ADDIS ABABA UNIVERSITY
OFFICE OF
RESEARCH AND GRADUATE PROGRAMS

PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *Leonotis ocymifolia*

BY

YESHITILA ASTERAYE

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PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *Leonotis ocymifolia*

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By

Yeshitila Asteraye

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PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *Leonotis ocymifolia*

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ABSTRACT

PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *Leonotis ocymifolia*

Advisor: Dr. Ashebir Fiseha

In this project, a phytochemical study has been undertaken on the leaves of *Leonotis ocymifolia* and the solvent extract of the plant has been conducted.

Leonotis ocymifolia is indigenous to Eastern and Southern Africa and is occasionally cultivated for its medicinal uses, which include acting as ascaricide, an anticancer drug, and as a treatment for ulcers, wounds, obesity and eczema.

The ethanol extract of the leaves of *Leonotis ocymifolia* led to the isolation of two compounds A and E. The structure of compound A was determined by means of spectroscopic methods (IR, ^{1}H NMR, ^{13}C NMR, and DEPT) but the structure of compound E could not be determined because of its small amount which led to see no carbon atom in its ^{13}C NMR spectrum.

1 INTRODUCTION

Organic chemistry as it stands today has developed largely from the chemistry of natural products. With the advent of modern spectroscopic techniques such as multidirectional spectroscopy, mass spectroscopy, and improved chromatographic techniques, a host of new organic substances from terrestrial and marine organisms are being discovered, many of which have interesting bioactivities [1].

The study of natural products has been and continues to be a major deriving force in the development of the fields of organic and medicinal chemistry. Higher plants used in traditional medicine provided some of the first prototype drugs used clinically in the treatment of a wide variety of diseases. The need to purify natural products from complex mixtures and to determine their structures has driven the development of even more sophisticated methods of separation of compounds and for their structural analysis by chemical and subsequently spectroscopic means [2].

1.1 *Leonotis ocymifolia*

The genus Leonotis (Lion's ear) consists of about 30 species of plants in the family Lamiaceae, all but one native to Southern Africa; the one other species *Leonotis neptifolia* is native to tropical Africa and Southern India [4-5]. One of these species and the plant of study in this project paper is *Leonotis ocymifolia.*

Leonotis ocymifolia is a striking, orange, autumn blooming perennial that is suitable for gardens. Its flowers dry very well and can keep their color for two years or more [6].

Three varieties of *Leonotis ocymifolia* are currently recognized. Only variety *ocymifolia* is considered here [7] (Figure 1).

Figure 1. Live Plant

Characteristic features of the plant are:

- the clothing hairs of stem and leaf lamina, very numerous on the lower leaf surface, mainly 2-celled, warty, up to 160 microns in length, thin-walled, curved;
- the glandular hairs of leaf and stem, with short unicellular stalk and multicellular head up to 60microns in diameter, visible at 7.5× magnification as almost sessile glands, shiny in the fresh leaf;
- the epidermal cells of the leaf lamina with sinuous walls and diacytic stomata; the absence of calcium oxalate crystals;
- the occasional orange pollen grains and orange clothing hairs of the corolla.

1.2 MEDICINAL USES of *Leonotis* SPECIES

Medicinal plants are known to provide a rich source of raw materials for traditional medicine in Africa, Asia and other parts of developing world, particularly those living in villages. The World Health Organization (WHO 1991) stated that of the people in developing countries about 80 % still relay on traditional plant derived medicines, mainly due to their lower price [3].

Leonotis has been used to treat various conditions including epilepsy, diabetes, bites and stings, skin diseases, muscular cramps, respiratory disorders, hypertension, headaches, viral hepatitis, intestinal worms, obesity and eczema. In 1992, *Leonotis leonurus* was shown to reduce breast tumors in mice [4].

Leonotis leonurus and *L. nepetifolia* are mildly narcotic and are sometimes smoked as a Marijuana substitute. They also have many medicinal uses. A plant infusion was once used by the Iroquois as a digestive aid or nerve tonic as well in many rituals.Users frequently report on the unpleasant taste and harshness of the smoke on the throat and lungs (due to the high carcinogen levels in the plant's foliage). This experience is less reported upon the smoking of the flowers. After a moderate dosage (3-4 grams, smoked) users report a sort of "floaty" feeling, lightheadedness, giddiness, mild euphoria, and reduced stress. Higher doses (8 grams+) may cause effects such as mild auditory and/or visual hallucinations and increased euphoria. *Leonotis ocymifolia* is used more or less interchangeably with *L. leonurus* in some areas, but specific uses include the treatment of diabetes, hypertension, anemia, eczema and other skin irritations. It is also utilized as a purgative and emmenagogue [6].

Leonotis ocymifolia is indigenous to Eastern and Southern Africa and is occasionally cultivated for its medicinal uses, which include acting as ascaricide, an anticancer drug, and as a treatment for ulcers and wounds [6-8]. Among those plants with claimed anti-fertility properties in traditional medicines in Ethiopia, *Leonotis ocymifolia*, locally known as "***Yeferes Zeng***" is one [9]. Its leaves are used in the treatment of hookworm, while the flowers and roots in the treatment of gout and leishmaniasis [10]. People living around the Bale area in Southern Ethiopia use the leaves of this plant to expel intestinal parasites [11]. In addition it was found that the ethanol and aqueous extracts of the leaves and roots of the plant showed anti-implantation and anti-fertility effects in rats [12].

Species of Leonotis are among the frequently used herbal remedies to treat various bronchial illness and epilepsy. The essential oils of the leaf and flower of *L. leonurus* and *L. ocymifolia* growing in the Eastern Cape of South Africa exhibited a broad spectrum anti-bacterial activity against gram-positive (Bacillus subtilus, Bacillus cereus, Micrococcus kristinal, Staphytococcus aurues, etc) and gram-positive bacteria with MIC values ranging from 1.25 to 0.039 mg/ ml [13].

1.3 CHEMICAL COMPOSITION OF *Leonotis ocymifolia*

Micro chemical tests indicated the presence of alkaloids, saponins and tannins but not cardiac or anthraquinone glycosides. Little is known of the secondary metabolites of this species. Analysis of Ethiopian collections of leaves of *L.ocymifolia* revealed the presence of five different labdane type diterpene lactones with the following different structures whose ^{13}C-NMR assignments have been made unambiguously [14].

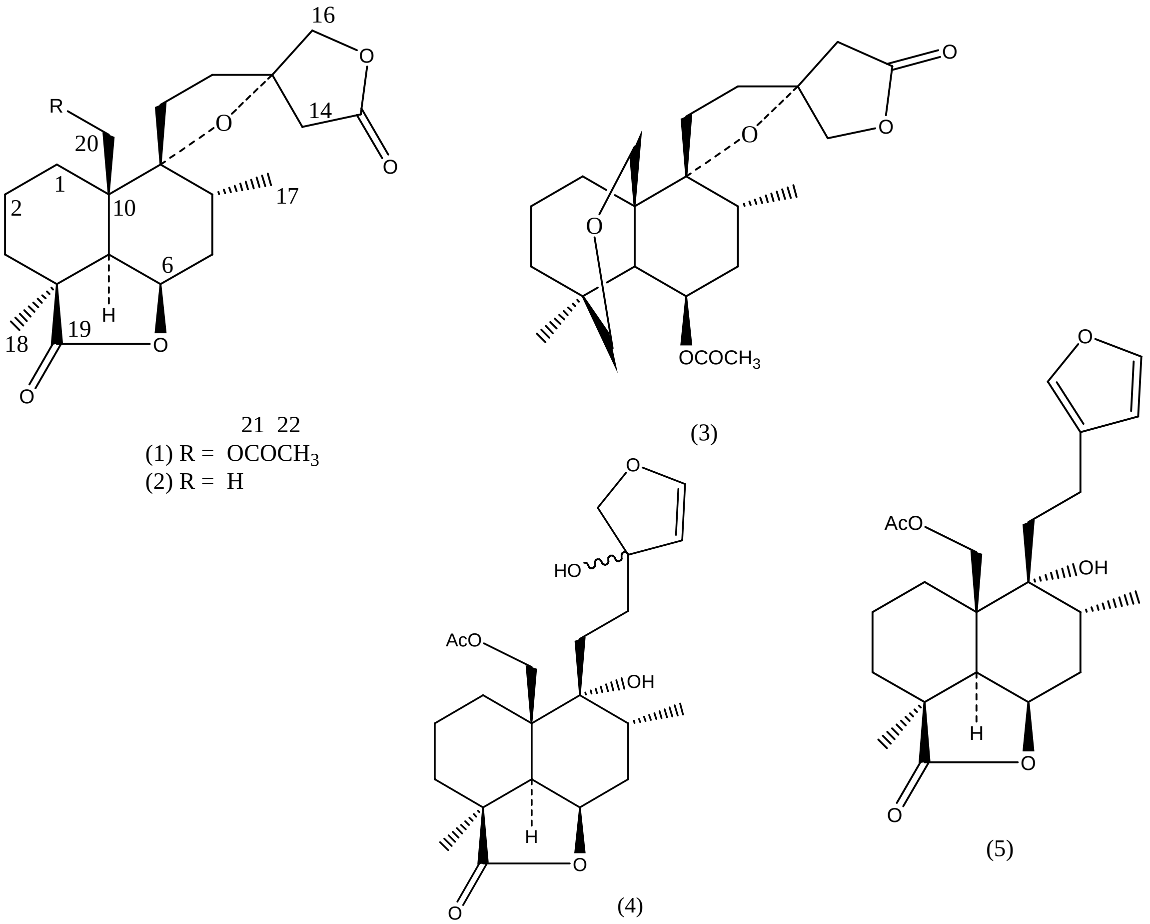
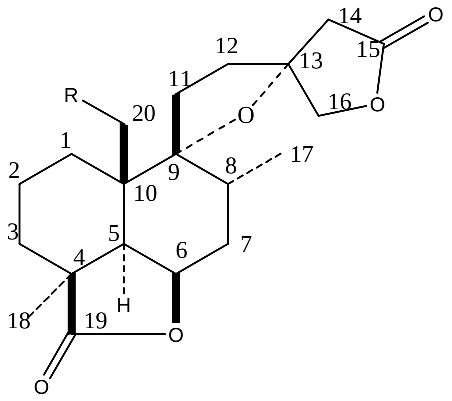


Figure 2. Diterpenes obtained from leaves of *L.ocymifolia*

The gum obtained on extraction of *L. dubia* leaves with acetone afforded (from ethyl acetate) a crystalline solid (Figure 3, **1**), which was shown by TLC to consist almost entirely of a single compound, Dubin, $C_{21}H_{30}O_6$, m. p. 187-188^{0}c , the structure of which was supported by spectroscopic evidences [15].

