PHYTOCHEMICAL INVESTIGATION ON THE AERIAL PART OF ANTHEMIS TIGREENSIS (CHLOROFORM EXTRACT)

A GRADUATE PROJECT SUBMITTED TO THE OFFICE OF RESEARCH AND GRADUATE PROGRAMME OF ADDIS ABABA UNIVERSITY

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BY

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SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATION ON THE AERIAL PART OF *ANTHEMIS TIGREENSIS* (CHLOROFORM EXTRACT)

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DECLARATION

I, the undersigned, declare that this project is my original work and has not been presented for a degree in any other University and that all sources of materials used for the project have been duly acknowledged.

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This is to confirm that **Aselefech sorsa** has incorporated the comments of the examining board in the final version of her M.Sc. project.

Sincerely yours,

_____

Dr. Nigist Asfaw

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Abstract

A. tigreensis in the family Asteraceae, in the genus *Anthemis* is the only species found in Ethiopia. *A. tigreensis* has medicinal value and is important in traditional medicine like the other species in the genus. *Anthemis nobilis* (Chamomile) has been known from Roman times as an antispasmodic and sedative in folk treatment of digestive and rheumatic disorders. It is also a popular herb tea. *A. cotula* shows antimicrobial activity against both Gram-negative and Gram-positive microorganisms. In local medicine, the roots of *A.tigreensis* are used against wet eczema.

In this work, two compounds, **At-3**(AntheindurolideA) and **At-17**(dihydro-5-(4-(tetrahydro-3-methylene-2-oxofuran-4-yl)-2-hydroxy-2-methylbut-3-enyl)-3-methylfuran-2(3H)-one were isolated from the aerial part of the plant. **At-3** was isolated before from *A. tigreensis* and other species of *Anthemis*. To the best of our knowledge compound **At-17** has not been reported before in the Natural Product Dictionary and literature. The structures were elucidated from 1D and 2D-NMR, UV and IR spectra data and by comparison of the data obtained with those reported for the compounds in the literature.

1. Introduction

1.1 Importance of Natural Product

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites**,** often are differentially distributed among limited taxonomic groups within the plant kingdom. Their functions, many of which remain unknown, are being elucidated with increasing frequency. The primary metabolites**,** in contrast, such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, are found in all plants and perform metabolic roles that are essential and usually evident.[1]

The term 'natural product' is commonly used for those organic compounds of natural origin that are unique to one organism, or common to small number of closely related organism. Natural products are secondary metabolites of an organism.[2]

Compounds and extracts derived from the natural product have found uses in medicine, agriculture, cosmetics, and food in ancient and modern societies around the world. Therefore, the ability to access natural products, understand their usefulness, and drive applications has been a major driving force in the field of natural product research.[3]

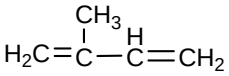
The study of natural products is now very much an interdisciplinary field embracing chemistry. Organic chemists, however, have long been interested in these novel phytochemicals and have investigated their chemical properties extensively since the 1850s. Studies of natural products stimulated development of the separation techniques, spectroscopic approaches to structure elucidation, and synthetic methodologies that now constitute the foundation of contemporary organic chemistry. Interest in natural products was not purely academic but rather was prompted by their great utility as dyes, polymers, fibers, glues, oils, waxes, flavoring agents, perfumes, and drugs.

1.2 Terpenes

Plant and animals produce an amazingly diverse range of chemicals. Most of these are based on carbon and so the chemistry of carbon came to be known as organic chemistry. These chemical products of plants and animals can be classified into primary and secondary metabolites. Primary metabolites are those which are common to all species and can be sub-divided into proteins, carbohydrates, lipids, and nucleic acids. These four groups of materials are defined according to the chemical structure of their members. The secondary metabolites are often referred to as "natural products". These can be sub-divided in to terpenoids, alkaloids, shikimates, and polyketides.

Terpenes are a large and varied class of hydrocarbons, produced primarily by a wide variety of plants and also by some insects such as swallowtail butterflies, which emit terpene from their osmeterium. They are the major components of resin, and of turpentine produced from resin. The name "terpene" is derived from the word "turpentine", the suffix "ene" indicating the presence of olefinic bonds. Terpenoids are oxygen- containing analogues of terpenes. They are thoroughly distributed in the plant kingdom, especially in those plants that have abundant chlorophyll.[4]

Among these are compounds which fall in the general class of terpenes, compounds made of 5-carbon unit, often called isoprene units, put together in a regular pattern, usually head-to-tail in terpenes up to 25 carbons.

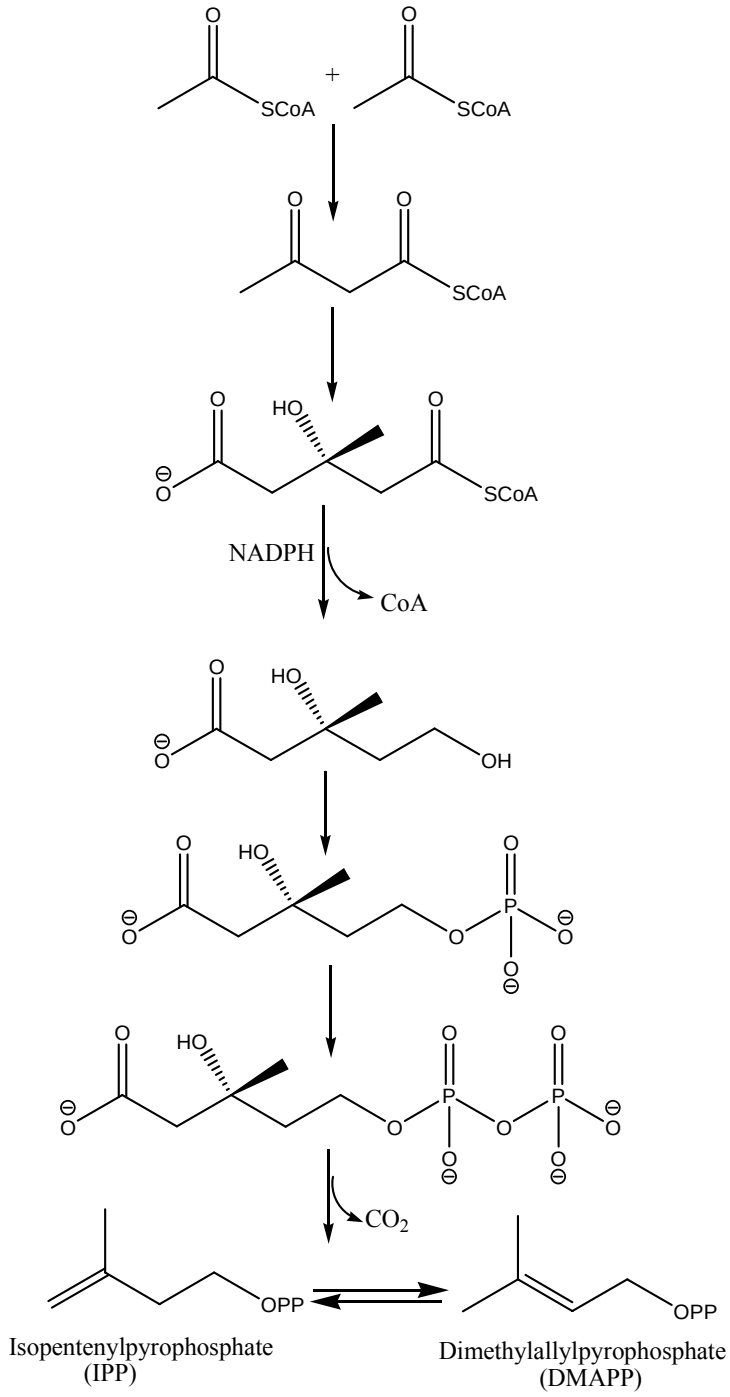


Isoprene

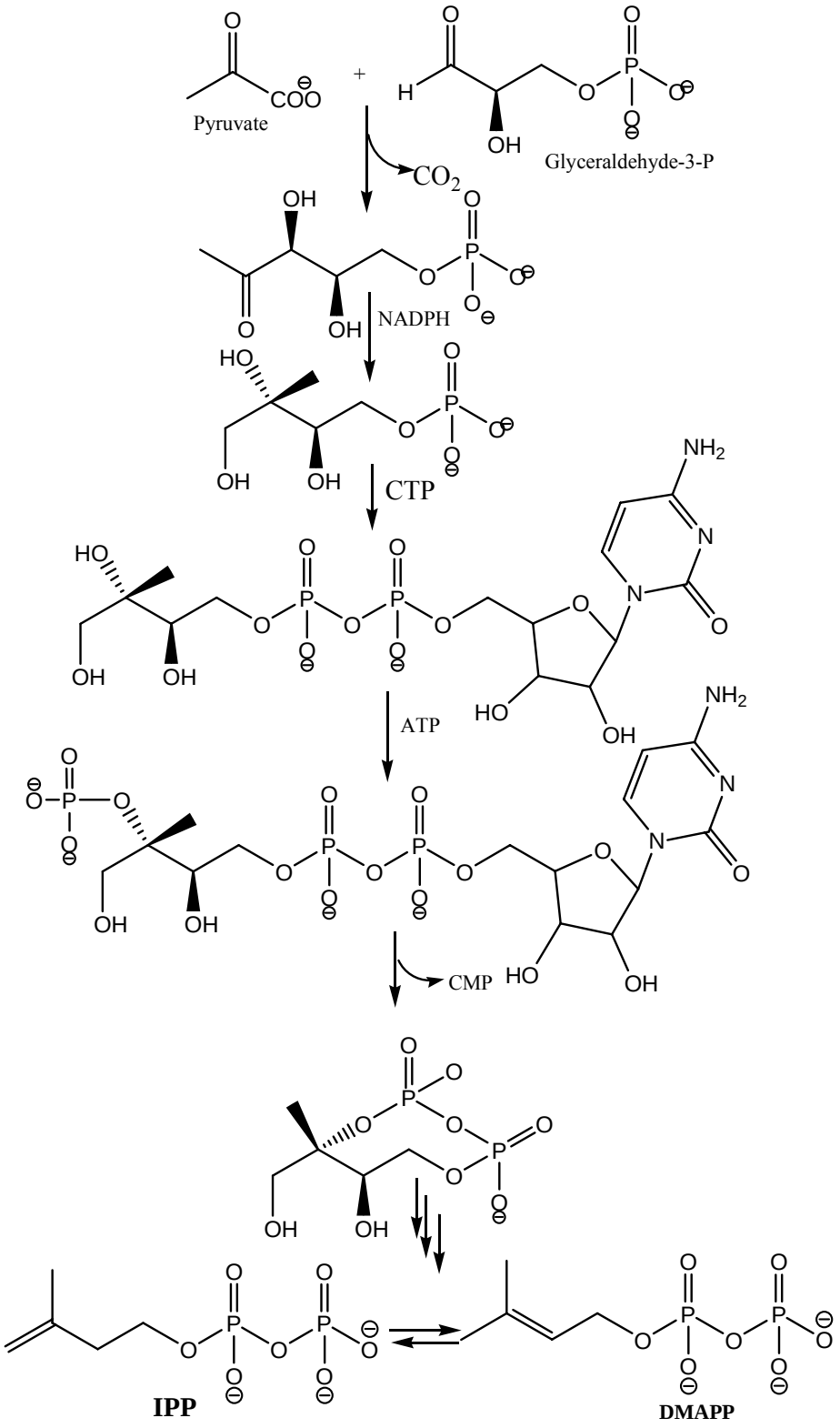
Terpenes containing 30 carbons or more are usually formed by the fusion of two smaller terpene precursors such that the head to tail "rule" appears to be violated. In overall, terpenes hold potential interest practical applications especially in the fragrance and flavors industries, as well as in the pharmaceutical and chemical industries.

1.2.1 Biosynthesis of terpenoids

Isopentenyl diphosphate (IPP), the universal building block for all isoprenoids is formed by two different biosynthetic routes: the well known acetate/mevalonate pathway (Scheme 1) and the non-mevalonate pathway starting from pyruvate and giyceraldehydes-3-phosphate (Scheme 2).[5] These five carbon intermediate are responsible for the formation of all terpenes.

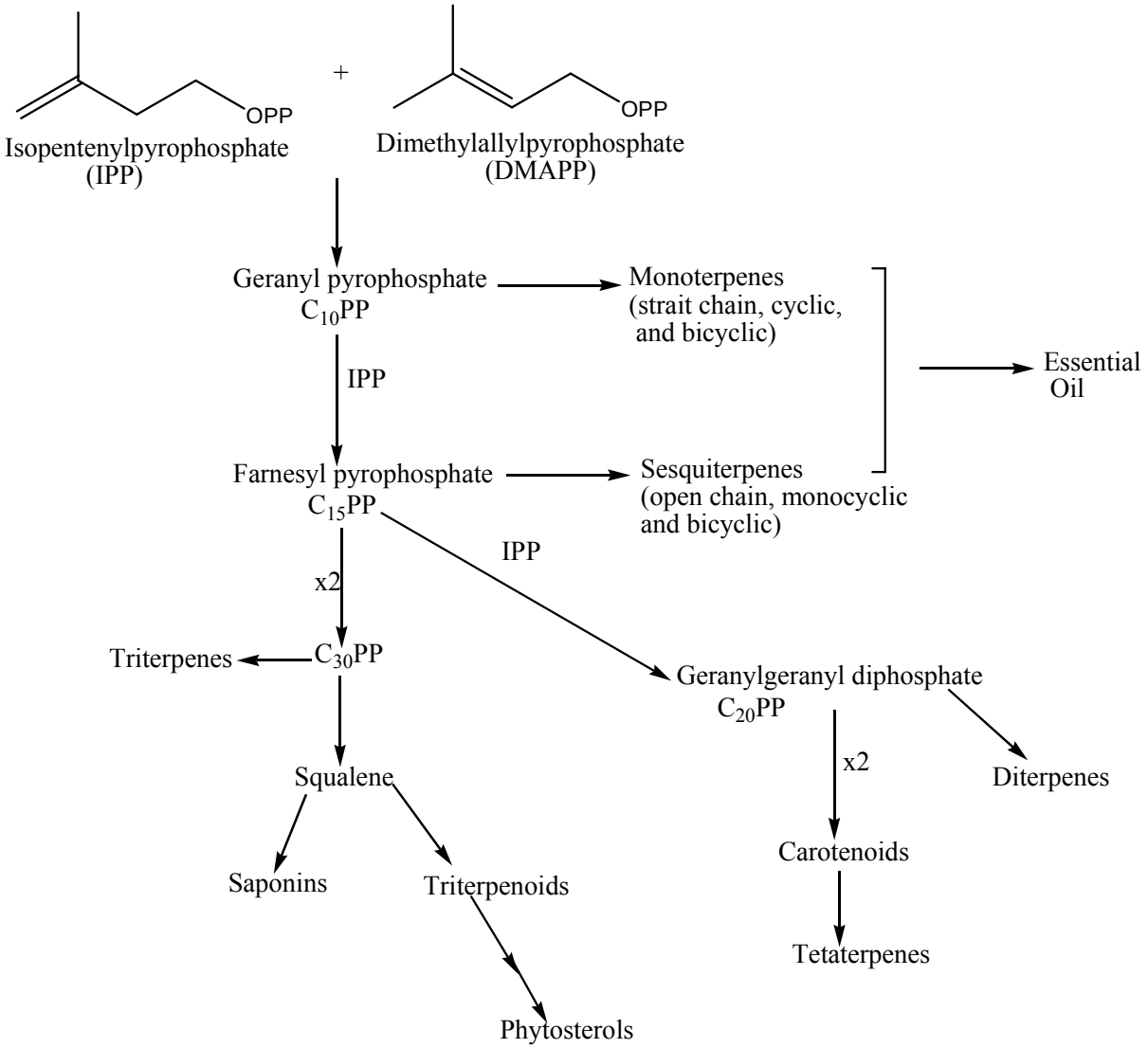


Scheme 1. Mevalonate-acetate pathway for the biosynthesis of isoprenoids.



Scheme 2. Non-mevalonate glyceraldehydes-3-phosphate/pyruvate pathway of isoprenoid biosynthesis.

