THE ISOLATION AND IDENTIFICATION OF SESQUITERPENE LACTONE CONSTITUENTS OF THE PLANTS *CENTAUREA CHEIROLOPHA WAGENİTZ* AND *CENTAUREA CUNEIFOLIA SM.*

by

İZLEM YETÜT

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APPROVED BY:

Prof. Dr. Belkıs HALFON
(Thesis Supervisor) .....

Assoc. Prof. İlknur DOĞAN .....

Prof. Dr. Sevil Öksüz .....

30th of April, 1998

DATE OF APPROVAL .....

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ABSTRACT

The aim of this study is the isolation and identification of the physiologically active terpenic constituents of the plants *Centaurea cuneifolia Sm.* (Compositae) and *Centaurea cheirolopha Wagenitz* (Compositae). Former studies with *Centaurea* species have resulted in the identification of terpenoids as well as flavonoids and other natural compounds.

Centaurea cuneifolia Sm. yielded two sesquiterpene lactones which have been identified on the basis of Infra Red and Nuclear Magnetic Resonance Spectra. The two sesquiterpene lactones identified are Dihydromelitensin and 8α-(3,4-dihydroxy-2-methylene butanoic acid) ester of Dihydromelitensin.

Centaurea cheirolopha Wagenitz yielded four sesquiterpene lactones. Infra Red, Nuclear Magnetic Resonance, and Mass Spectra have been used in the identification of the four sesquiterpene lactones, namely Aguarin B, Cynaropicrin, 8α-(2,3-dihydroxy-2-methyl propanoic acid) ester of Zaluzanin-C and 15-deschloro-15-hydroxychlorojanerin. Eventhough 15-deschloro-15-hydroxychlorojanerin has been formerly isolated from other plant species, this is the first study where it has been isolated from *Centaurea* species. The structure of this sesquiterpene lactone has been identified on the basis of ^{13}C NMR (Attached Proton Test-APT) in addition to the previous spectroscopic methods.

ÖZET

Bu çalışmanın amacı *Centaurea cheirolopha Wagenitz* ve *Centaurea cuneifolia Sm.* bitkilerinin fizyolojik aktivite gösterebilecek terpenik bileşiklerini ayırmak, saflaştırmak ve kimyasal yapılarını aydınlatmaktır.

Bundan önceki çalışmalar *Centaurea* türlerinin terpenik bileşiklerin yanısıra flavonoit ve diğer doğal bileşikleri de içerdiğini ortaya koymuştur.

Bu çalışmada *Centaurea cuneifolia Sm.* bitkisinden iki seskiterpen lakton elde edilmiştir. Bunların yapıları IR ve NMR spektroskopisi yöntemleri ile Dehydromelitensin ve Dehydromelitensin'in 8α-(3,4-dihidroksi-2-metilen bütanoik asit) esteri olarak belirlenmiştir.

Centaurea cheirolopha Wagenitz bitkisinden ise dört seskiterpen lakton elde edilmiştir. Bunlar Aguarin B, Cynaropicrin, Zaluzanin-C'nin 8α-(2,3-dihidroksi-2-metil propanoik asit) esteri ve *Centaurea* türleri içinde ilk defa bulunan 15-deschloro-15-hidroksiklorojanerindir. Bu seskiterpen laktonun yapısı IR, NMR ve Kütle Spektoskopik yöntemlerin yanısıra ^{13}C NMR (APT) spektrumu yardımıyla saptanmıştır.

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LIST OF SYMBOLS

APT	Attached Proton Test
CC	Column Chromatography
CC1	The First Compound Isolated from *Centaurea cheirolopha Wagenitz* (Compositae)
CC2	The Second Compound Isolated from *Centaurea cheirolopha Wagenitz* (Compositae)
CC3	The Third Compound Isolated from *Centaurea cheirolopha Wagenitz* (Compositae)
CC4	The Fourth Compound Isolated from *Centaurea cheirolopha Wagenitz* (Compositae)
CCU1	The First Compound Isolated from *Centaurea cuneifolia Sm.* (Compositae)
CCU2	The Second Compound Isolated from *Centaurea cuneifolia Sm.* (Compositae)
EI-MS	Electronic Ionization Mass Spectroscopy
GLC	Gas-Liquid Chromatography
HPLC	High Performance Liquid Chromatography
MPLC	Medium Performance Liquid Chromatography
TLC	Thin Layer Chromatography

1. INTRODUCTION

For many years terpenoids were an interesting subject for academic research. More recently with the advent of sophisticated methods of isolation, identification and structure determination, terpene research has expanded greatly and terpenes have emerged as one of the most diverse and intriguing groups of natural products.

Centaurea (Compositae) is a genus which has 172 species in Turkey [1], most of which are endemic. Some of these species have served as folk medicine. *Centaurea cyanus L.* species, which is widespread in the West and South-west Anatolia regions, is used for its antidiarrheatic activity. The flowers of *Centaurea behen L.* are good against stomach disorders. In North-west Anatolia *Centaurea calcitrapa L.* species serves as antipyretics, and in North-east Anatolia *Centaurea jacea L.* species is used for its purgative and antipyretic activities [2].

Centaurea cuneifolia Sm. is found in North-West Turkey in Edirne, Tekirdağ, Kırklareli, İstanbul, Çanakkale and Balıkesir; and in Greece and Bulgaria. *Centaurea cheirolopha Wagenitz* is found in South-Anatolia, Niğde, İçel, Adana, Hatay, Maraş, and also in Lebanon [1].

Centaurea species are known to be rich in sesquiterpene lactones of various types, many of which have been shown to be biologically active [3]. The sesquiterpene lactone constituents of *Centaurea cheirolopha Wagenitz* and *Centaurea cuneifolia Sm.* have not been studied before. Therefore these two plants were chosen and this study concerns the isolation and identification of the sesquiterpene lactone constituents of *Centaurea cuneifolia Sm.* and *Centaurea cheirolopha Wagenitz.*

2. THEORY

The study of naturally occuring compounds from plants has progressed to its present state of development by passage through several distinct stages. Most of the early investigations of natural compounds were centered around those materials- mostly vegetable drugs with recognized poisonous or medicinal properties or fragrant oils and food flavoring materials- that were readily accesible as articles of commerce or were used in a way or another by man. Since theories of chemical constitution and concepts of molecular structure did not develop until the latter part of the nineteenth century, little progress was made in the study of these compounds most of which are of quite complex structure.

By the time chemical theory had progressed to a stage at which concepts of molecular structure began to develop, a mass of experimental data had accumulated and the application of structural theories to the existing empirical knowledge led to rapid advances in the establishment of the structures of many natural products. During the development of natural products chemistry, the isolation and identification of new natural compounds continued but the chief concern of the organic chemist now became the application of developing structural concepts to the proof of the structures of the known compounds and the confirmation of structure by total synthesis.

Terpene chemistry began with investigations of the many essential oils that have been used by man, chiefly as perfumes and to some extent as flavoring materials in food, from the most remote times. Present-day investigations of the chemistry of simple terpenes involve largely in solution of the remaining questions of stereochemistry, the study of the many novel chemical transformations of these compounds and the development of improved methods for their synthesis. Pioneering work continues, for the immense area of the chemistry of higher (C_{15} to C_{40}) terpenoid and steroid compounds continues to provide limitless opportunities for important advances in organic chemistry.

It is becoming clear that new goals and purposes are now being added to the study of natural products, and a new phase of inquiry is beginning. The research in natural

products chemistry ranges far beyond the limits of the search for drugs or other compounds with practical uses, and even beyond the challenge presented by an unknown structure of a new compound. The structures of naturally occuring compounds are often reflections of genetic individuality, and many of them are now recognized as valuble indicators of the relationships between the living organisms from which they are derived and of the chemical transformations that take place in the cell [4].

2.1. Terpenoids

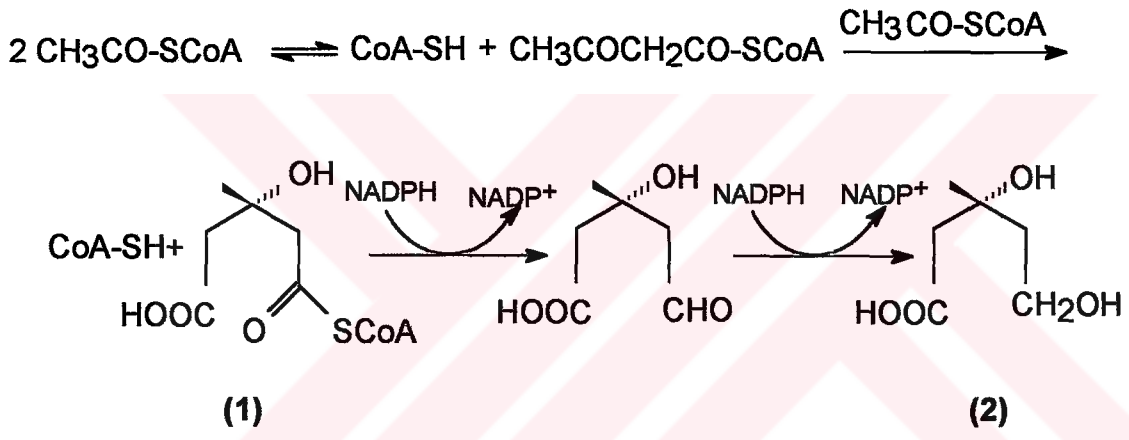
Chemically, terpenoids are generally lipid-soluble and are located in the cytoplasm of the plant cell.

A considerable number of quite different functions have been ascribed to plant terpenoids. Their growth regulating properties are very well documented and also some of them are found to be physiologically active.The important contribution of carotenoids to plant color is well known and it's almost certain that C_{40} terpenoids are also involved as accessory pigments in photosynthesis. The importance of mono and sesquiterpenes in providing plants with many of their distinctive smells and odours is also familiar to most scientists. Less is generally known of the role of terpenoids in the more subtle interactions between plants and animals, e.g. as agents of communication and defence among insects, but this's now an area of active research. Finally it should be mentioned that certain non-volatile terpenoids have been implicated as sex hormones among the fungi [5].

Among the terpenoids, sesquiterpene lactones are of special importance due to their anti-tumor, cytotoxic, phytotoxic, anti-bacterial and anti-fungal activities. Other properties possessed by these sesquiterpene lactones include their bitter or pungent taste and their ability to act as allergens [6].

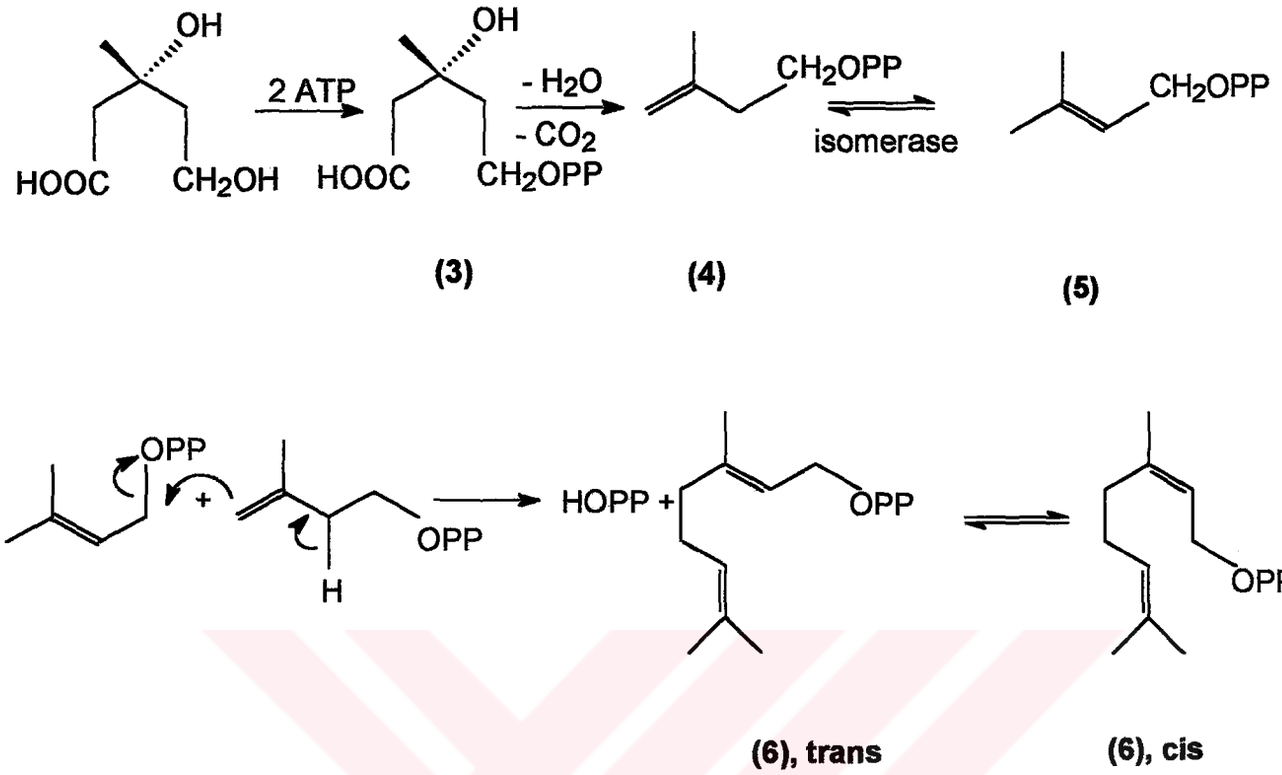
2.1.1. Biosynthesis of Terpenoids:

An enormous range of plant substances are covered by the word "terpenoid", a term which is used to indicate that all such substances have a common biosynthetic origin. Acetyl coenzyme-A (CH_3CO-SCoA), which is a complex thiol derivative, is a fundamental compound in the biosynthesis of many natural products (FIGURE 2.1). It's formed from the oxidative degradation of carbohydrate units. Condensation of two acetyl coenzyme-A units into 3-hydroxy-3-methyl glutaril coenzyme-A (1) and subsequent step-wise reduction to mevalonic acid (2) is shown below:



The steps involved in the transformation of mevalonic acid to the isopentane unit are believed to be as follows:

Phosphorylation of mevalonic acid with two moles of adenosine triphosphate (ATP) gives mevalonic acid 5-pyrophosphate (3) which decarboxylates and dehydrates to give 3-methyl-3-butenyl (isopentyl) pyrophosphate (Δ^3-IPP) (4) which is the precursor of isoprene units that generates the terpenoids. An enzymatic isomerization to 3-methyl-2-butenyl pyrophosphate (Δ^2-IPP) (5) and subsequent condensation of the two isomers produces geranyl prophosphate.(6)



Geranyl pyrophosphate serves as the precursor of cyclic monoterpenoids via the cis isomer. It condenses with Δ^3-IPP to farnesyl pyrophosphate, the biosynthetic precursor of sesquiterpenes. Farnesyl pyrophosphate in turn condenses with Δ^3-IPP to form geranyl-geranyl pyrophosphate, precursor to diterpenes and carotenoids. The triterpenoids are biosynthesized from squalene which is produced from the tail-to-tail linkage of two farnesyl pyrophosphate units (FIGURE 2.2) [4].

Terpenoids are classified according to whether they contain two (C_{10}), three (C_{15}), four (C_{20}), six (C_{30}) or eight (C_{40}) isoprene units. They range from essential oil components, the volatile mono- and sesquiterpenes (C_{10} and C_{15}), through the less volatile diterpenes (C_{20}) to the involatile triterpenoids and sterols (C_{30}) and carotenoid pigments (C_{40}) [5].

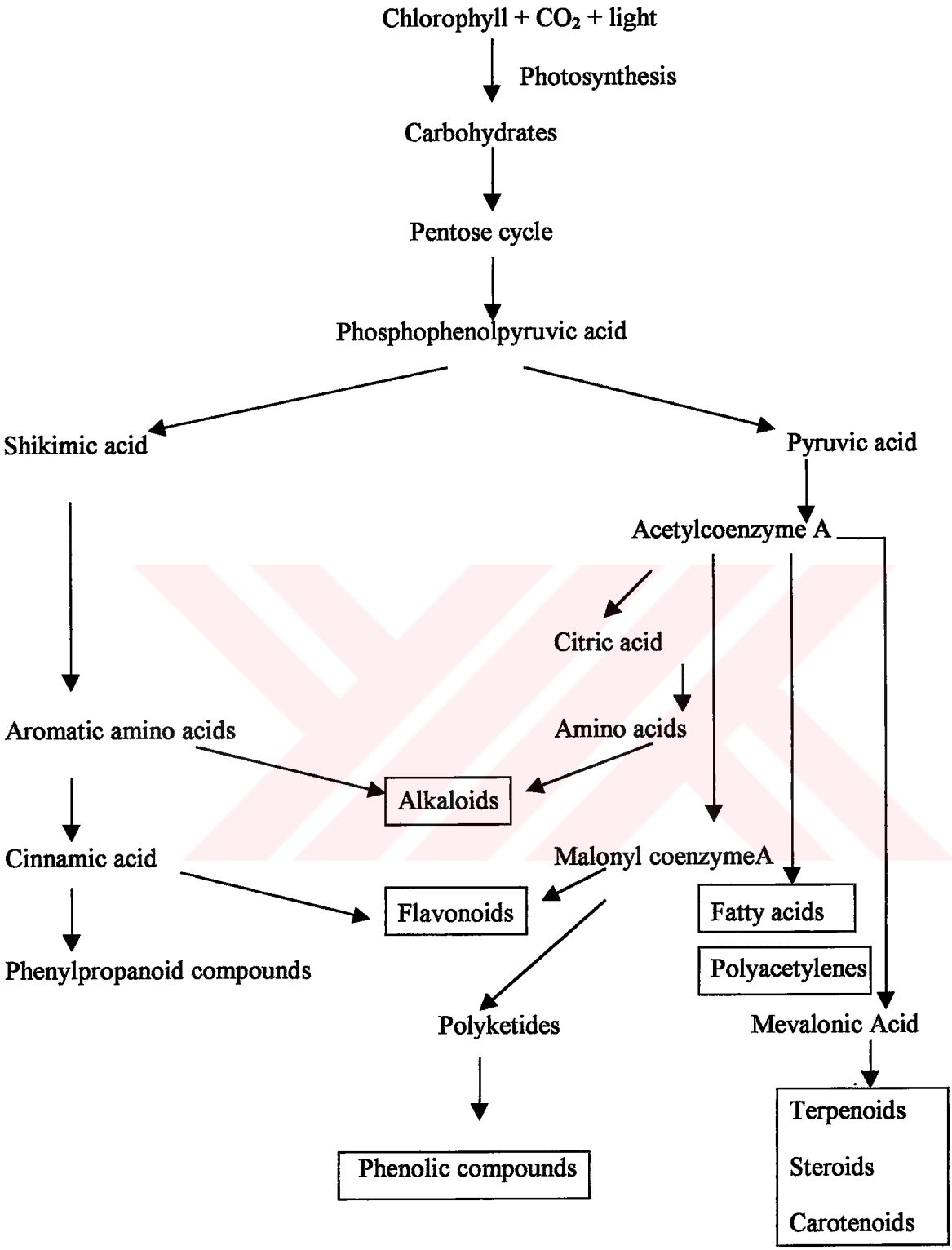


FIGURE 2.1 Biosynthetic Pathways of Secondary Plant Metabolism

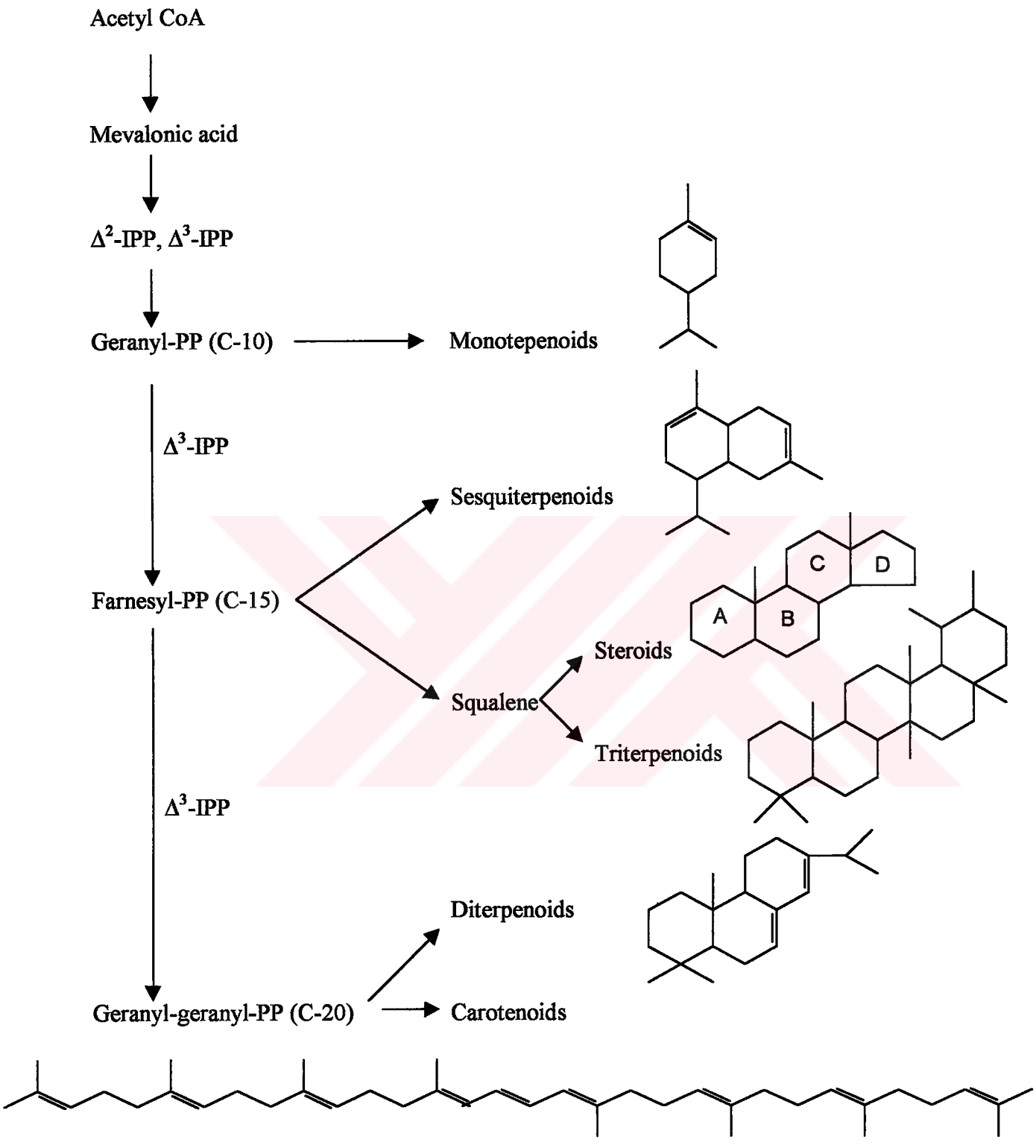
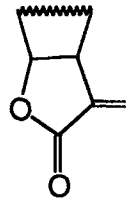