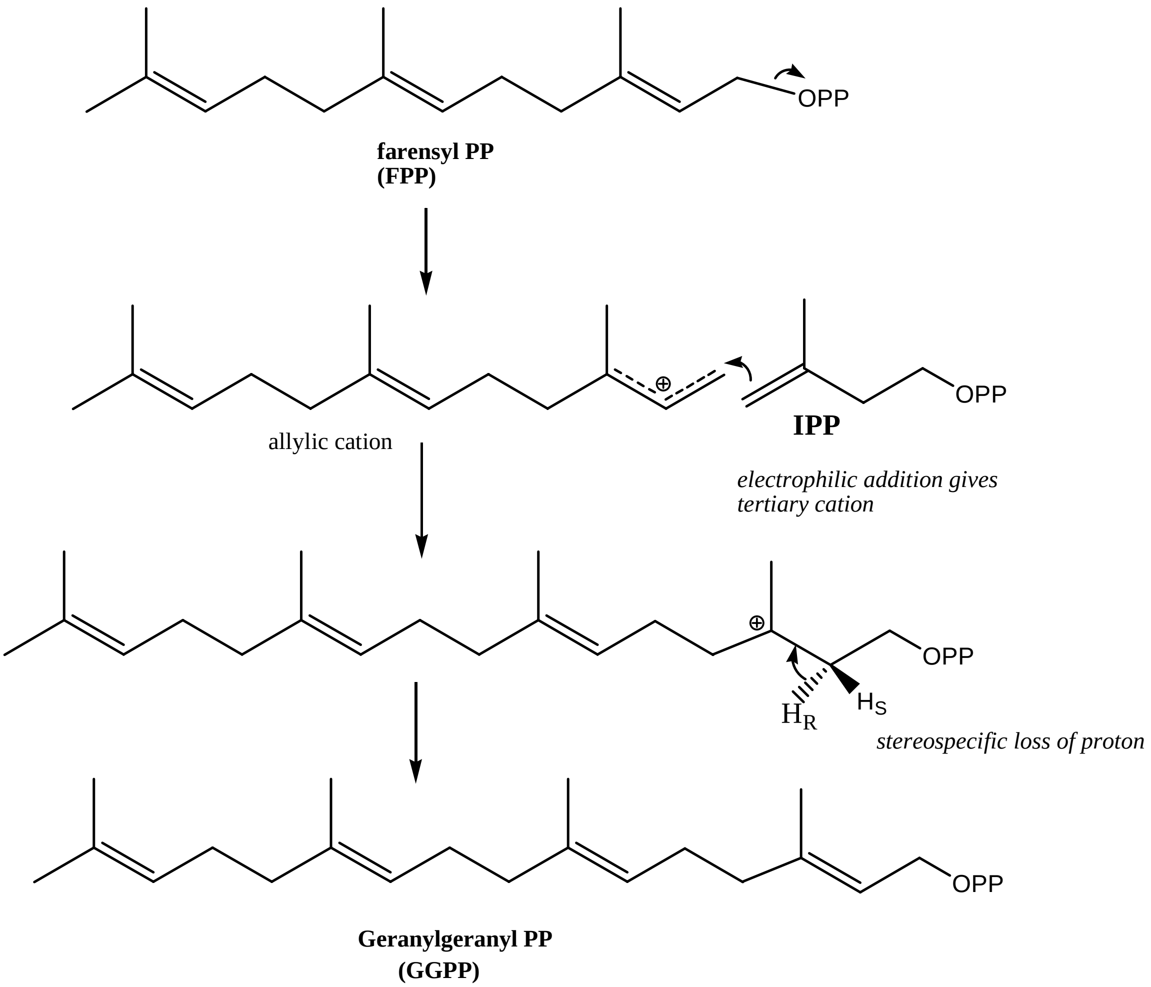


(1) R = OAc
(2) R = H

Figure 3. Diterpenes obtained from the *L. dubia* leaves with acetone.

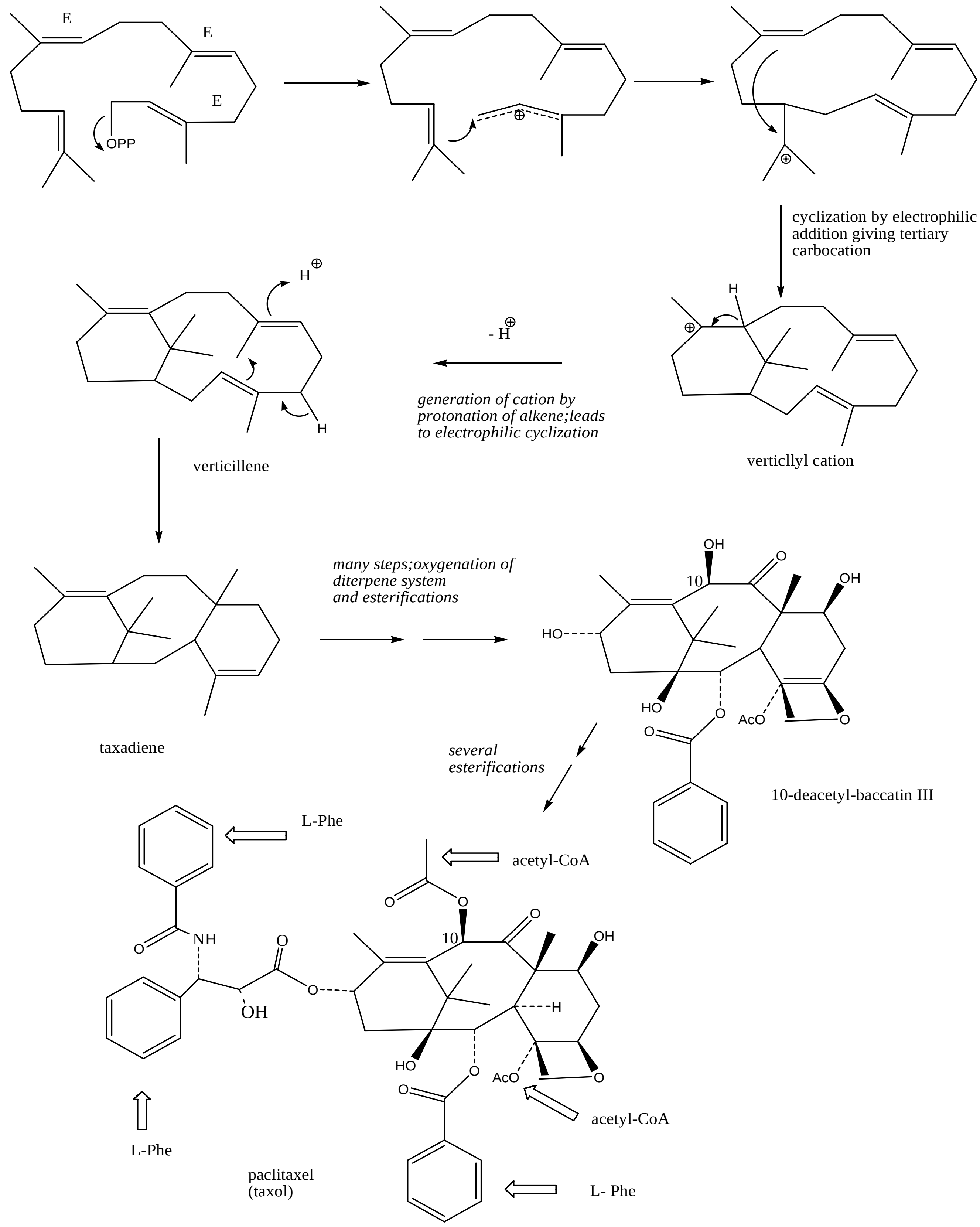
1.4 BIOSYNTHESIS OF DITERPENES [16]

The diterpenes arise from geranylgeranyl diphosphate (GGPP), which is formed by addition of a further IPP molecule, to farensyl diphosphate (FPP).



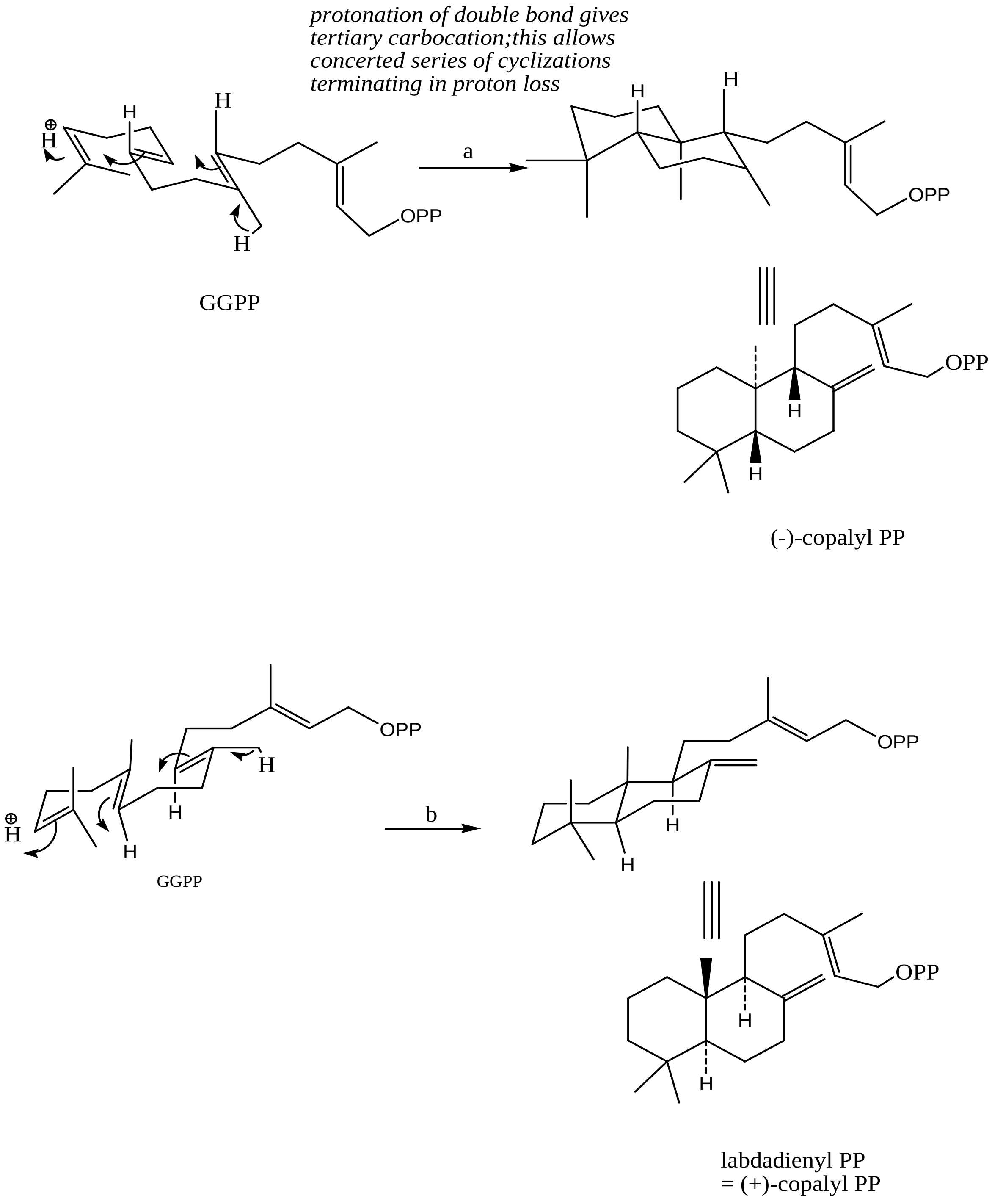
Scheme 1. Generation of GGPP

Cyclization reactions of GGPP mediated by carbocation formation, plus the potential for Wagner-Meerwein rearrangements, will allow many structural vriants of diterpenoids to be produced.



