2 (C_8-d), 38.0 (C_7-d), 82.9 (C_6-d), 31.5 (C_{11}-s), 15.0 (C_{16}-d), 20.5 (C_{17}-q), 32.4 (C_{13}-t), 172.0 (C_{12}-s).

27	1.14 (3H, d, J = 7.29 Hz, C_{18}-H), 2.90 (1H, brs, C_7-H), 4.73-4.90 (3H, m, C_{14}-H and C_{16}-H), 5.12 (1H, brs, C_{15}-H), 5.29 (1H, brs, C_{15}-H).	37.5 (C_2-t), 37.0 (C_3-t), 150.8 (C_4s), 109.1 (C_{15}-t), 50.8 (C_1-d), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 36.2 (C_9-t), 28.3 (C_{17}-t), 59.2 (C_{16}-d), 9.0 (C_{18}-q), 172.7 (C_{12}-s).
28	0.97 (3H, t, J = 7.5 Hz, C_{18}-H), 3.99 (1H, t, J = 9 Hz, C_6-H), 4.7 (1H, s, C_{14}-H), 4.85 (1H, s, C_{14}H), 5.06 (1H, d, J = 1.2 Hz, C_{15}-H), 5.3 (1H, d, J = 1.2 Hz, C_{15}-H), 6.75 (1H, dt, J = 3Hz; 9 Hz, C_{13}-H)	37.5 (C_2-t), 37.0 (C_3-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 150.7 (C_4-s), 109.5 (C_{15}-t), 85.0 (C_6-d), 41.9 (C_7-d), 36.5 (C_9-t), 129.0 (C_{11}-s), 142.5 (C_{13}-d), 29.6 (C_8-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s), 22.1 (C_{16}-t).
29	0.93 (3H, t, J = 7.5 Hz, C_{18}-H), 3.90 (1H, t, J = 9 Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 5.06 (1H, d, J = 1.5 Hz, C_{15}-H), 5.25 (1H, d, J = 1.5 Hz, C_{15}-H), 6.02 (1H, dt, J = 2.7 Hz; 7.5 Hz, C_{13}-H)	37.5 (C_2-t), 37.0 (C_3-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 150.6 (C_4-s), 109.4 (C_{15}-t), 85.2 (C_6-d), 48.5 (C_7-d), 36.5 (C_9-t), 129.0 (C_{11}-s), 142.5 (C_{13}-d), 29.6 (C_8-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s), 30.4 (C_{16}-t).
30	0.86-0.90 (3H, m, C_{11}-cyclopropyl-H), 0.90 (3H, t, J = 7.5 Hz, C_{18}-H), 3.98 (1H, t, J = 9 Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 5.06 (1H, d, J = 1.5 Hz, C_{15}-H), 5.25 (1H, d, J = 1.5 Hz, C_{15}-H), 6.02 (1H, dt, J = 2.7 Hz; 7.5 Hz, C_{13}-H)	37.5 (C_2-t), 37.0 (C_3-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 150.7 (C_4-s), 109.5 (C_{15}-t), 85.0 (C_6-d), 38.5 (C_7-d), 27.8 (C_8-t), 36.5 (C_9-t), 29.0 (C_{11}-s), 22.5 (C_{13}-d), 28.5(C_8-t), 11.6 (C_{18}-q), 29.7 (C_{17}-t), 171.9 (C_{12}-s).

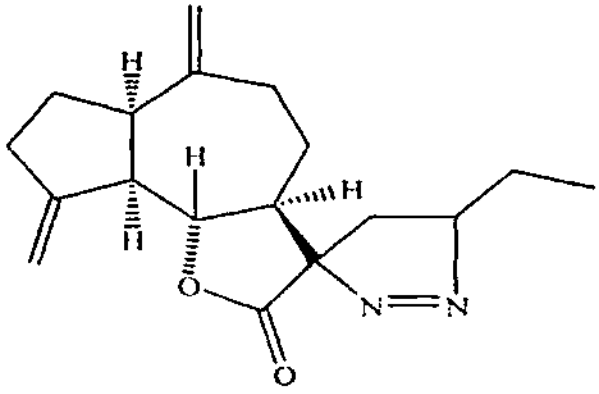
In order to prove the stereochemistry of N=N at C-11 in pyrazoline, its structure was compared to with that of spirocyclopropyl derivative of dehydrocostus lactone (21).

Looking into the structure of (21), the methyl group of C_{16} should be *trans* with respect to the C-C bond between C_7 and C_{11}. As this bond is β-oriented, therefore, methyl at C_{16} has to be below the plane of cyclopropane ring (Singh *et al* 1993). Since this cyclopropanes derivative is obtained from the decomposition of pyrazoline (22), having methyl *trans* to $C_7 - C_{11}$ bond, hence it must also be *trans* in the original pyrazoline assuring biradical mechanism of decomposition, thereby suggesting the stereostructure (26) for this pyrazoline.



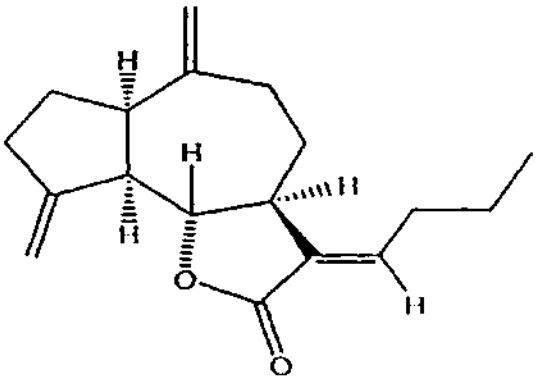
[26]

Further, in order to achieve C-alkylation at carbon-carbon double bond in conjugation with the lactone moiety, dehydrocostus lactone was treated with diazopropane to yield the pyrazoline product (27), whose structural authenticity was confirmed by the spectral data.

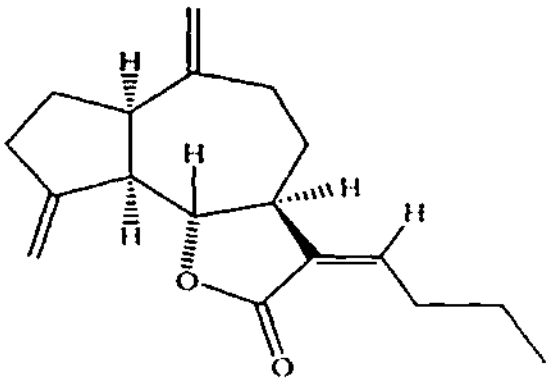


[27]

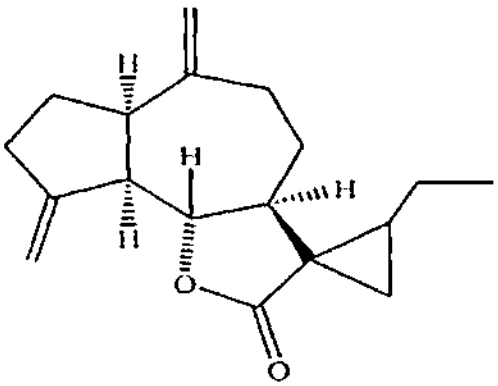
This pyrazoline as well, upon pyrolysis gave the desired compounds (28-30) through the intermediary of singlet and triplet biradicals.



[28]



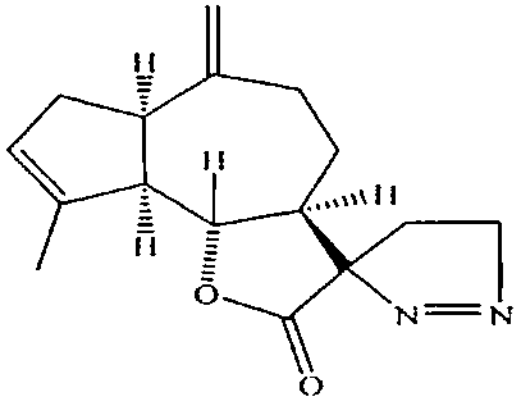
[29]



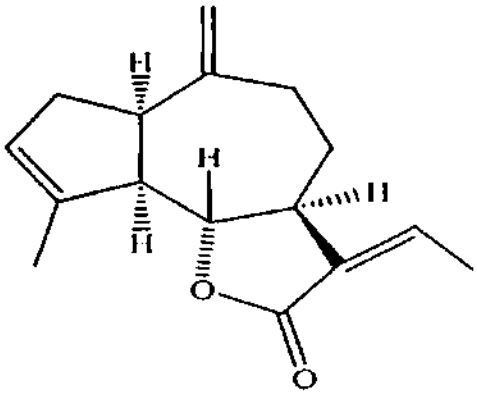
[30]

It is evident from the Table 3 that structural pairs 23, 24 and 28, 29 showed similar ^{1}H NMR data, *Z*-isomer (23) showed chemical shift at δ 5.95 for C_{13} in contrast to δ 6.74 in case of the *E*-isomer (24). Similarly, for compounds 28 and 29, the chemical shifts for C_{13} were observed at δ 6.02 and δ 6.75, respectively. These observations were further corroborated by detailed study of ^{13}C NMR spectra which affirm these two pairs of compounds to be geometrical isomers. The isomers showed almost same carbon shifts as in case of compounds 23 and 24. For compounds 28 and 29, signals for C_{16} appeared at δ 22.1 and 30.4, respectively. The structures were further supported by the fact that these assignments are similar to those for C_{16} in case of compounds 23 and 24 which appeared at δ 29.8 and 20.0 respectively.

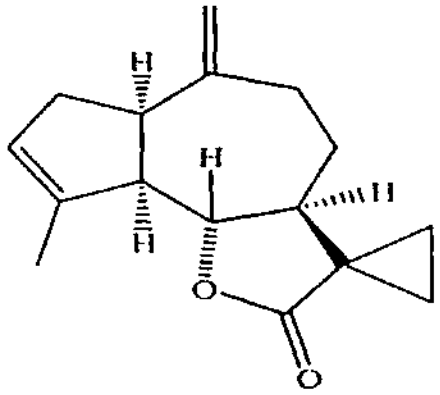
Similarly, pyrazoline derivative of isodehydrocostus lactone (12) was prepared by reaction with diazomethane and the microwave mediated decomposition of this pyrazoline (31) was attempted which afforded 13-methyl isodehydrocostus lactone (32) and 11-spirocyclopropyl derivative (33) (Table 4).



[31]

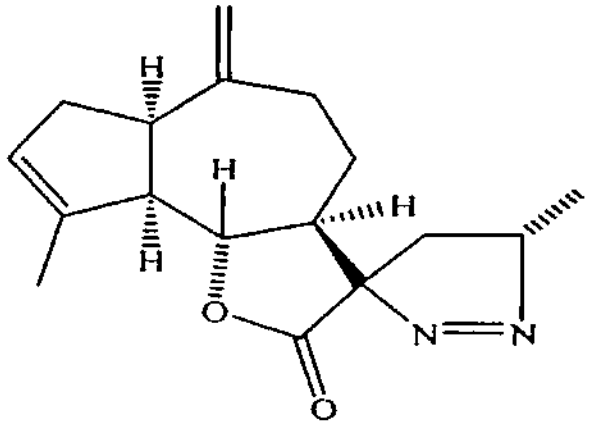


[32]



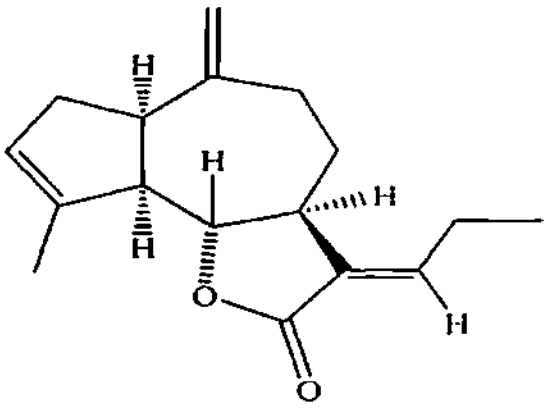
[33]

In order to introduce the methyl group at C-16, isodehydrocostus lactone (12) was treated with an ethereal solution of diazoethane to afford pyrazoline (34) with mp 165°C.

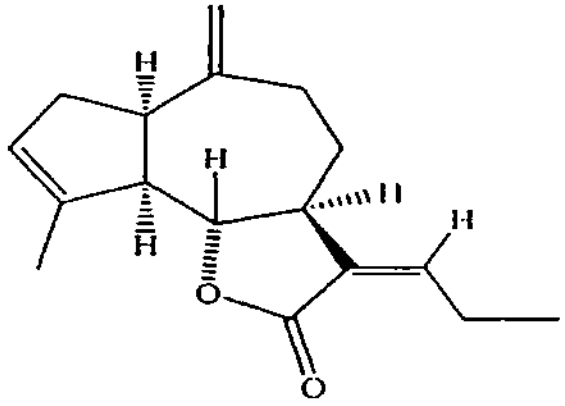


[34]

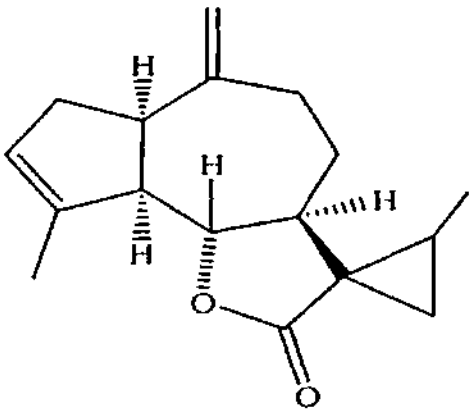
^{1}H NMR showed a multiplet at δ 4.70 due to two hydrogens and the signals for the C_6 – H and C_{16} – H appeared as a double doublet at 4.1 with coupling constant 10 Hz. Microwave assisted pyrolysis of this addend afforded the derivatives (35-37).



[35]

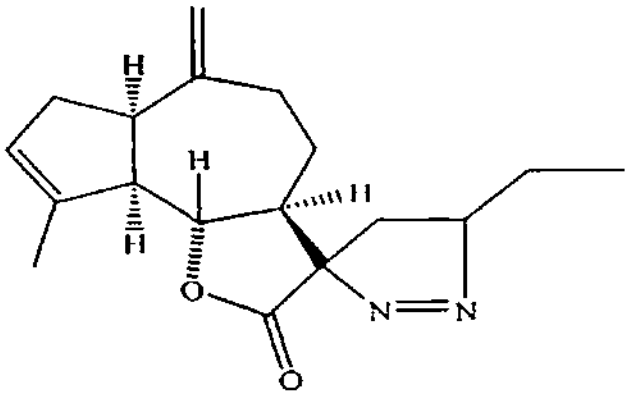


[36]



[37]

In order to extend this idea of isomerisation, it was thought worthwhile to extend the carbon chain at C_{13} position. For this, isodehydrocostus lactone was treated with ethereal solution of diazopropane leading to the formation of the corresponding pyrazoline (38), which on exposure to microwave irradiation, afforded the pyrolysis products (39-41).



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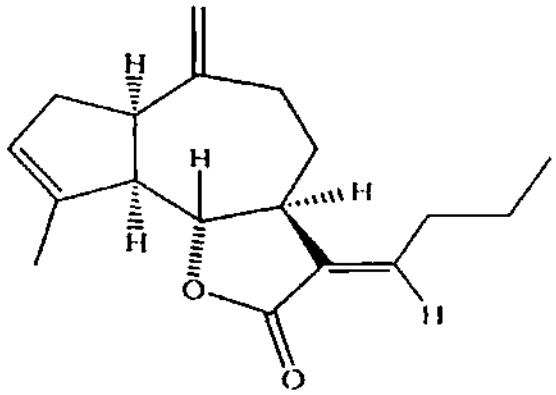
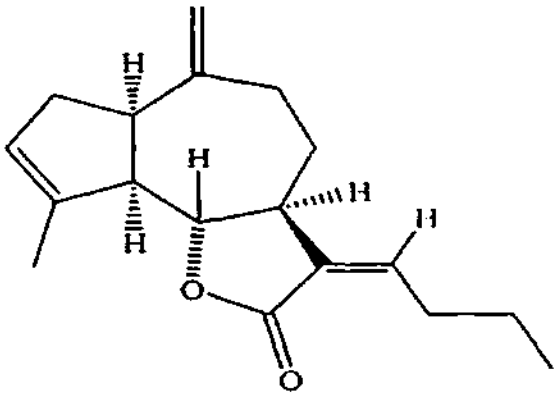
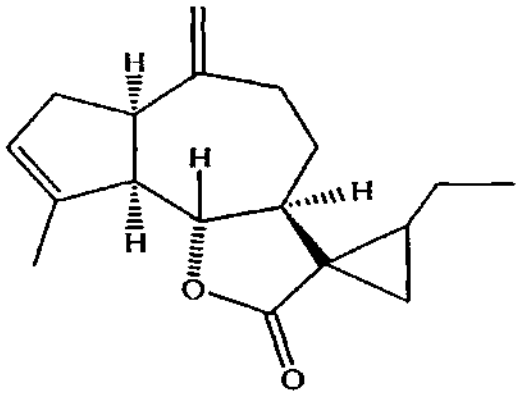


[39]



[40]



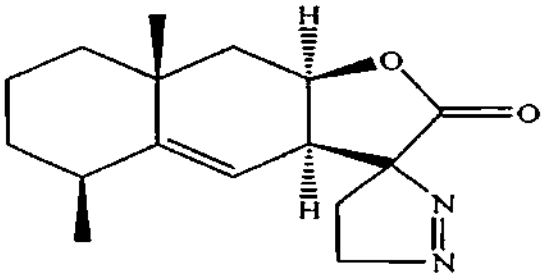
[41]

Table 4: Spectroscopic data of pyrazoline derivatives of isodehydrocostus lactone (12)

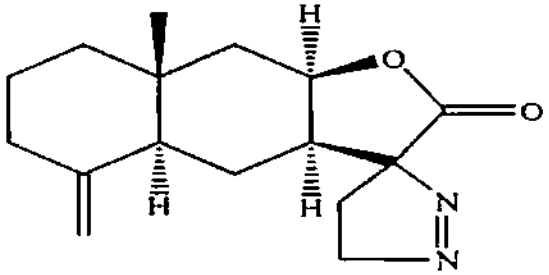
Compound No.	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
31	4.6 (1H, t, J= 9Hz, C_6-H), 4.72 (2H, br s, C_{14}-H), 4.88 (2 H, t, J=9 Hz, C_{16}-Hs). 5.48 and 5.60 (1H each, br s, C_{15}- Hs), 5.3 (1H, m, C_3-H), 4.15 (1H, t, J = 8 Hz, C_6-H), 1.5 (3H, brs, C_{15}-H).	48.5 (C_1-t), 35.8 (C_2-t), 125.0 (C_3-d), 142.6 (C_4-s), 53.0 (C_5-d), 79.5 (C_6-d), 35.2 (C_7-d), 36.0 (C_9-t), 148.5 (C_{10}-s), 108.8 (C_{11}-s), 171.2 (C_{12}-s), 33.5 (C_{13}-t), 77.8 (C_{14}-t), 20.9 (C_{15}-q), 108.8 (C_{16}-t).
34	1.54 (3H, d, J = 7.2 Hz, C_{17}-H), 1.70 (3H, bs, C_{15}-H), 4.70 (2H, m, C_6-H and C_{16}-H), 5.20 (2H, bs, C_{14}-H and C'$_{14}$-H), 5.62 (1H, brs, C_3-H)	19.35 (C_{17}-q), 25.84 (C_{13}-t), 30.09 (C_8-t), 30.60 (C_9-t), 32.25 (C_2-t), 36.44 (C_3-t), 47.26 (C_7-d), 52.05 (C_1-t), 100.57 (C_{11}-s), 109.65 (C_{15}-q), 109.70 (C_3-t), 112.65 (C_{14}-t), 149.00 (C_{10}-s), 151.03 (C_4-s), 172.50 (C_{12}-s), 52.4 (C_{16}-d), 20.0 (C_{17}-q)
35	1.3 (3H, t, J = 7.5 Hz, C_{17}-H), 1.5 (3H, brs, C_{15}-Hs), 4.2 (1H, t, J = 8 Hz, C_6-H), 4.78 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 5.24 (1H, brs, C_3-Hs), 6.84 (1H, dt, J = 3 Hz; 7.5 Hz, C_{13}-H)	37.5 (C_2-t), 123.5 (C_3-t), 142.9 (C_4-s), 109.1 (C_{15}-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 85.0 (C_6-d), 41.9 (C_7-d), 31.5 (C_8-t), 36.5 (C_9-t), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 14.3 (C_{17}-q), 169.8 (C_{12}-s), 20.2 (C_{16}-H).
36	1.6 (3H, brs, C_{15}-Hs), 1.03 (3H, t, J = 7.6 Hz, C_{17}-H), 3.86 (1H, t, J = 8 Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 5.03 (1H, s, C_{14}-Hs), 5.28 (1H, brs, C_3-H), 5.95 (1H, dt, J = 2 Hz; 7 Hz, C_{13}-H)	37.5 (C_2-t), 122.8 (C_3-t), 143.8 (C_4-s), 109.1 (C_{15}-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 35.8 (C_9-t), 32.0 (C_8-t), 48.5 (C_7-d), 85.0 (C_6-d), 169.8 (C_{12}-s), 130.1 (C_{11}-s), 141.5 (C_{13}-d), 30.4 (C_{16}-t), 14.3 (C_{17}-q).
37	0.81-0.92 (3H, m, C_{11}-cyclopropyl-Hs), 1.40 (3H, d, J = 7 Hz, C_{17}-H), 1.65 (3H, brs, C_{15}-Hs), 5.05 (1H, s, C_{14}-H), 5.25 (1H, s, C_3-H),	37.5 (C_2-t), 122.9 (C_3-t), 142.8 (C_4-s), 109.1 (C_{15}-t), 37.0 (C_3-t), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 36.1 (C_9-t), 28.2 (C_8-d), 38.0 (C_7-d), 31.5 (C_{11}-s), 15.0 (C_{16}-d), 20.5 (C_{17}-q), 32.4 (C_{13}-t), 172.0 (C_{12}-s).

38	1.14 (3H, t, J = 7.29 Hz, C_{18}-H), 2.90 (2H, brs, C_{17}-H), 4.73-4.90 (3H, m, C_{14}-H and C_{16}-H), 3.86 (1H, t, J = 8 Hz, C_6-H), 1.55 (3H, brs, C_{15}-H), 5.29 (1H, brs, C_3-H).	37.5 (C_2-t), 123.5 (C_3-d), 142.8 (C_4-s), 109.1 (C_{15}-t),50.8 (C_1-d), 148.5 (C_{10}-s), 109.0 (C_{14}-t), 36.2 (C_9-t), 28.3 (C_{17}-t), 59.2 (C_{16}-d), 9.0 (C_{18}-q), 172.7 (C_{12}-s).
39	0.97 (3H, t, J = 7.5 Hz, C_{18}-H), 3.99 (1H, t, J = 9 Hz, C_6-H), 4.7 (1H, s, C_{14}-H), 4.85 (1H, s, C_{14}-H), 1.56 (3H, brs, C_{15}-Hs), 5.3 (1H, d, J = 1.2 Hz, C_3-H), 6.75 (1H, dt, J = 3Hz; 9 Hz, C_{13}-H)	37.5 (C_2-t), 123.5 (C_3-d), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 142.7 (C_4-s), 15.5 (C_{15}-q), 85.0 (C_6-d), 41.9 (C_7-d), 36.5 (C_9-t), 129.0 (C_{11}-s), 142.5 (C_{13}-d), 29.6 (C_8-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s), 20.2 (C_{16}-H).
40	0.93 (3H, t, J = 7.5 Hz, C_{18}-H), 3.90 (1H, t, J = 9 Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 1.6 (3H, brs, C_{15}-Hs), 5.25 (1H, d, J = 1.5 Hz, C_3-H), 6.02 (1H, dt, J = 2.7 Hz; 7.5 Hz, C_{13}-H)	37.5 (C_2-t), 122.8 (C_3-t), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 142.6 (C_4-s), 15.4 (C_{15}-q), 85.2 (C_6-d), 48.5 (C_7-d), 36.5 (C_9-t), 129.0 (C_1-s), 142.5 (C_{13}-d), 31.1 (C_{16}-t), 29.6 (C_8-t), 23.0 (C_{17}-t), 14.2 (C_{18}-q), 170.2 (C_{12}-s).
41	0.86-0.90 (3H, m, C_{11}-cyclopropyl-H), 0.90 (3H, t, J = 7.5 Hz, C_{18}-H), 3.98 (1H, t, J = 9 Hz, C_6-H), 4.76 (1H, s, C_{14}-H), 4.86 (1H, s, C_{14}-H), 1.61 (3H, brs, C_{15}-Hs), 5.25 (1H, d, J = 1.5 Hz, C_3-H)	37.5 (C_2-t), 123.0 (C_3-d), 109.0 (C_{14}-t), 148.5 (C_{10}-s), 50.8 (C_1-d), 57.0 (C_5-d), 142.7 (C_4-s), 15.5 (C_{15}-q), 85.0 (C_6-d), 38.5 (C_7-d), 27.8 (C_8-t), 36.5 (C_9-t), 29.0 (C_{11}-s), 22.5 (C_{13}-d), 28.5(C_8-t), 11.6 (C_{18}-q), 29.7 (C_{17}-t), 171.9 (C_{12}-s), 20.2 (C_{16}-H).

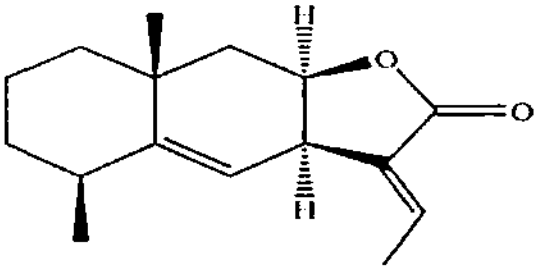
Similarly, different substituted and non-substituted pyrazolines of alantolactone (42) and that of isoalantolactone (43) on pyrolysis under microwave conditions gave the expected products (44-53) which were characterized on the basis of spectroscopic data (Table 5).



[42]



[43]



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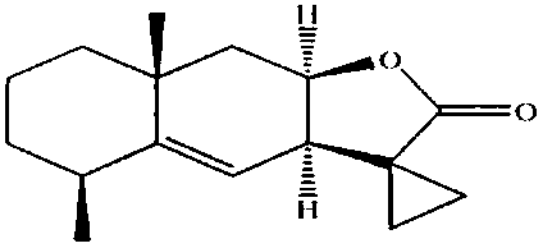
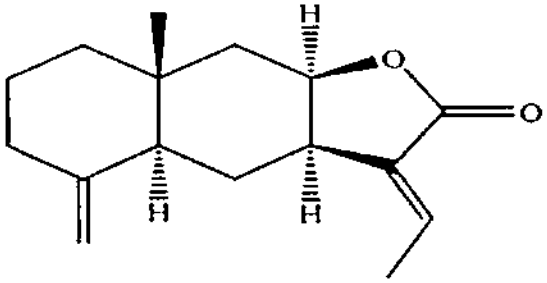


[45]



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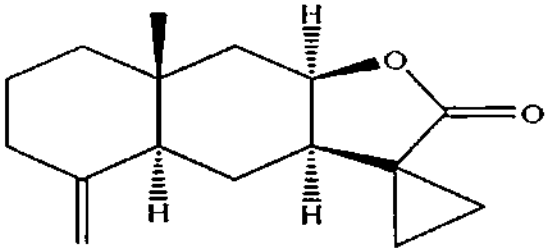

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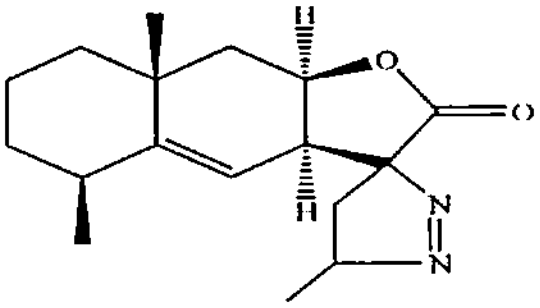

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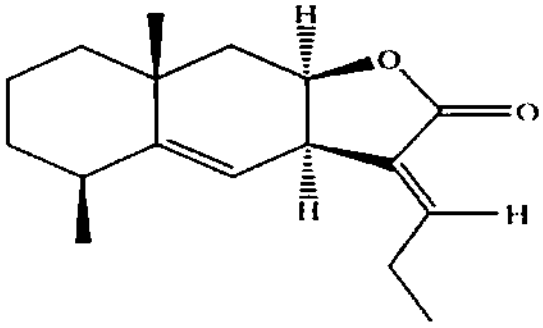

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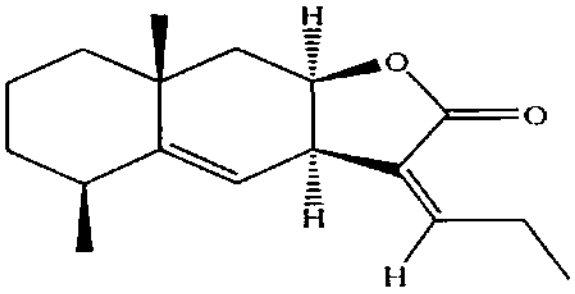
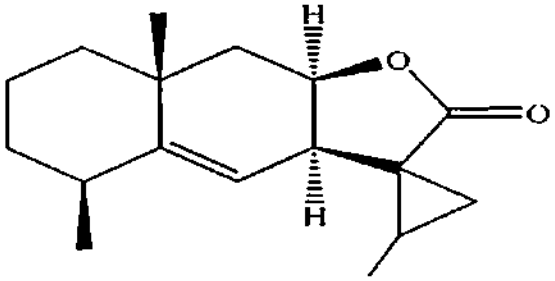


[50]



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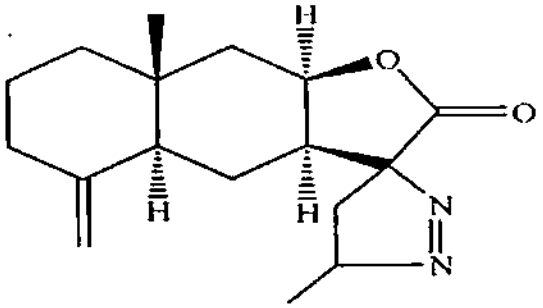
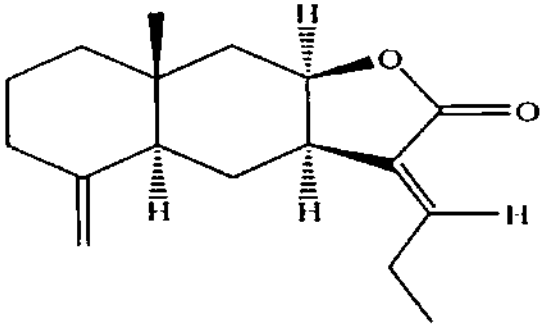


[52]



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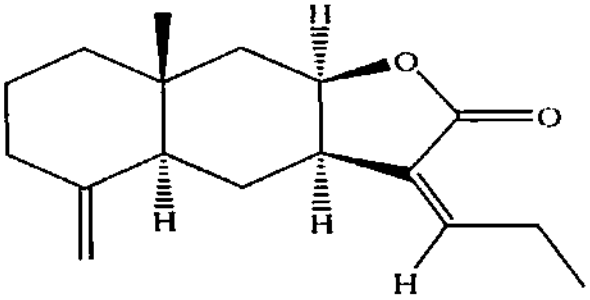

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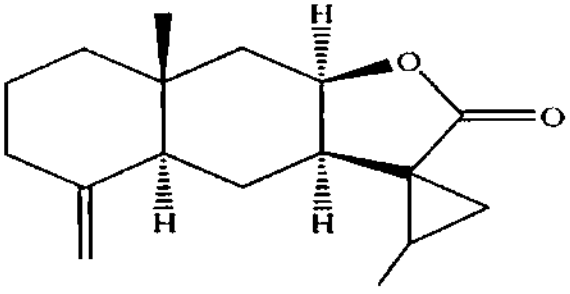
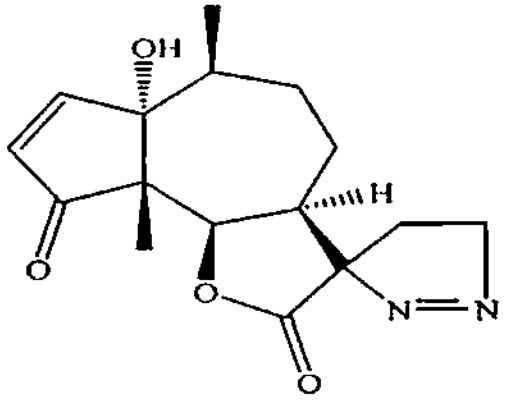
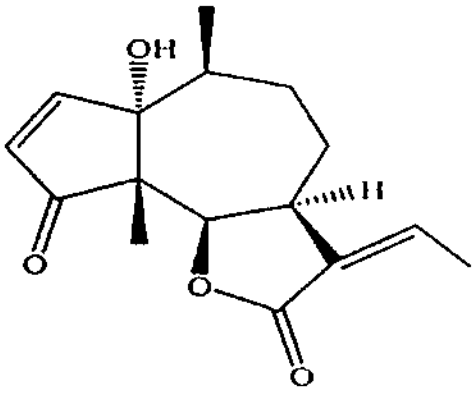


[55]

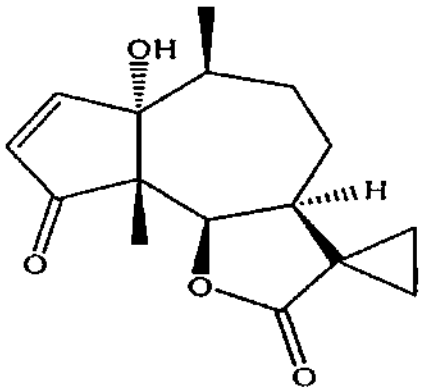
Similarly, the pyrazoline derivative of parthenin (56) was prepared and subsequently pyrolysed under microwave irradiations to afford 57 and 58.



