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PHYTOCHEMICAL INVESTIGATION ON THE PETROLEUM ETHER EXTRACT OF THE AERIAL PARTS OF *LAGGERA TOMENTOSA*

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PHYTOCHEMICAL INVESTIGATION ON THE PET-ETHER EXTRACTS OF THE AERIAL PARTS OF *LAGGERA TOMENTOSA*.

Abstract

Phytochemical investigation on the pet-ether extract of the aerial parts of *Laggera tomentosa* afforded two eudesmane derivatives, 4α-acetoxy-3α-angeloyloxy-7,11-dehydroeudesman-8-one and 4α-acetoxy-3α-(2',3'-epoxy-2'-methylbutyrate)-11-hydrroxy-6,7-dehydro-eudesman-8-one. 4α-acetoxy-3α-ang-eloyloxy-7,11-dehydroeu- desman-8-one has been isolated before from *L. tomentosa* and *Bluma alata*. 4α-acetoxy-3α-(2',3'-epoxy-2'-methylbutyrate)-11-hydroxy-6,7-dehydro-eudesman-8-one has been isolated before from *Pluchea odorata* but not isolated from *Laggera tomentosa*. Their structures have been established by spectroscopic means.

1. Introduction

1.1. Natural products

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 amino acids, carbohydrate, protein, and nucleic acids, are found in all plants and animals to perform metabolic roles that are essential and usually evident[1].

The secondary metabolites are often referred to as “natural products”. These can be subdivided into terpenoids, alkaloids, shikimates and polyketides. The classification is based on the means by which the materials were produced. The reaction path leading to a particular natural product is called the *biosynthetic pathway*, and the corresponding event is known as the *biogenesis*. Different plant and animal species can employ dramatically different biosynthetic pathways to produce the same metabolite [2]**.**

Natural products chemistry covers the chemistry of naturally occurring organic compounds, their biosynthesis, function in their own environment, metabolism, and more conventional branches of chemistry such as structure elucidation and synthesis [3].

1.2. Terpenes

Terpenes are widespread in nature, mainly in plants as constituents of essential oils. Many terpenes are hydrocarbons, but oxygen-containing compounds such as alcohols, aldehydes or ketones (terpenoids) are also found. Fortunately, despite of their structural diversity, they have a simple unifying feature by which they are defined and by which

they may be easily classified. Terpenes are a unique group of hydrocarbon based natural products whose structure may be derived from isoprene units [4].

1.2.1. Classification of Terpenes

Terpenes are classified based on the number of 5-carbon units (isoprene) they contain (Hemiterpenes C_5, Monoterpenes C_{10}, Sesquiterpenes C_{15}, Diterpenes C_{20}, Sesterpenes C_{25}, Triterpenes C_{30}, and Tertaterpenes C_{40}). Like all natural products, within this simple classification lies an enormous amount of structural diversity which leads to a wide variety of terpene like (terpenoid) compounds [5].

At a more homely level they have become articles of commerce in the perfumery food industries as being the basis of cosmetics, soaps, flavors, and coloring as well as being used in disinfectants, detergents, and in many medical preparations and vitamin supplements [6].

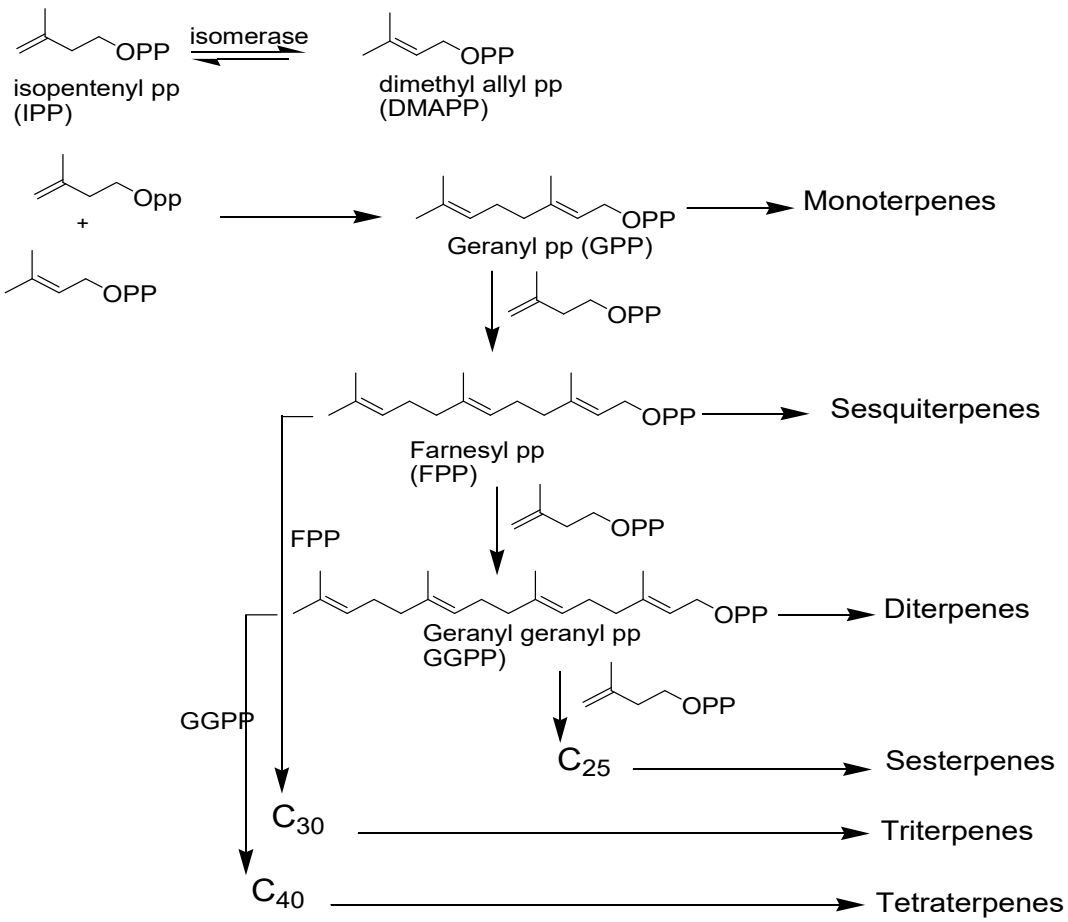
1.3. Biogenesis of Terpenes

Terpenes are derived biosynthetically from units of isoprene, which has the molecular formula C_5H_8. The basic molecular formula of terpenes are multiples of that, $(C_5H_8)n$ where n is the number of linked isoprene units. This is called the *isoprene rule* or the *C5 rule*. The isoprene units may be linked together "head to tail" to form linear chains or they may be arranged to form rings. One can consider the isoprene unit as one of nature's common building blocks.