Scheme 2. Cyclization reactions of GGPP

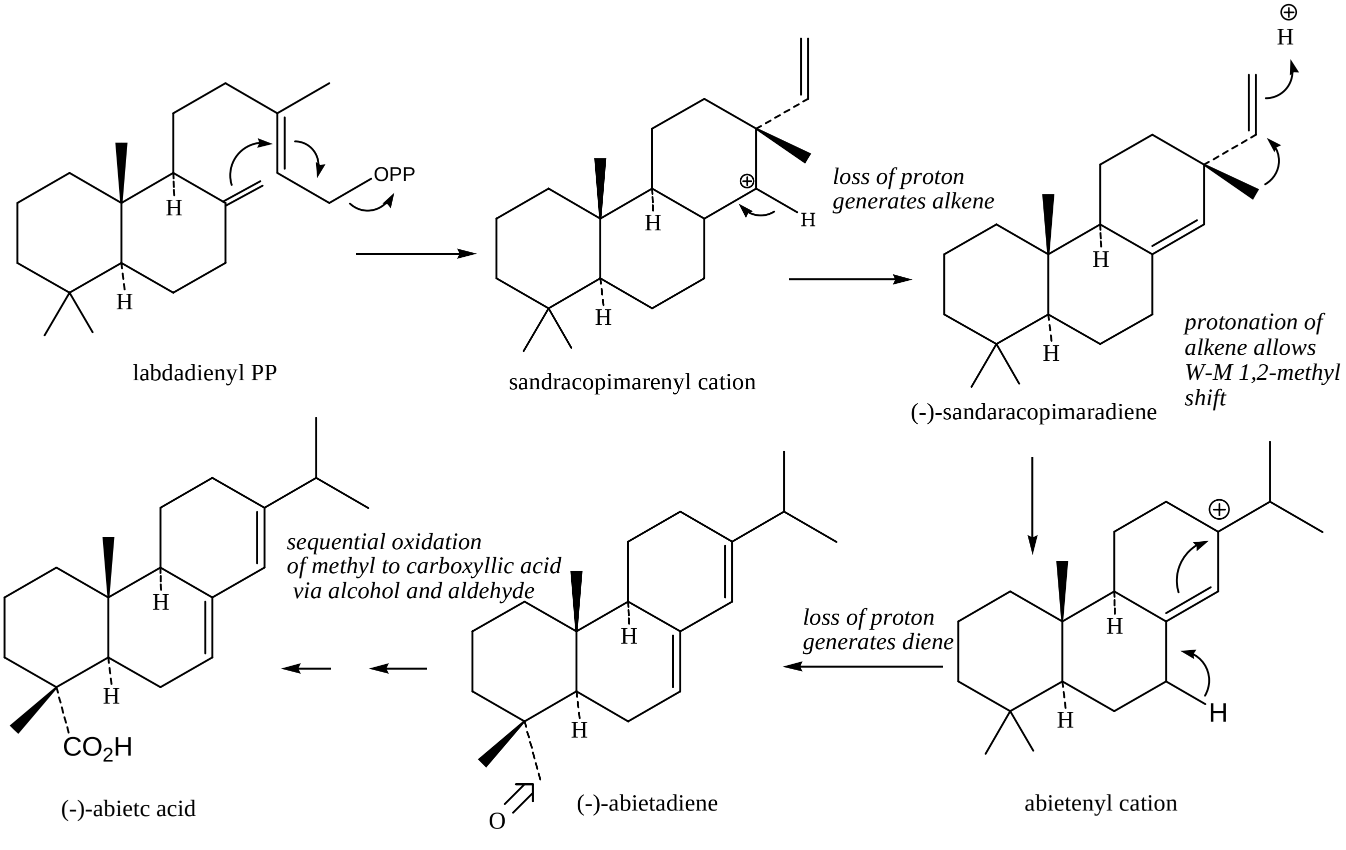
Protonation of GGPP can initiate a concerted cyclization sequence, terminated by loss of a proton from a methyl, yielding copalyl PP (Scheme 3a). The stereochemistry in this product is controlled by the folding of the substrate on the enzyme surface, though an alternative folding can lead to labdadienyl PP, the enantiomeric product having opposite configuration at the newly generated chiral centers (Scheme 3b).



protonation of double bond gives tertiary carbocation;this allows concerted series of cyclizations terminating in proton loss

Scheme 3. Concerted cyclization sequences of GGPP

The alternative stereochemistry typified by labdadienyl PP can be seen in the structure of abeitic acid (Scheme 4), the major component of the rosin fraction of turpentine from pines and other conifers. Initially, the tricycle system is built up by as in the pathway to ent-kaurine, via the same mechanism, but generating the enantiomeric series of compounds. The cation loses a proton to give sandaracopimaradiene, which undergoes a methyl migration to modify the side-chain, and further proton loss to form the diene abietadiene.

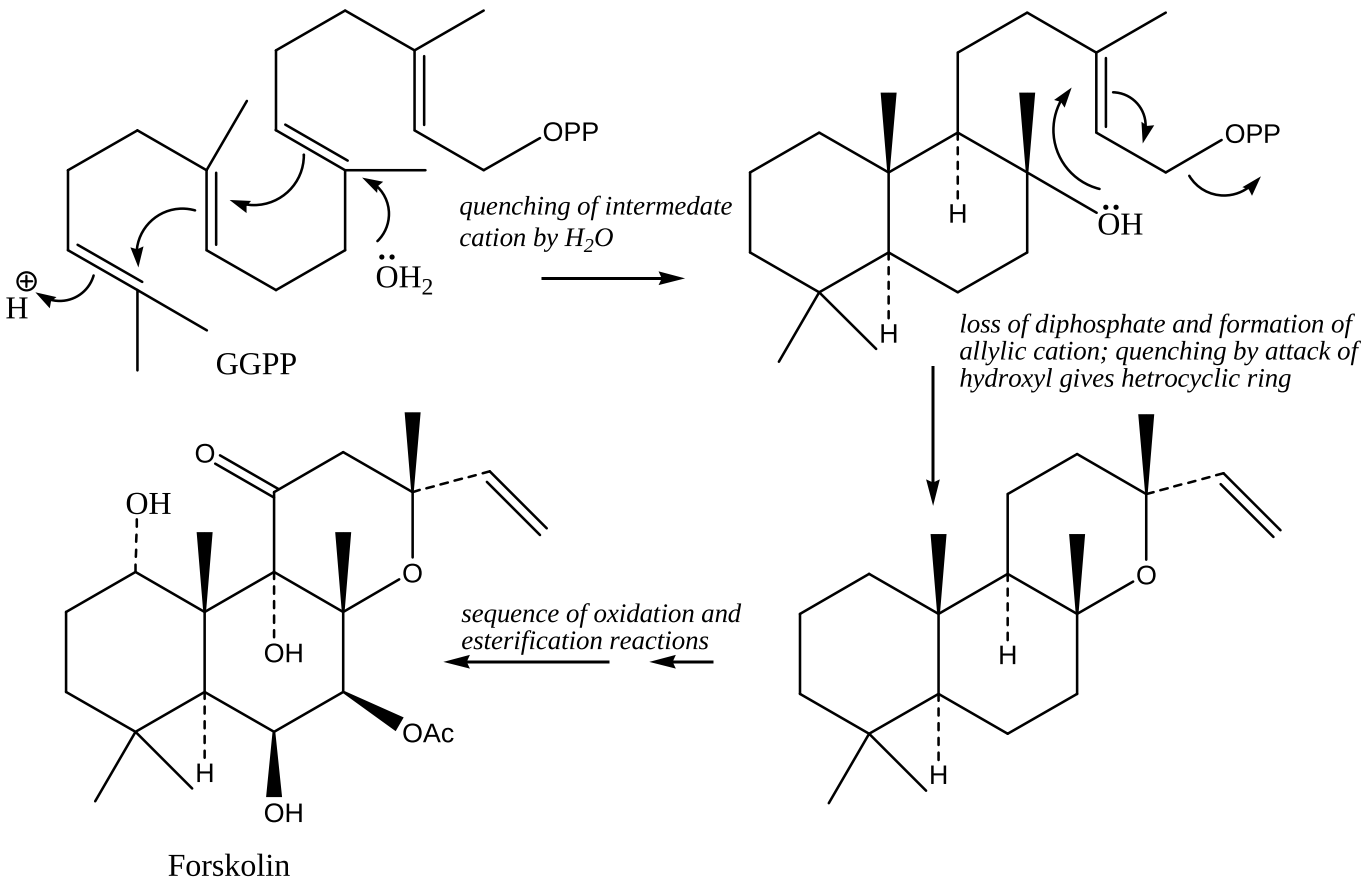


Scheme 4. Conversion of Labdadienyl PP to (-)-abietic acid

Extensive modification of the labdadienyl diterpene skeleton is responsible for generation of the ginkgolides, highly oxidized diterpene trilactones.

Several rearrangements, ring cleavages, and formation of lactone rings can broadly explain its origin. (Scheme 5), though this scheme is highly speculative and likely to be incorrect. Although detailed evidence is lacking, it is known that labdadienyl PP is a precursor, and most probably dehydroabietane also. The usual tert-butyl subsistent arises as consequence of the A ring cleavage.

In Forskolin, the third ring is heterocyclic rather than carboxylic. The basic skeleton of ForsKolin can be viewed as the result of quenching of the cation by water as opposed to proton loss, followed by S_N2 nucleophilic substitution on to the allylic cation generated by loss of diphosphate), a series of oxidative modifications will then lead to forsKolin. This compound has been isolated from *coleus forsKohlii*, a plant used in Indian traditional medicine and shown to have quite pronounced hypertensive and antispasmodic activities (Scheme 5).



Scheme 5. Conversion of GGPP to Forskolin.

2 OBJECTIVES OF THE STUDY

The main objective of this project is to:

1. isolate compounds from the dried leaves of *Leonotis ocymifolia* and
2. elucidate the structures of compounds isolated using spectroscopic methods (IR, NMR, and UV-VIS).