[56]



[57]



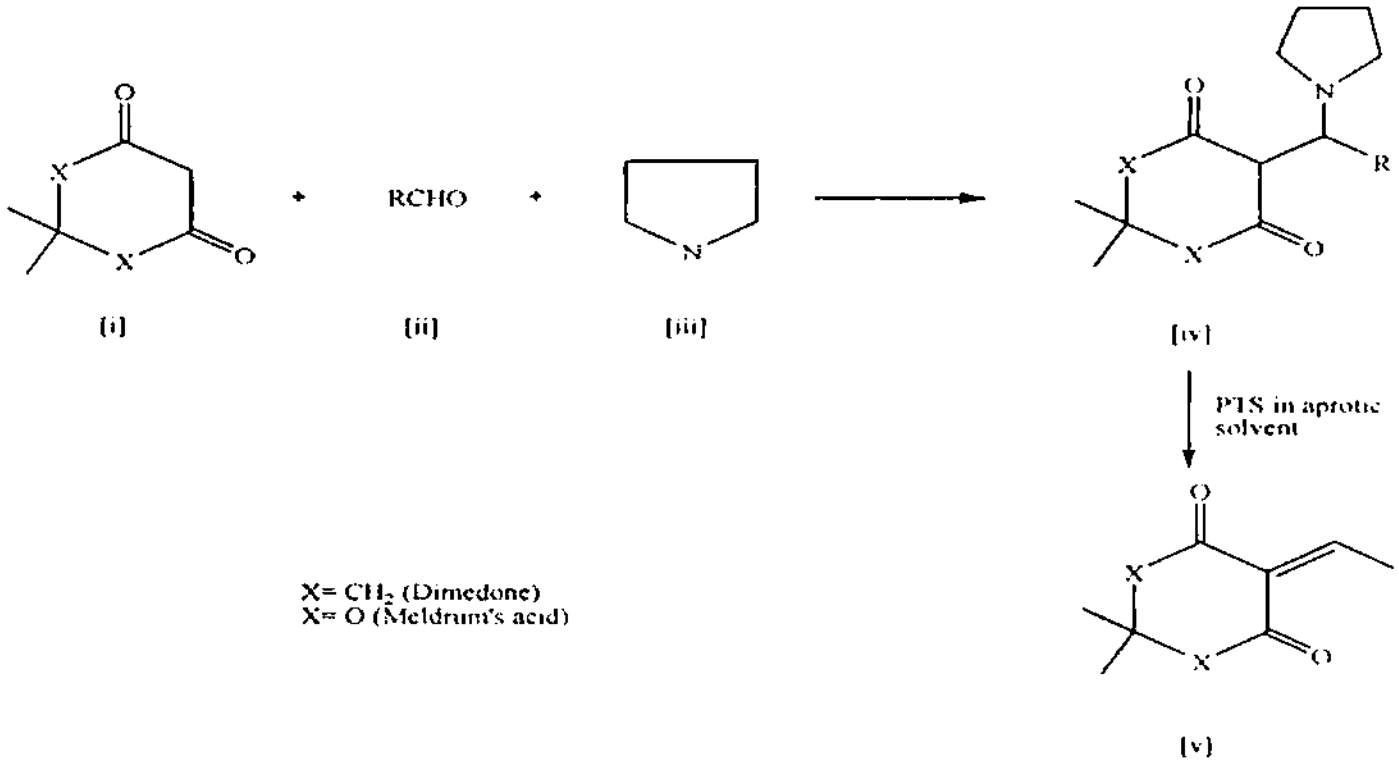
[58]

Table 5: IR and ^{1}H NMR data of some pyrazoline derivatives of alantolides and pseudoguaianolides

Compd No.	IR bands(ν_{max}/cm^{-1})	^{1}H NMR δ (ppm)
48	2929, 1754, 1455, 1373, 1197, 1126, 1037, 887	1.23 (3H, d, J = 7.76 Hz, C_{15}-H), 1.26 (3H, s, C_{14}-H), 1.57 (3H, d, J = 7.29 Hz, C_{17}-H), 4.78 (1H, m, C_8-H), 5.51 (1H, m, C_{16}-H)
49	2927, 1739, 1668, 1440, 1371, 11199 970, 867	1.05 (3H, t, J = 7.56 Hz, C_{17}-H), 1.11 (3H, d, J = 7 Hz, C_{15}-H), 1.25 (3H, s, C_{14}-H), 3.47 (1H, brs, C_7-H), 4.77 (1H, m, C_8-H), 5.08 (1H, d, J = 3.8 Hz, C_6-H), 6.14 (1H, dt, J = 1.6 Hz; 6 Hz, C_{13}-H)
50	2929, 12743, 1326, 1247, 1132, 975, 902	0.98 (3H, m, C_{11}-cyclopropyl-H), 1.14 (3H, d, J = 7.56 Hz, C_{15}-H), 1.23 (3H, d, J = 5 Hz, C_{17}-H),1.27 (3H, s, C_{14}-H),4.9 (1H, m, C_8-H),5.07(1H, d, J = 3 Hz,C_6-H)
51	2927, 1757, 1668, 1629, 1549, 1459, 1379, 1266, 1198, 1030, 977, 866	1.12 (3H, t, J = 7.3 Hz, C_{17}-H), 1.15 (3H, d, J = 6.6 Hz, C_{15}-H), 1.21 (3H, s, C_{14}-H), 3.47 (1H, brs, C_7-H), 4.87 (1H, m, C_8-H), 5.16 (1H, d, J = 3 Hz, C_6-H), 6.65 (1H, dt, J = 1.8 Hz; 7.8 Hz, C_{13}-H)
52	2933, 1768, 1644, 1441, 1160, 993, 944, 890	0.85 (3H, s, C_{14}-H), 1.55 (3H, d, J = 7.29 Hz, C_{17}-H), 4.45 (3H, brs, C_8-H), 4.74 – 4.85 (2H, m, C_{15}-H), 5.57 (1H, m, C_{16}-H)
53	2935, 1741, 1704, 1671, 1133, 1091, 991, 914, 883	0.84 (3H, s, C_{14}-H), 1.06 (3H, t, J = 7.56 Hz, C_{17}-H), 2.62 (1H, m, C_7-H), 4.46 (1H, d, J = 1.6 Hz, C_{15}-H), 4.48 (1H, brs, C_8-H), 4.76 (1H, d, J = 1.1 Hz, C_{15}-H), 6.12 (1H, t, J = 1.9 Hz, C_{13}-H)
54	2933, 1741, 1145, 954, 900	0.86 (3H, s, C_{14}-H), 0.99-1.16 (3H, m, C_{11}-cyclopropyl-H), 1.19 (3H, d, J = 5.9 Hz, C_{17}-H), 4.49 (1H, s, C_{15}-H), 4.67(1H, m, C_8-H), 4.76 (1H, s, C_{15}-H)
55	2927, 1747, 1683, 12216, 997, 960, 896, 773	0.83 (3H, s, C_{14}-H), 1.1 (3H, t, J = 7.6 Hz, C_{17}-H), 2.99-3.08 (2H, m, C_{16}-H), 2.98-3.05 (1H, m, C_7-H), 4.43 (1H, s, C_{15}-H), 4.48 (1H, m, C_8-H), 4.77 (1H, s, C_{15}-H), 6.63 (1H, t, J = 8 Hz, C_{13}-H)
56	3600, 3390, 1770, 1730, 1340, 980	1.2 (d, 3H, C_{10}-CH_3, J= 7.0 Hz), 1.43 (s, 3H, C_5-CH_3), 4.66 (m, 2H, -CH_2-N=N-), 5.67 (d, 1H, C_6-H, J= 7.0 Hz), 6.26 (d, 1H, C_3-H, J= 7.0 Hz), 7.7 (d, 1H, C_2-H, J= 7.0 Hz).

Decomposition of Mannich bases under microwave irradiation

It has been reported that synthesis of monoalkylidenes of Meldrum's acid follows a general reaction (Scheme III) using equimolar quantities of 1, 3- diketones (i), aldehyde (ii) and cyclic aminase (iii) to yield the Mannich bases (iv). There Mannich bases when subjected to elimination reactions under acidic conditions afforded desired monoaldylidenes (v).



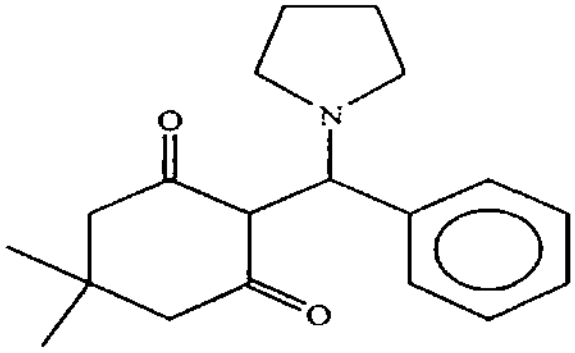
[i] [ii] [iii] [iv] [v]

SCHEME III

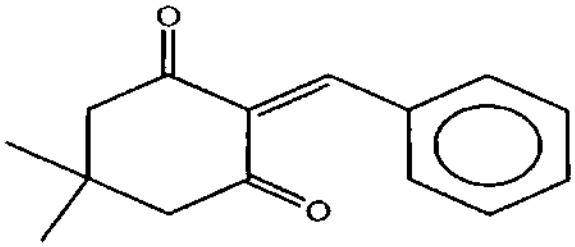
The reaction was quite independent as far straight chain, branched and aromatic aldehydes were used to prepare Mannich bases.

Traditionally, Knoevenagel reaction of active methylenes with various aldehydes is achieved through the intermediacy of Mannich bases. The Mannich bases are decomposed by using *p*-toluene sulfonic acid in dichloromethane. With reports suggesting successful observance of Knoevenagel condensation of 1,3-diones with aromatic aldehydes under microwave irradiation on solid matrix, which probably proceeds through the intermediacy of Mannich bases, it was thought worthwhile to confirm this by

attempting the decomposition of Mannich bases under microwave irradiation. The Mannich base (59) was adsorbed on silica gel as usual using dichloromethane as solvent and the free flowing powder was subjected to microwave irradiation for 5 minutes at 640W. Interestingly, the usual workup yielded the corresponding arylidene (60, 80%).



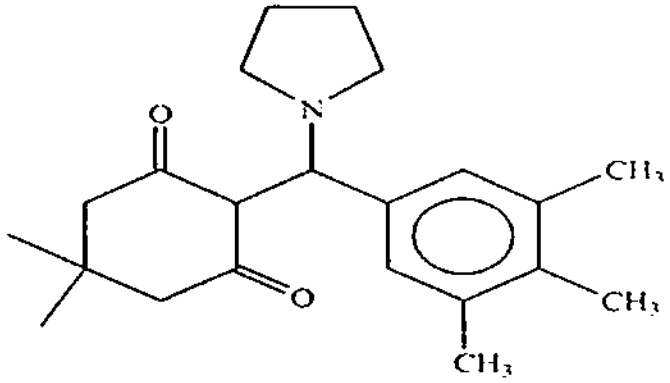
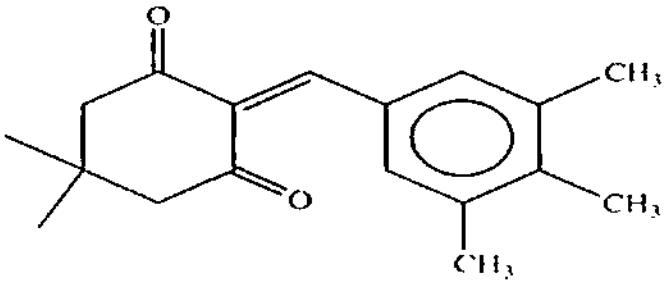
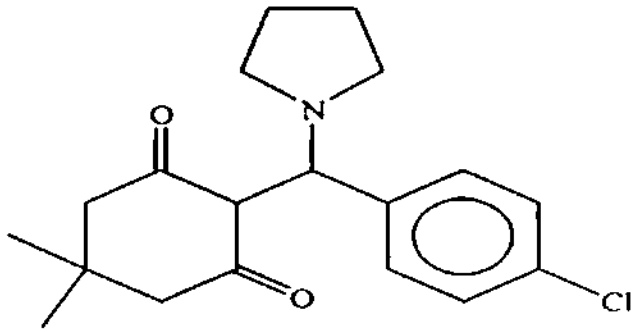
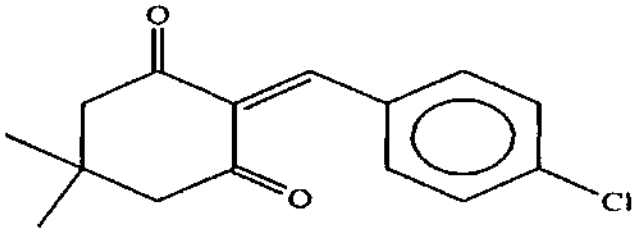
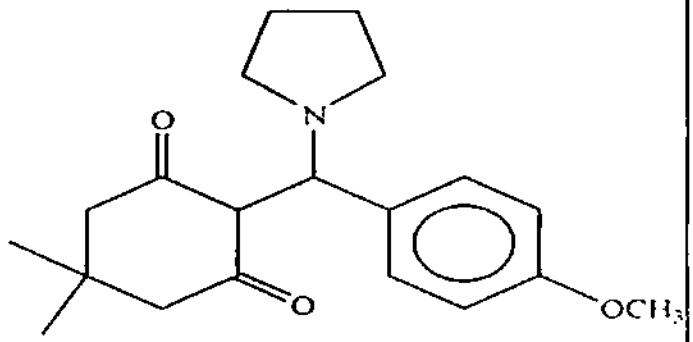
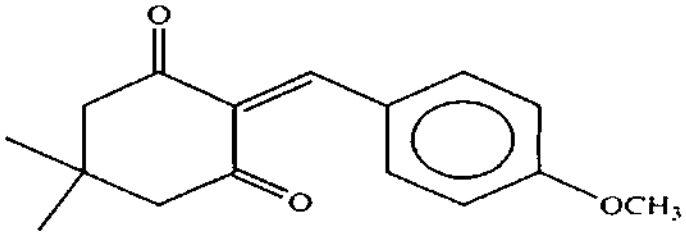
[59]

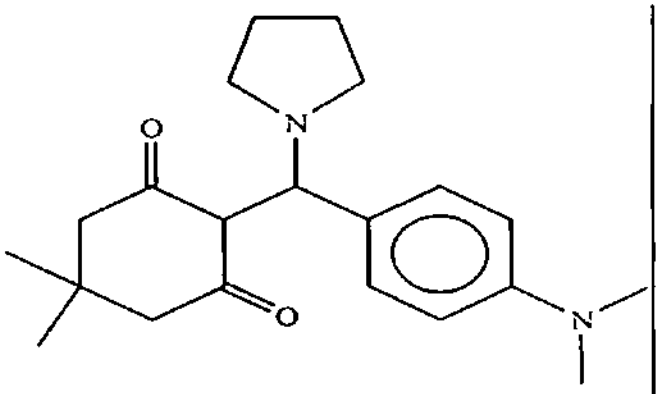


[60]

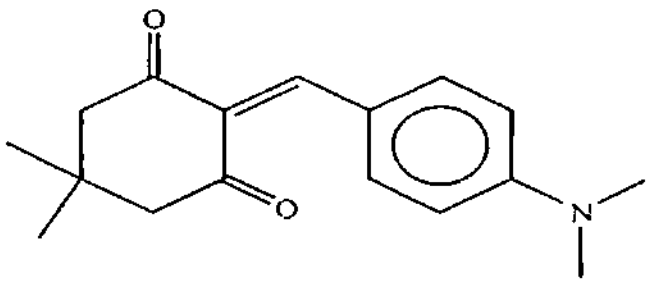
Similar results were obtained when other Mannich bases (61-65) prepared in our laboratory were subjected to same reaction conditions when they afforded the corresponding arylidenes (66-70). In order to confirm that the decomposition had occurred under the influence of microwaves and not due to adsorption procedure on silica gel, a blank experiment was conducted using all the ingredients except microwave irradiation. In this case, the formation of arylidene was not observed even after 1 hour of putting together the reactants in contact with silica gel. Table 6 and 7 present the structures of various arylidenes formed by the decomposition of Mannich bases along with their spectral data.

Table 6: Structures of various Mannich bases and their corresponding arylidenes

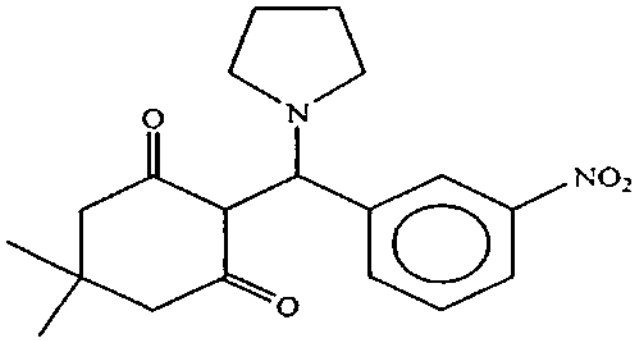
Structure of Mannich base	Structure of corresponding arylidene
 [61]	 [66]
 [62]	 [67]
 [63]	 [68]



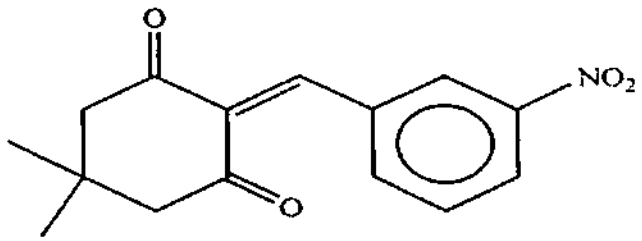
[64]



[69]



[65]



[70]

Table 7: Spectroscopic data of various arylidenes formed by decomposition of Mannich bases

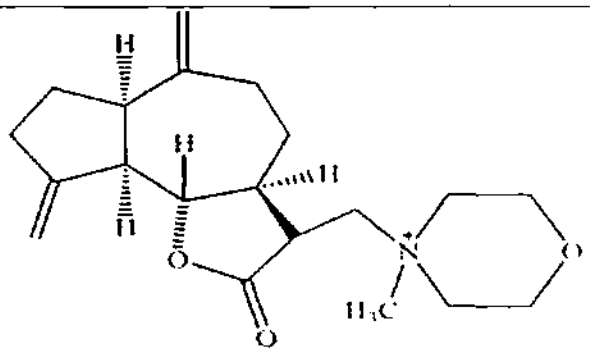
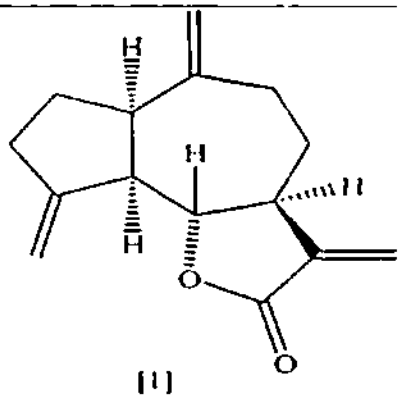
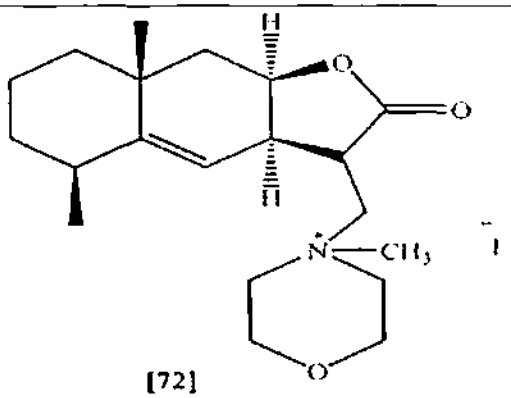
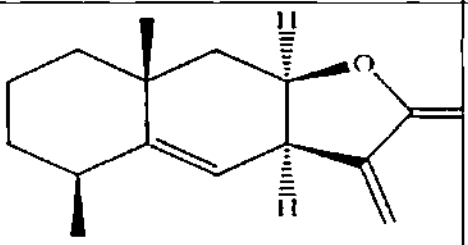
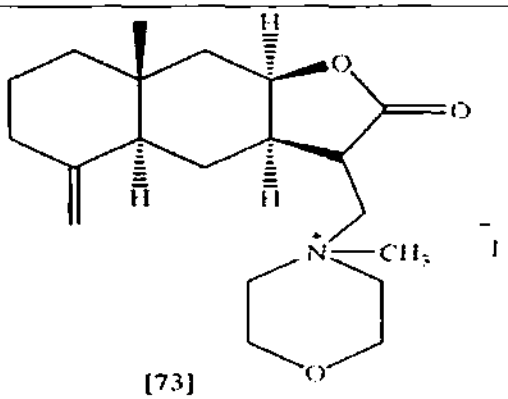
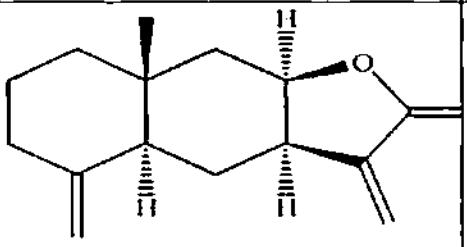
Compound no.	^{1}HNMR δ (ppm)	^{13}CNMR δ (ppm)
60	1.12 (3H, s), 1.11 (3H, s), 1.22 (3H, s), 2.47 (4H, bs), 5.57 (1H, s), 7.22 – 7.28 (5H, m).	194.3 (C_1-s), 52.8 (C_2-t), 30.3 (C_3-s), 52.8 (C_4-d), 194.4 (C_5-s), 250.9 (C_6-s), 146.0 (C_7-d), 135.3 (C_8-s), 126.5 (C_9-d), 128.5 (C_{10}-d), 128.1 (C_{11}-d), 128.5 (C_{12}-d), 126.3 (C_{13}-d), 26.5 (C_{14}-q), 26.6 (C_{15}-q)
66	1.12 (3H, s), 1.24 (3H, s), 2.32 – 2.48 (4H, m), 3.75, 3.83, 3.93 (3H each, s), 5.50 (1H, s), 6.34 (1H, s) and 6.63 (1H, s).	194.3 (C_1-s), 52.8 (C_2-t), 30.3 (C_3-s), 52.8 (C_4-d), 194.4 (C_5-s), 250.9 (C_6-s), 146.0 (C_7-d), 135.3 (C_8-s), 126.5 (C_9-d), 136.5 (C_{10}-s), 135.1 (C_{11}-s), 136.5 (C_{12}-s), 123.3 (C_{13}-d), 26.5 (C_{14}-q), 26.6 (C_{15}-q), 22.5 (C_{16}-q), 18.8 (C_{17}-q), 22.1 (C_{18}-q).
67	1.09 (3H, s), 1.21 (3H, s), 2.32 – 2.46 (4H, m), 5.66 (1H, s), 7.02 (2H, d, J = 9 Hz) and 7.20 (2H, d, J = 9 Hz)	194.3 (C_1-s), 52.8 (C_2-t), 30.5 (C_3-s), 52.7 (C_4-d), 194.3 (C_5-s), 140.8 (C_6-s), 146.1 (C_7-d), 132.9 (C_8-s), 127.8 (C_9-d), 128.6 (C_{10}-d), 133.2 (C_{11}-s), 128.5 (C_{12}-d), 127.5 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q)
68	1.09 (3H, s), 1.22 (3H, s), 2.32-2.41 (4H, s), 3.76 (3H, s), 5.48 (1H, s), 6.80 (2H, d, J = 9 Hz), 6.99 (2H, d, J = 9 Hz)	194.4 (C_1-s), 52.9 (C_2-t), 30.4 (C_3-s), 52.9 (C_4-d), 194.38 (C_5-s), 140.8 (C_6-s), 145.9 (C_7-d), 127.6 (C_8-s), 127.4 (C_9-d), 114.5 (C_{10}-d), 159.9 (C_{11}-s), 113.9 (C_{12}-d), 127.5 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q)
69	1.13 (3H, s), 1.25 (3H, s), 2.0-2.21 (4H, m), 5.56 (1H, s) and 7.44-8.23 (4H, m)	194.2 (C_1-s), 52.8 (C_2-t), 30.2 (C_3-s), 52.5 (C_4-d), 194.0 (C_5-s), 141.2 (C_6-s), 145.9 (C_7-d), 124.7 (C_8-s), 127.1 (C_9-d), 113.9 (C_{10}-d), 148.5 (C_{11}-s), 114.2 (C_{12}-d), 127.1 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q), 40.2 (q, N-CH_3), 40.3 (q, N-CH_3)
70	1.09 (6H, s), 2.37-2.56 (4H, m), 2.91 (3H, s), 3.01 (3H, s), 6.64 (24, d, J = 9 Hz), 6.26 (2H, d, J = 9 Hz), 6.02 (1H, s)	194.2 (C_1-s), 52.8 (C_2-t), 30.2 (C_3-s), 52.7 (C_4-d), 194.4 (C_5-s), 140.5 (C_6-s), 145.2 (C_7-d), 135.8 (C_8-s), 132.2 (C_9-d), 129.8 (C_{10}-d), 122.8 (C_{11}-d), 147.2 (C_{12}-s), 119.8 (C_{13}-d), 26.5 (C_{14}-q), 26.4 (C_{15}-q)

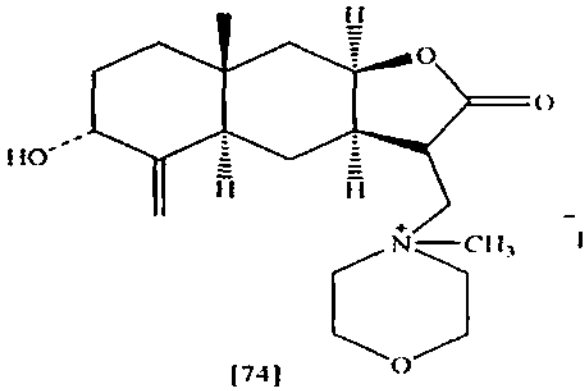
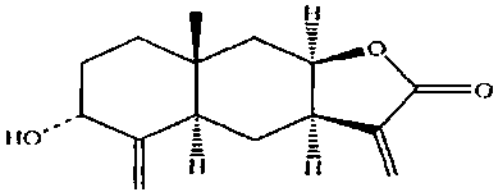
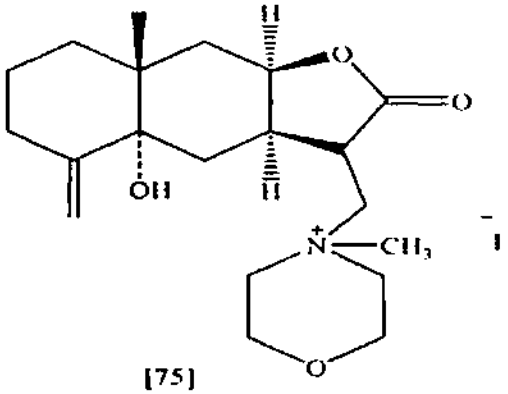
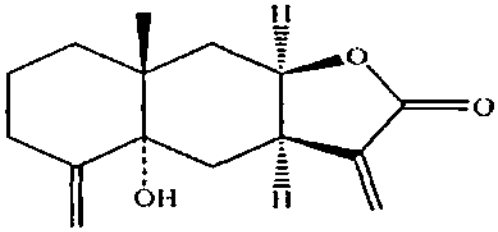
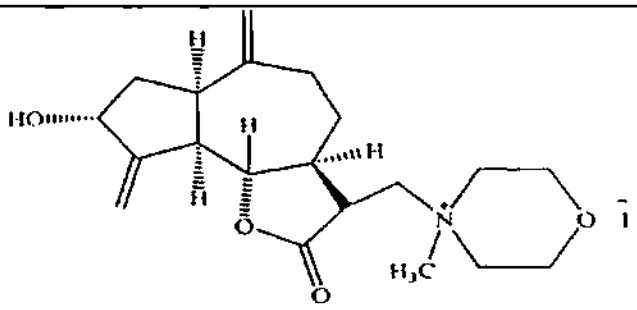
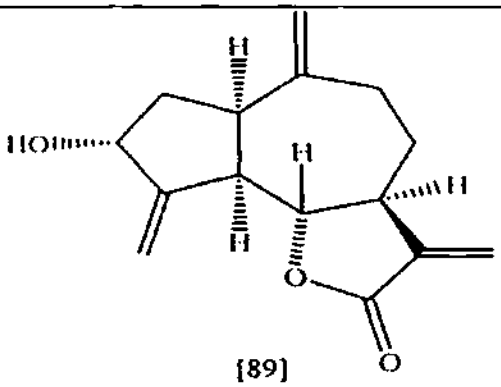
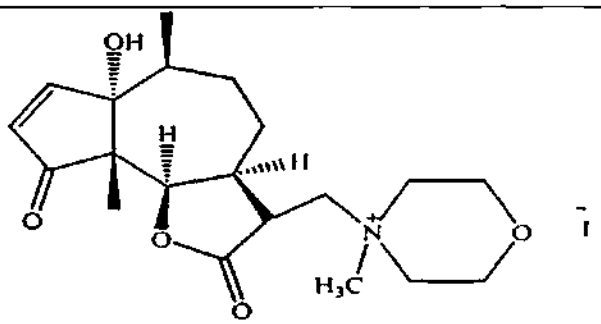
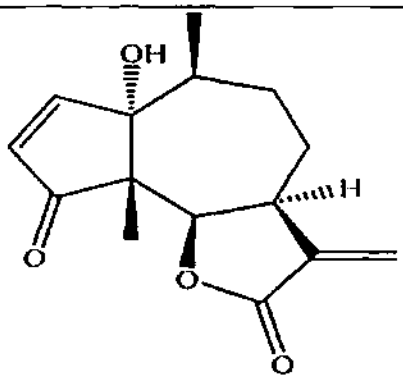
Microwave-assisted Hoffman elimination of quaternary ammonium salts

Usually, the quaternary ammonium salts of sesquiterpene lactones are decomposed into the parent lactone by treating with sodium hydrogen carbonate for 24 hours (Srivastava *et al* 1970). The reaction is very useful for the isolation and purification of these lactones with α-methylene-γ-lactone moiety having great structural and biological significance. In extending on sesquiterpene lactones, it became essential to generate this functionality from the Michael amine-adducts under mild conditions. For the above stated decomposition reaction conditions, atleast 24 hrs were required for the elimination to occur and the reaction failed in case of compounds with base labile groups. To avoid these hindrances, a method was devised involving the decomposition of morpholide adducts of sesquiterpene lactones on solid matrix under microwave irradiation. The method proved to be solvent free and environmentally benign.