FIGURE 2.2 Biosynthetic Formation of Terpenoids

2.1.1. Sesquiterpenes

The sesquiterpenes are naturally occurring compounds, which contain three isoprenoid units with fifteen carbon atoms. They may be acyclic or cyclic hydrocarbons, alcohols, ketones or lactones [7]. A large, structurally varied and botanically closely allied class of sesquiterpenes is a group of lactones found distributed throughout plants of the family Compositae [4]. Among the sesquiterpene lactones, over 2000 compounds are known today [5].

The classification of sesquiterpene lactones is based on their carbocyclic skeleton in which the suffix "olide" refers to the lactonic function. In sesquiterpene lactones formed by oxidation of the "head" methyl group, the lactonic function commonly represents a α-methylene γ-lactone moiety (7), or a modified functionality derived from 7. The α, β-unsaturated lactone is either cis- or trans-fused to the C6-C7 or C8-C7 positions of the carbocyclic skeleton. Structural modifications of the basic terpene skeleton involve the incorporation of an epoxide ring, hydroxyl groups (generally esterified), and/or a 5-carbon acid, such as tiglic or angelic acid. Some sesquiterpene lactones also contain covalently bound halogen atoms [8]. The most common ester groups attached to sesquiterpene lactones are listed in TABLE 2.1.



(7)

TABLE 2.1 Common Side Chains in Sesquiterpene Lactones

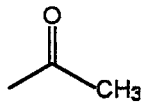
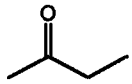
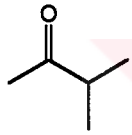
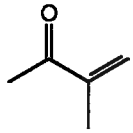
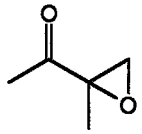
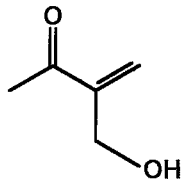
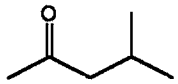
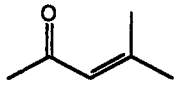
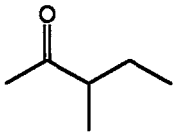
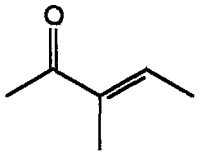
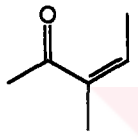
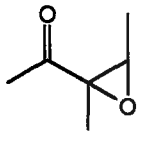
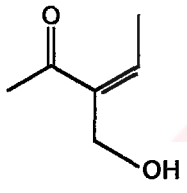
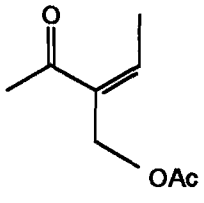
Structure of Side Chain	Type of Ester	Abbreviation
	Acetate	Ac
	Propionate	Pro
	Isobutyrate	i-But
	Methacrylate	Mac
	Epoxymethacrylate	Epoxymac
	4-Hydroxymethacrylate	Mac-4-OH
	Isovalerate	I-Val
	Senecioate	Sen

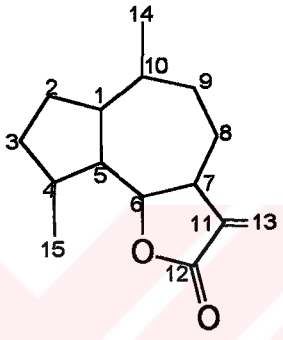
TABLE 2.1 Common Side Chains in Sesquiterpene Lactones (Continued)

Structure of Side Chain	Type of Ester	Abbreviation
	2-Methylbutanoate	2-Mebut
	Tiglate	Tig
	Angelate	Ang
	Epoxyangelate	Epoxyang
	Sarracianate	Sar
	Acetylsarracianate	Sarac

2.1.1.1. Skeleton Types.

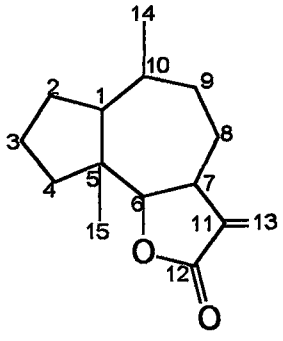
Most of the several hundred naturally occurring sesquiterpene lactones can be classified on the basis of four carboxylic skeletons; guaianolides, pseudo-guaianolides, eudesmanolides and germacranolides. They have different physiological activities.

Guaianolides: They are those sesquiterpene lactones that are based upon the guaiane skeleton.(8). The guaianolides may be lactonized at either C6 or C8 [9].



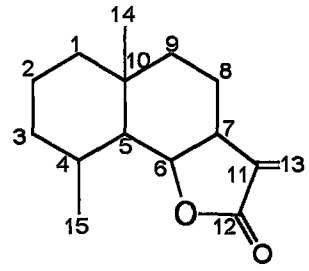
(8)

Pseudo-guaianolides: The largest class of sesquiterpene lactones is the pseudoguaianolides, which are based upon the 5/7 carboxylic ring system, which contains a methyl group at the C5 ring junction. The pseudoguaianolides may be lactonized at either C6 or C8 and may be cleaved between C3 and C4 or C4 and C5 (9) [9].



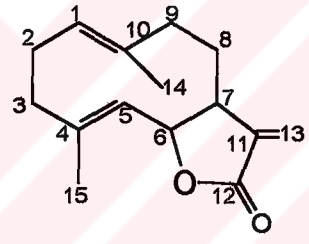
(9)

Eudesmanolides: They are those sesquiterpene lactones that are based on the eudesmane skeleton. They may be lactonized at either C6 or C8 (10) [9].



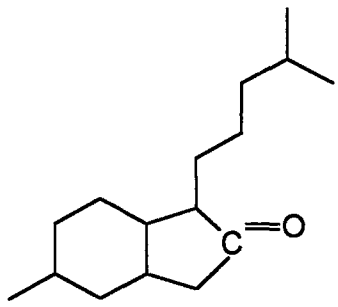
(10)

Germacranolides: They are the sesquiterpene lactones that are based upon a cyclodecadiene ring, which usually contains a trans,trans-diene system at the C4-C5 and C1-C10 positions (11) [9]. Germacranolides may be lactonized at either C6 or C8 and usually contain additional oxygen functions at a variety of positions.

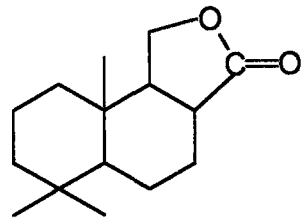


(11)

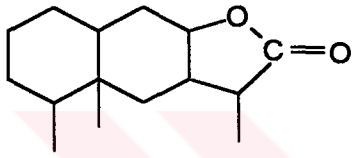
In addition to the four basic skeleton types which have been discussed above, a few other sesquiterpene lactone skeletons have been shown to exist. These are shown in FIGURE 2.3 [9].



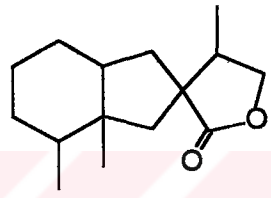
Bisabolenolide



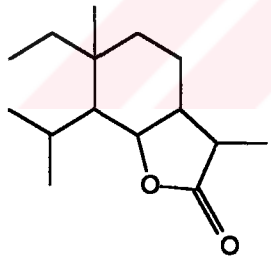
Drimanolide



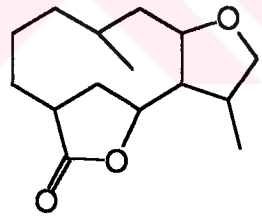
Eremophilenolide



Fukinanolide



Elemanolide



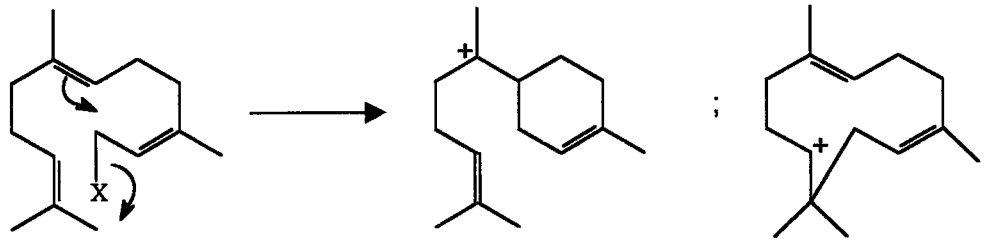
Germafurenolide

FIGURE 2.3 Minor Classes of Sesquiterpene Lactones

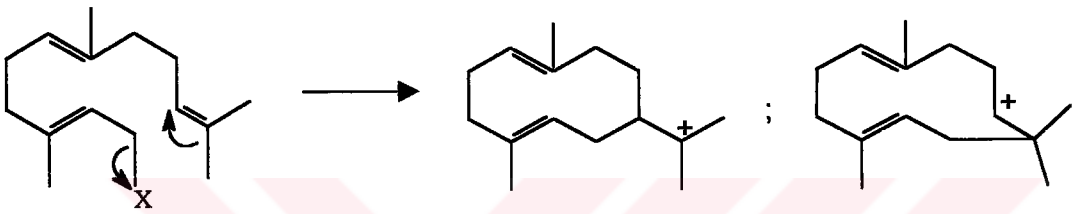
2.1.1.2. Biosynthesis of Sesquiterpene Lactones.

During the formation of the skeleton, farnesyl pyrophosphate turns into trans-trans and cis-trans farnesyl cation and it was found out that cyclization of farnesyl pyrophosphate leads to the formation of the germacradiene skeleton (FIGURE 2.4). From germacradiene other different skeletal types of sesquiterpene lactones are derived by either acid catalysis, thermolysis or photolysis (FIGURE 2.6) [8].

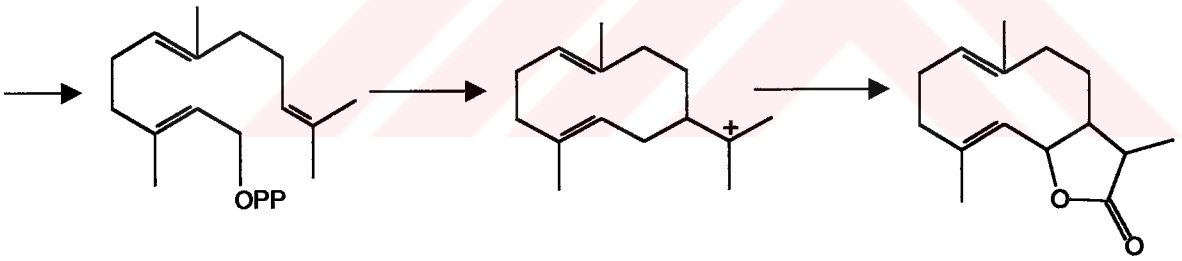
It is clear that the change of the isopropenyl group (equivalent to the initial isopropyl carbonium ion) to the lactone is an oxidation process. In sesquiterpene lactones the isopropyl side chain has been modified to the oxidation state of carboxylic acid. Introduction of oxygen into the ring provides the hydroxyl group with which the carboxyl group interacts to form the lactone ring. The manner and the stage of the biosynthetic steps in which the side chain oxidation occurs are not known. But formation of the carboxyl group of the sesquiterpene lactones provides a versatile and satisfactory explanation for the biosynthesis of the numerous oxidation states of the isopropyl residue. The formation of the carboxyl group is shown in FIGURE 2.5 [4].



cis-trans farnesyl pyrophosphate



trans-trans farnesyl pyrophosphate



farnesyl pyrophosphate Simple sesquiterpene skeleton Simple sesquiterpene lactone skeleton

FIGURE 2.4 Formation of Germacradiene Skeleton

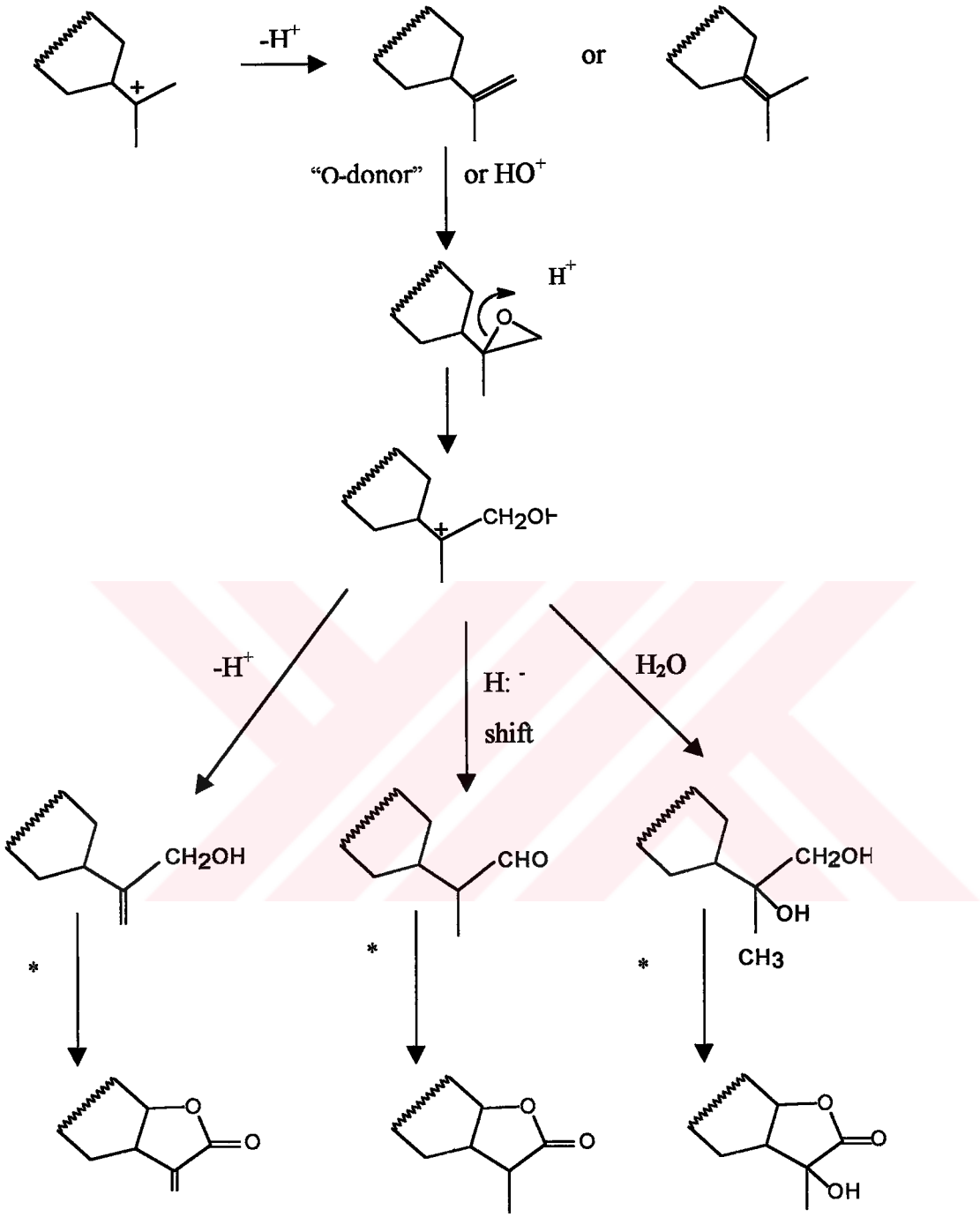


FIGURE 2.5 Formation of Carboxyl Group of the Sesquiterpene Lactones

* Following introduction of oxygen into the ring, and further oxidation

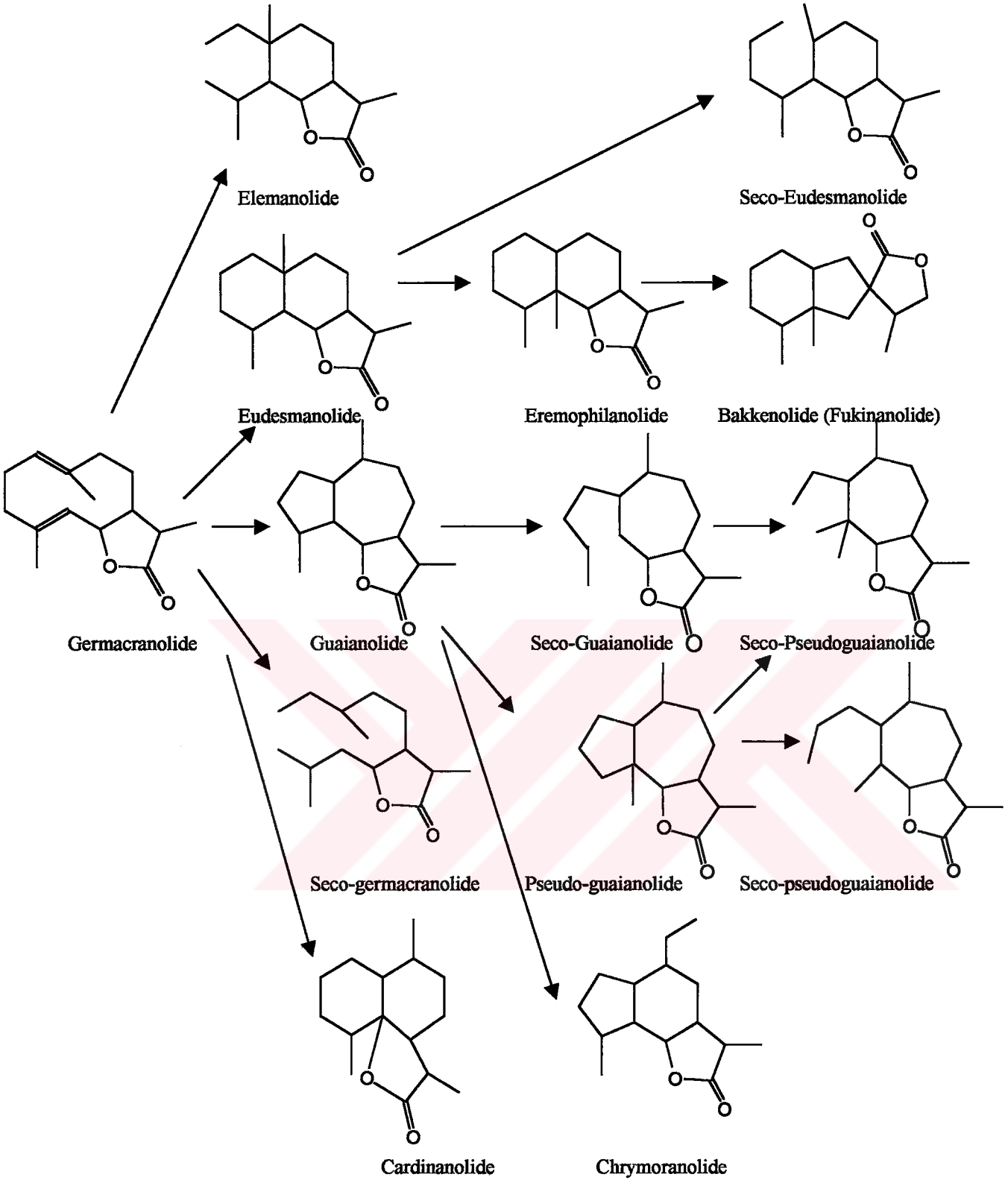


FIGURE 2.6 Biogenetic Relationships of Germecranolide Derived Sesquiterpene Lactones

2.1.2.3. Biological Activities.

Anti-tumor and cytotoxic activity: It was found that all the known cytotoxic sesquiterpenes contained a lactone function; all but one of these were α, β- unsaturated and the α-ethylenic linkage was exocyclic in every case. In a further study of the structure activity relationship among sesquiterpene lactones it was noted that the presence of a C_{11}-C_{13} exocyclic double bond conjugated to the γ-lactone was essential for cytotoxicity. Compounds having endocyclic double bonds gave unstable cysteine-adducts and were inactive. However sesquiterpene lactones which incorporated a cyclopentenone or α-methylene lactone (in addition to the α, β-methylene γ-lactone) appeared to produce enhanced cytotoxicity [6]. The in vitro activity (cytotoxicity) is enhanced by the presence of certain additional α, β-unsaturated carbonyl functions. For in vivo (antitumor) activity of germacronalides the presence of α, β-unsaturated ester side chain adjacent to the γ-lactone and either a primary or secondary allylic alcohol or both are necessary [10].