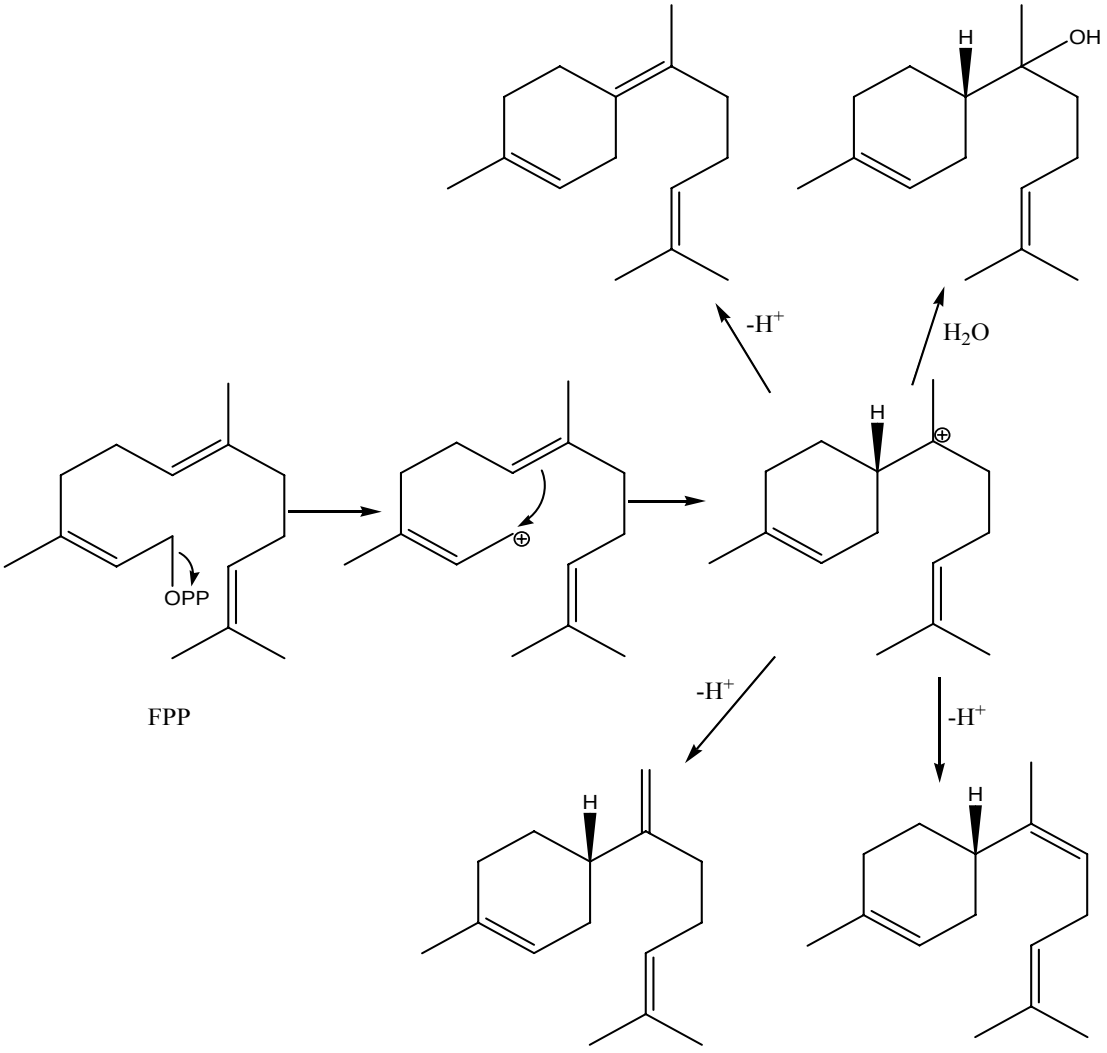
Using a simple five carbon building block, nature creates an array of terpenoid chemicals with an infinite variety of structural variation and vast range of biological functions. Terpenoid biosynthesis involves mostly head to tail addition of isopentenyl diphosphate (IPP, the active C_5 isoprene unit), to its isomer dimethylallyl diphosphate.[6]



Scheme3. Biosynthesis of various classes of terpenoids in plants.

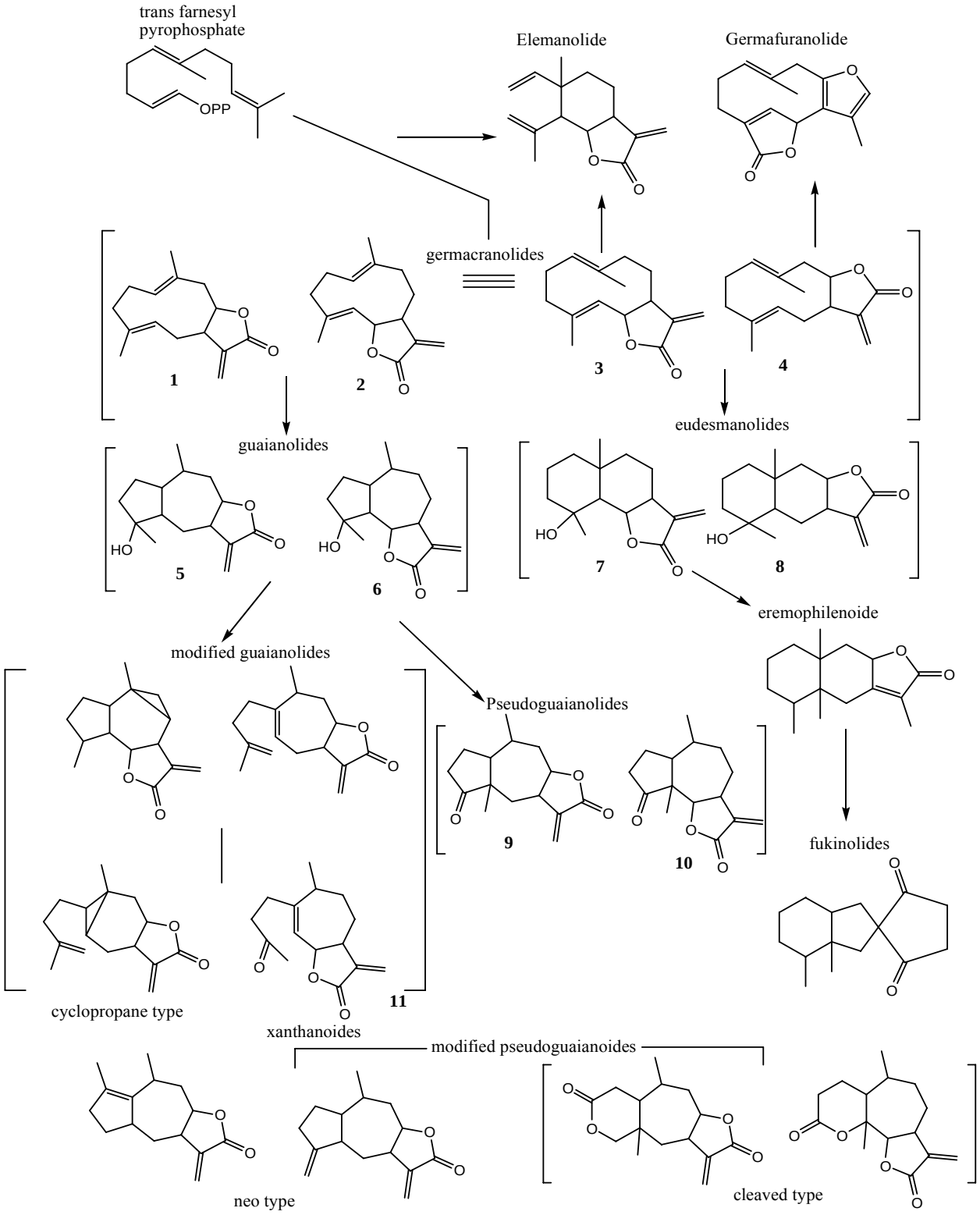
1.2.2 Biosynthesis of Sesquiterpenes

Sesquiterpenes are formed from three isoprene units and thus contain 15 carbon atoms. Sesquiterpene lactons (STLs) are typical terpenoids of the plant family Asteraceae.[7]



Scheme 4. Biosynthesis of sesquiterpenes.

These secondary metabolites are primarily classified on the basis of their carbocyclic skeletons into germacranolide (1, 2, 3, 4), guaianolides (5, 6), eudesmanolides (7, 8), pseudoguaianolides (9, 10), and xanthanolides 11 (Scheme 5).[8]



Scheme 5. Possible biogenetic relationship of different skeletal types of sesquiterpene lactones.

1.3 The genus *Anthemis*

The Asteraceae (also known by the older alternative name Compositae) is one of the largest families of vascular plants with about 1535 genera and about 23,000 species. It is widely distributed with the herbaceous species found predominantly in temperate regions and the trees occurring mainly at high altitudes in tropical areas.

The genus *Anthemis*, in the family Asteraceae, with more than 130 species is widely distributed in Europe, especially around the Mediterranean, in West, Southwest, and Central Asia, as well as in North Africa.[9] In Ethiopia there is only one *Anthemis* species namely, *Anthemis tigreensis* J. Gey ex A. Rich.[10]

According to the literature this is one of the best phytochemical investigated genera of the family Asteraceae. Polyacetylenes, flavonoides, and sesquiterpene lactones are the three main classes of secondary metabolites of the genus, several of which are herbal medicines, insecticides, and dyes.[11] *Anthemis nobilis* (Chamomile) has been known from Roman times as an antispasmodic and sedative in folk treatment of digestive and rheumatic disorders. It is also a popular herb tea.[12] The essential oil of *A. nobilis* possesses anti-inflammatory and sedative properties. *A. cotula* shows antimicrobial activity against both Gram-negative and Gram-positive microorganisms.[13]

The chemical studies of different *Anthemis* species have been done worldwide especially in Southern Europe. In most species, the four common chemical compounds are germacranolides **12**, eudesmanolides **13**, guaianolides **14,** and irregular sesquiterpene lactones **15** (Figure 1) and their derivatives.[14] Besides these, there are also other terpenes and terpene derivatives (Table 1), which are constituents of the genus.

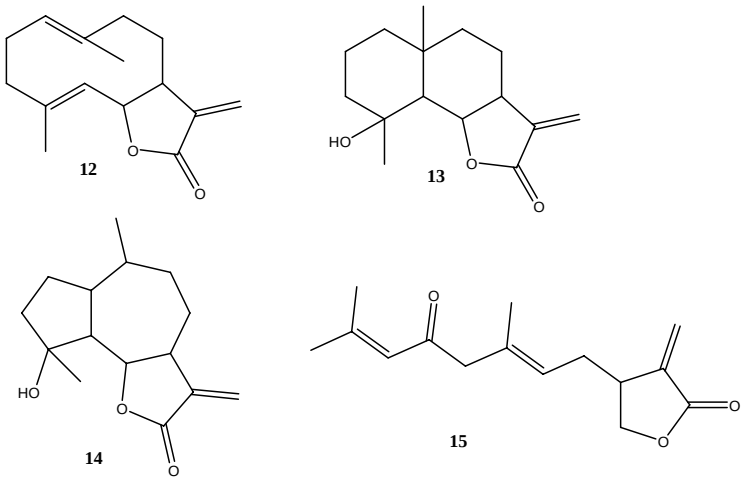


Figure 1. Some sesquiterpene lactone types from *Anthemis* species**.**

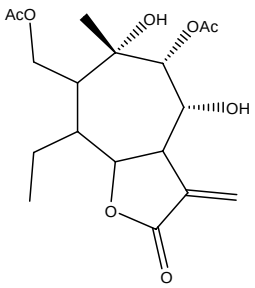
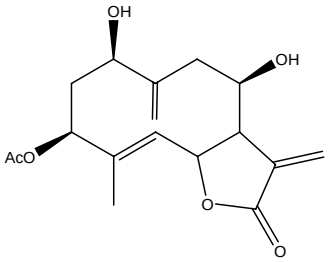
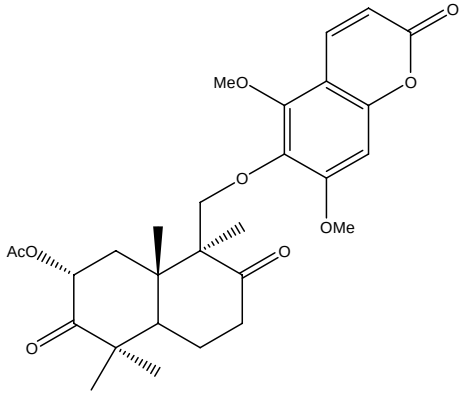
1.4 *Anthemis tigreensis*

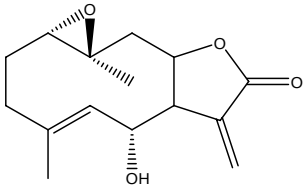
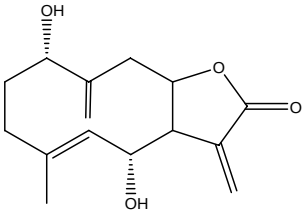
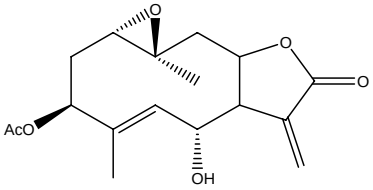
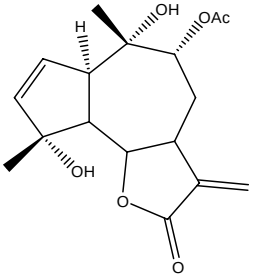
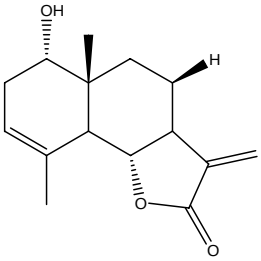
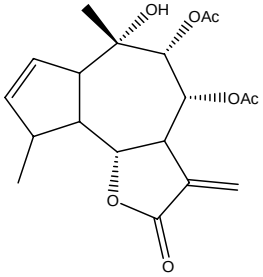
A. tigreensis, vernacular name 'embaha' (in Tigreegna), is a prostrate or sometimes erect perennial herb growing to 5-60 cm high. Its leaves are gray-green, alternate and divided into rounded segments. Its flower heads are white, attractive on long stalks. It is a common plant of higher altitudes and sometimes locally dominant in fallow fields. It grows on degraded area, mountain slopes, in grassland and seasonally water logged soils, at altitudes between 1800-4620m.

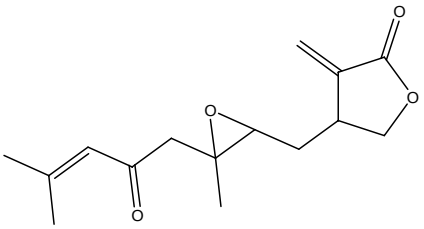
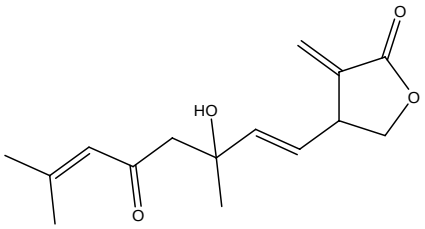
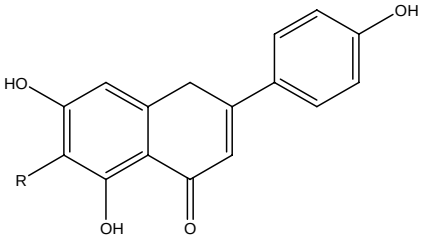
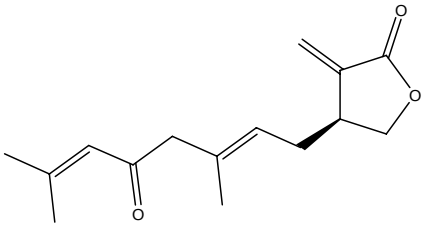
In Ethiopia, *A.tigreensis* is found in Tigray, Gojjam, Gonder, Wollo, Shoa, Arsi, Harrer, and Bale, extending to Kenya, Uganda, and Tanzania.[10] It flowers through out the year, but especially in masses after the short rains. Honeybees collect pollen and nectar from the flowers. In local medicine, the roots are used against wet eczema.[15]