In a typical procedure, the morpholide adduct was adsorbed on basic alumina and irradiated with microwave at 800 W for 5 minutes. Usual workup furnished the original sesquiterpene lactone in nearly quantitative yield. It is worthwhile to mention that under these conditions base labile groups, epoxides, aldehydes, esters etc. remained intact proving it to be a method of choice for the isolation and purification of sesquiterpene lactones having α-methylene-γ-butyro lactone moiety as far as reaction time, ease of workup and environmental impact is concerned. Table 8 represents the quaternary ammonium salts employed for the reaction and the parent lactones obtained along with the per cent yields of the products.

Table 8: Structures of quaternary ammonium salts and their elimination products along with per cent yields

Quaternary ammonium salt	Parent compound	% Yield
 [71]	 [1]	90
 [72]	 [2]	88
 [73]	 [3]	85

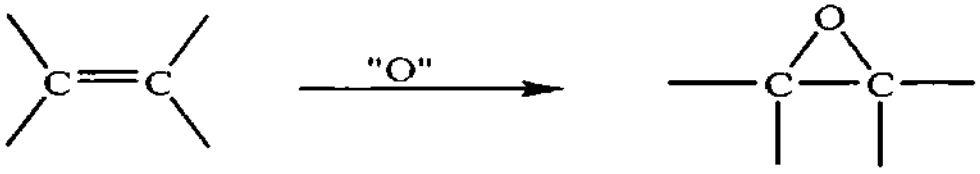
 [74]	 [93]	**82**
 [75]	 [92]	**83**
 [76]	 [89]	**88**
 [77]	 [8]	**92**

Oxidation reactions

Oxidation reactions are very important processes for biological systems and in organic chemistry. There are numerous oxidation reagents for organic compounds and new ones are added to this list almost everyday. It is well known that different organic substrates can be converted into varied oxidation products depending on the type of oxidant used. For instance, $Zn(ClCrO_3)_2.9H_2O$ (ZCC) is an oxidant which can be used under very mild conditions (Firouzabadi and Sharifi 1992). Pyridinium chlorochromate (PCC) will oxidize a primary alcohol to an aldehyde and stop at that stage (Corey and Suggs 1975, Solomons 1992). PCC also does not attack double bonds. *m*-Chloroperbenzoic acid (*m*-CPBA) is another useful reagent that attacks electron rich substrates such as simple alkenes, alkenes carrying functional groups such as ethers, alcohols, esters, ketones and amides. This results in an oxygen atom transfer to the substrate (Jensen and Slobodzian 2000, Meijer and Hogt 2006).

Epoxidations

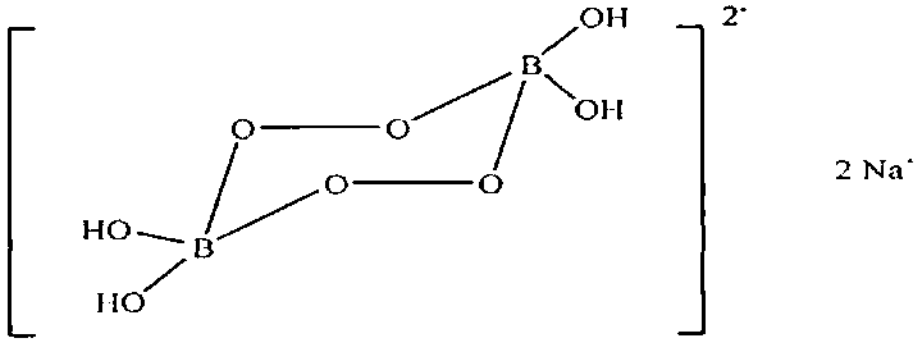
Alkenes, either derived from natural resources or generated as products of the chemical industry, are found in great abundance in the realm of organic molecules. One of the most useful transformations of the carbon-carbon double bond is epoxidation (Scheme V). By epoxidation, two adjacent carbon atoms are functionalized while either of these two adjacent carbons is activated towards nucleophilic attack, which makes the resulting epoxides useful and versatile intermediaries in organic chemistry. Furthermore, in asymmetric synthesis the epoxidation reaction is attractive since it can produce two chiral carbons in one step.



Due to increasing environmental demands, the use of classical stoichiometric oxidants for achieving this reaction is no longer optional. In

order to make the process cleaner, safer and more efficient, the development of new reagents for epoxidation has become mandatory.

Sodium perborate is a versatile reagent for functional group oxidations (Mckillop and Sanderson 2000). It is a cyclic disubstituted peroxide. In water, or in solvents with a significant aqueous component, this persalt functions mainly as a convenient source of mildly alkaline hydrogen peroxide, the borate helping somewhat to buffer, stabilize against decomposition and activate towards nucleophilic oxidations, through associated species such as $[B(OH)_3OOH]^-$. Its properties in aqueous solution are practically similar to those of a hydrogen peroxide solution.



[78]

Accordingly, sodium perborate is considered as a solid form of hydrogen peroxide used as a strong oxidizing agent in various industries. In comparison with the solution of hydrogen peroxide, solid form of sodium perborate compounds provide better conditions of stability and convenient handling.

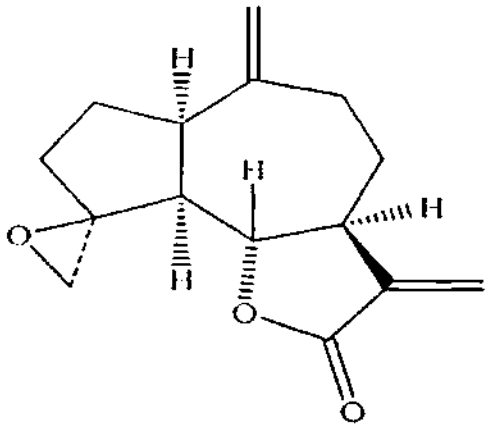
This cyclic peroxide only dissolves significantly in those non-aqueous media with which it reacts. It is often used in acetic acid or other carboxylic acids.

Sodium perborate finds a wide range of applications in oxidant chemistry including sulfide oxidations (Bower *et al* 1995), oxidation of amines, phenols, alcohols, aldehydes, ketones, nitriles, organoboranes, olefins, C-C cleavage and aromatic side chains (Hou and Tao 1996). Of these, epoxidation of olefins holds great significance since epoxidation has

long been an important synthetic transformation in synthetic chemistry. The importance of this reaction lies in the fact that it leads to versatile products that are of great relevance in both the pharmaceutical industry and academic laboratories.

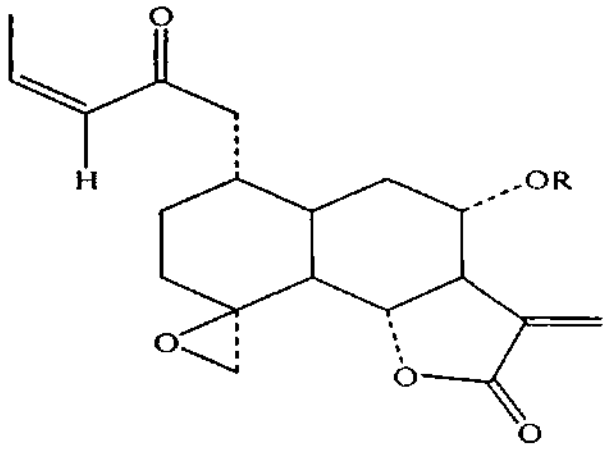
Thus, the wide range of applications of epoxidation reactions and the relevance of sodium perborate towards this reaction inspired as to attempt the epoxidation of some sesquiterpene lactones with this reagent.

For this, sodium perborate was dissolved in isopropanol: H_2O (3:1) and this was made to react with dehydrocostus lactone under stirring. Continuous monitoring of the reaction mixture by TLC revealed that after one and a half hour a two component mixture was furnished (TLC) that was subjected to CC in order to obtain the epoxide (79).

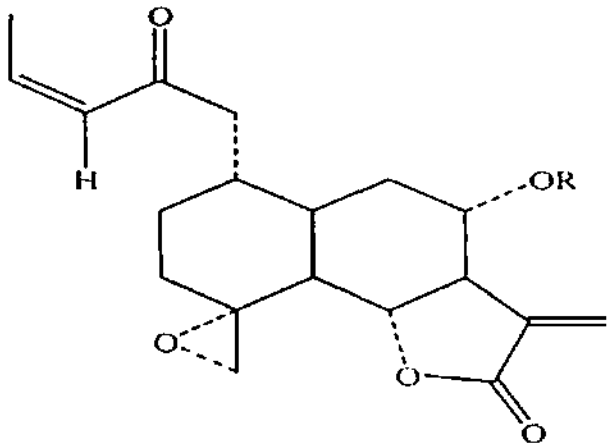


[79]

The stereochemistry of the epoxide (79) was ascertained from the chemical shift differences of the epoxy protons. A survey of literature revealed that the compound (80) showed the epoxy protons as two doublets at δ 3.43 and 2.75 ($\Delta\delta$ = 0.68) with a coupling constant of 5 Hz while the corresponding α-epoxide (81) showed epoxy signals at δ 2.58 and 2.67 ($\Delta\delta$ = 0.19).



[80]



[81]

The comparison of this data with that of epoxide (79) showed that the chemical shift difference ($\Delta\delta$) of epoxy hydrogens exhibited by (79) is 0.66 (δ 3.58 – 2.92) suggesting the β-sterochemistry for the epoxide in this compound.

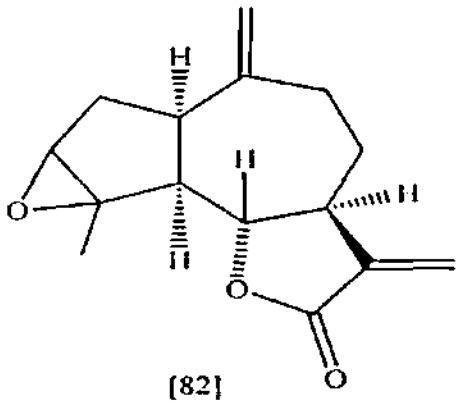
In order to select a more suitable solvent to enhance the yield or to alter the stereochemistry of the epoxide, dehydrocostus lactone was treated with sodium perborate using solvent system comprising acetic anhydride and dichloromethane in different proportions *viz.* (3:1, 1:1, 1:3, 9:1 and 1:9). Acetic anhydride and dichloromethane when used in the proportion of 9:1 proved to be best suited for the reaction affording a single epoxidised product. The mixture of the epoxide and unreacted dehydrocostus lactone was resolved by CC and the product was characterized spectrometric analysis and comparison of data with that of the already prepared sample.

As the chemical industry strives to improve process efficiency, safety and reduce environmental impact, Phase Transfer Catalysis (PTC) has become recognized as a useful tool to achieve these goals.The PTC methodology involves a substrate (which is soluble in the organic layer) and an anionic reagent (often a nucleophile), which is dissolved in the aqueous layer. The substrate and the anion are then brought together by a catalyst, which transports the anion into the organic phase where reaction can take place with the substrate.

Quaternary ammonium and phosponium salts with their unique capability to dissolve in both aqueous and organic liquids are the catalysts of choice for most phase transfer applications. The ammonium derivatives are the most commonly used, but the phosphonium based phase transfer catalysts offer other interesting properties as well, like higher thermal stability. Other phase transfer catalysts include crown ethers and polyethylene glycols (PEG).

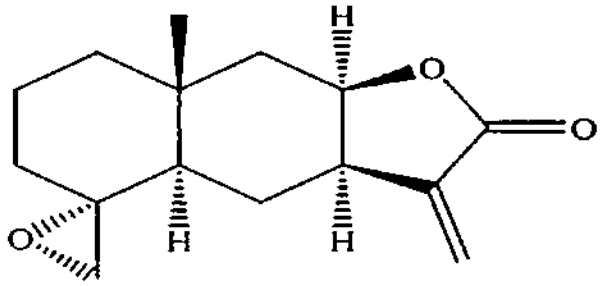
In another attempt to devise the optimum reaction conditions for epoxidation with sodium perborate, it was thought worthwhile to employ a phase transfer catalyst for the purpose. In this case, dehydrocostus lactone was dissolved in dichloromethane and a solution of sodium perborate was prepared in distilled water. Mixture of the two was stirred for 6 hours in the presence of catalytic amount of cetyl trimethyl ammonium bromide. Usual workup afforded a product which on spectral analysis proved to be the epoxy derivative of dehydrocostus lactone. However, the reaction gave fairly good results in terms of yield and reaction time ut were not comparable to the ones discussed above.

A similar reaction was attempted for isodehydrocostus lactone (12) in the solvent system acetic anhydride: dichloromethane (9:1) when a single compound (82) was obtained. It showed ^{1}HNMR signals (90 MHz, $CDCl_3$) at δ 1.25 (3H, s, C_{14}-H), 1.6 (3H, s, C_{15}-H), 3.1-3.5 (3H, m, C_3-H), 3.95 (1H, t, J= 9 Hz, C_6-H), 5.2 (1H, d, J= 2 Hz, C_{13}-H), 5.9 (1H, d, J= 2 Hz, C_{13}-H) proving it to be the epoxy derivative of isodehydrocostus lactone.



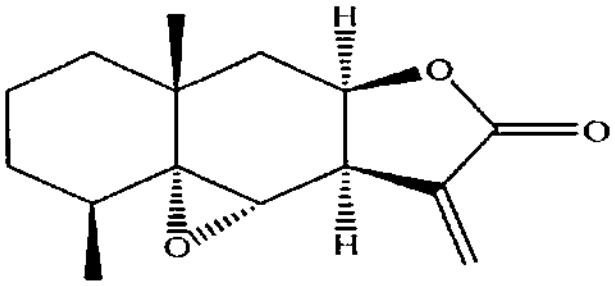
[82]

Epoxidation reaction with sodium perborate was also performed for isoalantolactone (3) using acetic anhydride-dichloromethane (9:1) as the solvent system. Usual work up afforded pure compound (83) in 82% yield. The IR spectrum showed signals at 1765, 1645, 1470, 1272, 942, 812 cm^{-1}. ^{1}HNMR spectrum exhibited all the signals shown by isoalantolactone with epoxy protons as a doublet at δ 2.80 due to C_{15}-Hs. The epoxidation at $\Delta^{4,15}$ was also supported by the fact that the IR and NMR spectra were found to be superimposable with those of the authentic samples prepared by the reaction of isoalantolactone with PBA.



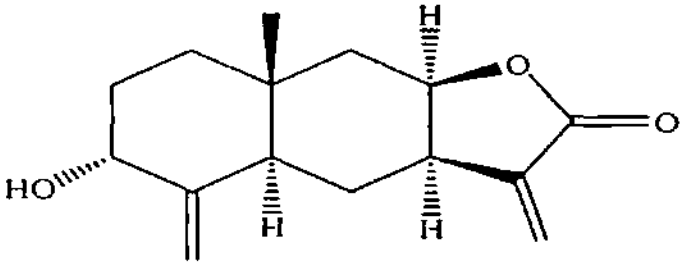
[83]

Similar reaction was carried out with alantolactone (2), when the corresponding epoxide (84) was formed. Compound (84) was identified on the basis of the spectral and chemical correlation method. IR signals were observed at 1760 (for lactone moiety, 1660, 1460, 1140 (C-O stretch) and 890 cm^{-1} (C=C bend). In ^{1}H NMR spectrum, a doublet at δ 1.04 was observed due to C_4-CH_3, a singlet at δ 1.10 due to C_{10}-CH_3, C_6-H was observed as a multiplet at δ 4.52 as a typical signal for the lactone and two doublets at δ 5.50 and 6.23 confirmed the presence of exomethylene group. Further, the chemical shift and shape of signal of C_6-H at δ 2.89 (d, 1H) suggested the compound to be 5α, 6α - epoxyalantolactone.

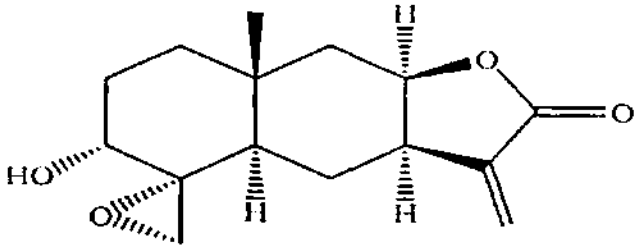


[84]

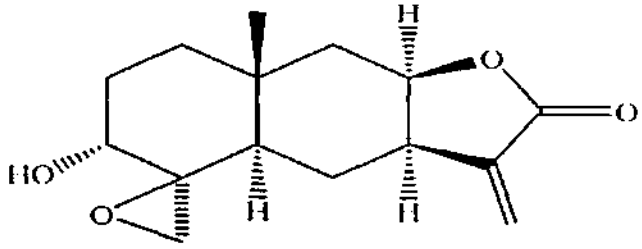
In another attempt, isotelekin (93) was treated with sodium perborate leading to the formation of two isomeric epoxides, which were identified as (85) and (86) on the basis of spectroscopic data. ^{1}H NMR spectrum of (85) showed signals at δ 2.79 and 2.62 (d, 1H each) due to C_{15}-Hs, whereas ^{1}H NMR spectrum of (86) showed only a broad singlet due to C_{15}-Hs at 2.74.



[93]



[85]

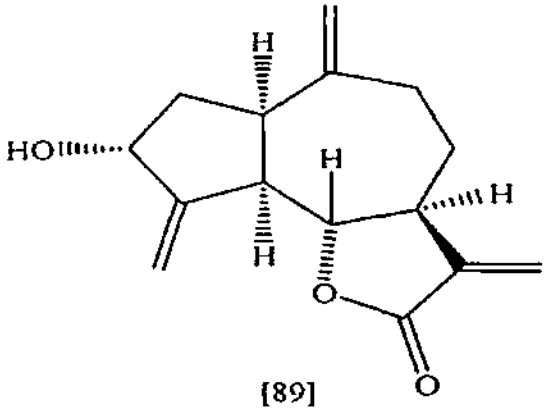


[86]

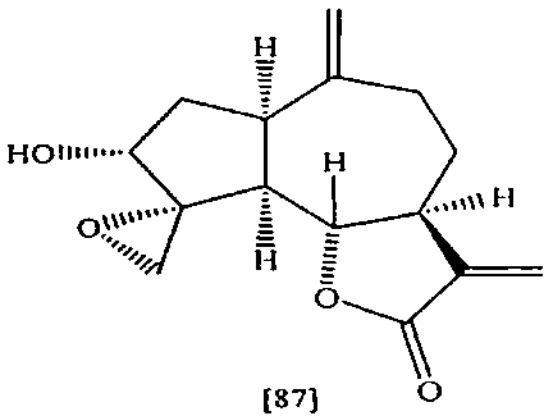
In order to confirm the stereochemistry of the epoxides formed in the above case, isotelekin (93) was treated with $VO(acac)_2$/TBHP, a

stereoselective reagent leading to the formation of epoxide having same stereochemistry as that of hydroxyl group, to yield a compound (85).

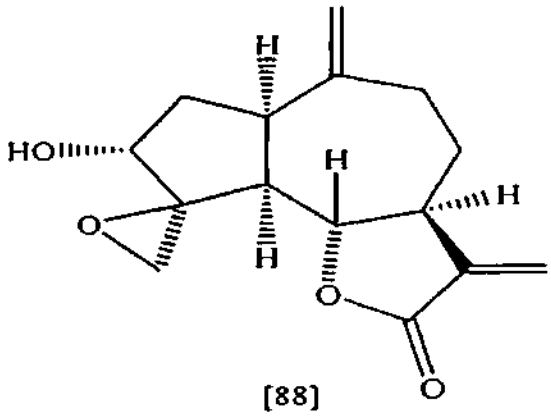
In a similar manner, isozaluzanin-C (89) on reaction with sodium perborate resulted in the formation of mixture of two isomers (87) and (88) with one as the major compound (85, TLC). The epoxide showed IR signals at 1760 cm^{-1} confirming the presence of intact lactone. Among the ^{1}HNMR signals, two doublets at δ 2.80 and 3.00 were obtained for C_{15}-Hs. In comparison to this, in the other isomer, the corresponding ^{1}HNMR signals due to C_{15}-Hs were observed at δ 3.40 and 2.71. The larger chemical shift difference is attributable to *syn* – orientation of epoxide and hydroxyl group. These findings together with supporting literature data, led to the assignment of stereostructures to (87) and (88).



[89]



[87]



[88]

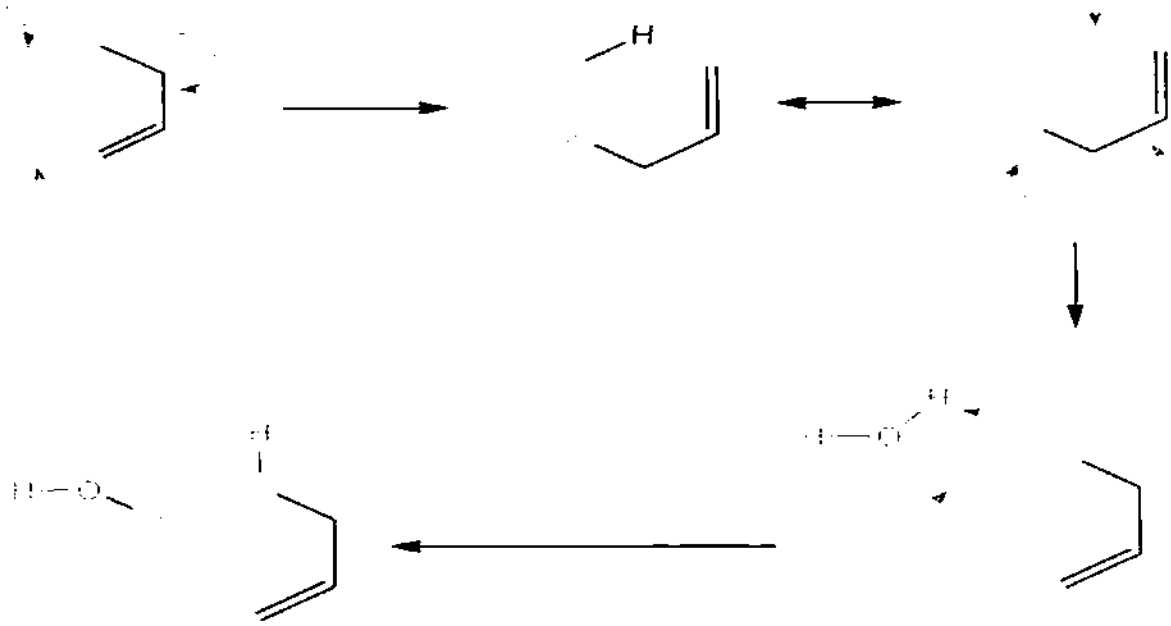
In order to further confirm these observations, isozaluzanin-C was further reacted with $VO(acac)_2$/TBHP which resulted in formation of a compound that showed IR and ^1H NMR identical to (87), thereby confirming the α–stereochemistry of the epoxide. Theses findings are indicative of the fact that the oxirane oxygen with the reagent of placed from the same side on which hydroxyl group is present.

Allylic oxidations

Allylic oxidation is a reaction of fundamental importance in organic chemistry with applications ranging from agricultural products to pharmaceuticals (Salvador and Clark 2001). It is an organic oxidation converting an allylic methylene group into an allylic alcohol or a carbonyl. This chemical transformation is also important from mechanistic point of view.

The allylic oxidation of unsaturated natural compounds such as Δ^5-steroids has traditionally been carried out with chromium reagents such as CrO_3-pyridine complex (Fullerton and Chen 1976), chromium trioxide and 3,5-dimethyl pyrazole (Salmond *et al* 1978), pyridinium chlorochromate and sodium dichromate in acetic acid. However, the large excess of reagent used in these procedures along with a difficult workup and production of environmentally hazardous chromium residues, makes these reactions increasingly unacceptable on a commercial scale.

Selenium dioxide is one representative of a group of oxidizing agents that can bring about the allylic oxidation with greater ease.



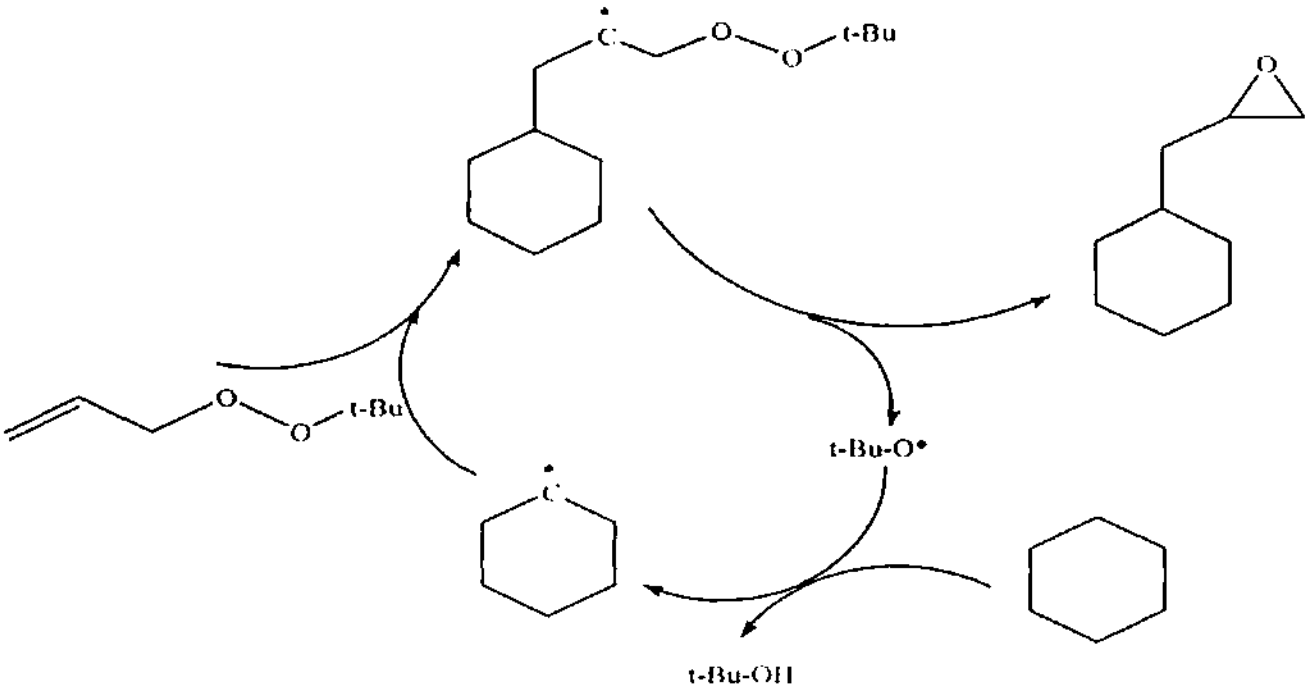
SCHEME IV

This type of reaction involves free radicals but in some cases a pericyclic concerted reaction mechanism is found for selenium oxide oxidations. The first step is an ene reaction, transferring the allylic proton to the selenium oxide, and the second step is a [2,3] sigmatropic reaction.