The germacranolide sesquiterpene lactones Molephantinin, Ambrosin and Eupahyssopin have been shown to exhibit in vivo antitumor activity. And the germacranolide sesquiterpene lactones that exhibit in vivo cytotoxic activity, are Alatolide, Baileyin, Phantomolin, Lipiferolide, Epitulipinolide Diepoxide, Eupaformonin , and Eremantholide A [11].

Microbial growth inhibitors (Antibiotics): Some sesquiterpene lactones have been shown to possess anti-bacterial, anti-fungal or anti-helminthic properties [6]. According to Lee antimicrobial activity is due to an α, β-unsaturated cyclopentanone ring and not to the α-methylene γ-lactone moiety [12]. Calzada, on the contrary claims that there is a clear relationship between antimicrobial activity and the presence of an α-methylene γ-lactone group. The mechanism of action for microbial activity is alkylation of nucleophilic centers (sulphydryl groups) in the microorganisms [13].

Costunolide promoted germination of sorghum, carrot, and cucumber but inhibited ryegrass, and wheat [14]. Mikanolide and Dihydromikanolide inhibit the growth in culture, of a bacterium, and also of a yeast [6].

Allergic contact dermatitis: Some sesquiterpene lactones have been shown to be a major class of allergens causing allergic contact dermatitis in humans. Over 80 sesquiterpene lactones were used in patch tests to determine their allergenic potential, and the presence of an α-methylene group, exocyclic to the γ-lactone, was shown to be the principal immunochemical requisite for the production of dermatitis [6].

All the known allergenic sesquiterpene lactones containing an exocyclic α-methylene function which may conjugate with sulphydryl groups of proteins in cells by a Michael-type addition, form complete antigens capable of producing cell-mediated contact allergic reactions [6].

Some sesquiterpene lactones reported to cause allergic contact dermatitis in humans are Pathenin, Alantolactone, Frullanolide, Costunolide and Arteglasin-A [6].

Vertebrate poisoning: Livestock poisoning from foraging on bitter tasting plants is well documented in agricultural literature. For example, *Hymenoxys odorata* (bitterweed) is an important livestock toxicant that affects primarily sheep and goats, and the major toxin involved in death of sheep is the sesquiterpene lactone Hymenovin in populations of *Hymenoxys odorata.* The lactone Tenulin imparted a bitter taste to milk after oral administration to a lactating cow [6].

These sesquiterpene lactones present in plants that are known to be toxic to livestock, have not necessarily been demonstrated to be the poisonous agents [6].

Plant growth inhibitors (phytotoxins): A variety of sesquiterpene lactones of different skeletal types have been reported to show plant growth regulatory activity [6].

The phytotoxicity of the eudesmanolide Alantolactone was demonstrated by its inhibitory effect on seed germination, seedling growth, rate of respiration and degradation of starch and protein in mung beans [14].

Anti-inflammatory and anti-hyperlipidemic action: Anti-inflammatory and anti-hyperlipidemic action may be explained by enzyme inhibition through addition to sulphydryl groups of enzymes. Both effects are enhanced by conjugated systems other than α-methylene γ-lactone function, such as an α, β-unsaturated cyclopentanone ring and an α-

epoxy cyclopentanone system in the sesquiterpene molecule, although their contribution is less then that of the α-methylene γ-lactone moiety [10].

2.1.2.4. Mechanism of Action of Sesquiterpene Lactones.

An examination of sesquiterpene lactones that exhibit growth inhibitory properties indicates that the following structural configurations are at least the principal requirements for biological activity:

- The presence of an exocyclic methylene conjugated to γ-lactone
- The presence of a functional group, such as an epoxide, hydroxyl, chlorohydrin, unsaturated ketone or O-acyl adjacent to the α-CH_2 of γ-lactone which can enhance the reactivity of the conjugated lactone toward biological nucleophiles.

The inhibitory action of sesquiterpene lactones results from the presence of highly electrophilic functional groups. These selectively alkylate by Michael-type addition to sulphydryl proteins, specifically thiol groups in preference to other nucleophiles [6].

2.1.3. Isolation and Identification Techniques

2.1.3.1. Methods of Separation and Purification.

The common techniques used for the separation and purification of plant constituents are column chromatography (CC) and preparative thin layer chromatography. The more sophisticated methods include Medium Performance Liquid Chromatography (MPLC), High Performance Liquid Chromatography (HPLC), and Gas-Liquid Chromatography (GLC).

Column chromatography is a very useful technique for the isolation of large quantities of compounds from crude plant extracts. Adsorbents, commonly used for separation are silica gel, kieselguhr, magnesol, cellulose, alumina, polyamide, sephadex

and ion exchange resins. The advent of the relatively new chromatographic media like polyamide and sephadex, has a great effect on the type and efficiency of separations achieved [15].

***Silica gel*:** This is an adsorbent, which can be commercially obtained easily. It requires activation before use; this can be achieved by heating, possibly in a vacuum, when the adsorbent loses water and other adsorbed materials. In recent years commercial varieties of silica have become available that appear to offer distinct advantages over the usual gels which, when dry form glassy solids and have to be crushed and sieved to obtain appropriate particle sizes. Silica gel is polar, therefore it performs a separation due to the polarities of the constituents [15].

Sephadex gel: Sephadex is a highly cross-linked dextran. Several different types of sephadex are available and all have the same chemical properties, but differ in degree of swelling, which makes them useful for chemical fractionation within various molecular size ranges. The gels are manufactured in bead form, and the degree of swelling is characterized by the amount of water taken by one gram of dry sephadex (water regain). Besides the several G types of sephadex there is LH-20 sephadex which is the hydroxypropyl ether of sephadex G-25. It will form a gel in both aqueous and organic solvents [15].

2.1.3.2. Identification Methods.

Once the constituents are isolated and purified, they are compared with known compounds on TLC plates. After that their spectral data are obtained and accordingly they are classified. The instruments used are infrared (IR), nuclear magnetic resonance (NMR) and mass spectrometers (MS).

3. EXPERIMENTAL

3.1. Chromatographic Methods

3.1.1. Column Chromatography (CC)

Columns with suitable dimensions were chosen for the elution of known amounts of extracts and filled with enough amounts of adsorbents. Two different types of adsorbents were used in the columns.

Silica gel column: CC was carried out on Kiesel gel 100 (0.063-0.200 mm, Merck) which was applied to the columns either as a slurry using a non-polar solvent or dry as it was. The extract dried on a minimum amount of silica gel, was placed on top of the adsorbent in the column. Then the columns were eluted with solvents with increasing polarities.

Sephadex column: The sephadex gel (Pharmacia and Film Corp.) was swollen in PE/ $CHCl_3$/ MeOH (7:4:1) for 24 hours and then poured into the column as a slurry. After the adsorbent settled down, the plant extract dissolved in a minimum amount of solvent, was applied on to the column by the help of a pipette. Solvents used for elution were PE/ $CHCl_3$/ MeOH (7:4:1) and MeOH combinations.

3.1.2. Thin Layer Chromatography (TLC)

The plates used were silica gel (Merck, Kiesel gel 60 F_{254}, 0.2 mm thick). All the chromatograms were viewed under an ultraviolet lamp. The spots were developed by spraying ceric sulphate solution, which was prepared by dissolving 2 g of cerium (IV)

sulphate in 100 ml of 10% sulphuric acid solution. Then the plates were heated in an oven at 85-90 ^{0}C for 10 minutes.

While performing TLC, different solvent systems were used due to the polarity difference of the isolated constituents.

3.2. Instruments

3.2.1. NMR Spectrophotometer

^{1}H NMR, ^{13}C NMR (APT) spectra were recorded on a Bruker AC-200L spectrometer operating at 200 MHz using deuterated chloroform and methanol solvents and TMS as internal standard, at the Chemistry Department of the Turkish Scientific and Research Institute (TUBITAK-Gebze).

3.2.2. Infrared Spectrophotometer

IR spectra were recorded on a Perkin Elmer 983 instrument at the Chemistry Department of the Turkish Scientific and Research Institute (TUBITAK-Gebze) and on a Perkin Elmer 1600 FTIR instrument at the Chemistry Department of the Boğaziçi University.

3.2.3. Mass Spectrometer

Mass spectra were recorded on a VG-ZABSPEC instrument (1000 Resolution) at the Chemistry Department of the Turkish Scientific and Research Institute (TUBITAK-Gebze).

3.3. Plant preparation and extraction

3.3.1. *Centaurea cheirolopha Wagenitz* (Compositae)

Centaurea cheirolopha Wagenitz was collected in June 1995 from Adana-Pozantı, Armut oluğu by Dr. Nur Tan and identified by Dr. K. Alpınar (University of İstanbul, Faculty of Pharmacy). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of İstanbul (ISTE:68303).

Dried and powdered aerial parts of the plant (1.5 kg) were extracted with petroleum ether, diethylether and ethanol (1:1:1) respectively at room temperature. The extracts were concentrated by distillation and evaporated to dryness. The diethylether residue was dissolved in a small amount of MeOH and the mixture was left in the refrigerator overnight to remove long-chain saturated hydrocarbons. The precipitate was removed by filtration and the residue (85 g) was chromatographed over a silica gel column eluting with the solvent systems of increasing polarities namely 100% PE, 5% Et_2O, 10% Et_2O, 25% Et_2O, 50% Et_2O, 75% Et_2O, 100% Et_2O, 100% EtOH, 5% H_2O. The fractions eluted with 50% Et_2O were combined and analyzed separately. The same procedure was also applied to the fractions eluted with 50% Et_2O, 75% Et_2O, 100% Et_2O, 100% EtOH.

3.3.2. *Centaurea cuneifolia Sm.* (Compositae)

Centaurea cuneifolia Sm. was collected in July 1980 from Kastro (Western part of Black Sea) by Dr. Belkıs Halfon and identified by Dr. A. Çarpıcı (University of İstanbul, Faculty of Science, Department of Botany). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of İstanbul (ISTE:45507).

Dried and powdered aerial parts (3 kg) of the plant were macerated with petroleum ether, diethyl ether (2:1) at room temperature. The extract was concentrated by distillation and evaporated to dryness.

3.4. Separation and Purification

3.4.1. *Centaurea cheirolopha Wagenitz* (Compositae)

3.95 g of 100% Et_2O extract was dissolved in diethylether, mixed with a small amount of silica gel and dried. The dried material was placed on a silica gel column .The column was eluted with solvent systems of increasing polarity.

Solvent systems	Fraction number
Pure CH_2Cl_2	1 - 13
Acetone / CH_2Cl_2 (5:95)	14 - 24
Acetone / CH_2Cl_2 (10:90)	25 - 33
Acetone / CH_2Cl_2 (25:25)	34 - 40
Acetone / CH_2Cl_2 (50:50)	41 - 47
Acetone / CH_2Cl_2 (75:25)	48 - 49
Pure Acetone	50 - 53
Acetone / MeOH (90:10)	54 - 61
Acetone / MeOH (75:25)	62 - 65
Acetone / MeOH (50:50)	66 – 69

69 fractions were collected and the volume of each faction was nearly 200 ml.The solvent of each fraction was evaporated to dryness by using vacuum distillation and each

one was applied on TLC. The plates were viewed under a UV lamp at 254 and 360 nm and then were sprayed with ceric sulphate and heated in an oven at 85-90 ^{0}C for 10 minutes.Similar fractions, as determined by UV and spray reagents on TLC, were combined.

Fractions 29, 33, 35 and 36 were purified by preparative chromatography and four pure compounds 29.1 (CC1), 33.7.2 (CC2), 35.1.2 (CC3) and 36.2.2 (CC4) were isolated.They were identified on the basis of ^{1}H NMR, ^{13}C APT, IR, and Mass spectral analysis.

Compound CC3 was also isolated from 50% Et_2O (2.10 g) and 100% EtOH (5.03 g) extracts of *Centaurea cheirolopha Wagenitz*. 75% Et_2O (3.84 g) extract also contains compound CC2.

3.4.2. *Centaurea Cuneifolia Sm.* (Compositae)

21.42 g of the plant extract was dissolved in ether, mixed with a small amount of silica gel and dried. The dried material was placed on a silica gel column .The column was eluted with solvent systems of increasing polarity.

Solvent systems	**Fraction number**
PE / Et_2O (95:5)	1
PE / Et_2O (90:10)	2
PE / Et_2O (75:25)	3
PE / Et_2O (50:50)	4
PE / Et_2O (25:75)	5 - 8
Pure Et_2O	9 - 29
Et_2O / MeOH (95:5)	30 - 39
Et_2O / MeOH (90:10)	40 - 51

Et_2O / MeOH (50:50)	52 - 57
Pure MeOH	58 - 91
Pure EtOH	92 - 96

96 fractions were collected and the volume of each fraction was nearly 200ml. The solvent of each fraction was evaporated to dryness by using vacuum distillation and each one was applied on TLC plates. The plates were viewed under a UV lamp at 254 and 360 nm and then were sprayed with ceric sulphate and heated in an oven at 85-90 ^{0}C for 10 minutes. Similar fractions, as determined by UV and spray reagents on TLC, were combined.

The 31st fraction was rechromatographed on a silica gel column. 50th and 51st fractions of this column were purified by preparative chromatography and two pure compounds 50.11.1 (CCU1) and 51.3.1 (CCU2) were identified on the basis of ^{1}H NMR, IR spectral analysis, and the comparison of the Rf values with authentic samples.

3.5. Physical and Spectral Properties of the Isolated Sesquiterpene Lactones

3.5.1. Sesquiterpene Lactones from *Centaurea cheirolopha Wagenitz*

CC1 (Aguarin B): 3β-hydroxy-8α-(2-methylpropenoyl)-4(15), 10(14), 11(13)-guaiatrien-12, 6α-olide

Rf: 0.70 (6 Benzene : 4 Et_2O, 6 times)

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (O-H stretching), 1760 (unsaturated γ-lactone), 1720 (α, β unsaturated ester), 1640 (unsaturation), 1380, 1270 (C-O stretching), 1160, 1020, 960, 770.

^{1}H NMR (200 MHz, CD_3OD) δ (ppm): H-1α 2.94 (m), H-2α 1.64 (ddd, J= 9, 10, 12 Hz), H-2β 1.98 (ddd, J= 7.5, 10, 12 Hz), H-3α 4.41 (br t), H-5α 2.79 (br t), H-6β 4.25 (dd, J= 9,

10 Hz), H-7α 3.18 (ddt, J= 9, 9, 3 Hz), H-8β 4.99 (ddd, J= 4, 5, 9 Hz), H-9α 2.63 (dd, J= 15, 5 Hz), H-9β 2.28 (dd, J= 15, 4 Hz), H-13 6.02 (d, J=3 Hz), H-13' 5.49 (d, J= 3 Hz), H-14 5.05 (br s), H-14' 4.79 (br s), H-15 5.34 (br s), H-15' 5.23 (br s), H-18 6.08 (br s), H-18' 5.63 (d, J= 1.5 Hz), H-19 1.88 (s)

EI-MS m/e (relative intensity): 330.1 $[M]^+$ (20), 262.1 $[M-C_4H_5O]^+$ (11), 244.1 $[262.1-H_2O]^+$ (100), 226.1 $[244.1-H_2O]^+$ (39), 216.1 (37), 197.1 (39), 183.1 (26), 173.1 (35), 159.1 (34), 148.1 (55), 135.1 (28), 129.1 (41), 119.1 (51), 105.1 (48), 97.0 (47), 91.0 (73), 85.0 (38), 79.0 (48), 77.0 (41)

CC2: 3β-hydroxy-8α-(2,3-dihydroxy-2-methylpropanoyl)-4(15), 10(14), 11(13)-guatrien-12, 6α-olide

Rf: 0.36 (6 Benzene : 4 Et_2O, 6 times)

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (O-H stretching), 1760 (α, β unsaturated γ-lactone), 1720 (α, β unsaturated ester), 1640 (unsaturation), 1420, 1280 (C-O stretching), 1160, 1060, 920, 760.

^{1}H NMR (200 MHz, CD_3OD) δ (ppm): H-1α 3.0 (m), H-2α 1.72 (ddd, J= 9, 10, 12 Hz), H-2β 2.25 (m), H-3α 4.57 (br t), H-5α 2.82 (br t), H-6β 4.28 (dd, J= 9, 10 Hz), H-7α 3.17 (ddt, J= 9, 9, 3 Hz), H-8β 5.22 (m), H-9α 2.70 (dd, J= 15, 5 Hz), H-9β 2.46 (dd, J= 15, 3 Hz), H-13 6.25 (d, J=4 Hz), H-13' 5.57 (d, J= 3 Hz), H-14 5.17 (br s), H-14' 5.09 (br s), H-15 5.50 (br s), H-15' 5.38 (br s), H-18 3.88 (d, J= 11 Hz), H-18' 3.65 (d, J= 11 Hz), H-19 1.56 (s).

EI-MS m/e (relative intensity): 364.1 $[M]^+$ (3), 262.1 $[M-C_4H_7O_3]^+$ (17), 244.1 $[262.1-H_2O]^+$ (100), 226.1 $[244.1-H_2O]^+$ (100), 216.1 (38), 199.1 (42), 181.1 (28), 171.1 (32), 159.0 (29), 148.0 (43), 129.1 (40), 119.1 (32), 105.1 (36), 103.1 $[C_4H_7O_3]^+$ (12), 93.0 (85), 91.0 (73), 82.9 (49), 85.0 (30), 79 (29), 77 (26), 69.0 (45), 57.0 (41).

CC3 (15-deschloro-15-hydroxychlorojanerin): 3β-hydroxy-4,4-hydroxy, hydroxymethyl-8α-(2-hydroxymethylpropenoyl)-10(14), 11(13)-guaiadien-12, 6α-olide

Rf: 0.38 (6 Benzene : 4 Et_2O, 6 times)

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (O-H stretching), 1760 (unsaturated γ-lactone), 1720 (α, β unsaturated ester), 1640 (unsaturation), 1280 (C-O stretching), 1160, 1060, 770.