Isoprene itself does not undergo the building process, but rather activated forms of isopentenylpyrophosphate (IPP) and dimethylallylpyrophosphate (DMAPP), are the components in the biosynthetic pathway [7].

Isopentenyl pyrophosphate and dimethylallyl pyrophosphate combine to yield Geranyl-pyrophosphate leading to monoterpenes. Similarly, compound derived from farnesyl-

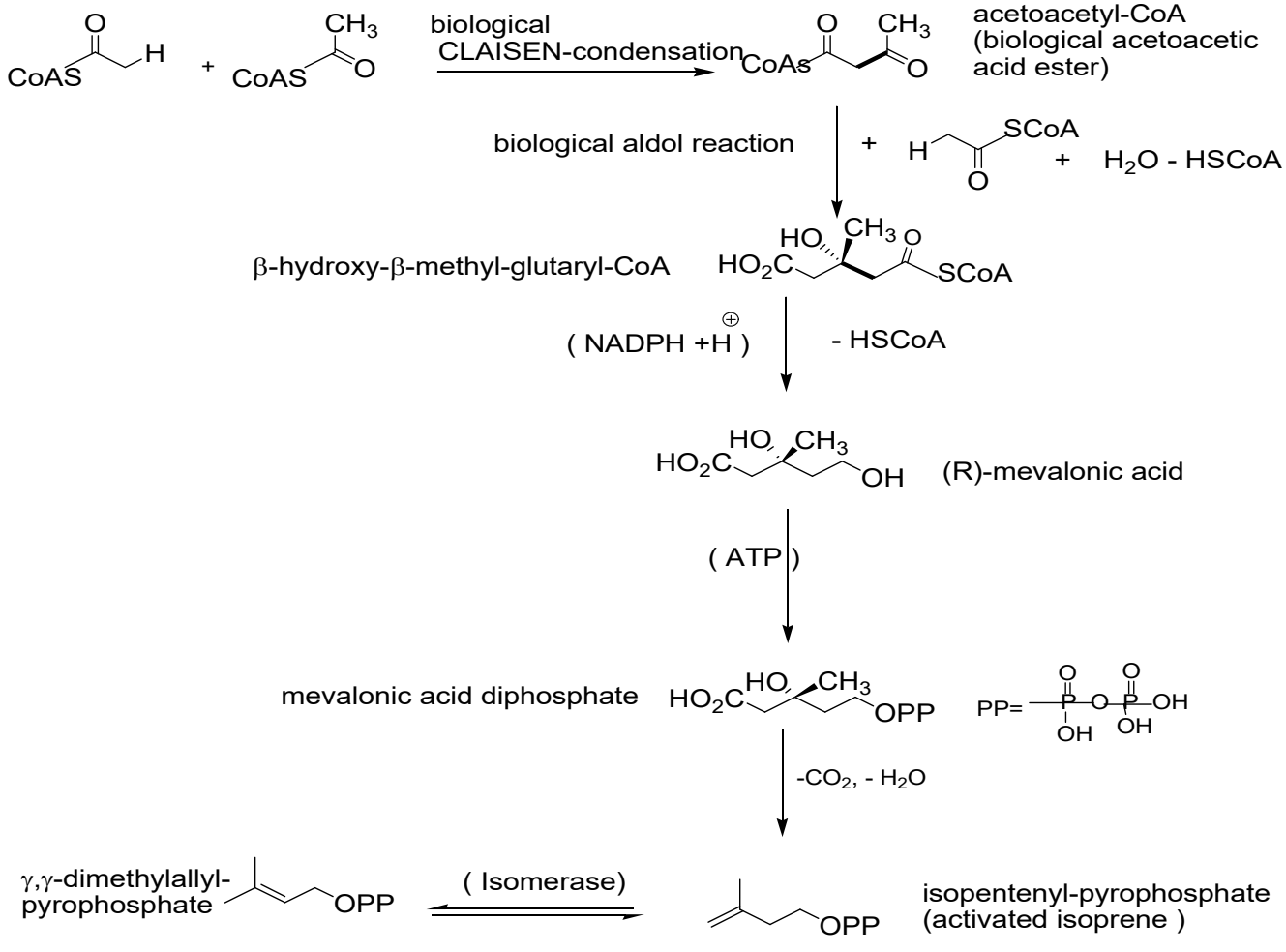
pyrophosphate lead to sesquiterpenes, and triterpenes are formed from two equivalents of farnesylpyrophosphate. These various combinations and oxidations give rise to large variety of terpenes [8]. This implies that the central pathway up to C_{25} compounds is formed by sequential addition of C_5 moieties derived from isopentenyl-pyrophosphate(IPP) to a starter unit derived from dimethylallylpyrophosphate (DMAPP).The parents of the C_{30} and C_{40} compounds are formed by reductive coupling of two FPP (i.e. C_{15}-residues) or GGPP (i.e. C_{20}-residues) respectively. This means that the condensing enzymes have involved to couple two equivalent units only and that generation of C_{25} or C_{35} compounds by condensation of two sizable unequal units is not possible [6].



Scheme 1. Biosynthesis of terpenes.

1.3.1 Biogenesis of isopentenylpyrophosphate

All terpenes can be derived from an isoprene unit. Isoprene itself does not function as the reactive biogenetic species. Isopentenylpyrophosphate and dimethylallyl-pyrophosphate are the reactive species involved in the formation of terpenes. Isopentenylpyrophosphate (IPP) is an intermediate in the classical, HMG-CoA reductase pathway used by organisms in the biosynthesis of tepenes. IPP is formed from Acetyl-CoA via Mevalonic acid. IPP can then be isomerized to dimethylallyl-pyrophosphate by the enzyme isopentenylpyrophosphate isomarase [9].