Selenium dioxide also introduces oxygen into a hydrocarbon molecule selectively and without structural rearrangement (Stephenson and Speth 1979). Though selenium dioxide is the most reliable reagent for the direct insertion of oxygen into an allylic carbon-hydrogen bond, formation of organo selenium compounds as byproducts of the reaction, high reaction temperature and limited use of solvents restricts its use for allylic oxidation.

t-Butyl hydroperoxide (TBHP) is another reagent of great significance in the oxidation reactions. TBHP is widely used as an oxidizing agent, also at large industrial scale. It is used to affect regiospecific, and asymmetric oxidations. Polar solvents slow down the rates of oxidation using TBHP, while higher alkyl substitution of alkenes increases the oxidation rates. It is used in oxidations of various substrates to give epoxides, ketones, aldehydes, sulfoxides, phosphinooxide and N-oxides. The major examples are the

Sharpless asymmetric epoxidation and oxidations in the presence of transition metals.



SCHEME V

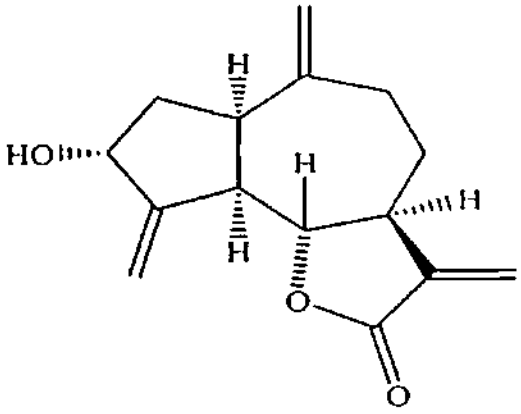
Seeing the utility of TBHP in oxidation reactions and need to circumvent the drawbacks of selenium dioxide alone as an oxidizing agent, a combination of the two has proved to be an ideal reagent for carrying out allylic oxidations (Umbreit and Sharpless 1977). Moreover, products of allylic oxidation using selenium dioxide in combination with TBHP are formed in higher yields than those obtained with stoichiometric selenium dioxide alone.

Several oxidations of olefins have been reported by various workers by using selenium dioxide in combination with TBHP (Sharpless and Verhoeven 1979) and SeO_2/TBHP coupled with silica gel (Chhabra *et al* 1981, Singh *et al* 1997, Singh *et al* 1998). A combination of the two reagents in dichloromethane has been reported to cause selective oxidation of primary allylic alcohols to the corresponding α, β-unsaturated aldehydes where the secondary allylic, benzylic and saturated alcohols remain unreacted (Kalsi *et*

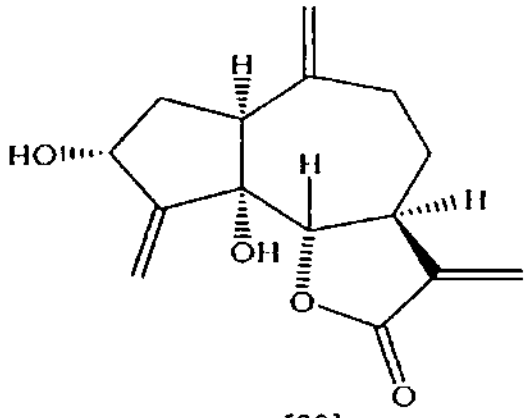
al 1992). This system is more selective than any known selenium dioxide mediated oxidation procedure.

During the present investigation, allylic oxidation of some sesquiterpene lactones with SeO_2/TBHP has been carried out by conventional and non-conventional methologies and the rates and per cent yields of both types of reactions have been compared.

Dehydrocostus lactone (1) was reacted with selenium dioxide in combination with TBHP in dichloromethane for 8 hours. In dehydrocostus lactone, several points of attack are available. However, the reaction under mild and controlled conditions afforded a compound (89, mp 143°C, 42%) which on comparison of spectrometric data with authentic sample proved to be identical with the naturally occurring isozaluzanin C. Further reaction furnished another compound, a diol (90, 23%) whose ^1H NMR and ^{13}C NMR revealed that oxidation had occurred at both C_3 and C_5 positions.



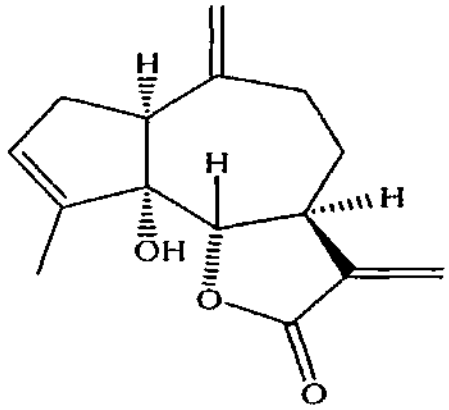
[89]



[90]

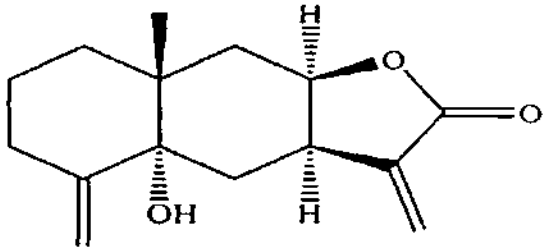
This reaction was then attempted under microwave irradiated conditions. Initial screening by TLC spotting technique revealed higher success rates as far as the reaction time is concerned. The reaction was then scaled up to gram level by adsorbing a solution of SeO_2/TBHP in dichloromethane onto silica gel along with the starting compound. An irradiation time of 7 minutes led to the completion of reaction with yields of products obtained on work up and CC, to be comparable to those obtained by the conventional method.

In case of isodehydrocostus lactone, the treatment with SeO_2/TBHP for 10 hours afforded a single hydroxyl derivative (91). When analysed for the ^1H NMR spectrum, both the compounds showed all the characteristic peaks of isodehydrocostus lactone along with the presence of one and two hydroxyl groups respectively showing that oxidation had occurred in a similar fashion as in case of dehydrocostus lactone (Table 10).

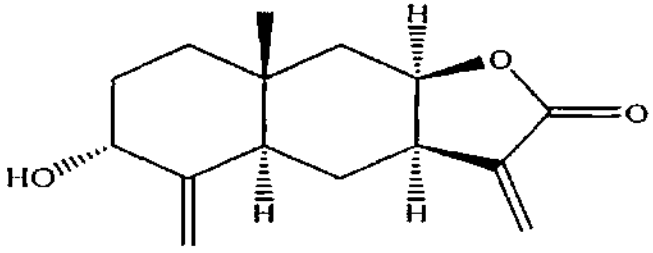


[91]

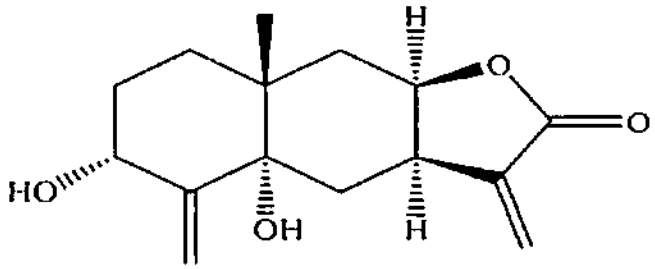
When a similar reaction was carried out with isoalantolactone, a reaction time of 6 hours with selenium dioxide and TBHP in dichloromethane followed by CC afforded 3 products. Comparison of the spectral data of these compounds with authentic samples proved their structures to be (92, 93 and 94).



[92]



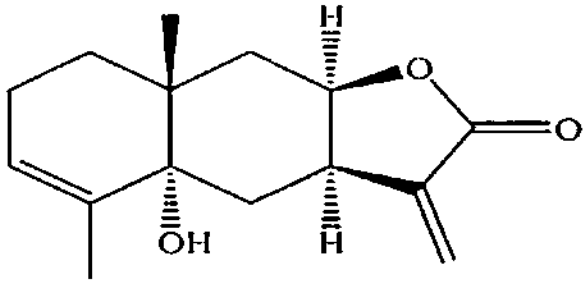
[93]



[94]

The same reaction when carried out under the microwave irradiation gave similar results with reaction time as low as 8 minutes.

Oxidation of the isomeric isoalantolactone afforded the respective alcohol (95). These reactions were also carried out under the influence of microwaves (Table 9).



[95]

Table 9: Comparison of reaction time for allylic oxidation by conventional and microwave methods

S. No.	Reactant No.	Product No.	Reaction time	
			Conventional method	Microwave method
1.	[1]	[89], [90]	8 h	7 min
2.	[12]	[91]	10 h	5 min
3.	[3]	[92], [93], [94]	6 h	8 min
4.	[13]	[95]	8 h	6 min

Table 10: Spectral data of products obtained by allylic oxidation of double bond isomers of dehydrocostus lactone and isoalantolactone

Compound No.	^{1}HNMR δ (ppm)	^{13}CNMR δ (ppm)
91	6.22 (1H, d, J = 3.2 Hz, C_{13}-H_a), 5.50 (14, d, J = 3.2 Hz, C_{13}-H_b), 4.90 (1H, brs, C_{14}-H_a), 4.82 (1H, brs, C_{14}-H_b), 5.3 (3H, m, C_3-H), 1.93 (3H, s, C_{15}-H), 4.09 (1H, t, J = 8 Hz, C_6-H).	40.04 (C_1), 30.24 (C_2), 125.0 (C_3-d), 145.3 (C_4-5), 90.0 (C_5-d), 87.5 (C_6-d), 42.0 (C_7-d), 36.0 (C_9-t), 148.5 (C_{10}-s), 32.5 (C_{11}-s), 171.2 (C_{12}-s), 120.5 (C_{13}-t), 109.2 (C_{14}-t), 14.8 (C_{15}-q).
95	2.95 (1H, m, C_7-H), 4.1 (1H, m, C_8-H), 5.40, 6.25 (1H each, br s, C_{13}-H), 1.08 (3H, s, C_{14}-H), 1.71 (3H, s, C_4-Me), 5.37 (3H, s, C_3-Me)	30.2 (C_1-t), 22.1 (C_2-t), 123.1 (C_3-d), 135.2 (C_4-s), 72.9 (C_5-d), 36.8 (C_6-t), 39.1 (C_7-d), 76.5 (C_8-d), 46.8 (C_9-t), 36.5 (C_{10}-s), 138.5 (C_{11}-s), 169.8 (C_{12}-s), 124.1 (C_{13}-t), 16.8 (C_{14}-q), 15.5 (C_{15}-q).

Reduction reactions

Reduction is one of the frequently used reactions in organic synthesis and a vast variety of reducing agents have been introduced for this achievement. Several powerful and mild reducing agents have been developed to introduce selectivity in the reduction of functional groups. Most commonly used reagents in synthetic organic laboratories are lithium aluminium hydride and sodium borohyride. Lithium aluminium hydride is an exceedingly powerful reducing agent capable of reducing practically all organic functional groups. Consequently, it is quite difficult to apply this reagent for the selective reduction of multifunctional molecules. On the other hand, sodium borohydride is relatively a mild reducing agent, primarily used for the reduction of reactive functional groups in protic solvents. Consequently, the rate of reductions is sometimes slow and a relatively low chemoselectivity is accompanied with the reactions.

In order to control the reducing power of sodium borohydride, hundreds of substituted boron hydrides have been reported in chemical literature and many of them are commercially available now. The rapid development in this field has been realized by:

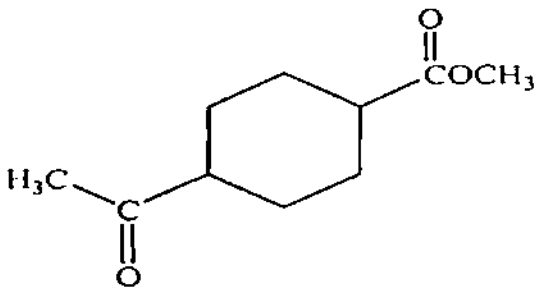
i) substitution of the hydride(s) with other groups which may exert marked steric and electronic influences upon the reactivity of the substituted complex ion (Rao 1991),
ii) variation in the alkali metal cation and metal cation in the complex hydride (Narsimhan and Balakumar 1998),
iii) use of ligands to alter behaviour of the metal hydrides (Zeynizadeh and Zahmatkesh 2004),
iv) combination of borohydrides with metal, metal salts, lewis acids, mixed solvent systems or some other agents (Zeynizadeh and Behyar 2005, Constantino *et al* 1998, Ganem and Osby 1986).

On the other hand, the economical demands and the existing state of environment have generated a need for paradigm shift to perform chemical

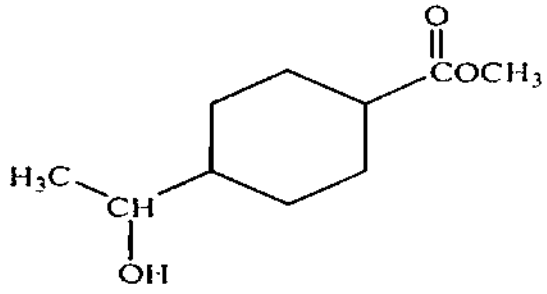
reactions by using ecologically safe reagents or media; and not to forget – organic reaction carried out in dry media have recently received careful attention with several advantages over the solution reactions.

Literature review shows that though the reduction of carbonyl compounds has been an important synthetic reaction, this transformation under solvent free conditions has been rarely investigated. Parthenin possesses two conjugated double bonds; one in conjugation with the lactone and the other with the carbonyl group. Therefore, to expand the above mentioned strategies and in continuous efforts to use microwave radiations to mediate chemical transformations, reduction reactions of parthenin with several reducing agents have been attempted and a comparative study of the reactions by conventional and microwave methods has been carried out.

Sodium tetrahydridoborate ($NaBH_4$) is the preferred reducing agent for chemoselective reductions of aldehydes and ketones. It can be easily modified to form either a stronger or more selective reducing agent (Periasamy and Thirumalaikumar 2000). It is a mild reducing agent that is relatively safe to handle. Furthermore, sodium borohydride reacts so slowly with hydroxylic solvents that reduction with it may be conducted in alcoholic solvents. Because of its greater selectivity, it is capable of reducing aldehydes or ketones that contain other functional groups. For example, keto ester (96) is reduced to the corresponding hydroxy ester (97) by sodium borohydride (Basappa *et al* 2005).

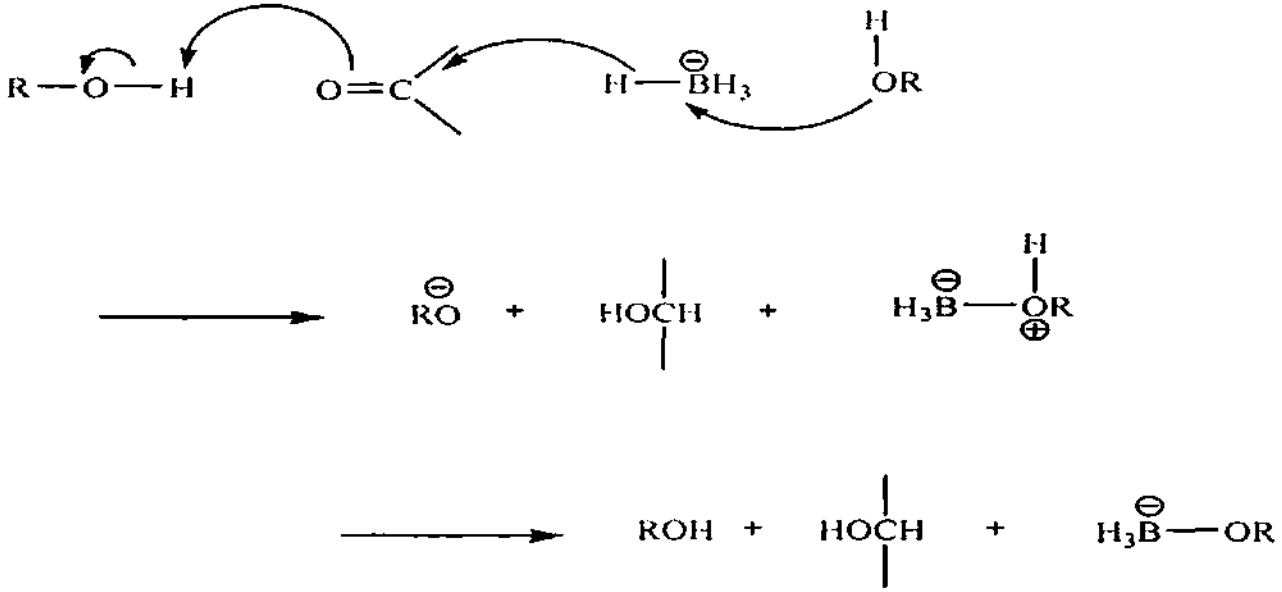


[96]



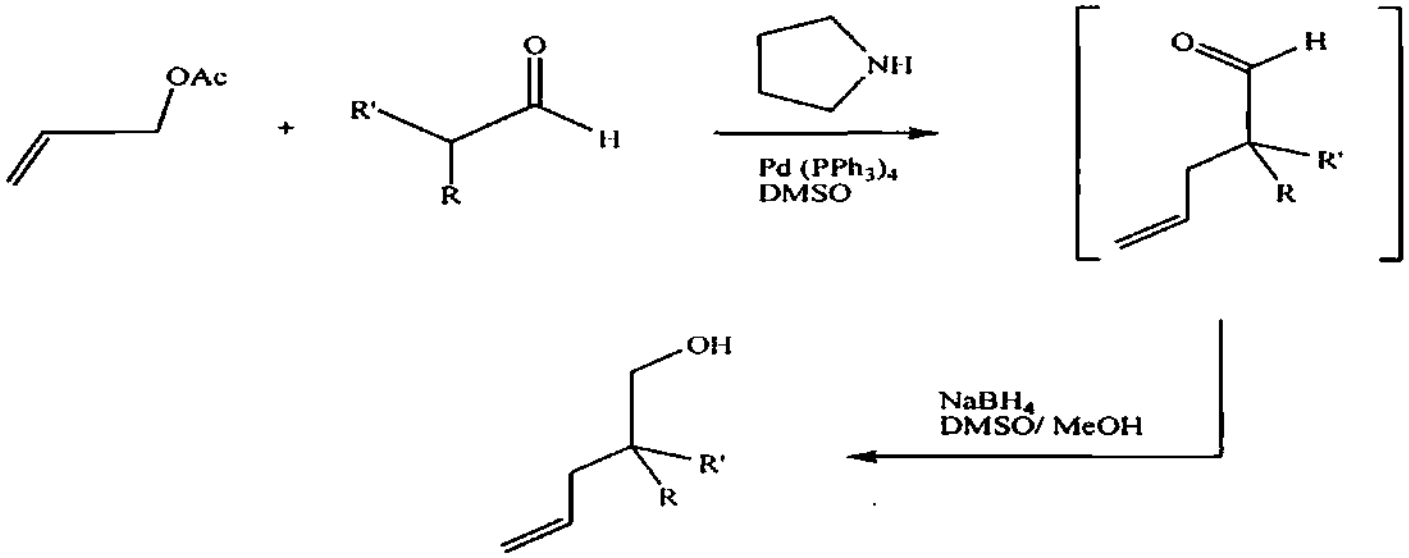
[97]

With regard to mechanism, sodium borohydride reductions can be considered as nucleophilic additions of hydride ion (H^-) to the carbonyl function, even though no free hydride ions are believed to be present. Experimental evidence indicates that a solvent molecule bonds to the boron atom as it transfers a hydride to the carbonyl carbon, while another solvent molecule transfers a proton to the carbonyl oxygen. All four hyrides on the boron are usable (Scheme VI).

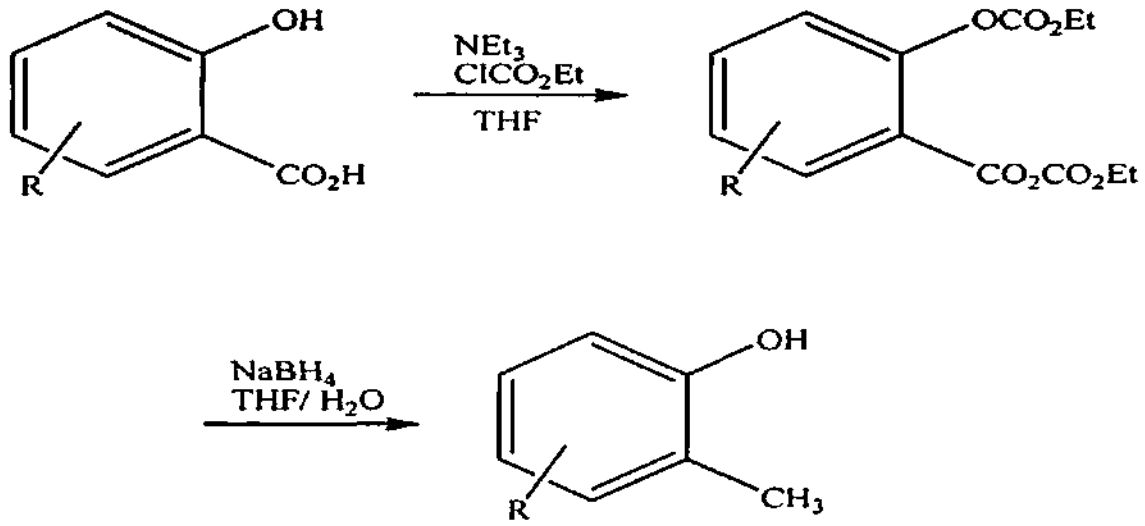


SCHEME VI

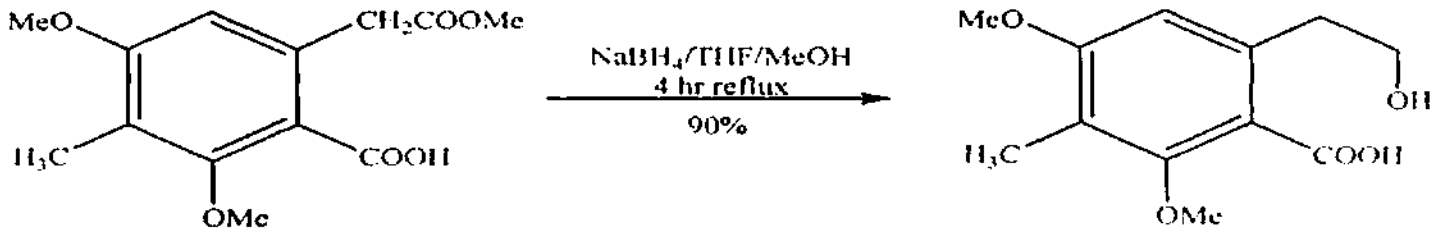
Several reduction reactions have been successfully achieved using sodium borohydride. It has been used in combination with palladium and enamine catalysts to direct allylic alkylation of aldehydes and ketones (Ibrahem and Cordova 2006).



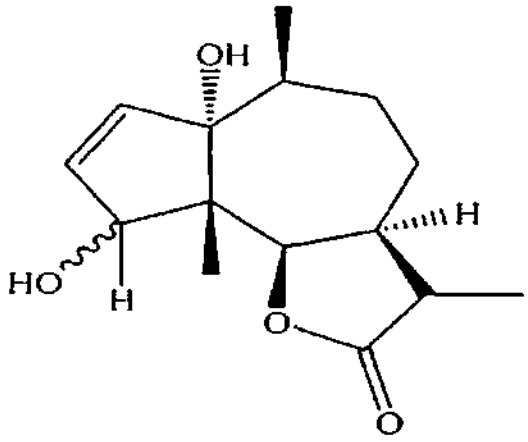
Salicylic acids and alcohols have been reduced to 2-methyl phenols by simple two step procedure (Mazzini and Salvadori 2005).



Sodium borohydride–THF–methanol system has been successfully applied for the reduction of methyl esters involving important methoxy acids (Saeed and Ashraf 2006).

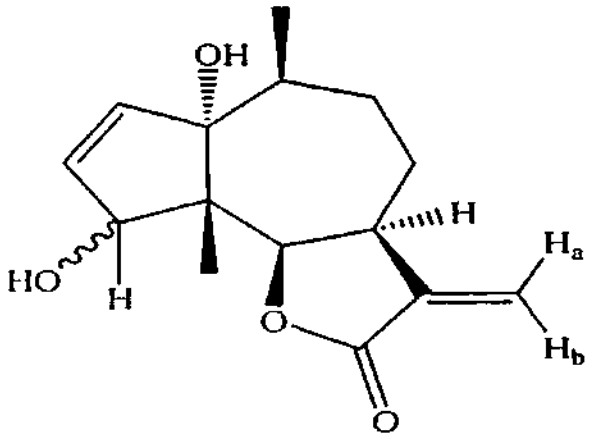


The reaction of parthenin with sodium borohydirde afforded a mixture of two products which were identified by their spectral data. According to the ^{1}H NMR spectrum, one of the products showed a doublet at δ 3.75 with a coupling constant of 3.5 Hz. A multiplet was seen due to two hydrogen atoms at δ 5.1. Other signals included a doublet at 1.2 with J = 7.2 Hz, another doublet at δ 1.11 with J = 7.56 Hz, a singlet δ 1.27 and a doublet at 5.03 with coupling constant 7.83 Hz. In accordance with this spectral data, the proposed structure of this product is (98).



[98]

Similar spectrum was obtained for the other product as well, with the only difference being that in place of a doublet at δ 1.2, two doublets were obtained at δ 5.62 and δ 6.26. Also the multiplet at δ 5.1 was replaced by another multiplet due to exchangeable hydrogen at δ 3.54. Accordingly the proposed structure of the second product is (99).



[99]

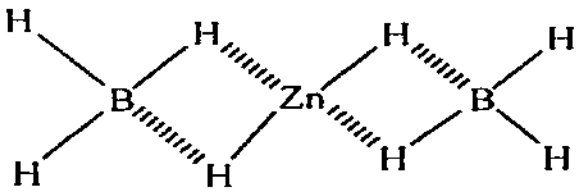
Although numerous literature references are available on the synthetic applications of various metal borohydrides, only sodium borohydride has gained commercial status, inspite of its poor solubility in organic solvents and lesser reactivity. Moreover, the reagent is inevitably used in excess quantities. To overcome these drawbacks, soluble metal borohydrides such as lithium borohydride, calcium borohydride and zinc borohydride have been developed. Among these reagents zinc borohydride is unique because:

i) Zn^{2+} is a soft lewis acid as compared to Ca^{2+}, Li^{+} and Na^{+} which are hard acids, and

ii) ii) Zn^{2+} has a better coordinating ability and is thus expected to impart selectivity in hydride transfer reactions.

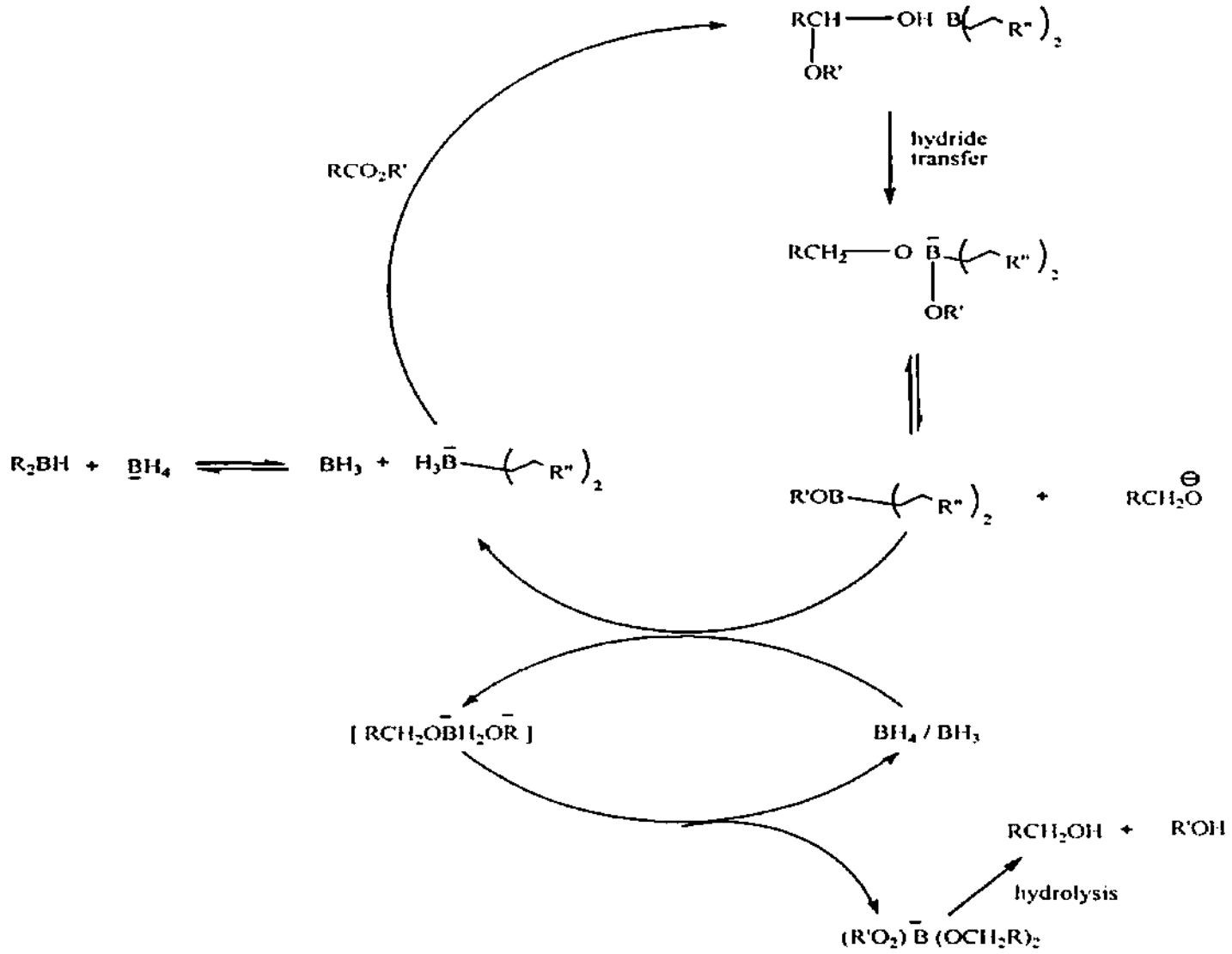
The supported-reagent technique has attracted considerable interest in recent years. Silica gel supported zinc borohydride has been employed for the region- and stereoselectice reduction reactions. Indeed, literature reports on $Zn(BH_4)_2$ indicate that the chemoselective reduction of β–ketoesters to the corresponding β-hydroxy esters can be easily achieved with better isomeric control because of the better coordinating ability of zinc with the carbonyl group of the ester (Oishi and Nakata 1984). This reaction has been utilized in the synthesis of certain natural products and in prostaglandin synthesis. Ranu and Das (1991) has reported $Zn(BH_4)_2$ to be a mild reducing agent capable of reducing aldehydes in the presence of ketones, and ketones in the presence of enones.