^{1}H NMR (200 MHz, CD_3OD) δ (ppm): H-2α 1.56 (m), H-3α 3.52 (m), H-5α 2.3 (br t), H-6β 4.88 (dd, J= 9,10 Hz), H-7α 3.20 (ddt, J= 10, 10, 3 Hz), H-8β 5.15 (m), H-9α 2.90 (dd, J= 15, 5 Hz), H-13 6.18 (d, J=4 Hz), H-13' 5.66 (d, J= 4Hz), H-14 5.13 (d= 2 Hz), H-14' 4.80 (d, J=2 Hz), H-15 4.24 (d, J= 12 Hz), H-15' 3.80 (d, J=12 Hz), H-18 6.35 (br s), H-18' 6.02 (d, J= 2 Hz), H-19 4.38 (br s).

EI-MS m/e (relative intensity): 381.1 $[M+1]^+$ (1), 296.1 $[M+1-C_4H_5O_2]^+$ (19), 278.1 $[296.1-H_2O]^+$ (11), 260.1 $[278.1-H_2O]^+$ (18), 242.1 $[260.1-H_2O]^+$ (18), 229.1 (25), 214.1 (14), 203.1 (15), 189.1 (20), 173.1 (20), 159.1 (11), 147.1 (20), 131.1 (10), 117.1 (14), 105.1 (9), 91.0 (18), 85.0 $[C_4H_5O_2]^+$ (100), 79.0 (13), 77 (6), 69 (13), 57.0 (28).

^{13}C APT (50.32 MHz,$CDCl_3$+CD_3OD) δ (ppm): C-1 46.74 (-), C-2 35.28 (+), C-3 74.65 (-), C-4 84.91 (+), C-5 58.48 (-), C-6 77.89 (-), C-7 48.08 (-), C-8 76.34 (+), C-9 38.91 (+), C-10 140.46 (+), C-11 137.82 (+), C-12 169.7 (+), C-13 122.74 (+), C-14 117.57 (+), C-15 78.53 (+), C-16 165.94 (+), C-17 143.42 (+), C-18 125.98 (+), C-19 61.04 (+).

CC4 (Cynaropicrin): 3-hydroxy-8α-(2-hydroxymethylpropenoyl)-4(15), 10(14), 11(13)-guaiatrien-12, 6α-olide

Rf: 0.25 (6 Benzene : 4 Et_2O, 6 times)

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (O-H stretching), 1760 (unsaturated γ-lactone), 1710 (α, β unsaturated ester), 1660 (unsaturation), 1420, 1275 (C-O stretching), 1060, 920.

^{1}H NMR (200 MHz, CD_3OD) δ (ppm): H-1α 2.98 (m), H-2α 1.74 (m), H-2β 2.24 (m), H-3α 4.57 (br t), H-5α 2.85 (br t), H-6β 4.26 (dd, J= 9,10 Hz), H-7α 3.20 (ddt, J= 10, 10, 3 Hz), H-8β 5.14 (m), H-9α 2.72 (dd, J= 14, 4 Hz), H-9β 2.40 (dd, J= 14, 5 Hz), H-13 6.23 (d, J=4 Hz), H-13' 5.63 (d, J=3 Hz), H-14 5.15 (br s), H-14' 4.94 (br s), H-15 5.49 (br s), H-15' 5.37 (br s), H-18 6.34 (br s), H-18' 5.96 (d, J=1.5 Hz), H-19 4.38 (br s)

EI-MS m/e (relative intensity): 346.1 $[M]^+$ (8), 262.1 $[M-C_4H_5O_2]^+$ (21), 244.1 $[262.1-H_2O]^+$ (51), 226.1 $[244.1-H_2O]^+$ (39), 216.1 (20), 197.1 (28), 183.1 (13), 169.1 (17), 159.1

(19), 148.1 (36), 129.1 (25), 119.1 (36), 105.1 (29), 97.0 (19), 91.0 (46), 85.0 $[C_4H_5O_2]^+$ (100), 79.0 (28), 69 (30), 57.0 (45)

3.5.2. Sesquiterpene Lactones from *Centaurea cuneifolia Sm.*

CCU1 (Dehydromelitensin): 8α-15-dihydroxy-1, 3, 11(13)-elematrien-12, 6α-olide

Rf: 0.51 (2 Benzene : 5 Et_2O : 0.25 MeOH, 3 times)

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (O-H stretching), 1760 (α, β unsaturated γ-lactone), 1640 (unsaturation), 1270 (C-O stretching), 1020, 960, 770.

^{1}H NMR (200 MHz, CD_3OD): H-1 5.78 (dd, J=11, 16 Hz), H-2 5.08 (d, J=11 Hz), H-2' 5.02 (d, J= 16 Hz), H-3 5.41 (br s), H-3' 4.95 (br s), H-5α 2.51 (d, J= 12 Hz), H-6β 4.15 (t, J= 12 Hz), H-7α 2.64 (tt, J= 12, 3 Hz), H-8β 4.18 (m), H-9α 1.87 (dd, J= 14, 5 Hz), H-9β 1.61 (dd, J= 14, 5 Hz), H-13 6.18 (d, J=4 Hz), H-13' 5.99 (d, J= 3 Hz), H-14 1.10 (s), H-15 4.09 (d, J=13 Hz), H-15' 3.97 (d, J=13 Hz).

CCU2: 15-hydroxy-8α-(3,4-dihydroxy-2-methylenebutanoyl)-1, 3, 11(13)-elematrien-12, 6α-olide

Rf: 0.23 (2 Benzene : 5 Et_2O : 0.25 MeOH, 3 times)

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (O-H stretching), 1770 (α, β unsaturated γ-lactone), 1715 (α, β unsaturated ester),1650 (unsaturation), 1420, 1215 (C-O stretching), 1050, 760.

^{1}H NMR (200 MHz, CD_3OD): H-1 5.76 (dd, J=11, 16 Hz), H-2 5.08 (d, J=11 Hz), H-2' 5.03 (d, J=16 Hz), H-3 5.42 (br s), H-3' 4.96 (br s), H-5α 2.58 (d, J=12 Hz), H-6β 4.25 (dd, J=11, 12 Hz), H-7α 2.96 (tt, J=11, 3 Hz), H-8β 5.29 (dt, J= 4, 11 Hz), H-9α 2.03 (dd, J= 14, 4 Hz), H-9β 1.64 (d, J=14 Hz), H-13 6.14 (d, J=3 Hz), H-13' 5.55 (d, J=3 Hz), H-14 1.16 (s), H-15 4.00 (d, J_{AB}=14 Hz), H-15' 4.08 (d, J_{AB}=14 Hz), H-18 4.63 (dd, J=4, 7 Hz), H-19 3.83 (dd, J=11, 4 Hz), H-19' 3.58 (dd, J=11, 7 Hz), H-20 6.38 (br s), H-20' 6.06 (br s).

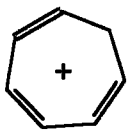
4. RESULTS AND DISCUSSION

4.1. General Information on Spectral Data

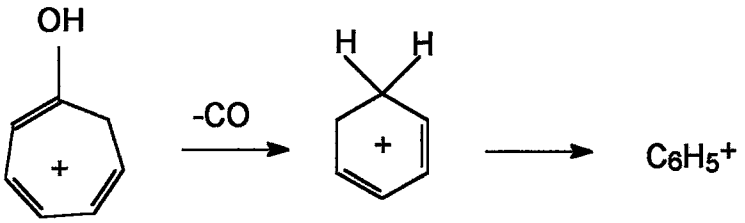
IR spectra of sesquiterpene lactones show a characteristic carbonyl peak (1740-1780cm^{-1}) due to γ-lactone carbonyl, in addition to possible ketone (1710cm^{-1}), hydroxyl (3400 cm^{-1}), double bond (1600cm^{-1}) and other functionalities.

In the ^{1}H NMR spectra of sesquiterpene lactones three characteristic methyl peaks are expected. The C-10 methyl of guainolides may be a secondary methyl group (0.8-1.10 ppm, d, J=7 Hz), vinylic (1.5-2.10 ppm, s) or an exocyclic methylene (4.8-4.9 ppm, two broad singlets or multiplet with small J value). The C-10 methyl of elemanolides appears as a singlet at 0.8-1.1 ppm. The C-4 methyl may be a secondary methyl group (0.8-1.10 ppm, d, J=7 Hz), vinylic (1.5-2.10 ppm, s) or an exocyclic methylene (5.0-5.3 ppm, two broad singlets). The C-11 methyl group in the lactone ring may be again secondary, vinylic or exocyclic methylene. The C-11 methylene protons appear as two characteristic doublets (J=1-4 Hz) at 5.40-6.40 ppm. The low field absorption being due to the proton oriented toward the lactone carbonyl group.This coupling is due to allylic interactions between C-7 and C-13 methylene protons. The C-6 proton appears at about 3.85-4.70 ppm as a doublet of doublets (dd), due to axial-axial coupling with the trans C-5 and trans C-7 protons, if the guainolide is lactonized at C-6. If the ring closure is at C-8, the C-8 proton appears as a three fold doublet (ddd) with the same chemical shift.

In the Mass spectrum of the sesquiterpene lactones, generally a tropylium ion m/e 91 (12) is formed. The peak at m/e 107 (13) which is represented as the hydroxytropylium ion can also be observed. Loss of CO from this ion gives m/e 79 (14) and loss of H leads to the phenyl cation at m/e 77 (15).



(12)

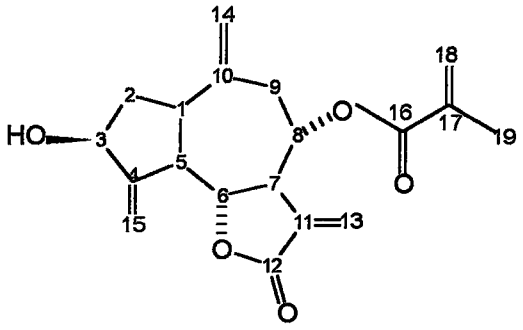


(13) (14) (15)

4.2. Experimental Results and Discussion

4.2.1. *Centaurea cheirolopha Wagenitz*

4.2.1.1. Structure Determination of CC1 (Aguarin B).



(16)

The IR spectrum (FIGURE 4.1) of CC1 (16) showed peaks at 1760 cm^{-1} (α, β-unsaturated γ-lactone C=O stretching), 1720 cm^{-1} (α, β unsaturated ester C=O stretching), 1270 cm^{-1} (C-O stretching of ester functionality), 1640 cm^{-1} (C=C stretching) and finally

3440 cm^{-1} (O-H stretching) indicating the presence of α, β unsaturated γ-lactone, α, β unsaturated ester and hydroxyl groups respectively.

The ^1H NMR spectrum (FIGURE 4.2) of CC1 in CD_3OD showed two doublets at δ 6.02 (H-13, d, J=3 Hz) and δ 5.49 (H-13', d, J=3 Hz) indicating the presence of typical exocyclic methylene protons of the lactone ring due to allylic coupling with H-7. The chemical shifts of methylene protons at C-14 appear at δ 5.05 (H-14, br s) and a broad singlet expected at δ 4.79 belonging to the H-14' overlapping with MeOH peak. The signals appearing at δ 5.34 (H-15, br s) and δ 5.23 (H-15', br s) indicate the presence of exocyclic methylene at the position C-4. The lactone proton H-6β appeared at δ 4.25 (dd, J= 9, 10 Hz) due to axial-axial coupling with the trans H-5 and trans H-7 protons. The splitting of H-6 is also evidence for lactone trans-ring closure at C-6. The signal of H-7α appeared at δ 3.18 (ddt, J= 9, 9, 3 Hz). The chemical shift of H-3α at δ 4.41 (t,br) is characteristic of protons with neighbouring β-OH functions. The H-8β signal appeared at δ 4.99 (ddd, J= 4, 5, 9). A pair of broad singlets at δ 6.08 and δ 5.63 and a vinylic methyl singlet at δ 1.88 indicated the presence of a methacrylate group. In the mass spectrum peaks at m/e 261.1 $[M-C_4H_5O]^+$ and 119.1 corroborated the presence of this group. The rest of the spectral data are listed in TABLE 4.2.

The EI Mass spectrum m/e (relative intensity) 330.1 ($C_{19}H_{22}O_5$) $[M]^+$ (20) supports the deduced structure by means of its molecular weight. The EI Mass spectrum (FIGURE 4.3) of CC1 showed:

330.1 $[M]^+$ (20)

85 $[C_4H_5O_2]^+$ (38)

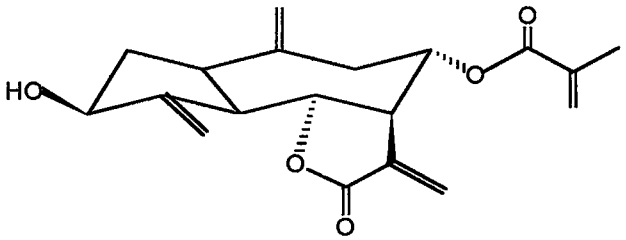
262.1 $[M-C_4H_5O]^+$ (11)

244.1 $[262.1-H_2O]^+$ (100)

226.1 $[244.1-H_2O]^+$ (39)

peaks besides the typical m/e 105.1 peak of the methyl tropylium ion, m/e 91 $[105.1-CH_3]^+$ tropylium ion, m/e 79 $[91-CH_3]^+$ and m/e 77 phenyl cation.

The compound CC1 was identified by comparison of its spectral data by those of similar compounds in the literature. The data agree with those of Aguarin B, the conformational structure is shown below (16), which has formerly been isolated from *Centaurea behen, Centaurea canariensis,Centaurea arguta, Centaurea glastifolia, Centaurea solstitialis subsp.schouwi* [16, 17, 18, 19, 20].



(16)