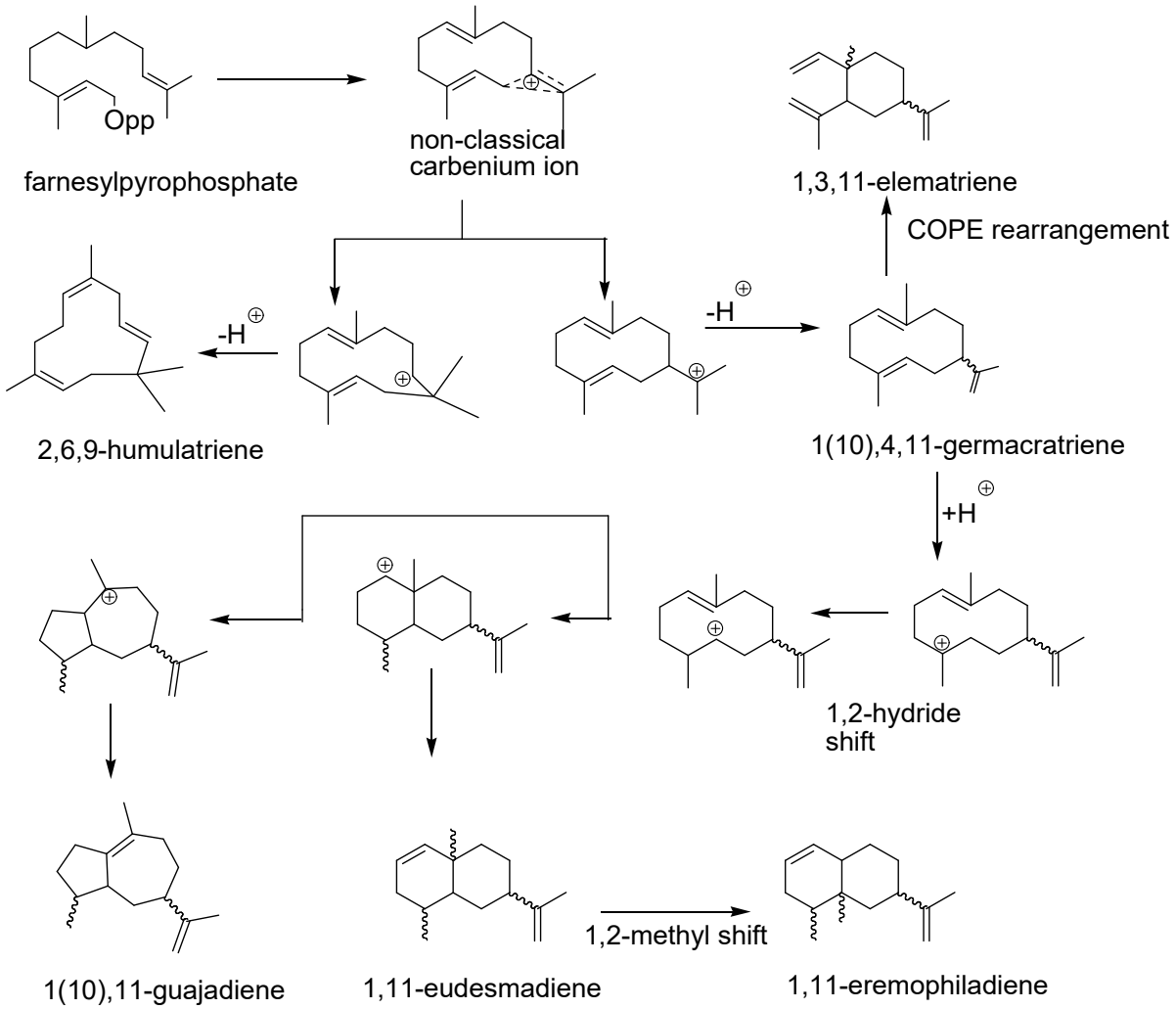
Scheme 2. Biogenesis of ispentenylpyrophosphate

1.3.2 Sesquiterpenes

Sesquiterpenes are defined as the group of 15 carbon compounds derived by the assembly of three isoprene units and they are found mainly in higher plants but also in invertebrates. Sesquiterpenes with monoterpenes are an important constituent of essential oils in plants. They are the most diverse group of isoprene. In plants, they function as pheromones and juvenile hormones. Sesquiterpene structures present several acyclic, mono-, bi-, tri-, and tetra cyclic systems [10].

1.3.3 Biogenesis of Sesquiterpenes

Addition of further C_5 isoprenediphosphate (IPP) unit to geranyldiphosphate in an extension of the prenyltransferase reaction leads to the fundamental sesquiterpene precursor, farnesyldiphosphate (FPP). FPP can then give rise to linear and cyclic sesquiterpenes. They can be cyclized by subsequent rearrangement of the resulting carbonium ions [11].



Scheme 3. Biogenesis of sesquiterpenes

1.4. The Genus *Laggera*

The Asteraceae (also known by the older alternative name composite) is one of the largest families of vascular plants with about 1535 genera and about 23,000 species [12]. It is widely distributed with the herbaceous species found predominantly in temperate regions and the larger trees mainly at high altitudes in tropical areas [13].
The genus *Laggera*, in the family Asteraceae has about 20 species. In Ethiopia there are six Laggera species, namely *L. Crispata* (Vahl) Hepper and Wood, *L. braunii* (vatke), *L. Elatior* (R.E.Fries), *L. crassifollia* (Sch. Bip exa. Rich) oliv and Hern., *L. alata* (D. Don) oliv., *L. tomentosa* (Sch. Bip exa. Rich) oliv and Hern [14].

The *Laggera* species have been used in ethanomedical practices in different countries. In Asia and Africa *Laggera alata* (D. Don) SCH.-BIP. ex. OLIVER and *Laggera pterodonta* (DC) BENTH are employed as traditional herbal medicines because of their anti-inflammatory and antibacterial activities. The green herbal medicinal plant *Laggera pterodonta* shows anti-leukemia, anti-phlegm, and anti-bronchitis activities. Recently, much attention has been paid to *Laggera* species and their chemical contents because of their multifaceted activities. Extensive studies of *Laggera* have led to the identification of many compounds, such as monoterpenes, sesquiterpenes, cyclitols, and flavonoids [15].

The chemical studies of different *Laggera* species have been done before. In most species, eudesmane and their derivatives are the common chemical compounds. Beside to these, there are other terpenes and their derivatives, which are constituents of the genus.

