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PHYTOCHEMICAL INVESTIGATION ON

THE STEM BARK OF *EKEBERGIA CAPENSIS* (SOMBO)

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In Partial Fulfillment of the Requirements for Master of Science Degree in Chemistry

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Dedicated to Zelalem Tefera ---------

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Abstract

Phytochemical Investigation

On

The stem bark of *Ekebergia capensis*

By

Negera Abdissa

Advisor: Dr. Ashebir Fiseha

The stem bark of *Ekebergia capensis* is used traditionally by people of east Wollega for the treatment of Lung TB and its smoke is used to attract Bees if bee hives are activated with it. From the stem bark of *Ekebergia capensis***,** one flavonoid **EC-3** was isolated along with two acyclic triterpenoids namely 2, 3, 22, 23- tetrahydroxy- 2, 6, 10, 15, 19, 23- hexamethyl- 6, 10, 14, 18- tetracosatetraene (**EC-1**) and 2- hydroxymethyl 2, 3, 22, 23- tetrahydroxy-6, 10, 15, 19, 25- pentamethyl- 6, 10, 14, 18- tetracosatetraene (**EC-2**). The structural determination was accomplished by means of spectroscopic methods (IR, UV, 1D and 2D) NMR.

1. Introduction

1.1. General

Natural product chemistry is in its different aspects an ancient science. The preparation of foodstuffs, colouring matters, fibers, toxins, medicinals and stimulants are example of activities as old as mankind. When chemists in the late 18^{th} century took the final jump from the world of myths in to modern science, the properties of extract obtained from natural aroused great curiosity among scientists [1, 2].

Natural product is a chemical compound or a substance produced by living organisms found in nature that usually has a pharmacological activity for use in pharmaceutical drug discovery and design. Scientists began to separate purity, and finally analyses the compound in the living cells. Separation methods were developed and without doubt natural product chemistry has brought a great stimulus to the development of the refined techniques we have today, such as the various analytical and preparative chromatographic methods [2, 3].

The combination of natural products to the developments of medicine could be demonstrated by the amount of plant derived drugs being used. In general 40% of modern drugs are said to be of natural origin. The natural product from living organisms can be generalized in to primary and secondary metabolites. The former refers to the photosynthetic processes producing simple and widely distributed, low molecular weight carboxylic acid, carbohydrates, fats, proteins, and nucleic acids involved in the life process [4, 5].

Secondary metabolites are, in principle, non essential to life but they definitely contribute to the species fitness of survival [6]. Natural product chemistry as defined today concerns mainly the formation, structure and properties of secondary metabolites [7]. The World Health organization (WHO) estimates that around 80% of the world population in developing countries relies on traditional medicines for primary health care needs, of which major proportion corresponds to plant extracts or their active principles [8].

1.2. Genus *Ekebergia*

Meliaceae is a tropical and sub-tropical families of trees and shrubs having well-represented families with 51 genera and 800 species worldwide [9]. The major genera of meliaceae family are *Aglaia, Anthocarapa, Cabralea, Calodecarya, Carapa, Cedrela, Chukrasia, Cipadessa, Dysoxylum, Ekebergia, Guarea, Heckeldora, Khaya, Lansium, Lepidotrichilia, Lovoa, Malleastrum, Melia, Munronia, Naregamia, Neobeguea, Owenia, Ruagea, Sandoricum, Schmardaea, Soymida, Swietenia, Synoum, Toona, Trichilia* [10].

Ekebergia was named after Captain C.G. Ekeberg, whose sponsorship, in the 18th century, made it possible for Anders Sparman (the author of the tree species) to visit Africa [9]. *Ekebergia* is small genus of African tropical and sub-tropical trees and shrubs belonging to the family of meliaceae. The genus *Ekebergia* is rich in secondary metabolites, like Terpenoids, Limonoids and Coumarins from *E. capensis* [11-15], Stilbenes from *E. benguelensis* [16] and Coumarins from *E. Pterophylla* [17].

2. Secondary metabolites

2.1. Terpenes

2.1.1. Terms and Significance

The biological functions of terpenes have not yet been fully investigated. Many plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to expel certain animals using these plants as food [2, 12]. Less volatile but strongly bitter-tasting or toxic terpenes also protect some plants from being eaten by animals (antifeedants). In addition, terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants. Many insects metabolize terpenes they have received with their plant food to growth hormones and pheromones. Pheromones are signal compounds (sociohormones) that insects and other organisms excrete in order to communicate with others like them, e.g. to warn (alarm pheromones), to mark food resources and their location (trace pheromones), as well of assembly places (aggregation pheromones) and to attract sexual partners for copulation (sexual pheromones) [2].

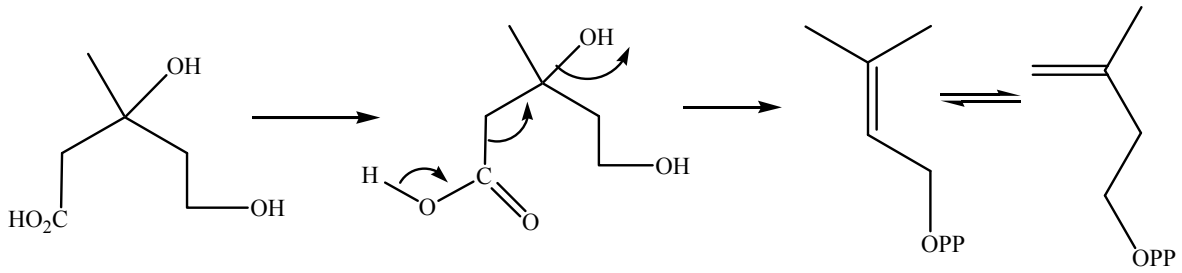
2.1.2. General structure

About 30,000 terpenes are known at present in the literature [2]. Their basic structure follows a general principle: *2-Methylbutane* residues, usually also referred to as *isoprene* units, (C5)n, build up the carbon skeleton of terpenes. In nature, terpenes occur predominantly as hydrocarbons, alcohols and their glycosides, ethers, aldehydes, ketones, carboxylic acids and esters [2, 19].

2.1.3. Biosynthesis of terpenoids

The recognition of biosynthetic principles is the most significant development in natural product chemistry. During the last century a great number of new structures were determined. In the earlier phase organic chemists were just content to solve the structure of natural products and group them according to origin, pharmacological activity or structure, but rather soon the mass of information suggested the need for amore coherent view of biogenesis.

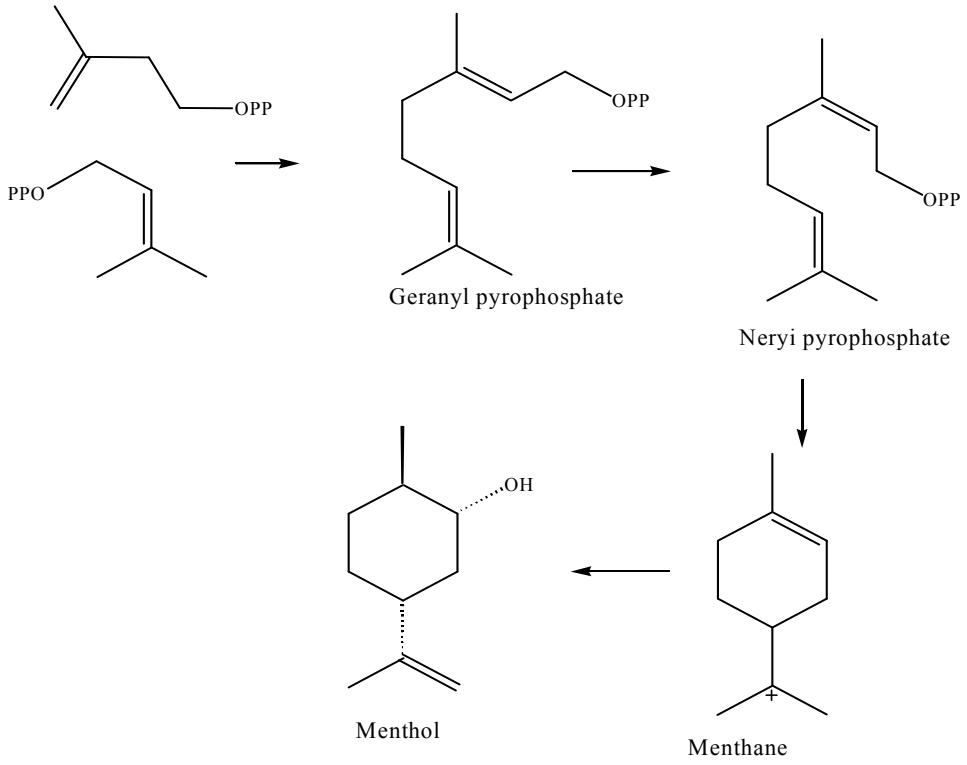
The biosynthetic routes of the various classes of compounds were mapped out precursors and intermediates were identified. The structure of carbon skeletons, the heterocyclic units and substitution patterns are defined by such considerations. Isoprene itself does not function as the reactive biogenetic species [2]. Isopentenyl and dimethyl allyl pyrophosphates are the reactive species involved in the formation of terpens. These are formed from mevalonic acid (scheme1) by phosphorylation followed by ATP assisted loss of water and carbon dioxide to give isopentenyl pyrophosphate (IPP). Isomerizaation of the double bond gives dimethyl allyl pyrophosphate (DMAPP) [5].



Scheme 1. Isoprenoid biosynthesis

2.1.3.1. Mono terpenes

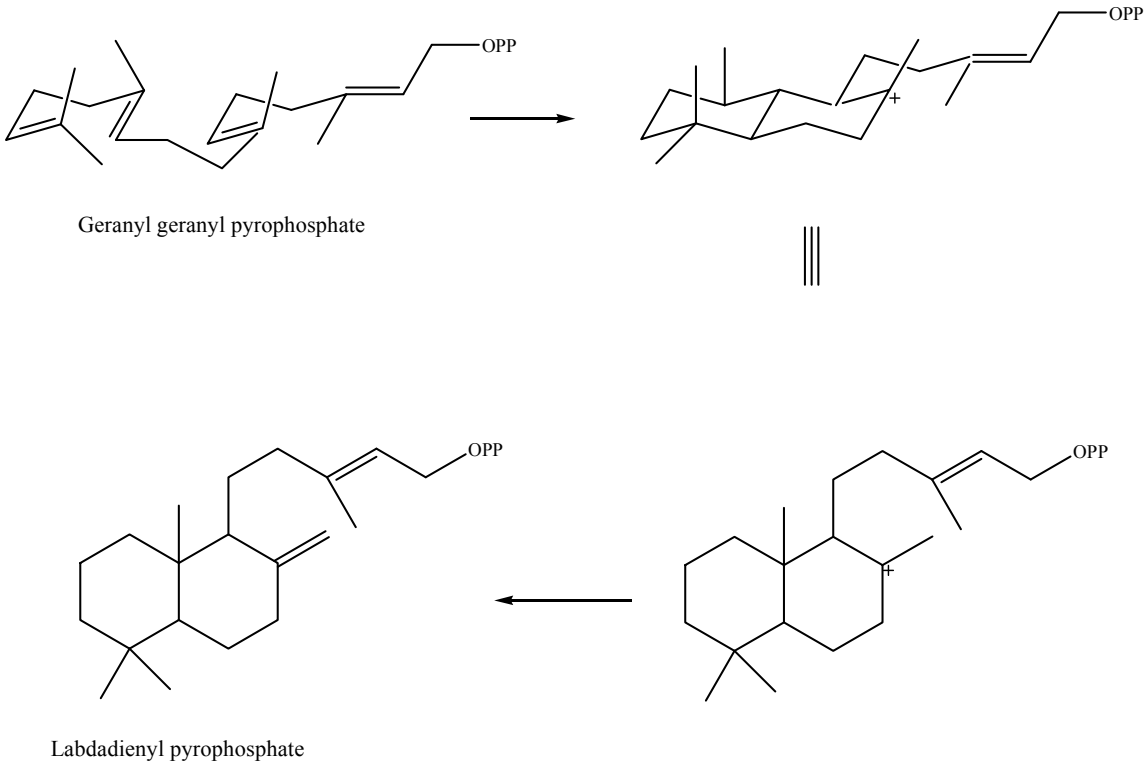
The biosynthesis of mono terpenes involves the dimerization of two isoprene units, in head-to-tail fashion, to form geranyl pyrophosphate. Isomerization to cis olefin, neryl pyrophosphate, sets the stage for cationic cyclization to give the menthane skeleton. Loss of proton and hydration of endocyclic olefin gives menthol [5].



Scheme 2. Biogenesis of monoterpens

2.1.3.2. Diterpenes

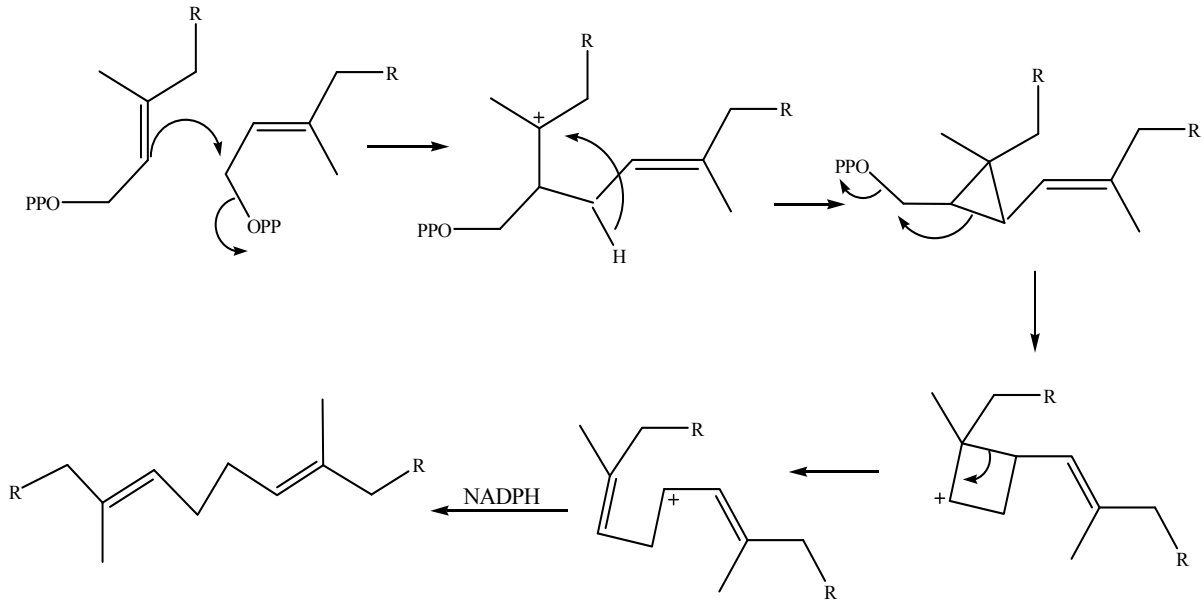
Diterpene contains 20 carbon skeleton formed from four isoprene units. The common precursor is the linear trans-trans geranyl geranyl pyrophosphate (scheme3), whose cyclization can be affected in many ways. The cyclization normally proceeds directly to abicyclic trans-decaline system which then undergoes a variety of different transformations. The straight forward loss of proton gives labdadienyl pyrophosphate, which functions as the intermediate to several structural types of diterpenes [5].



Scheme 3. Biogenesis of diterpenes

2.1.3.3. Triterpenes and higher

The mechanisms of biosynthesis of higher terpenes are remained a challenging problem until the isolation of a cyclopropane containing intermediate pre squalene pyrophosphate [20]. The two farnesyl units are joined together; the carbocation undergoes cyclization to the cyclopropane (pre squalene pyrophosphate) through loss of proton, as indicated in **scheme 4.** The pyrophosphate in the cyclopropylmethanol functions as a powerful leaving group, giving rise to a rearranged carbocation with cyclo butane skeleton. Being still a high-energy species, this undergoes rapid ring opening to the much more stable allylic cation which is finally trapped by NADPH, the biological hydride reductant, to give triterpene [5].



Scheme 4. Biogenesis of triterpenes

2.2. Flavonoids

The flavonoids are colouring substances contributing to the beauty of the flowers and fruits in nature. The flavones give yellow or orange colours. The occurrence of this numerous class of oxygen heterocyclic compounds is restricted to higher plants and ferns. Biologically the flavonoids play a major role in relation to insects pollinating or feeding on plants. The flavonoids are structurally characterized as having two hydroxylated aromatic rings, A and B, joined by a three carbon fragments. Several substructures can be distinguished; chalcone, flavones, isoflavones, aurones, and anthocyanidins [21- 23].

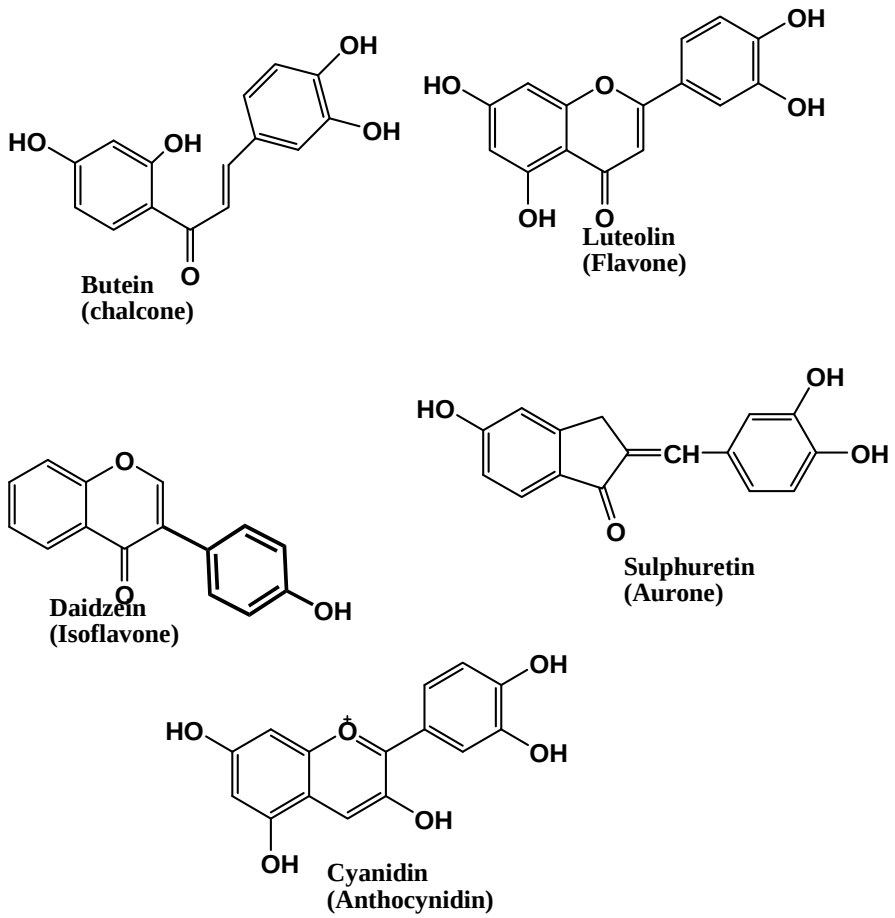
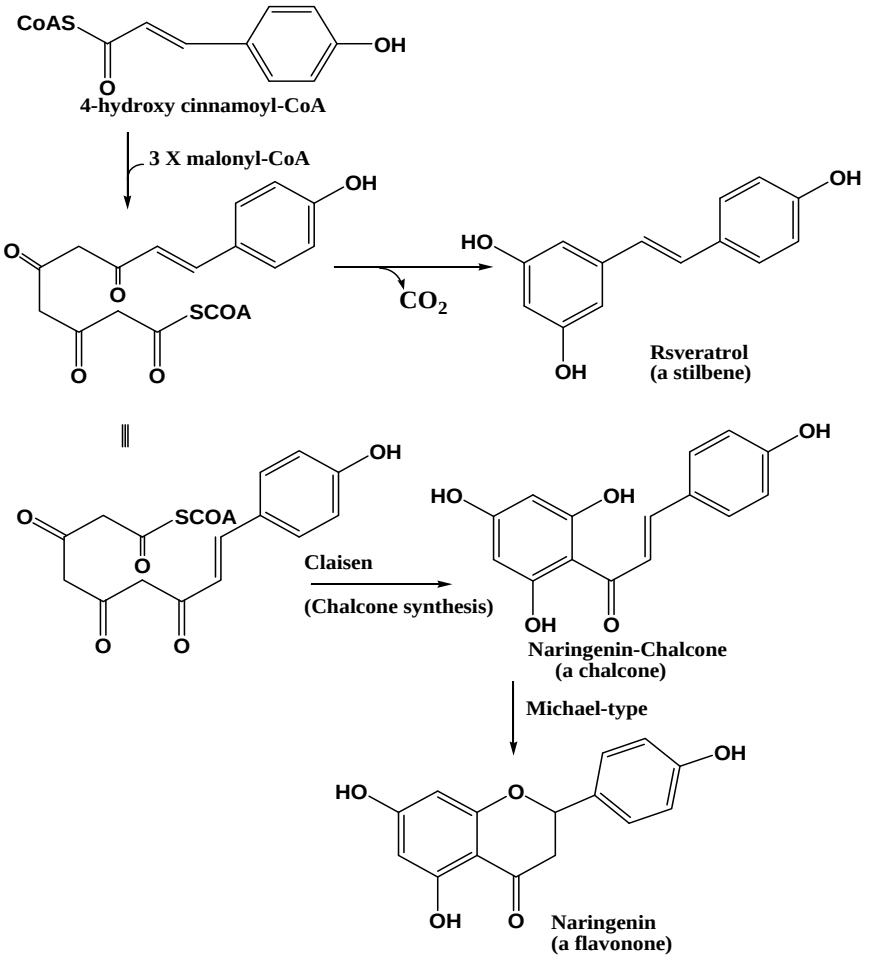


Figure 1. Structures of flavonoids.