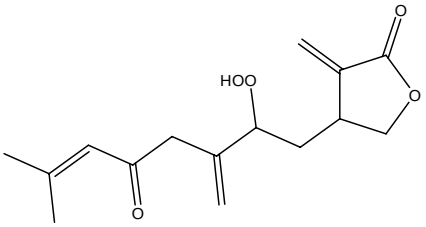
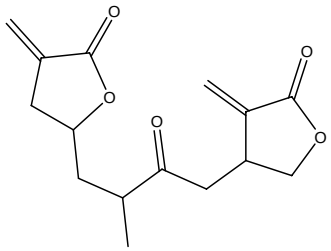
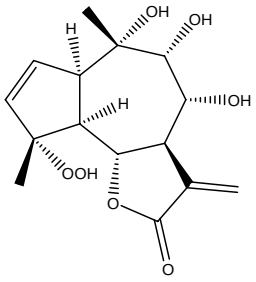
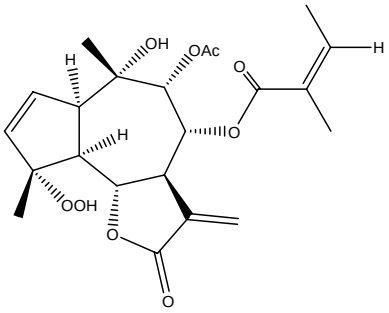
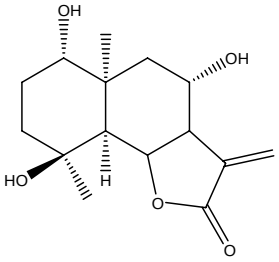
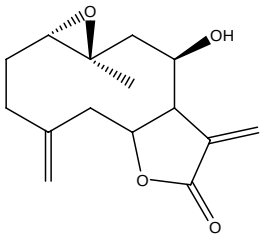
A literature survey indicated that there is two sesquiterpene lactons were isolated from petroleum ether and chloroform extract of the *A.tigreensis* namely 8-(tetrahydro-3'-methylene-2'-oxofuran-4'-yl)-2, 6-dimethylocta-2, 6-dienal and antheindurolide A, respectively. Chemical investigation on the essential oil of the plant indicated that borneol (11%) and bornyl acetate were the major components. Other compounds identified were, α-Pinene, Camphene, 3-Carene, 1, 8 Cineol, γ-Cadinene, Caryophyllene oxide and Cubenol.[16]

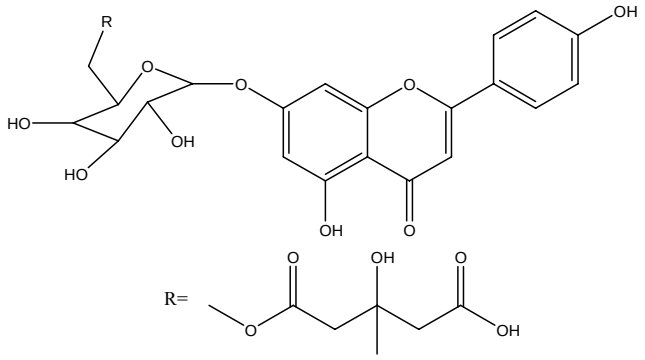
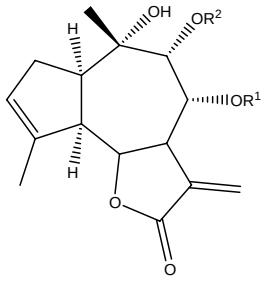
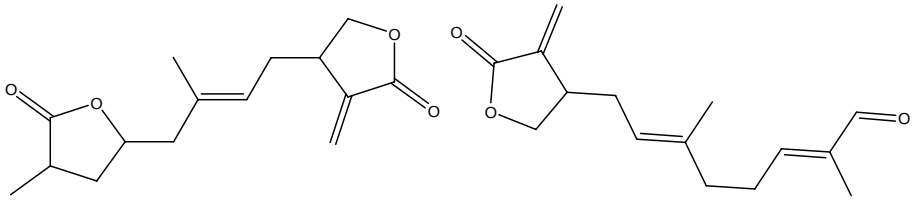
Table 1. Some components of *Anthemis* species.

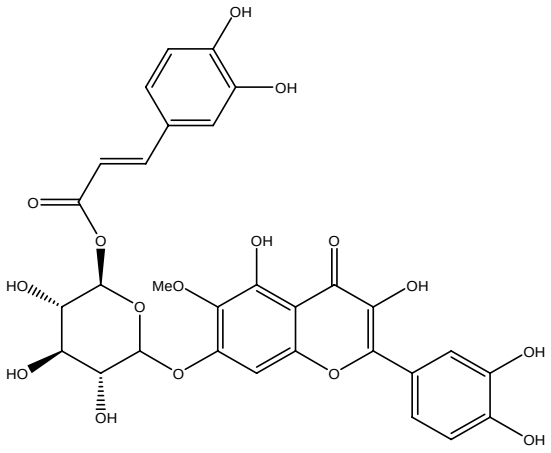
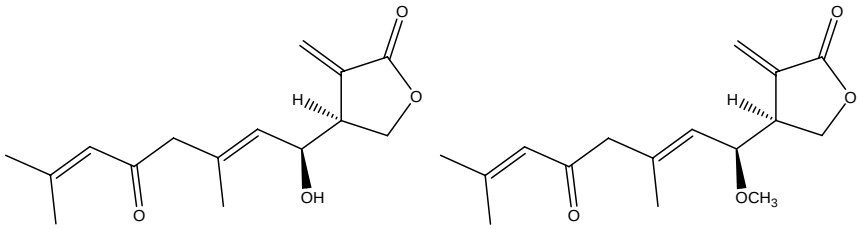
Species	**Compound**	**Ref**
A.aetnensis	 2,9-diacetoxy-8,10-dihydroxyguaia-3, 11(13)-diene-6,12-olide  1-β-hydroxyarbysculin  7-acetoxypectanone	17

A. altissima	 1α,10β-epoxy-6α-hydroxy-1,10H-inunolide  1-epi-tatridin	18
A. carpatica	 Germacranolide  Anthemolide A	19
	 Douglanin  9α-acetoxycumambrin A	20

A.cotula	 Anthecotuloide-5,6-oxide (new compound)  6-hydroxy-4,5-dehydro-5,6-dihydro anthecotuloide (new compound)  R=H Apigenin $R=OCH_3$ Hispidulin	11
	 Anthecotuloide	14

A.cotula	 5-hydroperoxy-6,13-dehydro-5,6-dihydro-anthecotuloide  Antheindurolide B	21
A.cretica	 Anthemolide B  8-O-angeloyl-9-O-acetyl-anthemolide B	22
A.melanolepis	 Melanolepins B  Melanolepins C	9

A.nobile	 Chamaemeloside	23
A.plutonia	 9α-acetoxycmmambrin B	24
A.tigreensis	 Antheindurolide 8-(tetrahydro-3'-methylene-2'-oxofuran-4'-yl)-2,6-dimethylocta-2,6-dienal	16

A. tinctoria	 Tinctosid	25
A.auriculata	 1-hydroxyanthecotuloide 1-methoxyanthecotuloide	26

1.5 Objective of the study

The objective of this work is to elucidate structures of compounds isolated from the aerial part of *A.tigreensis.* Studies on other species of the genus *Anthemis* have revealed that the plant have constituents that are important in medicine and perfumery. Similar chemical investigation on *A.tigreensis* will result in the identification of bioactive and fragrance constituents. The study will corroborate the therapeutic and perfumery potential of the plant. This will also lead to the domestication and conservation of the plant in the world. Therefore, in these studies the specific objective is to isolate and characterize the constituents of the chloroform extract of the plant.

2. Result and discussion

2.1 Solvent extraction of *Anthemis tigreensis*

The dried and powdered plant was extracted with chloroform. **At-3** and **At-17** were isolated and characterized from the 1D and 2D-NMR, UV, and IR spectra data. The extraction of the plant and isolation of the compounds is described in detail in the experimental part.

2.1.1 Characterization of At-3

In the IR (KBr) spectrum (Appendix 1), the absorption band at 2983cm^{-1} and 2919cm^{-1} showed the presence of C-H asymmetric stretch for CH_3 and CH_2 stretch, respectively. A strong absorption band at 1755cm^{-1} indicated the presence of γ-lactone functional group. A weak band at 1655cm^{-1} showed the presence of alkene C=C stretch. The presence of absorption bands between1300-1000cm^{-1} illustrated C-O stretches of the ester functional groups. A band at 817 cm^{-1} showed the presence of a trisubstituted double bond.

The UV spectrum (Appendix 2) displayed absorption band at λ_{max} (in $CHCl_3$) 241nm indicating the presence of a conjugated system.

The ^{1}H NMR spectrum (Appendix 3, Table 2), showed peaks at δ 1.25(3H, *d*, *J* = 7 Hz), and δ 1.65(3H, *s*) integrating for two methyl protons. A methylene group peaks appeared at δ 1.95 and 2.10(1H, *m*) each integrating for one proton. Other methylene signals appear at δ 2.25(2H, *m*) and 2.38(2H, *m*) but not the two protons are at the same carbon as showed in HSQC (Appendix 7). Peaks at δ 2.65(1H, *m, J* = 7.5 Hz), and 3.11(1H, *br m*) correspond to methine protons. Oxymethine signals appeared at δ 4.60(1H, *br m*). Oxymethylene signals showed at δ 3.95(1H, *dd*, *J* = 5 Hz) and 4.40(1H, *t*, *J* = 9Hz). Therminal methylene protons appeared at δ 5.63 and 6.21(1H, *d*, *J* = 2.6 Hz). Peak at δ 5.18(1H, *t*, *J* = 6 Hz) correspond to olefinic methine.

Table 2. ^{1}H and ^{1}H-^{1}H COSY spectral data of compound **At-3**.

Hydrogen on Carbon number	**^{1}H δ (ppm)**	**COSY**
1	1.95(*m*), 2.10(*m*)	H-1→H-10, H-2
2	4.60(*br m*)	H-2→H-1, H-3
3	2.25(*m*), 2.38(*m*)	H-3→H-2, H-15
5	5.18(*t*)	H-5→H-6, H-15
6	2.25(*m*), 2.38(*m*)	H-6→H-7, H-5
7	3.11(*br m*)	H-7→H-6, H-8, H-13
8	3.95(*dd*), 4.40(*t*)	H-8→H-7
10	2.65(*m*)	H-10→H-1, H-14
13	5.63(*d*), 6.21(*d*)	H-13→H-7
14	1.25(*d*)	H-14→H-10
15	1.65(*s*)	H-15→H-3, H-5

The ^{13}C NMR and DEPT-135 (Appendix 4, 5, Table 3), indicated that **At-3** has 15 carbons. The spectra show two methyl carbons at δ15.8 and 16.7. Three aliphatic methylene signals appear at δ 32.1, 34.9, and 45.2. Oxymethylene signal appeared at δ 70.5. Peaks at δ 33.7, and 38.5 represented for aliphatic methine. Oxymethine signal appeared at δ 76.4. Therminal methylene showed at δ 122.4. An olefinic methine is attested by the signal at δ 123.2. Two olefinic quaternary carbons appeared at δ 137.8, and 134.2. The presence of ester functional group was shown by the peaks at δ 170.7, and 179.8.

Table 3. ^{13}C and HMBC data of compound **At-3**.

Carbon number	**^{13}C δ(ppm)**	**DEPT**	**HMBC**
1	34.9	CH_2	H-1↔C-2, C-3, C-9, C-10, C-14
2	76.4	CH	H-2↔C-4, C-9, C-10
3	45.2	CH_2	H-3↔C-1, C-2, C-4, C-5, C-15
4	134.2	-	-
5	123.2	CH	H-5↔C-3, C-6, C-15
6	32.1	CH_2	H-6↔C-5, C-7, C-8, C-11
7	38.5	CH	H-7↔C-6, C-11
8	70.5	CH_2	H-8↔C-6, C-7, C-11, C-12
9	179.8	-	-
10	33.7	CH	H-1↔C-1, C-2, C-9, C-14
11	137.8	-	-
12	170.7	-	-
13	122.4	CH_2	H-13↔C-6, C-7, C-11, C-12
14	15.8	CH_3	H-14↔C-1, C-9, C-10
15	16.7	CH_3	H-15↔C-3, C-4, C-5

The HMBC spectrum (Appendix 6, Table 3), of the compound indicated that protons on methyl C-14 correlate with the carbonyl C-9, aliphatic C-10, and methylene C-1. Proton of methylene C-1 correlates with methine C-10, methyl C-14, carbonyl C-9, methine carbon C-2, and methylene C-3. The methine proton of C-2 correlates with carbonyl C-9, methine carbon C-10, and quaternary carbon C-4. Protons of methylene C-3 also correlate with methine C-2, methylene C-1, quaternary C-4, methine C-5, and methyl C-15. These observations lead to fragment **I** (Figure 2). Protons of C-3 correlate with quaternary carbon C-4, methyl C-15, methine C-5, methylene C-1, and methine carbon C-2. Methyl protons of C-15 correlate with quaternary carbon C-4, methylene C-3, and methine C-5. Methine proton on C-5 correlates with methylene C-3, methyl C-15, and methylene C-6. These observations lead to fragment **II**.

Protons of C-6 correlate with methine C-5, aliphatic methine C-7, quaternary carbon C-11, and C-12. Methylene protons at C-8 correlate with ester carbonyl C-12, olefinic quaternary C-11, methylene C-6, and methine C-7. The terminal methylene C-13 protons correlate with methylene C-6, methine C-7, quaternary carbon C-11, and C-12. These analyses lead to fragment **III.**

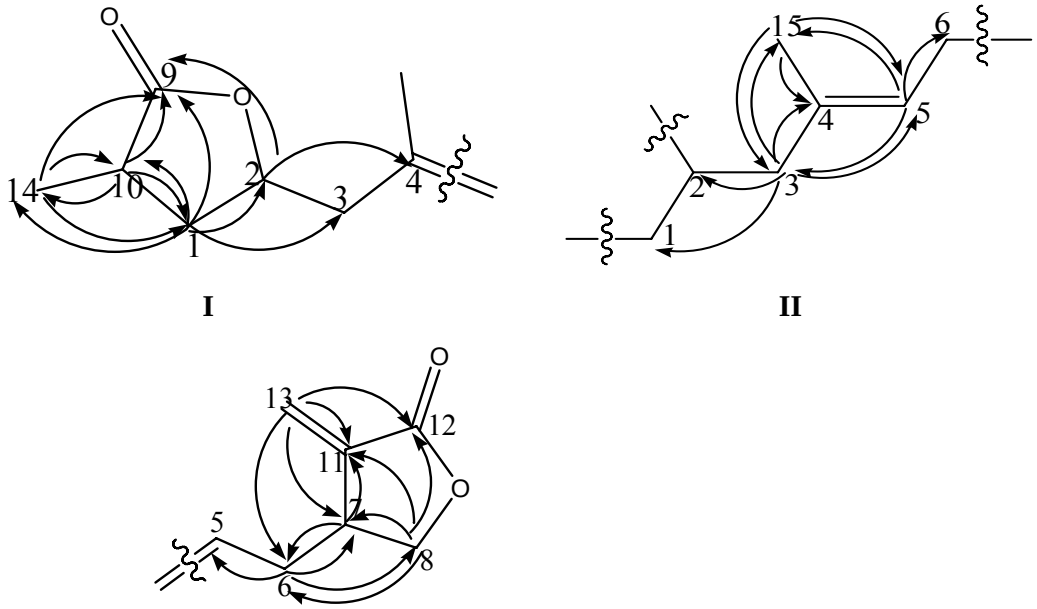
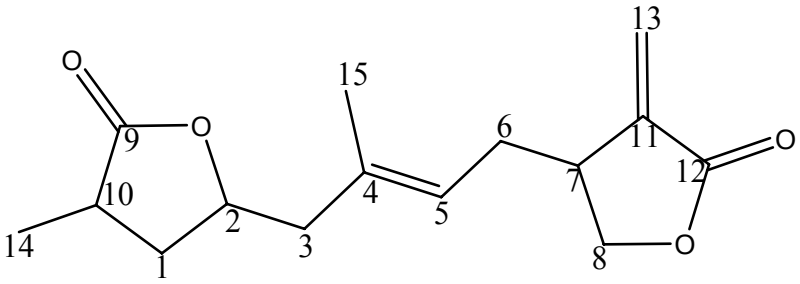


Figure 2. The proposed fragments for **At-3** with HMBC correlation.

Combination of fragment **I, II, III** (Figure 2) resulted in the proposed structure **16** for the compound **At-3**. The ^{1}H and ^{13}C data obtained for **At-3** is in agreement with those reported for Antheindurolide A from *A. pseudocotula* (Table 4) and *A. tigreensis*.[16]



Antheidurolide A **(16)**

Table 4. ^{1}H and ^{13}C spectra data of **At-3** compared with literature report.