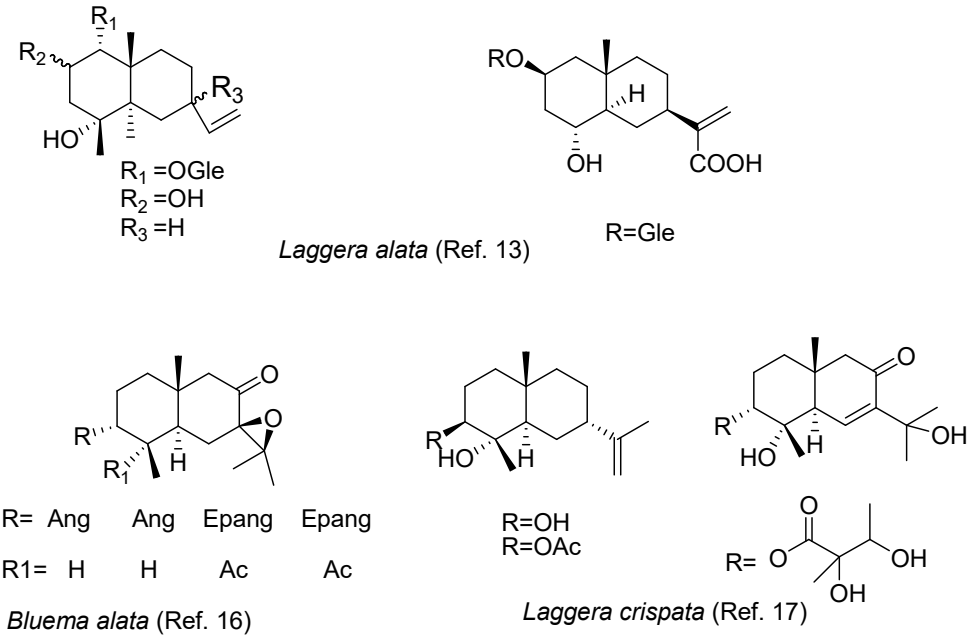


Figure 1 Some components of crude extract of *Laggera* species.

1.5. *Laggera Tomentosa*

Laggera tomentosa (known locally as “Keskese”) is a perennial fragrant bushy herb (0.5-1.2 m high) and endemic to Ethiopia. It grows on dry hill, mountain slopes at altitude of 2345-2950 m. In Ethiopia, it is found in Tigray, Gonder, Gojjam, Wollo, Shoa and Arsi [14].

Locally, the plant is used in traditional medicine. The juice of the crushed plant is ingested as a treatment for stomachache, and is used against migraine. It can also be used as a fumigant and for cleansing milk containers.

Chemical investigation on the essential oil of *L. tomentosa* has been reported before [19]. However, only few compounds have been isolated from the solvent extract of *Laggera tomentosa* [2, 11, and 20].

2. Objectives of the project

The main objective of the project is to isolate and characterize the chemical constituents of the petroleum-ether extract of the aerial parts of *Laggera tomentosa*. The plant was selected for the study because it is endemic to Ethiopia and important in traditional medicine.

3. Results and Discussion

Two compounds LTP-F4 and LTP-F6 were isolated and characterized from the petroleum-ether extract of *Laggera tomentosa*. Structure elucidations of the compounds were based on the spectroscopic data obtained for the compounds and in comparison with the data in the literature for similar compounds.

3.1. Characterization of LTP-F4

The UV spectrum **(**Appendix 1**)** revealed the absorption band at 259.4 nm indicating that the molecule has an α-β unsaturated carbonyl group and due to $\pi \longrightarrow \pi^*$. The ^{1}H NMR (Appendix 2) showed a quartet peak at δ 6.08 integrating for one proton accounted for the presence of olefinic proton on C-3'. A doublet peak observed at δ 1.99 integrated for three protons of methyl group attached to olefinic carbon(C-3').

The three singlet peaks at δ 1.80, 2.01, and 1.91 integrated for three protons each attributed to methyl groups attached to olefinic C-11, C-11 and C-2' respectively. A singlet peak at δ 1.41 assignable to three protons of methyl group attached to oxygen-substituted quaternary carbon (C-4). A singlet peak at δ 0.99 showed for three protons of methyl group attached to a quaternary carbon (C-10). A doublet of doublet at δ 5.94 integrated for one proton that attached to oxygen substituted-tertiary carbon (C-3). A multiplet peak at δ 1.93 & 1.66 integrated for two protons indicating diastereotopic protons of methylene group (C-2). The doublet of doublet peaks at δ 2.69 & 2.27 integrated for one proton each attributed to diastereotopic protons on C-6. A doublet peak at δ 2.26 integrated for two protons that attached to C-9. A doublet of doublet peak at δ 3.17 integrated for one proton attached to tertiary carbon (C-5).

Table 1. ^{1}H NMR, ^{13}C NMR spectral data of LTP-F4

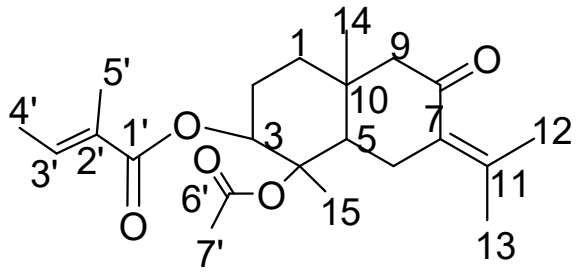
H or C	shift (ppm)	
1	1.57 m	37.66
2	1.93 m	25.61
	1.66 m	
3	5.94 dd	73.88
4	-	87.37
5	3.19 dd	44.75
6	2.69 dd	25.80
	2.27dd	
7	-	129.97
8	-	201.95
9	2.26 d	59.99
10	-	36.95
11	-	144.43
12	1.80 s	22.53
13	2.01 s	22.77
14	0.99 s	19.38
15	1.41 s	16.75
1’	-	166.99
2’	-	127.99
3’	6.08 q	137.79
4’	2.00 d	15.76
5’	1.91 s	20.67
6’	-	170.32
7’	1.99 s	23.34

The proton decoupled ^{13}C NMR (Appendix 3) spectrum of LTP-F4 showed well resolved resonance of the 22 carbon atoms. The multiplicity of each carbon atom was determined using DEPT experiment, which revealed the presence of seven methyl groups, four methylene groups, three methine groups, eight quaternary carbons (three carbonyl carbons, three vinylic carbons and two saturated carbons), indicating 32 hydrogen atoms attached to carbon atoms.

Table 2. ^{1}H NMR and ^{13}C NMR spectral of LTP-F4 compared with the data reported by Guilhon and Muller (Ref. 21).