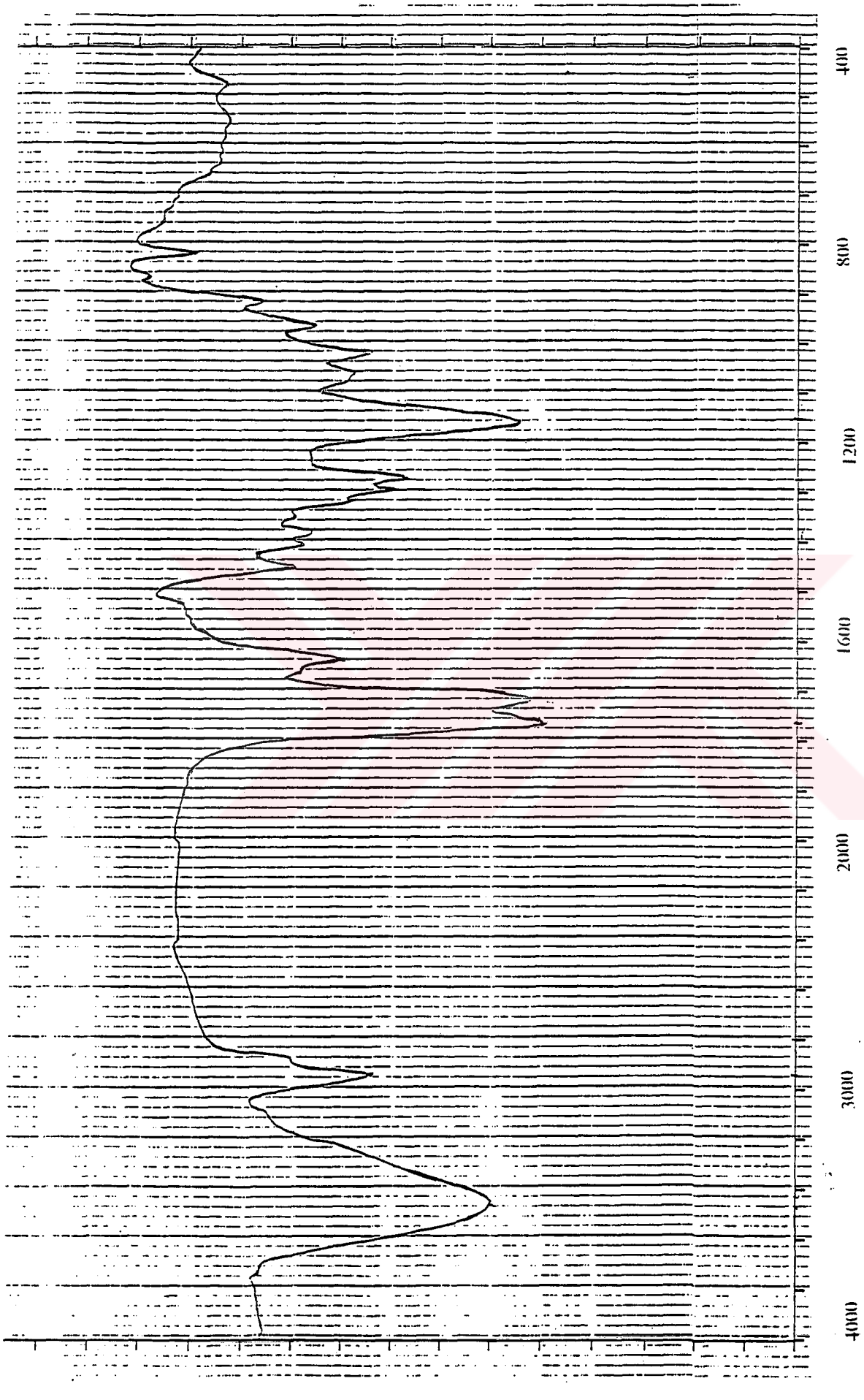


FIGURE 4.1 The IR Spectrum of CC1

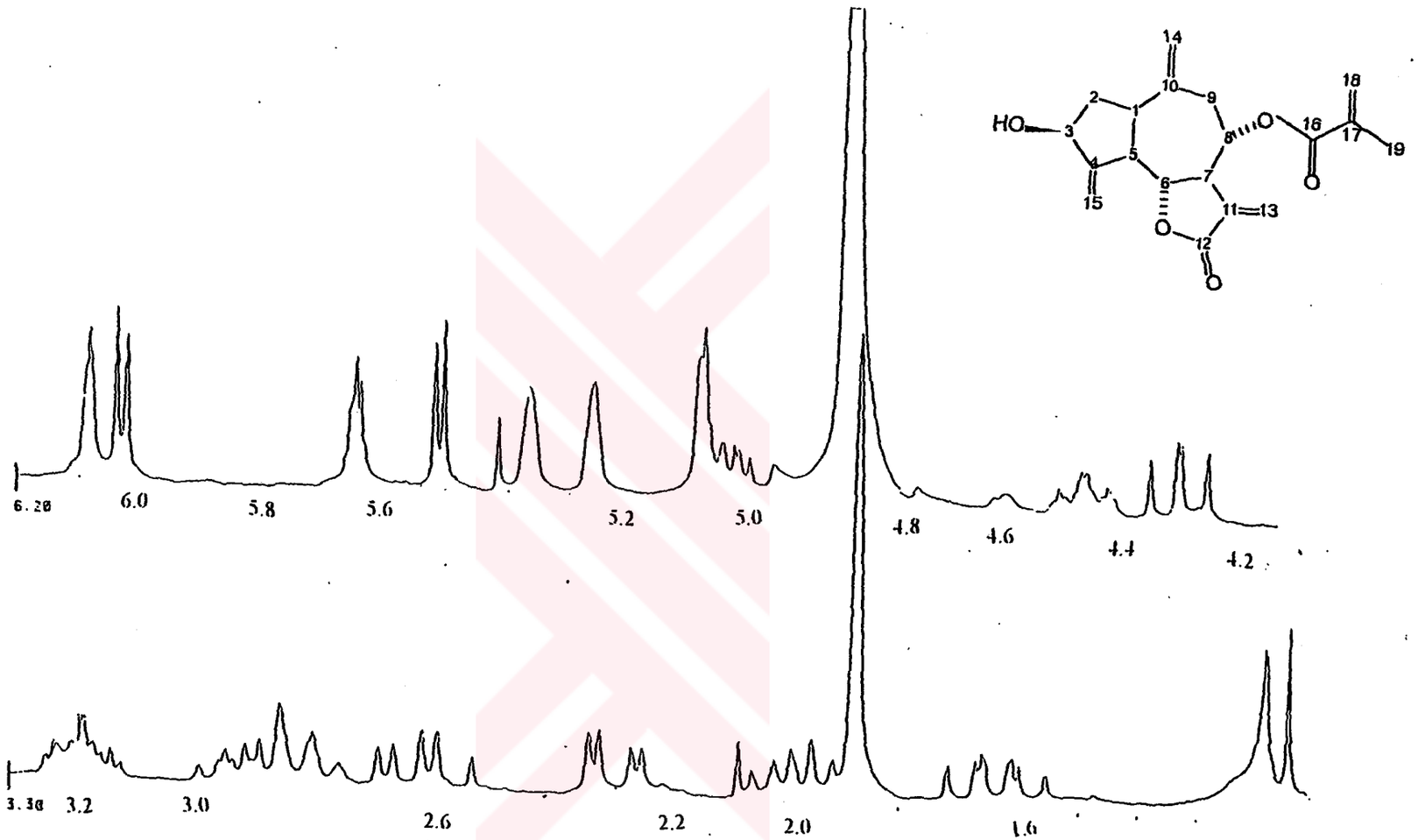


FIGURE 4.2 The ^{1}H NMR Spectrum of CC1

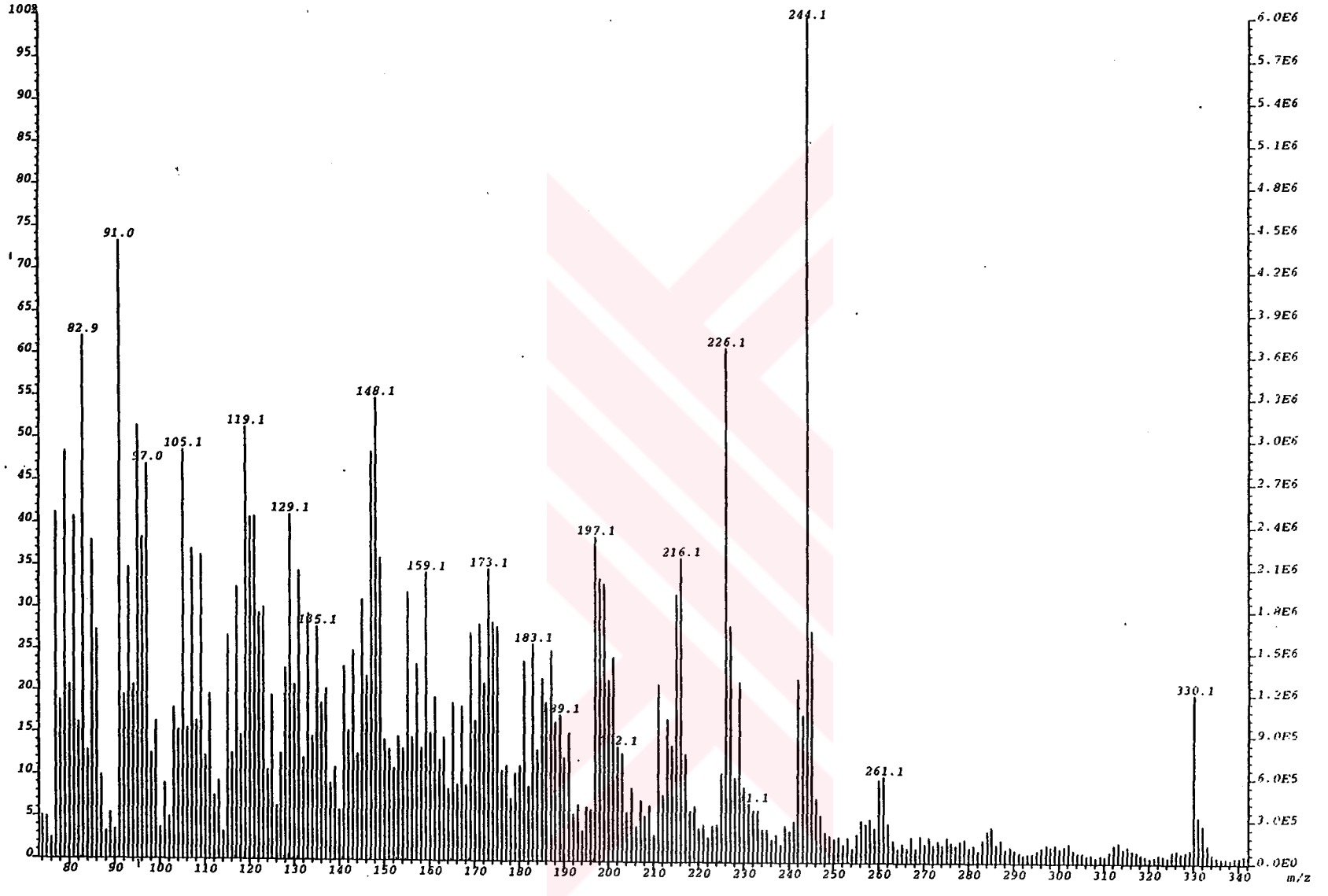
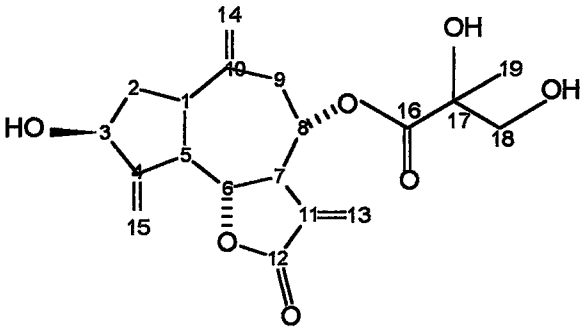


FIGURE 4.3 The EI Mass Spectrum of CC1

4.2.1.2. Structure Determination of CC2.



(17)

The compound CC2 (17) exhibited the typical IR absorption bands of an α, β-unsaturated γ-lactone C=O group (1760 cm^{-1}), acyl group (1720 cm^{-1} and 1280 cm^{-1}), hydroxyl group (3440 cm^{-1}) and unsaturation (1640 cm^{-1}) (FIGURE 4.4).

The ^1H NMR spectrum (FIGURE 4.5) of CC2 in $CDCl_3$ showed two doublets at δ 6.25 (H-13, d, J=4Hz) and δ5.57 (H-13', d, J=3 Hz) indicating the presence of typical exocyclic methylene protons of the lactone ring due to allylic coupling with H-7. The chemical shifts of methylene protons at C-14 appear at δ 5.17 (H-14, br s) and δ 5.09 (H-14', br s) and the signals appearing at δ 5.50 (H-15, br s) and δ 5. 38 (H-15', br s) indicate the presence of exocyclic methylene at the position C-4. The lactone proton H-6β appeared at δ 4.28 (dd, J=9, 10 Hz) due to axial-axial coupling with the trans H-5 and trans H-7 protons. The splitting of H-6 is also evidence for lactone ring closure at C-6. The signal of H-7α appeared at δ 3.17 (ddt, J=9, 9, 3 Hz). The chemical shift of H-3α at δ 4.57 (br t, J=7 Hz) is characteristic of protons with neighbouring β-OH functions The chemical shift of H-5α appears at δ 2.82 as broad triplet. A pair of doublets at δ 3.88 and δ 3.65 (J=11 Hz) indicated the presence of diastereotopic protons at C-18 with neighbouring OH group and a methyl singlet at δ 1.56 suggested that the acyl group is an α, β dihydroxyisobutyryl moiety. In the mass spectrum peaks at m/e 244.1 $[M-C_4H_8O_4]^+$ and 119.1 $[C_4H_8O_4]^+$ confirmed the presence of this group. The rest of the spectral data are listed in TABLE 4.2.

The EI Mass spectrum m/e (relative intensity) 364.1 ($C_{19}H_{24}O_7$) $[M]^+$ (3) supports the deduced structure by means of its molecular weight. The EI Mass spectrum (FIGURE 4.6) of CC2 showed :

364.1 $[M]^+$ (3)

119.1 $[C_4H_8O_4]^+$ (32)

262.1 $[M- C_4H_7O_3]^+$ (17)

244.1 $[M- C_4H_8O_4]^+$ (96)

227.1 $[244.1-H_2O]^+$ (100)

peaks besides the typical m/e 105.1 peak of the methyl tropylium ion, m/e 91 $[105.1-CH_3]^+$ tropylium ion, m/e 79 $[91-CH_3]^+$ and m/e 77 phenyl cation.

The above data are in agreement with those of 3β-hydroxy-8α-(2,3-dihydroxy,2-methylpropanoyl)-4(15), 10(14), 11(13)-guatrien-12,6α-olide as cited in the literature [21].

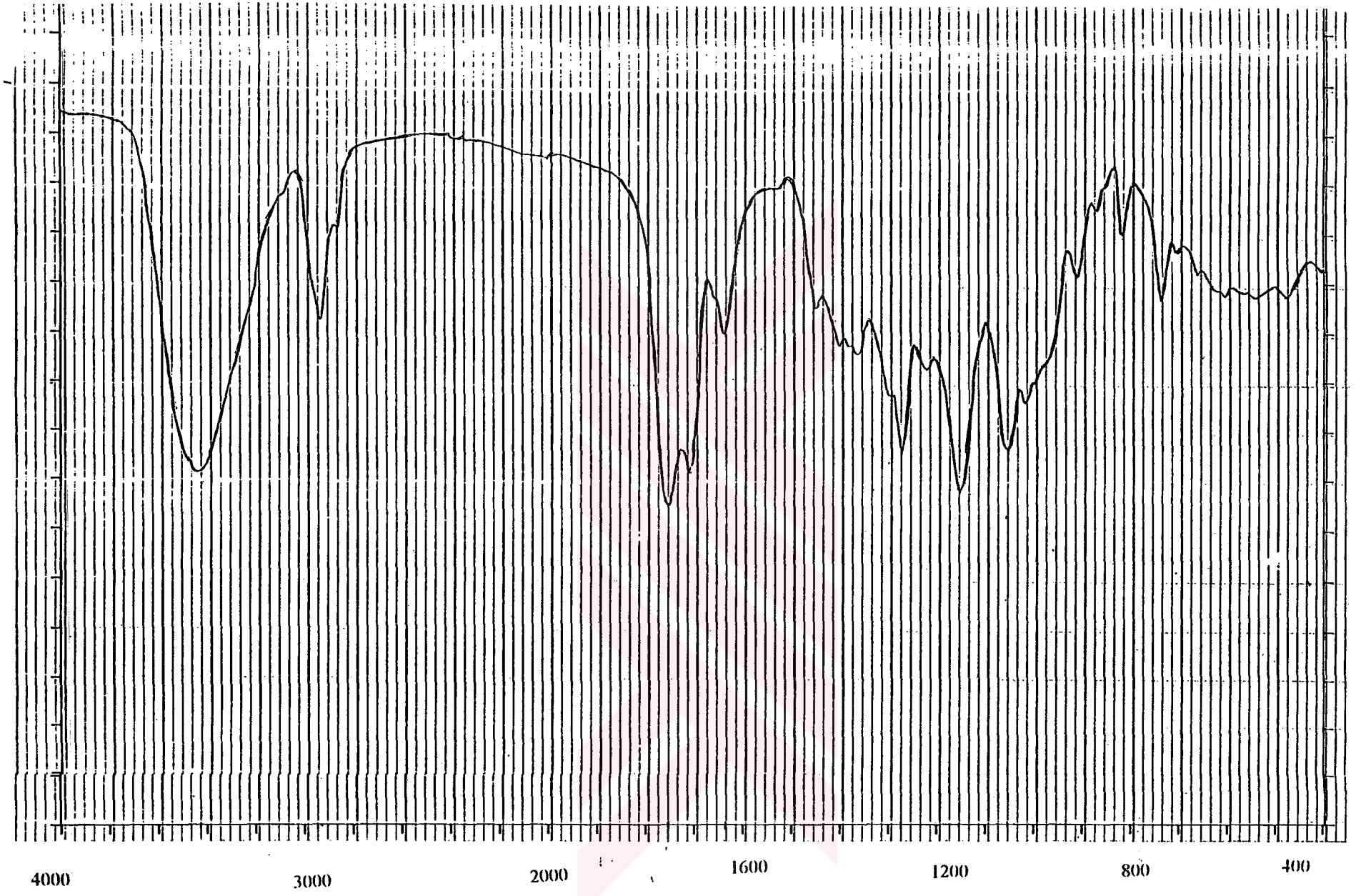


FIGURE 4.4 The IR Spectrum of CC2

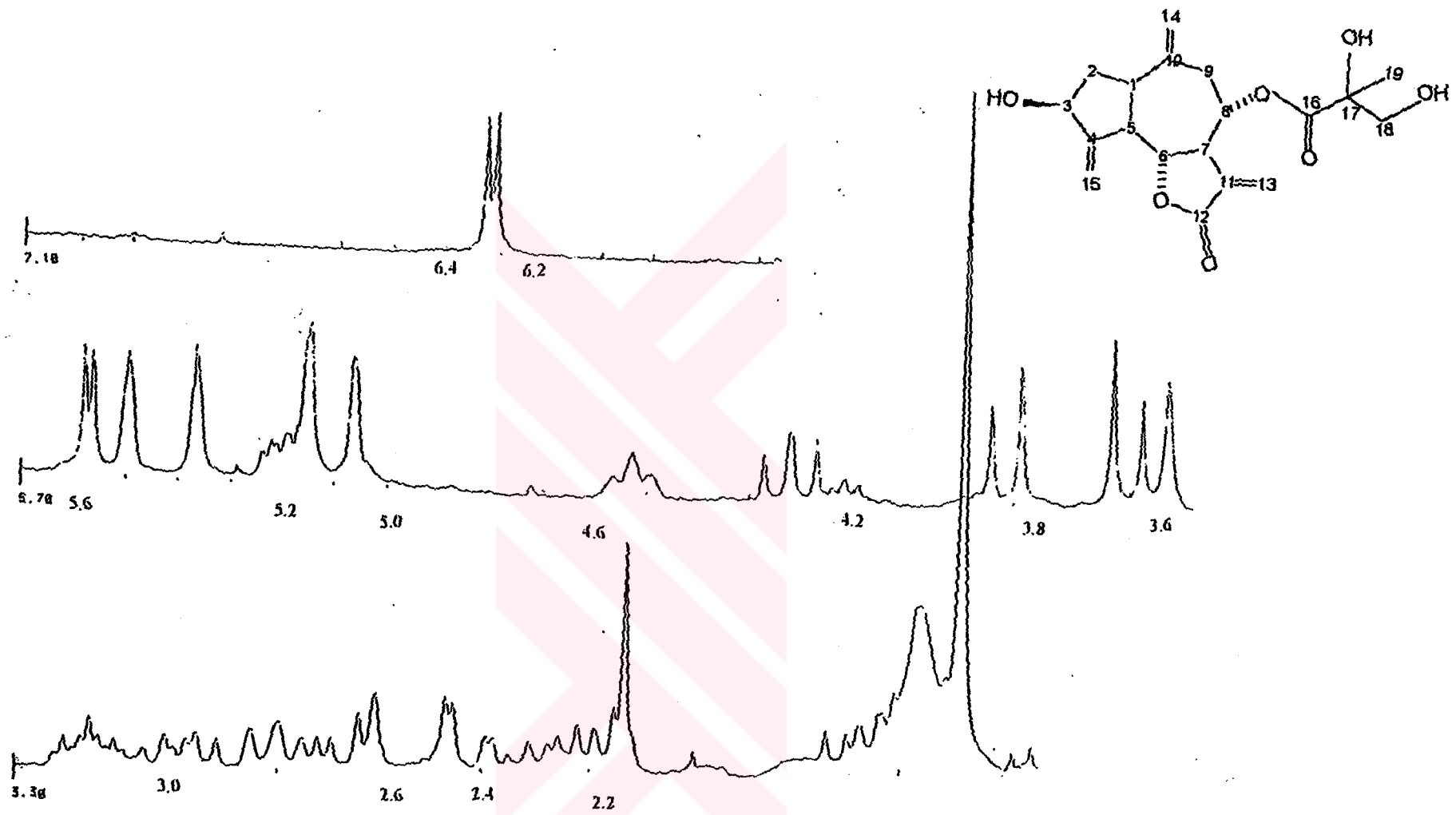


FIGURE 4.5 The ^{1}H NMR Spectrum of CC2

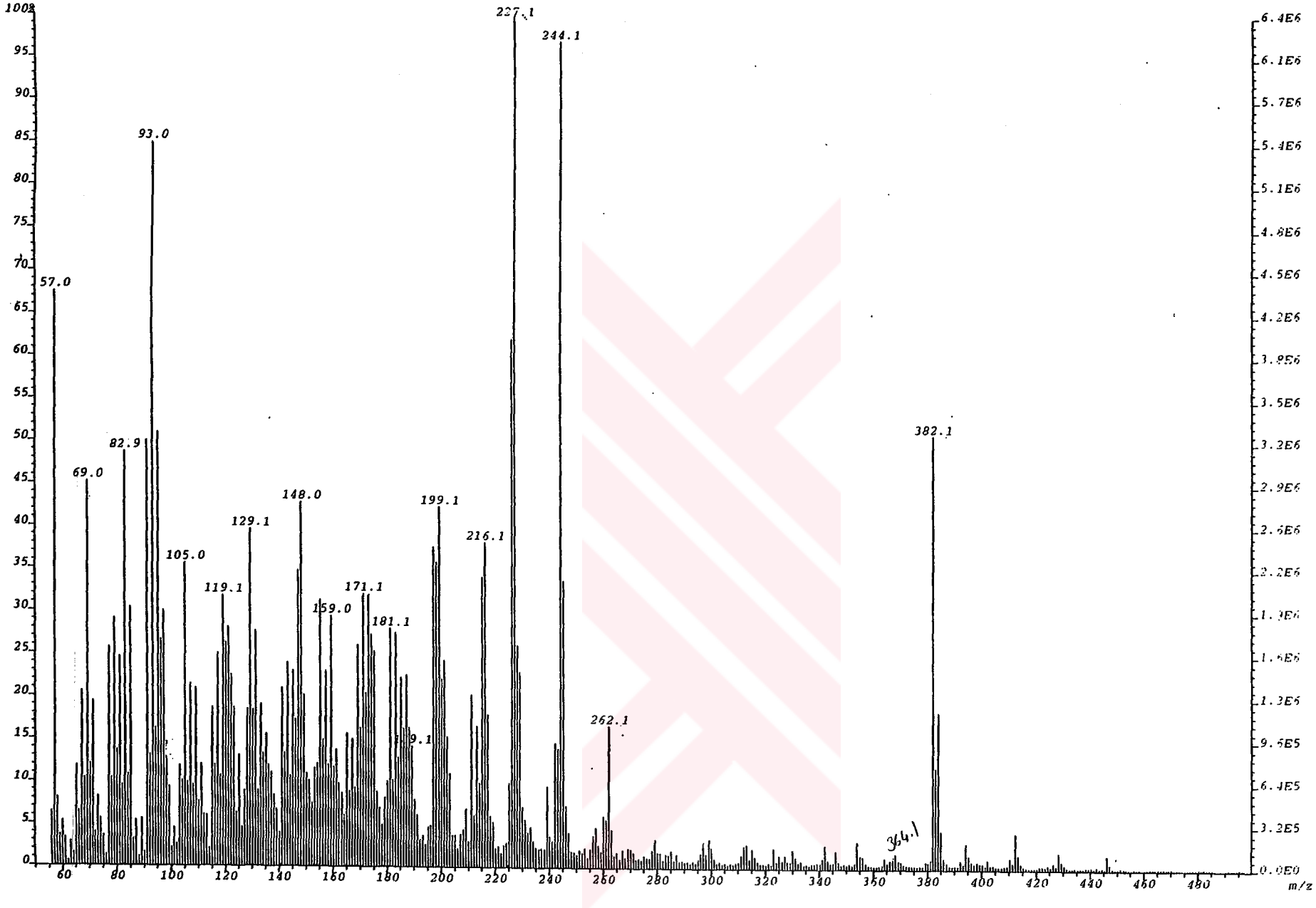
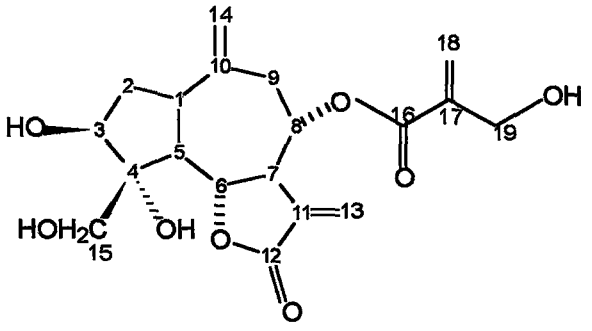


FIGURE 4.6 The EI Mass Spectrum of CC2

4.2.1.3. Structure Determination of CC3.



(18)

The compound CC3 (18) exhibited the typical IR absorption bands of an α, β unsaturated γ-lactone C=O group (1760 cm^{-1}), acyl group (1720 cm^{-1} and 1280 cm^{-1}), hydroxyl groups (3440 cm^{-1}) and unsaturation (1640 cm^{-1}) (FIGURE 4.7).