2.2.1. Biogenesis of Flavonoids

Flavonoids are products from cinnamoyl-CoA starter units with chain extension using three molecules of malonyl-CoA. This initially gives a polyketide which according to the nature of the enzyme responsible, can be folded in two different ways. This allows aldol or claisen-like reaction to occur. Enzymes stilben synthase and chalcone synthase couple a cinnamoyl-CoA unit with three malonyl-CoAunit giving stilbenes, e.g. resveratrol or chalcones e.g. naringenin-chalcone respectively. Most of chalcones contain six membered heterocyclic rings, formed by Michael-type nucleophilic attack of a phenol group on to unsaturated ketone giving a flavonone [22, 23].



Scheme 5. Biosynthesis of Flavonoids.

3. *Ekebergia capensis*

3.1. Botanical Background

Ekebergia capensis is some times called Cape Ash belong to the family of meliaceae. This is a tropical and sub-tropical family of trees and shrubs, which is an ever green, medium-sized to large tree 7-20m tall. The species name *capensis* means from the cape but used in reference to Africa, since this tree occurs naturally from this region [9]. It is wide-spread in tropical Africa from Senegal to South Africa and east wards to Ethiopia, mainly Tigray, Welo, Gonder, Gojam, Wollega, Shawa, Arsi, Ilubabor, Kefa, Bale, Harerge and Sidamo regions [24].

E. capensis is covered with flowers every year, where the conditions are favorable, although in unfavorable localities trees may flower separately and only once in several years. For example: In South Africa, trees flower from September to November, and fruiting occurs from December to April; in Zambia, flowers appear between August and October and fruits November to January. Pollination is by bees and ants. Fruits are eaten by monkeys and birds, which helps to disperse the seed. Cape ash makes a good shade in the garden. It has been used as stunning street. The light and soft wood of cape ash is easy to work with, and with its straw color it makes attractive furniture. The stem bark smoke has good ouder and used to attract Bees when Bee hive are activated with it [9, 10].

Many parts of the *E. capensis* have medicinal values including, the bark contains 7.23% tannin and is used for tanning lather, the bark is used as an emetic, and used for treating dysentery and lung T.B. Decoction from roots are used to treat headaches and heart burns [10]. Leaves are used as a remedy for intestinal worms, branches are burned in the field to ward off the evil sprits and its wood is used to facilitate childbirth [15].

3.2. Secondary metabolites from *E. capensis*

Phytochemical study on the *E. capensis* leads to the isolation and characterization of different class of secondary metabolites. Previous phytochemical investigation on *Ekebergia capensis* species have shown that, different parts of *E. capensis* are rich in secondary metabolites.

Phytochemical investigation on the hexane extract of seeds of *E. capensis* led to the isolation of limonoids, capensolactones **1** and methyl 3α -hydroxy 3-deoxyangolensate **2** and diacylate angolensate derivative **3** [13,14]. Phytochemical investigation on the stem bark of *Ekebergia capensis* led to the isolation of two acyclic tritepenoids, 2,3,22,23-tetrahydroxy- 2,6,10,15,19,23- hexamethyl-6,10,14,18- tetracosatetraene **4** and 2-hydroxymethyl 2,3,22,23-tetrahydroxy-6,10,15,19,25,-pentamethyl-6,10,14,18-tetracosatetraene **5** along with known cyclic triterpenoids, Oleanonic acid **6**, 3-epi oleanoic acid **7** [11,12]. Phytochemical investigation on wood of *E. capensis* results β-sitosterol **8**, oleanonic acid **6**, 3-epioleanolic acid **7**, 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene **4**, and 7- hydroxyl-6- methoxy coumarin **9** [15].

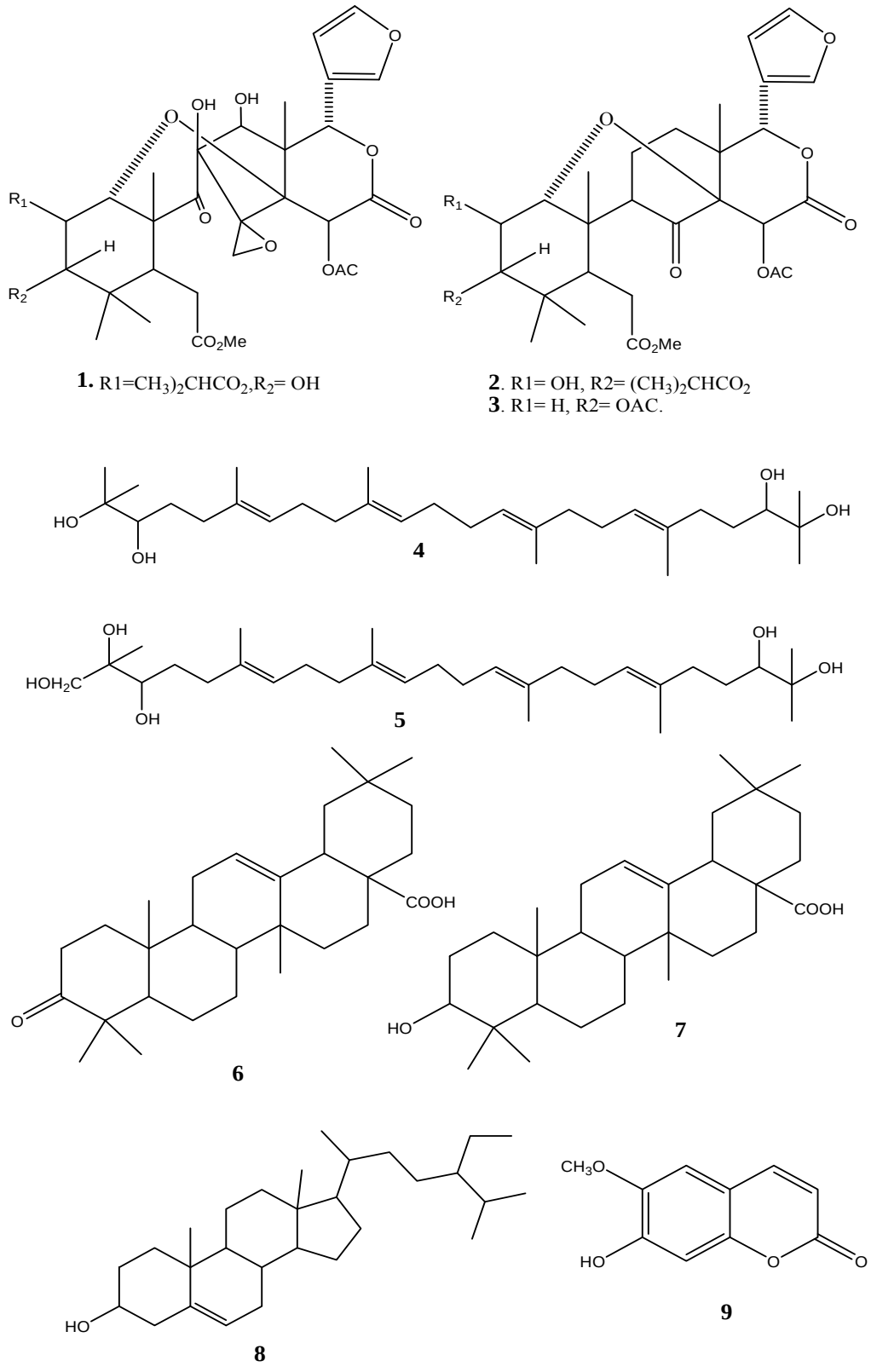


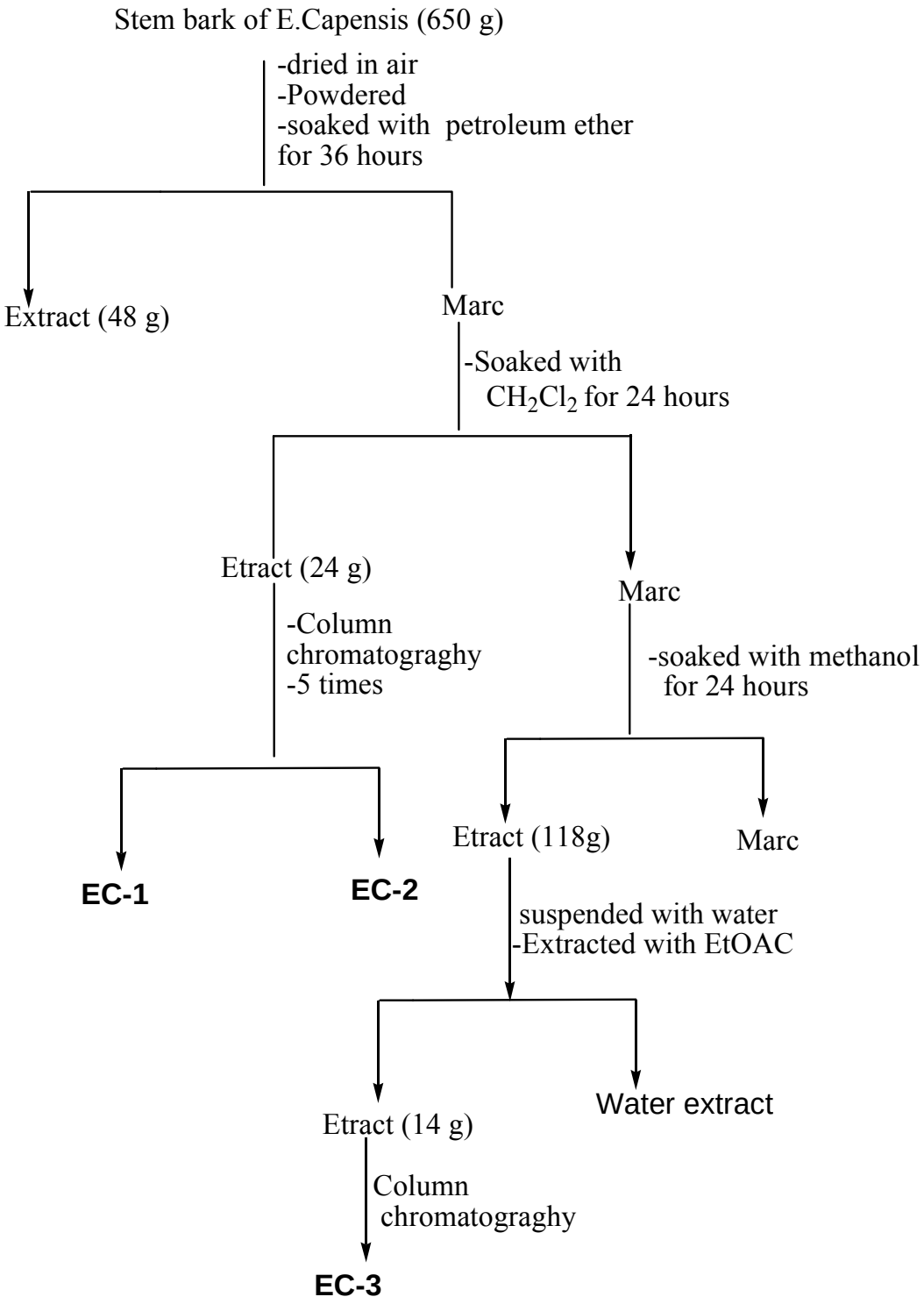
Figure 2. Secondary metabolites from *E. capensis*

4. Objective of the study

The main objective of the study of this project was isolation and structural elucidation of the constituents of the stem bark of *Ekebergia capensis*. The plant was selected for this study because it is important in traditional medicines for the treatments of chronic cough or lung TB and traditionally people activate Bee hive with its smoke.

5. Result and discussions

The air dried and powdered stem bark of the *Ekebergia capensis* was extracted with dichloromethane. This extract when developed on TLC, sprayed with 1% vanillin sulphuric acid, and after heating for a few minutes has shown the characteristic color change that indicates the presence of terpenes. One gram of this extract was subjected to silica gel, resulted two acyclic triterpenes. And the ethyl acetate extract of the marc after extraction with dichloromethane resulted in a flavonoid. This study has resulted in the isolation and characterization of three compounds.



Scheme 6. Method used to extract plant material

5.1. Characterization of compound EC-1

The compound EC-1 was obtained as colorless oil, soluble in chloroform, not UV active and showed a characteristic colour change to pale red on TLC plate upon spraying 1% vanillin sulphuric acid and after heating for a few minutes. This compound has Rf value 0.45 using chloroform: methanol (9:1) as solvent systems. In the IR spectrum of the compound **EC-1** (Appendix 1.1), the absorption band at 3417 cm^{-1} showed the O-H stretching that indicates the presence of a hydroxyl group. The strong absorption band at 2944 cm^{-1} showed the presence of the C-H stretching for methyl groups. The absorption band at 1694 cm^{-1} showed the presence of the olefinic C=C stretching. The absorption band at 1072 cm^{-1} showed the presence of the C-O bond stretching.

The ^{1}H NMR spectrums (Appendix 1.2) of EC-1 in $CDCl_3$ suggested the presence of four methyl signals at δ1.15 (6H, *s*) and δ 1.20 (6H, *s*) , four olefinic protons at δ 5.14 (2H, *m*) and δ 5.19 (2H, *m*) , four methyl groups at δ 1.60 (6H, *bs*) and δ 1.62 (6H, *bs*) attached to sp^2 carbons, eight methylene groups at δ 2.02 (8H, *m*) and δ 2.09 (8H, *m*) attached to sp^2 carbons, two methylene protons at δ 1.42 (2H, *m*) and δ 1.58 (2H, *m*) adjacent to chiral center and two methine protons at δ 3.35 (2H, *dd*) attached to carbon containing electronegative atom oxygen.

The ^{13}C NMR spectrum (appendix 1.3), analyzed with the aids of DEPT-135 (Appendix 1.4), showed three quaternary carbons, which were attributable to two olefinic, one oxygenated carbon. In addition, three methines, five methylenes and four methyl carbons were observed. Out of the four methyl carbons, two were in the olefinic region.

Table 1. Proton Decoupled ^{13}C NMR and DEPT spectral data of Compound **EC-1**

Carbon No.	^{13}C NMR δ(in ppm)	DEPT δ(in ppm)	Remark
1.	23.3	23.3	CH_3
2.	73.1	-	C (Quaternary carbon)
3.	78.3	78.3	CH
4.	29.7	29.7	CH_2
5.	36.8	36.8	CH_2
6.	134.8	-	C (Quaternary carbon)
7.	125.1	125.1	CH
8.	26.5	26.5	CH_2
9.	39.7	39.7	CH_2
10.	134.9	-	C (Quaternary carbon)
11.	124.5	124.5	CH
12.	28.2	28.2	CH_2
13.	28.2	28.2	CH_2
14.	124.5	124.5	CH
15.	135.0	-	C (Quaternary carbon)
16.	39.7	39.7	CH_2
17.	26.5	26.5	CH_2
18.	125.1	125.1	CH
19.	135.0	-	C (Quaternary carbon)
20.	36.8	36.9	CH_2
21.	29.7	29.7	CH_2
22.	78.3	78.3	CH
23.	73.0	-	C (Quaternary carbon)
24.	23.3	23.3	CH_3
25.	26.4	26.4	CH_3
26.	16.0	16.0	CH_3
27.	15.9	15.9	CH_3
28.	15.9	15.9	CH_3
29.	16.0	16.0	CH_3
30.	26.4	26.4	CH_3

From comparison of the ^{13}C NMR spectral data of **EC-1** with literature, compound **EC-1** closely resembles 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (compound **4**) .

Table 2. Proton Decoupled ^{13}C NMR spectral data of Compound **EC-1** (base skeleton) compared with literature.

Carbon No.	**EC-1**	**Compound 4(literature)**
1.	23.3	23.4
2.	73.1	73.0
3.	78.3	78.3
4.	29.7	29.7
5.	36.8	36.8
6.	134.8	134.9
7.	125.1	125.2
8.	26.5	26.6
9.	39.7	39.7
10.	134.9	134.9
11.	124.5	124.5
12.	28.2	28.3
13.	28.2	28.3
14.	124.5	124.5
15.	135.0	135.0
16.	39.7	39.7
17.	26.5	26.6
18.	125.1	125.2
19.	135.0	135.0
20.	36.8	36.8
21.	29.7	29.7
22.	78.3	78.3
23.	73.0	73.0
24.	23.3	23.4
25.	26.4	26.4
26.	16.0	16.0
27.	15.9	15.9
28.	15.9	15.9
29.	16.0	16.0
30.	26.4	26.4

Heteronuclar Single Quantum Correlation (HSQC) experiment (Appendix 1.5) also correlates the chemical shift of proton with directly bonded carbon atom. The HSQC NMR spectrum showed some selected correlations such that, the two diastereotopic methylene protons at δ 1.42 and δ 1.58 correlate with that of carbon at δ 29.70. In the same manner, the HSQC spectrum also showed different kinds of correlations of the protons with that of carbons as shown below in **table 4**.

Table 3. Heteronuclar Single Quantum Correlation (HSQC) spectra of EC-1

Carbon No.	Proton No	^{1}H NMRδ (in ppm)	Appearance
1.	H-1	1.15	*s*
3.	H-3	3.35	*dd*
4.	H-4	1.42 and 1.58	*m*
5.	H-5	2.09 and 2.28	*m*
7.	H-7	5.19	*m*
8.	H-8	2.09	*m*
9.	H-9	2.02	*m*
11.	H-11	5.14	*m*
12.	H-12	2.02	*m*
13.	H-13	2.02	*m*
14.	H-14	5.14	*m*
16.	H-16	2.02	*m*
17.	H-17	2.09	*m*
18.	H-18	5.19	*m*
20.	H-20	2.09 and 2.23	*m*
21.	H-21	1.42 and 1.58	*m*
22.	H-22	3.35	*dd*
24.	H-24	1.15	*s*
25.	H-25	1.20	*s*
26	H-26	1.62	*bs*
27.	H-27	1.60	*bs*
28.	H-28	1.60	*bs*
29	H-29	1.62	*bs*
30.	H-30	1.20	*s*

The ^{1}H-^{1}H correlation spectroscopy (Homonucllear COSY) experiment (Appendix 1.6) showed the correlations between H-4 at δ 1.42 and δ 1.58 and H-3, H-21 at δ 1.42 and δ 1.58 and H-22, indicating that the two methylene protons are diastereotopic. Similarly, there are also other strong couplings which were observed from COSY experiment as shown below in **table 5**.