Carbon and hydrogen number	**^{1}H δ(ppm)**	**Literature ^{1}H δ(ppm)**	**^{13}C δ(ppm)**	**Literature ^{13}C δ(ppm)**
1	1.95(*m*) 2.10(*m*)	1.91(*ddd*) 2.05(*ddd*)	34.9	34.6
2	4.60(*br m*)	4.55(*dddd*)	76.4	76.2
3	2.25(*m*) 2.38(*m*)	2.18(*br dd*) 2.32(*br dd*)	45.2	44.9
4	-	-	134.2	137.6
5	5.18(*t*)	5.14(*br dd*)	123.2	123.0
6	2.25(*m*) 2.38(*m*)	2.22(*br dd*) 2.31(*br dd*)	32.1	31.8
7	3.11(*br m*)	3.05(*m*)	38.5	38.2
8	3.95(*dd*) 4.40(*t*)	3.89(*dd*) 4.34(*dd*)	70.5	70.3
9	-	-	179.8	179.7
10	2.65(*m*)	2.60(*dd q*)	33.7	33.5
11	-	-	137.8	134.0
12	-	-	170.7	170.6
13	5.63(*d*) 6.21(*d*)	5.60(*d*) 6.14(*d*)	122.4	122.1
14	1.25(*d*)	1.19(*d*)	15.8	15.5
15	1.65(*s*)	1.69(*br s*)	16.7	16.4

2.1.2 Characterization of At-17

The IR (NaCl) spectrum (Appendix 9), of the compound displayed absorption band at 3481cm^{-1} which showed the presence of alcohol functional group. Absorption band at 3097cm^{-1} indicated the presence of alkene C-H stretch. Absorption band at 2921cm^{-1} and 2850cm^{-1} demonstrated the presence of alkane CH_3 and CH_2 stretch, respectively. Absorption band at 1762cm^{-1} indicated the presence of γ-lactone functional group. Absorption band at 1662cm^{-1} showed the presence of alkene C=C stretch. The absorption bands between 1300-1000, i.e 1261cm^{-1}, 1180cm^{-1}, 1110cm^{-1} demonstrated the presence of C-C-O stretch of ester functional group. Absorption band at 1008cm^{-1} indicated the presence of C-O stretch of tertiary alcohol.

The UV spectrum (Appendix 10) displayed absorption band at λ_{max} (in $CHCl_3$) 241nm indicating the presence of a conjugated system.

The H^1 NMR spectrum (Appendix 11, Table 5) of the compound showed peaks at δ 1.31(3H, *d, J* = 7.2 Hz), and δ 1.39 (3H, *s*) integrating for two methyl protons. A methylene signals appeared at δ1.81 and δ 1.97 (1H, *dd, J* = 10 Hz & 4.4 Hz) each integrating for one proton. Other methylene signals appeared at δ 2.09 (1H, *m*) and δ 2.11 (1H, *m*) each integrating for one proton. A broad singlet peak was also observed at δ 2.34 due to O-H exchangeable proton. Oxymethylene signals appeared at δ 4.06(1H, *dd, J* = 8.4 Hz & 8 Hz) and δ4.55 (1H, *t, J* = 8.8 Hz & 9.2 Hz). Terminal methylene showed signals at δ 5.63 and δ 6.36 (1H, *d, J* = 2.4Hz). Aliphatic methine proton peaks appeared at δ 2.72 (1H, *m*) and δ 3.78 (1H, *br m*). Peak at δ 4.70 (1H, *br m*) correspond to Oxymethine proton. Furthermore the two overlapped signal appeared at δ5.79 assigned for the two olefinic methine protons.

Table 5. ^{1}H and ^{1}H-^{1}H COSY spectra data of compound **At-17**.

Hydrogen on Carbon number	**^{1}H δ(ppm)**	**COSY**
1	2.09(*m*), 2.11(*m*)	$H^{1}\leftrightarrow H^{10}$, $H^{1}\leftrightarrow H^{2}$
2	4.70(*br m*)	$H^{2}\leftrightarrow H^{1}$, $H^{2}\leftrightarrow H^{3}$
3	1.81(*dd*), 1.97(*dd*)	$H^{3}\leftrightarrow H^{2}$
5	5.79(*m*)	$H^{5}\leftrightarrow H^{6}$
6	5.79(*m*)	$H^{6}\leftrightarrow H^{5}$, $H^{6}\leftrightarrow H^{7}$
7	3.78(*br m*)	$H^{7}\leftrightarrow H^{6}$, $H^{7}\leftrightarrow H^{8}$, $H^{7}\leftrightarrow H^{13}$
8	4.06(*dd*), 4.55(*t*)	$H^{8}\leftrightarrow H^{7}$
10	2.72(*m*)	$H^{10}\leftrightarrow H^{14}$, $H^{10}\leftrightarrow H^{1}$
13	5.63 (*d*), 6.36 (*d*)	$H^{13}\leftrightarrow H^{7}$
14	1.31(*d*)	$H^{14}\leftrightarrow H^{10}$
15	1.39*(s)*	–

The ^{13}C NMR and DEPT-135 (Appendix 12, 13, Table 6) indicated that **At-17** has 15 carbon atoms. The spectra showed two aliphatic methyl carbons at δ 15.8 and 29.2, two aliphatic methylene carbons at δ 36.5 and 47.3, one oxymethylene carbon atom at δ 70.3, one a terminal methylene carbon at δ 123.4, two aliphatic methine at δ 33.7 and 42.5, two olefinic methine at δ 125.6 and 140.8, one oxymethine at δ 75.5, and one oxygenated quaternary carbon at δ 72.2, one olefinic quaternary carbon at δ 137.3, two ester carbonyl carbons at δ 179 and 170.

Table 6. ^{13}C and HMBC spectra data of compound **At-17**.

Carbon number	**^{13}C (ppm)**	**DEPT**	**HMBC**
1	36.5	CH_2	$H^1 \rightarrow C^{14}$, C^{10}, C^3, C^2, C^9
2	75.5	CH	_
3	47.3	CH2	$H^3 \rightarrow C^{15}$, C^1, C^4, C^2, C^5
4	72.2	_	_
5	140.8	CH	$H^5 \rightarrow C^7$, C^4, C^6
6	125.6	CH	$H^6 \rightarrow C^7$, C^4, C^5
7	42.5	CH	$H^7 \rightarrow C^{11}$
8	70.3	CH_2	$H^8 \rightarrow C^7$, C^6, C^{12}, C^{11}
9	179	_	_
10	33.7	CH	$H^{10} \rightarrow C^{14}$, C^9
11	137.3	_	_
12	170	_	_
13	123.4	CH_2	$H^{13} \rightarrow C^7$, C^{11}, C^{12}
14	15.8	CH_3	$H^{14} \rightarrow C^9$, C^1, C^{10}
15	29.2	CH_3	$H^{15} \rightarrow C^3$, C^4, C^5

The HMBC spectrum (Appendix 14, Table 6) of the compound indicated that proton of C-10 correlated with the methyl carbon C-14 at δ 15.8, and carbonyl carbon of ester C-9. Proton of C-1 correlated with methyl carbon C-14, methine carbon C-10, aliphatic methylene carbon C-3, oxymethine carbon C-2 and carbonyl carbon of ester C-9. Protons of C-3 correlated with the methyl carbon C-15 at δ 29.2, aliphatic methylene carbon C-1, oxygenated quaternary carbon C-4, oxymethine carbon C-2, and olefinic methine carbon C-5. Protons of C-14 correlated with carbonyl carbon of ester C-9, aliphatic methylene carbon C-1, methine carbon C-10. These observations together with HSQC (Appendix 15) led to partial structure **I** (Figure 3). Protons of C-15 correlated with aliphatic methylene carbon C-3, oxygenated quaternary carbon C-4, and olefinic methine carbon C-5. Proton of C-5 correlated with aliphatic methine carbon C-7, oxygenated quaternary carbon C-4, olefinic methine carbon C-6.

Proton of C-6 correlated with aliphatic methine carbon C-7, oxygenated quaternary carbon C-4, olefinic methine carbon C-5, and ester carbonyl carbon C-12. These observations resulted in fragment unit **II**. Protons of C-13 correlated with vinylic quaternary carbon C-11, ester carbonyl carbon C-12, and methine carbon C-7. Diasterotopic protons C-8 correlated with methine carbon C-7, olefinic methine carbon C-6, and ester carbonyl carbon C-12. These analyses gave fragment unit.

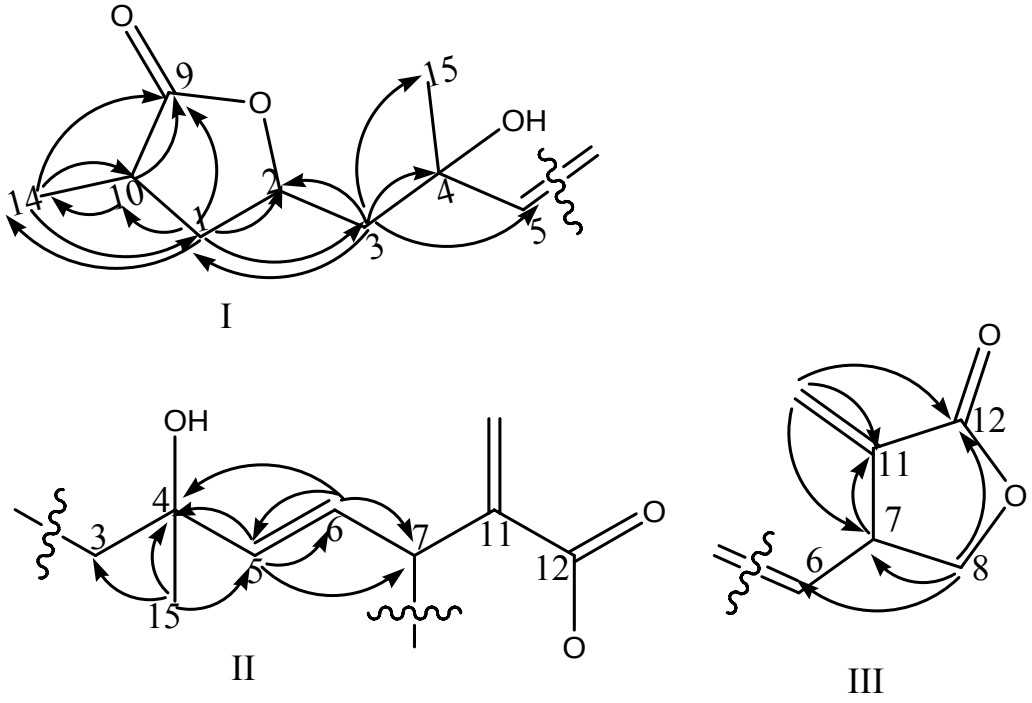
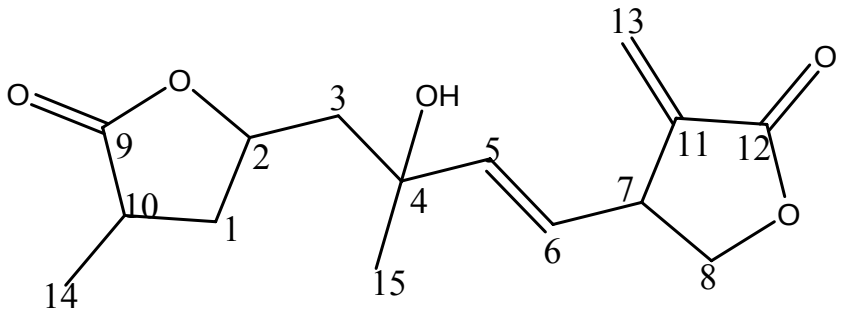


Figure 3. The proposed fragments of **At-17** with HMBC correlation.

Combination of fragment **I**, **II**, and **III** (Figure 3) gave the proposed structure **17** for compound **At-17. At-17** is not registered in Natural Product Dictionary. To the best of our knowledge it is reported for the first time.



Dihydro-5-(4-(tetrahydro-3-methylene-2-oxofuran-4-yl)-2-hydroxy-2-methylbut-3-enyl)-3- methylfuran-2(3H)-one (**17**)

3. Experimental

A. tigreensis is perennial herb, and floret white. It was collected in February 9, 2006 4Km from Debresina towards Addis Ababa close to Debresina high school. Edges of the farm land 9^0 50′ 46.1″ N 39^0 45′ 16.7″ E with altitude of 2700m. The plant was authenticated by Prof. Sebsebe Demissew of the Biology Department, Addis Ababa University, and deposited in The National Herbarium, Biology Department, AAU with a Voucher specimen No. 6511.

3.1 Extraction and Isolation

The whole plant was dried and powdered. 860g of the powdered plant was soaked in 2.5 L chloroform (40-60 ^{0} C) at room temperature for 24 hrs. The extract was filterer and then concentrated under reduced pressure (35 ^{0} C) to yield 20.5g (2.4%).

The chloroform extract was applied on a column chromatography packed with 200g silica gel. Isolation was carried out using the solvents chloroform and ethyl acetate with increasing polarity. A total of 16 fractions were obtained. Fractions that showed the same R_f value and the same characteristic color on TLC were combined (Table 7).

Table 7. Chloroform extract fractions.

Solvent system	**Ratio**	**Fractions**	**Volume (ml)**	**Fractions combined**	**Code**
Chloroform-ethyl acetate	9:1	1-4	150ml	1-4	CE1
”	8:2	5-9	150ml	5-9	CE2
”	1:1	10-14	150ml	10-14	CE3
Ethyl acetate	1	15	150ml		CE4
”	1	16	150ml		CE5

CE = Chloroform extract

Fraction 16 (CE-5) was applied on a sephadex column eluted with chloroform and methanol (2:1). The chlorophyll part was removed and concentrated under reduced pressure (on a rotavapor) to yield 128mg. It was applied on CC silica gel (30g) and further fractionated in to 34 fractions of 10ml each.

Table 8. Fractions of CE5.

Solvent system	**Ratio**	**Fractions**	**Volume (ml)**	**Combined fraction**	**Code**
Cloroform-ethyl acetate	4:1	1-6	10ml	3-6	CE5-1
”	3.5:1.5	7-12	10ml		CE5-2
”	1:1	13-22	10ml	19-22	CE5-3
Ethyl acetate	1	23-25	10ml	23-25	CE5-4
Ethyl acetate-methanol	9:1	26-34	10ml	31-34	CE5-5

Based on TLC analysis, fractions that showed the same characteristics of spots were combined. Fraction 23-25 (74mg) was have four compounds that have the same R_f value in different solvent system, but slightly different R_f value in a ratio 9.7:0.3 chloroform and methanol solvent mixture. They have similar color of spot when the TLC was sprayed with 0.5% vanillin in H_2SO_4. 74mg of the four compounds was applied on a preparative TLC (2mm layer thickness) and developed in 9.7:0.3 chloroform and methanol mixture. Fraction 23-25(CE5-4) further fractionated into 32 fractions.

3.1.1 Isolation of At-3

Fraction 3 was concentrated on a rotavapor, and repeated rinsing of this fraction with petroleum ether resulted in 10mg of pure compound **At-3**.

At-3 is a white solid compound of melting point 58-59. IR Vmax cm^{-1} 2983, 2919, 1755, 1655, 1046, 1164, 1255. ^{1}H NMR (400MHz, $CDCl_3$) δ 1.95(1H, *m*, H-1); 2.10(1H, *m*, H-1); 4.60(1H, *br m*, H-2); 2.25(1H, *m*, H-3); 2.38(1H, *m*, H-3); 5.18(1H, *t*, H-5); 2.25(1H, *m*, H-6); 2.38(1H, *m*, H-6); 3.11(1H, *br m*, H-7); 3.95(1H, *dd*, H-8); 4.40(1H, *t*, H-8); 2.65(1H, *m*, H-10); 5.63(1H, *d*, H-13); 6.21(H, *d*, H-13); 1.25(3H, *d*, H-14); 1.65(3H, *s*, H-15). UV λ_{max} ($CDCl_3$) 241nm. The ^{13}C NMR data and spectrum are shown in Table 3 and Appendix 4 respectively.

3.1.2 Isolation of At-17

Fraction 17-23 was regrouped and concentrated on a rotavapor. Repeated rinsing of this faction with petroleum ether resulted in 6mg of **At-17.**

At-17 is a colorless oily substance that showed R_f value of 1.6 in solvent ratio of 9.5:0.5 chloroform and methanol respectively. IR Vmax cm^{-1} 3481, 3097, 2921, 2850, 1762, 1662, 1261, 1180, 1110, 1008. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.09(1H, *m*, H-1); 2.11(1H, *m*, H-1); 4.70(1H, *br m*, H-2); 1.81(1H, *dd*, H-3); 1.97(1H, *dd*, H-3); 5.79(1H, *m*, H-5); 5.79(1H, *m*, H-6); 3.78(1H, *br m*, H-7); 4.06(1H, *dd*, H-8); 4.55(1H, *t*, H-8); 2.72(1H, *m*, H-10); 5.63(1H, *d*, H-13); 6.36(1H, *d*, H-13); 1.31(3H, *d*, H-14); 1.39(3H, *s*, H-15). UV λ_{max} ($CDCl_3$) 241nm. The ^{13}C NMR data and spectrum are shown in Table 6 and Appendix 12 respectively.