H or C	LTP-F4	shift (ppm)	Ref. 21	
1	1.57 m	37.66	-	37.7
2	1.66 m	25.61		25.8
	1.93 m			
3	5.94 dd	73.88	5.93 dd	74.0
4	-	87.37	-	87.40
5	3.19 dd	44.75	3.16 dd	44.9
6	2.27 dd	25.80	2.69 dd	25.6
	2.69 dd			
7	-	129.97	-	129.9
8	-	201.95	-	201.8
9	2.26 d	59.99	2.26 d	59.9
			2.2 d	
10	-	36.95	-	36.9
11	-	144.43	-	144.5
12	1.80 s	22.53	1.96 d	23.3
13	2.01 s	22.77	1.77 d	22.5
14	0.99 s	19.38	0.95	19.4
15	1.41 s	16.75	1.38	16.7
1’	-	166.99	-	167
2’	-	127.99	-	128
3’	6.08 q	137.79	6.05	137.7
4’	2.00 d	15.76	1.96 d	15.7
5’	1.92 s	20.67	1.89 d	20.6
6’	-	170.32	-	170.3
7’	1.99 s	23.34	1.994 s	22.7

By comparing, the ^{1}H NMR and ^{13}C NMR spectral data of LTP-F4 with the data reported by Guilhon and Muller (Ref. 21), the structure of **1** was proposed for LTP-F4.



1

Moreover, the above prediction is also supported by using its 2D NMR spectral data as follows.

^{1}H-^{1}H correlation spectroscopy (COSY) (Appendix 4, Table 3) showed strong correlation between H-3 at δ 5.94 (dd) and H-2 at δ 1.93 (m) & 1.66 (m) showing that methyl protons at C-2 are diastereotopic and the oxygen carrying C-3 is chiral. Similarly, strong coupling between H-5 at δ 3.19(dd) and H-6 at δ 2.69(dd) & 2.27(dd) indicating methylene protons at C-6 are diastereotopic. There are also coupling between H-3′ at δ 6.07 and H-4′ at δ 2.00.

Table 3. ^{1}H-^{1}H COSY spectra data of compound LTP-F4

Position	H	COSY
1	1.57 m	H-2a, H-2b
2a	1.93 m	H-1, H-2b, H-3
2b	1.66 m	H-1, H-2a, H-3
3	5.94 dd	H-2a, H-2b
5	3.19 dd	H-6a, H-6b
6a	2.69 dd	H-5, H-6b
6b	2.27 dd	H-5, H-6a
3’	6.08 q	H-4’
4’	2.00 d	H-3’

Heteronuclear Multiple-Quantum Correlation (HMQC) (Appendix 5) experiments correlate the chemical shift of proton with the chemical shift of directly bonded carbon. It illustrates the three most important significant when applied to route structural problem. First, the ability to transfer the assignment of protons on to proton spectrum, extending characterization of the molecule. Second, the well dispersion of protons resonance according to heteronuclear. For example, the region between 1-2.1 ppm contains a number of overlapped signals which are difficult to assign each signal of protons. Clearly the picture is evident when emerges from heteronuclear carbon dimension. Third, it is possible to identify the correlation between geminal vicinal protons. In COSY, some times difficult to identify between geminal and vicinal protons owning to the lack of differentiation between geminal and vicinal protons.

Heteronuclear Multiple-Bond Correlation (HMBC) (Appendix 6 and table 4) experiment gave information about correlation of hydrogens and carbons that are two or three bonds away. It shows the correlation between protonated carbons and non-protonated carbons that are one or two bonds away from one another. It helps to draw the partial structure of the molecule.

Table 4. HMBC spectra data of compound LTP-F4

Position	H	C	HMBC
1	1.57 m	37.66	C-3, C-9, C-14
2	1.93 m	25.61	C-4
3	5.94 dd	73.88	C-4, C-1’
5	3.19 dd	44.75	C-4, C-6, C-9, C-10, C-1
6	2.69 dd	25.80	C-5, C-7, C-8, C-10, C-11
9	2.26 d	59.99	C-5, C-1, C-14
12	1.80 s	22.53	C-13, C-7, C-11
13	2.01 s	22.77	C-12, C-7, C-11
14	0.99 s	19.38	C-1, C-5, C-9
15	1.41 s	16.75	C-3, C-4, C-5
3’	6.08 q	127.99	C-1’, C-4’, C-5’
4’	2.00 d	15.76	C-3’, C-2’
5’	1.91 s	20.67	C-2’, C-3’, C-1’
7’	1.99 s	23.34	C-6’

Based on COSY and HMBC correlations the partial structures (1, 2 & 3) shown in figure 2 can be drawn.

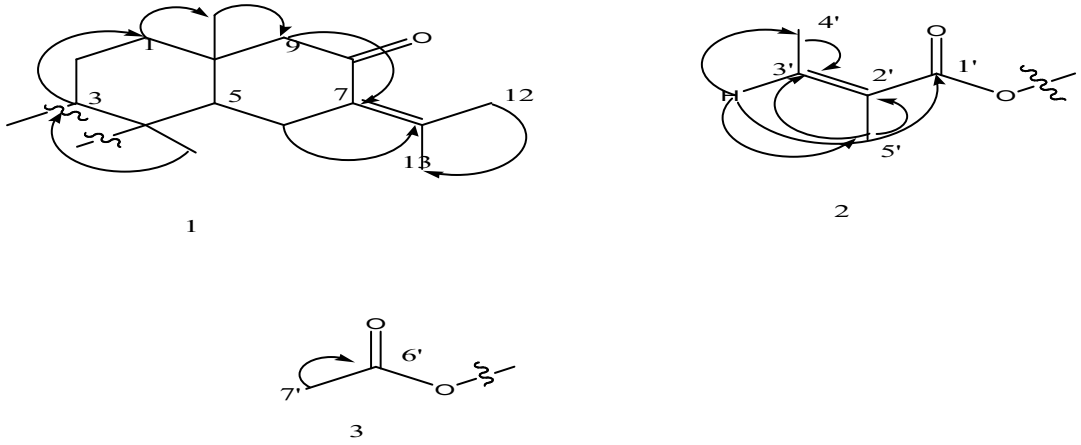
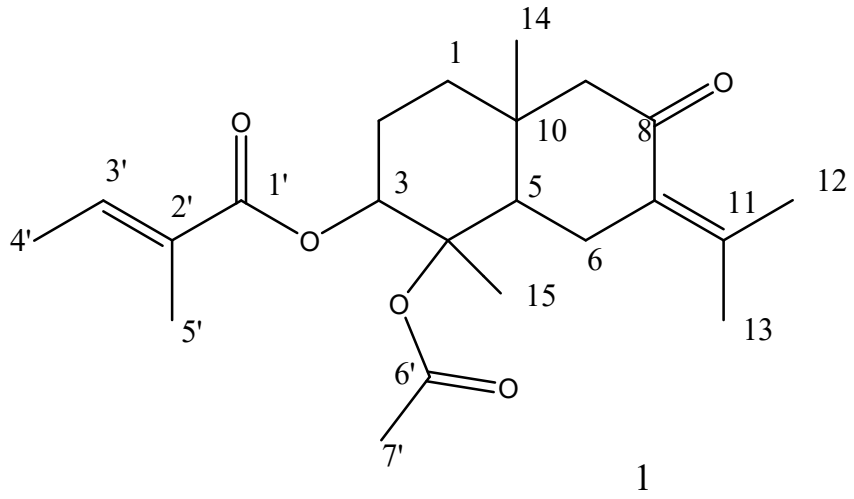