It would appear from the preceding reports that $Zn(BH_4)_2$ is a mild reagent with only a limited scope. However, the unique properties of $Zn(BH_4)_2$ come to light with subjected to tandem reduction – hydroboration.



[100]

Scheme VII represents the mechanism of action of zinc borohydride.



SCHEME VII

Thus, a similar reaction of parthenin was carried out using zinc borohydride as the reducing agent. A single product was obtained in this case which showed a ^1H NMR spectrum that was superimposable on the spectrum obtained for compound (98).

The alcohol thus obtained was subjected to oxidation with pyridinium chlorochromate to give back the keto product whose spectral data was identical with dihydroparthenin, thus confirming the structure of the reduced reaction product.

From the aforementioned observations, it can be inferred that zinc borohydride is a more selective reducing agent as compared to sodium borohydride. In case of sodium borohydride, reduction reaction occurs at both the exocyclic double bond of the γ-lactone moiety as well as the cyclopentenone ring whereas in case of zinc borohydride, the reduction occurs only at the carbonyl group of the cyclopentenone ring thus leading to the formation of a single alcoholic product.

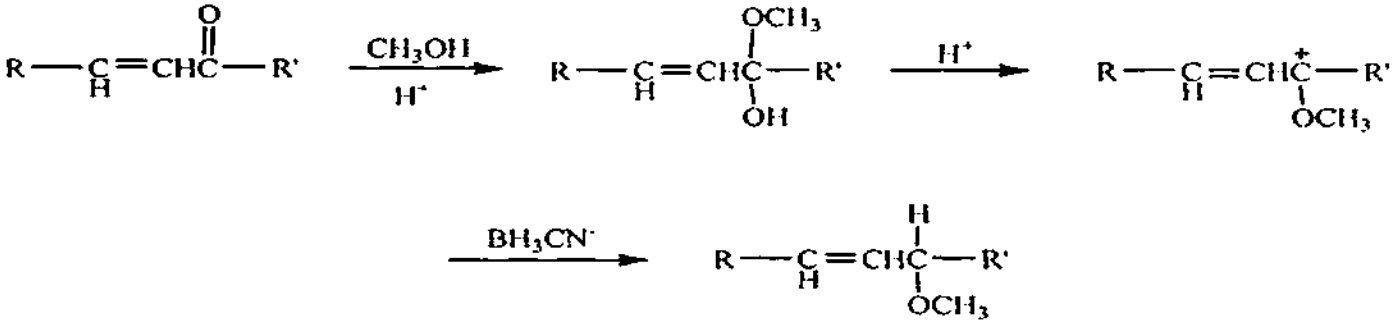
Based on these observations, it was thought worthwhile to study the selectivity of some other reducing agents towards the reactive moieties of parthenin.

The problems often encountered in the reduction of α, β-unsaturated carbonyl systems with hydride reagents are legion. In addition to affording the corresponding allylic alcohols, the reductions are often accompanied by such side reactions as concomitant reduction of the double bonds leading to saturated alcohols or less commonly, reduction to enols which eventually give the saturated ketones. Such divergence in reduction products has been attributed to completing 1,2 vs. 1,4 attack by hydride, but predictions concerning the mode of attack for a particular substrate are difficult.

Sodium cyanoborohydride is a selective reducing agent used for a variety of chemical reductions, including aldehyde, ketones, oximes, enamines, reductive alkylations of amines and hydriazines. The utility of sodium cyanoborohydride as a reducing agent is greatly enhanced by its stability under acid conditions, and its solubility in a protic solvent. It is a milder and more selective reducing agent as compared to sodium

borohydride. It has been observed that in this case 1,4-addition competes favourably when the double bond is further conjugated with an aromatic ring or contained in a 5- or 6- membered ring (Hutchins and Kandasamy 1975).

Also, the carbonyl reducing capabilities of cyanoborohydride have been noted to vary greatly with pH. In basic or neutral media, aldehydes and ketones are practically inert towards the reagent and adequate reduction rates are only obtained under acidic conditions where the carbonyl carbon is rendered more electrophilic by protonation.



SCHEME VIII

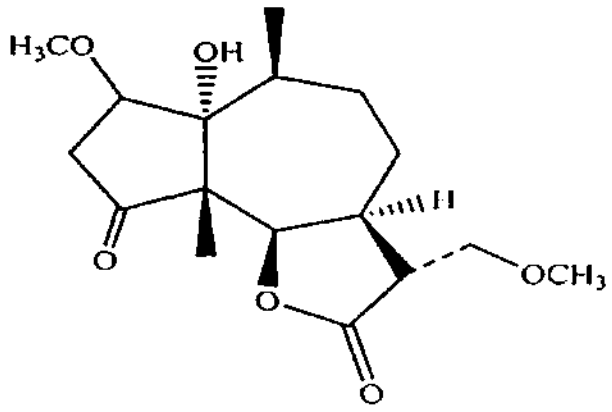
When parthenin was treated with sodium cyanoborohydride, it led to the formation of 3 compounds whose structures were established on the basis of their ^{1}H NMR and ^{13}C NMR analysis.

One of the compounds showed ^{1}H NMR peaks similar to a previously obtained product from the sodium borohydride reduction confirming its structure to be (98).

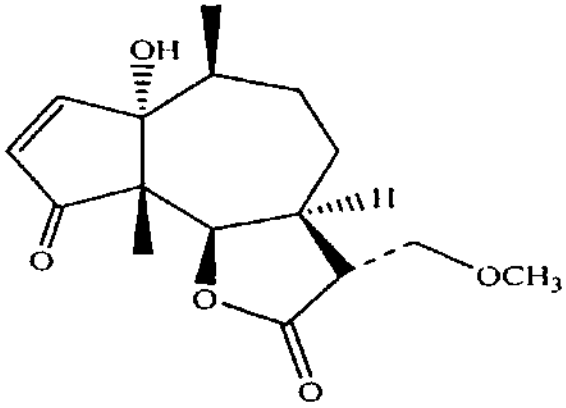
Another compound (101) obtained gave ^{1}H NMR peaks ($CDCl_3$, 270 MHz) at δ (ppm) 1.14 (3H, s, C_5-Me), 1.16 (3H, d, J= 8.37 Hz, C_{10}-Me), 3.34 (3H, s, -CH_2-OCH_3), 3.47 (3H, s, =CH-OCH_3), 3.62 (2H, d, J= 5.37 Hz, CH_2-OCH_3), 4.95 (1H, d, J= 8.10 Hz, C_6-H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ (ppm) at: 84.20 (C_1-s), 76.28 (C_2-d), 37.12 (C_3-t), 213.40 (C_4-s), 57.80 (C_5-s), 80.09 (C_6-d), 37.63 (C_7-t), 30.02 (C_8-t), 26.27 (C_9-t), 49.72 (C_{10}-d), 43.68 (C_{11}-d), 171.16 (C_{12}-s), 71.44 (C_{13}-t), 16.42 (C_{14}-q), 14.23 (C_{15}-q), 58.94 (C_{16}-q), 60.25 (C_{17}-q).

The third compound (102) showed ^1H NMR signals ($CDCl_3$, 270 MHz) δ (ppm) at 1.15 (3H, s, C_5-Me), 1.3 (3H, d, J= 7.0 Hz, C_{10}-Me), 6.15 (1H, d, C3-H, J= 6.0 Hz), 7.62 (1H, C2-H, J= 5.95 Hz), 3.5 (3H, s, $-CH_2-OCH_3$), 5.03 (1H, d, J= 7.83 Hz, C_6-H). The structure was further supported by ^{13}C NMR ($CDCl_3$, 300 MHz) δ (ppm) at 84.20 (C_1-s), 155.8 (C_2-d), 132.12 (C_3-t), 213.40 (C_4-s), 68.80 (C_5-s), 80.09 (C_6-d), 41.63 (C_7-t), 30.02 (C_8-t), 26.27 (C_9-t), 40.72 (C_{10}-d), 47.68 (C_{11}-d), 177.16 (C_{12}-s), 74.44 (C_{13}-t), 13.42 (C_{14}-q), 7.23 (C_{15}-q), 74.94 (C_{16}-t), 60.25 (C_{17}-q).

Based on these spectroscopic observations, following structures have been proposed for the two compounds.



[101]



[102]

Unlike analogous reductions with borohydride, reduction with sodium cyanoborohydride in acidic methanol afforded the corresponding methyl ethers in addition to the allylic alcohol. Ostensibly, these products arise from protonation and ionization of the initially produced alcohol followed by solvent capture. The stereochemistry of methoxy group on C-13 was assigned as α-oriented from the coupling constant $J_{11,13}$ (12Hz).

Another reagent of great synthetic significance is lithium tri-*tert* butoxy aluminohydride. In general, the tri-alkoxy aluminohydrides of lithium are prepared by treatment of lithium aluminium hydride with alcohols. This is based on the general reaction.

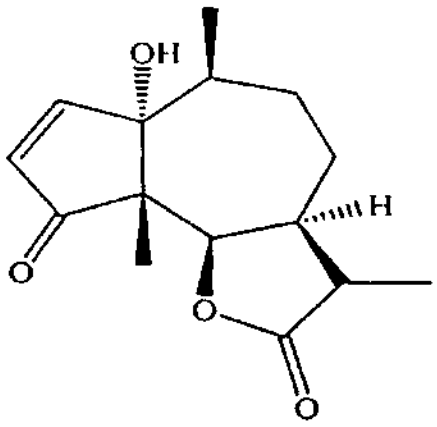
$$LiOR + Al(OR)_3 \longrightarrow LiAl(OR)_4$$

However, the addition of four moles of *t*-butyl alcohol results in the formation of only three moles of hydrogen. The fourth mole of hydrogen is evolved only on extended treatment at elevated temperature.

The reaction product, lithium tri-*tert* butoxy aluminohydride, is only slightly soluble in ethyl ether but is readily soluble in tetrahydrofuran (Brown and Mcfarlin 1958).

Thus, the reaction of parthenin with this reducing agent was carried out in THF which furnished a mixture of two products that were identified by spectral analysis. One of the products was found to be identical in structure to (98). A minor product (103) obtained in this reaction showed ^{1}H NMR signals ($CDCl_3$, 270 MHz) δ (ppm) at 7.50 (d, 1H, J= 5.9 Hz, C_2-H), 6.17 (d, 1H, J= 5.9 Hz, C_3-H), 5.01 (d, 2H, J= 7.90 Hz, C_6-H), 3.54 (m, 1H, C_7-H), 1.70 (s, 3H, C_5-Me), 1.13 (d, 3H, J= 7.54 Hz, C_{10}-Me).

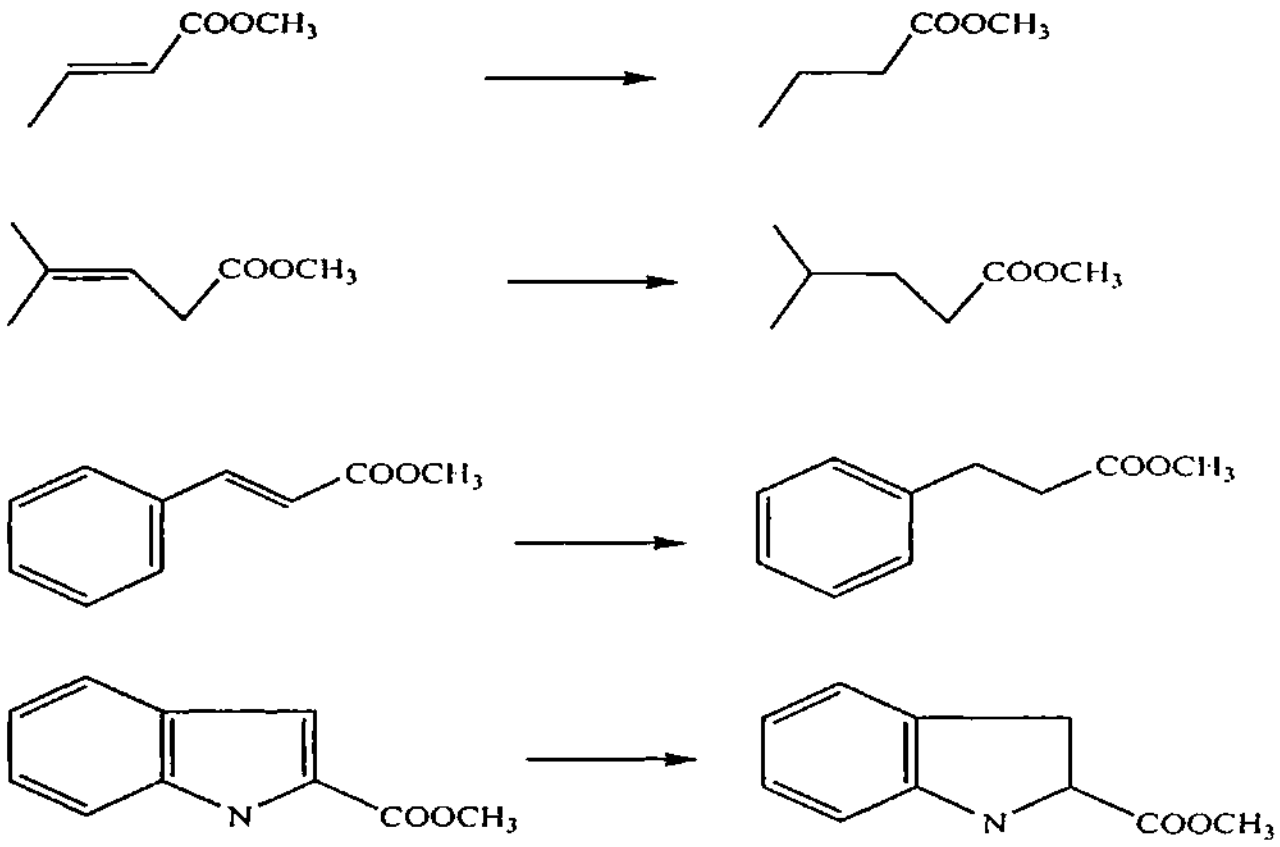
The spectral data of this compound surprisingly revealed that C_2=C_3 remained intact while methylene C=C conjugated to lactone underwent reduction. The stereochemistry for C_{11} methyl was confirmed by comparison of its spectral data with that of dihydroparthenin.



[103]

Magnesium in methanol (Mg/MeOH) system is an extremely versatile, efficient, economical and convenient reducing agent for various reactions useful in organic synthesis such as reductive cyclization, reductive elimination, reductive cleavage, reduction of a conjugated double bond, desulfonylation and reduction of various functional groups.

It has been reported that various α, β-unsaturated esters undergo double bond reduction selectively with magnesium in dry methanol to give corresponding saturated compounds in fairly good yields (Scheme IX).



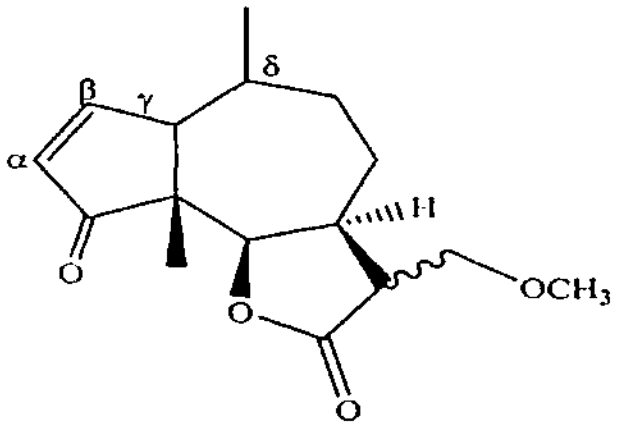
SCHEME IX

Lactones being internal esters, it was thought worth while to treat parthenin with magnesium in dry methanol. Michael addition rather than reduction at unsaturated centers was noticed leading to the formation of two products which were identified by NMR analysis to be (101) and (102).

However, when a similar reaction was carried out with anhydroparthenin, a pure liquid compound was obtained which showed IR bands at 1750 and 1650 cm^{-1} suggesting the presence of carbonyl group and a double bond. ^1H NMR spectrum showed a doublet signal due to three hydrogens at δ 1.20 with J = 7 Hz. It showed two signals at δ 1.35 and δ 3.30.

Other signals included doublets at δ 3.65 with J = 6.5 Hz and δ 4.5 with J = 8 Hz. The presence of a double bond in conjugation with carbonyl was shown by the presence of signals at δ 6.45 and δ 8.1 with J = 6.3 Hz and 6 Hz, respectively.

These spectroscopic observations coupled with ^{13}C NMR data suggested the following structure for the compund:



[104]

Thus, anhydroparthenin, when subjected to the action of active Mg in dry methanol yielded methoxy ambrosin by the reduction of γ-δ double bond and Michael addition to the α-methylene-γ-lactone moiety.

Biological activity

A vast and diverse assortment of organic compounds is produced by plants and the great majority of these do not appear to participate directly in growth and development. These substances are often differentially distributed among limited taxonomic groups within the plant kingdom and are traditionally referred to as secondary metabolites. Recognition of the biological properties of myriad natural products has fuelled the current focus on search for new chemicals. Sesquiterpene lactones are one of the very important groups of natural plant products known for their biological activities. Recently, focus of the present research in the area of sesquiterpene lactones, has been to explore their herbicidal potential. In order to substantiate their plant growth regulating properties, present studies were

undertaken to alter the basic parent skeletons (natural compounds), dehydrocostus lactone from *Saussurea lappa* and *Cichorium intybus*, alantolactone and isoalantolactone from *Inula racemosa* and parthenin from *Parthenium hysterophorus*.

These transformations included oxidation, reduction, isomerization and formation of adducts. Initially screening of newly synthesized compounds for their biological activity was tested on germination and seedling growth of pea (*Pisum sativum*) seeds, seed germination in Hoagland solution served as controls, while GA_3 (25 and 50 μg ml^{-1}) and ABA (10 and 20 μg ml^{-1}), both terpenoid derivatives were used as standards for their growth promoting and inhibitory activities. Based on the data generated from pea studies, tested compounds were categorized for their growth promotory and inhibitory activities. The compounds identified as potential inhibitors were tested for their weedicide activity using seed germination assay. Seeds of *Avena fatua* and *Phlaris minor* weeds were used.

***Pisum sativum* (Pea)**

The effect of natural components (2 and 3) isolated from *Inula racemosa* and their structure analogues on germination and seedling growth behaviour of pea seeds are shown in Figures 1 and 2 and Table 11. Compared to control, various tested compounds inhibited seed germination and seedling growth in terms of length of root, shoot and accumulation of fresh and dry mass of seedlings. Seed germination ranged between 95-100 per cent in response to various treatments at lower concentrations (50-300 μg ml^{-1}). However, at 500 μg ml^{-1} concentrations, germination was reduced by 72-73 per cent in response to compounds 2, 43 and 83, while it ranged between 80-90 per cent with remaining compounds as compared to controls. However, the degree of inhibition was not marked when compared with ABA treatments, which reduced germination percentage to 43 and 22, respectively, in response to 10 and 20 μg ml^{-1} concentrations as compared to control (Figure 1).

Unlike germination, the inhibitory effect of these compounds was more pronounced on length and accumulation of fresh and dry mass of seedlings (Table 11).

Further, length of shoot was inhibited to a greater extent as compared to root. The magnitude of inhibition was maximum in response to compounds 3 followed by 2, 13, 43, 42, 83 and 84.

These compounds also affected the initiation of secondary roots (Figure 2). In controls, about 22-23 secondary roots developed per seedling. All the tested compounds of this series reduced the number of secondary roots with maximum inhibition being caused by compound 3 which was by about 82 per cent over control, followed by compounds 2, 13, 42, 43, 84 and 81.

Table 11 and Figures 3 and 4 show effect of natural component isolated from *Saussurea lappa* and its structural analogues (compounds 1, 12, 19-23, 25, 79, 82, 88) on seed germination and various seedling growth parameters of pea.

Seed germination: Germination of seeds in response to 50 and 100 μg concentrations of different compounds of this series was almost comparable with control (Figure 3). However, at higher concentration germination was reduced by about 4-28 per cent in response to compounds 20, 21, 23, 25, 79, 82 and 88 per cent as compared to control. Maximum inhibition of seed germination was obtained in response to compound 82. But the magnitude of inhibition was for less than obtained in response to ABA treatments.

Seedling growth: Like germination, these compounds also exerted their effect on seedling growth (Table 11). The shoot length of seedlings increased in response to 50 μg ml^{-1} concentration of compound numbers 20, 21, 23, 25 and reduced in response to higher concentrations of all the compounds.

However, the magnitude of this increase was less as compared to GA_3 treatments. The root length of seedlings treated with lower concentration of compounds 23 and 25 remained almost comparable with controls and reduced in response to all other treatments. Maximum inhibition of root and shoot length was observed in response to compound no. 22. There was also

large variation in accumulation of dry mass of control and treated seedlings (Table 11). However, the inhibitory effect of these tested compounds was less marked as compared to ABA treatments.

Figure 4 shows the effect of these compounds on development of secondary roots compared to controls, all the tested compounds reduced the number of secondary branches. The inhibitory effect increased with increasing concentrations.

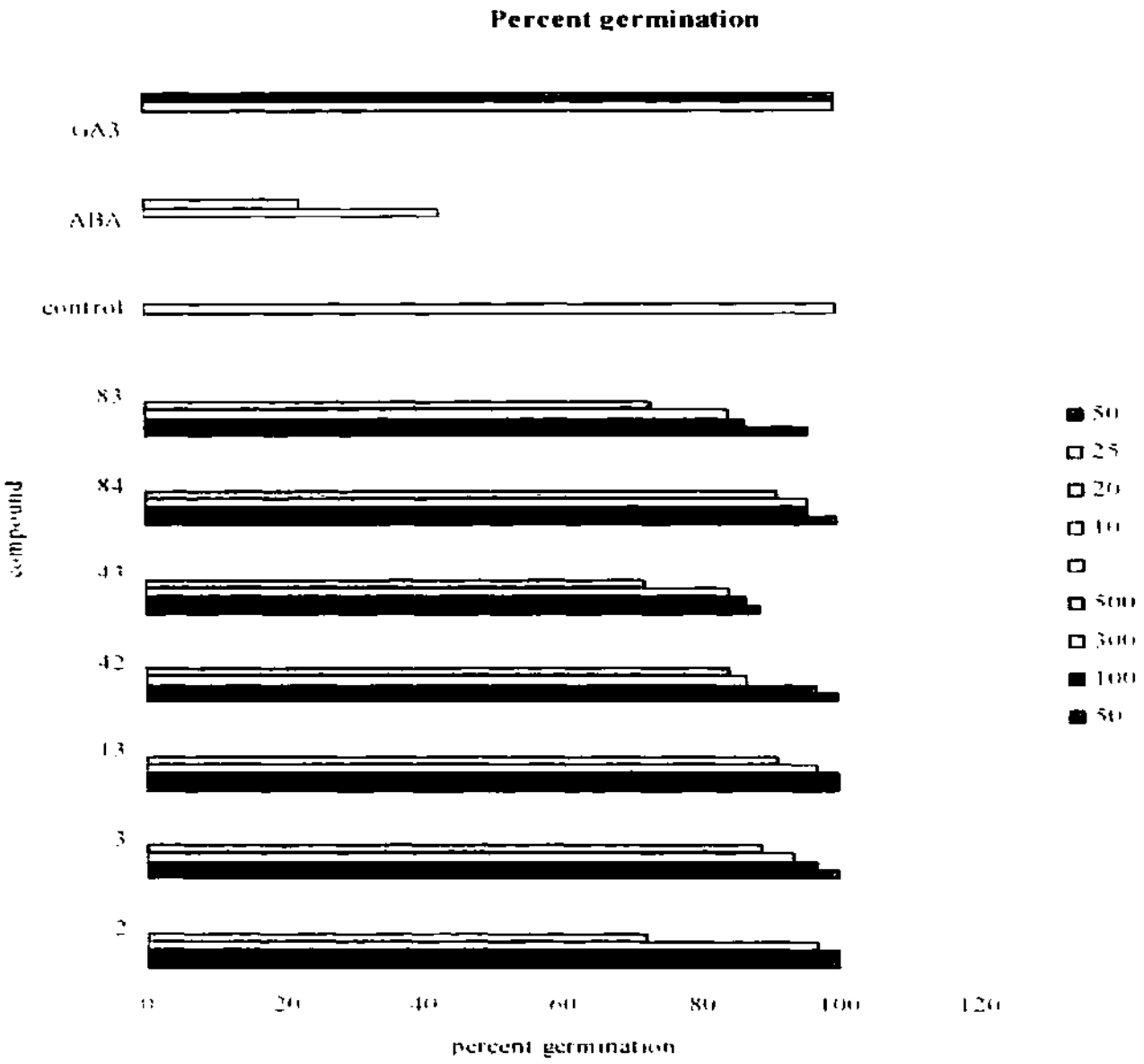
Table 11 and Figures 5 and 6 show the effect of parthenin isolated from *Parthenium hysterophorus*, and its structural analogues (compounds 8, 56, 98, 99, 101, 102, 104) on germination and seedling growth of pea seeds.

Seed germination: All the compounds of this series except 56 and 104, significantly, reduced germination of seeds (Figure 5) compared to control (100%) germination of seeds, was reduced to 42 and 22 per cent, respectively in response to 300 and 500 $\mu g\ ml^{-1}$ concentrations of compounds 98 and 101. Compound 104 was found least effective in eliciting the decrease in seed germination.

Seedling growth: All these tested compounds also reduced length of root and shoot with concomitant reduction in fresh and dry mass of seedlings (Table 11). Maximum inhibition of length of root and shoot was observed in response to compound 101 followed by 98. At 500 $\mu g\ ml^{-1}$ concentration, complete inhibition of extension growth was observed with compound 101.

Further, the magnitude of inhibition caused by these compounds was almost comparable with ABA treatments. Further, all the tested compounds of this series except compound no. 56 were found more inhibitory than compounds from *Inula racemosa* and *Saussurea lappa*.