4. Conclusion

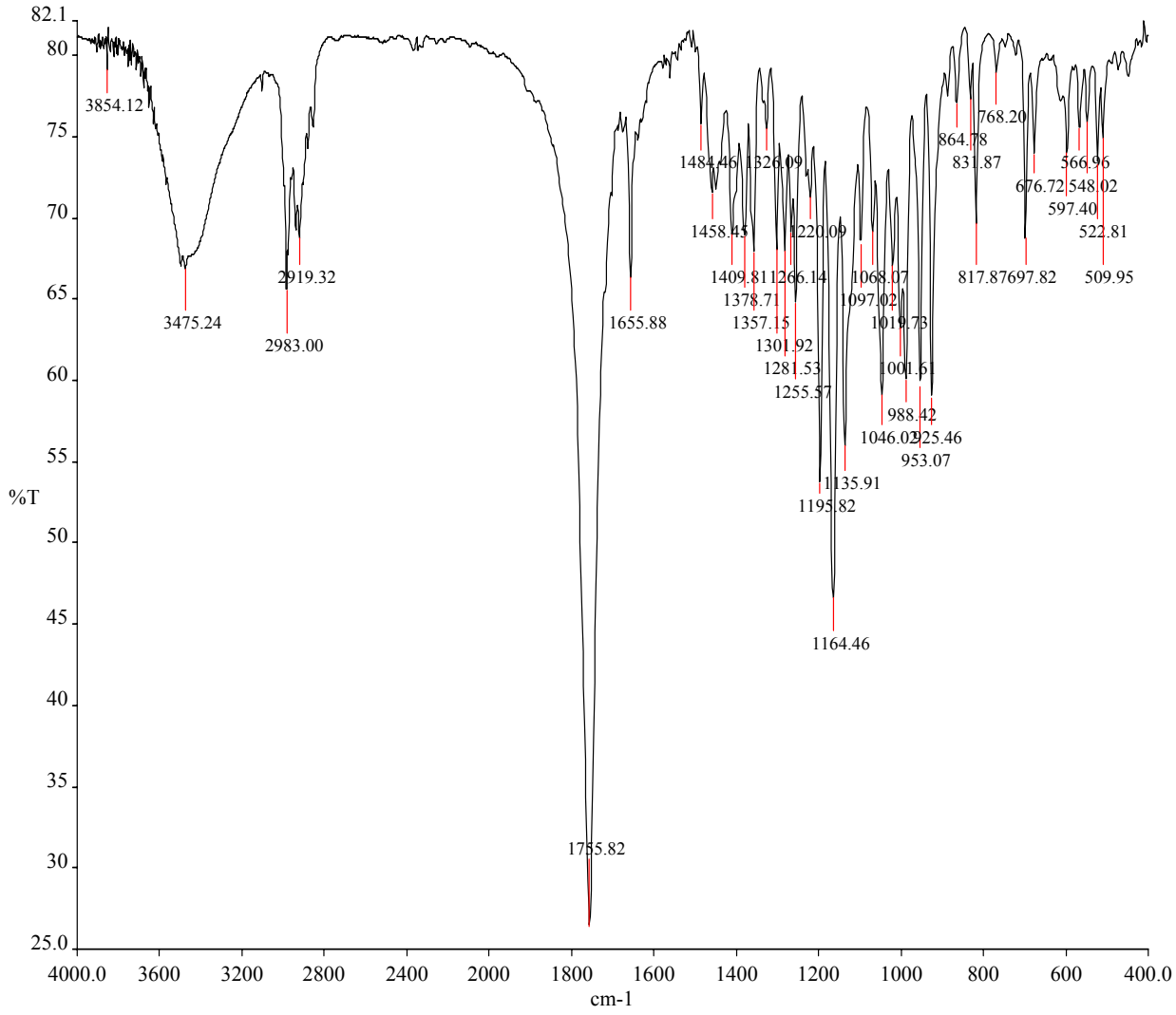
This investigation was conducted because of the importance of compounds in the genus *Anthemis*, especially the sesquiterpene lactones which are of great interest for their cytotoxic properties. Since many sesquiterpene lactones are also antimicrobial agents, it is possible that they also exert their action by altering the microbial composition. Two sesquiterpene lactones were isolated and characterized from *Anthemis tigreensis*. To the best of our knowledge compound **At-17** is reported for the first time.

5. Reference

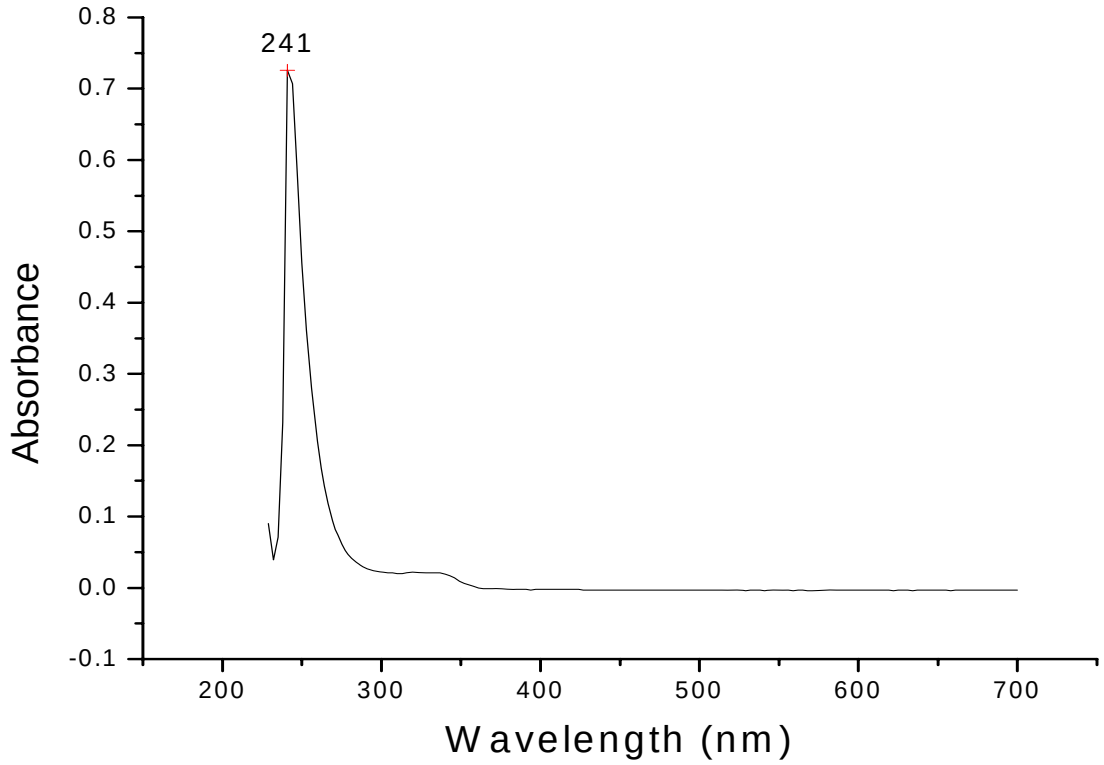
1. Croteau, R.; Kutchan, T. M.; Lewis, N.G. Natural Products (Secondary Metabolites). *Amarican Society of Plant Physiologists* **2000**, 1250-1251.
2. Mann, J.Secondary Metabolism 2nd ed., Clarendon press, Oxford, **1987**.
3. Sarker, S. D.; Latif, Z.; Gray, A. I. Natural Product Isolation 2nd ed., Humana press, Totowa, New Jersey, **2006**.
4. http://en.wikipedia.org/wiki/Terpene
5. Jelena, H. C.; Michel, R. CO_2 as main carbon source for isoprinoid biosynthesis via the mevalonet-independent methylerythirol 4-phosphate route in the marine diatoms *Phaeodactylum tricornutum* and *Nitzschia ovalis*. Phytochemistry **2000**, 53, 21.
6. Shanker, D.V.; Bhalla, R.; Luthra, R. An overview of the non-Mevalonate pathway for terpenoid biasynthesis in plants. *J.Biosci*. **2003**, 28, 638.
7. Dewick, P.M.; Medicinal natural products 2nd ed., John Wiley and Sons, LTD, England, **2001**, 194.
8. file://A:\Nutrition Toxicology Sesquiterpene Lactons.htm
9. Saroglou, V.; Karioti, A.; Heilmann, J.; Kypriotakis, Z.; Skaltsa, H. Sesquiterpene Lactons from *Anthemis melanolepis*. *Helvetica Chimica Acta* **2007**, 90,171-172.
10. Mesfin, T. Flora of Ethiopia and Eritrea, The national Herbarium, Addis Ababa Universty V.4, P.2, 167.
11. Vuckovic, I.; Vujisic, L.; Vajs, V.; Tesevic, V.; Janackovic, P.; Milosavljevic, S. Phytochemical investigation on *Anthemis cotula*. *Serb. Chem. Soc*. **2006**, 71,127-129.
12. http://en.wikipedia.org/wiki/*Anthemis*
13. Javidnia, K.; Miri, R.; Kamalinejad, M.; Sarkarzadeh, H.; Jamalian, A. *Flavour Fragr. J.* **2004**, 19, 213.
14. John, V.; Hans, B.; Susannah, A.; Wilhelm, B. *Org. Biomol. Chem.* **2003**, 1, 1503.
15. Fichtl, R.; Admasu, A. Honeybee Flora of Ethiopia, 1st ed., Margraf verlag, Weikershein German, **1994**, 33.

16. Sindu, D., Msc Thesis, Phytochemical Studies on *Anthemis tigreensis*. Addis Ababa University, Addis Ababa Ethiopia, **2005**.
17. Bruno, M.; Bondi, M.; Vasallo, N.; Gedris, T.E.; Herz, W.Guaianolides and other terpenoides from *Anthemis aetenesis*. *Phytochemistry* **1997**, 45, 375.
18. Maria, K.; Anastasia, K.; Spyros, S.; Helen, S. *J. Nat. Prod*. **2003**, 66, 699.
19. Vajs, V.; Todorovic, N.; Bulatovic, V.; Menkovic, N.; Macura, S.; Juranic, N.; Milosavojevic, S. Further Sesquiterpene Lactons from *Anthemis carpatica*. Phytochemistry **2000**, 54,629.
20. Jordanka, S.; Milka, T.; Ljuba, E. *Z. Naturforsech* **2002**, 57c, 769.
21. Staneva, J. D.; Todorova, M. N.; Evstatieva, L. N. *Biochemical systematics and Ecology* **2005**, 33, 97.
22. Vlatka, V.; Bulatovic,V.; Fodulovic-Savikin, K.; Menkovic, N.; Menkovic, N.; Macura, S.; Juranic, N.; Milosavljevic, S. Highly oxygenated guaianolides from *Anthemis cretica* subsp. *Cretica*. *Phytochemistry* **1999**, 50, 287.
23. Tschan, G. M.; Koenig, G. M.; Wright-Anthony, D.; Sticher, Otto. Chmaemeloside, a new flavonoid glycoside from *Chamaemelum* nobile. *Phytochemistry* **1996**, 41, 643.
24. Bruno, M.; Maggio, A.; Arnold, N. A.; Diaz, J.G.; Herz, W. Sesquiterpene lactones from *Anthemis plutonia*. *Phytochemistry* **1998**, 49, 1739.
25. Masterova, I.; Grancai, D.; Grancaiova, Z.; Pour, M.; Uhik, K. A New Flavonoid: Tinctosid from Anthemis tinctoria. *Pharmazie* **2005**, 12, 956.
26. Theodori, R.; Karioti, A.; Rancic, A.; Skaltsa, H. Linear Sesquiterpene Lactones from Anthemis auriculata and Their Antibacterial Activity. **2006**, 69, 663.

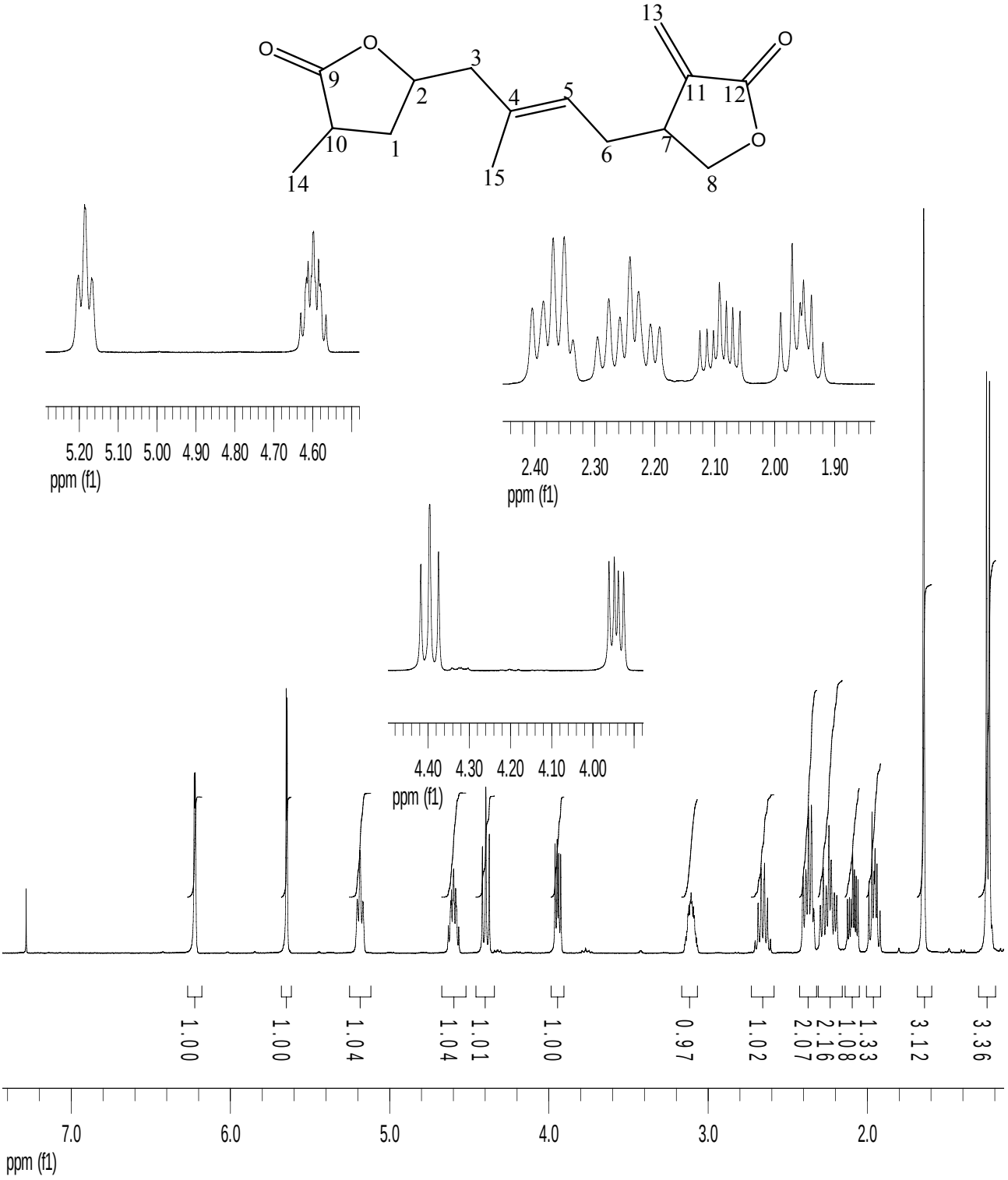
Appendix 1. IR spectrum of **At-3**.



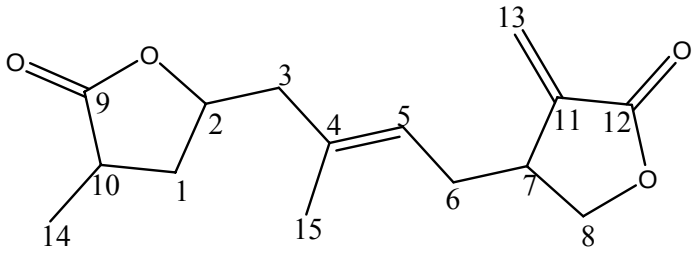
Appendix 2. UV-visible absorption spectrum of **At-3**.