Figure 2. The proposed fragments of LTP-F4

Combination of fragments (Fig. 2) gave the full structure of **1** for compound **LTP-F4.**



This compound has been isolated before from *Bluma alata* [21] and *Laggera tomentosa* [11].

3.2 Characterization of LTP-F6

Characterization of LTP-F6 was done by using spectroscopic techniques. The UV spectrum (Appendix 7) revealed an absorption band at 284 nm. This is due the presence of an α-β

The ^{1}H NMR (Appendix 8) showed multiplet peaks at δ 1.61 (1H, m) and δ 1.57(1H, m) attributed to two protons attached to C-1. This shows diastereotopic protons of methylene group. The peaks at δ 2.03 (1H, m) and δ2.05 (1H, m) assignable to two protons on C-2, that are diastereotopic protons of methylene group. The broad triplet peak at δ 5.91 (1H, br t) integrated for one proton of methine group attached to oxygen substituted tertiary carbon. The doublet peak at δ 3.11 (1H, d) accounted for one proton attached to tertiary carbon. The doublet peak at δ 7.19 (1H, d) integrated for one proton of methine (C-6) group. The doublet peaks at δ 2.32 (1H, d) and 2.36 (1H, d) integrated for two protons of methylene group, that are not chemically equivalent due to diastereotopic property. The singlet peak at δ 1.49, 1.53, 1.03, and 1.61 each integrated for three protons of methyl groups. The quartet peak at δ 3.07 (1H, q) integrated for one proton of methine group (C-3'). The doublet peak at δ 1.34 (3H, d) integrated for three protons on the allylic carbon (C-4'). The singlet peak at δ 1.56 (3H, s) and 2.09 (3H, s) each integrated for three protons of the methyl groups.

Table 5. ^{1}H NMR, ^{13}C NMR and spectral data of LTP-F6

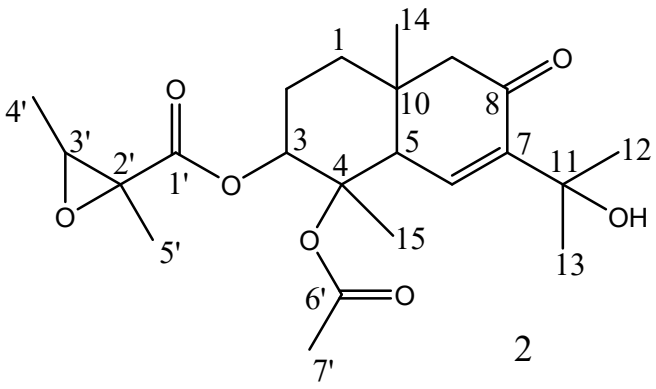
position	H	C
1	1.57 m	32.10
	1.61 m	
2	2.03 m	23.05
	2.05 m	
3	5.91 br t	73.20
4	-	81.55
5	3.11 d	48.90
6	7.19 d	143.40
7	-	142.55
8	-	197.24
9	2.32 d	58.08
	2.36 d	
10	-	38.94
11	-	83.47
12	1.53 s	24.49
13	1.49 s	24.54
14	1.03 s	18.40
15	1.61 s	18.98
1’	-	168.25
2’	-	60.04
3’	3.07 q	59.65
4’	1.34 d	13.92
5’	1.56 s	19.36
6’	-	169.55
7’	2.09 s	22.25

The proton decoupled ^{13}C NMR spectrum (Appendix 9, Table 5) of LTP-F6 showed 22 carbon atoms. The multiplicity of each carbon atom was determined using DEPT-135 experiment, which indicates the presence of seven methyl groups, three methylene groups, four methine groups and eight quaternary carbons indicating 31 hydrogen atoms attached to carbon atoms and two hydrogen atoms of hydroxyl groups are present.

Table 6. ^{1}H NMR and ^{13}C NMR spectral data of LTP-F6 compared with data reported by Nakanishi et al [22] and Arriaga et al [23].

Position	H	H [22 & 23]	C	C [22]
1	1.61 & 1.57 m	1.2 & 1.8 m	32.10	32.90
2	2.05 & 2.03 m	1.8 m	23.05	23.00
3	5.91 br t	5.93 dd	73.20	73.70
4	-	-	81.55	82.90
5	3.11 d	3.10 d	48.90	-
6	7.19 d	6.97 d	143.70	-
7	-	-	142.55	-
8	-	-	197.24	-
9	2.32 & 2.36 d	2.36 br s	58.08	-
10	-	-	38.94	-
11	-	-	83.47	-
12	1.53 s	1.48 s	24.50	-
13	1.49 s	-	24.55	-
14	1.03 s	1.02 s	18.40	-
15	1.61 s	1.57 s	18.98	19.1
1’	-	-	168.25	168.2
2’	-	-	60.04	59.9
3’	3.07 q	3.08 q	59.65	59.6
4’	1.34 d	1.34 d	13.91	13.9
5’	1.56 s	1.60 s	19.36	19.4
6’	-	-	169.55	169.3
7’	2.09 s	2.04 s	22.2	22.2

From ^{1}H NMR, ^{13}C (Appendix 8 & 9) of LTP-F6 and (table 6); it was proposed that the structure of the molecule is **2.**



2

The above prediction is supported by using its 2D NMR spectral data as follows

Table 7. ^{1}H-^{1}H COSY spectra data of compound LTP-F6.