Table 4. ^{1}H NMR and some selected ^{1}H-^{1}H COSY spectral data of compound **EC-1**

Carbon No.	^{1}H NMRδ (in ppm)	^{1}H-^{1}H COSY
1.	1.15	
3.	3.35	H-3↔H-4
4.	1.42 and 1.58	H-4↔H-3,H-5
5.	2.09 and 2.28	H-5↔H-4
7.	5.19	H-7↔H-8
8.	2.09	H-8↔H-7
9.	2.02	
11.	5.14	H-11↔H-12
12.	2.02	H-12↔H-11
13.	2.02	
14.	5.14	
16.	2.02	
17.	2.09	
18.	5.19	
20.	2.09 and 2.23	H-20↔H-21
21.	1.42 and 1.58	H-21↔H-20,H-22
22.	3.35	H-22↔H-21
24.	1.15	
25.	1.20	
26	1.62	
27.	1.60	
28.	1.60	
29	1.62	
30.	1.20	

Based on the HMBC experiment (Appendix 1.7) the correlations of the protons with carbons which are two or three bonds away can be shown structurally as follows:

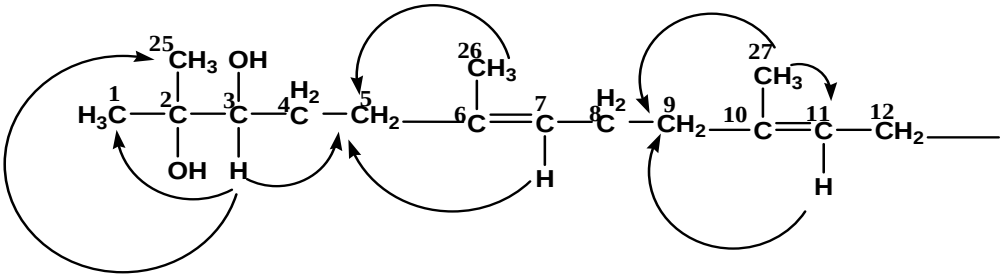


Figure 3. Observed HMBC correlations of compound **EC-1**.

Based on spectroscopic data and literature, the compound **EC-1** is a known triterpene with the following structure.

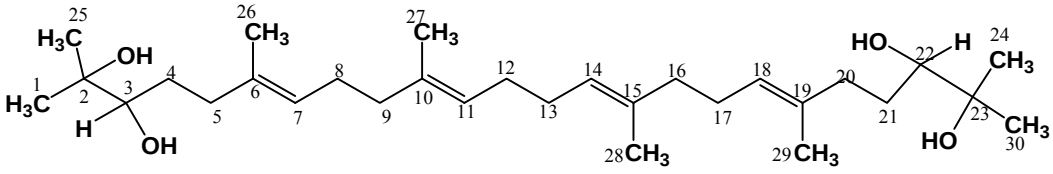


Figure 4. 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene(**EC-1**)

5.2. Characterization of compound EC-2

The spectral data of EC-2 were very similar to those of EC-1. This suggested that EC-2 was an acyclic triterpene derivative of EC-1. The compound EC-2 was obtained as acolourless oil, soluble in chloroform, not UV active and showed a characteristic colour change to pale red on TLC plate upon spraying 1% vanillin sulphuric acid and after heating for a few minutes. This compound has Rf value O.36 using chloroform: methanol (9:1) as solvent systems.

In the IR spectrum of the compound **EC-2** (Appendix 2.1), the absorption band at 3400 cm^{-1} showed the O-H stretching that indicates the presence of a hydroxyl group. The strong absorption band at 2925 cm^{-1} showed the presence of the C-H stretching for methyl groups. The absorption band at 2854 cm^{-1} showed the presence of C-H stretching of the methylene groups. The absorption band at 1666 cm^{-1} showed the presence of the olefinic C=C stretching. The situation was also similar in case of the ^{1}H NMR data (appendix 2.2). The two degenerate signals at δ 1.15 and 1.20 due to four terminal methyl groups present in compound EC-1 were replaced by three signals at δ 1.07, 1.15, and 1.20 due to three methyl groups in compound EC-2. Typical AB type signals at δ 3.50 and 3.61 due to methylene protons of a hydroxymethyl group were observed.

Table 5. Comparision of ^{1}H NMR spectral data of EC-1 and EC-2

Carbon No.	Proton No	^{1}H NMR δ (in ppm)	
		EC-1	**EC-2**
1.	H-1	1.15	1.07
3.	H-3	3.35	3.61
4.	H-4	1.42 and 1.58	1.53
5.	H-5	2.09 and 2.28	2.09 and 2.25
7.	H-7	5.19	5.19
8.	H-8	2.09	2.09
9.	H-9	2.02	2.02
11.	H-11	5.14	5.14
12.	H-12	2.02	2.02
13.	H-13	2.02	2.02
14.	H-14	5.14	5.14
16.	H-6	2.02	2.02
17.	H-17	2.09	2.09
18.	H-18	5.19	5.19
20.	H-20	2.09 and 2.23	2.09 and 2.25
21.	H-21	1.42 and 1.58	1.41and 1.53
22.	H-22	3.35	3.35
24.	H-24	1.15	1.15
25.	H-25	1.20	3.50 and 3.61
26	H-26	1.62	1.62
27.	H-27	1.60	1.60
28.	H-28	1.60	1.60
29	H-29	1.62	1.62
30.	H-30	1.20	1.20

The ^{13}C NMR spectrum (appendix 2.3), as depicted in **table 7**, most of the signals in EC-2 corresponding to those found in EC-1 are split in to two, and in addition, a signal at δ 69.1 attributable to the methylene carbon of a hydroxy methyl group is present. These results suggest that EC-2 is an asymmetrical compound.

Table 6. Comparision of ^{13}C NMR spectral data of EC-1 and EC-2

EC-1		**EC-2**	
Carbon No.	^{13}C NMR δ(in ppm)	Carbon No.	^{13}C NMR δ(in ppm)
C-1, C-24	23.3	C-1	19.5
		C-24	23.1
C-2, C-23	73.1	C-2	74.4
		C-23	73.2
C-3, C-22	78.3	C-3	75.6
		C-22	78.2
C-4, C-21	29.7	C-4	29.3
		C-21	29.8
C-5, C-20	36.8	C-5	36.3
		C-20	36.8
C-6, C-10	134.8	C-6	134.8
		C-10	134.9
C-7, C-18	125.1	C-7	124.9
		C-18	125.0
C-8, C-17	26.5	C-8, C-17	26.5
C-9, C-16	39.7	C-9, C-16	39.6
C-11, C-14	124.5	C-11, C-14	124.4
C-12, C-13	28.2	C-12, C-13	28.2
C-15, C-19	135.0	C-15, C-19	134.9
C-25, C-30	26.4	C-25	69.1
		C-30	26.4
C-26, C-29	16.0	C-26, C-29	16.0
C-27, C-28	15.9	C-27, C-28	15.9

Table 7. Proton Decoupled ^{13}C NMR and DEPT spectral data of Compound **EC-2**

Carbon No.	^{13}C NMR δ(in ppm)	DEPT δ(in ppm)	Remark
1.	19.5	19.5	CH_3
2.	74.4	-	C (Quaternary carbon)
3.	75.6	75.6	CH
4.	29.3	29.3	CH_2
5.	36.3	36.3	CH_2
6.	134.8	-	C (Quaternary carbon)
7.	124.9	124.9	CH
8.	26.5	26.5	CH_2
9.	39.6	39.6	CH_2
10.	134.9	-	C (Quaternary carbon)
11.	124.4	124.4	CH
12.	28.2	28.2	CH_2
13.	28.2	28.2	CH_2
14.	124.4	124.4	CH
15.	134.9	-	C (Quaternary carbon)
16.	39.6	39.6	CH_2
17.	26.5	26.5	CH_2
18.	125.0	125.0	CH
19.	134.9	-	C (Quaternary carbon)
20.	36.8	36.8	CH_2
21.	29.8	29.8	CH_2
22.	78.2	78.2	CH
23.	73.2	-	C (Quaternary carbon)
24.	23.1	23.1	CH_3
25.	69.1	69.1	CH_2
26.	16.0	15.9	CH_3
27.	15.9	15.9	CH_3
28.	15.9	16.0	CH_3
29.	16.0	16.0	CH_3
30.	26.4	26.4	CH_3

Table 8. Proton Decoupled ^{13}C NMR spectral data of Compound **EC-2** (base skeleton) compared with literature [11, 12].

Carbon No.	**EC-2**	**Compound-5**
1.	19.5	19.6
2.	74.4	74.1
3.	75.6	75.9
4.	29.3	29.2
5.	36.3	36.4
6.	134.8	134.9
7.	124.9	125.1
8.	26.5	26.5
9.	39.6	39.6
10.	134.9	135.0
11.	124.4	124.5
12.	28.2	28.2
13.	28.2	28.2
14.	124.4	124.6
15.	134.9	135.0
16.	39.6	39.7
17.	26.5	26.5
18.	125.0	125.2
19.	134.9	135.0
20.	36.8	36.8
21.	29.8	29.7
22.	78.2	78.3
23.	73.2	73.0
24.	23.1	23.3
25.	69.1	69.2
26.	16.0	16.0
27.	15.9	15.9
28.	15.9	15.9
29.	16.0	16.0
30.	26.4	26.4

From comparison of the ^{13}C NMR spectral data of **EC-2** with literature, compound **EC-2** closely resembles 2-hydroxymethyl 2,3,22,23-tetrahydroxy-6,10,15,19,25,-pentamethyl-6,10,14,18-tetracosatetraene.

Heteronuclar Single Quantum Correlation (HSQC) experiment (Appendix 1.5) also correlates the chemical shift of proton with directly bonded carbon atom. The HSQC NMR spectrum showed some selected correlations such that, the two diastereotopic methylene protons at δ 1.41 and δ 1.53 correlate with that of carbon at δ 29.8. In the same manner, the HSQC spectrum also showed different kinds of correlations of the protons with that of carbons as shown below in **table 8**.

Table 9. Heteronuclar Single Quantum Correlation (HSQC) spectra of EC-2

Carbon No.	Proton No	^{1}H NMR δ (in ppm)	Appearance
1.	H-1	1.07	*s*
3.	H-3	3.61	*dd*
4.	H-4	1.53	*m*
5.	H-5	2.09 and 2.25	*m*
7.	H-7	5.19	*m*
8.	H-8	2.09	*m*
9.	H-9	2.02	*m*
11.	H-11	5.14	*m*
12.	H-12	2.02	*m*
13.	H-13	2.02	*m*
14.	H-14	5.14	*m*
16.	H-6	2.02	*m*
17.	H-17	2.09	*m*
18.	H-18	5.19	*m*
20.	H-20	2.09 and 2.25	*m*
21.	H-21	1.41and 1.53	*m*
22.	H-22	3.35	*dd*
24.	H-24	1.15	*s*
25.	H-25	3.50 and 3.61	*d* and *bd*
26	H-26	1.62	*bs*
27.	H-27	1.60	*bs*
28.	H-28	1.60	*bs*
29	H-29	1.62	*bs*
30.	H-30	1.20	*s*

The ^{1}H-^{1}H correlation spectroscopy (COSY) experiment (Appendix 1.6) showed the correlations between H-3 and H-4, H-21 at δ 1.41 and δ 1.53 and H-22, indicating that the two methylene protons are diastereotopic. Similarly, there are also other strong couplings which were observed from COSY experiment as shown below in **table 9**.

Table 10. ^{1}H NMR and some selected ^{1}H-^{1}H COSY spectral data of compound **EC-2**

Carbon No.	^{1}H NMRδ (in ppm)	^{1}H-^{1}H COSY
1.	1.07	
3.	3.61	H-3↔H-4
4.	1.53	H-4↔H-3,H-5
5.	2.09 and 2.25	H-5↔H-4
7.	5.19	H-7↔H-8
8.	2.09	H-8↔H-7
9.	2.02	H-9↔H-8
11.	5.14	H-11↔H-12
12.	2.02	H-12↔H-11,H-13
13.	2.02	H-13↔H-14,H-12
14.	5.14	H-14↔H-13
16.	2.02	H-16↔H-17
17.	2.09	H-17↔H-18
18.	5.19	H-18↔H-17,H-16
20.	2.09 and 2.25	H-20↔H-21
21.	1.41and 1.53	H-21↔H-20,H-22
22.	3.35	H-22↔H-21
24.	1.15	
25.	3.50 and3.61	
26	1.62	
27.	1.60	
28.	1.60	
29	1.62	
30.	1.20	

Table 11. Observed correlations in HMBC spectral data of compound **EC-2**

Carbon No.	^{13}C NMR δ (ppm)	HMBC
1.	19.5	H-1↔C-1,C-2, C-3, C-25
2.	74.4	
3.	75.6	H-3↔ C-1
4.	29.3	
5.	36.3	H-5↔ C-26 C-7
6.	134.8	
7.	124.9	H-7↔ C-8, C-26
8.	26.5	H-8↔C-7,C-9,C-26, C-27
9.	39.6	H-9↔C-8,C-10,C-27
10.	134.9	
11.	124.4	H-11↔C-9,C-12,C-13,C-27
12.	28.2	H-12↔C-11,C-13,C-14
13.	28.2	H-13↔C-12,C-14
14.	124.4	H-14↔C-13,C-16,C-28
15.	134.9	
16.	39.6	H-16↔C-17,C-18,C-28
17.	26.5	H17↔C-16,C-18
18.	125.0	H-18↔C-17,C-29
19.	134.9	
20.	36.8	H-20↔C-18,C-22,C-29
21.	29.8	H-21↔C-29
22.	78.2	H-22↔C-23,C-24
23.	73.2	
24.	23.1	H-24↔ C-23,C-30
25.	69.1	H-25↔ C-1,C-2,C-3
26.	16.0	H-26↔ C-5,C-6,C-7
27.	15.9	H-27 ↔C-8,C-9,C-11
28.	15.9	H-28↔ C-14,C-16
29.	16.0	H-29↔ C-18,C-19,C-20
30.	26.4	H-30 ↔C-22,C-23,C-24,C-30

Based on the HMBC experiment (Appendix 1.7) the correlations of the protons with carbons, which are two or three bonds away, can be shown structurally as follows:

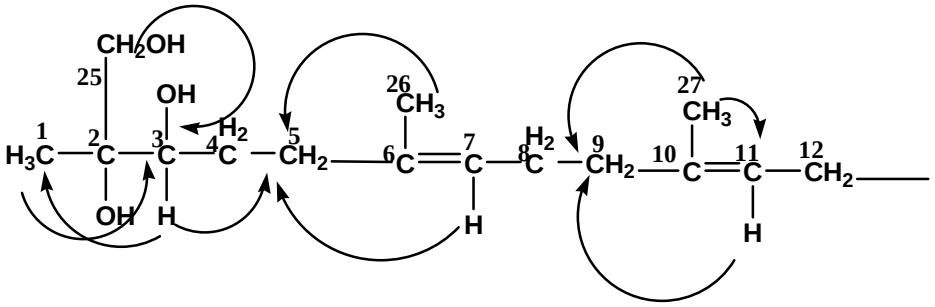


Figure 5. Observed HMBC correlations of compound EC-2

Based on spectroscopic data and literature, the compound **EC-2** is a known triterpene with the following structure.

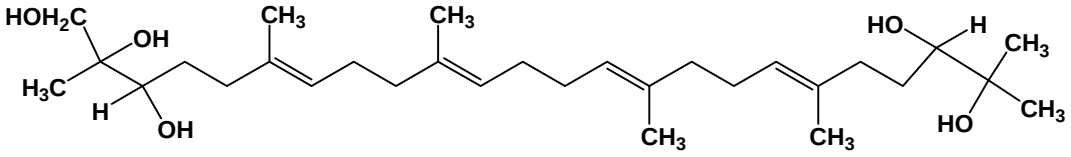


Figure: 6. 2-hydroxymethyl 2,3,22,23-tetrahydroxy-6,10,15,19,25,-pentamethyl-6,10,14,18-tetracosatetraene(**EC-2**)

5.3. Characterization of compound EC-3

Compound EC-3 was obtained as yellow solid, in soluble in $CHCl_3$ with m.p 210-215 ^oC. In the IR (KBr) spectrum (Appendix 3.1) absorption band at 1629 cm^{-1} showed the presence aromatic C=C stretching vibration and 1448 cm^{-1} showed C-O stretching. Absorption at 3368 cm^{-1} is due to OH. The UV-Vis spectrum at λ_{max} (ethanol) (Appendix 3.2) showed the absorption band at 300 nm indicated that the molecule has conjugation.

The ^1H NMR spectrum (Appendix 3.3) of EC-3 in DMSO suggested the presence of one methylene signal at δ 2.50 and 2.75 (2H, *dd*), two methine protons at δ 4.02 (1H, *unre*) and 4.70 (1H, *unre*), two doublet aromatic protons at δ 5.75 (1H, *d*) and 5.95 (1H, *d*). The spectrum also showed one broad singlet signal aromatic protons at δ 6.40 (2H, *bs*).

The ^{13}C NMR spectrum (Appendix 3.4), analyzed with the aid of DEPT (Appendix 3.5), showed eight quaternary carbons, which were attributable to three oxygenated aromatic carbon at δ 156.2, 156.7 and 157.0, each of them are meta directed with respect to each other, and three oxygenated ortho-meta aromatic carbons at δ 132.6, 145.9 and 145.9, of which two are symmetric, two methine carbons at δ 65.5 and 78.6 attached to oxygen atom and one methylene carbon at δ 28.6.

Table 12. Proton Decoupled ^{13}C NMR and DEPT spectral data of Compound **EC-3**

Carbon No.	^{13}C NMR δ(in ppm)	DEPT δ(in ppm)	Remark
2	78.6	78.6	CH
3	65.5	65.5	CH
4	28.6	28.6	CH_2
4a	99.1	-	C (Quaternary carbon)
5	156.2	-	C (Quaternary carbon)
6	95.6	95.6	CH
7	157.0	-	C (Quaternary carbon)
8	94.6	94.6	CH
8a	156.7	-	C (Quaternary carbon)
1’	130.2	-	C (Quaternary carbon)
2’	106.5	106.5	CH
3’	145.9	-	C (Quaternary carbon)
4’	132.6	-	C (Quaternary carbon)
5’	145.9	-	C (Quaternary carbon)
6’	106.5	106.5	CH

Heteronuclar Single Quantum Correlation (HSQC) experiment (Appendix 3.6) also correlates the chemical shift of proton with directly bonded carbon atom. The HSQC NMR spectrum showed some selected correlations such that, the diastereotopic methylene protons at δ 2.50 and δ 2.75 correlate with that of carbon at δ 28.6. In the same manner, the HSQC spectrum also showed different kinds of correlations of the protons with that of carbons as shown below in **table12.**

Table 13. Heteronuclar Single Quantum Correlation (HSQC) spectra of EC-3

Carbon No.	Proton No	^{1}H NMR δ (in ppm)	Multiplicity
2	H-2	4.70	*unre*
3	H-3	4.02	*unre*
4	Ha-4 and Hb-4	2.50 and 2.75	*dd*
6	H-6	5.75	*d*
8	H-8	5.95	*d*
2’	H-2’	6.40	*bs*
6’	H-6’	6.40	*bs*

The ^{1}H-^{1}H correlation spectroscopy (COSY) experiment (Appendix 1.7) showed the correlations between H-2 and the two aromatic protons at δ 6.40 indicating distant coupling of protons. Similarly, there are also other strong couplings which were observed from COSY experiment as shown below in table 13.