3. RESULTS AND DISCUSSION

Dried leaves of *Leonotis ocymifolia* were extracted with ethanol for 48 hours at room temperature. The solvent was removed using rotary evaporator to give 22g residue. Preliminary separation of the extracts gave 10mg of a diterpene (Compound A) from fraction A. compound A was characterized using IR, UV-VIS, 1D and 2D NMR spectroscopy. Characterization of compound E was attempted using only IR and ^{1}H NMR data. Unambiguous identification of this compound was not possible due to lack of other data.

3.1 CHARACTERIZATION OF COMPOUND A

Compound A (Figure 5), is a deep red crystalline solid obtained from fraction A of Ethanol extract. Its TLC plate gave deep yellow color when sprayed with Sulphuric acid (vanillin), which is a characteristic of terpenes.

In the IR spectrum (Appendix A) a broad band at 3447cm^{-1} spectrum shows that the compound has a hydroxyl functional group. The methyl C-H stretching in this compound is indicated by a sharp peak at 2920cm^{-1}. Two sharp peaks at 2360cm^{-1} and 2342cm^{-1} show that there is a saturated aliphatic nitrile group attached. Three sharp peaks at 1654cm^{-1}, 1559cm^{-1} and 1457cm^{-1} are indicative of aromatic ring. Another sharp peak at 966cm^{-1} is for a methyl group attached to a quaternary carbon. Therefore the IR spectrum depicts the presence of hydroxyl functionality, nitrile group, an aromatic group and a methyl group attached to a quaternary carbon in the compound isolated.

The UV spectrum of compound A (Appendix H) shows absorbance peaks at 280nm and 350nm which may support that the compound is both aromatic and has olefinic carbons. This region of absorbance may tell us the possibility of conjugation.

The presence of an aromatic group in compound A is also supported by its ^1H NMR spectrum (Appendix B). The ^1H NMR spectrum depicts two clearly separated regions of protons, one in the aromatic (olefinic) region δ 6.0- δ 6.7 and the other in the aliphatic region δ (1.0- 2.2). There are seven protons in the region from δ 6.0 to δ 6.7. A multiplet at δ 6.66 is integrated for two protons. A doublet at δ 6.37 (J= 8.4Hz) is integrated for one proton in the aromatic region. Another doublet at δ 6.27 (J= 8.4Hz) is integrated to 1H. At δ 6.17 there is a multiplet integrated to three protons which are almost in the same chemical shift region. A sharp singlet at δ 2.15 with 1H is the proton of the hydroxyl group. A triplet with an integration of two protons at δ 2.05 is for the protons of a methylene group. A sharp singlet at δ 1.99 is integrated to six protons that are for two methyl groups in the same chemical environment. At δ 1.74 a singlet peak is integrated to three protons. A multiplet with an integration of 2H is also shown at δ 1.64. This peak should be for a methylene group. Another multiplet at δ 1.48 with two protons also indicates protons of a methylene group. A singlet with a proton is found at δ1.28ppm. The sharp singlet at δ 1.05 with six protons should be for protons of two methyl groups in the same chemical environment. At δ 0.91there is a multiplet with an integration of 1H.

δ (ppm)	No of Hydrogen, Multiplicity	Remark
0.91	1H, m	H-10
1.05	6H, s	H-4, H-5
1.28	1H, m	H-9
1.48	2H,m	H-7
1.64	2H, m	H-6
1.74	3H, s	H-3
1.99	6H, s	H-1, H-2
2.05	2H, t (J= 12Hz)	H-8
2.19	1H, s	-OH
6.17	3H, m	H-19, H-22, H-23
6.27	1H, d (J= 8.4Hz)	H-17
6.37	1H, d (J= 8.4Hz)	H-16
6.66	2H, m	H-18, H-20

Table 1. ^{1}H NMR data of compound A.

The ^{13}C spectrum of compound A (Appendix C, Table 2) also strengthens the fact that there are two different chemical shift regions, one from δ 120- 140 which is for the aromatic group and (or olefinic carbons) and the other from δ 12-40 which is for the aliphatic group. The ^{13}C spectrum of compound A is therefore in line with the ^{1}H NMR spectrum. The region from δ 12 to δ 40 has 12 carbons. The longest peak with two carbons at δ 28.99 is for two carbon atoms that overlapped. There is a peak at δ 76.78. The region from δ120 to δ140 is that of the aromatic and olefinic carbons of compound A. In the ^{13}C spectrum, there are 11 carbons in the aromatic region. Therefore, compound A has a total of 24 carbon atoms.

The DEPT (Appendix D) showed 16 peaks corresponding to 17 carbons. Compound A has five methyl groups at δ 12.85, 12.91, 21.75 and 28.99. The longest peak at δ 28.99 tells us there is an overlapping of two methyl carbons which are the same in chemical shift. There are three methylene groups at δ19.30, 33.14 and 39.71. There are nine methine groups, out of which two are in the aliphatic region at δ 14.19 and 30.99 and seven are in the aromatic region at δ 125.12, 126.72, 130.04, 130.85, 132.50, 137.31, and137.80.

The difference in the number of carbon atoms of the ^{13}C spectrum and the DEPT spectrum is seven. Therefore, compound A has seven quaternary carbon atoms at δ 29.79, 34.39, 76.78, 129.42, 136.21, 136.52, and 138.05.

Carbon		H-attached to the carbon (No of H, Multiplicity, δ ppm	Remark
No	δ (ppm)		
1	28.99	3H, s, 1.99	$-CH_3$
2	28.99	3H, s, 1.99	$-CH_3$
3	21.75	3H, s, 1.74	$-CH_3$
4	12.85	3H, s, 1.05	$-CH_3$
5	12.91	3H, s, 1.05	$-CH_3$
6	33.14	2H, m, 1.64	$-CH_2-$
7	19.30	2H, m, 1.48	$-CH_2-$
8	39.71	2H, t, 2.05	$-CH_2-$
9	30.99	1H, m, 1.28	-CH-
10	14.19	1H, m, 0.91	-CH-
11	29.79	--------------	quaternary
12	34.39	--------------	quaternary
13	76.78	--------------	quaternary
14	138.05	--------------	quaternary
15	136.52	-------------	quaternary
16	132.50	1H, d, 6.37	=CH-
17	130.85	1H,d, 6.27	=CH-
18	137.80	1H, m, 6.66	=CH-
19	126.72	1H, m, 6.17	=CH-
20	137.31	1H, m, 6.66	=CH-
21	136.21	--------------	quaternary
22	130.04	1H, m, 6.17	=CH-
23	125.12	1H, m, 6.17	=CH-
24	129.42	--------------	quaternary

Table 2. ^{13}C NMR data of compound A.

In the ^{1}H- ^{1}H COrrelation SpectroscopY (COSY) spectrum of compound A (Appendix E), a strong correlation is observed between the six methyl protons (δ 1.05, s) of C-4 & C-5 (δ 12.85 and 12.91 respectively) and the proton at C-23 (1H,d, δ 6.27). A correlation is also observed between H-20 (δ 6.66, m) and H-19 (δ 6.17, m), between H-18 (δ 6.66, m) and H-19 (δ 6.17, m).

Heteronuclear Single Quantum Correlation (HSQC) experiment correlates the chemical shift of protons with the chemical shift of directly bonded carbon atom. HSQC spectral data of compound A (Appendix F) shows the six protons (δ 1.05, 6H, s) attached to C-4 and C-5 (δ 12.85 and 12.91 respectively), 2 protons. The two protons at δ1.64 (m) are connected to C-6 (δ 33.14). The other two protons (δ 1.48, 2H, m) are correlated to C-7 (δ 19.30). Of the three protons (δ 6.17, 3H, m), one is correlated to C-23 (δ 125.12), the other to C-22 (δ 130.04) and the third to C-19 (δ126.72). The doublet with an integration of 1H (δ 6.37) is correlated to C-16 (δ 132.50).

Heteronuclear Multiple Bond Correlation (HMBC) experiment gives information about couplings of hydrogens and carbons two or three bonds away from each other. In the HMBC spectrum of compound A (Appendix G), the six protons (δ 1.99, 6H, s) are correlated to C-11 (δ 29.79), C-17 (δ 130.85), C-9 (δ 30.99, C-3 (δ 21.75), C-6 (δ 33.14). The six protons at C-4 (δ12.85) and C-5 (δ 12.91) are correlated to C-10 (δ 14.19) and C-23 (δ 125.12). At δ 6.66 (2H, m), there is correlation between these protons with C-18 (δ 137.80) and C-20 (δ 137.31). The three protons at δ 6.17 (H-19, H-22 & H-23) correlated to C-14 (δ 138.05), C-18 (δ 137.80), C-20 (δ 137.31) and C-15 (δ 136.52). All the above correlations can be shown in the following figure.

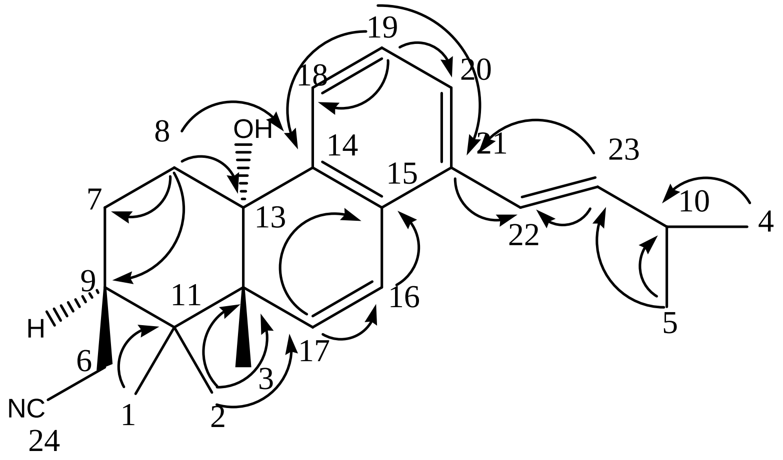


Figure 4. HMBC Correlations of compound A

From all the above data, the following structure suggested for compound A.

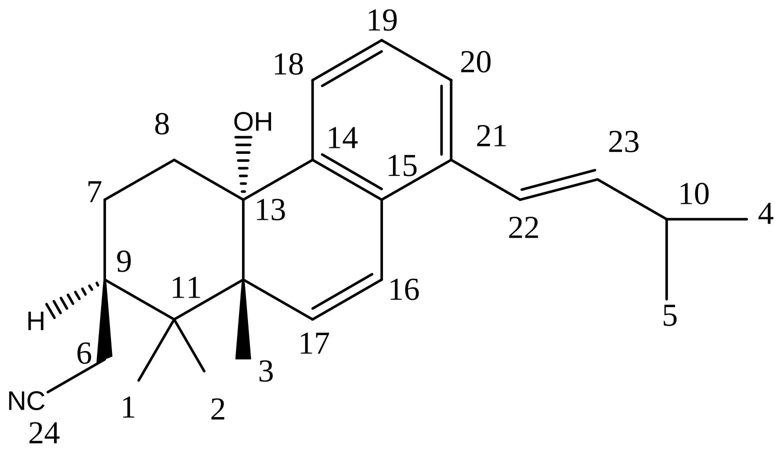


Figure 5. Suggested structure of compound A.