Position	H	COSY
1a	1.61 m	H-1b, H-2a, H-2b
1b	1.57 m	H-1a, H-2a, H-2b
2a	2.05 m	H-2b, H-1a, H-1b, H-3
3	5.91 br t	H-2a, H-2b
5	3.11 d	H-6
6	7.19 d	H-5
9a	2.32 d	H-9b
9b	2.36 d	H-9a
3’	3.07 q	H-4’
4’	1.34 d	H-3’

^{1}H-^{1}H correlation spectroscopy (COSY) (Appendix 10, Table 7) showed strong correlation between H-3 at δ 5.91 and H-2 at δ 2.03; H-1 at δ 1.61 and H-2 at δ2.03; H-5 at δ3.11 and H-6 at δ7.19; H-3’ at δ3.07 and H-4’ at δ1.34.

These indicating that C-1, C-2 and C-3 are in the same region. C-3’ and C-4’ are bonded with one another in other than this region.

Hetereonuclear Multiple-Quantum Correlation (HMQC) (Appendix 11) experiment is one of the most commonly employed proton-detected single-bond correlation experiment. It provides a simple map of connectivities in which a crosspeak correlates two attached nuclei. It identify between geminal and vicinal protons. Because the geminal protons at different chemical shift correlate with a single carbon while vicinal protons of different chemical shift correlate with carbons with different chemical shift. So it simplifies the problem faced during protons assignment on proton spectrum.

Heteronuclear Multiple-Bond Correlation (HMBC) (Appendix 12 and table 8) experiment gave information about correlation of carbons and neighboring hydrogens that are two or three bonds away.

Table 8. HMBC spectral data of compound LTP-F6

Position	H	C	HMBC
1	1.57 m	32.10	C-5, C-3, C-4
2	2.03 m	23.05	C-10, C-1’
3	5.91 br t	73.20	C-1’, C-1, C-4, C-5
5	3.11 d	48.90	C-15, C-10, C-4, C-7, C-6
6	7.19 d	143.40	C-10, C-8, C-11, C-4, C-7
9	2.36 d	58.08	C-14, C-10, C-5, C-8, C-7
12	1.53 s	24.49	C-13, C-11, C-7
13	1.49 s	24.54	C-12, C-11, C-7
14	1.03 s	18.40	C-1, C-10, C-5, C-9
15	1.61 s	18.98	C-5, C-3, C-4
3’	3.07 q	59.65	C-4’
4’	1.34 d	13.91	C-3’, C-2’
5’	1.56 s	19.36	C-3’, C-2’, C-1’
7’	2.09 s	22.25	C-6’

Based on COSY and HMBC correlations the following fragments (I, II and III) can be drawn.

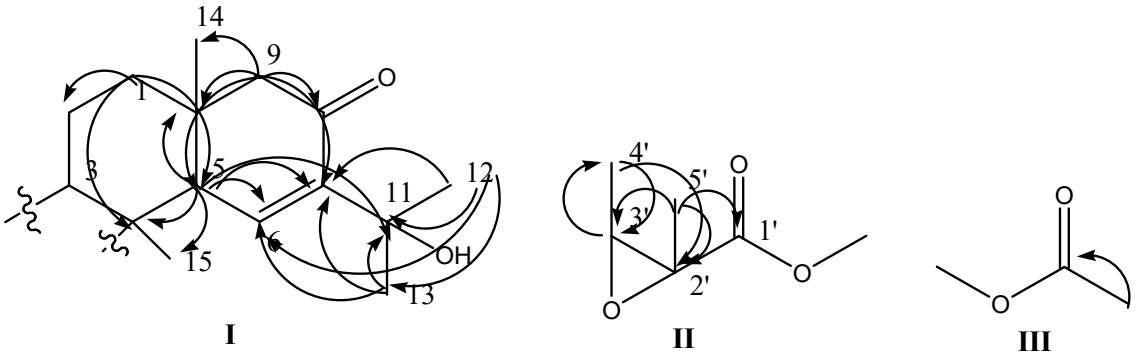
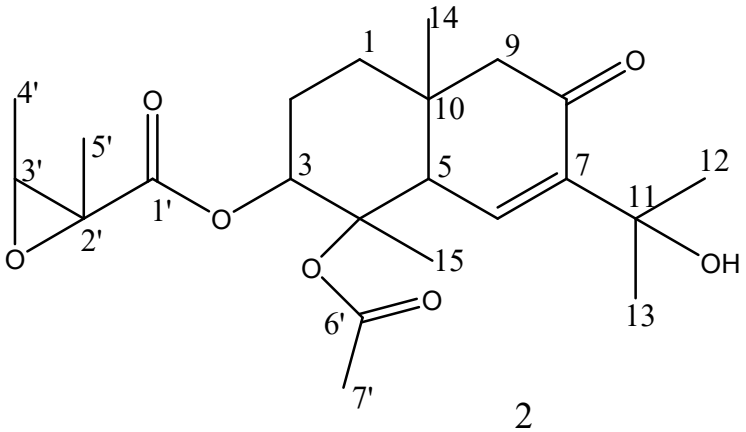


Figure 3. The proposed fragments of LTP-F6

Combinations of the fragments gave the proposed structure 2.



2

This compound has been isolated before from *Pluchea odorata* [23] but not from *Laggera tomentosa.*

4. Experimental

4.1. General

UV spectrum (in $CHCl_3$) was measured at room temperature.

^1H, ^{13}C, and 2D NMR experiments were conducted on a Bruker Advance 400 MHz spectrometer. The purity of compounds was monitored on silica gel GF_{254}. Analytical thin layer chromatographs were run on silica gel (Merck) coated on aluminum foil, 0.2mm thickness. The spots were detected by their UV fluorescence and by spraying with 0.5% vanillin in sulfuric acid solution.

Silica gel 60 (Merck), particle size 0.063-0.200 (70-230 mesh ASTM used for column chromatography.