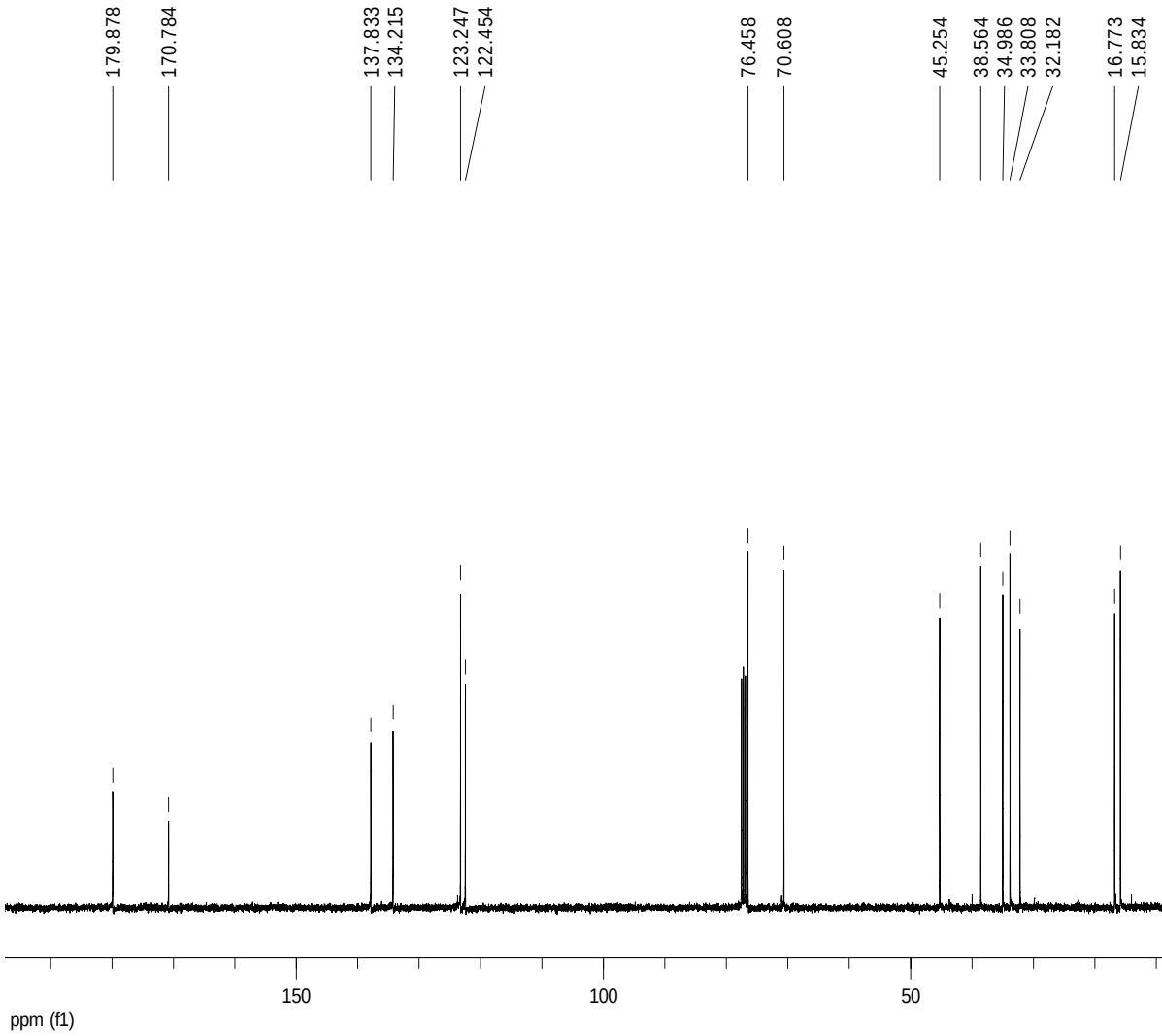


Appendix 3. ^{1}H-NMR spectrum of **At-3**.

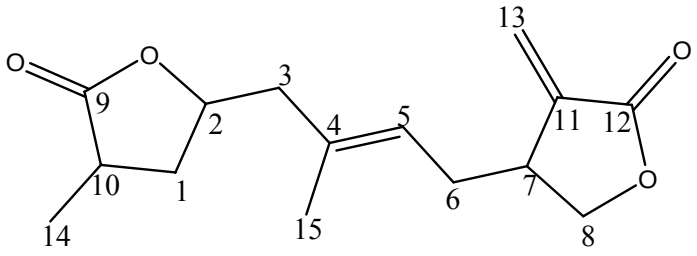


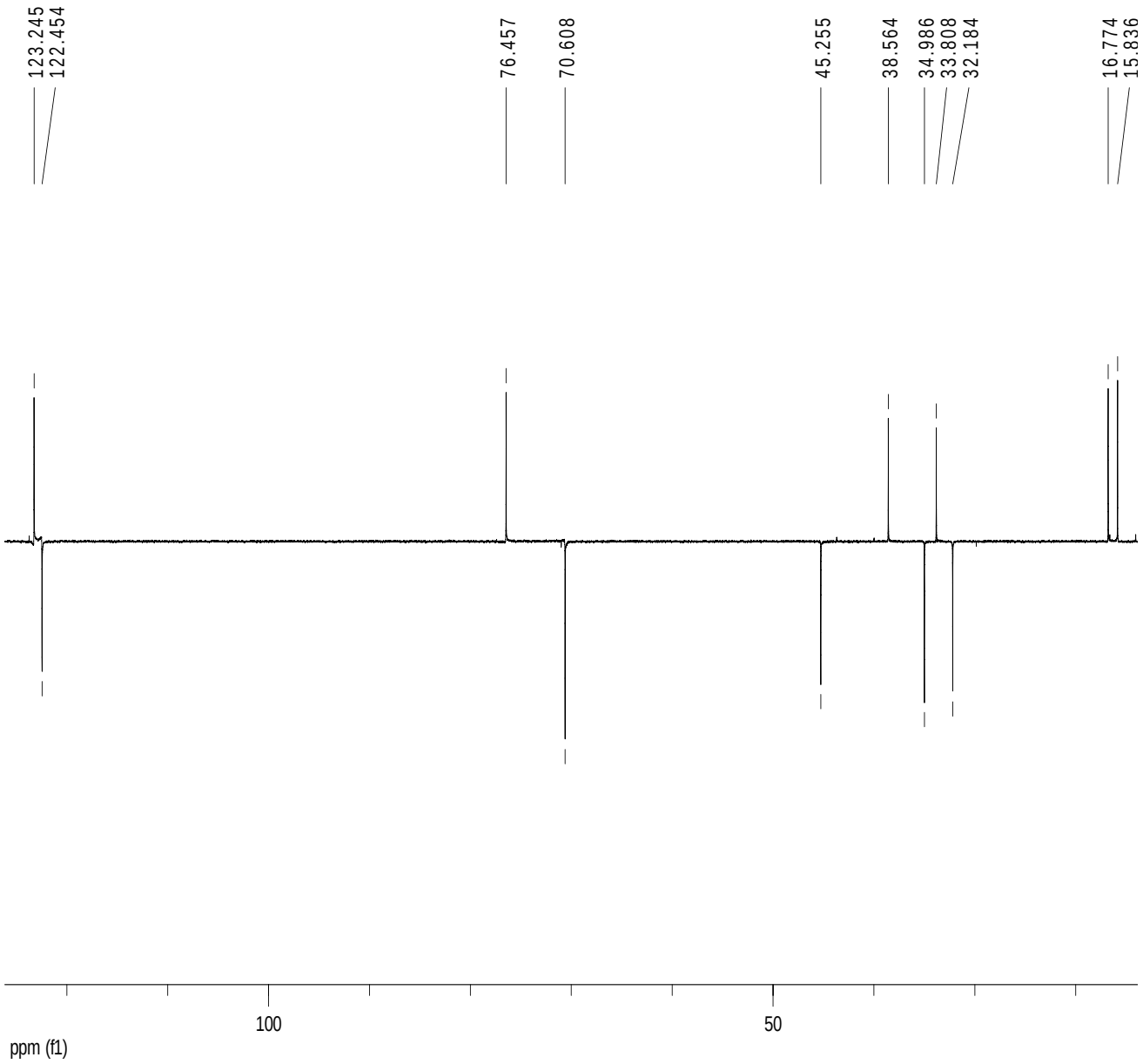
Appendix 4. [13] C-NMR spectrum of **At-3**.



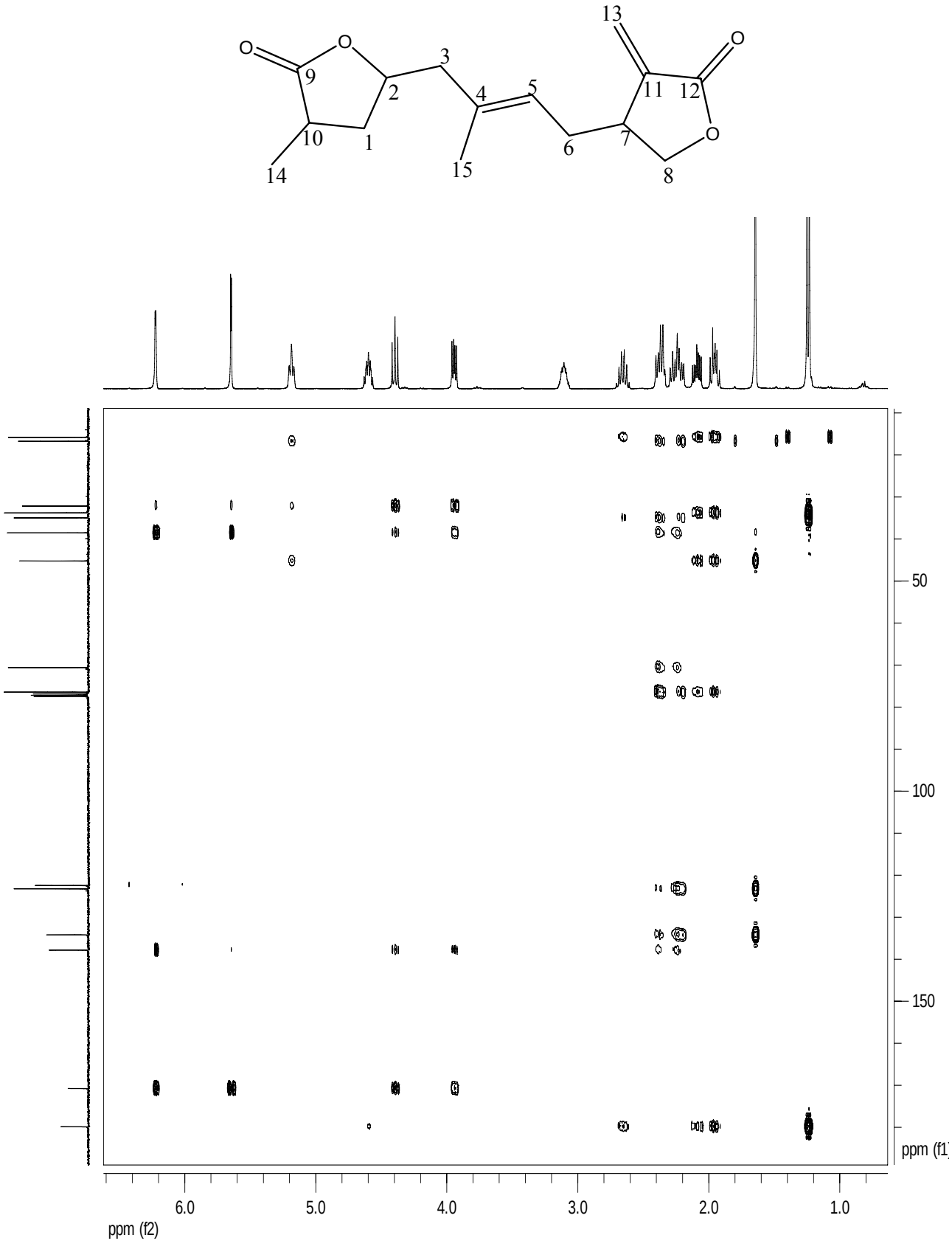


Appendix 5. DEPT-135 spectrum of **At-3**.

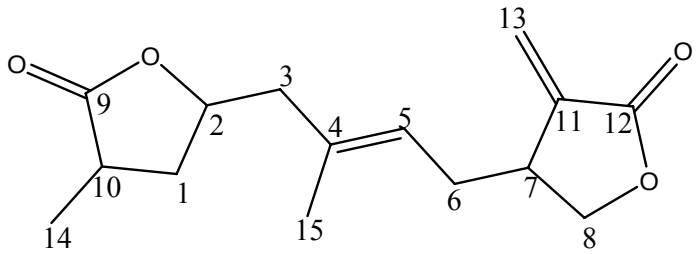


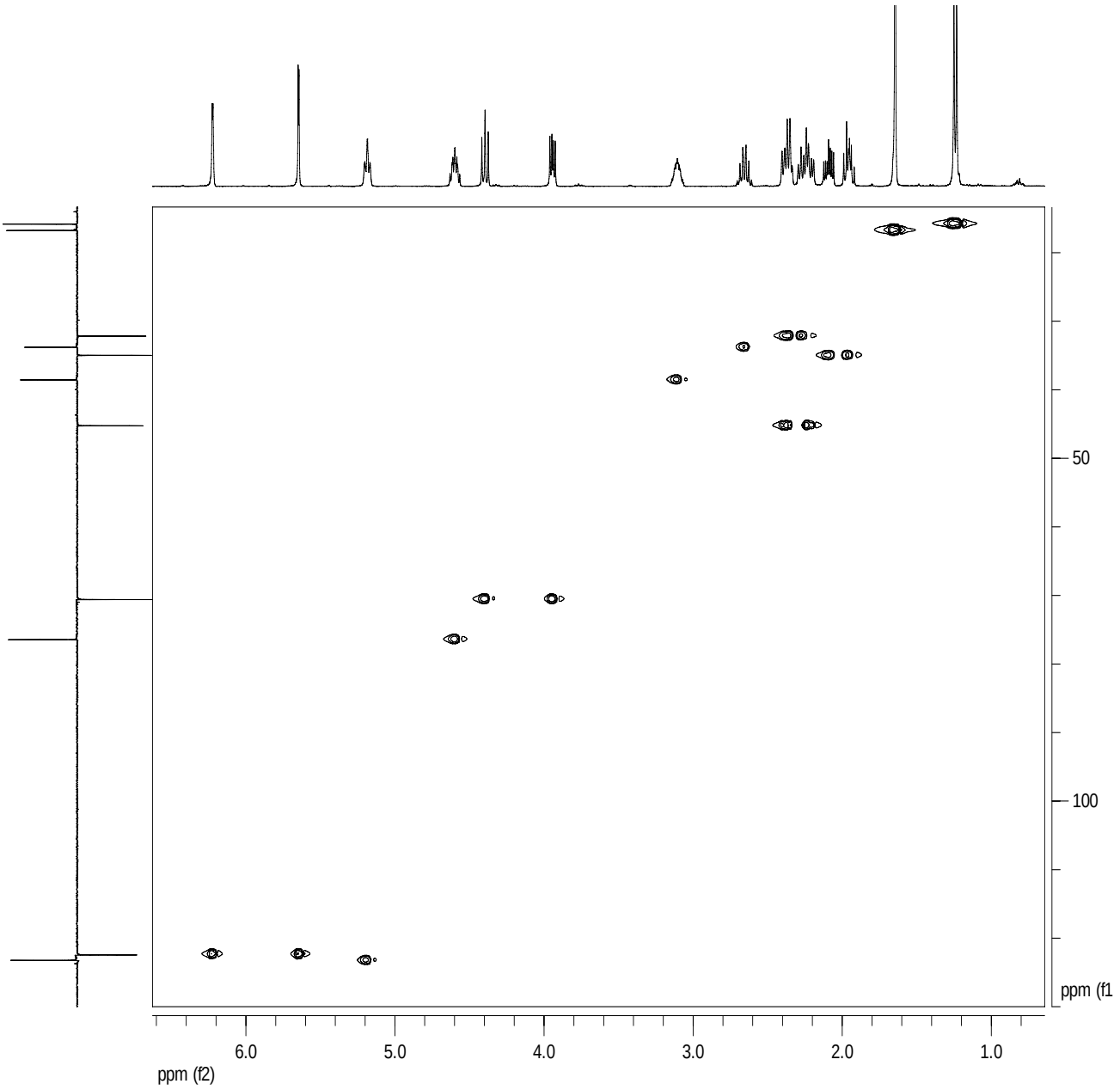


Appendix 6. HMBC spectrum of **At-3**.