3.2 Partial Characterization of Compound A

Only IR (Appendix I) and ^{1}H NMR spectra (Appendix J) were recorded for compound E. From its IR spectrum, a broad peak at 3421cm^{-1} is due to a hydroxyl group. A sharp peak at 2921cm^{-1} shows aliphatic C-H stretching. A broader band is also observed at 1718cm^{-1}.This may probably be due to a carbonyl carbon attached to a heteroatom. The peak at 1458cm^{-1} indicates the presence of α, β-unsaturated groups in the compound. At 965cm^{-1}, there is a methyl group attached to a quaternary carbon atom.

The ^{1}H NMR spectrum of compound E (Appendix J) showed peaks at δ 0.87 (3H, d), δ 1.28 (3H, s), and δ 1.98 (3H, s) that represent methyl protons. A sharp peak appeared at δ 1.65 (2H, s). Another signal appeared at δ 2.41 (1H, d). A peak at δ 3.51 (2H, s) appeared for oxymethylene protons. Two peaks at δ 4.26 and δ 4.02 were both integrated to 1H each. Alkene protons appeared at δ 6.17 (1H, d) and δ 6.47 (1H, d).

The ^{13}C NMR spectrum of Compound E has no observable carbons. Hence, it has been difficult to characterize and elucidate the structure of Compound E.

4 EXPERIMENTAL SECTION

4.1 MATERIALS AND METHODS

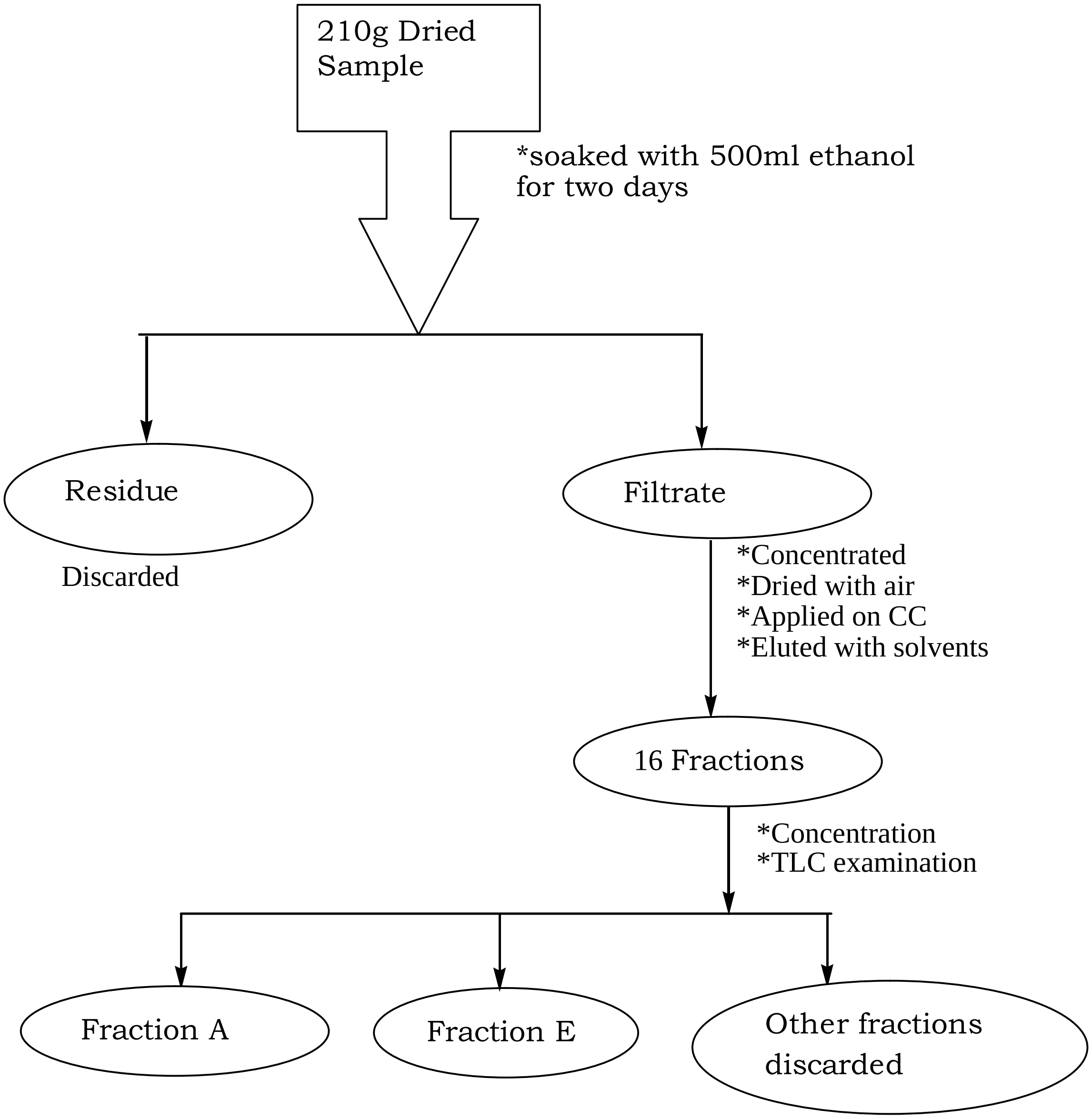
Melting points were determined on a Bock-Monoscop-NR174 apparatus. The UV-VIS spectrum was run in GENESY's spectrometer with methanol at room temperature. The IR spectrum for Compound A was run as KBr pellets on Perkin-Elmer BX infrared spectrometer in the range 4000cm^{-1} - 400cm^{-1}. The ^{1}H NMR,^{13}C and Dept spectra were recorded on a Bruker Advanced 400 MHz spectrometer with $CDCl_3$ as a solvent and TMS as internal standard.

The purity of compounds was monitored on silica gel plates. Analytical thin layer chromatography was run on silica gel (Merck) coated on aluminum foil, 0.2 mm thickness. The spots were detected by their UV fluorescence and by spraying with Sulphuric acid (vanillin). Column chromatography was performed on silica gel 60 (Merck), particle size 0.063-0.200mm (70-230 mesh ASTM).

4.2 SAMPLE COLLECTION

The leaves of *Leonotis ocymifolia* were collected in December 2005 from Kotebe region, Addis Ababa, Ethiopia during the flowering stage of the plant. A voucher specimen (YAT-001) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Faculty of Science.

4.3 METHOD OF EXTRACTION



4.4 ISOLATION

110 gm of silica gel was measured and mixed with 200mL of n-Hexane. Then the mixture was packed into a column. 10mL of n-Hexane was added to the 22 gm dried sample of the crude extract. This concentrated sample was then applied on to the top of packed silica gel using a dropper. Elution was done with 100mL pure n-Hexane, followed by 100ML n-Hexane: EtOAc (1:1), 100mL pure EtOAc and then 100mL EtOAc: MeOH (1:1). A total of 16 fractions were collected as follows.

Fraction	Solvent	Volume	Ratio
A	n-Hexane	100mL	Purely n-Hexane
B	n-Hexane		
C	n-Hexane		
D	n-Hexane		
E	n-Hexane:EtOAc	100mL	1:1
F	n-Hexane:EtOAc		
G	n-Hexane:EtOAc		
H	n-Hexane:EtOAc		
I	EtOAc	100mL	Purely EtOAc
J	EtOAc		
K	EtOAc		
L	EtOAc		
M	EtOAc: MeOH	100mL	1:1
N	EtOAc: MeOH		
O	EtOAc: MeOH		
P	EtOAc: MeOH		

Table 3. Solvent systems and fractions collected from crude extract.

The fractions collected other than A and E were discarded because their TLC results did not show spots. Fractions A and E showed pure spots. The solvent system used for the TLC examination of fraction A was n-Hexane: EtOAc (4.5: 0.5), and for fraction E was n-Hexane: EtOAc (1: 4). The two fractions were left in a hood for 12hrs. Deep red thread like crystals were observed in fraction A, while yellow crystals were observed in the fraction containing E. Faction A was labeled compound A and its amount was 10mg. Fraction E was labeled compound E. Its amount was only 5mg. The IR, NMR (^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC) and UV- VIS spectra for compound A were recorded and was characterized.

Compound A is a deep red crystalline solid with sweet smell, **R$_f$** 0.56 (n-Hexane: EtOAc, 4.5:0.5), **mp** 167-170^{0}C. **IR** V$_{max}$ (KBr) cm.$^{-1}$: 3447.90 (-OH), 2920.31 (-CH_3), 2360.58 and 2343.22 (aliphatic nitrile, –C≡N), 1654.02, 1559.89 and 1507.87 (C=C), 1457.91 and 1364.14 (CH_2).966.05cm-1(Methyl attached to quaternary carbon).

^{1}H NMR δ_{ppm} (400HZ, $CDCl_3$) 0.90 (1H, m, H-10), 1.05 (6H, s, Me-4 &Me-5) 1.28(1H, s, H-9), 1.48 (2H, m, G2-7), 1.64 (2H, m, H2-6) 1.74 (3H, s, Me-3), 1.99 (6H, s, Me-1, Me-2), 2.05 (2H, t, H2-8), 2.19 (1H, s, -OH), 6.17 (3H, m, H-19, H-22, H-23), 6,27 (1H, d, H-17), 6.37 (1H, d, H-16), 6.66 (2H, m, H-18 & H-20).

^{13}C NMR (100Hz, $CDCl_3$) δppm 12.85 (C-4), 12.91 (C-5) 14.19 (C-10), 19.30 (C-7), 21.75 (C-3), 28.99 (C-1 & C-2), 29.79 (C-11, quaternary), 30.99 (C-9), 33.14 (C-6), 34.39 (C-12, quaternary), 39.71 (C-8), 76.78 (C-13, quaternary), 125.12(C-23), 126.72 (C-19), 129.42 (C-24, quaternary), 130.04 (C-22), 130.85 (C-17), 132.50 (C-16), 136.21 (C-21, quaternary), 136.52 (C-15, quaternary), 137.31 (C-20), 137.80 (C-18), 138.05 (C-14, quaternary).