Table 14. ^{1}H NMR and some selected ^{1}H-^{1}H COSY spectral data of compound **EC-3**

Carbon No.	^{1}H NMR δ (in ppm)	^{1}H-^{1}H COSY
2	4.70	H-2↔H-3,Ha-4,Hb-4,H-2',H-6'
3	4.02	H-3↔H-2, Ha-4,Hb-4
4	2.50 and 2.75	Ha-4↔H-2,H-3 and Hb-4↔ H-2,H-3
6	5.75	H-6↔H-8
8	5.95	H-8↔H-6
2’	6.40	H-2'↔H-2
6’	6.40	H-6'↔H-2

From ^{1}H-^{1}H cosy, ^{13}C NMR, DEPT, and ^{1}H NMR spectral data's, the partial structure suggested below.

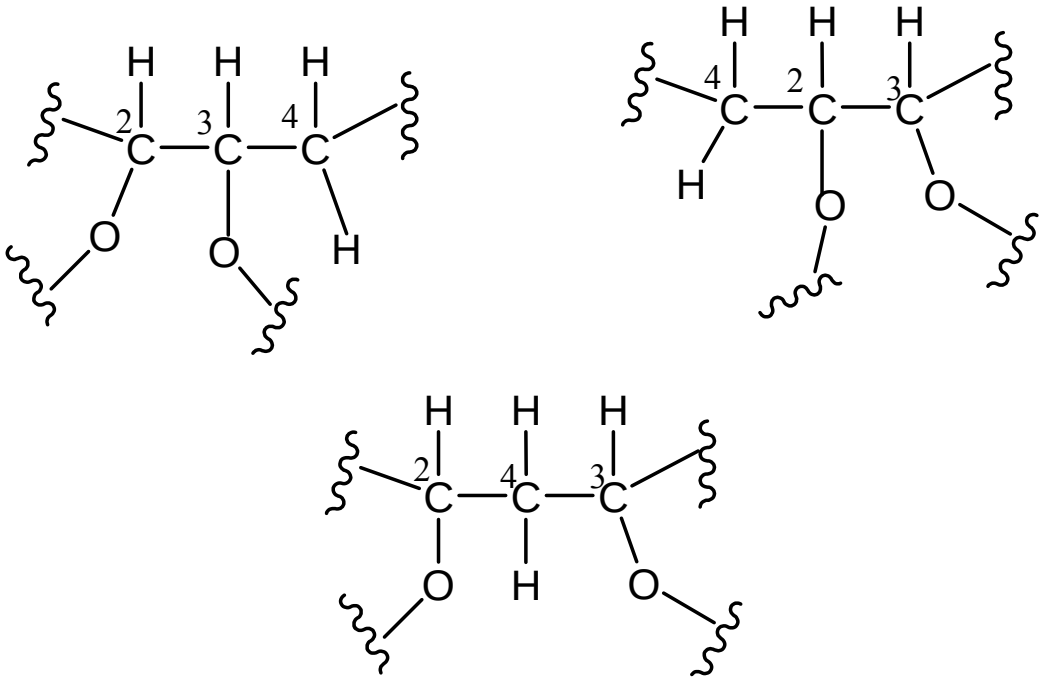


Figure 7. suggested partial structure based on ^{1}H-^{1}H cosy, ^{13}C NMR, DEPT, and ^{1}H NMR spectral data of EC-3

Differentiating the suggested partial structures based on proton multiplicity is difficult, since the protons are not well resolved. To identify the correct partial structures, finding the fourth carbon which is attached to any of the three carbons is necessary. From HMBC spectral data (Appendix 3.8) methine proton H-3 and methylene protons H-4 correlates with quaternary aromatic carbon C-4a at δ 99.1 indicating that C-4a is attached to either C-4 at δ 28.6 or C-3 at δ 65.5. In addition to this the HMBC spectrum also showed correlation between the methine proton H-2 at δ 4.70 with the aromatic quaternary carbon C-1' at δ 130.2 and two aromatic carbons C-2' and C-6' at δ 106.5.

Table 15. Observed correlations in HMBC spectral data of compound **EC-3**

Carbon No.	^{13}C NMR δ (ppm)	HMBC
2	78.6	H-2↔C-8a,C-4, C-3, C-1',C-2',C-6'
3	65.5	H-3↔C-4a,C-1'
4	28.6	Ha-4 and Hb-4↔C-2, C-3,C-4a, C-5,C-8a
4a	99.1	
5	156.2	
6	95.6	H-6↔C-4a,C-5,C-7,C-8
7	157.0	
8	94.6	
8a	156.7	
1’	130.2	
2’	106.5	H-2'↔C-2,C-1',C-3' C-4',C-6'
3’	145.9	
4’	132.6	
5’	145.9	
6’	106.5	H-6'↔C-2,C-1',C-2',C-4',C-5'

Based on this information, the suggested partial structures could be;

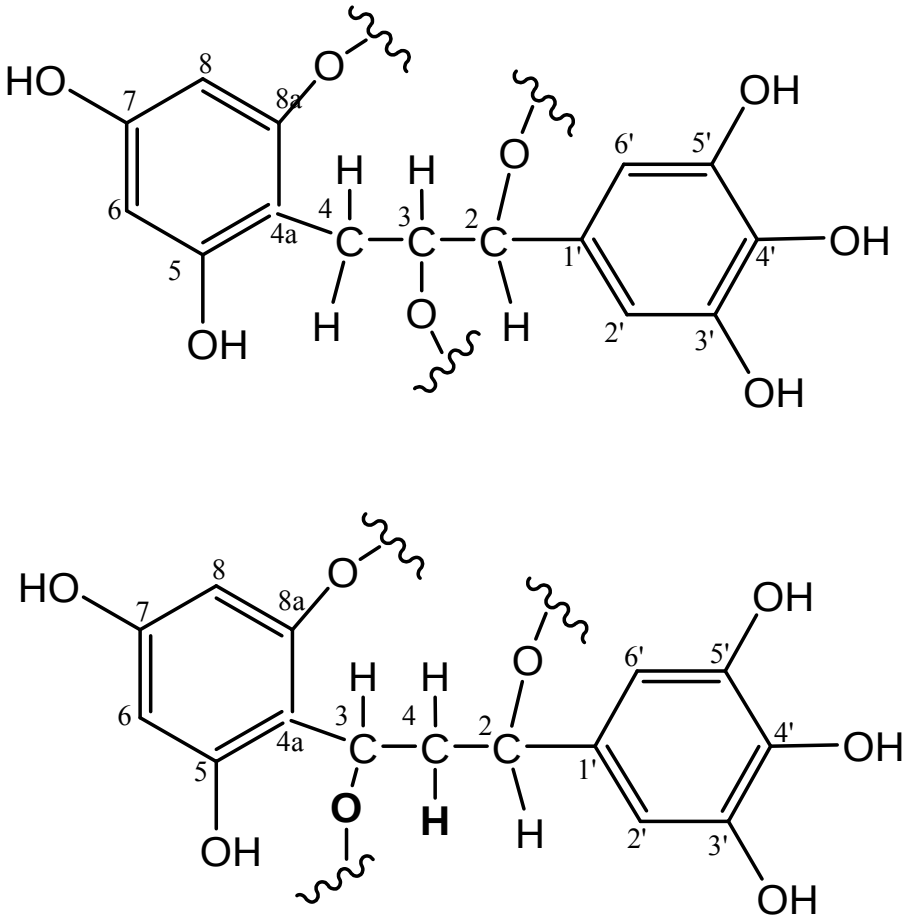


Figure 8. suggested partial structure based on ^{1}H-^{1}H cosy, ^{13}C NMR, DEPT, ^{1}H NMR and HMBC spectral data.

The critical thinking to suggest whether it is hetrocyclic ring or hydroxylated compound, most of the compound containing 15 carbon atoms and having yellow coloured solid compound are probably flavonoid compounds. In addition to this, the methine proton H-2 correlates with one of the three oxygenated aromatic carbon at δ 156.7. So the suggested partial structures could be the hetrocyclic ring as indicated in the **Fig. 9** below.

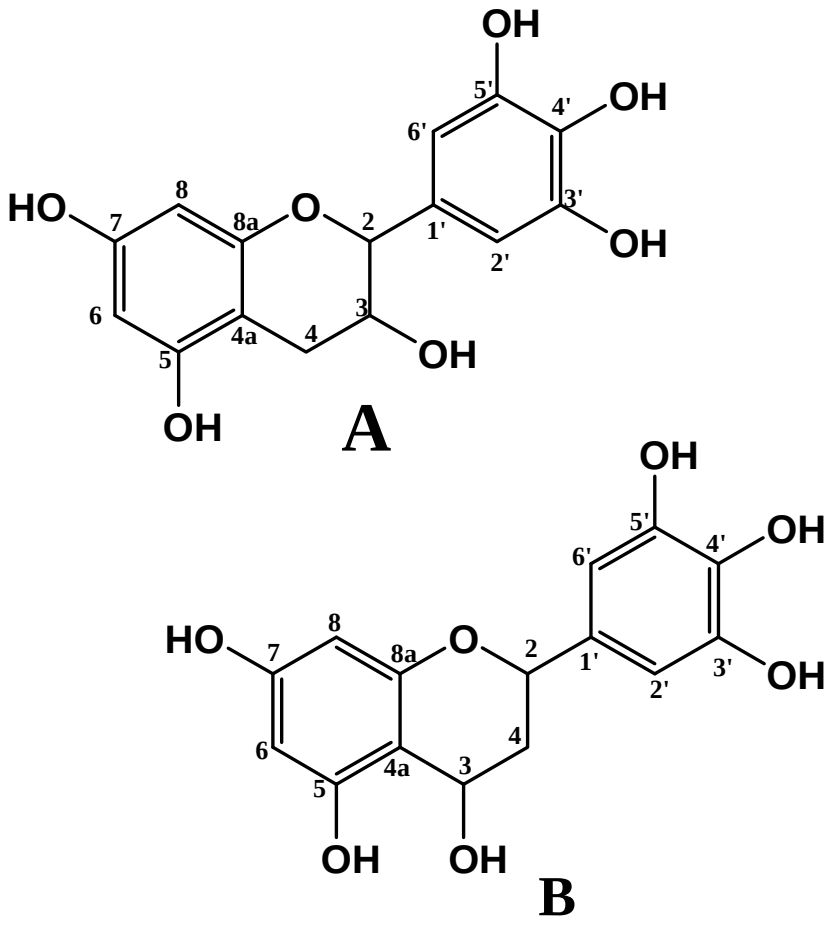


Figure 9. Two possible structures for EC-3

To differentiate between these two suggested structures, the HMBC spectra data showed weak correlations between methine proton H-3 with quaternary carbon C-1' and the methylene protons Ha-4 and Hb-4 with quaternary carbons C-5 and C-8a. Based on all of the spectral data and suggested information, the proposed structure of compound EC-3 was found to be structure **A**.

6. Experimental

6.1. General

^{1}H, ^{13}C, DEPT and 2D NMR spectras were recorded on a Bruker Advance 400MHz spectrometer in $CDCl_3$ and deuterated DMSO with TMS as internal solvent. The ultra-violet and visible spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer (200-800nm). Infrared spectra were obtained on Perkin-Elmer BX infrared spectrometer (400-4000cm^{-1}) using KBr. Melting points were recorded using Thomas Hoover capillary melting point apparatus. Analytical thin layer chromatograms were run on a ready made 0.2 mm thick layer of Merck silica gel 60 F_{254} coated on aluminum foil. Compounds on TLC were detected after spraying 1% vanillin sulphuric acid and after heating for a few minutes.

6.2. Plant Material

Ekebergia capensis (sombo) is evergreen tall tree occurring in east wollega. The stem bark has been used for treatment of lung TB. The stem bark of E. Capensis was collected from oromia regional state, East wollega administrative zone, kiremu Werda, Kiremu (01) particularly from Homi-Abaloya, which is 460 km away from Addis Ababa on April 2008. Voucher specimen is deposited in the National Herbarium, Department of Biology, Addis Ababa University (Voucher no. : MG 03/05).

6.3. Extraction and Isolation

The air dried and powdered plant material (650 g) was first soaked with petroleum ether for 36 hours and the extract was collected.

6.3.1. Dichloromethane Extract

The solvent free marc was then soaked with dichloromethane of for 24 hours and the extract was collected. This filtrate was evaporated under reduced pressure using the Rotavapor and afforded 24 g gum residue, which on TLC solvent system chloroform: methanol (9:1) showed two colored spots, having Rf value of 0.36 and 0.45. One gram of this crude extract was applied to silica gel (30 g) column chromatography, which was packed with chloroform (100%). The column was eluted with the following solvent system and 38 fractions each 25 ml were collected.

1. chloroform (100%) 1-5 fractions
2. chloroform: methanol (9.5:0.5) 6-10 fractions
3. chloroform: methanol (9:1) 11-16 fractions
4. chloroform: methanol (8.5:1.5) 17-21 fractions
5. chloroform: methanol (8:2) 22-28 fractions
6. chloroform: methanol (7:3) 29-33 fractions
7. chloroform: methanol (6:4) 34-38 fractions

Out of 38 fractions which were collected using the solvent system increased polarity, only these fractions from 13-18 showed two characteristic colored spots on TLC up on spraying 1% vanillin H_2SO_4 and after heating for a few minute. These fractions were mixed together and packed repeatedly for five times with chloroform: methanol (9:1) solvent systems and compound **EC-1** and **EC-2** were obtained.

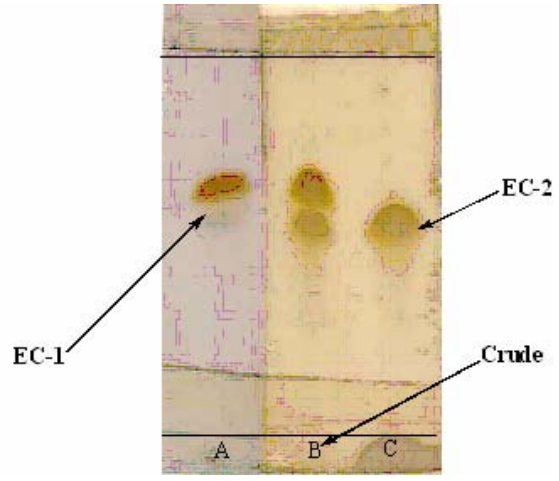


Figure 10. TLC of dichloromethane extract

6.3.2. Ethyl acetate Extract

After extracted with dichloromethane, the solvent free mark was soaked with methanol. The extract was collected and 118 g yellow solid residue was obtained upon drying it. The methanol extract (118 g) was suspended with distilled water and then extracted with ethyl acetate affording 14 g yellow solid residues which on TLC of solvent system chloroform: methanol (8:2) showed two colored spots having Rf value of 0.16 and 0.4. One gram of this extract was dissolved in ethyl acetate and applied to silica gel (30 g) column chromatography.

The column was eluted with the following solvent system and 44 fractions each 20 ml were collected.

1. chloroform (100%) 1-7 fractions
2. chloroform: methanol (9.5:0.5) 8-15 fractions
3. chloroform: methanol (9:1) 16-20 fractions
4. chloroform: methanol (8.5:1.5) 21-28 fractions
5. chloroform: methanol (8:2) 29-37 fractions
6. chloroform: methanol (7:3) 38-44 fractions

Out of 44 fraction, only fractions from 30-43 showed one spot having yellow color. These fractions were pure and after the removing solvent using Rota vapor affording 120 mg of compound **EC-3**.

Figure 11. TLC of compound EC-3

6.4. Spectral Data

Compound EC-1: colorless oil, IRν_{max} (KBr) cm-^{1}: 3572, 3464 (OH), 1670 (C=C), 1452, 1388, 1162, 1078; ^{1}H NMR (400MHz,$CDCl_3$); δ 1.15, 1.20 (each 6H, *s*, 1,24, 25, 30- Me), 1.42 (2H, *m*, 4, 21), 1.58 (2H, *m*, Hb- 4, 21), 1.60 (6H, *bs*, 27, 28- me), 1.62 (6H, *bs*, 26, 29- Me), 2.02 (8H, *m*, H- 2, 9, 12.13, 16), 2.09 (6H, *m*, Ha- 5, 20 and H_2- 8, 17), 2.23 (2H, *m*, Hb- 5, 20), 3.35 (2H, *dd*, H-3,22), 5.14 (2H, *m*, H- 11, 14), 5.19 (2H, *m*, H-7, 18); ^{13}C NMR (125MHz,$CDCl_3$) δ 15.9 (C- 27, C-28), 16.0 (C-26, C-29), 23.3 (C-1,C- 24), 26.40 (C-25, C-30), 26.50 (C-8, C-17), 28.22 (C-12, C-13), 29.70 (C-4, C-21), 36.82 (C-5, C-20), 39.65 (C-9,C-16), 73.05 (C 2, C-23), 78.28 (C-3, C-22), 124.45 (C-11, C-14), 125.08 (C-7, C-18), 134.81 (C-6, C-10), 134.96 (C-15, C-19).

Compound EC-2 colorless oil, IRν_{max} (KBr) cm-^{1}; 3464 (OH), 675 C=C), 1454, 388, 1160, 1076; ^{1}H NMR (400 MHz, $CDCl_3$); δ 1.07 (3H, s, 1-Me), 1.15 (3H, *s*, 24-Me), 1.20 (3H, *s*, 30-Me), 1.41 (1H, *m*, Ha-21), 1.53 (3H, *m*, H_2-4 and Hb-21), 1.60 (6H, *bs*, 27, 28-Me), 1.62 (6H, *bs*, 26, 29-Me), 2.02 (8H, *m*, H_2-9, 12, 13, 16), 2.09 (6H, *m*, Ha-5, 20 and H_2-8, 17), 2.23 (2H, *m*, Hb-5, 20), 3.35 (1H, *dd*, H-22), 3.50 (1H, *d*, Ha-25), 3.61 (2H, *bd*, Hb-25 and H-3), 5.14 (2H, *m*, H-11, 14), 5.19 (2H, *m*, H-7, 18); ^{13}CNMR (125MHz, $CDCl_3$) δ 15.96 (C-26, 27), 16.0 (C-28, 29), 19.48 (C-1), 23.11 (C-24), 26.42 (C-30), 26.46 (C-8, 17), 28.18 (C-12, 13), 29.32 (C-4), 29.79 (C-21), 36.33 (C-5), 36.80 (C-20), 39.63 (C-9, 16), 69.06 (C-25), 73.23 (C-23), 74.44 (C-2), 75.62 (C-3), 78.24 (C-22), 124.42 (C-11), 124.44 (C-14), 124.90 (C-7), 124.96 (C-18), 134.76 (C-6), 134.93 (C-10, 15, 19).

Compound EC-3 solid m. p 257-260; IRν_{max} (KBr) cm^{-1}; 3464 (OH), 1675 (C=C), 1484, 1388, 1160; ^{1}H NMR (400 MHz, DMSO), δ 2.50 (1H, *dd*, Ha-4), 2.75 (1H, *dd*, Hb-4), 4.02 (1H, *unre*, H-3), 4.70 (1H, *unre*, H- 2), 5.75 (1H, *d*, H-6), 5.95 (1H, *d*, H- 8), 6.40 (2H, *bs*, H-2' and H- 6'); ^{13}CNMR (125MHz, DMSO). δ 28.6 (C-4), 65.5 (C-3), 78.6 (C-2), 94.6 (C-8), 95.6 (C-6), 99.1 (C-4a), 106.5 (C-2' and C-6'), 130.2 (C-1'), 132.6 (C-4'), 145.9 (C-3' and C-5'), 156.2 (C-5), 156.7 (C-8a), 157.0 (C-7).