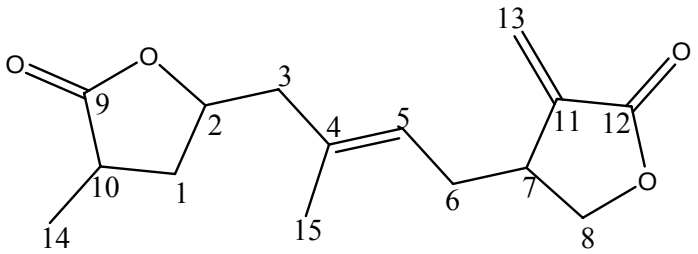


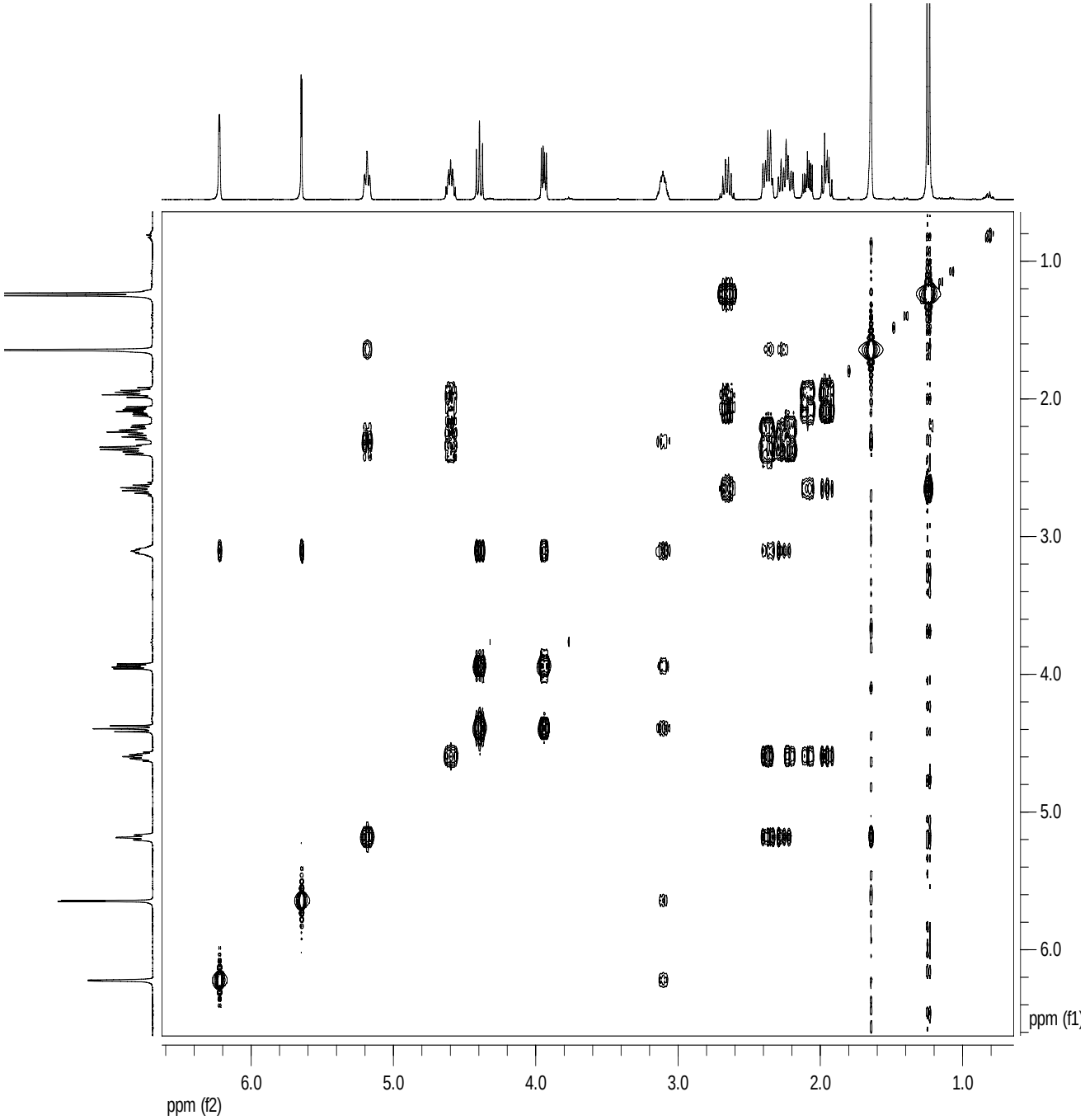
Appendix 7. HSQC spectrum of **At-3**.



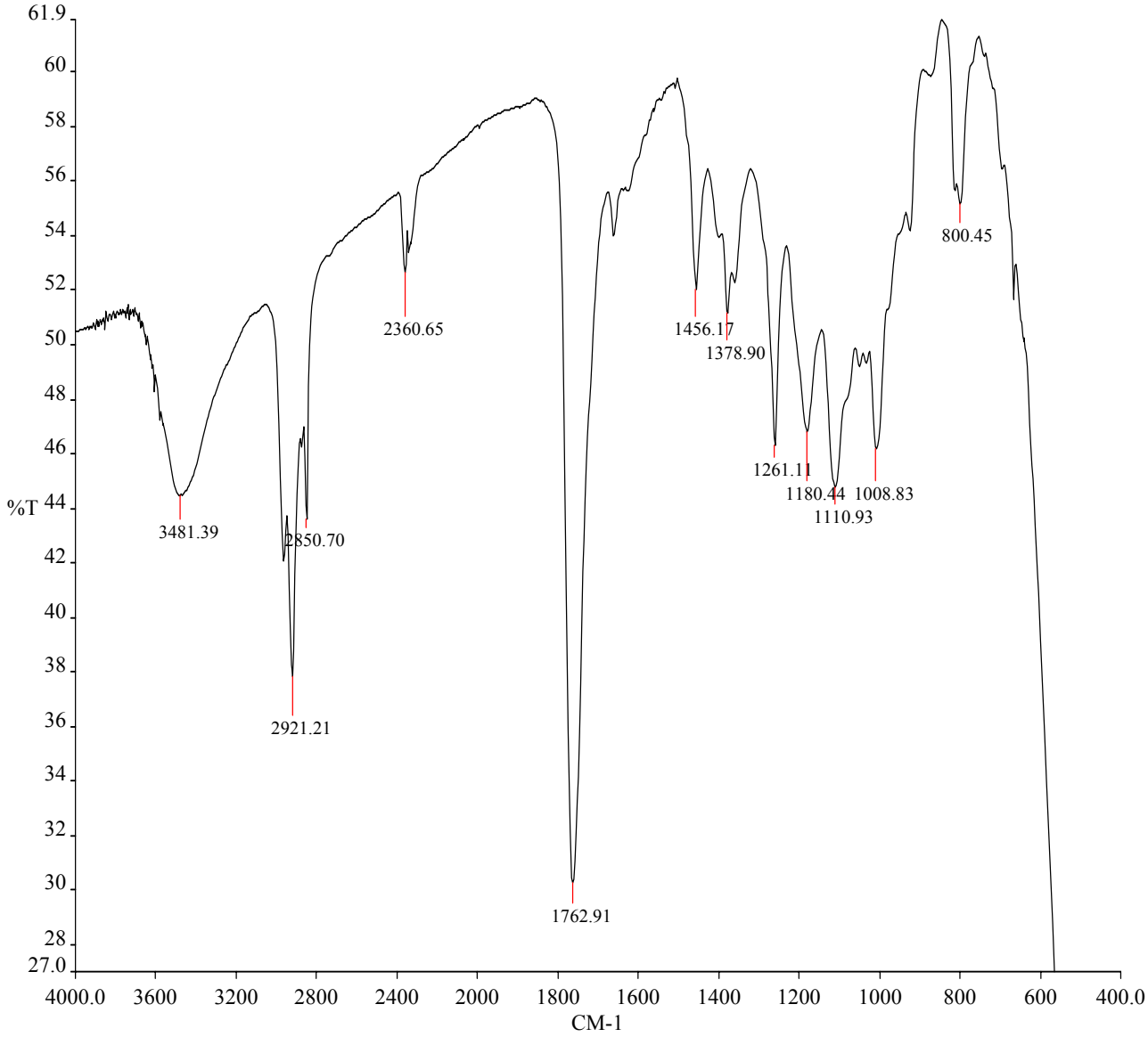


Appendix 8. COSY spectrum of **At-3**.

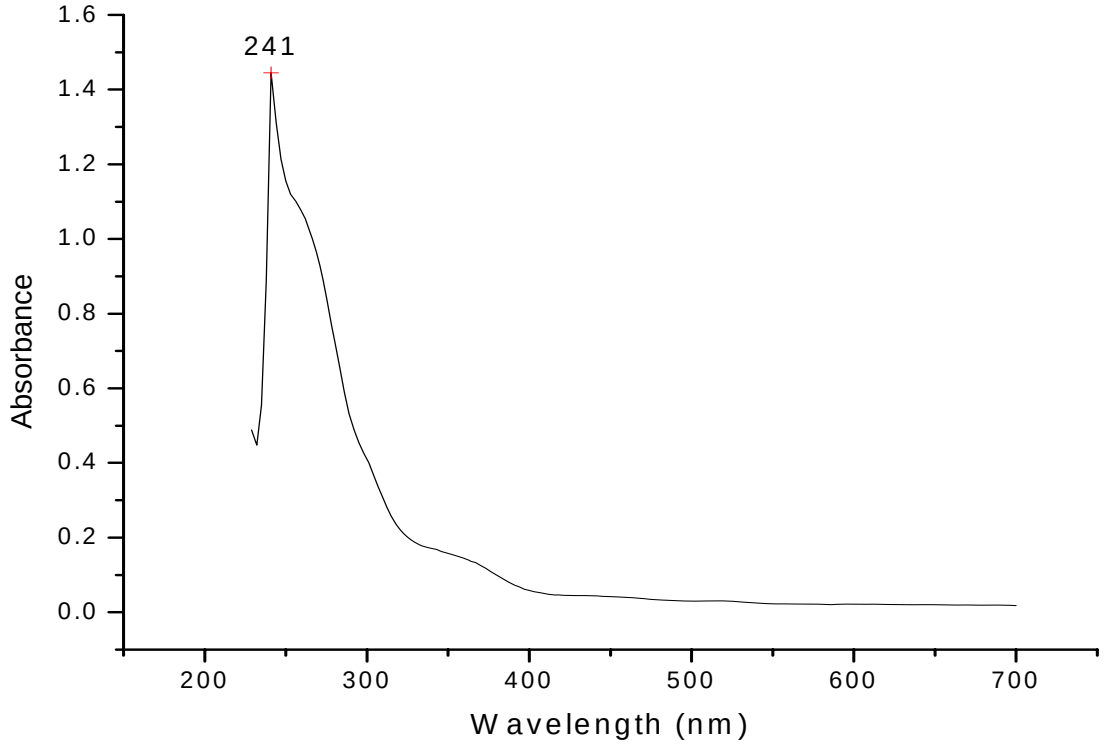




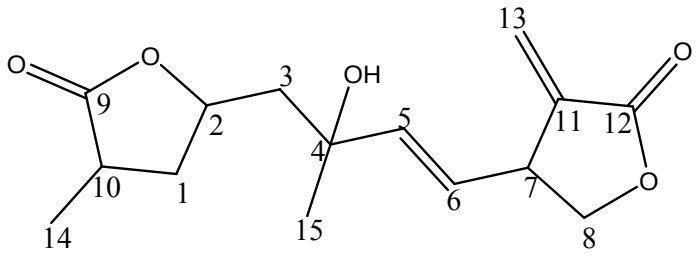
Appendix 9. IR spectrum of **At-17**.

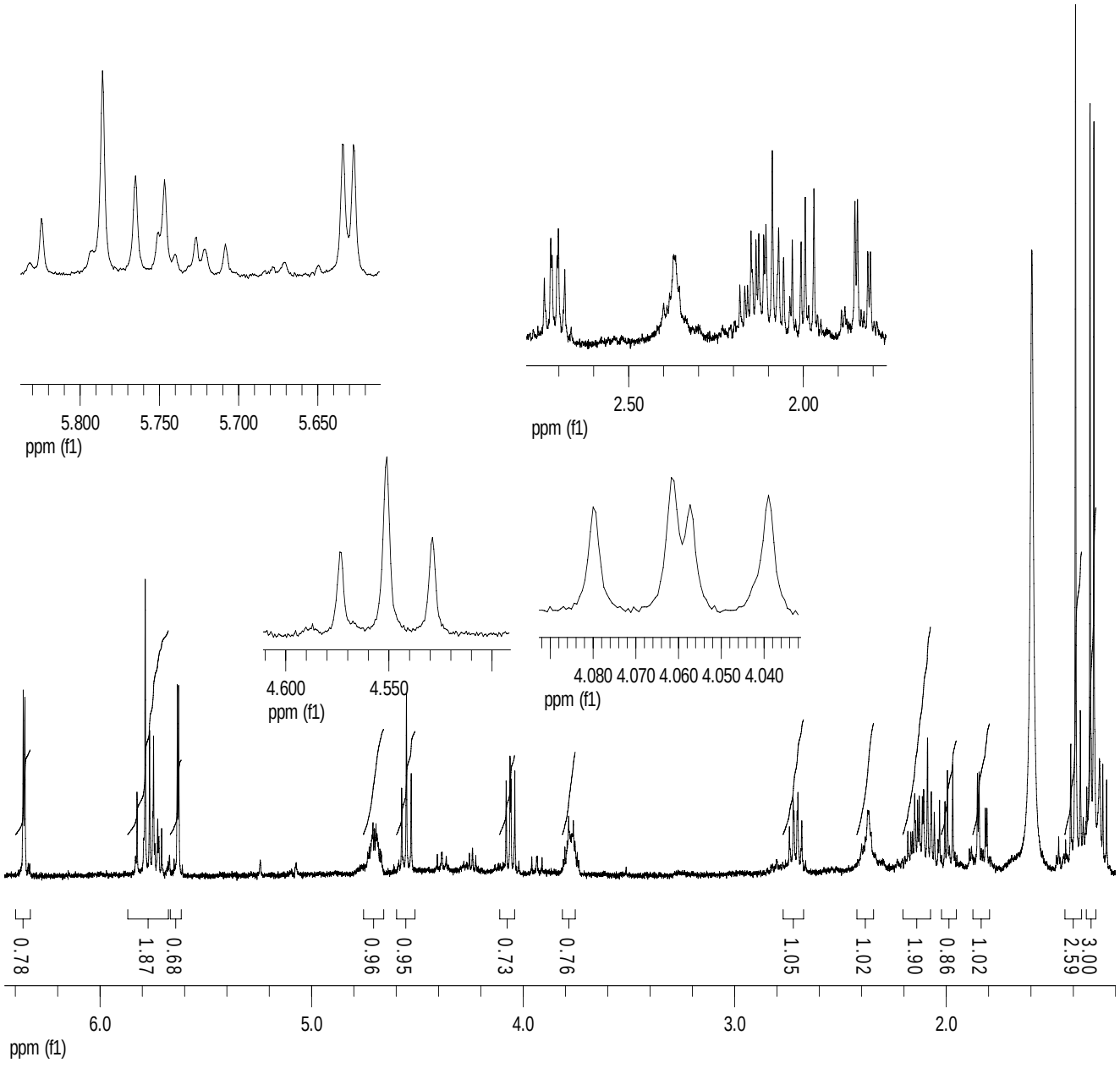


Appendix 10. UV-visible absorption spectrum of **At-17**.