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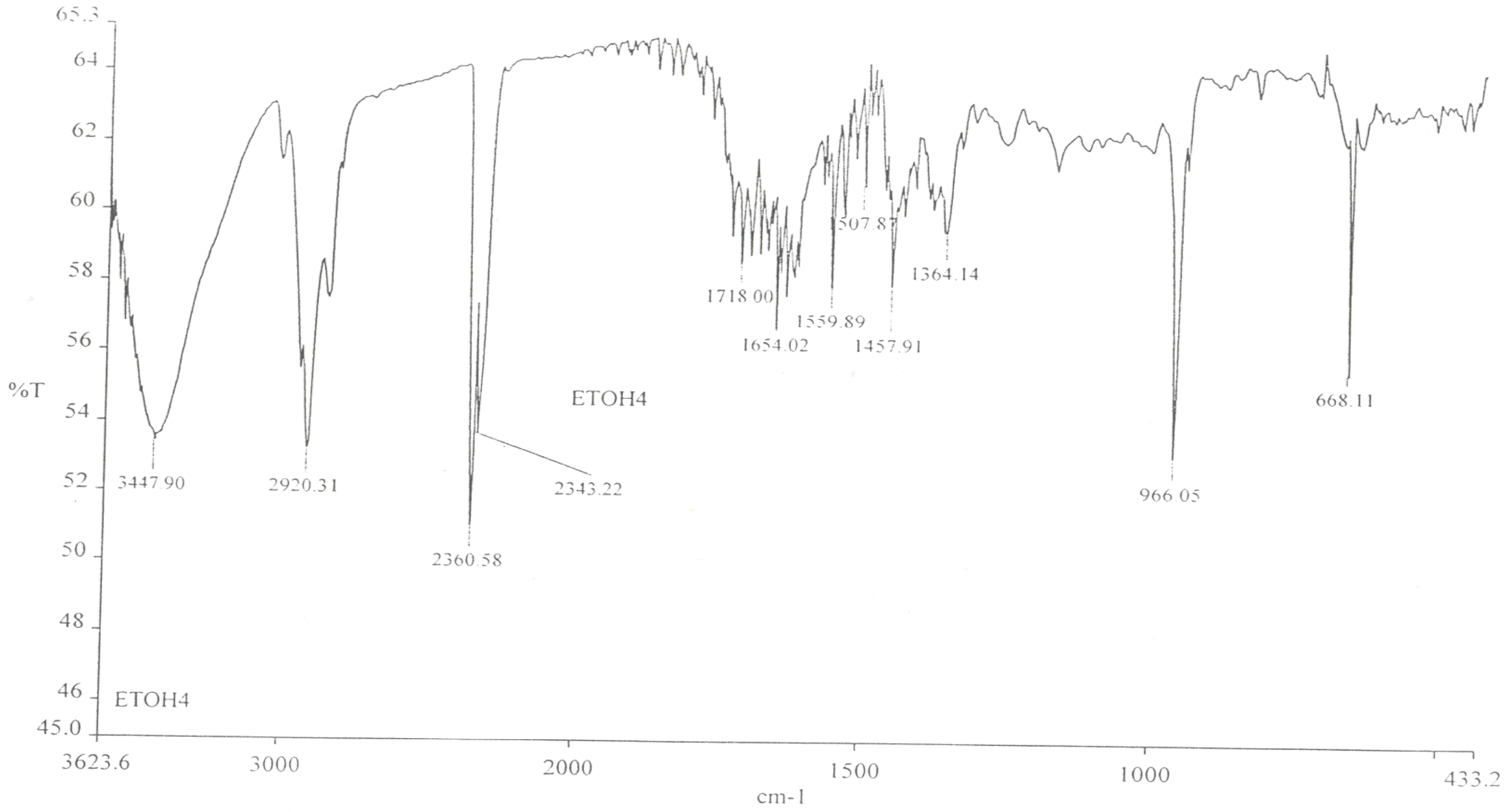
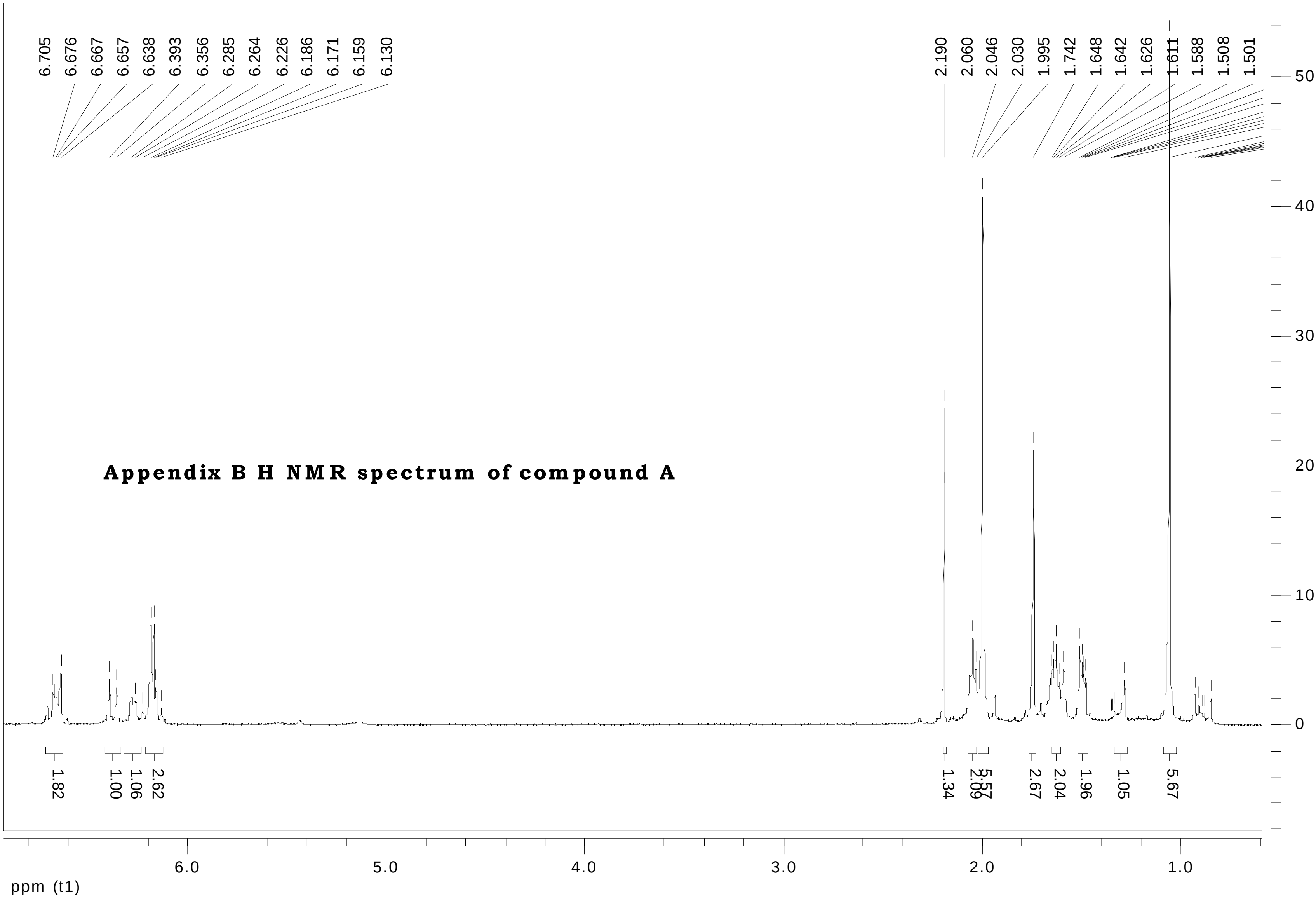
APPENDICES