7. Conclusions

The stem bark of *Ekeberigia capensis* is used for treatment of chronic cough or lung TB, and the smoke of its stem bark has good odour and used to attract bees if bee hives are activated with it traditionally in east wollega. In this project, the dichloromethane extract of stem bark of *E. capensis* gives two known triterpenoids, namely 2, 3, 22, 23- tetrahydroxy- 2, 6, 10, 15, 19, 23- hexamethyl- 6, 10, 14, 18- tetracosatetraene (**EC-1**) and 2- hydroxymethyl 2, 3, 22, 23- tetrahydroxy-6, 10, 15, 19, 25- pentamethyl- 6, 10, 14, 18- tetracosatetraene(**EC-2**), and the ethyl acetate extract results one flavonoid, **EC-3**.

8. References

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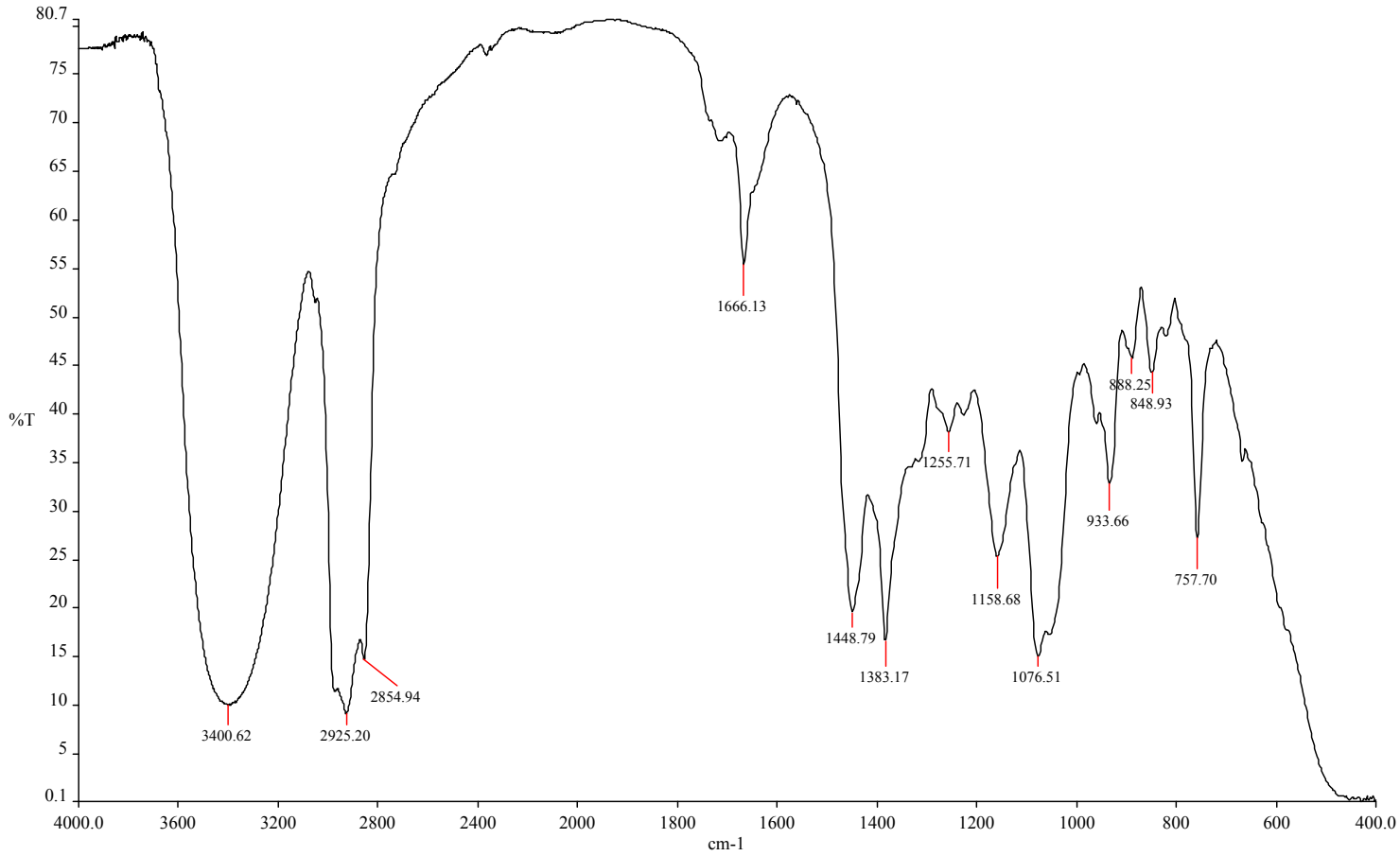
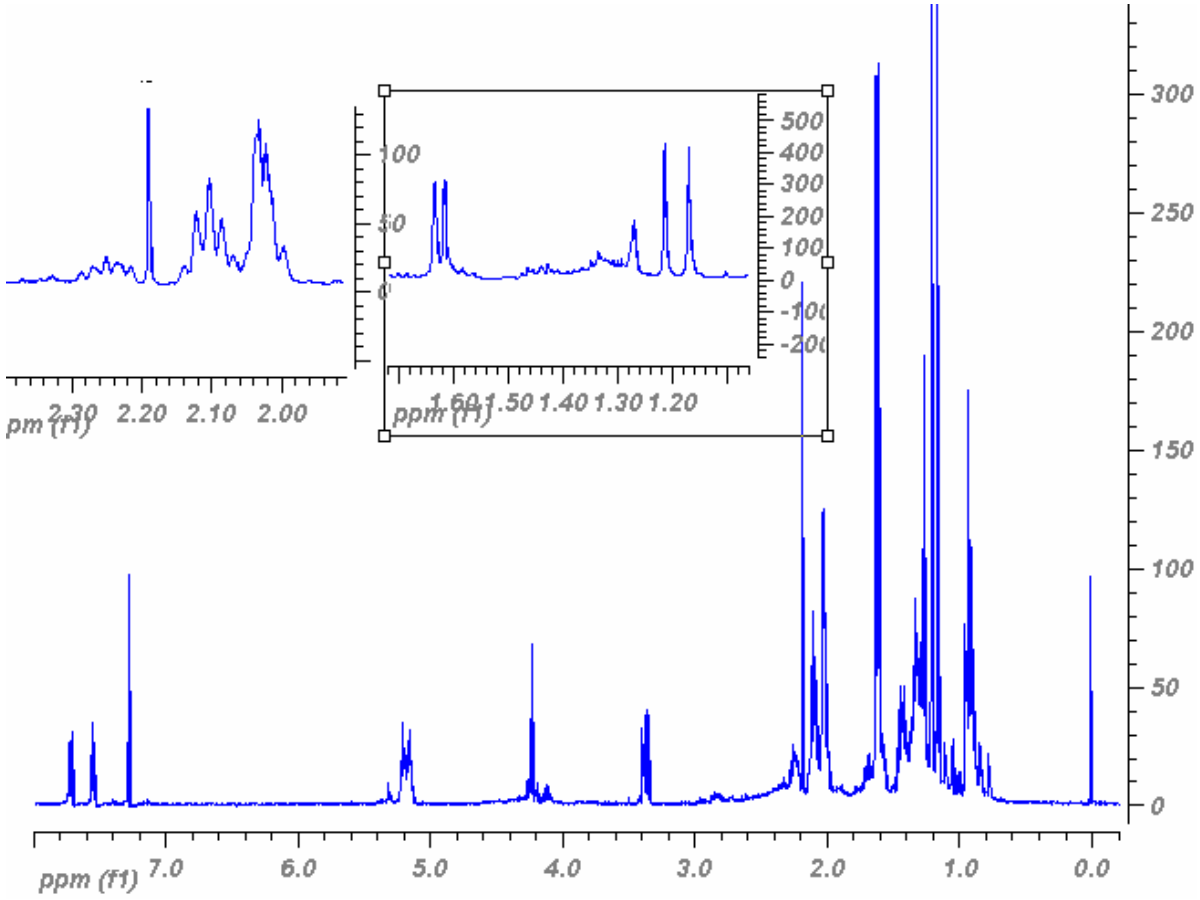
APPENDICES

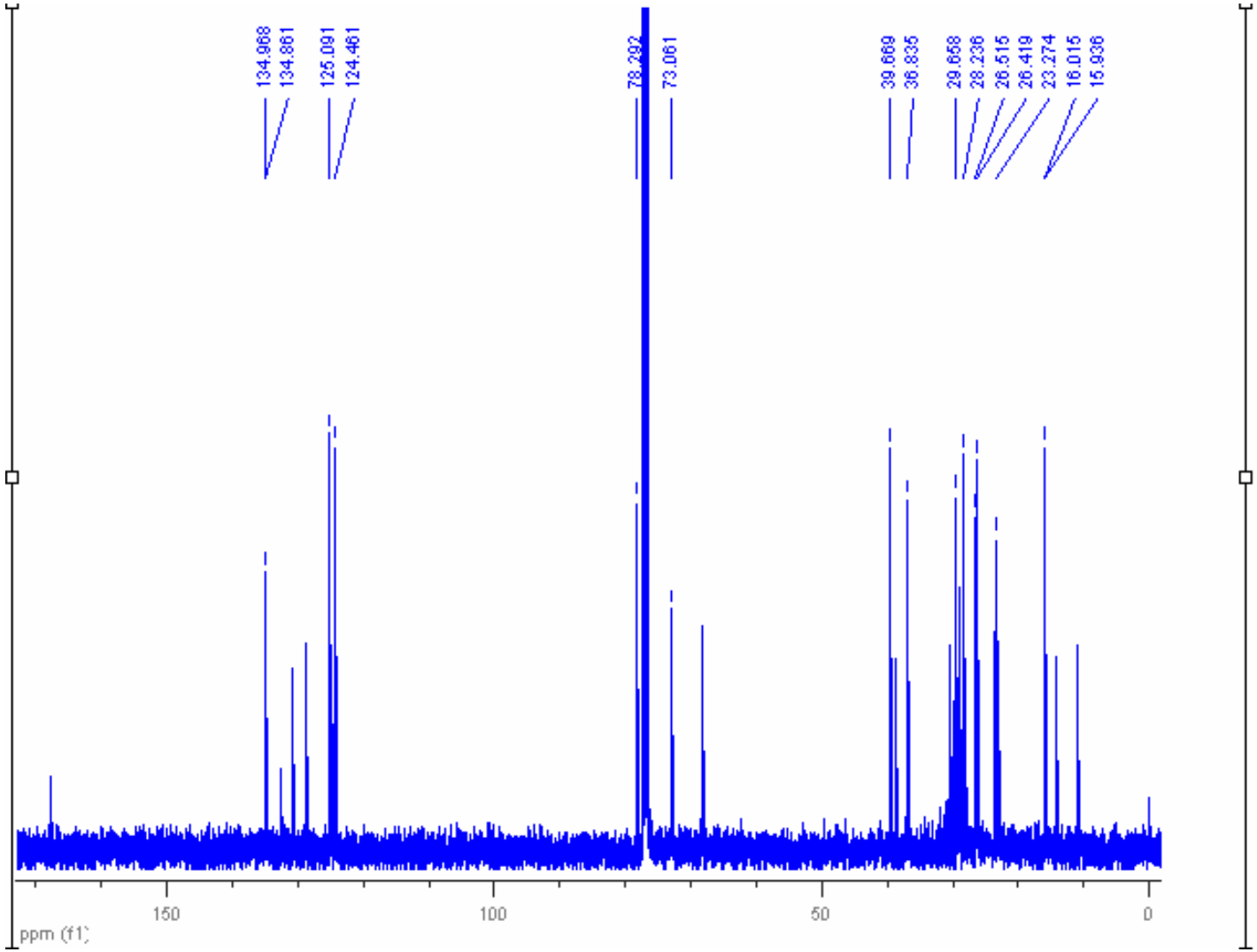
Appendix 1.1. IR spectra of EC-1



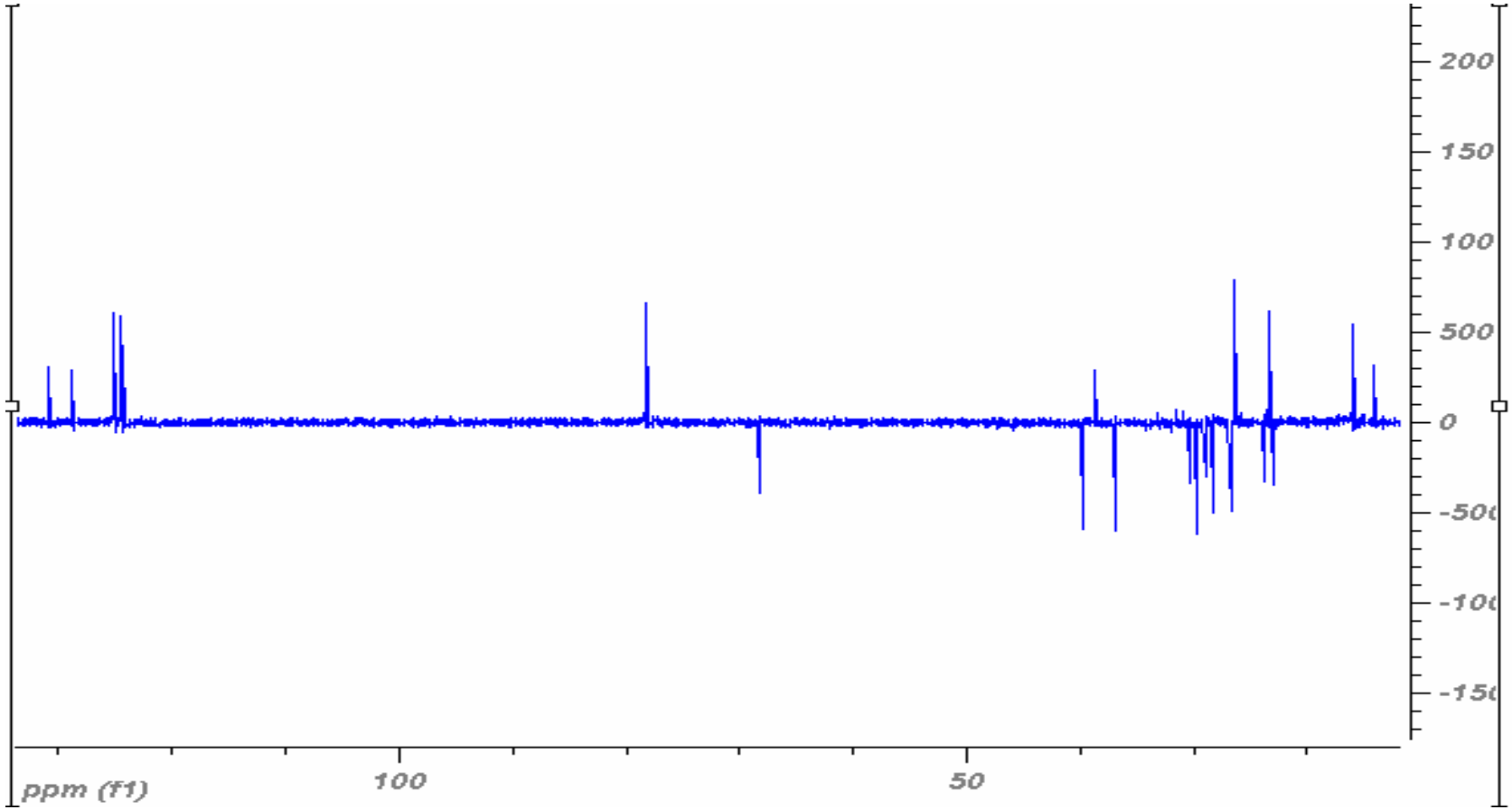
Appendix 1.2. ^{1}H NMR of EC-1



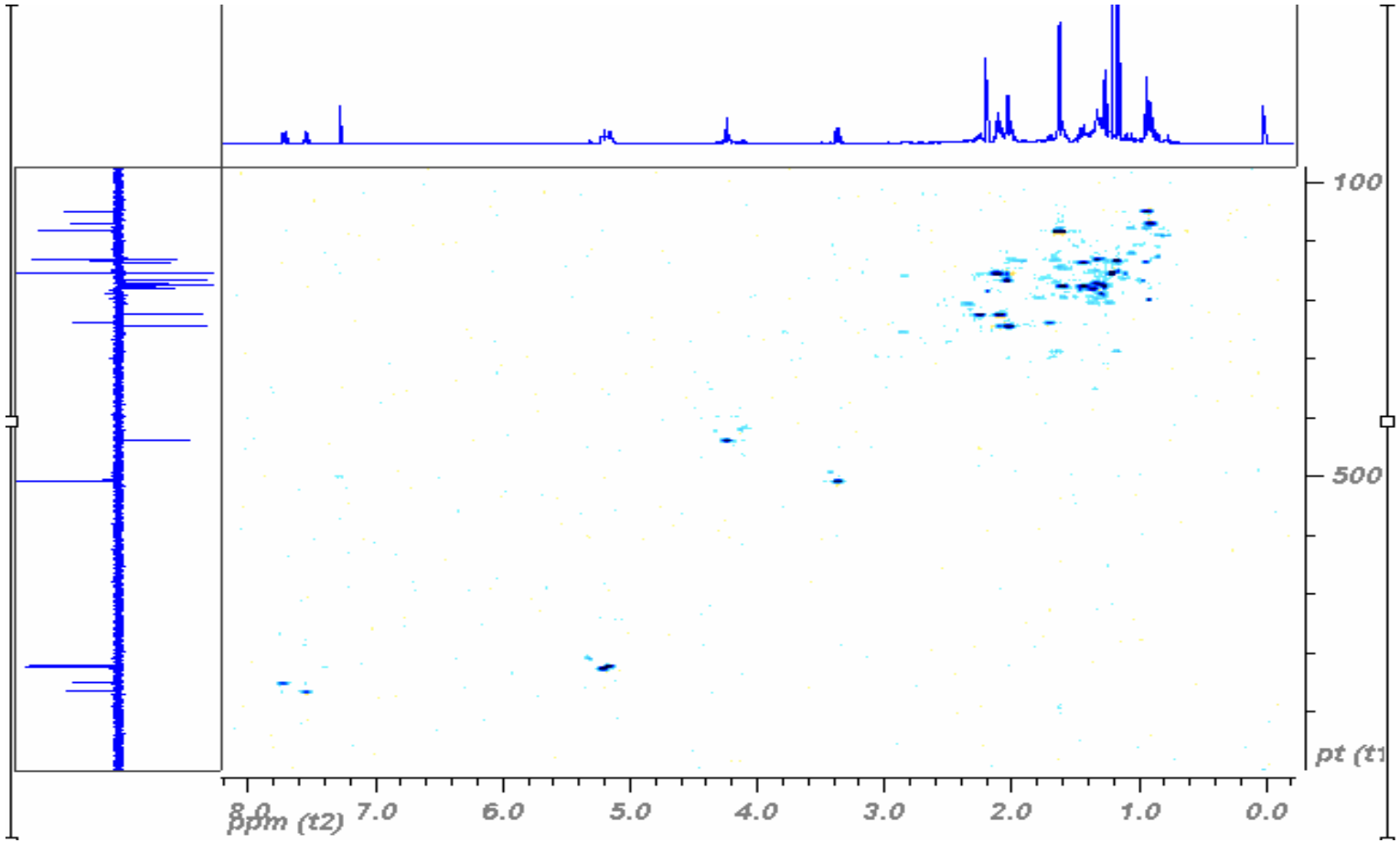
Appendix 1.3. ^{13}C NMR spectra of EC-1