Figure 6 shows the effect of tested compounds on number of secondary roots. In controls, about 22-23 roots developed on primary root. The initiation of secondary roots and their inhibitory effect increased with increasing concentrations. The magnitude of inhibition of rooting by different compounds at various tested concentrations ranged between 61 to 86 per cent. The inhibition of initiation of secondary roots in response to ABA treatments was



gure 1: Effect of different concentrations of eudesmanolides on per cent germination of pea seeds

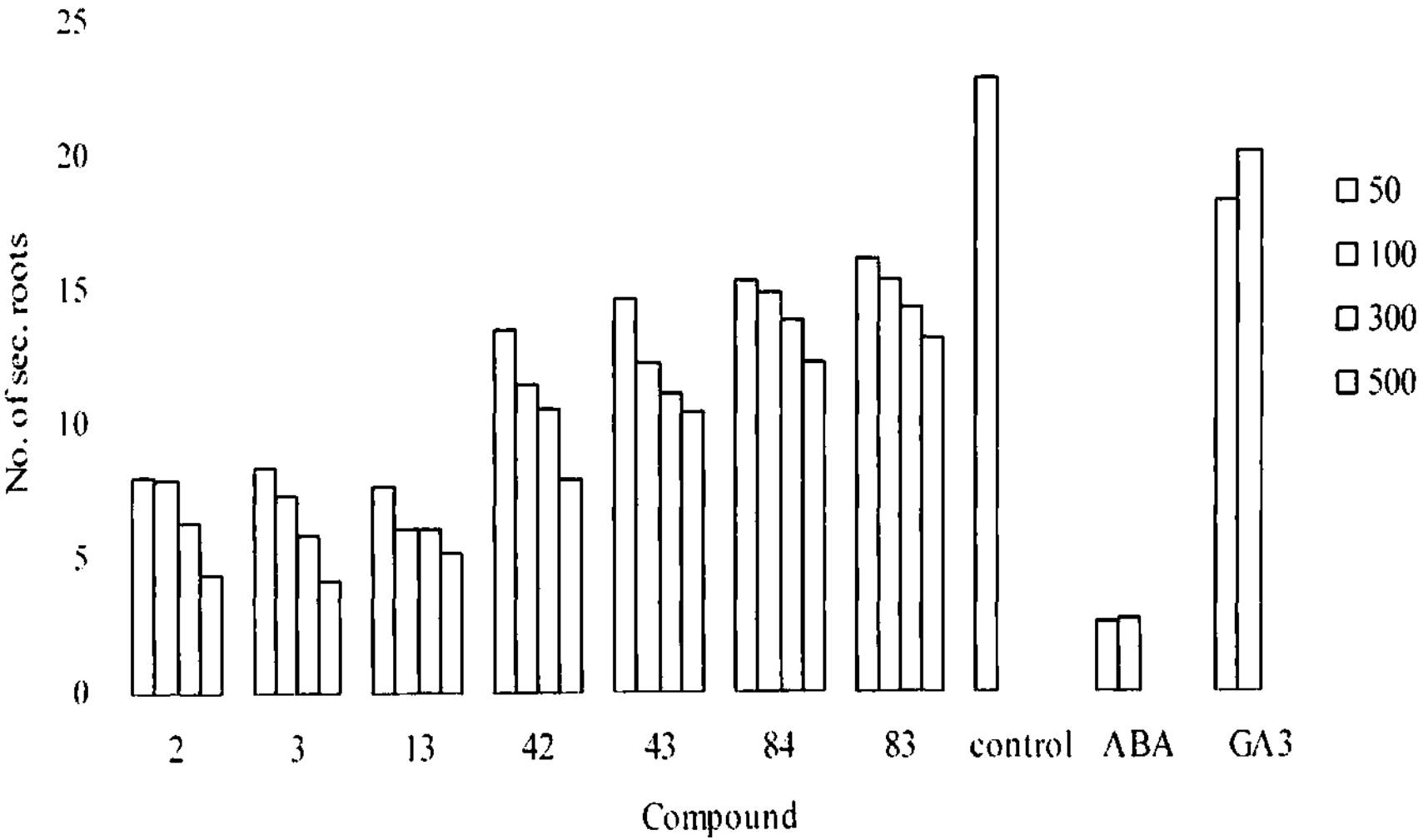


Figure 2: Effect of different concentrations of eudesmanolides and their derivatives on number of secondary roots in pea Seedlings

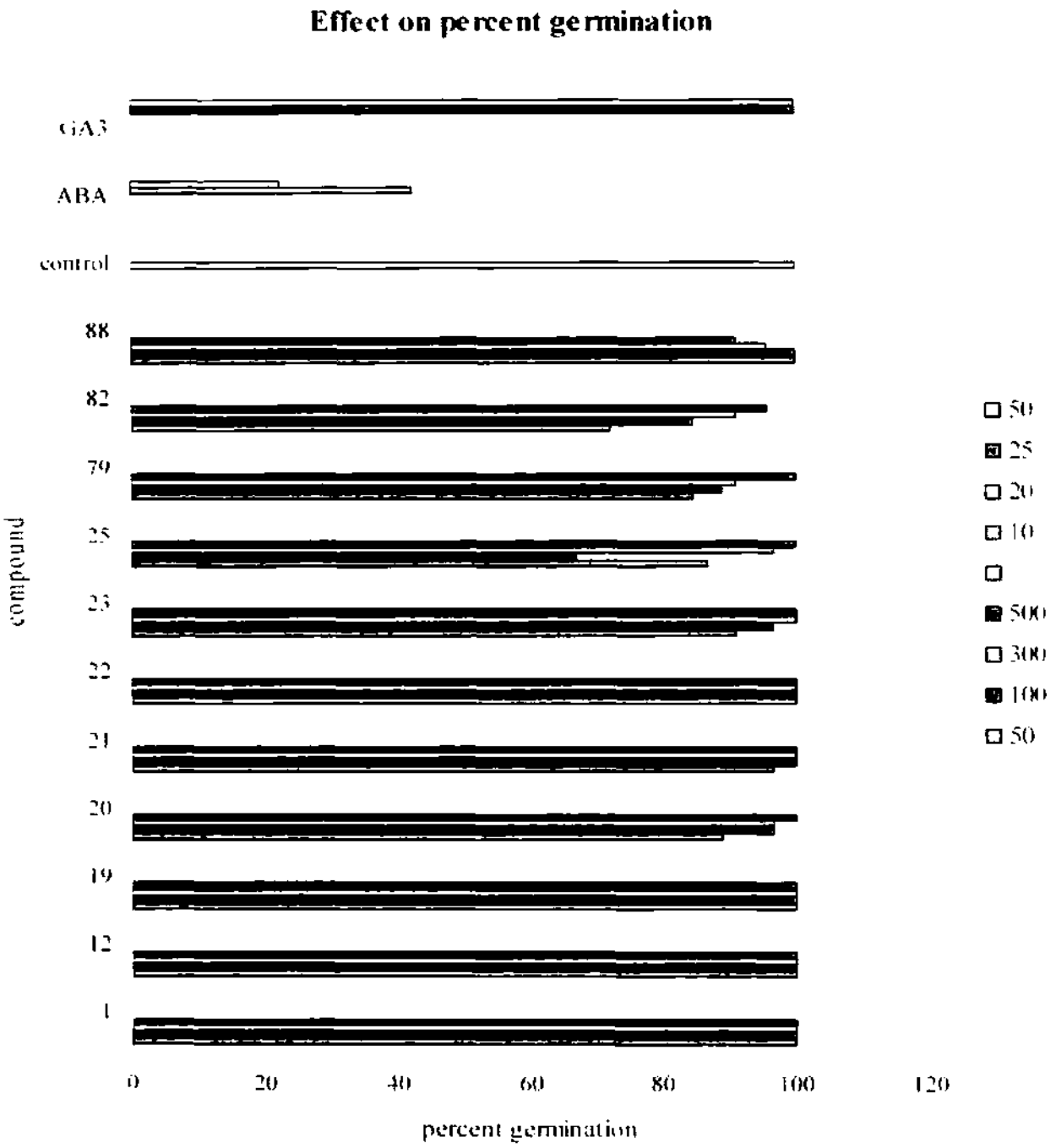


Figure 3: Effect of different concentrations of guaianolides on per cent germination of seeds of *Pisum sativum*

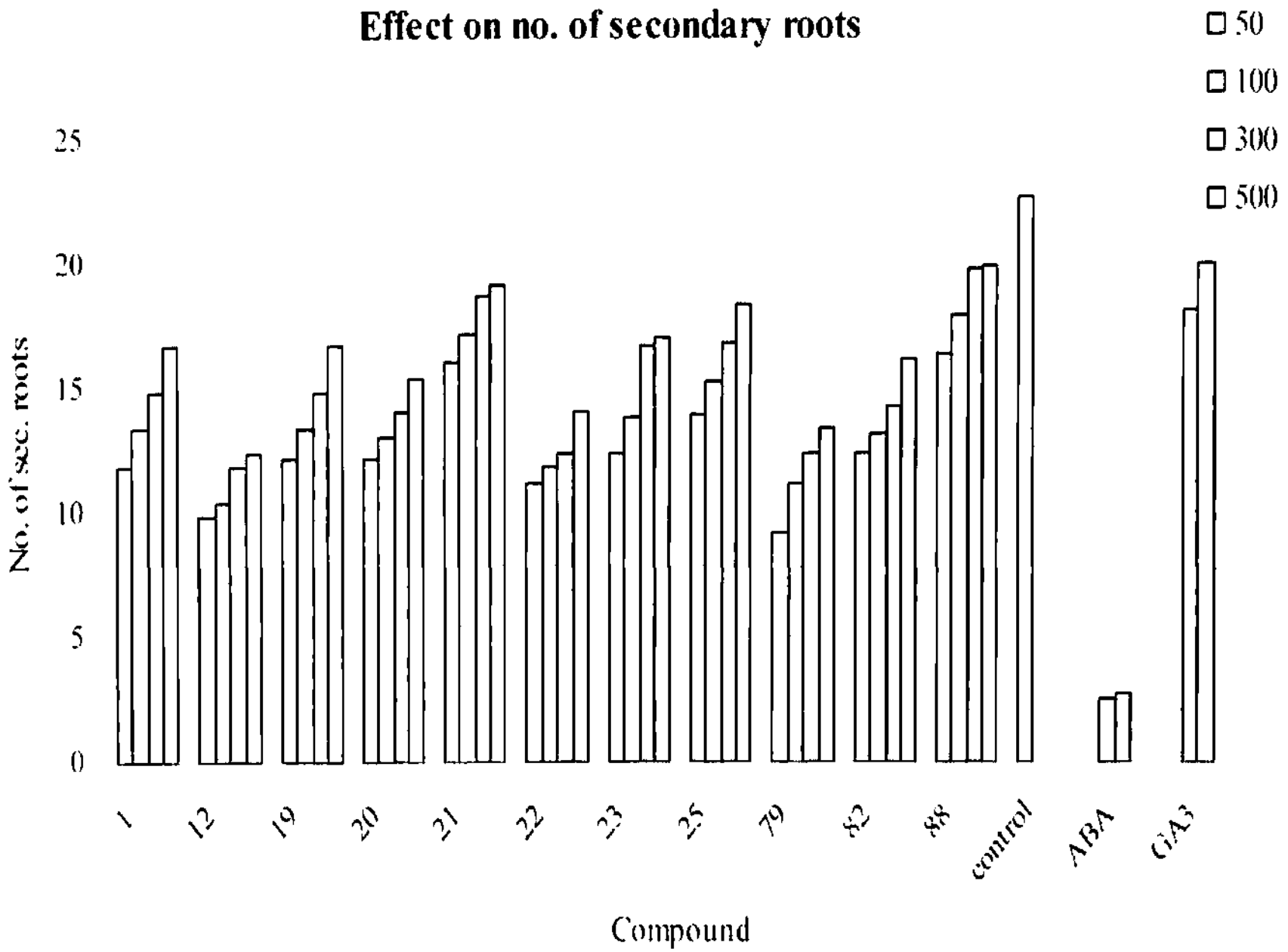


Figure 4: Effect of different concentrations of guaianolides and their derivatives on number of secondary roots in pea seedlings

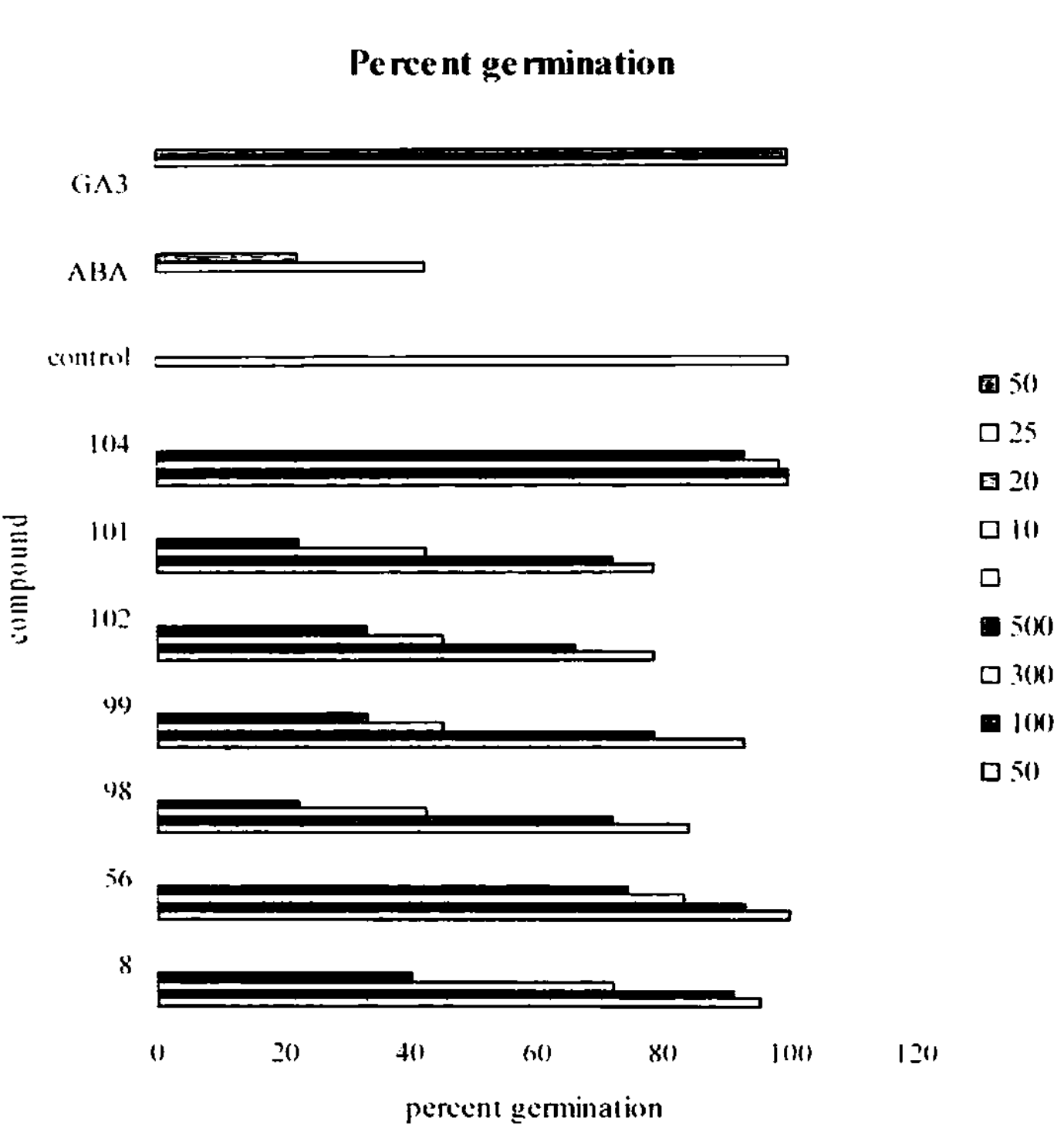


Figure 5: Effect of different concentrations of pseudoguaianolides on per cent germination of seeds of *Pisum sativum*

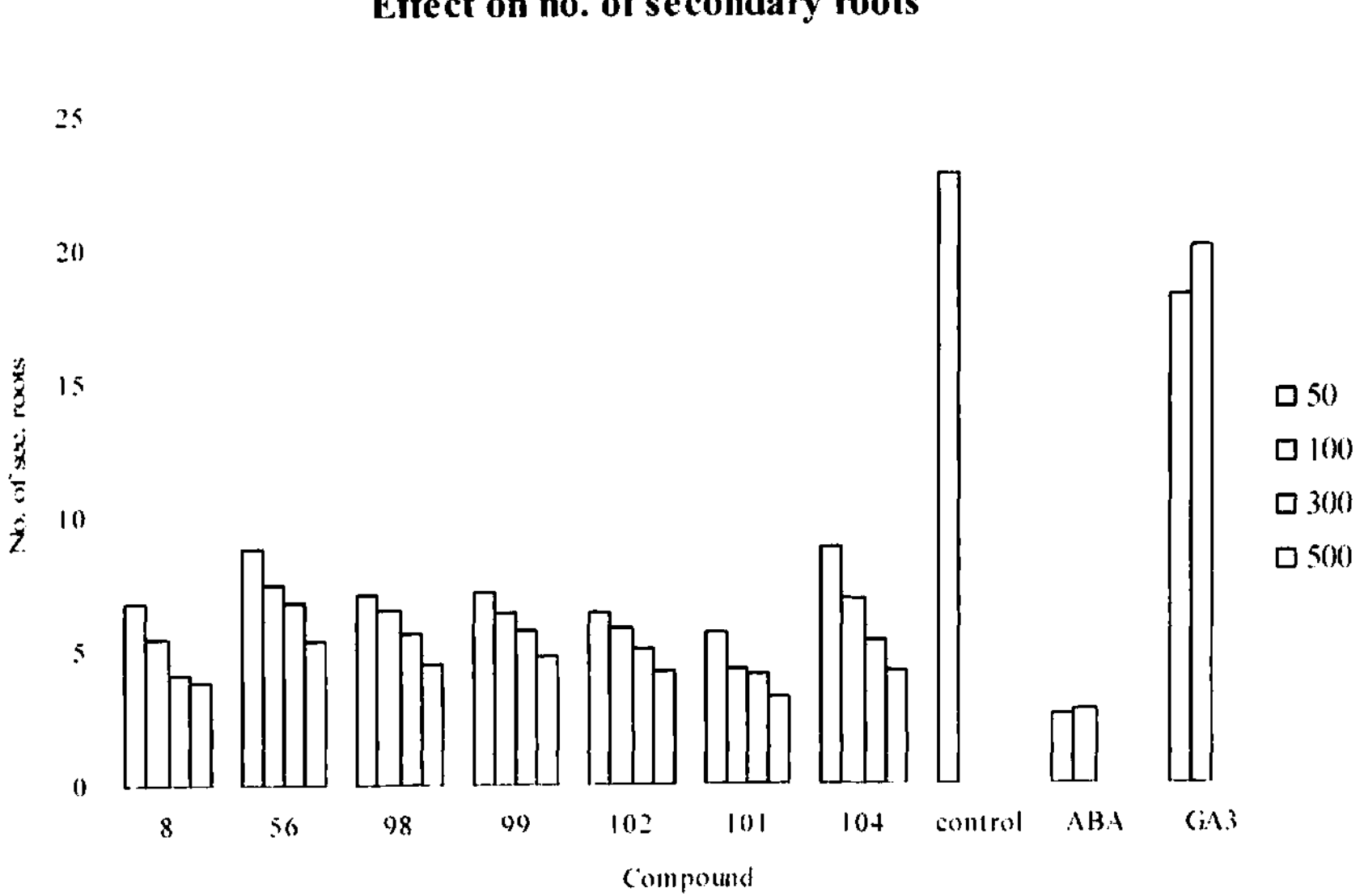
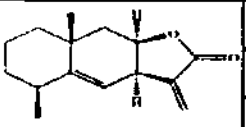
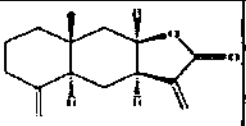
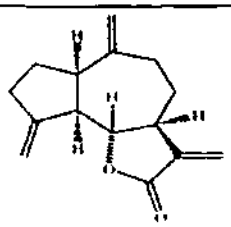
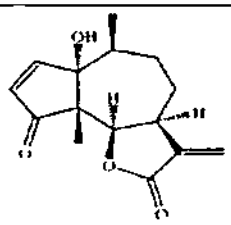
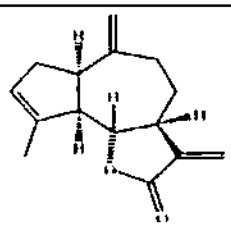
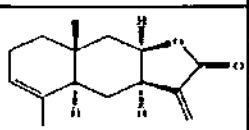
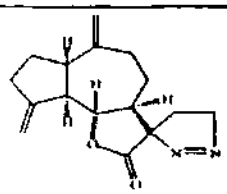
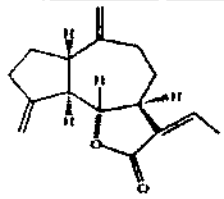
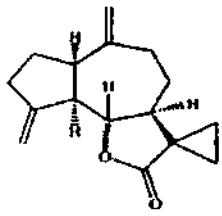
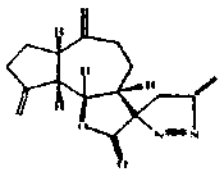
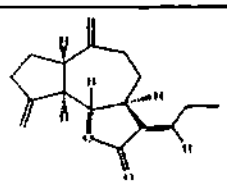
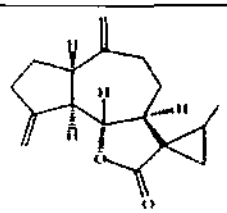
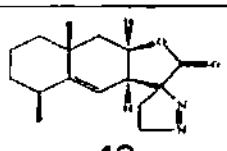
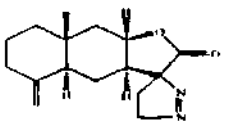
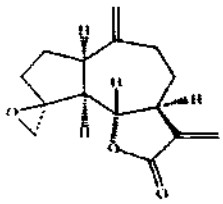
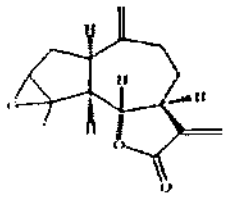
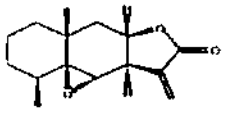
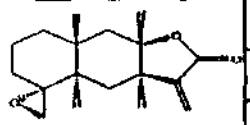
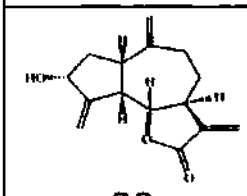
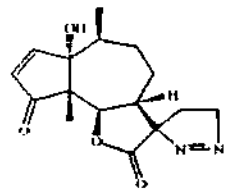


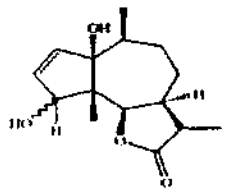
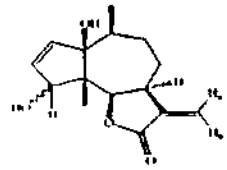
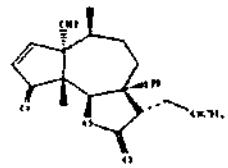
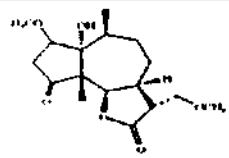
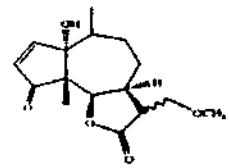
Figure 6: Effect of different concentrations of pseudoguaianolides and their derivatives on number of secondary roots in pea seedlings

Table 11: Effect of various sesquiterpene lactones on the seedling growth of *Pisum sativum*

Compound	Conc. ($\mu g\ ml^{-1}$)	% germ.	Root length (cm)	Shoot length (cm)	Fresh wt. (g)	Dry wt. (g)
 2	50	100	9.115	1.734	2.415	0.095
	100	100	8.825	1.185	1.704	0.068
	300	96.6	5.625	0.995	1.115	0.012
	500	72.1	3.66	0.66	0.667	0.005
 3	50	100	7.928	1.34	1.74	0.083
	100	96.6	6.525	0.982	1.18	0.015
	300	93.33	4.115	0.855	0.985	0.005
	500	88.89	2.686	0.625	0.425	0.005
 1	50	100	13.75	7.32	5.25	1.86
	100	100	13.82	6.46	4.58	1.75
	300	100	13.62	5.18	3.98	1.07
	500	100	13.25	4.32	3.04	0.90
 8	50	95.56	6.58	1.12	1.55	0.027
	100	91.11	4.925	0.825	0.925	0.020
	300	72.10	3.18	0.76	0.58	0.020
	500	40.20	1.998	0.48	0.398	0.005
 12	50	100	9.125	5.18	2.995	0.15
	100	100	8.87	4.325	1.862	0.085
	300	100	7.462	2.568	0.982	0.009
	500	100	5.82	1.125	0.625	0.001
 13	50	100	9.325	1.815	2.562	0.18
	100	100	8.86	1.26	1.815	0.081
	300	96.60	5.628	0.892	1.012	0.035
	500	91.11	3.98	0.75	0.775	0.001

 19	50	100	12.21	8.18	4.18	0.162
	100	100	11.42	7.525	3.232	0.093
	300	100	9.625	5.86	1.965	0.048
	500	100	7.82	3.928	1.18	0.011
 20	50	88.89	11.12	8.68	3.80	0.18
	100	96.60	10.85	7.112	3.115	0.093
	300	96.60	7.962	5.925	2.98	0.058
	500	100	7.81	5.18	2.52	0.051
 21	50	96.6	12.85	8.80	3.95	0.112
	100	100	10.965	7.86	3.18	0.089
	300	100	8.052	6.12	3.012	0.075
	500	100	7.82	4.98	2.465	0.050
 22	50	100	10.63	5.115	2.925	0.072
	100	100	9.18	4.822	2.52	0.056
	300	100	7.52	4.132	2.36	0.048
	500	100	5.865	3.825	1.925	0.037
 23	50	91.11	13.76	9.16	4.25	0.112
	100	96.6	11.325	8.12	3.682	0.098
	300	100	9.865	7.625	3.085	0.092
	500	100	8.52	5.24	2.95	0.075
 25	50	86.67	13.78	9.45	4.56	0.125
	100	66.89	11.48	8.18	3.85	0.091
	300	96.60	9.62	7.65	3.012	0.087
	500	100	8.34	5.44	2.98	0.078
 42	50	100	10.86	5.60	2.85	0.075
	100	96.6	9.24	4.822	2.48	0.062
	300	86.67	7.86	4.122	2.14	0.043
	500	84.44	5.425	3.854	1.88	0.022

 43	50	88.89	10.15	5.081	2.35	0.06
	100	86.67	8.184	4.65	2.22	0.052
	300	84.44	6.424	4.082	1.89	0.028
	500	72.1	4.635	3.142	1.56	0.012
 79	50	84.44	9.22	4.56	1.56	0.019
	100	88.89	8.84	3.84	1.04	0.012
	300	91.11	6.86	3.12	0.88	-
	500	100	4.685	2.58	0.68	-
 82	50	72.1	9.26	4.98	2.00	0.018
	100	84.44	8.18	4.12	1.18	0.012
	300	91.11	7.24	3.63	0.98	0.003
	500	95.56	5.85	2.98	0.85	0.001
 84	50	100	11.82	7.32	3.89	0.04
	100	95.56	11.75	6.18	3.13	0.032
	300	95.56	10.95	4.312	2.158	0.020
	500	91.11	8.625	2.856	1.15	0.015
 83	50	95.56	10.86	6.48	2.56	0.025
	100	86.67	10.04	6.092	2.18	0.019
	300	84.40	8.86	5.14	1.75	0.008
	500	73.30	7.94	3.232	0.928	0.002
 88	50	100	10.18	5.64	2.195	0.015
	100	100	8.894	4.325	1.826	0.011
	300	95.56	7.432	2.462	0.998	0.001
	500	91.11	6.48	1.185	0.724	0.001
 56	50	100	12.12	7.142	3.95	1.025
	100	93.33	11.42	6.388	3.48	0.982
	300	83.33	10.26	4.265	2.232	0.12
	500	74.44	9.125	2.226	1.515	0.085

 98	50	84.44	6.12	1.28	1.52	0.091
	100	72.1	4.48	0.994	0.95	0.083
	300	42.6	3.094	0.765	0.52	0.042
	500	22.4	1.125	0.32	0.388	0.018
 99	50	93.33	8.125	2.125	1.323	0.084
	100	78.7	7.262	1.994	0.998	0.076
	300	45.5	5.148	1.265	0.622	0.059
	500	33.37	4.32	0.98	0.562	0.042
 102	50	78.70	4.52	0.98	0.842	0.061
	100	66.40	3.184	0.725	0.65	0.043
	300	45.50	2.26	0.52	0.425	0.025
	500	33.37	1.65	0.389	0.385	0.018
 101	50	78.70	3.984	0.85	0.725	0.059
	100	72.10	2.126	0.625	0.45	0.021
	300	42.60	1.125	0.43	0.392	0.012
	500	22.40	-	-	-	-
 104	50	100	8.92	2.185	1.34	0.081
	100	100	6.428	1.927	1.012	0.076
	300	98.80	5.384	1.034	0.724	0.055
	500	93.33	4.26	0.985	0.532	0.039
Control		100	13.75	8.58	4.23	1.185
ABA	10	42.6	3.15	0.95	0.752	0.052
	20	22.4	1.18	0.48	0.389	0.015
GA	25	100	13.12	13.56	5.05	2.095
	50	100	14.15	15.92	6.212	3.312

by about 88 per cent as compared to control. GA_3 treatments did not exert any marked effect on number of secondary roots.

Studies on weedicidal activity

Studies on the effect of natural components isolated from three different plant species and their structural analogues on germination and seedling growth of pea seeds revealed that these exhibited mixed response. Some compounds (1, 2, 3, 12, 19, 20, 21-25, 79, 87) did not exert any marked effect on inhibition of germination at 50 to 300 $\mu g\ ml^{-1}$ concentrations, though 500 $\mu g\ ml^{-1}$ concentration caused 10-16 per cent inhibition of germination as compared to control with 100 per cent germination. Compounds which inhibited the germination above 25 per cent were selected for testing their weedicidal activity. All these compounds were pseudoguaianolides (compound number 8, 56, 98, 99. 101, 102, 14) and their potential as weedicidal compounds was screened in comparison to the standard Isoproturon C (100% inhibition at 20 $\mu g\ ml^{-1}$ in both weeds).

Seed germination: Figures 7 and 8 show the effect of selected compounds on germination of *Avena fatua* and *Phlaris minor.* In controls, germination was 100 per cent. All the tested compounds inhibited seed germination in both the weeds. The effect of compounds 56, 98, 99 and 104 was not much marked in inhibiting the germination. On the contrary drastic reduction in germination was observed in seeds of both weeds in response to compounds 8, 101 and 102. The per cent inhibition of germination was more than 70 in both weeds in response to 300 $\mu g\ ml^{-1}$ concentration of these compounds. Though the magnitude of inhibition was higher than obtained with 10 $\mu g\ ml^{-1}$ ABA treatment, the compounds were found to be less effective than the standard at 25 and 50 $\mu g\ ml^{-1}$ concentration. Germination of seeds was completely checked at 500 $\mu g\ ml^{-1}$ concentration with compounds 8 and 101 and 20 $\mu g\ ml^{-1}$ ABA.

Seedling growth: Like germination, these selected compounds significantly suppressed seedling growth measured in terms of changes in root and shoot length and accumulation of fresh and dry mass of seedlings (Tables 12 and 13). Compared to controls, length of root and shoot was inhibited by more

than 50 per cent in response to 300 and 500 μg ml^{-1} concentration of compounds 56, 98, 99 and 104. The inhibiting effect of compounds 8, 101 and 102 was more drastic on these parameters. However, the magnitude of response varied with species. In *Avena fatua* average inhibition of length of root and shoot was about 76-85 and 91 per cent, respectively over control, in response to 8, 101 and 102 compounds, while it ranged between 50-68 for root and 75-84 per cent for shoot in response to these chemicals in *Phlaris minor*. The magnitude of inhibition of length of shoot and root obtained with these compounds was comparable with that obtained in response to 10 μg ml^{-1} ABA treatment but was lower than that observed in case of isoproturon C at 50 μg ml^{-1} concentration (Table 13).

These compounds also resulted in reduction in accumulation of seedling fresh and dry mass in both the weeds which closely paralleled with reduction in seedling length. Compounds 8, 101 and 102 were found to be more inhibitory compared to controls, the reduction in accumulation of fresh and dry mass of seedlings ranged between 90 and 97 per cent, respectively in *Avena fatua* and 67-74 and 87-93 per cent in *Phlaris minor*. The magnitude of inhibition, with these chemicals was comparable with that obtained with 10 μg ml^{-1} ABA treatment (Tables 12 and 13).