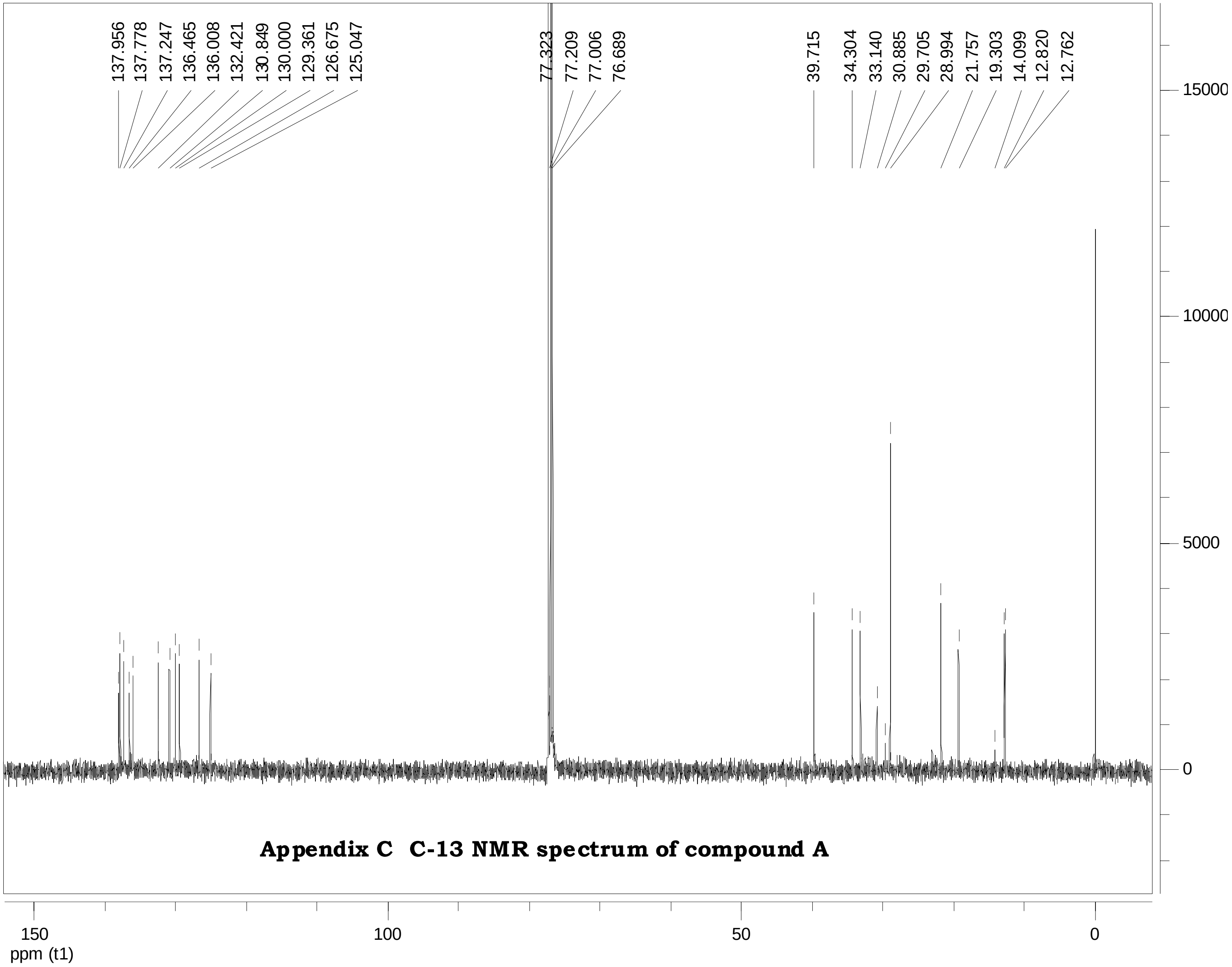


IR Spectrum of Compound A

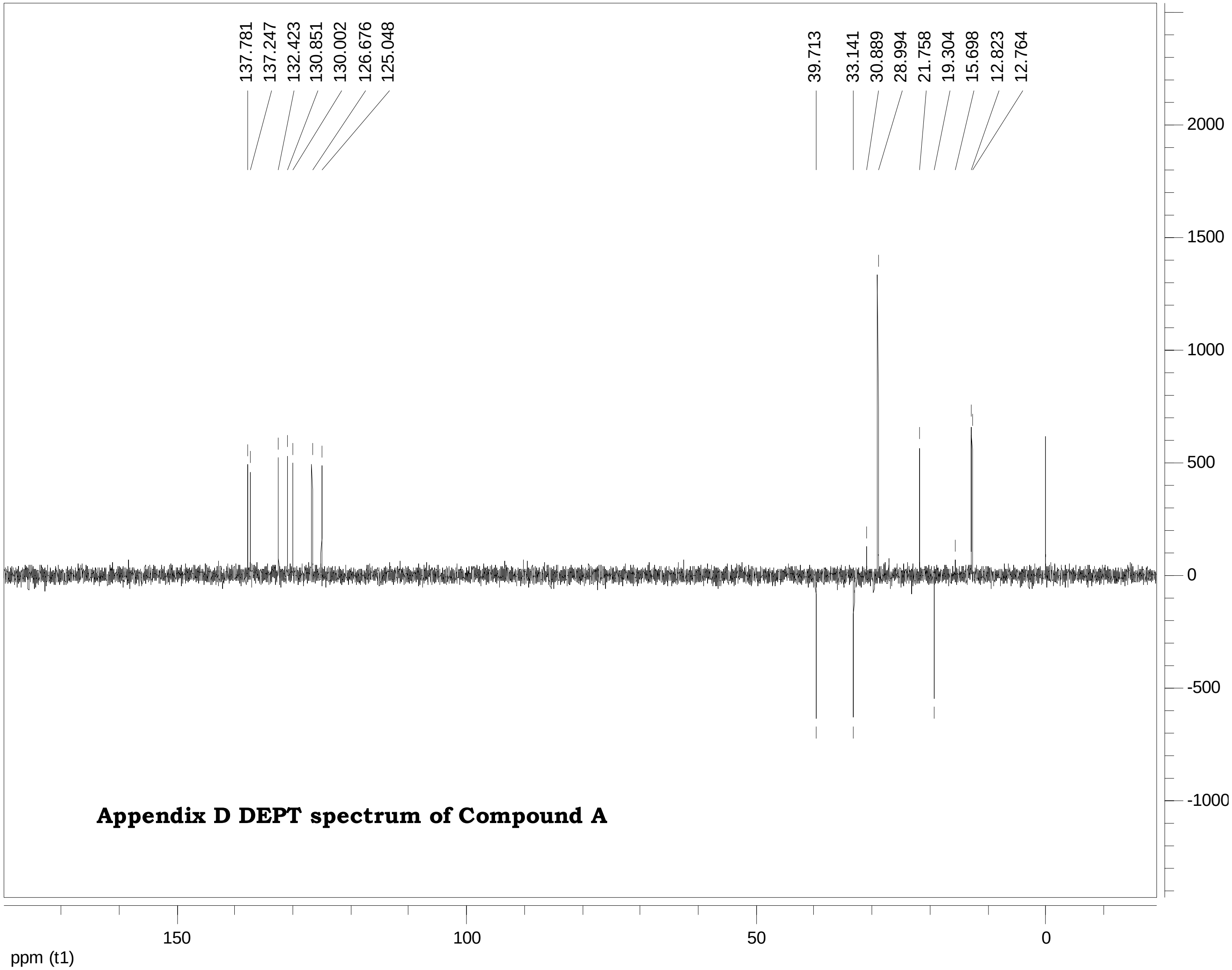
Appendix A



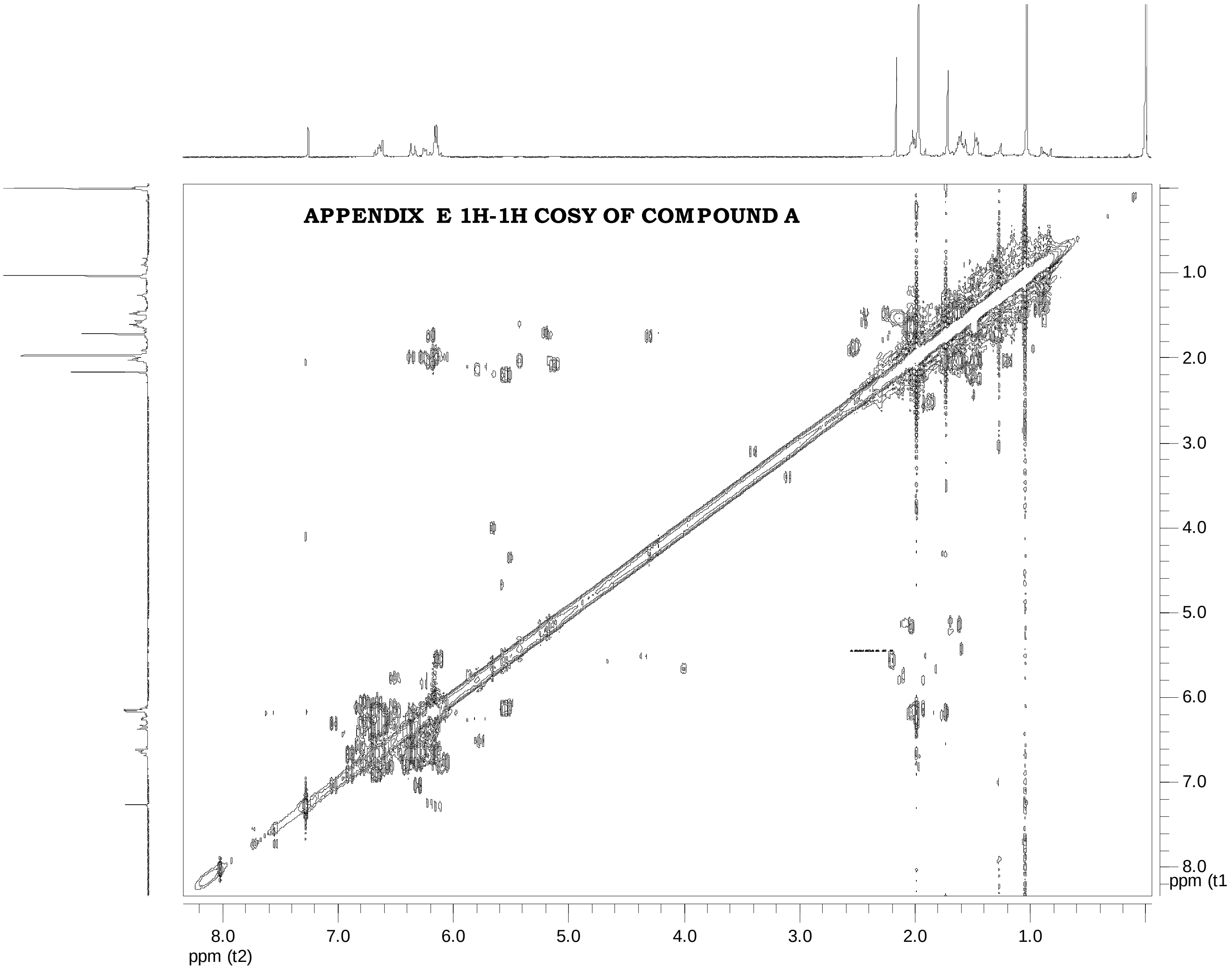
Appendix B H NMR spectrum of compound A



Appendix C C-13 NMR spectrum of compound A

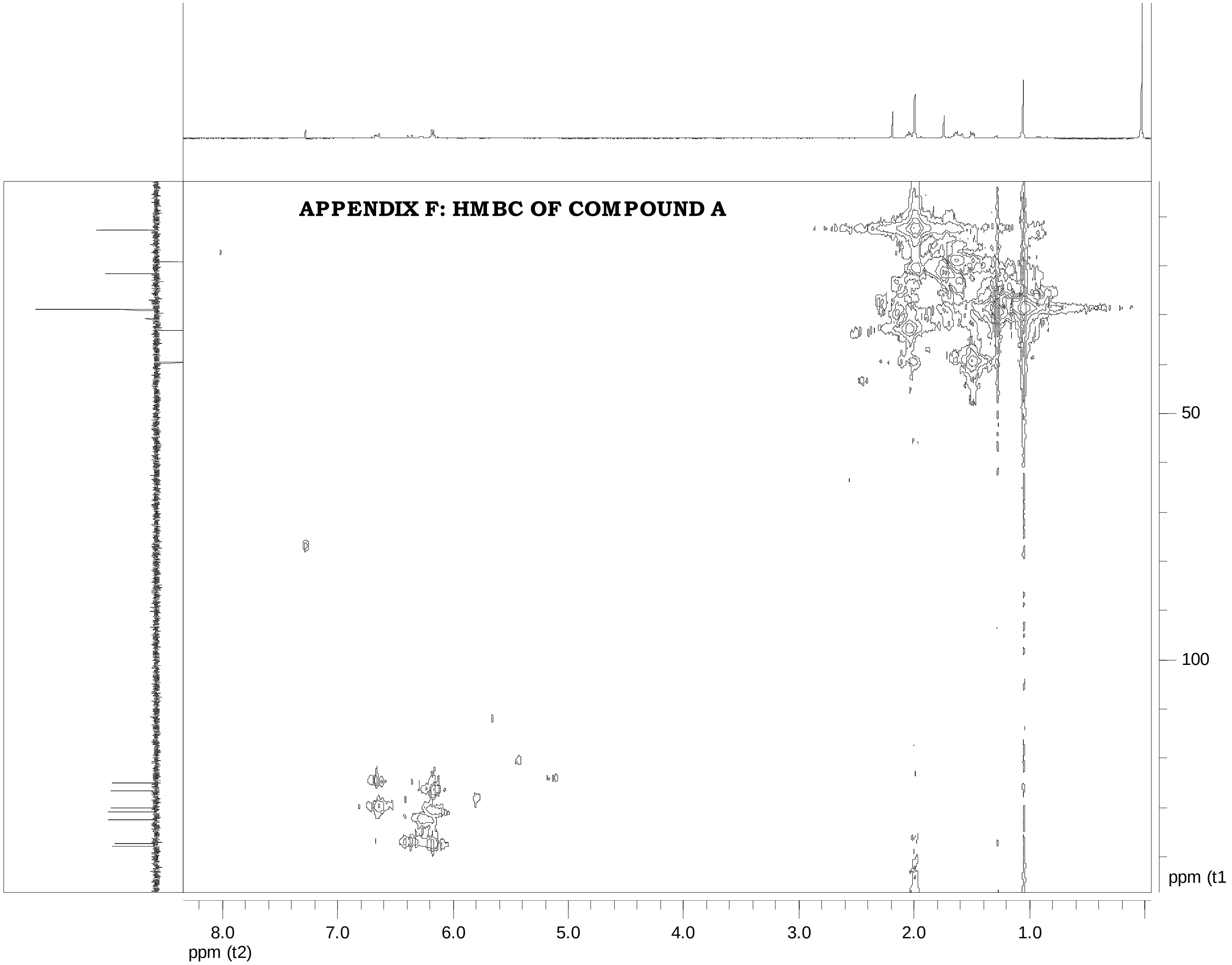


Appendix D DEPT spectrum of Compound A