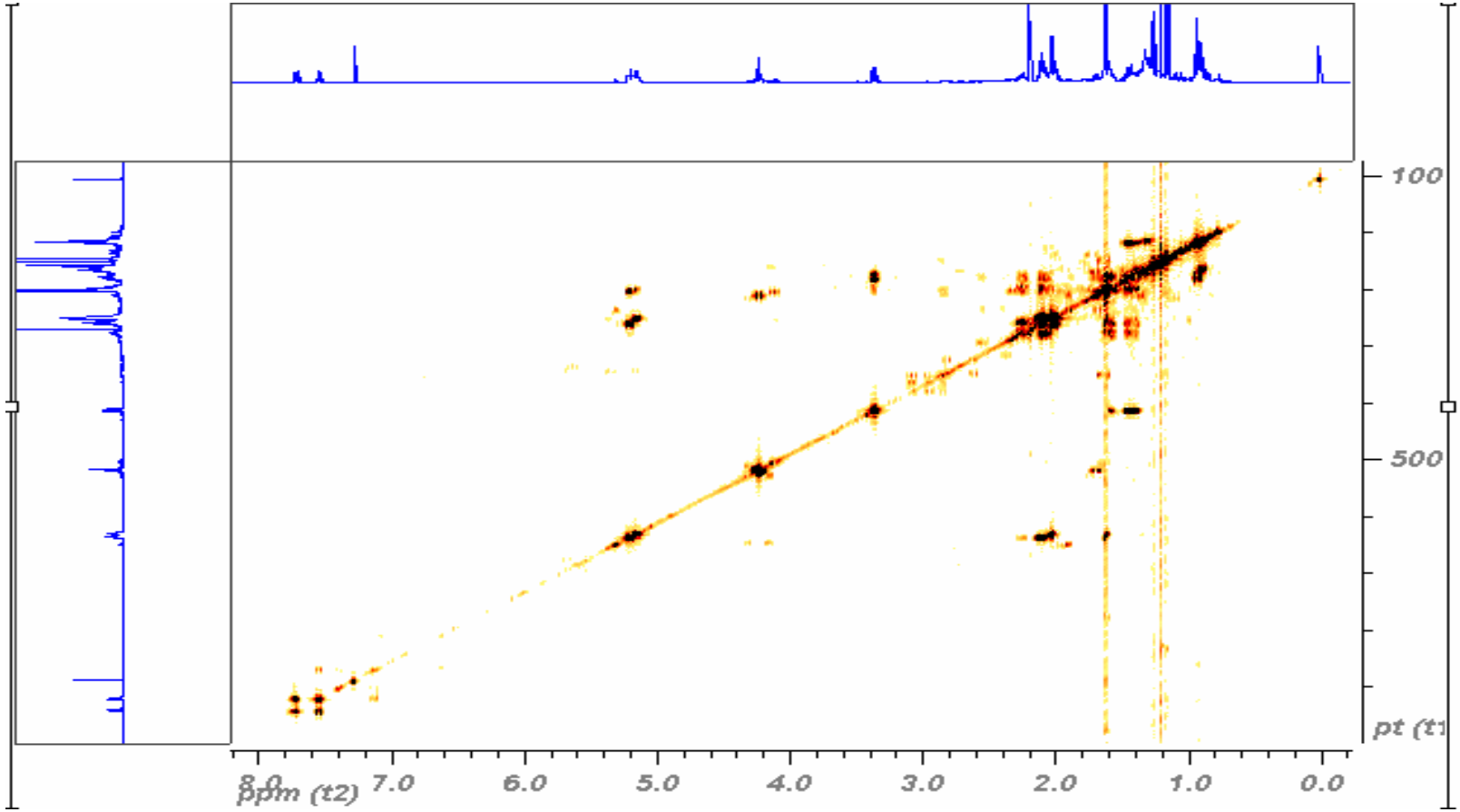
Appendix 1.4. DEPT NMR of EC-1



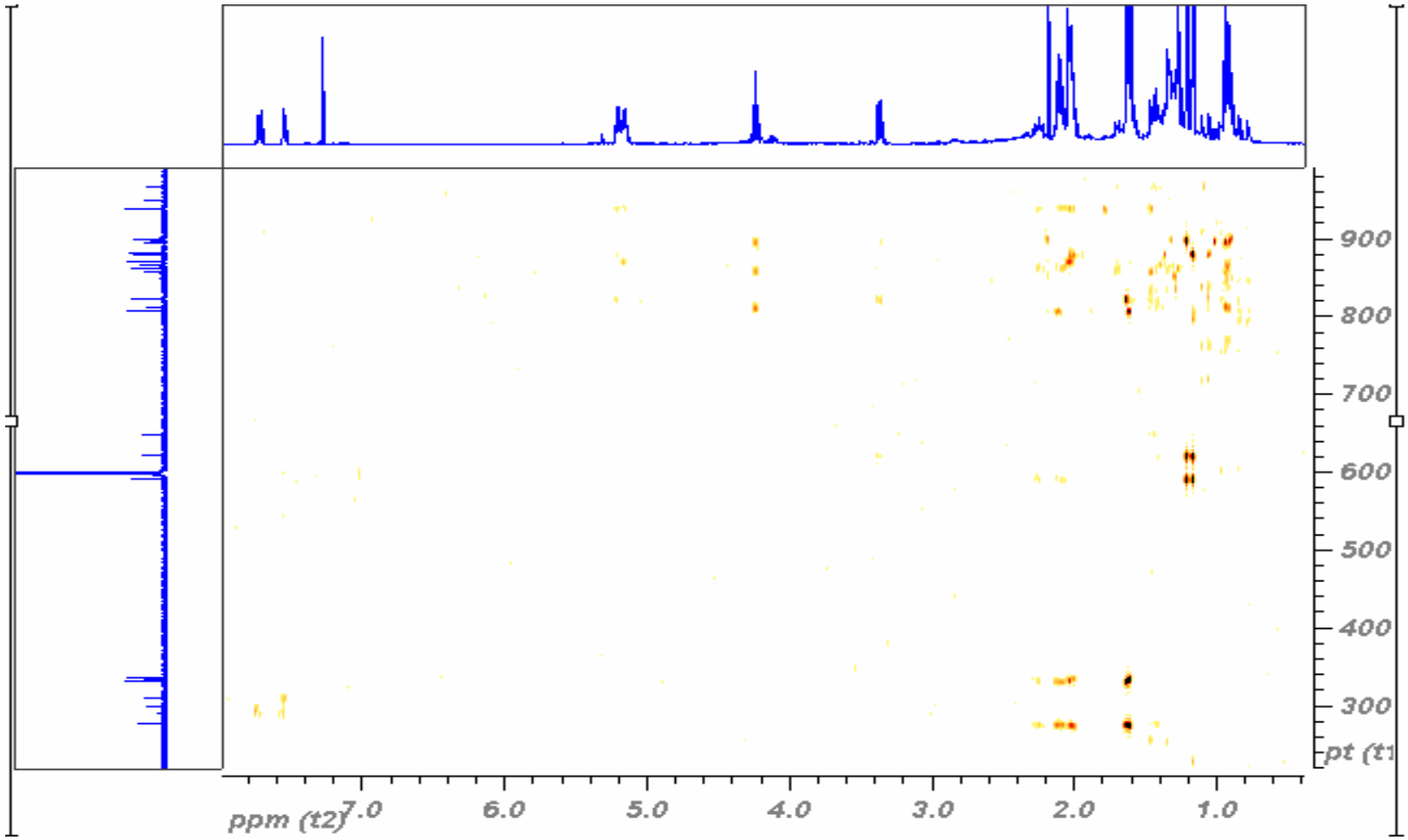
Appendix 1.5. HSQC of EC-1



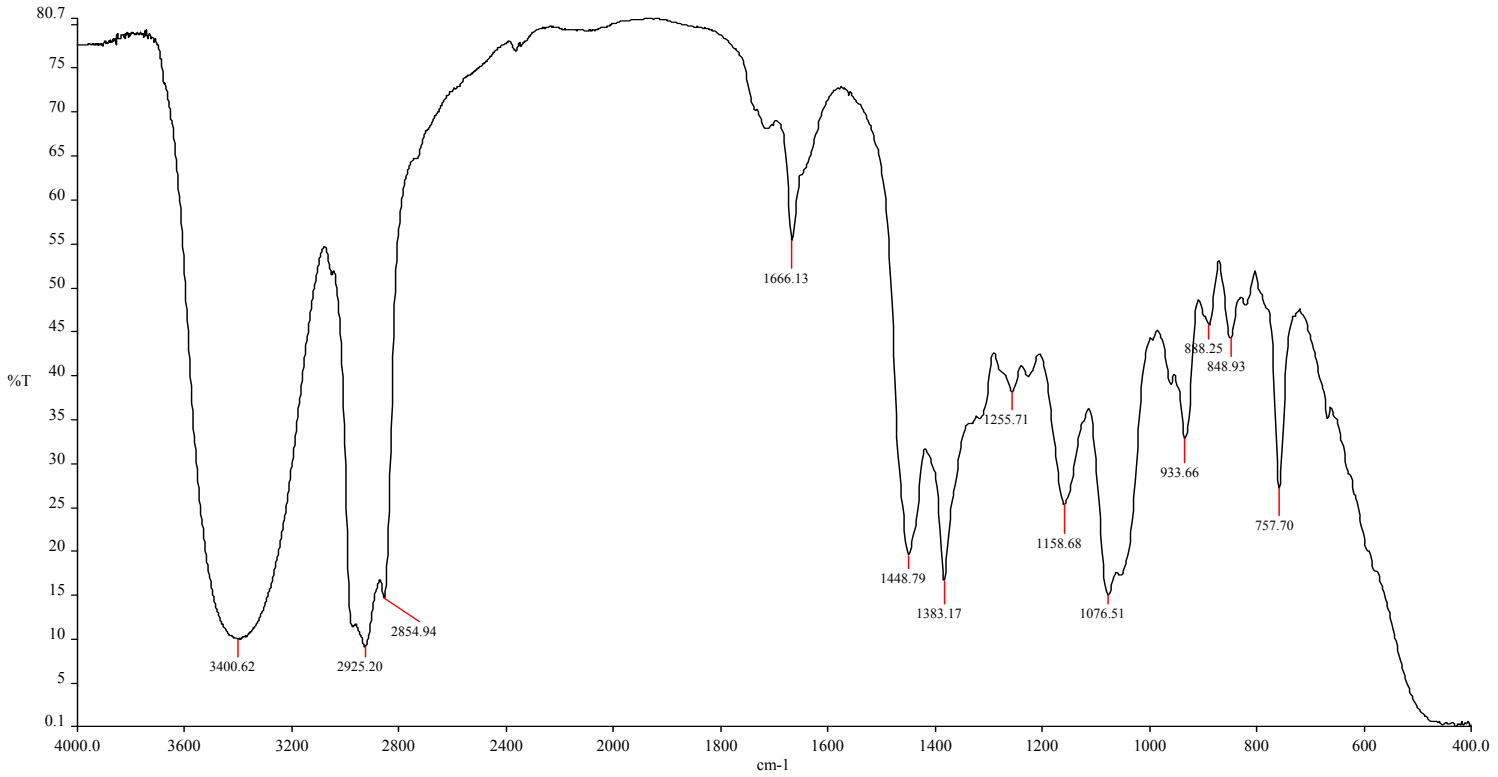
Appendix 1.6. H-H spectra of cosy of EC-1