Chlorophyll content: Since above tested compounds resulted significant reduction in seedling growth. Seedling growth initially depends on the mobilization of storage reserves from seeds followed by photosynthetic fixation of CO_2. Since significant reduction in dry mass of seedlings has been observed in both the seeds, attempt has also been made to study the effect of selected compounds (8, 56, 98, 99, 101, 102, 104) on total chlorophyll content of leaves of *Avena fatua* and *Phlaris minor* (Figures 9 and 10), compared to controls, these compounds reduced content of chlorophyll and the inhibitory effect increased with increasing concentrations. The effect of 56 and 104 compounds at 50 μgml^{-1} concentration was less marked. Maximum reduction in chlorophyll content was observed in leaves of both weeds with compound 102 at 500 μg ml^{-1} concentration.

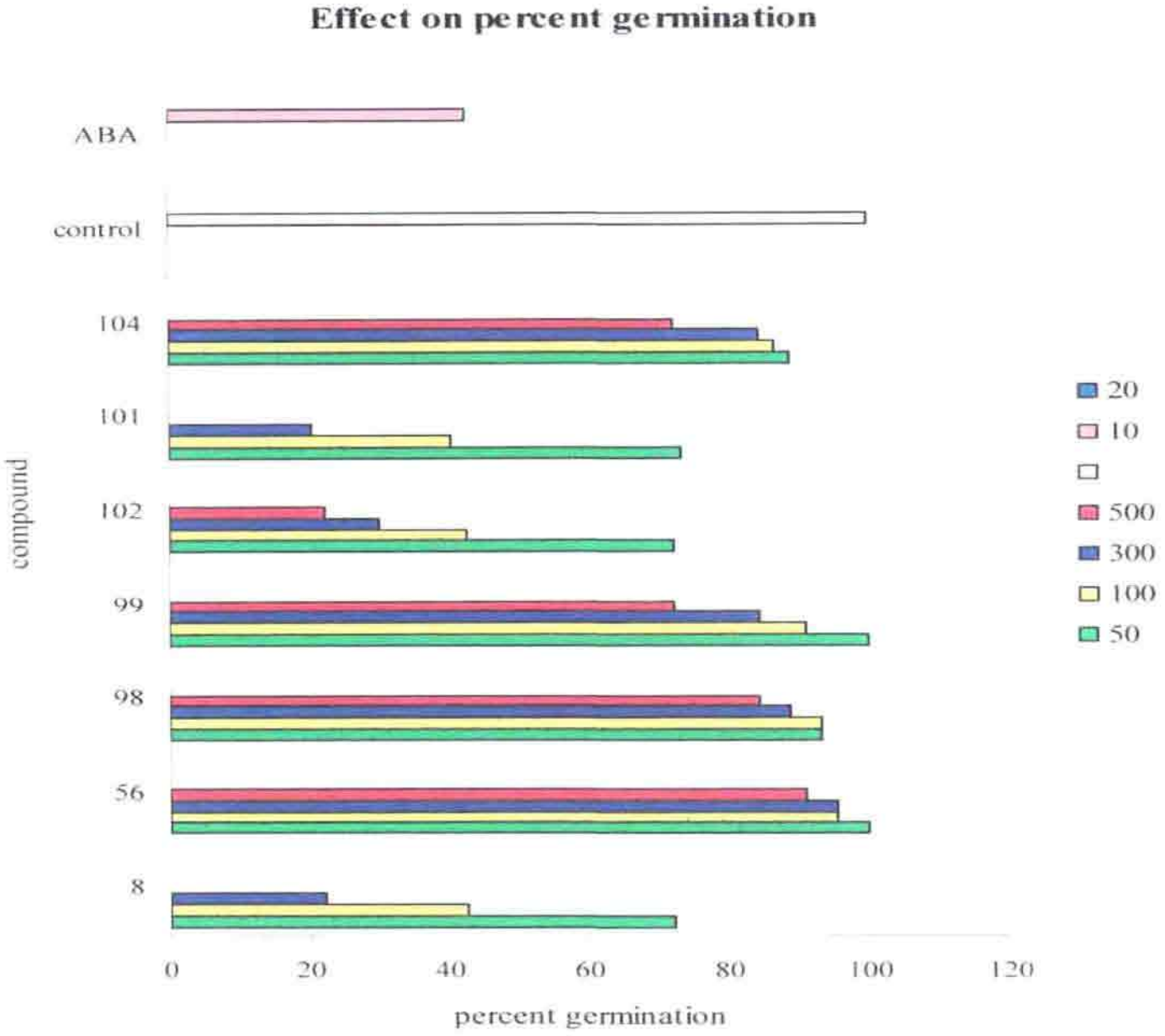


Figure 7: Effect of different concentrations of pseudoguaianolides on per cent germination of seeds of *Avena fatua*

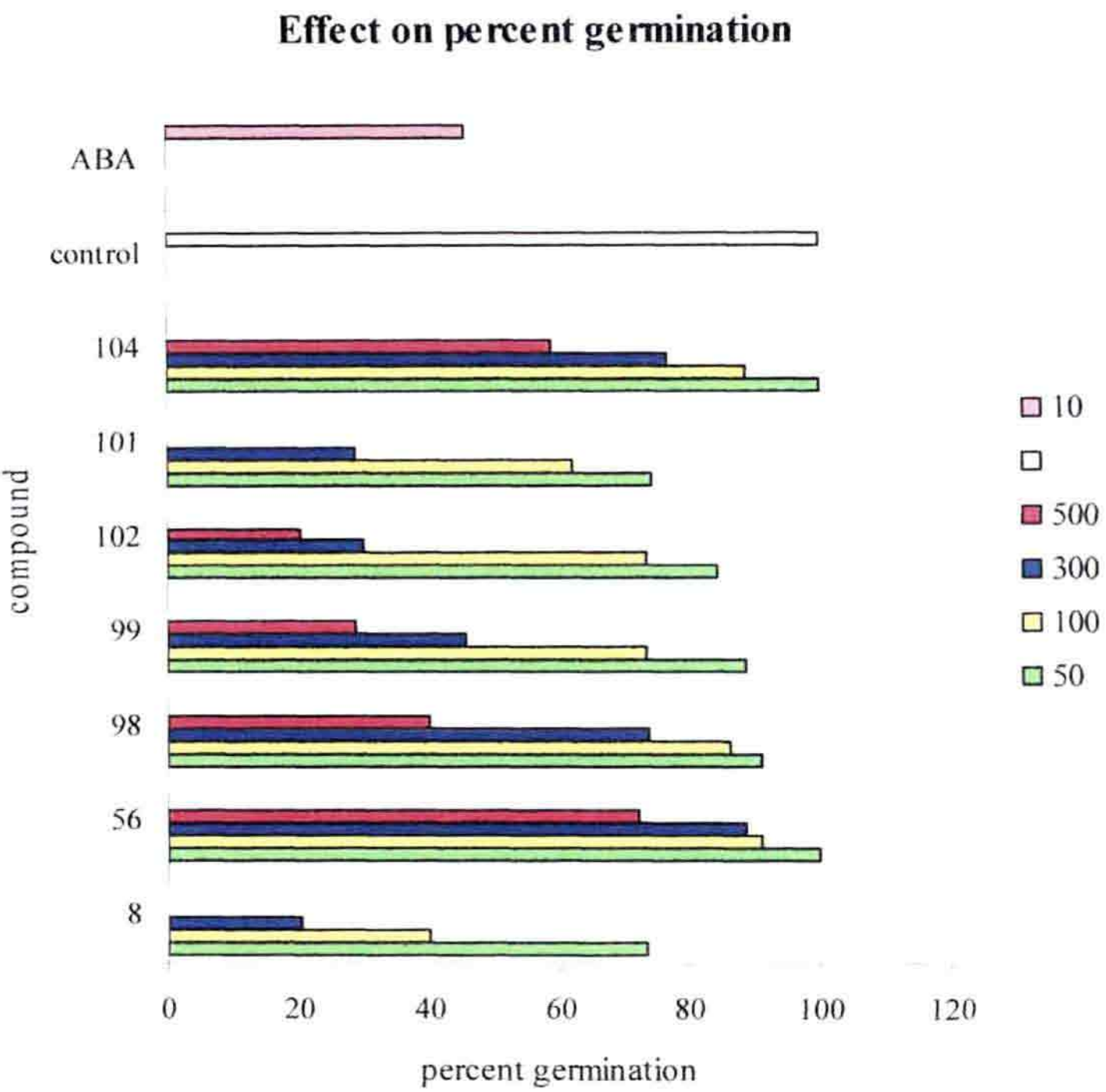


Figure 8: Effect of different concentrations of pseudoguaianolides on per cent germination of seeds of *Phlaris minor*

Table 12: Effect of some pseudoguaianolides on seedling growth of weeds

Compound No.	Concentration	*Avena fatua*				
		% germ	R.L.	S.L.	FW	DW
8	50	72.1	5.24	3.24	1.56	0.18
	100	42.6	4.28	1.83	1.08	0.085
	300	22.4	2.11	1.028	0.83	0.012
	500	0	-	-	-	-
56	50	100	6.25	4.464	3.45	0.98
	100	95.56	5.95	3.18	3.012	0.71
	300	95.56	4.18	2.265	2.83	0.56
	500	91.11	2.256	1.183	1.46	0.25
98	50	93.33	5.65	3.28	1.468	0.35
	100	93.33	4.347	2.57	1.31	0.28
	300	88.89	2.18	2.12	0.98	0.15
	500	84.44	1.46	1.83	0.46	0.09
99	50	100	6.448	4.18	3.28	0.915
	100	91.11	5.32	3.12	1.56	0.47
	300	84.44	4.57	1.98	1.40	0.31
	500	72.1	2.83	0.95	0.95	0.092
102	50	72.1	4.56	2.15	1.95	0.86
	100	42.6	3.48	1.93	1.32	0.56
	300	30.1	2.79	1.46	1.09	0.49
	500	22.4	1.36	0.95	0.86	0.012
101	50	73.3	3.49	1.985	1.56	0.73
	100	40.2	3.15	1.56	1.15	0.41
	300	20.3	2.46	1.012	0.98	0.001
	500	0	-	-	-	-
104	50	88.89	6.25	4.95	3.56	0.08
	100	86.67	4.48	4.18	1.78	0.76
	300	84.44	4.91	3.63	1.12	0.26
	500	72.1	3.46	2.12	1.06	0.018
Control		100	8.832	4.36	3.96	0.48
ABA	10	42.6	2.052	0.93	0.725	0.001
	20	0	-	-	-	-

Table 13: Effect of some pseudoguaianolides on seedling growth of weeds

Compound No.	Concentration	*Phlaris minor*				
		% germ	R.L.	S.L.	FW	DW
8	50	73.3	8.83	3.96	2.34	0.93
	100	40.2	7.42	2.85	2.18	0.56
	300	20.3	6.51	2.15	1.95	0.159
	500	-	-	-	-	-
56	50	100	11.93	7.62	4.25	1.012
	100	91.11	10.64	6.48	4.058	0.986
	300	88.84	9.38	5.47	3.83	0.732
	500	72.1	7.46	3.98	2.48	0.46
98	50	91.11	8.48	3.48	2.23	0.89
	100	86.42	7.36	2.56	2.15	0.50
	300	73.88	6.21	2.08	1.86	0.156
	500	40.20	4.98	1.56	1.52	0.09
99	50	88.84	7.42	4.56	2.57	0.85
	100	73.30	6.58	3.83	2.25	0.80
	300	45.62	5.26	2.42	1.75	0.148
	500	28.84	4.83	1.95	1.62	0.092
102	50	84.44	8.32	3.64	2.25	0.85
	100	73.30	7.48	3.43	2.18	0.78
	300	30.20	5.37	2.98	1.83	0.23
	500	20.30	4.29	2.12	1.78	0.152
101	50	74.44	6.93	2.14	1.98	0.16
	100	62.10	5.27	1.98	1.76	0.141
	300	28.80	4.18	1.43	1.43	0.085
	500	-	-	-	-	-
104	50	100	9.42	4.48	3.88	1.00
	100	88.89	8.98	4.12	3.12	0.95
	300	76.80	7.63	3.83	2.25	0.92
	500	58.80	6.41	3.26	2.18	0.85
Control			12.93	8.83	5.47	1.185
ABA	10		3.96	1.085	1.18	0.008
	20	0	-	-	-	-

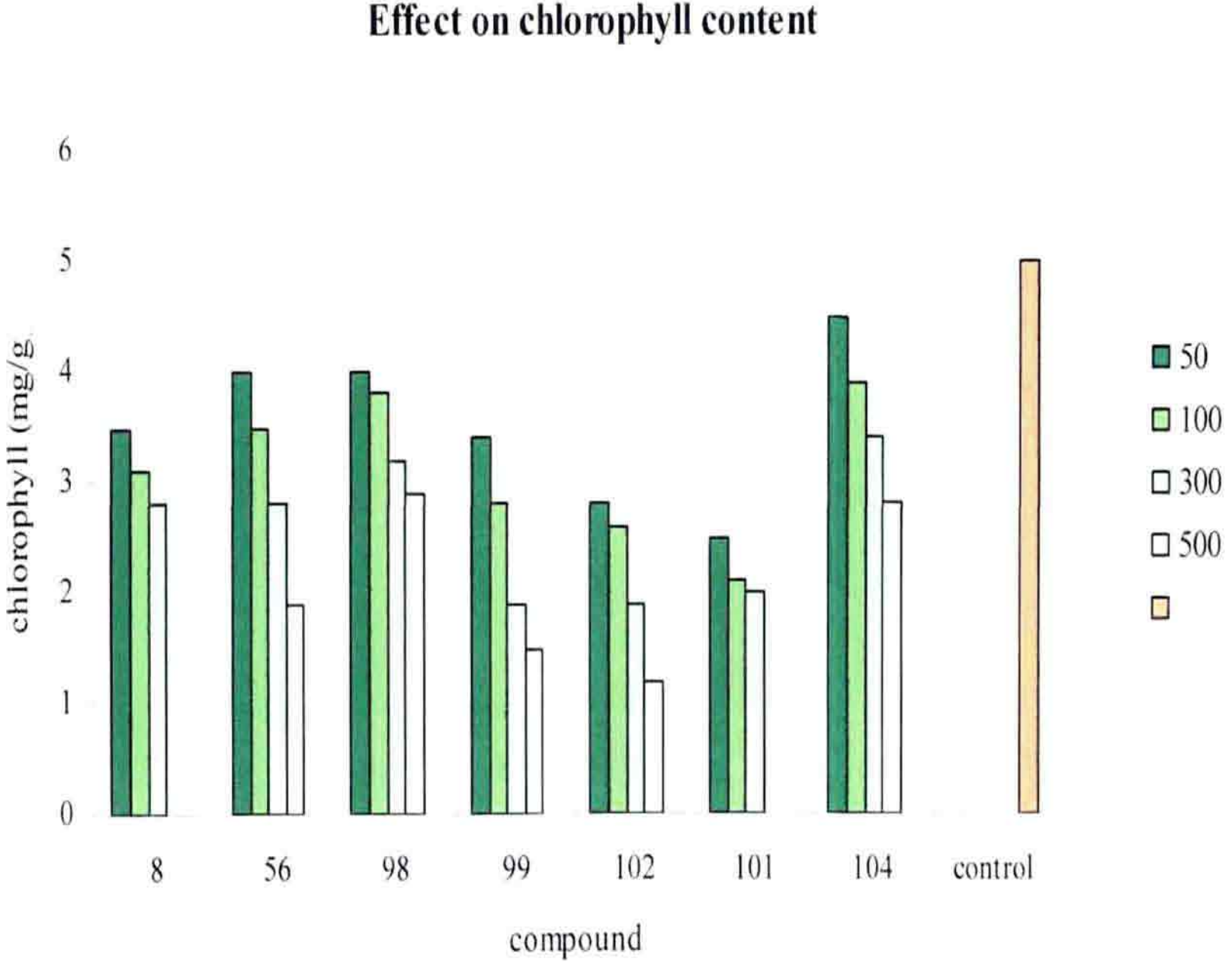


Figure 9: Effect of different concentrations of various compounds on chlorophyll content of seedlings of *Avena fatua*

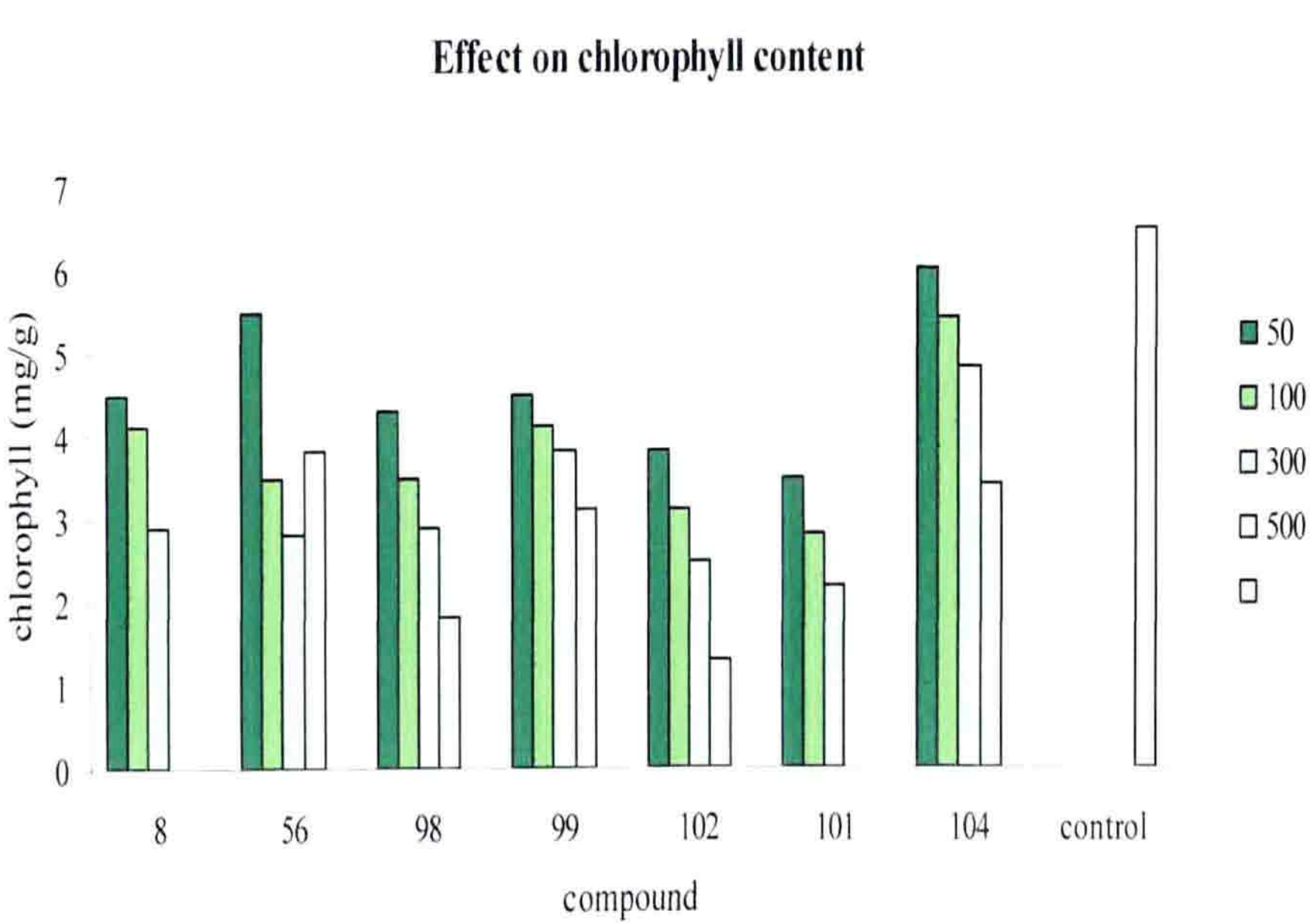


Figure 10: Effect of different concentrations of various compounds on chlorophyll content of seedlings of *Phlaris minor*

Among all the tested compounds, parthenin and its methoxy derivatives (101 and 102) were found to be most effective in inhibiting seed germination and seedling growth both in case of pea and the two weed species. The effectiveness can be related to the combined impact of the exomethylene group in conjugation with the lactone carbonyl and the intact cyclopentenone moiety. The inhibitory potential of the two methoxy derivativesreflects the importance of additional Michael receptor group in parthenin where the transformation to methoxy groups rendered the resulting compounds to be more lipophilic as compared to the parent compound, thereby, bringing out the significance of lipophilicity as one of the criteria for altering the growth regulating affects of these compounds.

However, the reasons for the impaired growth caused by these compounds cannot be solely determined based on these experiments. Possibly, these compounds may be acting through a reaction with -SH groups of amino acids present in proteins, a pathway utilized by sesquiterpene lactones in general. The effect is to interfere with normal plant functioning, resulting in impaired growth. Parthenin is also known to bring about some physiological changes such as damage of cell membrane, loss of dehydrogenase activity in roots and decreased water content in leaves of target plants (Pandey 1996).

The inhibitory effects of the alantolides (2 and 3) and their derivatives may be attributed to the unique carbon skeleton backbone with two 6-memebered rings in conjunction with the α-methylene-γ-lactone. The significance of the exomethylene at C_4 is brought to light from the higher effectiveness of isoalantolactone in causing retardation of growth as compared to its double bond isomer as well as alantolactone.

With regard to the studies carried out on dehydrocostus lactone (1) and its analogues, it was concluded that introduction of an additional carbon atom at C_{13} caused an increase in activity. Also the introduction of cyclopropyl ring led to increase in activity and the effect was further ameliorated with the addition of a methyl group. However, a further increase

in chain length seemed to have a retarding affect on the activity of the compounds.

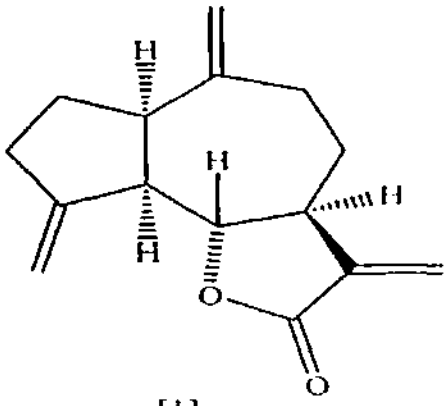
In case of the pyrazoline derivatives, there was no significant change in activity as compared to the controls. The possible reason for this may be the probable decomposition of the pyrazolines within the plant systems to inactive compounds. Among the epoxy derivatives, the low activity in all the cases may be attributed to the increase in polarity with the addition of oxygen atoms, which resulted in decrease in lipophilic character, thereby, leading to decrease in activity.

On the whole, this study suggests that among the various classes of sesquiterpene lactones *viz.* eudesmanolides, guaianolides and pseudoguaianolides, pseudoguaianolides may be good candidates for the development of new natural product-based herbicides. In this way, small modifications can induce conformational and reactivity changes that modulate or change the bioactivity.

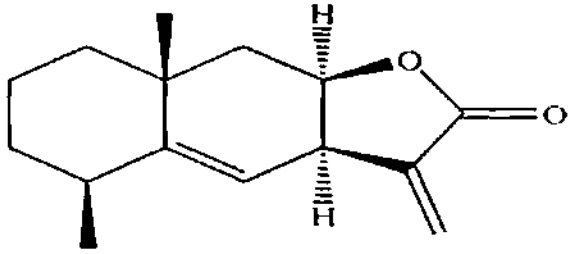
CHAPTER 5

SUMMARY

The work presented in this dissertation includes the extraction and purification of sesquiterpene lactones from *viz.* dehydrocostus lactone (1) from *Saussurea lappa*, alantolactone (2) and isoalantolactone (3) from *Inula racemosa*, parthenin (4) from *Parthenium hysterophorus* and lactucin (5), 8-deoxylactucin (6) and lactucopicrin (7) from *Cichorium intybus* through Soxhlet extraction followed by CC.



[1]



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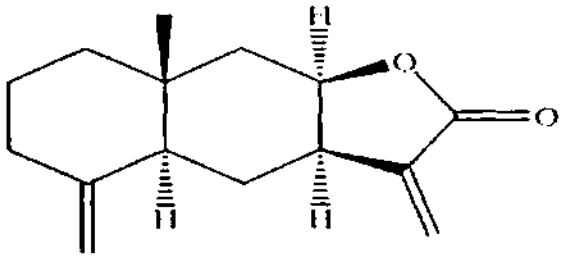
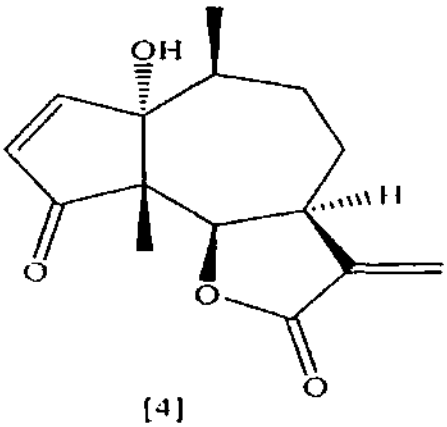


[3]



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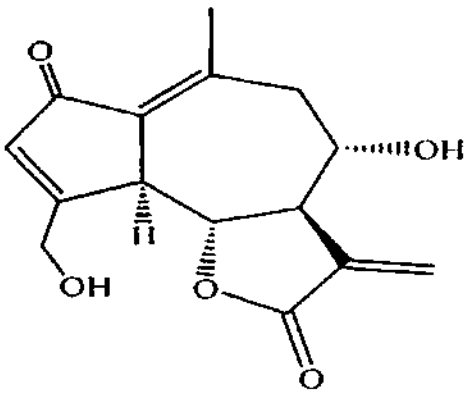

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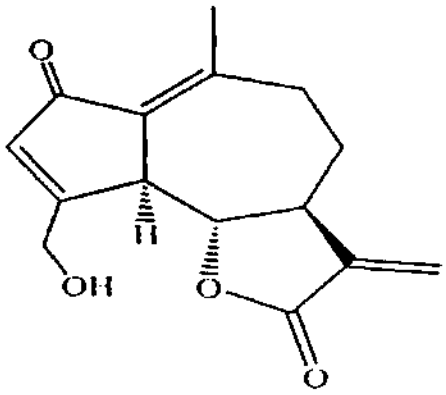

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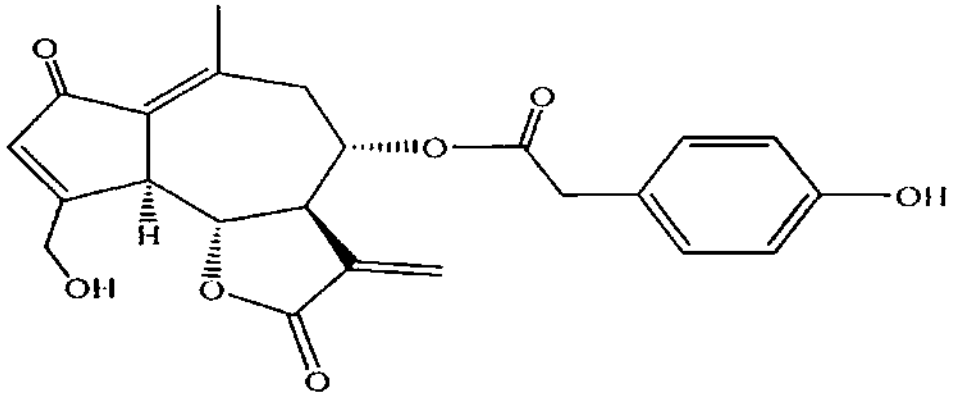
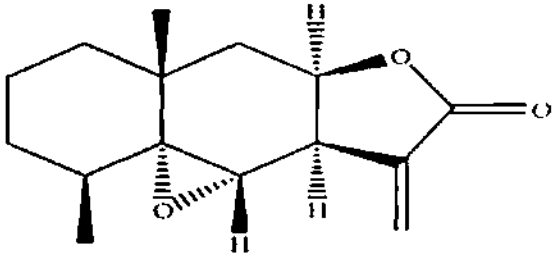


[7]

In the process, four new alantolides have been isolated from *Inula racemosa* by PTLC (8-11).



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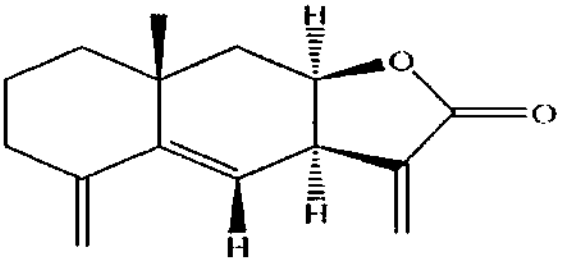

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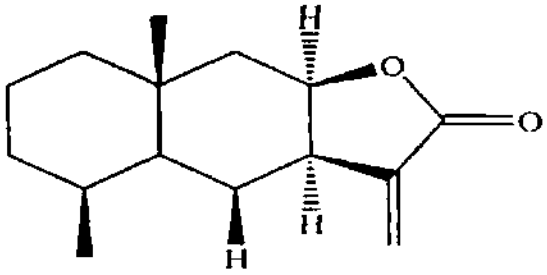

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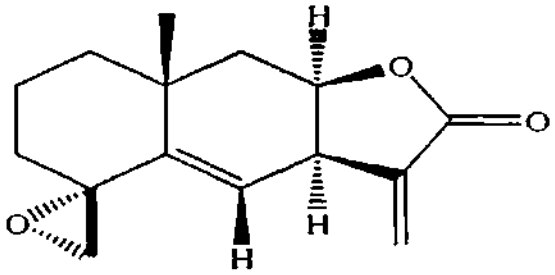


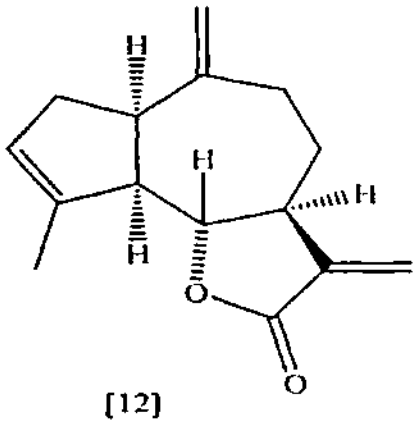
[11]

During the extraction of sesquiterpenoids from the roasted roots of chicory, isodehydrocostus lactone (12) has been isolated but the natural occurrence of the compound in the plant material is not confirmed.