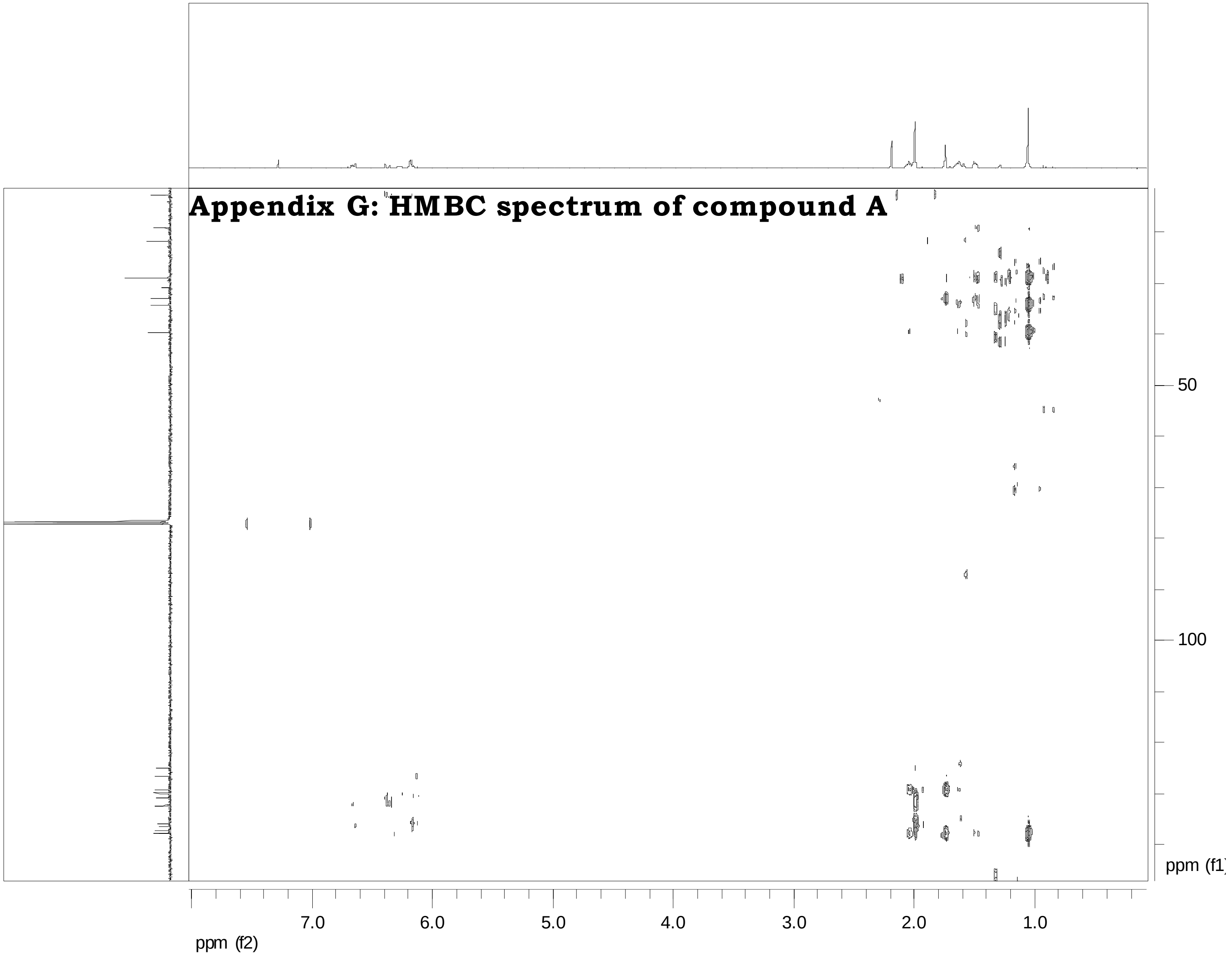


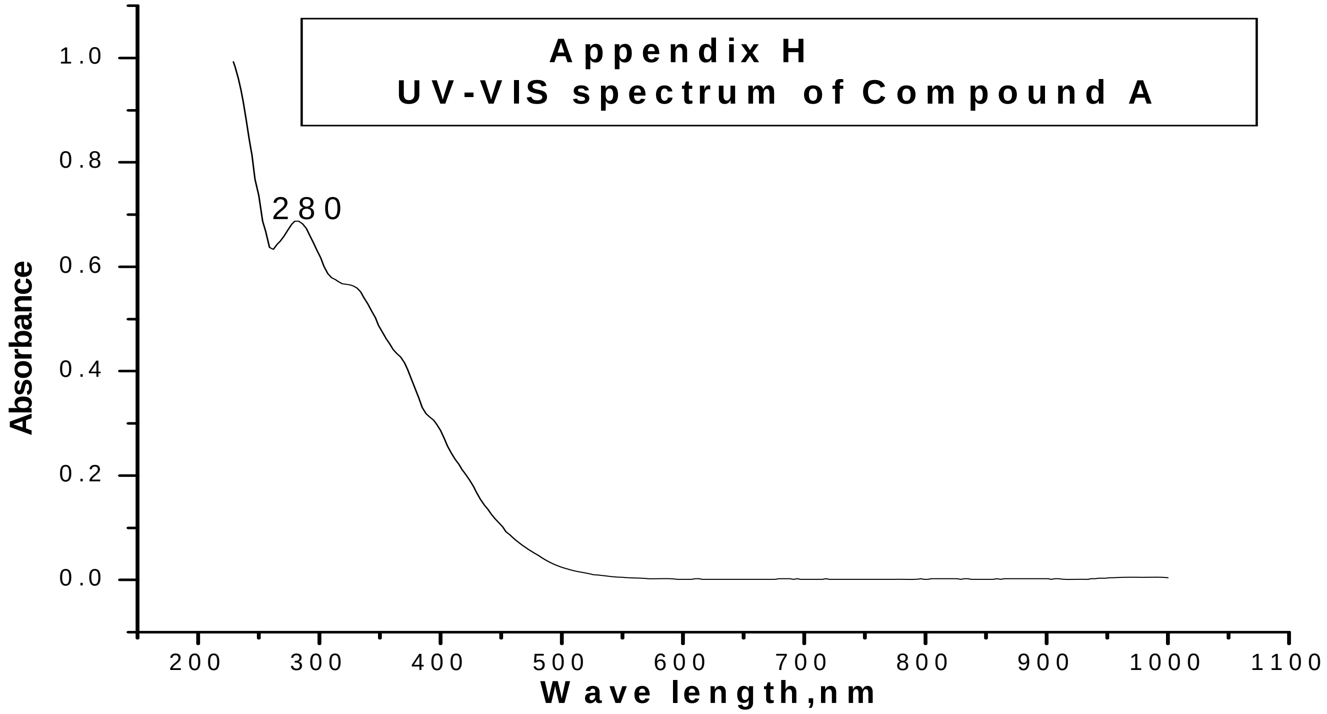
APPENDIX E 1H-1H COSY OF COMPOUND A

APPENDIX F: HMBC OF COMPOUND A

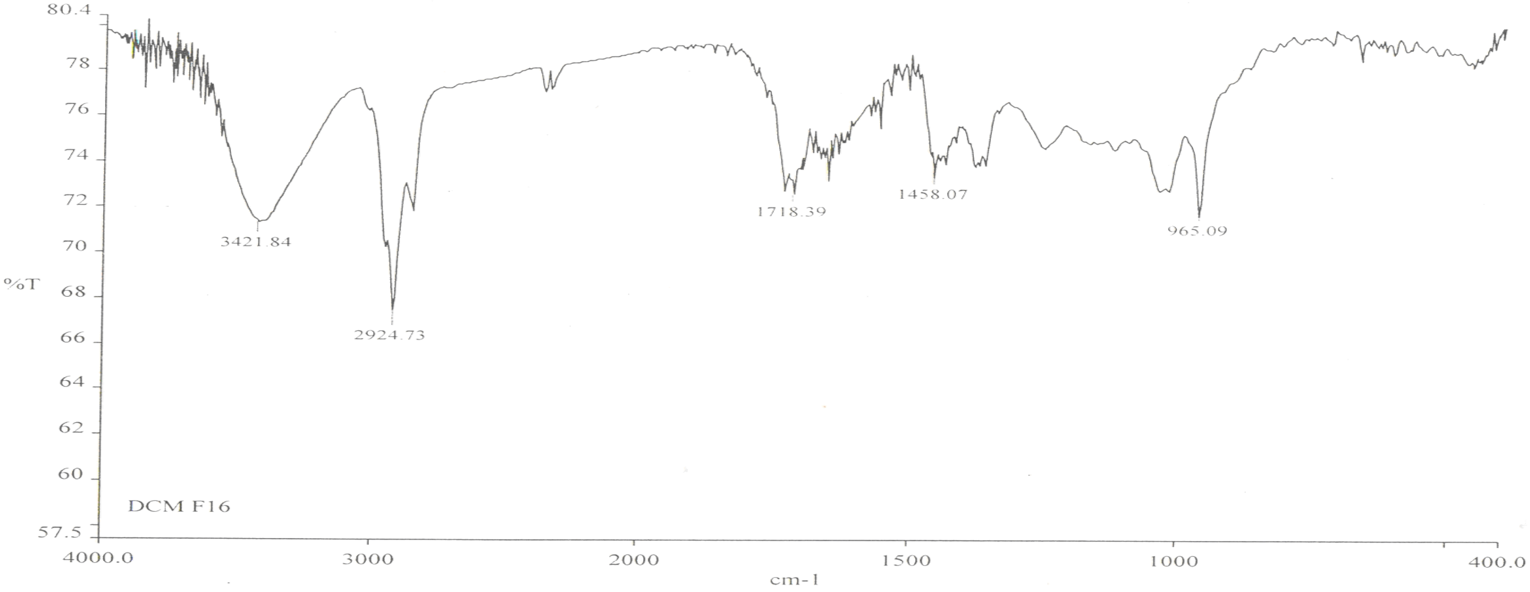


Appendix G: HMBC spectrum of compound A





Appendix H
UV-VIS spectrum of Compound A



Appendix I IR Spectrum of Compound E

Appendix J H-NMR spectrum of compound E