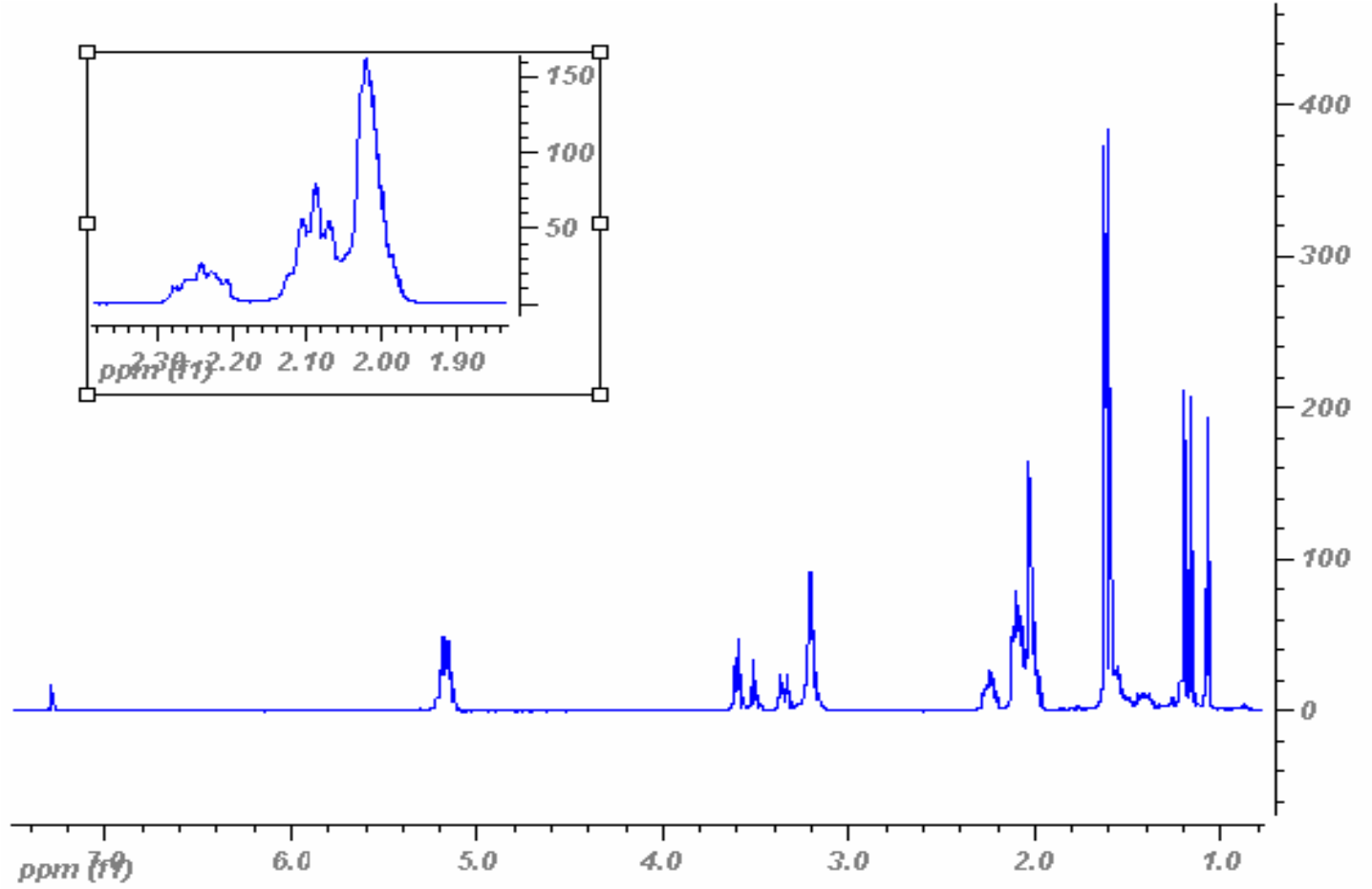
Appendix 1.7. HMBC of EC-1



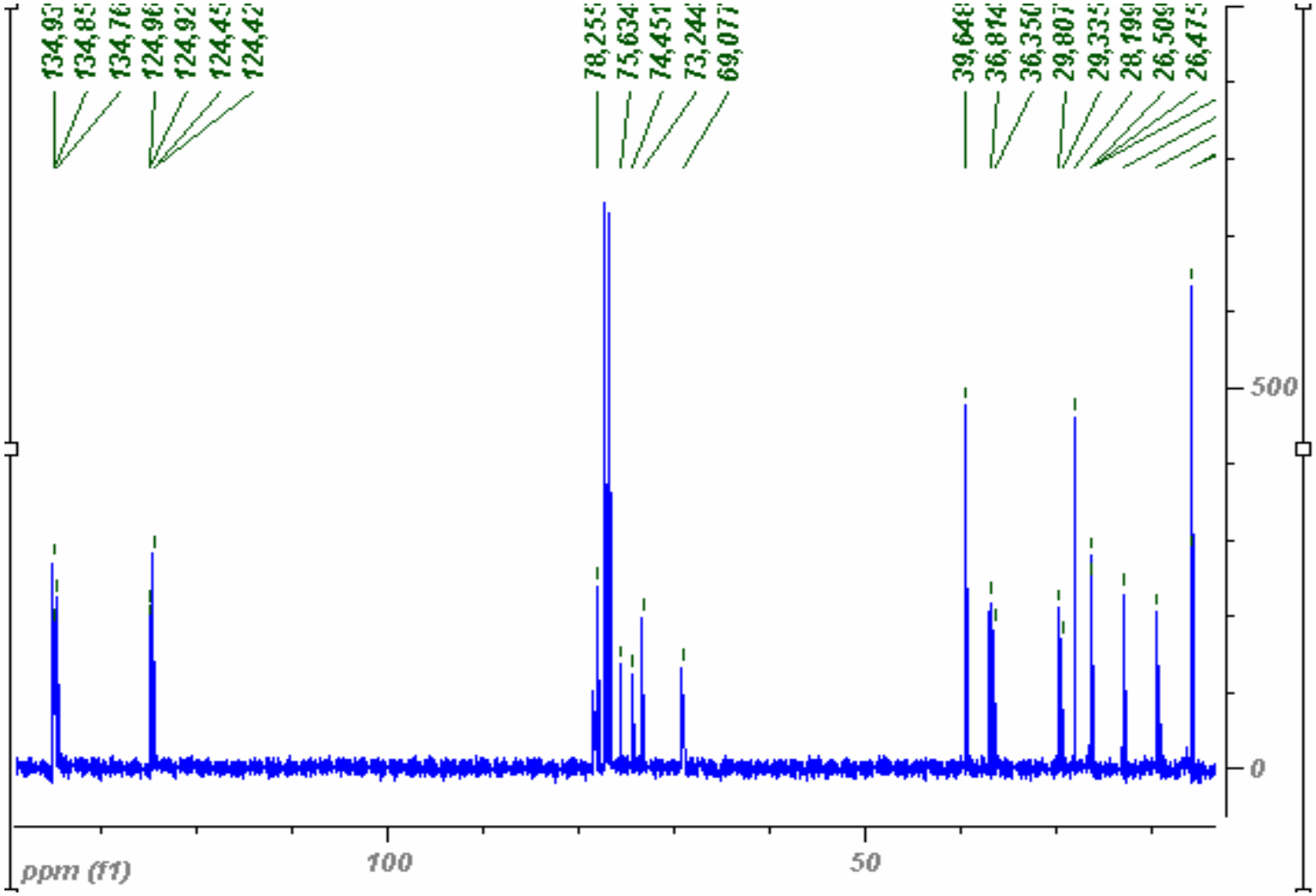
Appendix 2.1. IR spectra of EC-2



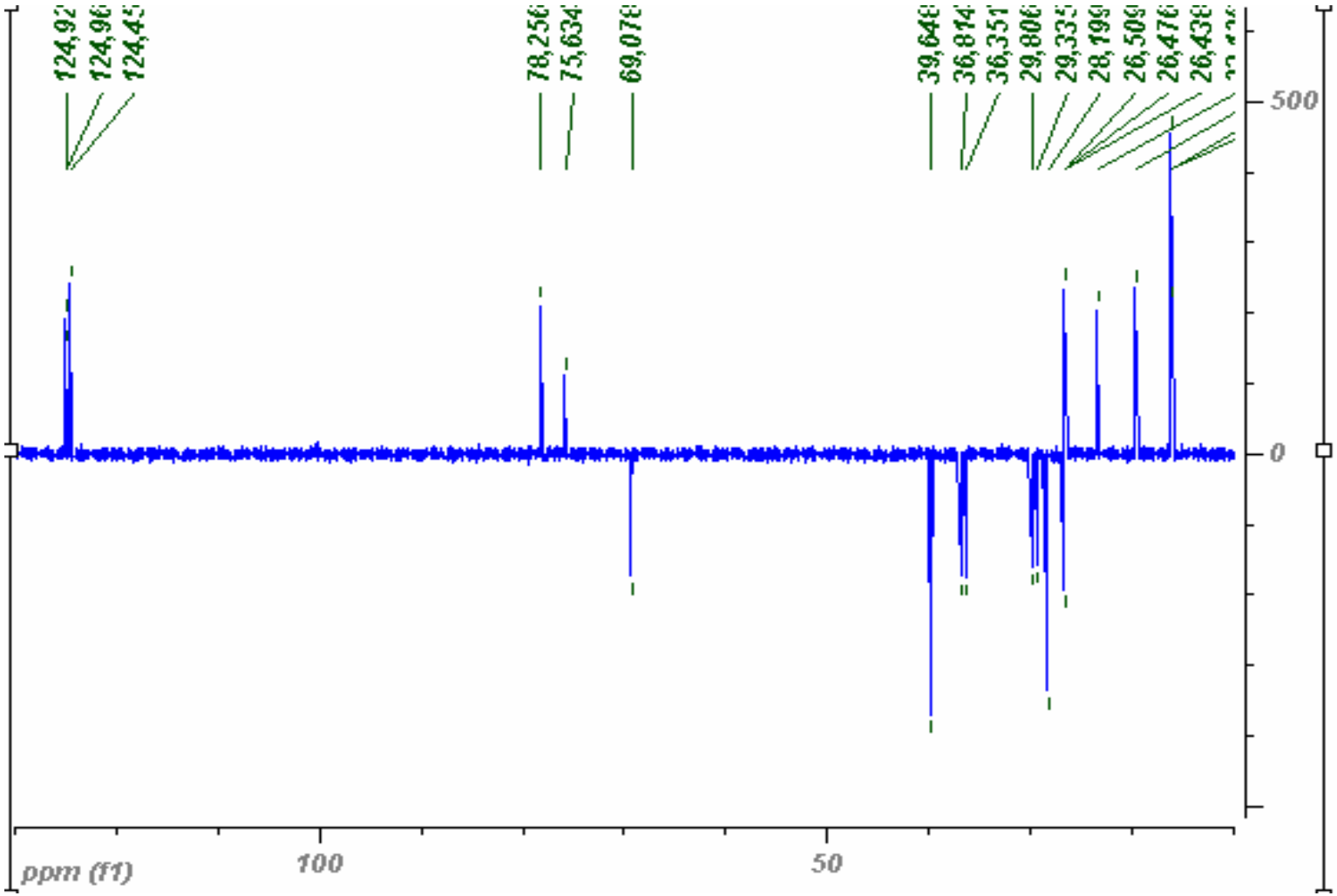
Appendix 2.2. ^{1}H NMR spectra of EC-2



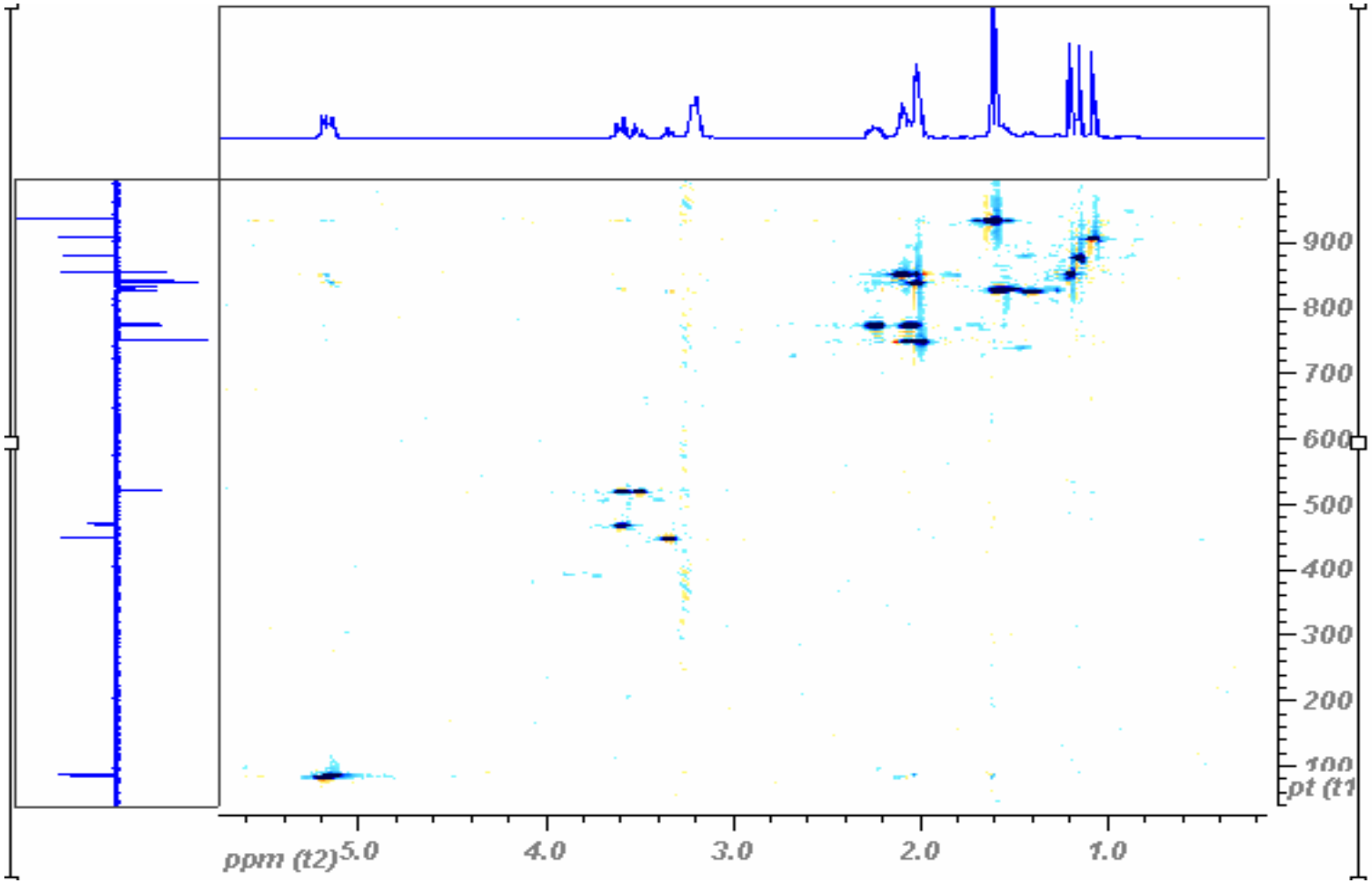
Appendix 2.3. ^{13}C NMR spectra of EC-2



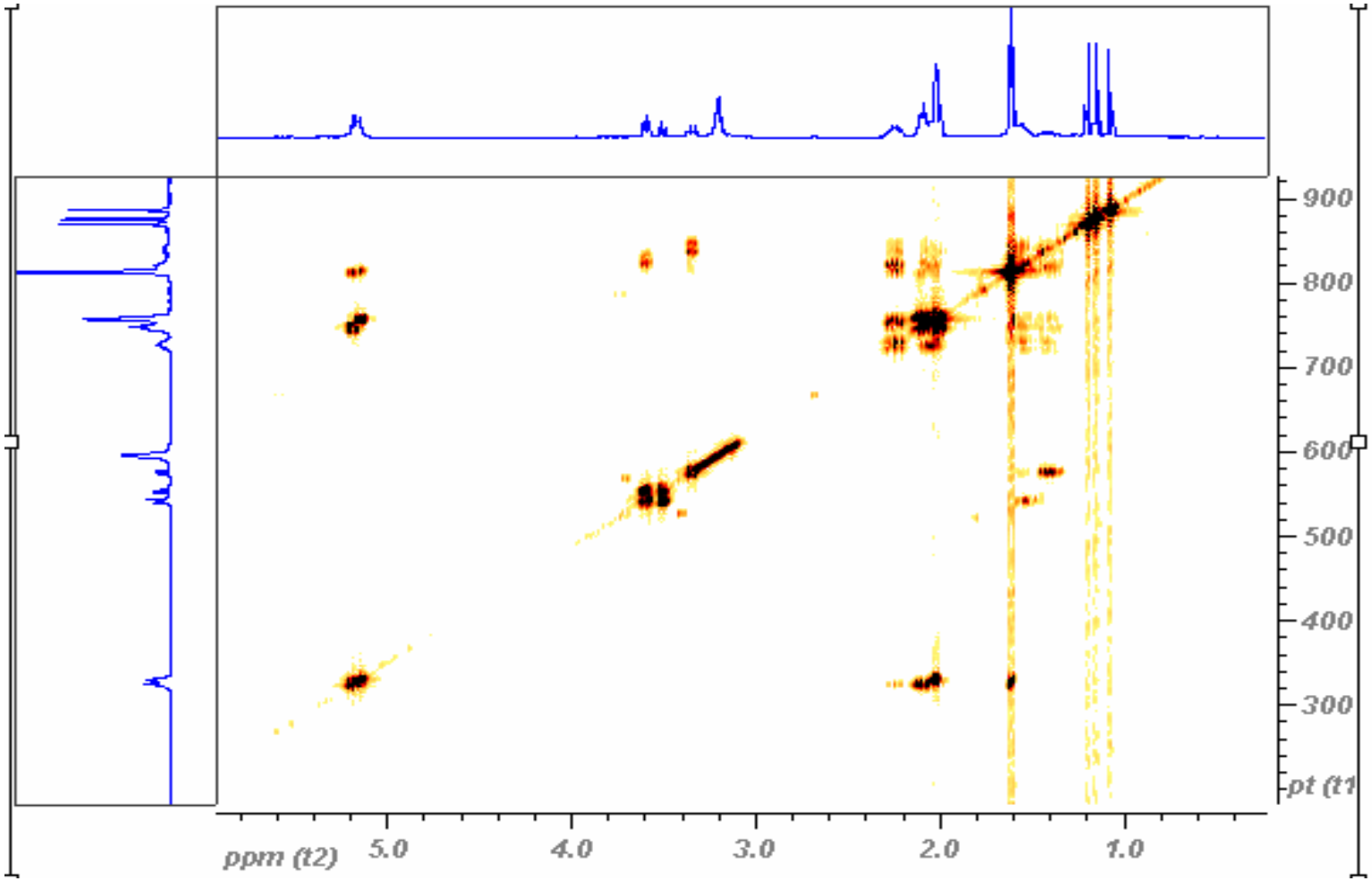
Appendix 2.4. ^{13}C DEPT NMR spectra of EC-2