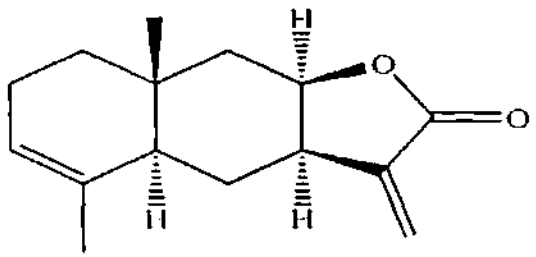
During the research, microwave energy has been extensively used to carry out different types of chemical reactions and the rates of the reactions have been compared with those of the conventional methods. Different categories of reactions carried out under microwave irradiated conditions include:

- Double bond isomerisation
- Decomposition of pyrazolines
- Decomposition of Mannich bases
- Hoffmann elimination of quaternary ammonium salts

Double bond isomerisation has been successfully achieved by using microwave energy with substantial increase in rate of reaction as compared to the conventional method employing iodine in benzene. The reaction was attempted on dehydrocostus lactone (1) and isoalantolactone (3) and their corresponding double bond isomers (12 and 13) were obtained.

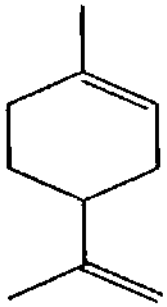


[12]

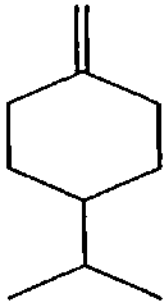


[13]

Similarly, a few monoterpenes (14 and 15) were also irradiated with microwaves to afford their respective isomers (16-18).



[14]



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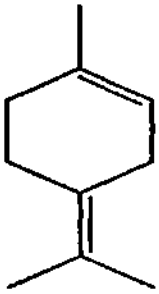
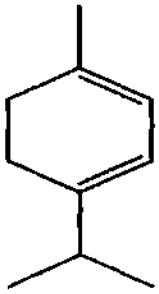
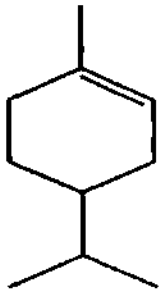


[16]

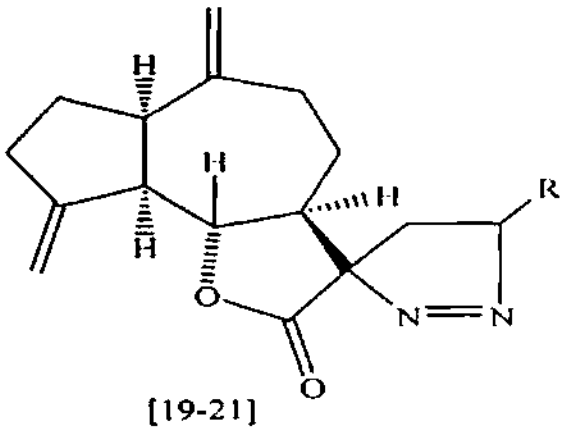


[17]



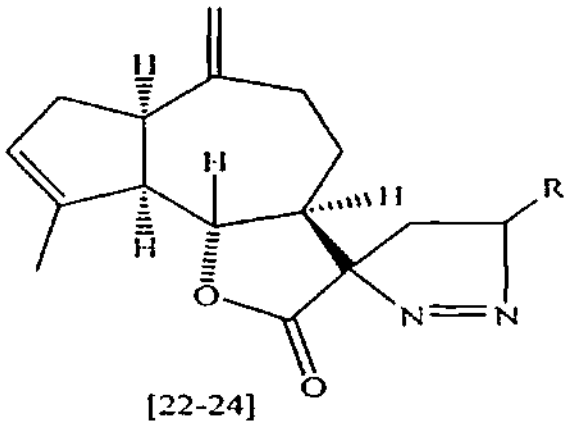
[18]

Pyrazoline derivatives of various sesquiterpene lactones were prepared using diazomethane, diazoethane and diazopropane.



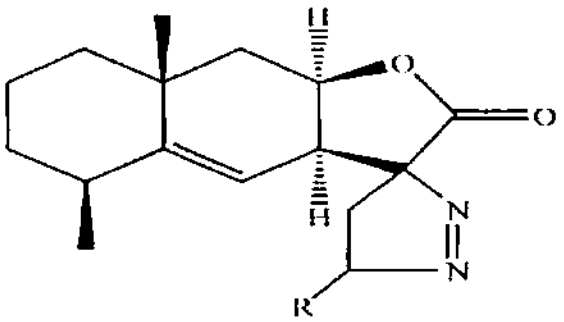
[19-21]

19. R= H
20. R= CH_3
21. R= C_2H_5



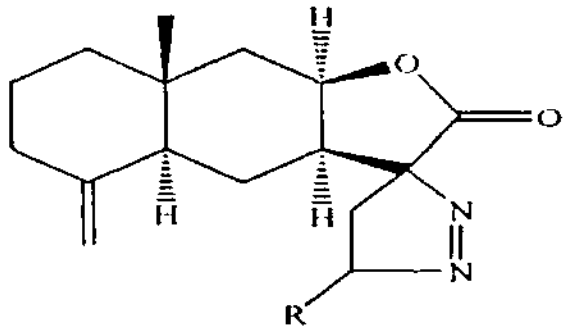
[22-24]

22. R= H
23. R= CH_3
24. R= C_2H_5



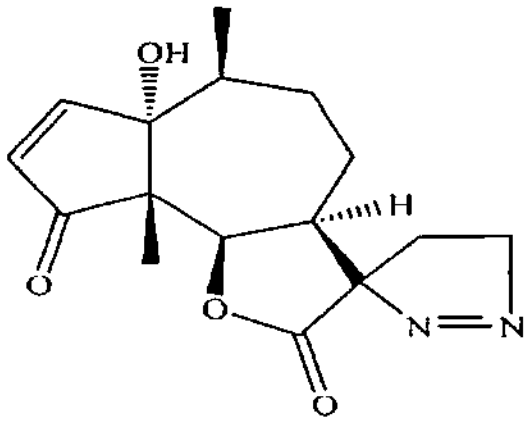
[25-27]

25. R= H
26. R= CH_3
27. R= C_2H_5



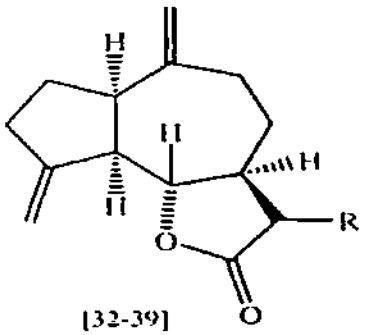
[28-30]

28. R= H
29. R= CH_3
30. R= C_2H_5

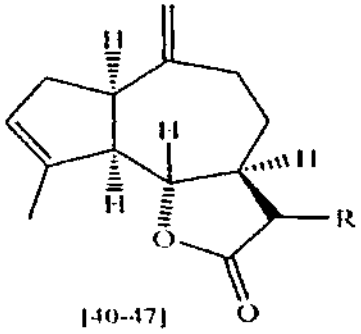


[31]

All the pyrazoline derivatives were decomposed on solid matrix using microwave radiations to afford the corresponding olefinic and cyclopropyl derivatives (32-65).



[32-39]



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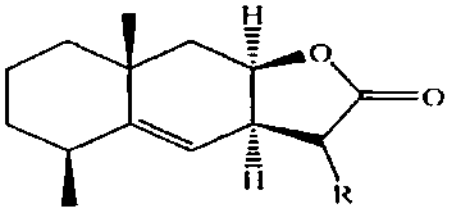
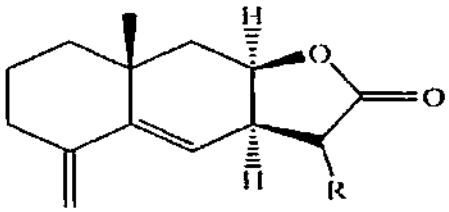
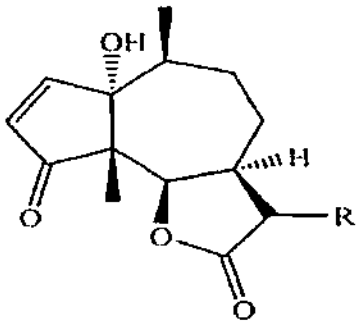


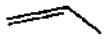
[48-55]



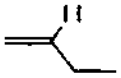
[56-63]

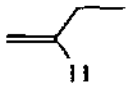


[64-65]

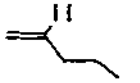
32, 40, 48, 56, 64. R= 

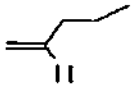
33, 41, 49, 57, 65. R= 

34, 42, 50, 58. R= 

35, 43, 51, 59. R = 

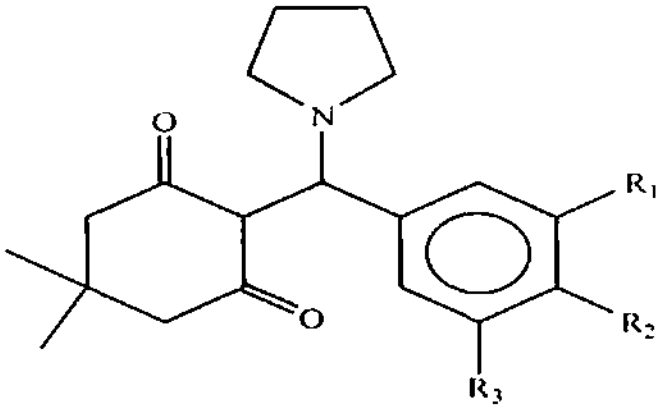
36, 44, 52, 60. R= 

37, 45, 53, 61. R= 

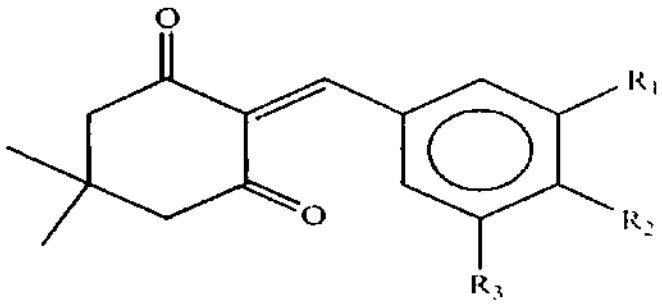
38, 46, 54, 62. R= 

39, 47, 55, 63. R= 

Several Mannich bases (66-71) were also irradiated with microwaves to furnish the respective arylidenes (72-77) with promising yields.



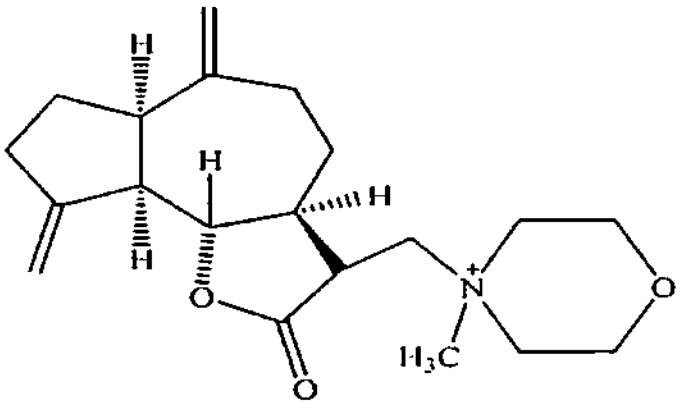
[66-71]



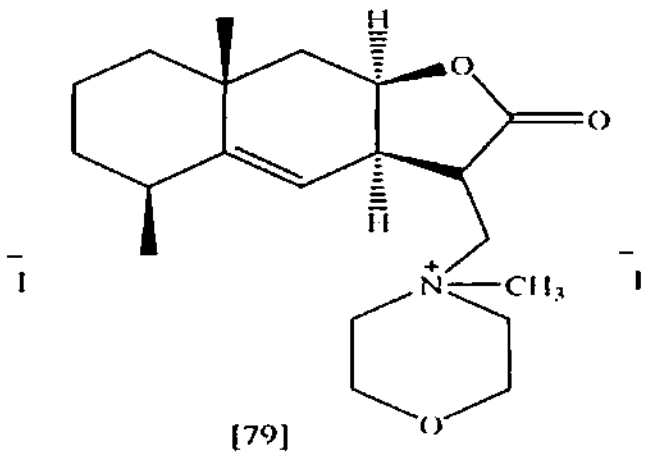
[72-77]

	R_1	R_2	R_3
66, 72.	H	H	H
67, 73.	CH_3	CH_3	CH_3
68, 74.	H	Cl	H
69, 75.	H	OCH_3	H
70, 76.	H	$-\overset{+}{N}$	H
71, 77.	NO_2	H	H

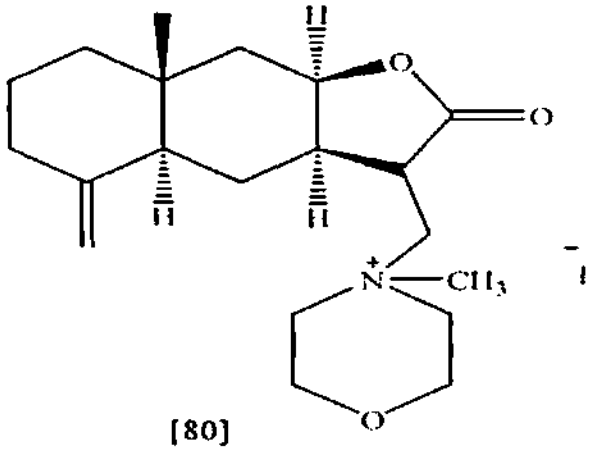
The quaternary ammonium salts of sesquiterpene lactones were decomposed into the parent lactones (78-84).



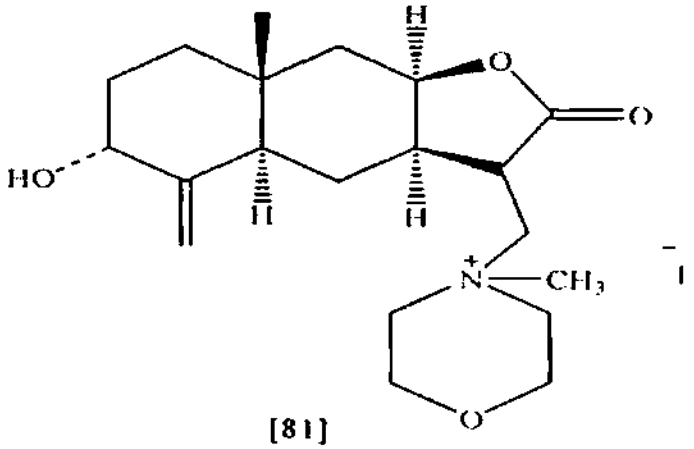
[78]



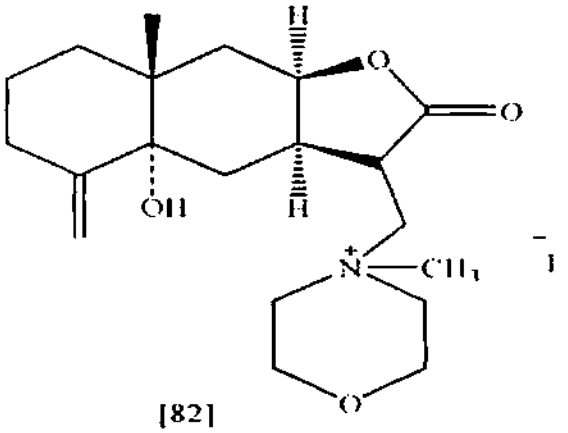
[79]



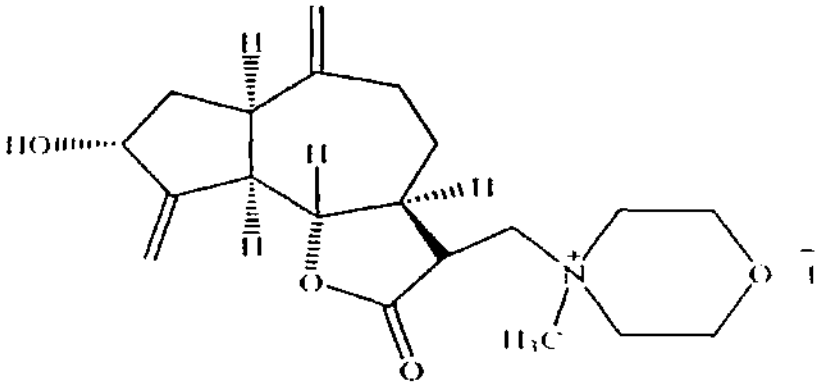
[80]



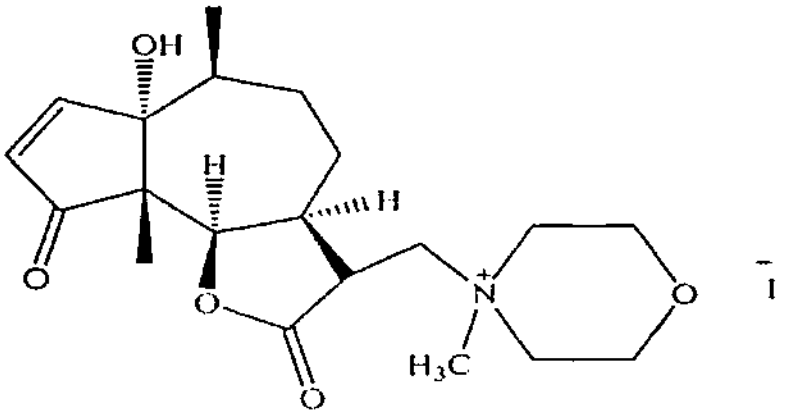
[81]



[82]

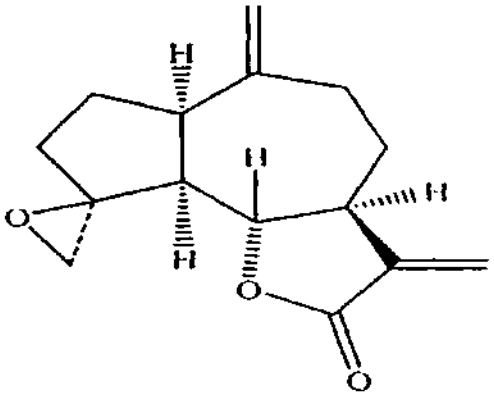


[83]

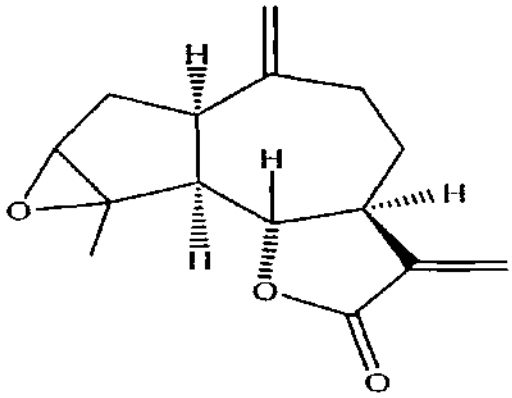


[84]

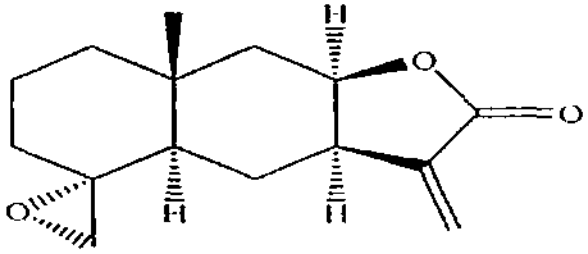
Epoxidations: Sodium perborate has been employed to carry out the epoxidation of various sesquiterpene lactones to afford the epoxy derivatives (85-88). The reaction was carried out using acetic anhydride in combination with dichloromethane in different proportions, isopropanol and water and in the presence of PTC. Acetic anhydride and dichloromethane in the ratio 9:1 proved to be the most suitable solvent system for the reaction.



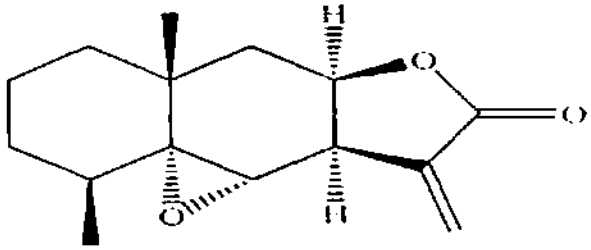
[85]



[86]

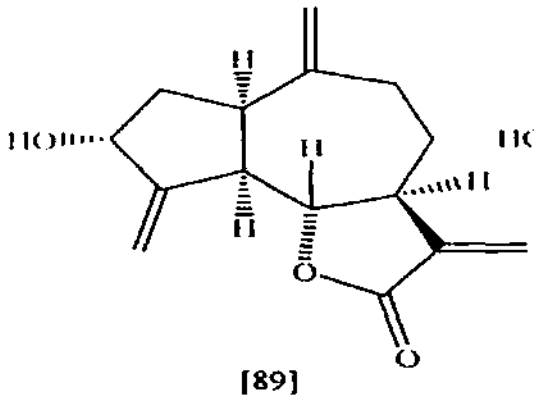


[87]



[88]

Allylic oxidations: Selenium dioxide in combination with TBHP is a well known reagent for allylic oxidation. It has been used to carry out the oxidation of several sesquiterpene lactones leading to the formation of allylic alcohols (89-95). Same reaction was also attempted under microwave irradiated conditions which proved to be highly efficient in terms of considerable reduction in reaction times.



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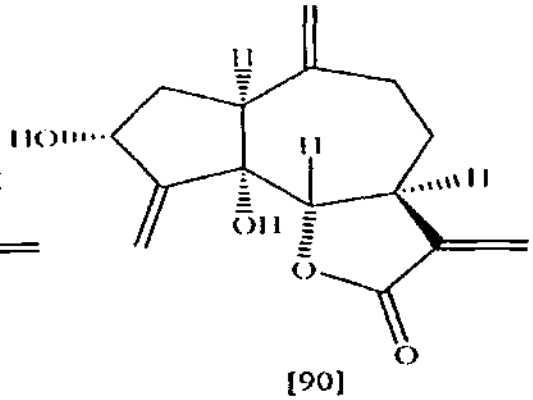
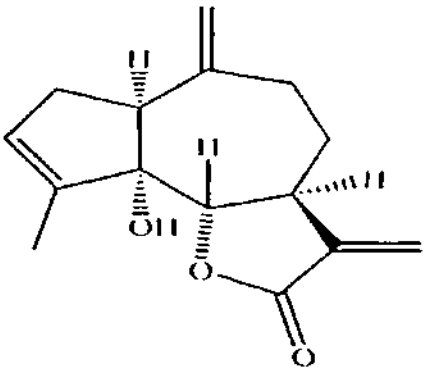


[90]



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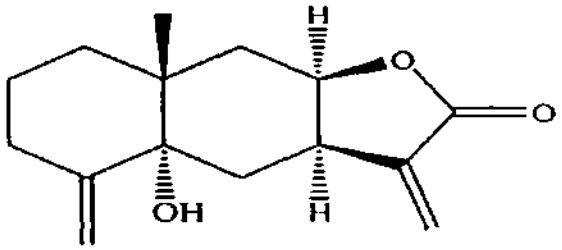

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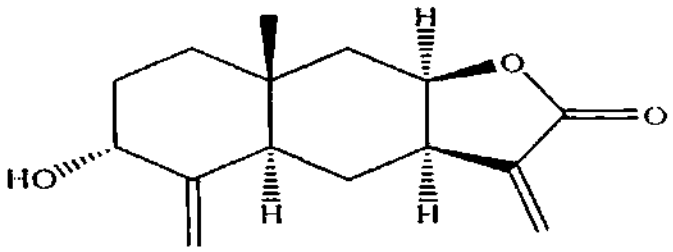

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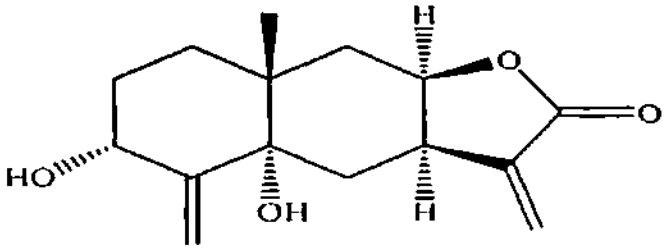

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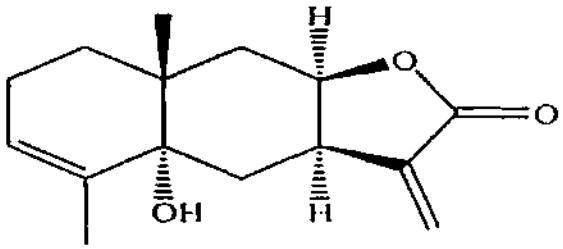
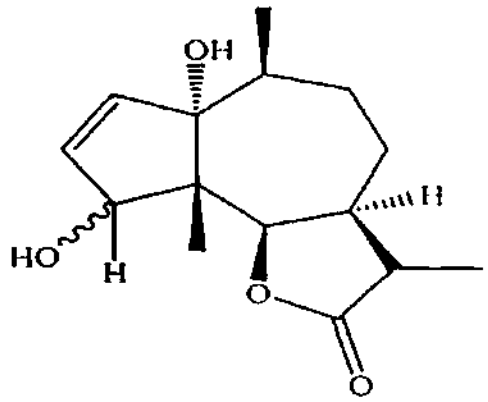
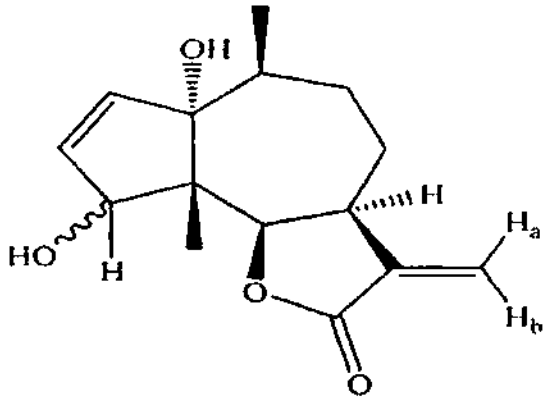


[95]

Owing to the presence of two conjugated double bonds in parthenin, one in conjugation with the lactone and the other with the carbonyl group, it attracts attention as a suitable substrate for reduction. Thus, reduction reactions of parthenin were attempted using a number of reducing agents. Reduction of parthenin with sodium borohydride afforded two products (96 and 97). A similar reaction with zinc borohydride yielded a single product (96). The reaction with sodium cyanoborohydride, in addition to the hydroxyl derivative (96), furnished two methoxy analogues of parthenin (98 and 99). Lithium tri-*tert*-butoxy aluminohydride reduced parthenin to two compounds (96 and 100). Reduction with magnesium in methanol was attempted for parthenin and anhydroparthenin. The reaction with parthenin formed mono- and di-methoxy derivatives (98 and 99) and the reaction with anhydroparthenin yielded (101).



[96]



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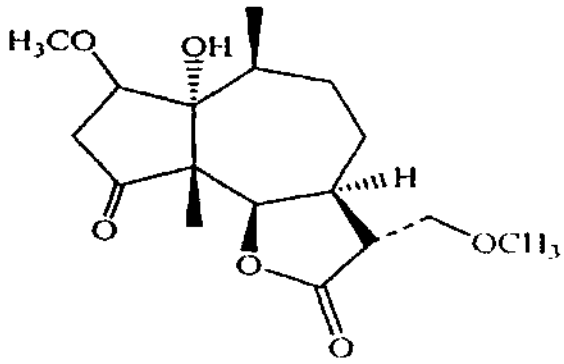

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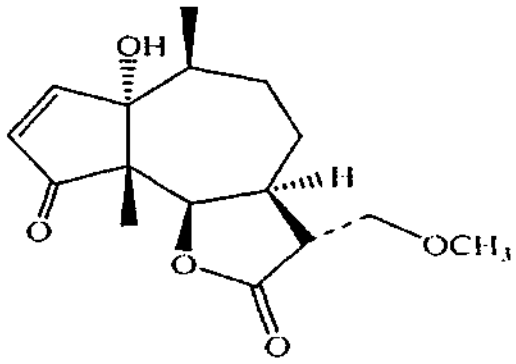

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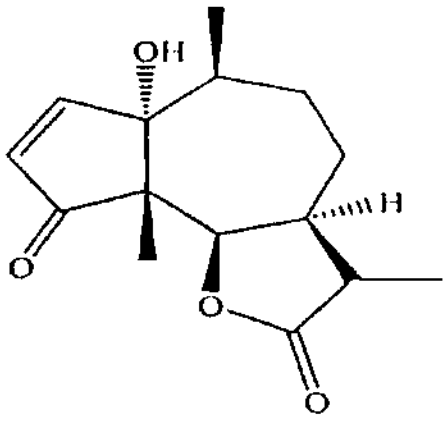
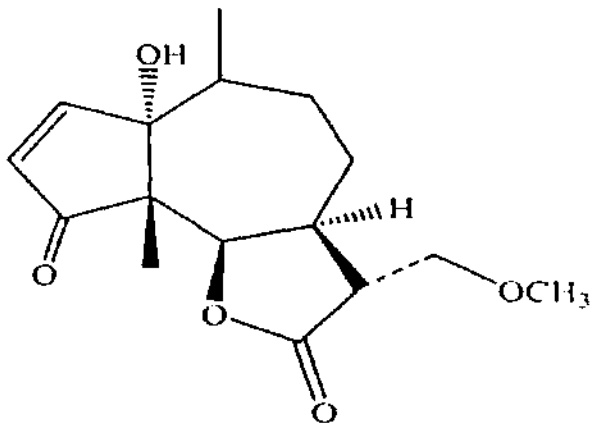


[100]



[101]

All the natural compounds isolated during the study, along with their synthetic analogues were screened for their plant growth regulatory effects on the seeds of *Pisum sativum*. Parthenin and its derivatives which showed maximum growth inhibitory effects on the pea seeds were tested for their weedicidal potential on two weed species *Avena fatua* and *Phlaris minor*. The

study revealed that parthenin and its methoxy derivatives possess promising potential as herbicides.

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