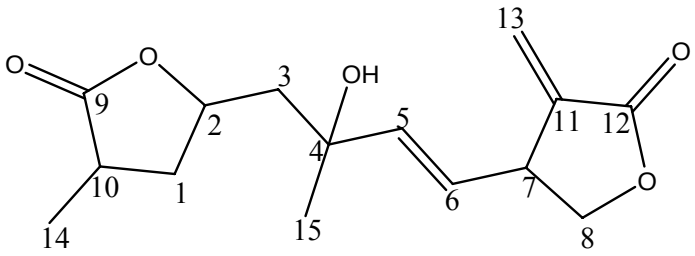


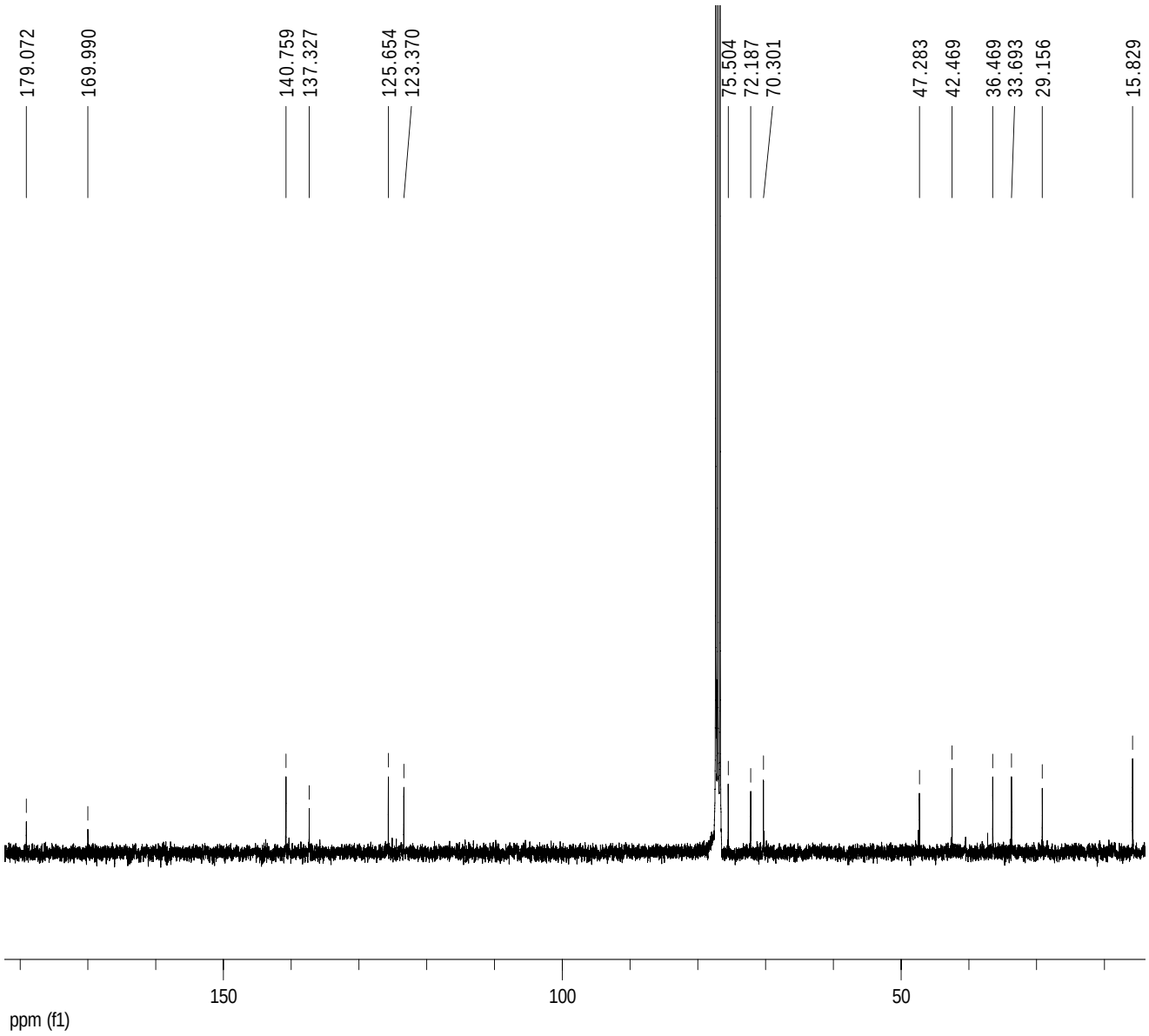
Appendix 11. ^{1}H NMR spectrum of **At-17**.



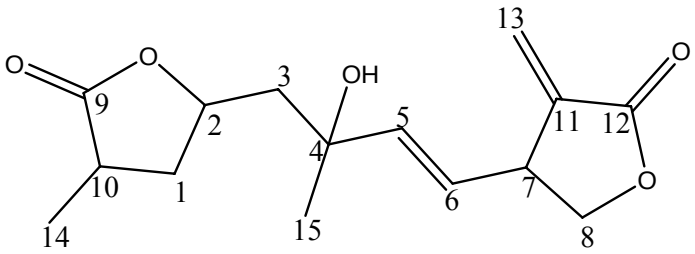


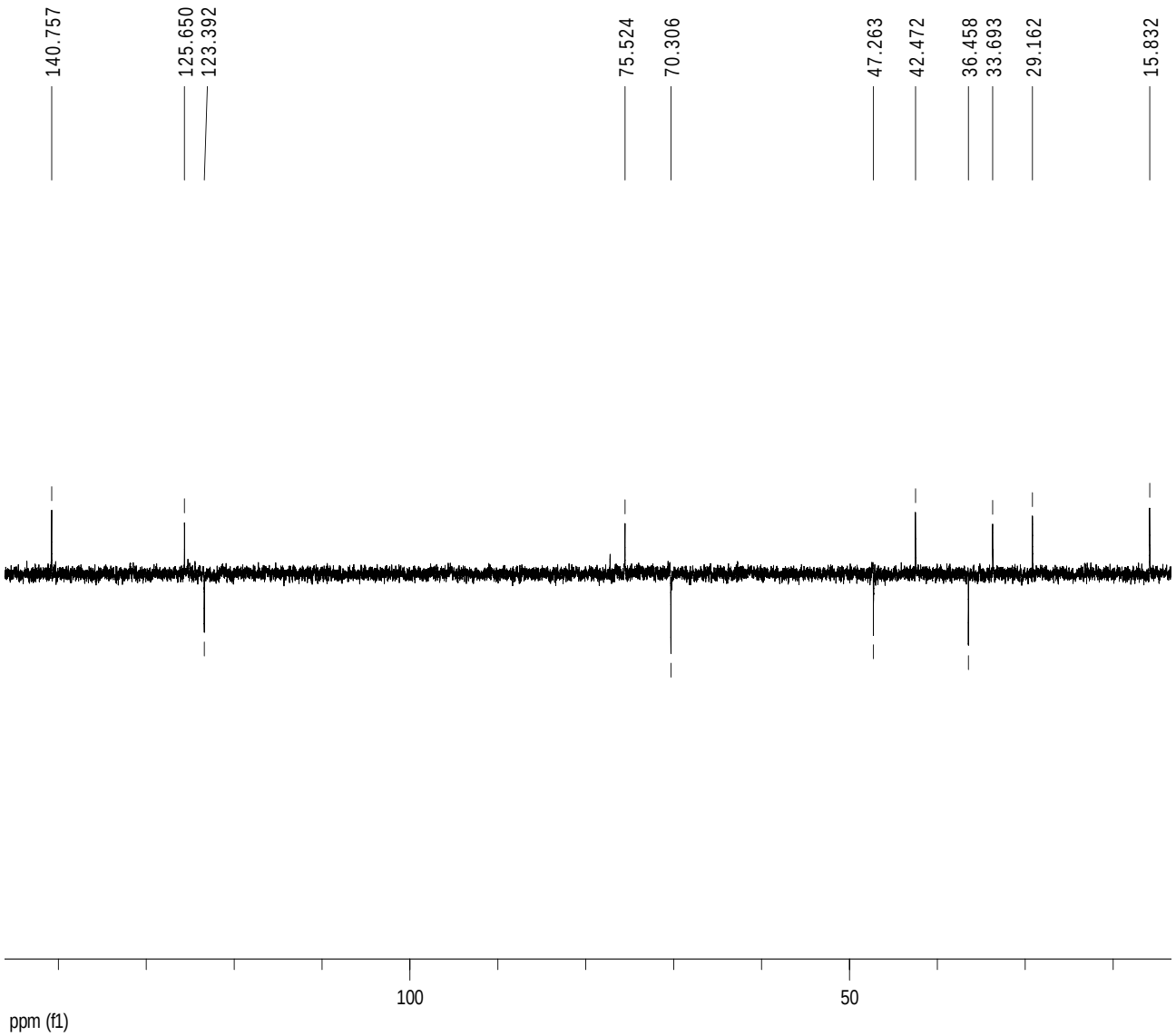
Appendix 12. ^{13}C NMR spectrum of **At-17**.



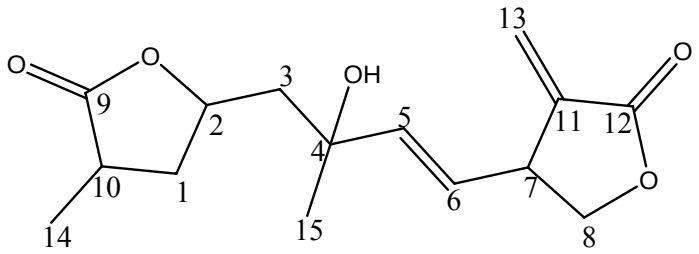


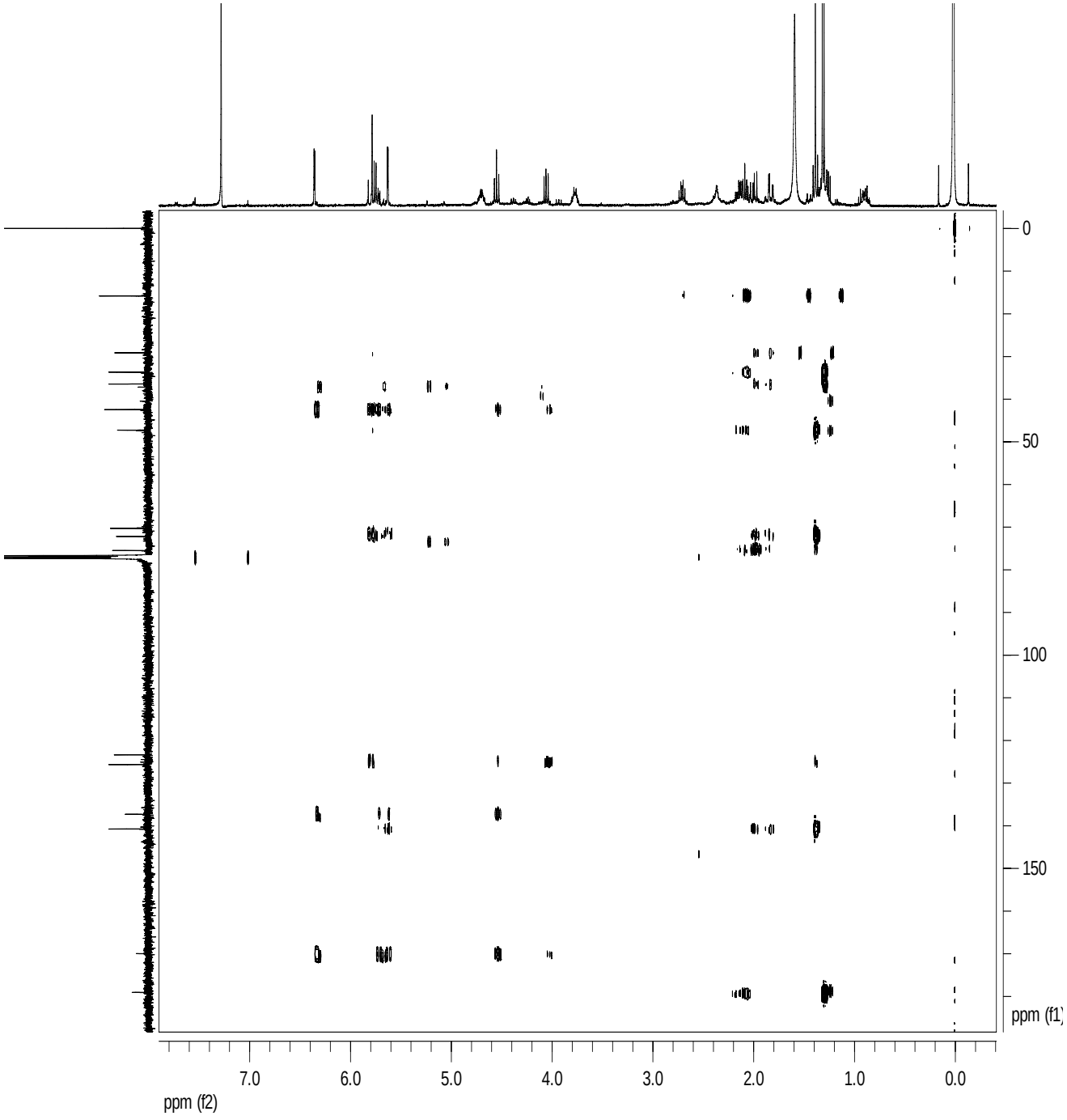
Appendix 13. DEPT-135 spectrum of **At-17**.



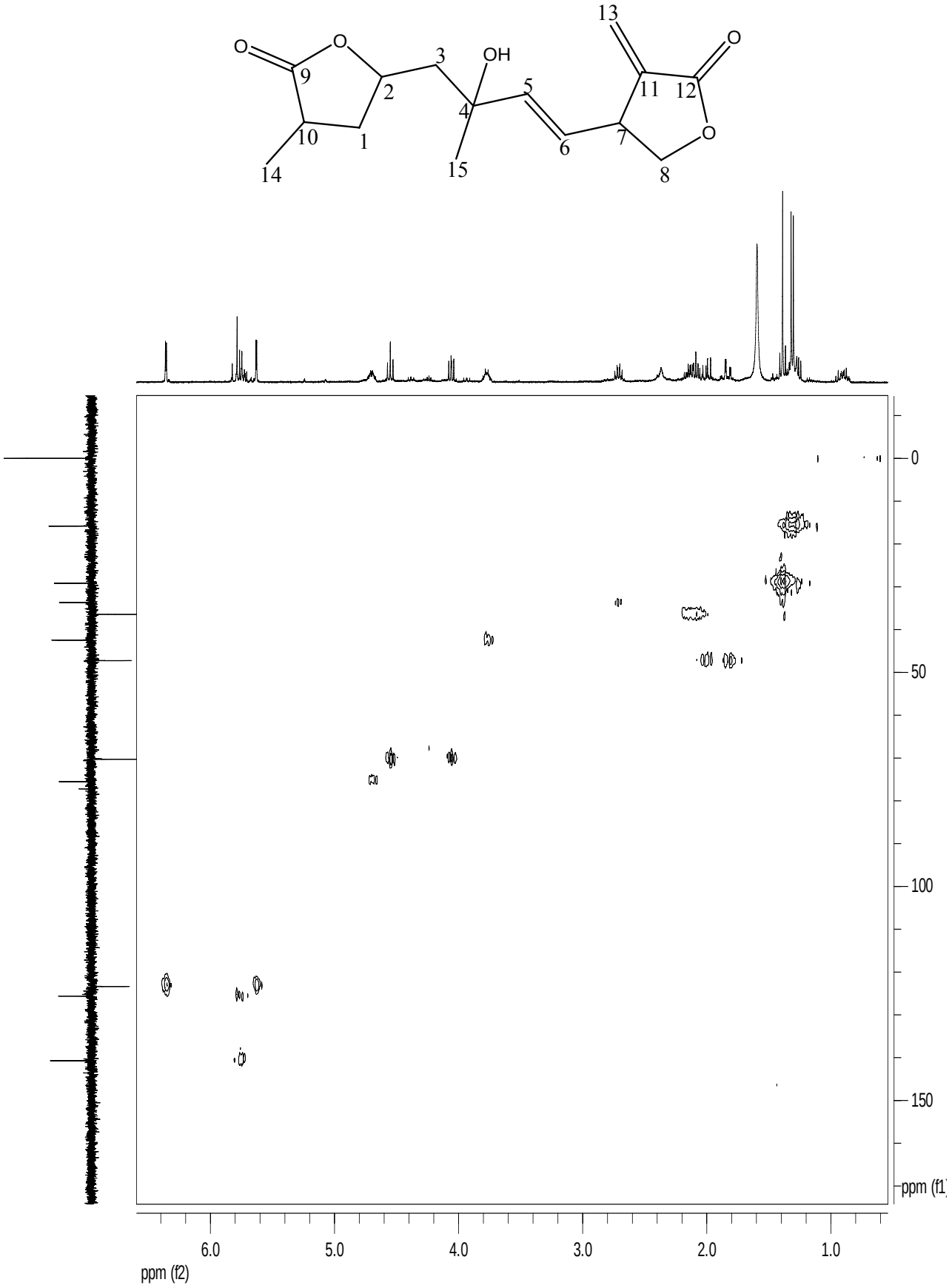


Appendix 14. HMBC spectrum of **At-17**





Appendix 15. HSQC spectrum of **At-17**.



Appendix 16. COSY spectrum of **At-17**.