The ^1H NMR spectrum (FIGURE 4.8) of CC3 in $CDCl_3$ + CD_3OD showed two doublets at δ 6.18 (H-13, d, J=4 Hz) and δ 5.66 (H-13', d, J=4 Hz) indicating the presence of exocyclic methylene protons of the lactone ring due to allylic coupling with H-7. The chemical shifts of methylene protons at C-14 appear at δ 5.13 (H-14, d, J=2 Hz) and δ 4.80 (H-14', d, J=2 Hz). The absence of the signals of exocyclic methylene protons (H-15 and H-15') and the presence of two nice doublets with large J value (J=12 Hz) at δ 3.80 and δ 4.24 indicate that the customary C4-methylene is substituted by HO-C4-CH_2OH. The lactone proton H-6β appeared at δ 4.88 (dd, J=9, 10 Hz) due to axial-axial coupling with the trans H-5 and trans H-7 protons. The splitting of H-6 is also evidence for lactone ring closure at C-6. The signal of H-7α appeared at δ 3.20 (ddt, J=10, 10, 3 Hz). The splitting of H-3α at δ 3.52 is different from that of guaianolides which have exocyclic methylene group at C-4. Therefore this signal also proves the presence of CH_2OH group at C-4. The H-8β signal appeared at δ 5.15 (m) and it indicates the presence of α-O functionality at C-8. Two singlets at δ 6.35 (br s) and δ 6.02 (d, J=2 Hz) are evidence for ester side chain which contains methylene group at C-17. The broad singlet expected at δ 4.38 belonging to the C-19 protons remains under the MeOH peak. The rest of the spectral data are listed in TABLE 4.1.

The ^{13}C APT (Attached Proton Test) spectrum (FIGURE 4.9) of CC3 supported the structure giving one saturated quaternary, three unsaturated quaternary, three methylene, six methine (three of them with neighbouring oxygen functionality), four secondary (two of them with oxygen functionality) and one carbonyl carbon signal. The other expected carbonyl signal is not observed probably due to insufficiency of material.

The EI Mass spectrum m/e (relative intensity) 380.1 ($C_{19}H_{24}O_8$) $[M]^+$ supports the deduced structure by means of its molecular weight. The EI Mass spectrum (FIGURE 4.10) of CC3 showed :

381.1 $[M+1]^+$ (1)

85 $[C_4H_5O_2]^+$ (100)

296.1 $[M+1-C_4H_5O_2]^+$ (19)

278.1 $[296.1-H_2O]^+$ (11)

260.1.1 $[278.1-H_2O]^+$ (18)

242.1.1 $[260.1-H_2O]^+$ (18)

peaks besides the typical m/e 105.1 peak of the methyl tropylium ion, m/e 91 $[105.1-CH_3]^+$ tropylium ion, m/e 79 $[91-CH_3]^+$ and m/e 77 phenyl cation.

The compound CC3 was assigned to structure 15-deschloro-15 hydroxychlorojanerin on the basis of above spectral data, which are confirmed by the literature [22].

TABLE 4.1 The ^{1}H NMR and ^{13}C NMR (APT) Data for CC3 (18)

H	δ (ppm)	C	δ (ppm)	APT	
1α	*	1	46.74	-	d
2α	1.56 m	2	35.28	+	t
2β	*	3	74.65	-	d
3α	3.52 (m)	4	84.91	+	s
5α	2.3 (br t)	5	58.48	-	d
6β	4.88 (dd, J= 9, 10 Hz)	6	77.89	-	d
7α	3.20 (ddt, J= 10, 10, 3 Hz)	7	48.08	-	d
8β	5.15 (m)	8	76.34	-	d
9α	2.90 (dd, J= 15, 5 Hz)	9	38.91	+	t
9β	*	10	140.46	+	s
13	6.18 (d, J= 4 Hz)	11	137.82	+	s
13'	5.66 (d, J= 4 Hz)	12	169.7**	+	s
14	5.13 (d, J= 2 Hz)	13	122.74	+	t
14'	4.80 (d, J= 2 Hz)	14	117.57	+	t
15	4.24 (d, J= 12 Hz)	15	78.53	+	t
15'	3.80 (d, J= 12 Hz)	16	165.94	+	s
18	6.35 (br s)	17	143.42	+	s
18'	6.02 (d, J= 2 Hz)	18	125.98	+	t
19	4.38 (br s)	19	61.04	+	t

* Complicated signals

** Literature value

FIGURE 4.7 The IR Spectrum of CC3

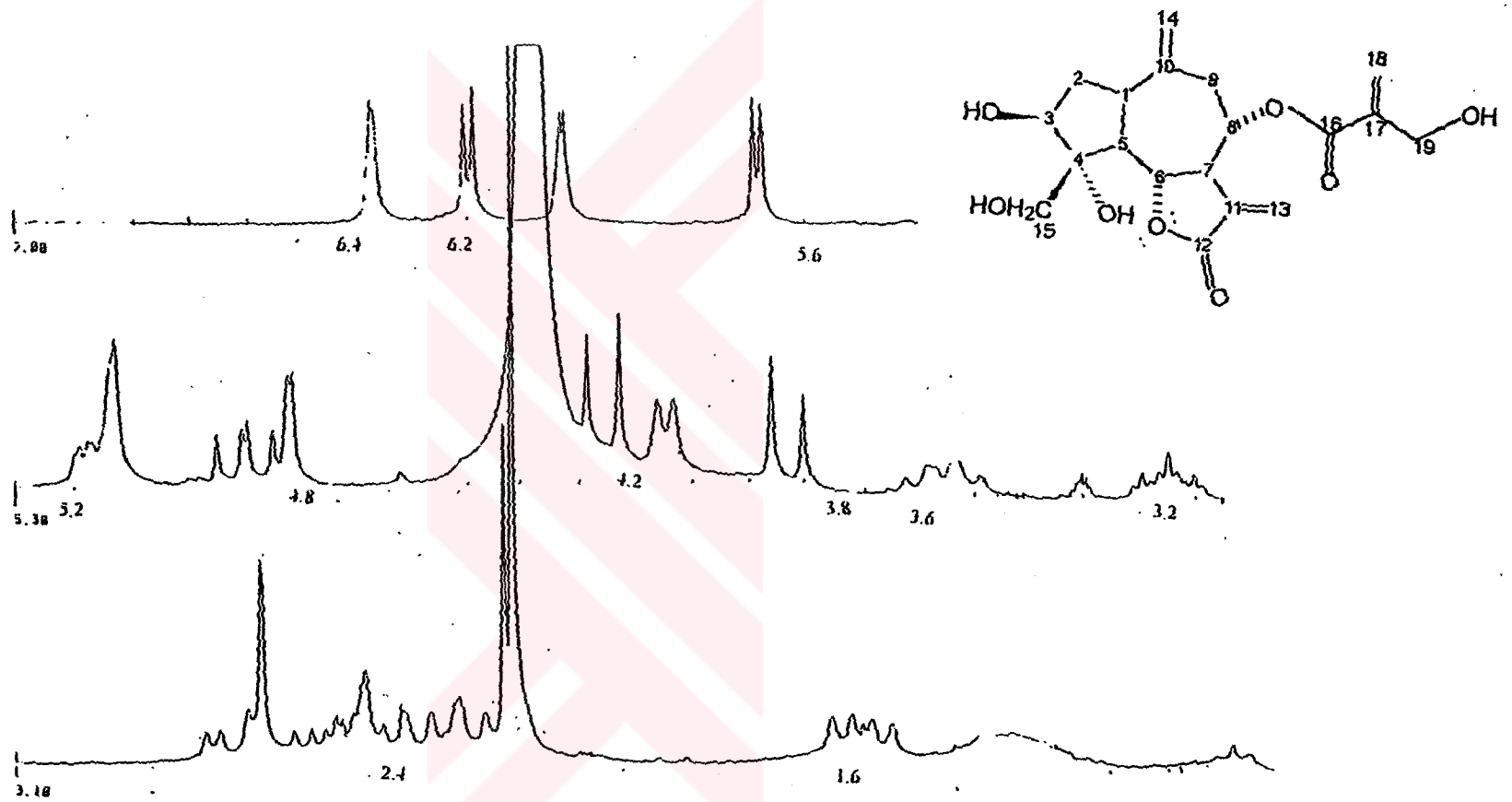


FIGURE 4.8 The ^1H NMR Spectrum of CC3

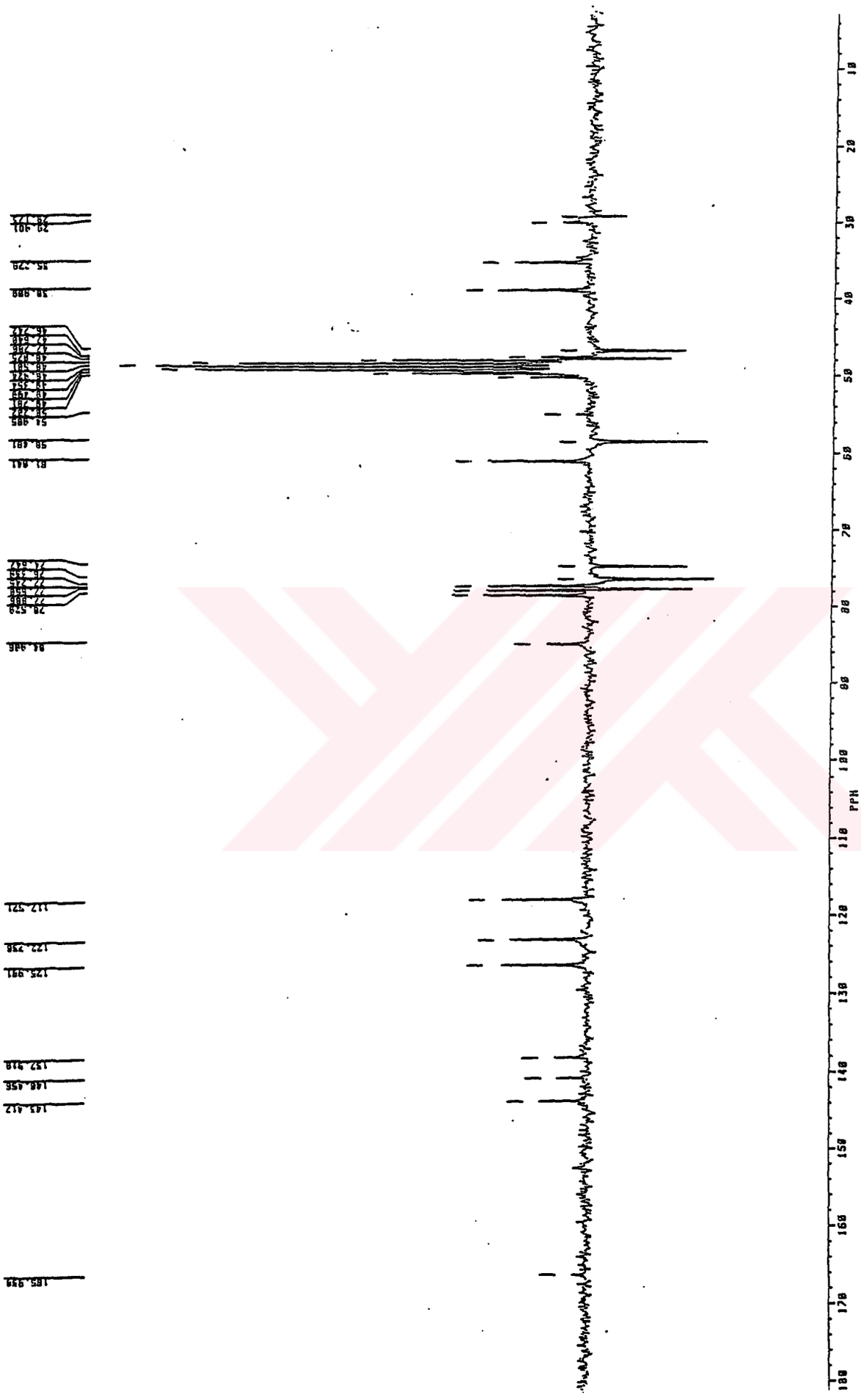


FIGURE 4.9 The ^{13}C NMR Spectrum of CC3

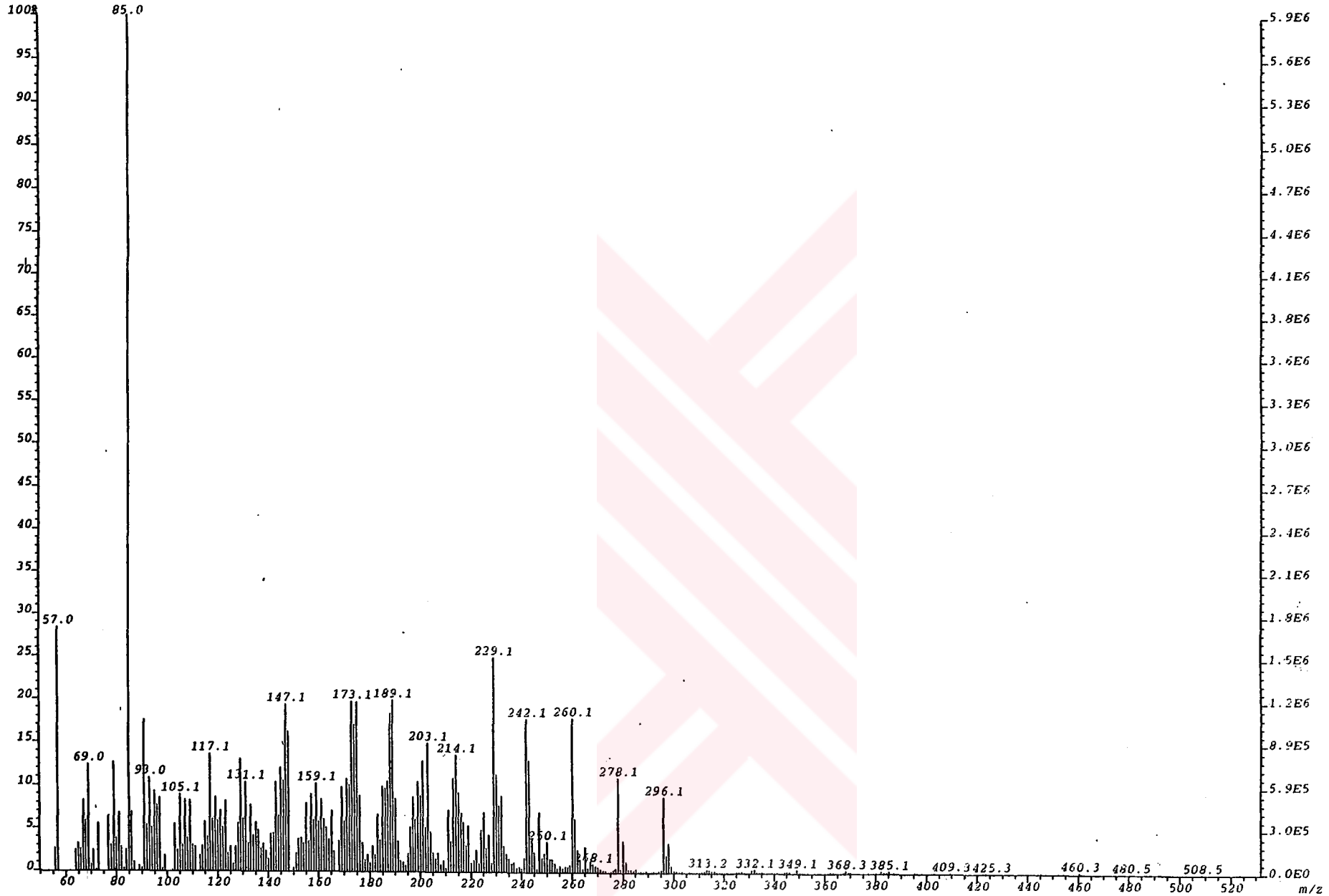
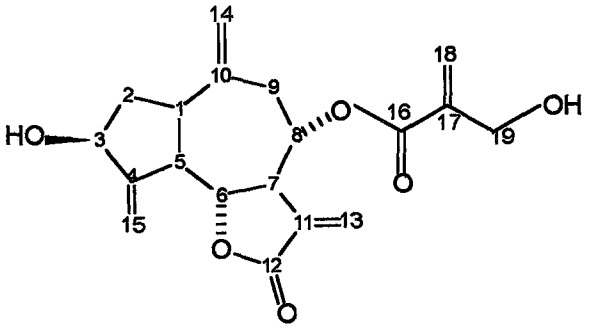


FIGURE 4.10 The EI Mass Spectrum of CC3

4.2.1.4. Structure Determination of CC4 (Cynaropicrin).



(19)

The compound CC4 (19) exhibited the typical IR absorption bands of an α, β-unsaturated γ-lactone C=O group (1760 cm^{-1}), acyl group (1710 cm^{-1} and 1275 cm^{-1}), hydroxyl groups (3400 cm^{-1}) and unsaturation (1660 cm^{-1}) (FIGURE 4.11).

The ^1H NMR spectrum (FIGURE 4.12) of CC4 in $CDCl_3$ showed two doublets at δ 6.23 (H-13, d, J=4 Hz) and δ 5.63 (H-13', d, J=3 Hz) indicating the presence of exocyclic methylene protons of the lactone ring. The chemical shifts of methylene protons at C-14 appear at δ 5.15 (H-14, br s) and δ 4.94 (H-14', br s) and the signals appearing at δ 5.49 (H-15, br s) and δ 5. 37 (H-15', br s) indicate the presence of exocyclic methylene at the position C-4. The lactone proton H-6β appeared at δ 4.26 (dd, J=9, 10 Hz) due to axial-axial coupling with the trans H-5 and trans H-7 protons. The splitting of H-6 is also evidence for lactone ring closure at C-6. The signal of H-7α appeared at δ 3.20 (ddt, J=10, 10, 3 Hz). The chemical shift of H-3α at δ 4.57 (br t, J=7 Hz) is characteristic of protons with neighbouring β-OH functions. The signals at δ 2.72 (H-9α, dd, J=15, 4 Hz) and δ 2.40 (H-9β, dd, J=15, 5 Hz) indicates the presence of α-O functionality at C-8. Two doublets at δ 6.34 (br s) and δ 5.96 (br s) are evidence for ester side chain which contains methylene group at C-17. The broad singlet at δ 4.38 belongs to the C-19 protons. The rest of the spectral data are listed in TABLE 4.2

The EI Mass spectrum m/e (relative intensity) 346.1 ($C_{19}H_{22}O_6$) $[M]^+$ (8) supports the deduced structure by means of its molecular weight. The EI Mass spectrum (FIGURE 4.13) of CC4 showed :

346.1 $[M]^+$ (8)

85 $[C_4H_5O_2]^+$ (100)

262.1 [M-4-hydroxymethacrylate]$^+$ (21)

244.1 $[262.1\text{-}H_2O]^+$ (51)

226.1 $[244.1\text{-}H_2O]^+$ (39)

peaks besides the typical m/e 105.1 peak of the methyl tropylium ion, m/e 91 $[105.1\text{-}CH_3]^+$ tropylium ion, m/e 79 $[91\text{-}CH_3]^+$ and m/e 77 phenyl cation.

The spectral data for compound CC4 are nearly identical with the data cited in the literature for Cynaropicrin, which has been isolated from *Centaurea behen* [16], *Centaurea canariensis* [17], *Centaurea arguta* [18], *Centaurea solstitialis subsp.schouwi* [20], *Centaurea kotschy* [21], *Centaurea hermanii* [23], *Centaurea calcitrapa* [24], *Centaurea clementei* [25], *Cheirolophus sempervirens(L)* [26], *Centaurea scoparia* [27] and. *Centaurea bella* [28].

Centaurea solstitialis has been reported to cause "Parkinson-like" disease in horses. One reason for this has been found to be due to the presence cynaropicrin. Cynaropicrin in this plant was found to be toxic in concentration dependent manner and may be responsible for the ability of the plant to cause neurodegenerative changes in the brain of horses [29]. An other litearature was stated that this plant was also found to inhibit smooth muscle contractility in a time dependent, non-specific and irreversible manner. This toxic effect is due specifically to the presence of potentially reactive α-methylene function in Cynaropicrin [30].