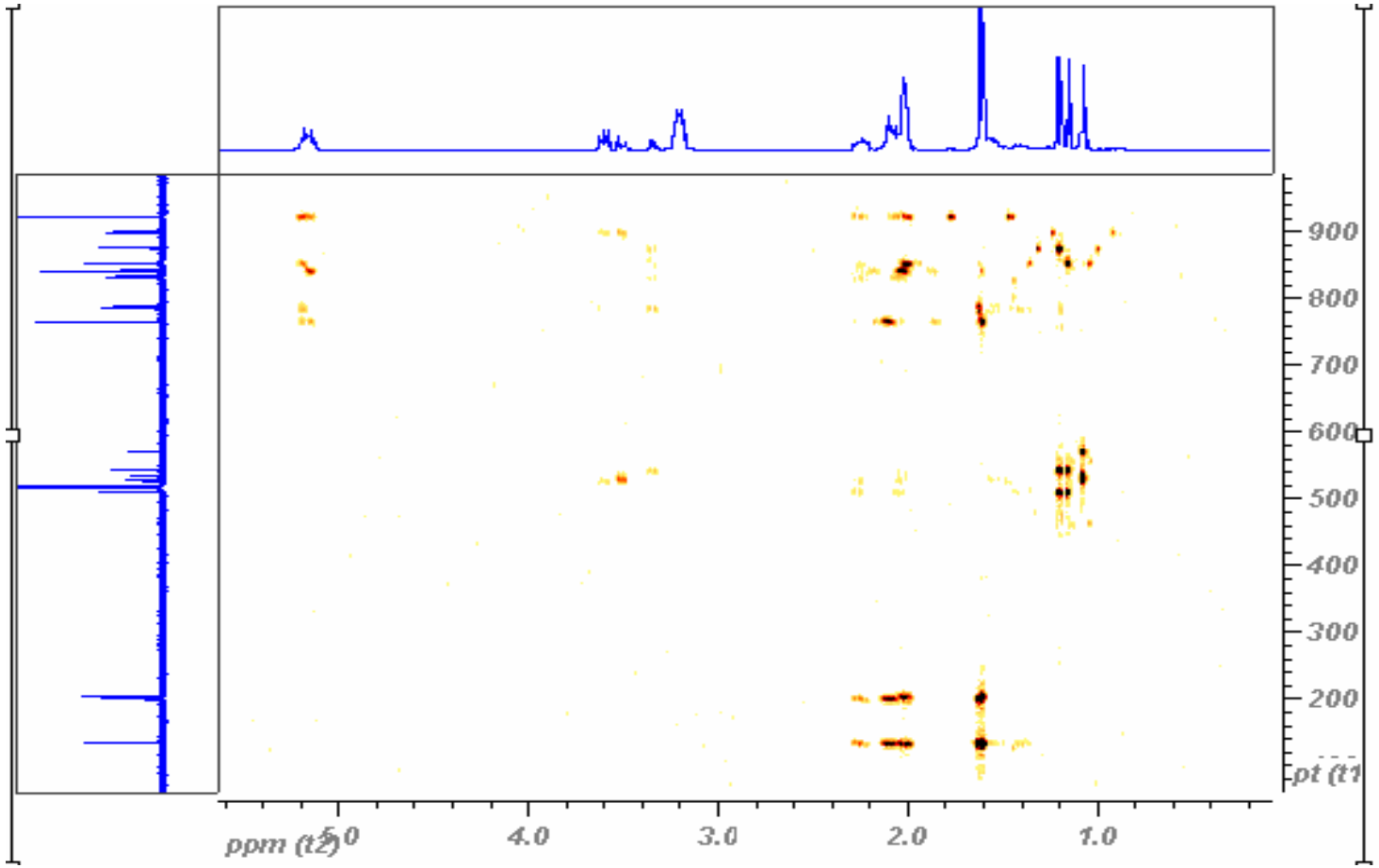
Appendix 2.5. HSQC spectra of EC-2



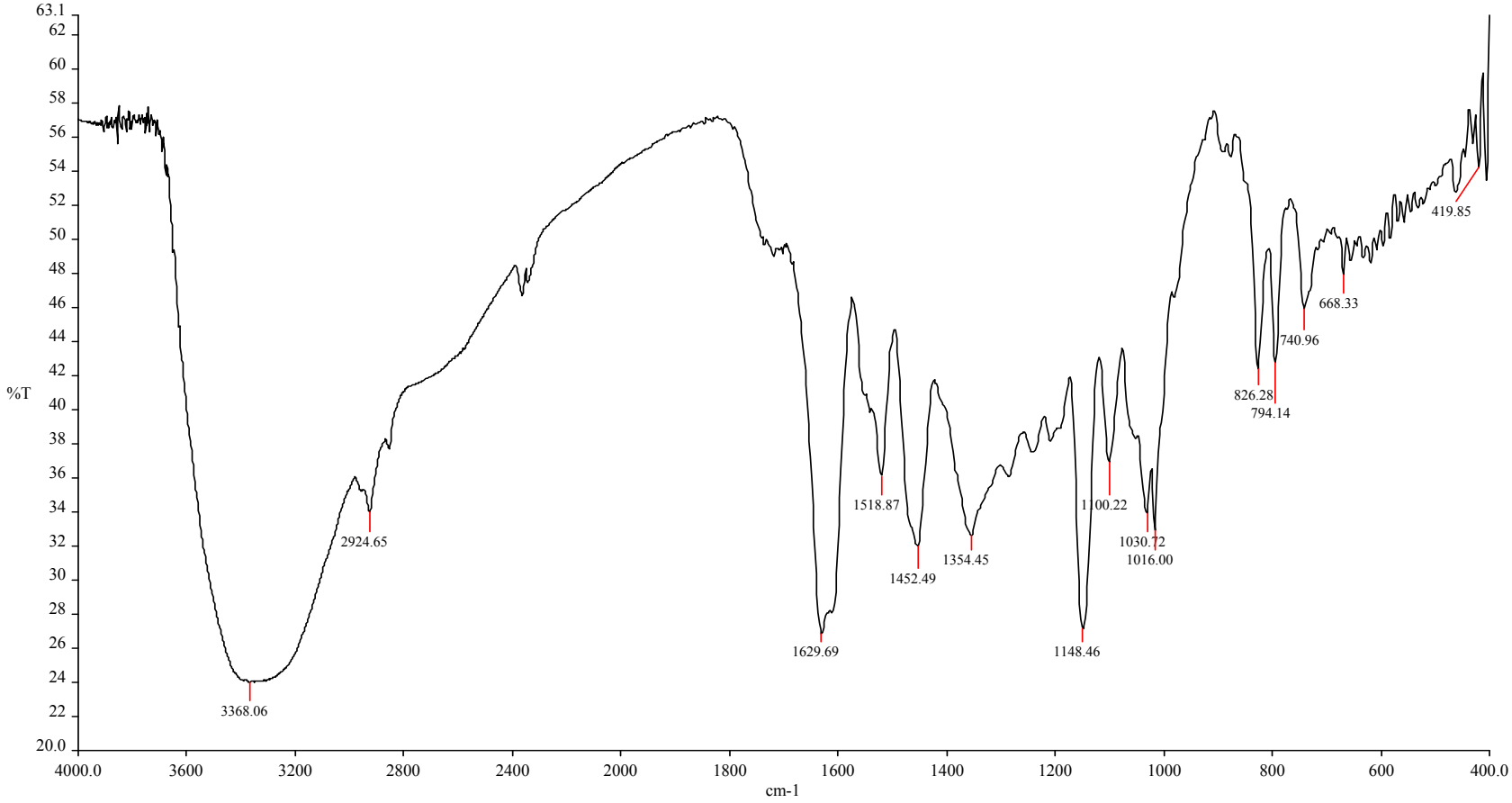
Appendix 2.6. H-H Cosy spectra of EC-2



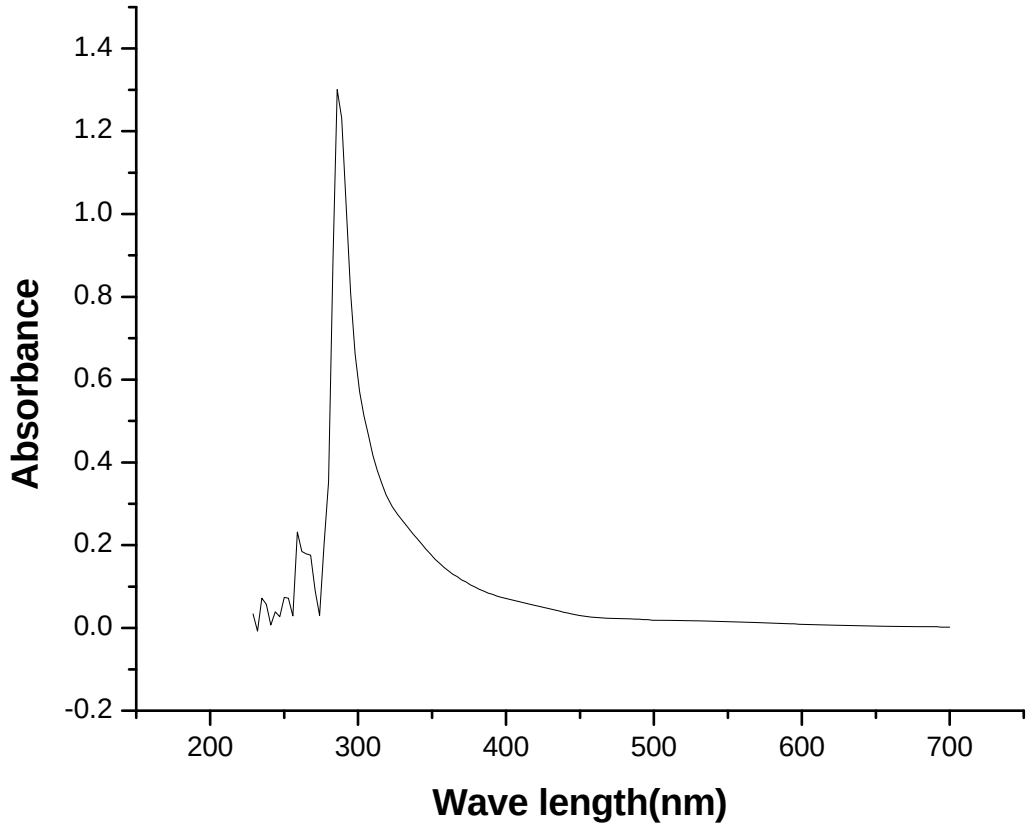
Appendix 2.7. HMBC spectra of EC-2



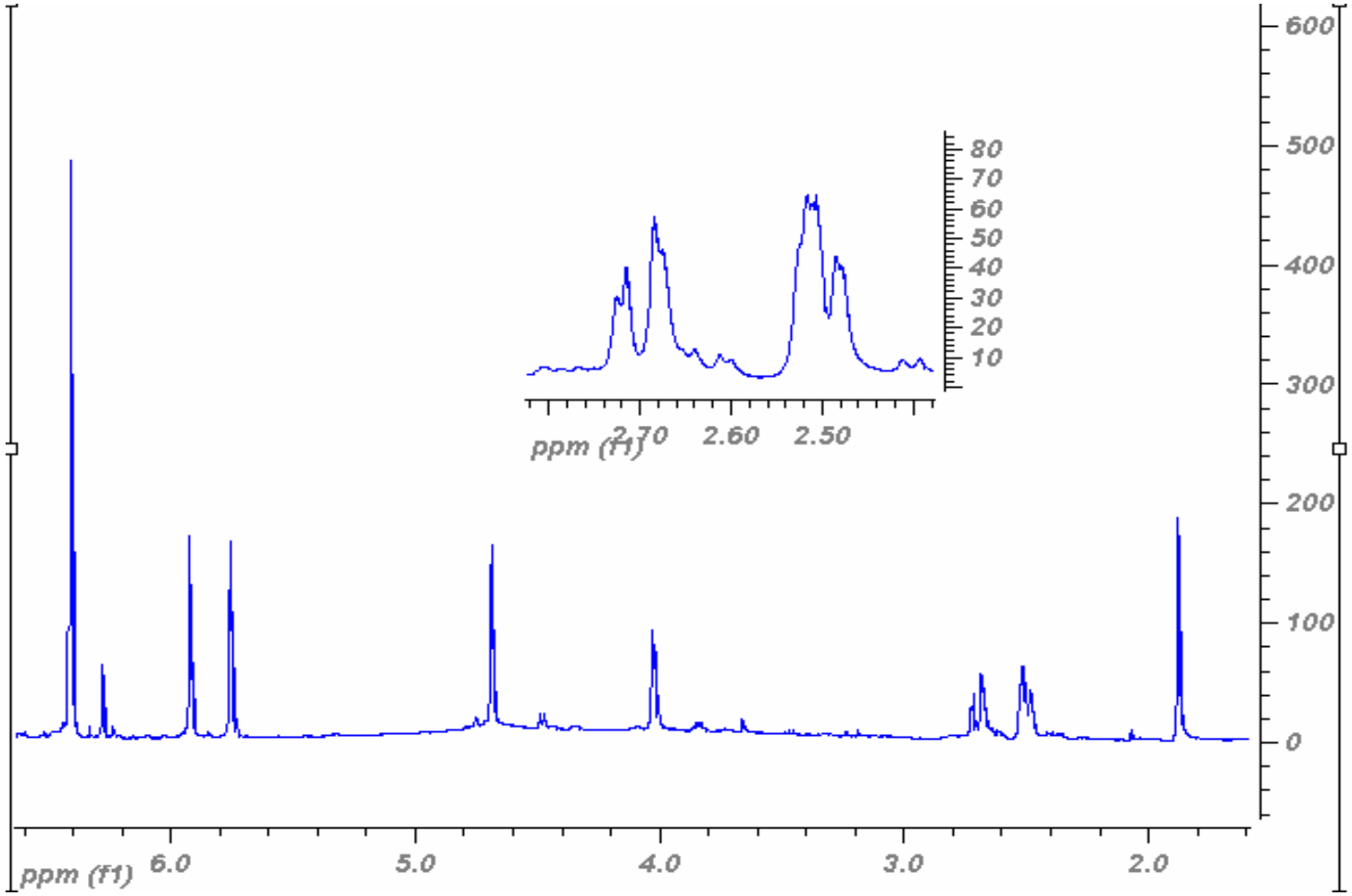
Appendix 3. 1. IR spectra of EC-3



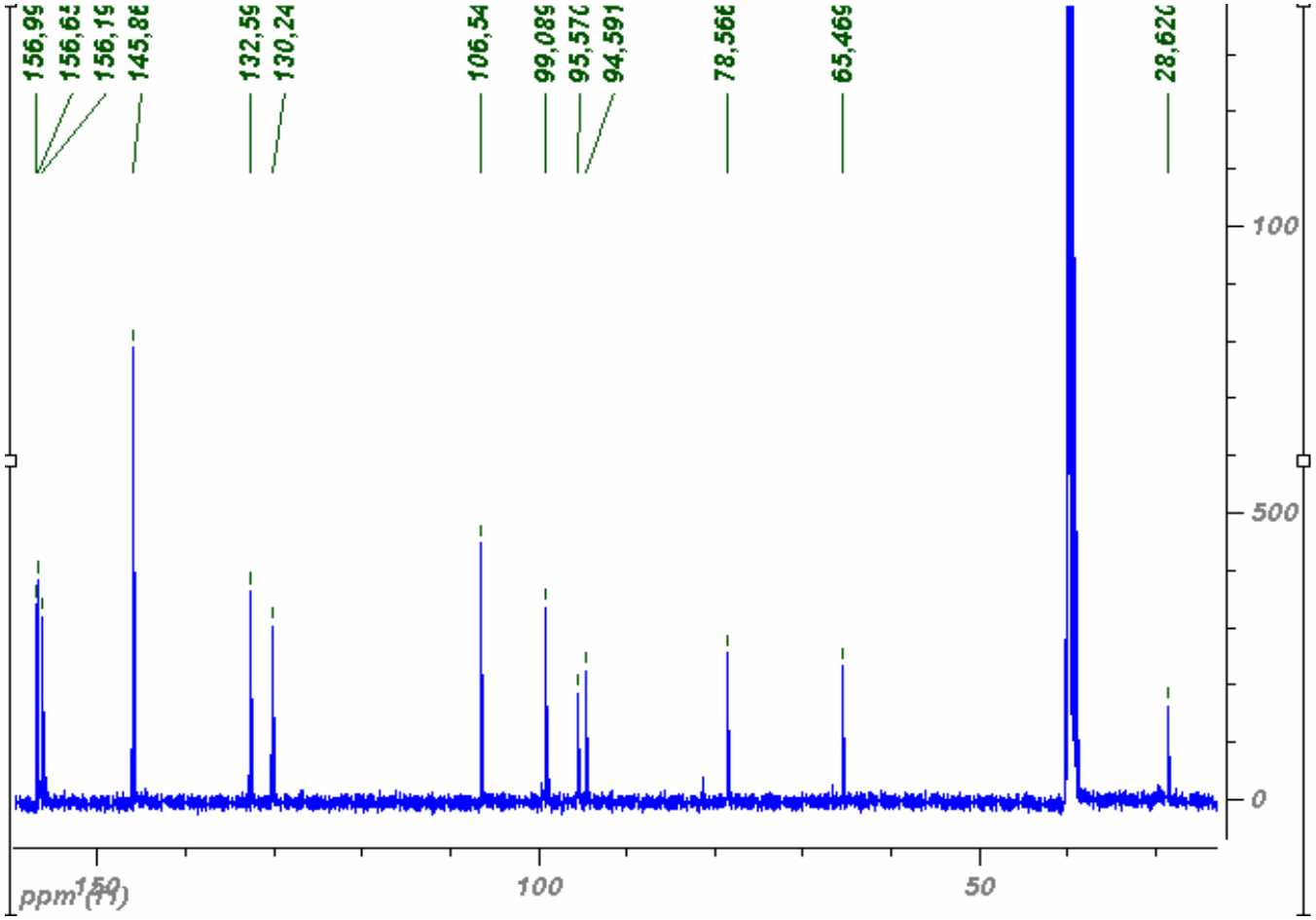
Appendix: 3.2. UV- spectrum of EC-3



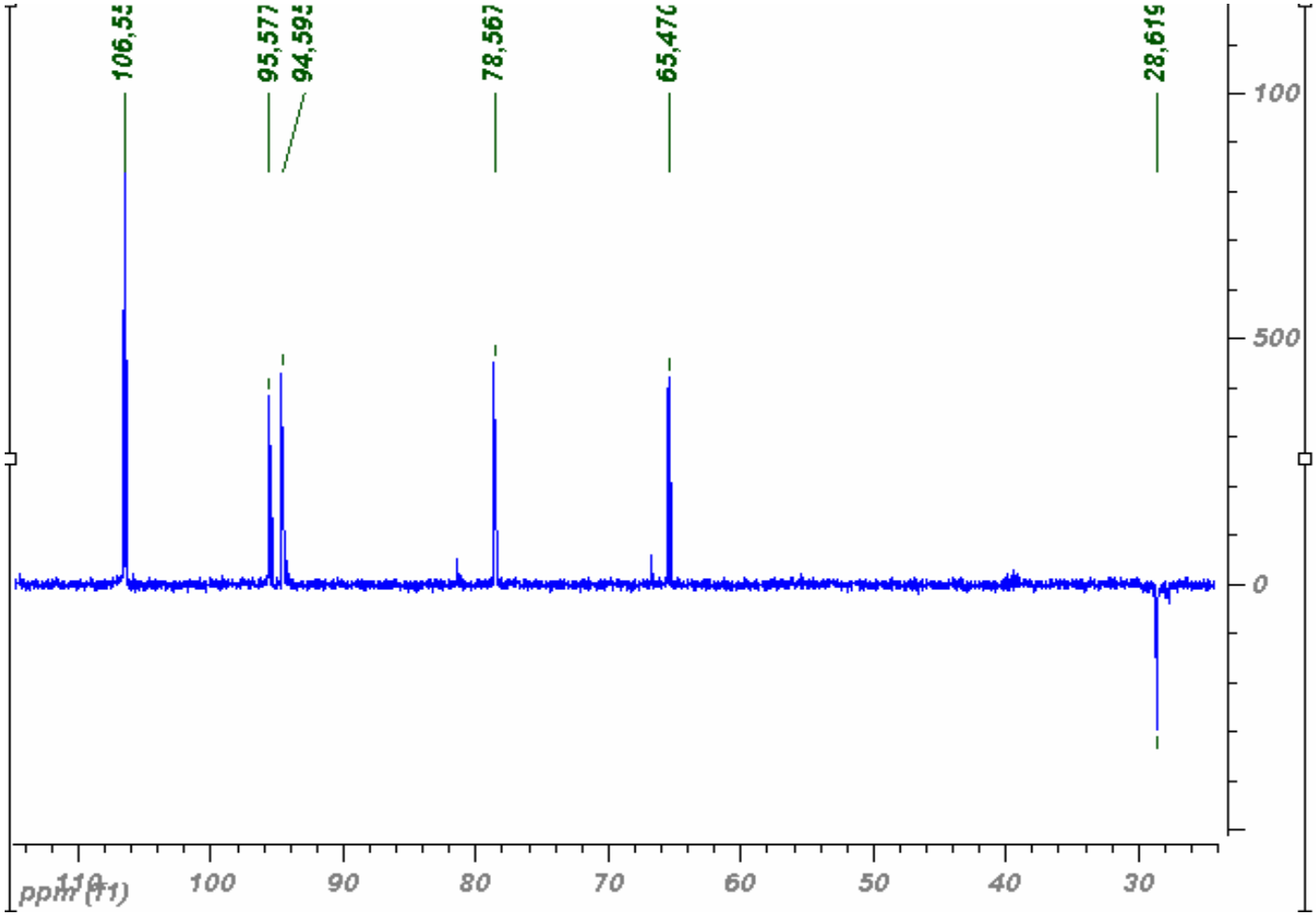
Appendix 3.3. ^{1}H NMR spectra of EC-3



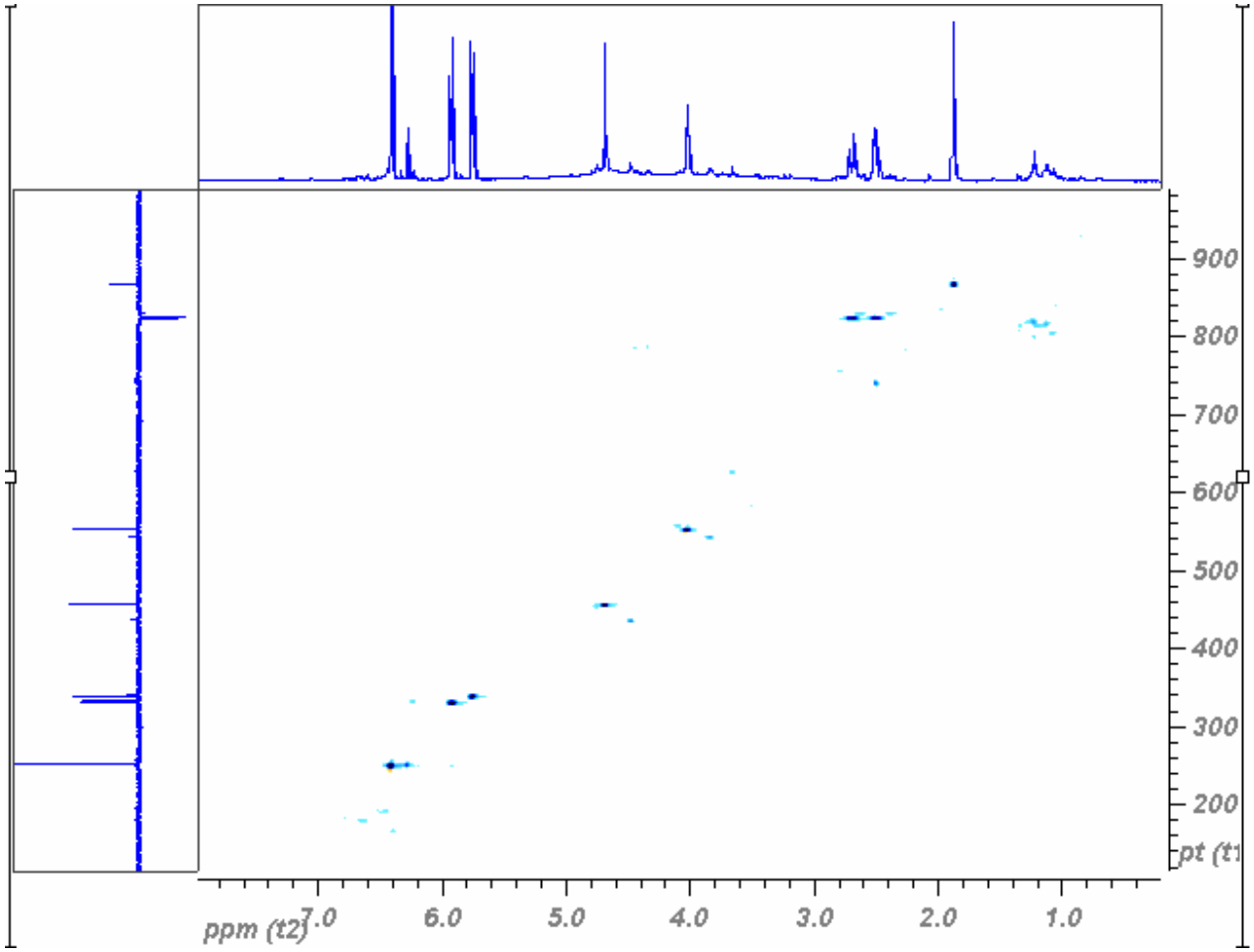
Appendix 3. 4. ^{13}C NMR spectra of EC-3



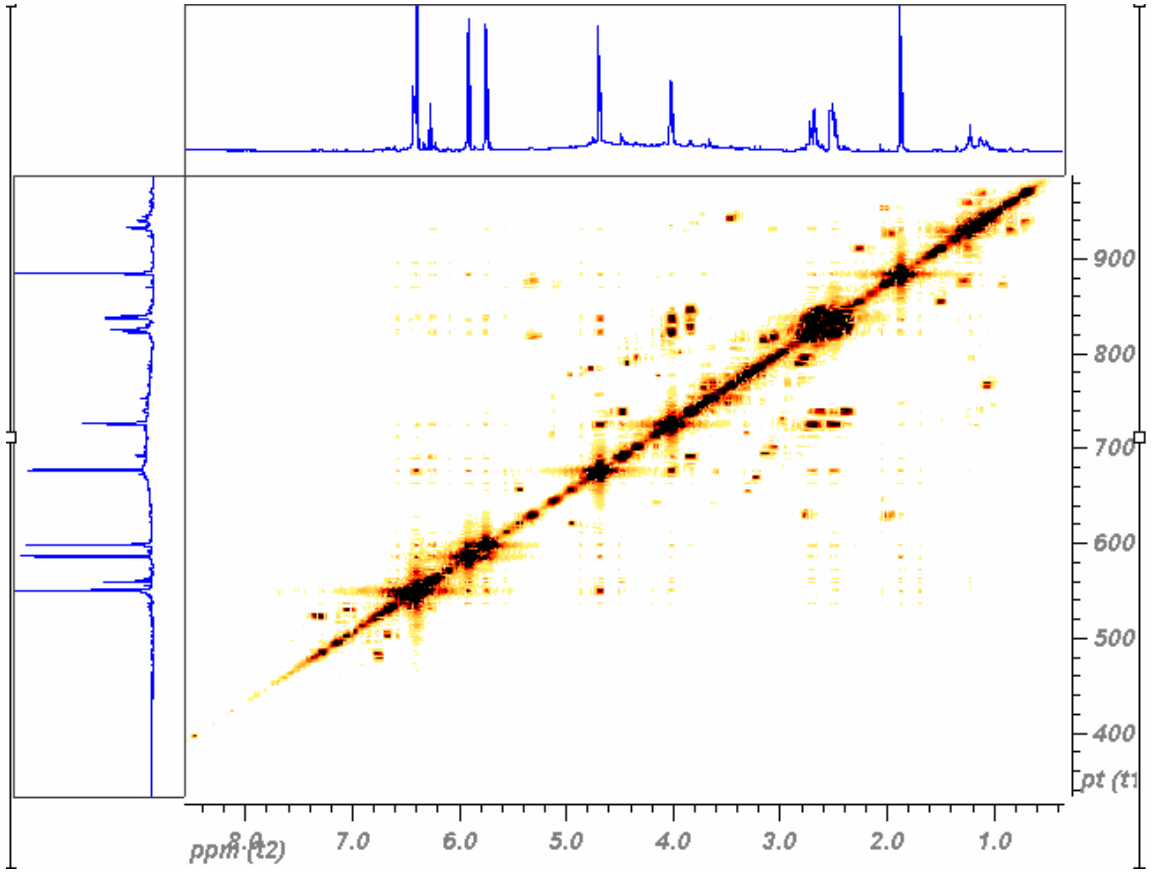
Appendix 3. 5. ^{13}C DEPT spectra of EC-3



Appendix: 3.6. HMQC spectra of EC-3



Appendix 2.7. H-H Cosy spectra of EC-3



Appendix: 3.8. HMBC spectra of EC-3