TABLE 4.2 ^{1}H NMR Data for CC1 (16), CC2 (17) and CC4 (19)

H	CC1 δ (ppm)	CC2 δ (ppm)	CC4 δ (ppm)
1α	2.94(m)	3.0 (m)	2.98(m)
2α	1.64 (ddd, J= 9, 10, 12 Hz)	1.72 (ddd, J= 9, 10, 12 Hz)	1.74 (m)
2β	1.98 (ddd, J= 7.5, 10, 12 Hz)	2.25 (m)	2.24 (m)
3α	4.41 (br t)	4.57 (br t)	4.57 (br t)
5α	2.79 (br t)	2.82 (br t)	2.85 (br t)
6β	4.25 (dd, J= 9, 10 Hz)	4.28 (dd, J= 9, 10 Hz)	4.26 (dd, J= 9, 10 Hz)
7α	3.18 (ddt, J= 9, 9, 3 Hz)	3.17 (ddt, J= 9, 9, 3 Hz)	3.20 (ddt, J= 10, 10, 3 Hz)
8β	4.99 (ddd, J= 4, 5, 9 Hz)	5.22 (m)	5.14 (m)
9α	2.63 (dd, J= 15, 5 Hz)	2.70 (dd, J= 15, 5 Hz)	2.72 (dd, J= 14, 4 Hz)
9β	2.28 (dd, J= 15, 4 Hz)	2.46 (dd, J= 15, 3 Hz)	2.40 (dd, J= 14, 5 Hz)
13	6.02 (d, J= 3 Hz)	6.25 (d, J= 4 Hz)	6.23 (d, J= 4 Hz)
13'	5.49 (d, J= 3 Hz)	5.57 (d, J= 3 Hz)	5.63 (d, J= 3 Hz)
14	5.05 (br s)	5.17 (br s)	5.15 (br s)
14'	4.79 (br s)	5.09 (br s)	4.94 (br s)
15	5.34 (br s)	5.50 (br s)	5.49 (br s)
15'	5.23 (br s)	5.38 (br s)	5.37 (br s)
18	6.08 (br s)	3.88 (d, J=11 Hz)	6.34 (br s)
18'	5.63 (d, J= 1.5 Hz)	3.65 (d, J= 11 Hz)	5.96 (d, J= 1.5 Hz)
19	1.88 (s)	1.56 (s)	4.38 (br s)

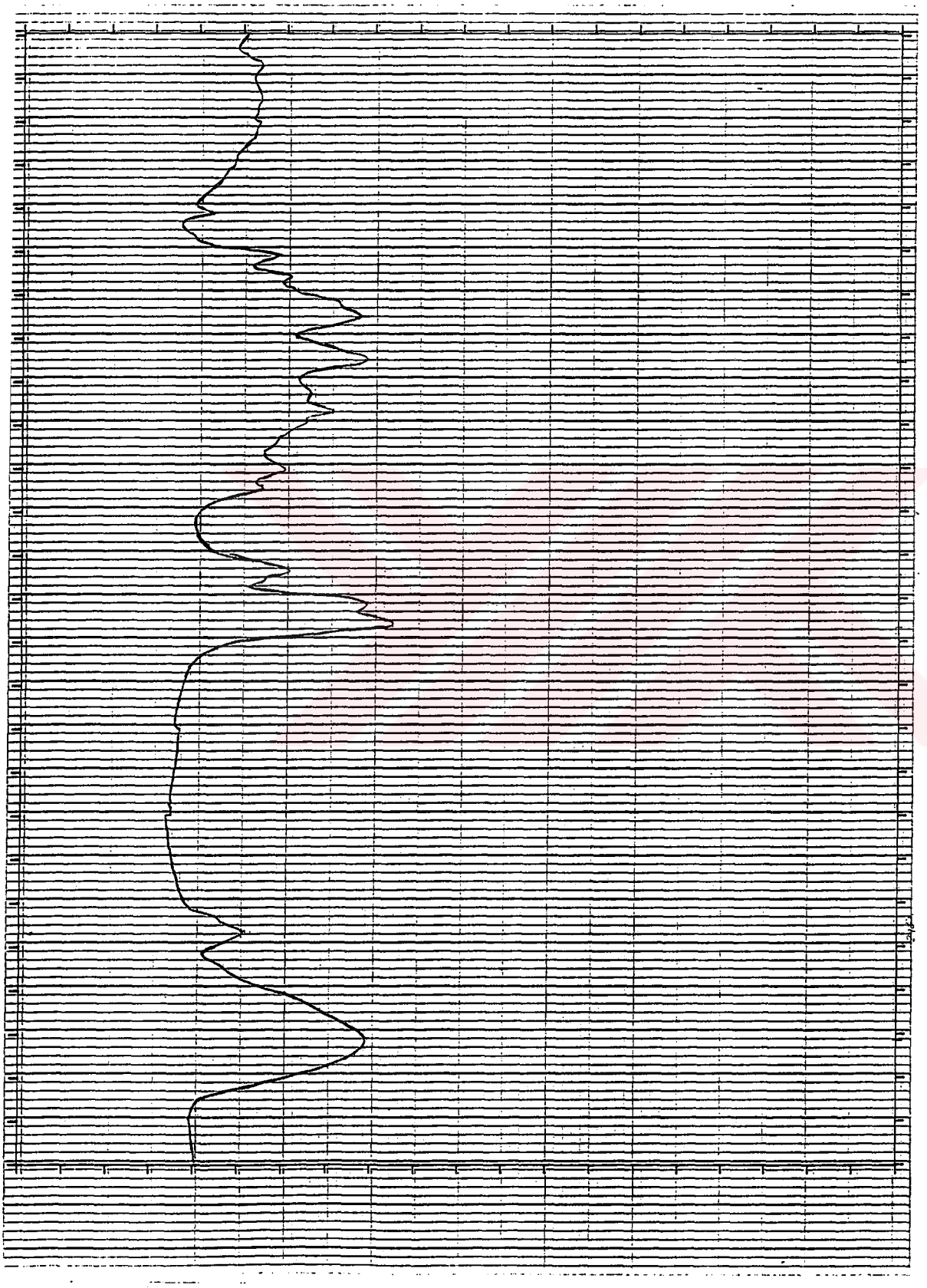


FIGURE 4.11 The IR Spectrum of CC4

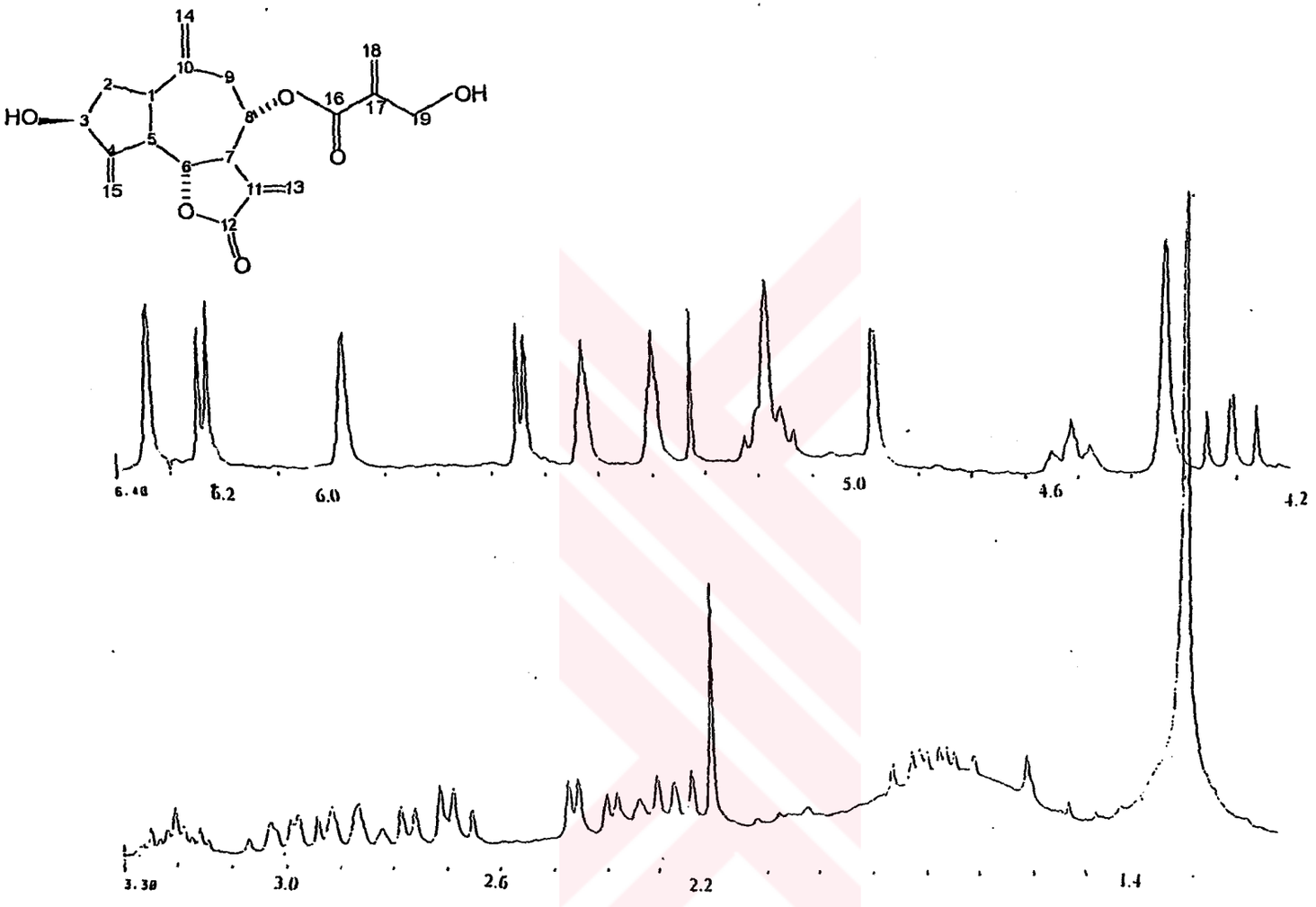


FIGURE 4.12 The ^{1}H NMR Spectrum of CC4

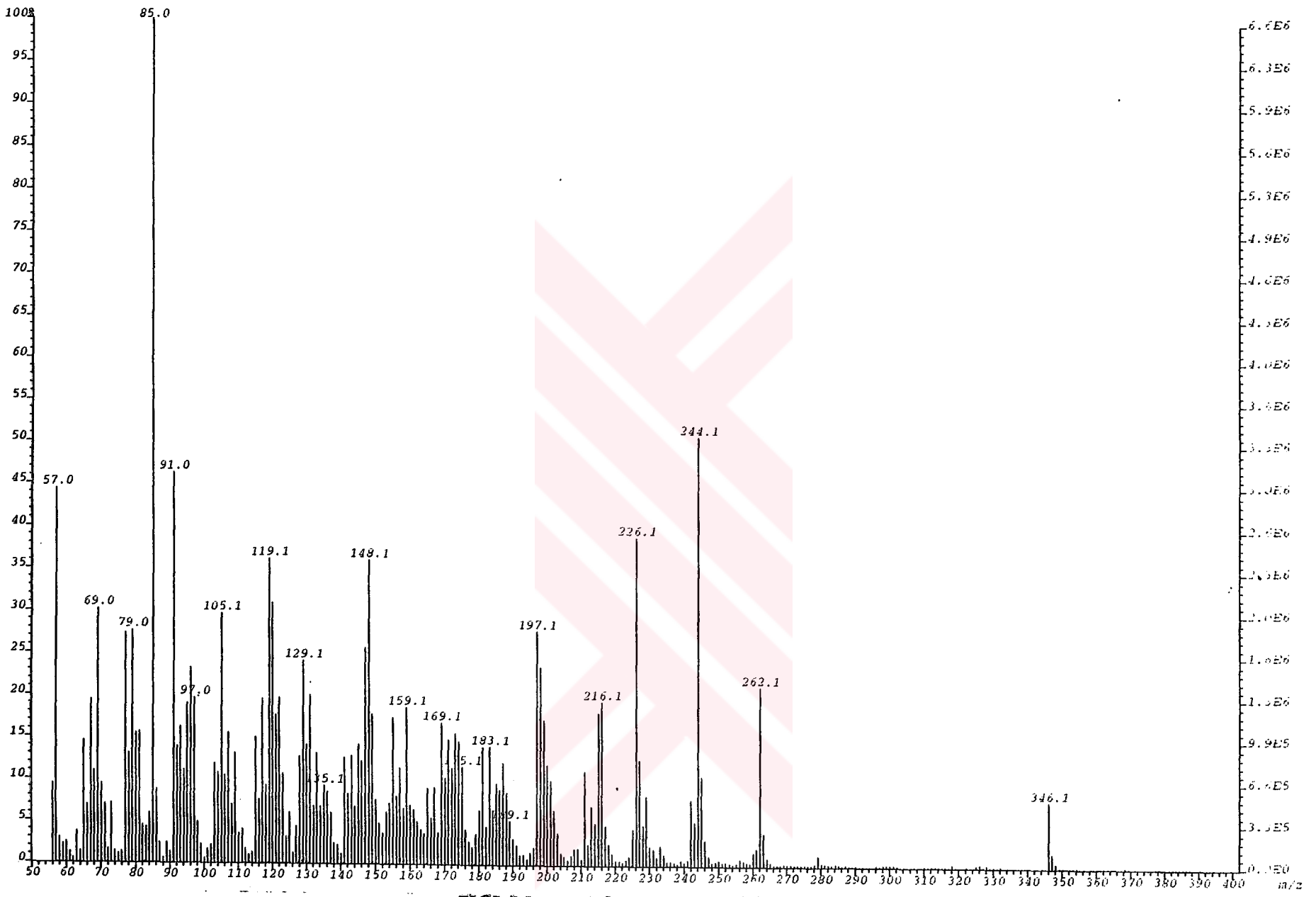
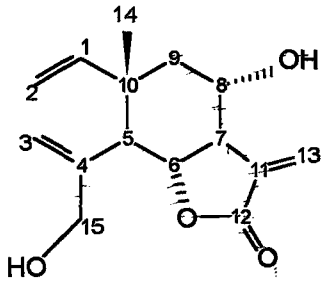


FIGURE 4.13 The EI Mass Spectrum of CC4

4.2.2. *Centaurea cuneifolia Sm.*

4.2.2.1. Structure Determination of CCU1 (Dehydromelitensin).



(20)

The compound CCU1 (20) exhibited the typical IR absorption bands of an α, β unsaturated γ-lactone C=O group (1760cm^{-1}), C-O stretching (1270cm^{-1}) hydroxyl groups (3440 cm^{-1}) and unsaturation (1640 cm^{-1}) (FIGURE 4.14).

The ^{1}H NMR spectrum (FIGURE 4.15) of CCU1 in $CDCl_3$ showed two doublets at δ 6.18 (H-13, d, J= 4Hz) and δ 5.99 (H-13', d, J= 3 Hz) indicating the presence of exocyclic methylene protons of the lactone ring due to allylic coupling with H-7. The chemical shift of methyl protons at C-14 is at δ 1.10 (H-14, s) and the expected signal for the third methyl group is absent. Instead, the presence of signals appearing at δ 3.97 (H-15, d, J=13 Hz) and δ 4.09 (H-15', d, expected J=13 Hz) (overlapping with H-6) indicate the presence of hydroxyl group attached to methylene at C-4. The signal of H-5 appeared at δ 2.51 (d, J=12 Hz). The lactone proton H-6 appeared at δ 4.15 (t, J=12 Hz) due to axial-axial coupling with the trans H-5 and trans H-7 protons. The splitting of H-6 is also evidence for lactone ring closure at C-6. The signal of H-7α appeared at δ 2.64 (tt, J=12, 3 Hz). The stereochemistry of this proton is decided from its coupling constant. A pair of singlets at δ 5.41 (br s) and δ 4.95 (br s) belongs to H-3 and H-3' respectively. A pair of overlapping doublets at δ 5.08 (d, J= 11 Hz) and δ 5.02 (d, J=16 Hz) belongs to H-2 and H-2' respectively. The signals at δ 1.87 (H-9a, dd, J=14, 5 Hz) and δ 1.61 (H-9β, dd, J=14, 5 Hz) indicates the presence of α-OH functionality at C-8. The signal of H-1 appeared at δ 5.76 (dd, J=11, 16 Hz).The rest of the spectral data are listed in TABLE 4.3.

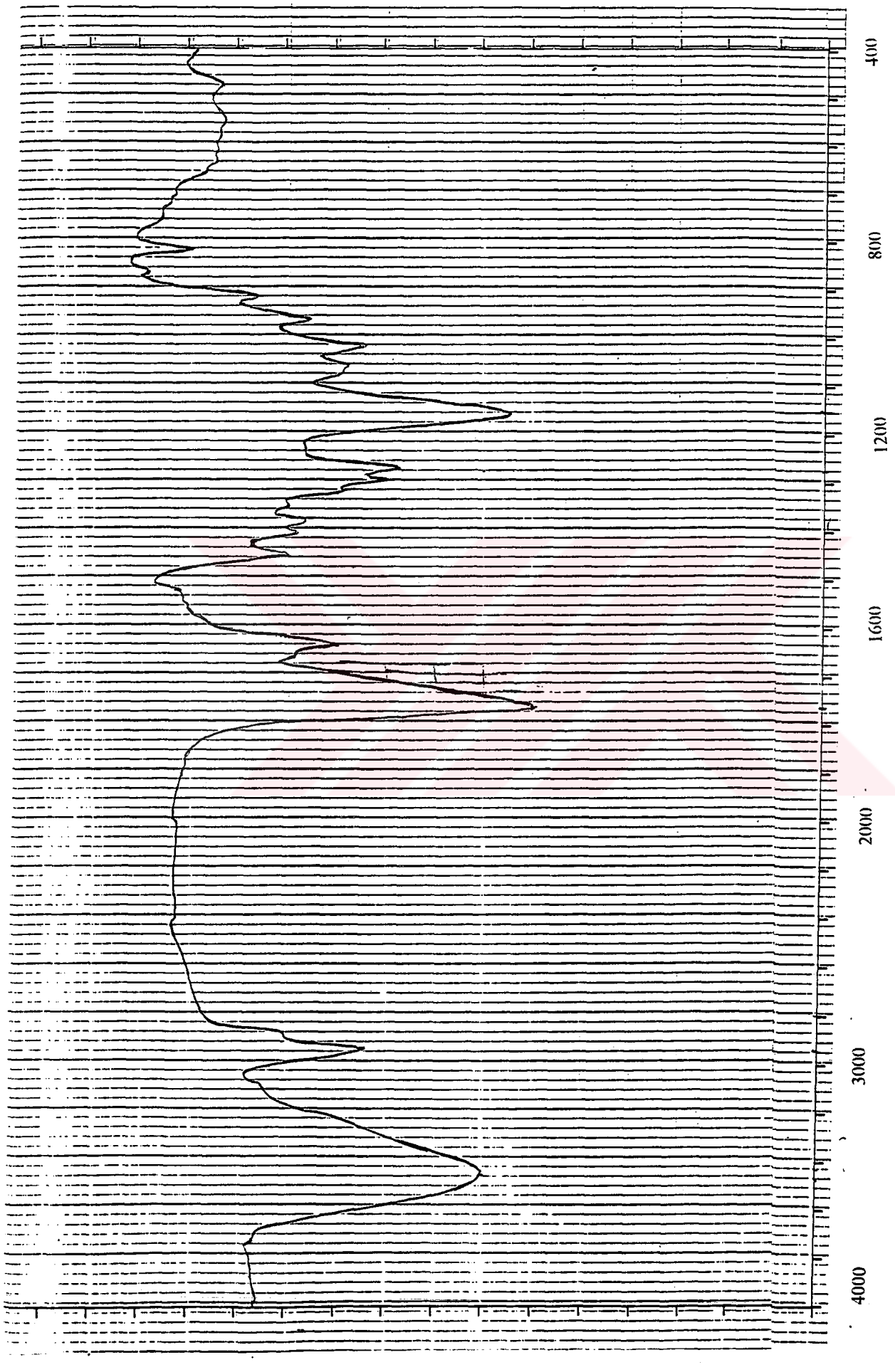


FIGURE 4.14 The IR Spectrum of CCU1

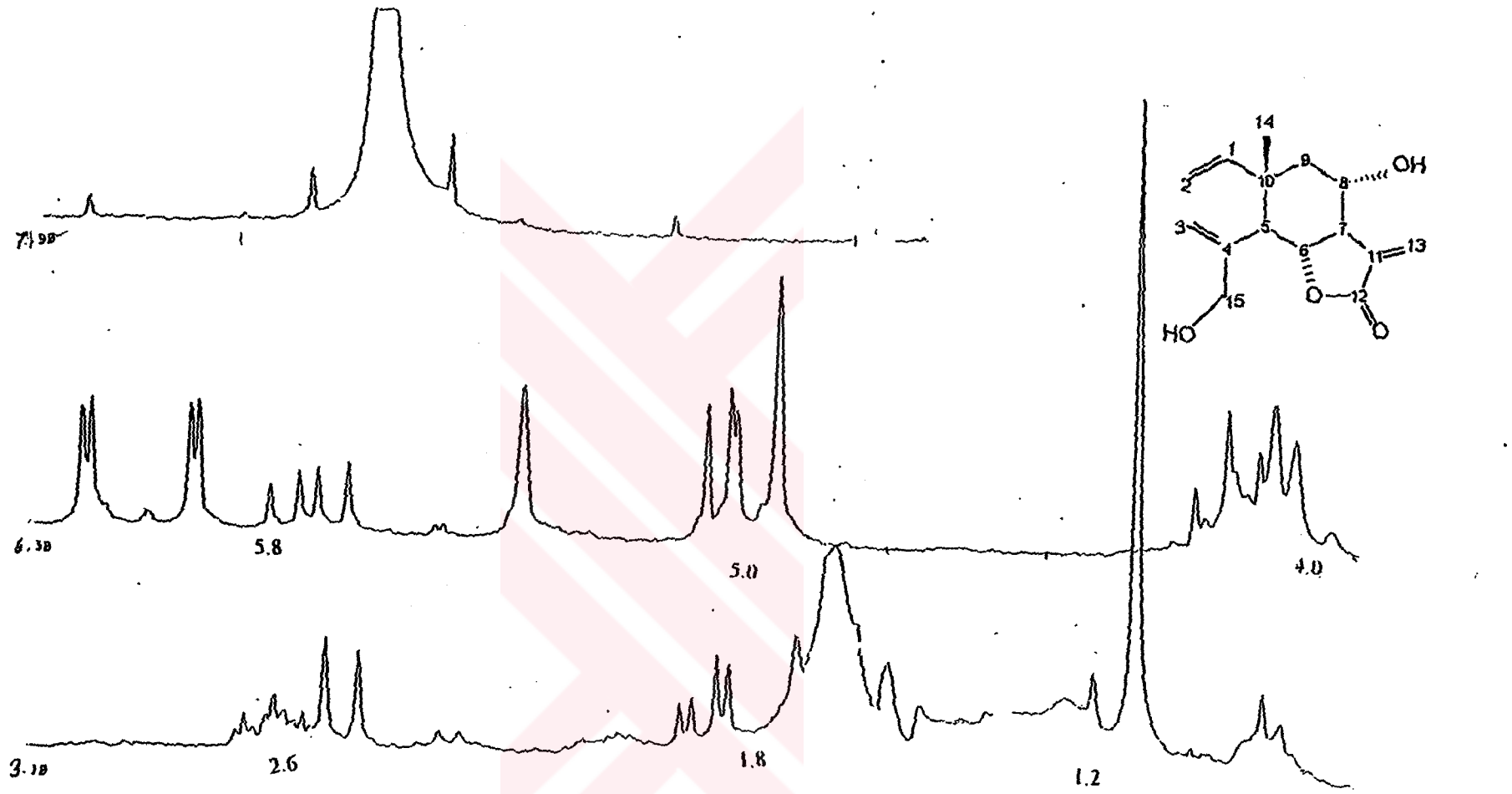
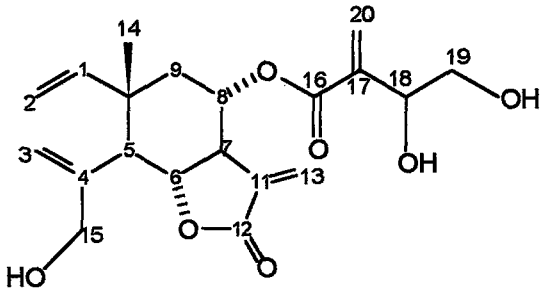


FIGURE 4.15 The ^{1}H NMR Spectrum of CCU1

4.2.2.2. Structure Determination of CCU2.



(21)

The IR spectrum (FIGURE 4.16) of CCU2 (21) showed peaks at 1770 cm^{-1} (α, β unsaturated γ-lactone C=O stretching), 1715 cm^{-1} (α, β unsaturated ester C=O stretching), 1215 cm^{-1} (C-O stretching of ester functionality), 1645cm^{-1} (C=C stretching) and finally 3400 cm^{-1} (O-H stretching) indicating the presence of α, β unsaturated γ-lactone, α, β unsaturated ester and hydroxyl groups respectively.

The ^1H NMR spectrum (FIGURE 4.17) of CCU2 in $CDCl_3$ showed two doublets at δ 6.14 (H-13, d, J=3Hz) and δ 5.55 (H-13', d, J=3 Hz) indicating the presence of exocyclic methylene protons of the lactone ring due to allylic coupling with H-7. The methyl protons at C-14 appear at δ 1.16 (H-14, s) and the chemical shifts of diastereotopic C-15 protons was calculated for the AB system are δ 4.00 (H-15, d, J_{AB}=14 Hz) and δ 4.08 (H-15', d, J_{AB}=14 Hz) which indicates the presence of hydroxyl group at the C-15. The signal of H-5 appeared at δ 2.58 (d, J=12 Hz). The lactone proton H-6 appeared at δ 4.25 (dd, J=11, 12 Hz) due to axial-axial coupling with the trans H-5 and trans H-7 protons. The splitting of H-6 is also evidence for lactone ring closure at C-6. The signal of H-7α appeared at δ 2.96 (tt, J=11, 3 Hz). A pair of singlets at δ 5.42 (br s) and δ 4.96 (br s) belongs to H-3 and H-3' respectively. The H-8β signal appeared at δ 5.29 (td, J=4, 11 Hz). A pair of doublets at δ 5.08 (d, J=11 Hz) and δ 5.03 (d, J=16 Hz) belongs to H-2 and H-2' respectively. The signals at δ 2.03 (H-9, dd, J=14, 4 Hz) and δ 1.64 (H-9', d, J=14 Hz) indicates the presence of α-OH functionality at C-8. The signal of H-1 appeared at δ 5.76 (dd, J=11, 16 Hz). Two singlets at δ 6.38 (br s) and δ 6.06 (br s) are evidence for ester side chain which contains methylene group at C-17. The multiplet (dd, J=4, 7 Hz) at δ 4.63 belongs to the C-18 proton. The signals of diastereotopic protons at C-19 appear at δ 3.83 (H-19, dd, J=11, 4

Hz) and δ 3.58 (H-19', dd, J=11, 7 Hz) which indicate the presence of neighbouring hydroxyl group. The rest of the spectral data are listed in TABLE 4.3.

This compound was initially isolated and identified from *Centaurea cineraria subsp.umbrosa* and the chemical shifts are assigned by decoupling experiments in the orginal literature [31].

TABLE 4.3 ^{1}H NMR Data for CCU1 (20) and CCU2 (21)

H	CCU1 δ (ppm)	CCU2 δ (ppm)
1	5.78 (dd, J= 11, 16 Hz)	5.76 (dd, J= 11, 16 Hz)
2	5.08 (d, J= 11 Hz)	5.08 (d, J=11 Hz)
2'	5.02 (d, J= 16 Hz)	5.03 (d, J= 16 Hz)
3	5.41 (br s)	5.42 (br s)
3'	4.95 (br s)	4.96 (br s)
5	2.51 (d, J= 12 Hz)	2.58 (d, J= 12 Hz)
6β	4.15 (t, J= 12 Hz)	4.25 (dd, J= 11,12 Hz)
7α	2.64 (tt, J= 12, 3 Hz)	2.96 (tt, J= 11, 3 Hz)
8β	4.18 (m)	5.29 (td, J= 4, 11 Hz)
9α	1.87 (dd, J= 14, 5 Hz)	2.03 (dd, J= 14, 4 Hz)
9β	1.61 (dd, J= 14, 5 Hz)	1.64 (d, J= 14 Hz)
13	6.18 (d, J= 4 Hz)	6.14 (d, J= 3 Hz)
13'	5.99 (d, J= 3 Hz)	5.55 (d, J= 3 Hz)
14	1.10 (s)	1.16 (s)
15	4.09 (d, J=13 Hz)	4.00 (d, J_{AB}=14 Hz)
15'	3.97 (d, J=13 Hz)	4.08 (d, J_{AB}=14 Hz)
18		4.63 (dd, J=4, 7 Hz)
19		3.83 (dd, J= 11, 4 Hz)
19'		3.58 (dd, J= 11, 7 Hz)
20		6.38 (br s)
20'		6.06 (br s)

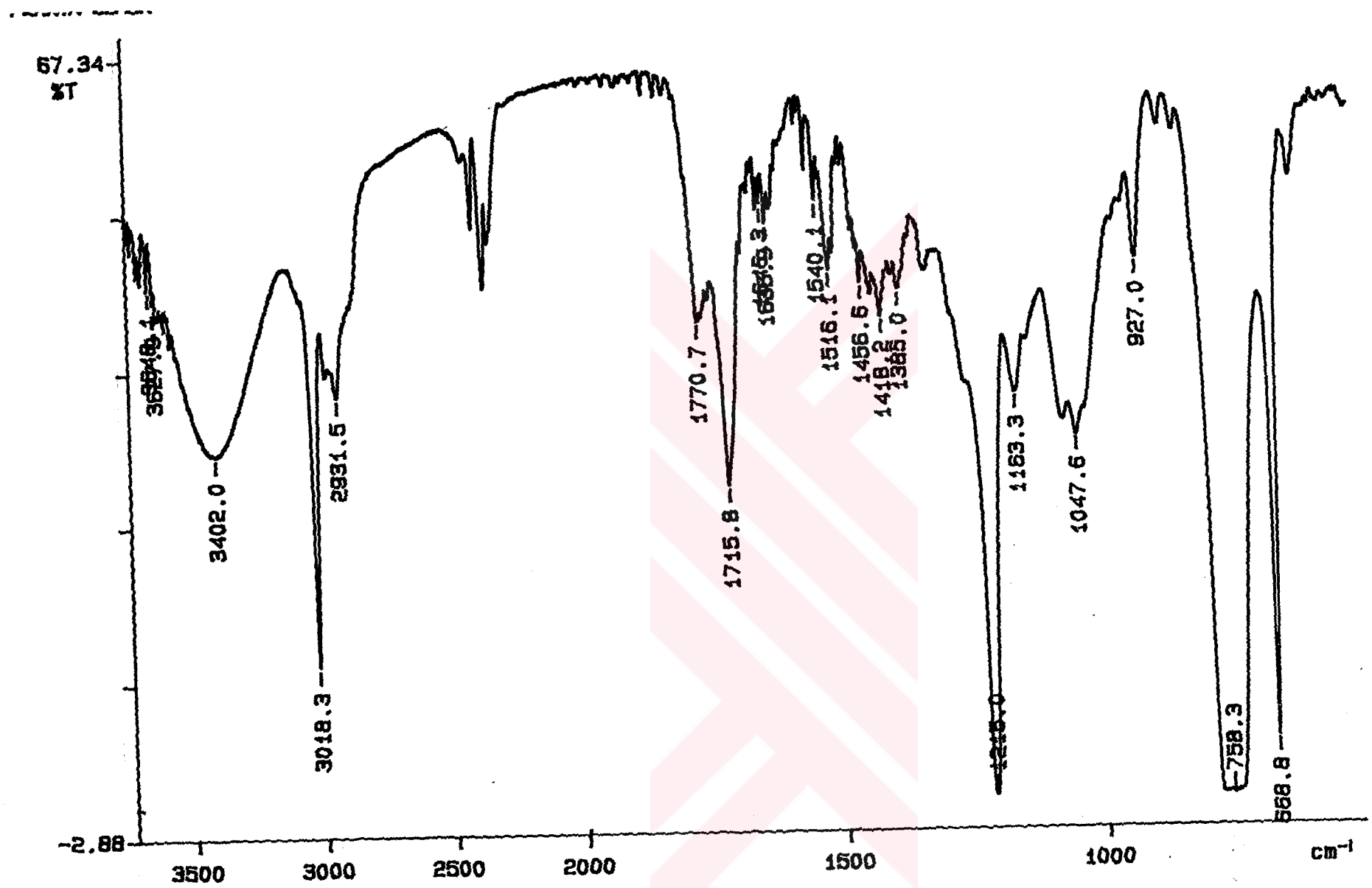


FIGURE 4.16 The IR Spectrum of CCU2

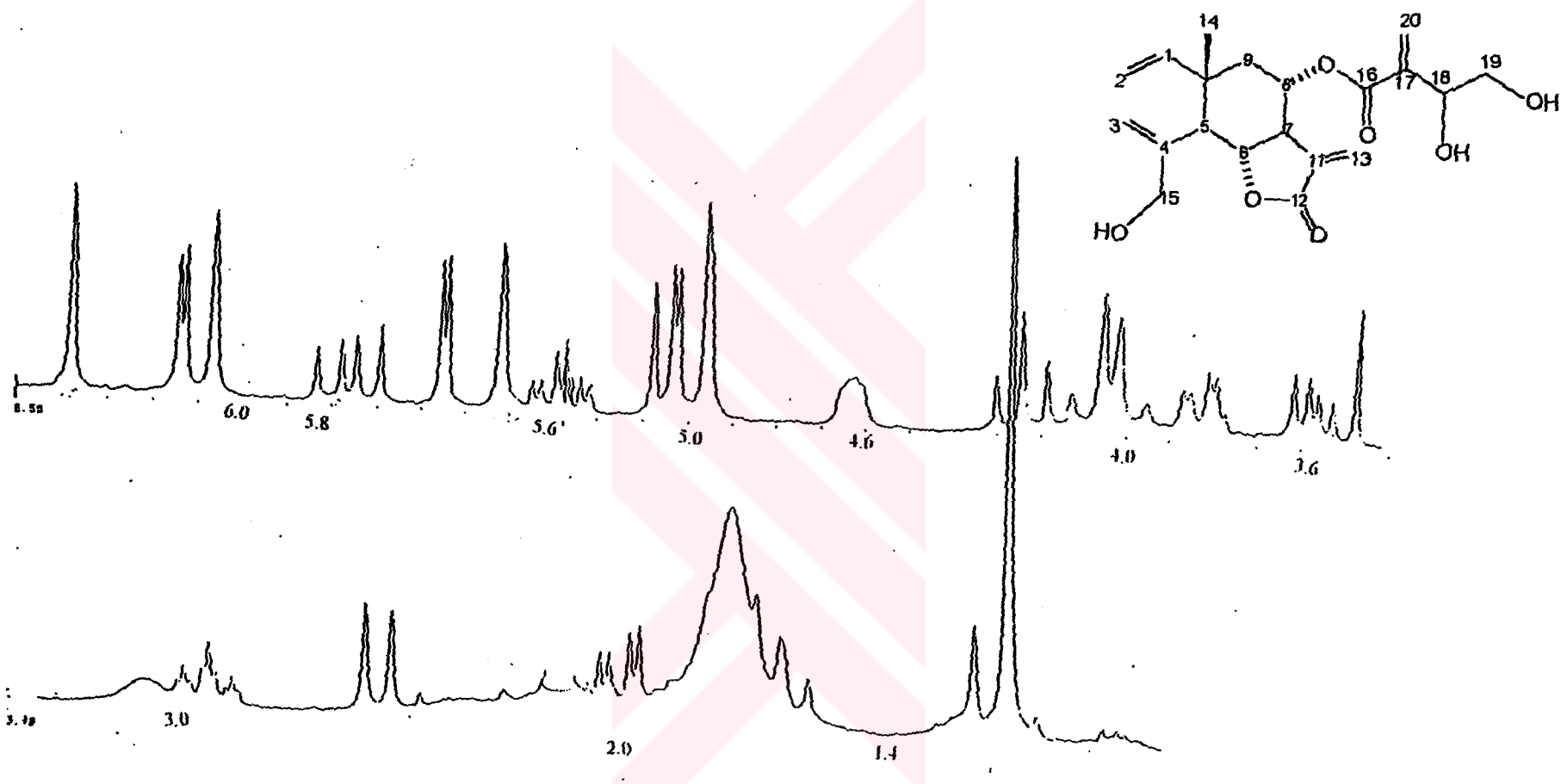


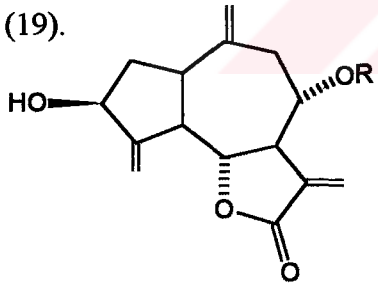
FIGURE 4.17 The ^{1}H NMR Spectrum of CCU2

5. CONCLUSION

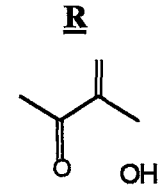
Terpenoids are natural products which are widely distributed in plants. Their physiological activities have been very well documented. Among the endemic plants in Turkey, *Centaurea* (Compositae) species are known to be rich in terpenoids, especially sesquiterpene lactones many of which have been shown to be physiologically active. The cytotoxic, antitumor, allergenic and antibiotic activities associated with sesquiterpene lactones, are related to the presence of the α-methylene-γ-lactone moiety.

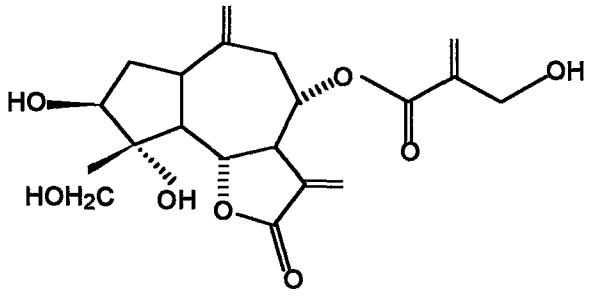
In this study the organic components of two *Centaurea* species *Centaurea cuneifolia Sm.* and *Centaurea cheirolopha Wagenitz* have been isolated by using chromatographic techniques and analyzed by spectroscopic means.

Centaurea cheirolopha Wagenitz yielded four sesquiterpene lactones of guaianolide type. These are Aguarin B (16), 8α-(2,3-dihydroxy-2-methyl propanoic acid) ester of Zaluzanin-C (17), 15-deschloro-15-hydroxychlorojanerin (18) and Cynaropicrin (19).



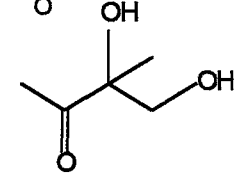
16



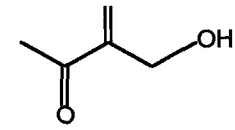


18

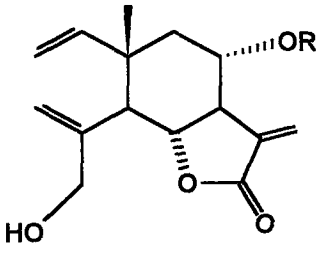
17

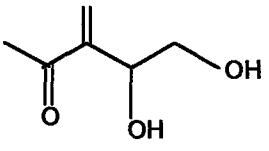


19



Centaurea cuneifolia Sm. yielded two sesquiterpene lactones with elemanolide skeletons, Dehydromelitensin (20) and 8α-(3,4-dihydroxy-2-methylene butanoic acid) ester of Dehydromelitensin (21).



	R
20	H
21	

The sesquiterpene lactones isolated from *Centaurea cheirolopha Wagenitz* and *Centaurea cuneifolia Sm.* contain γ-lactone with exocyclic methylene. Due to the presence of this group in each one, these compounds are expected to show physiological activities. The presence of additional α, β-unsaturated ester side chains adjacent to the γ-lactone moieties in Cynaropicrin, Aguarin B, 15-deschloro-15-hydroxychlorojanerin and 8α-(3,4-dihydroxy-2-methylene butanoic acid) ester of Dehydromelitensin, should also enhance the reactivities of these lactones toward biological nucleophiles.

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