4.2. Coding system

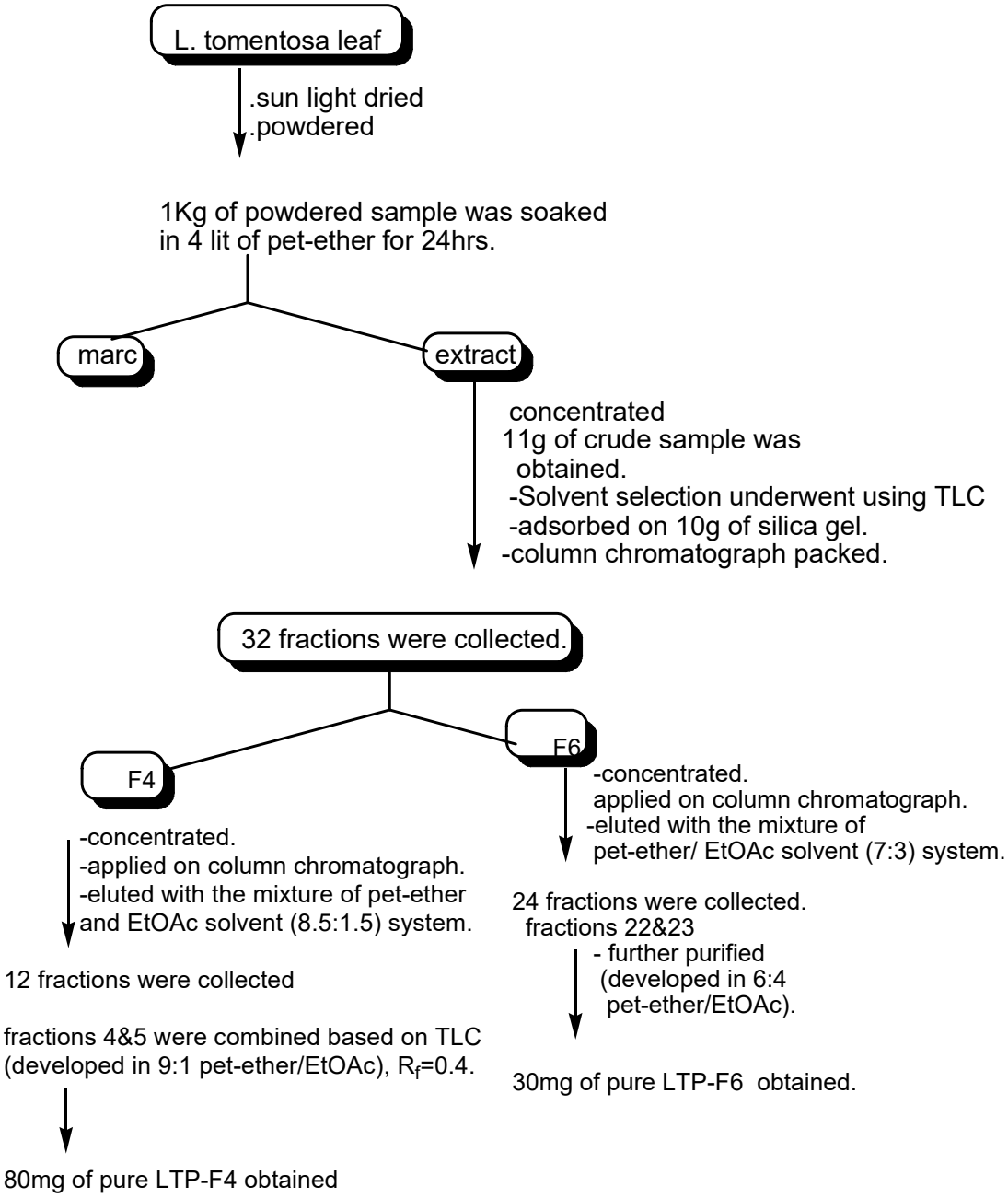
L stands for the genus name *Laggera*, T stands for species name *tomentosa*, P stands for pet-ether extract. F stands for fraction, 6 & 4 numbers.

4.3 Sample collection

Laggera tomentosa was collected from Daletti, Western Shoa of Ethiopia (26Km far from Addis Ababa near Alemgena) on February 22, 2009. A voucher specimen (SD 6487) is deposited at the National Herbarium (ETH), Department of Biology, Addis Ababa University, Addis Ababa.

4.4. Extraction

The aerial parts of *Laggera tomentosa* was dried and grounded into powder. 1Kg of the powdered *L. tomentosa* was extracted with petroleum ether (40-60 ^0C) for 24 hrs.The solvent was removed under reduced pressure to obtain 11g of black gummy material.



Scheme 4. Isolation of LTP-F4 and LTP-F6

4.5. Isolation and analysis.

The pet-ether extract of *L. tomentosa* (11g) was adsorbed on silica gel (10 g) by dissolving the sample with chloroform. After removal of the solvent, the dry sample was applied on a column packed with 100g of silica gel. The crude extract was separated by column chromatography, using pet-ether, increasing the degree of polarity by addition of ethylacetate (EtOAc). A total of 32 fractions were collected. The fractions were combined into 14 fractions based on TLC and R_f value analysis.

4.5.1. Isolation of LTP-F4

Fraction (4) was concentrated under reduced pressure and applied on a column packed with silica gel (50 g). Using pet-ether and ethyl acetate in the ratio of 8.5:1.5 as a solvent, 12 fractions were collected. Fractions 4 & 5 were combined based on TLC analysis (developed in 9:1 ratio of pet-ether/EtOAc) that showed R_f value of 0.4 in this solvent system. Concentrating this under reduced pressure yielded 80mg of pure **LTP-F4**.

LTP-F4 is a white solid compound. R_f= 0.40 in pet-ether/EtOAc (9:1) and it's optical rotation, $[a]_D^T$ = +23.8 (c=0.02, $CHCl_3$, λ_{max}= 589nm, T=22.8 ^{0}c). ^{1}H NMR (400MHz, $CDCl_3$) ; 1.57 (2H, m, H-1), 2.28 (2H, m, H-2), 5.94 (1H, dd, H-3), 3.19 (1H, dd, H-5), 2.69(1H, dd, H-6), 2.26 (2H, d, H-9), 1.80 (3H, s, H-12), 2.01 (3H, s, H-13), 0.99 (3H, s, H-14), 1.41 (3H, s, H-15), 6.08 (1H, q, H-3), 2.00 (3H, s, H-4'), 1.92 (3H, s, H-5′), and 1.99 (3H, s, H-7′).

4.5.2. Isolation of LTP-F6

F6 was concentrated under reduced pressure and applied on column packed with 40g of silica gel. Using pet-ether and ethyl acetate in the ratio of 9:1 as a solvent, 24 fractions were collected. Fractions (22 & 23) were combined based on TLC analysis (developed in 6:4 ratio of pet-ether/EtOAc) that showed R_f value of 0.48 in this solvent system. Concentrating this under reduced pressure yielded 30mg of pure LTP-F6.

LTP-F6 is a colorless solid compound R_f =0.48 inpet-ether/EtOAc (6:4) and it's optical rotation, $[a]_D^T$ =+58.9 (c=0.004, $CHCl_3$, λ_{max}=589nm, T=22.3 ^{0}c). ^{1}H NMR (400MHz, $CDCl_3$); 1.61(1H, m, H-1), 1.57(1H, m, H-1), 2.03(2H, m,H-2), 5.91 (1H, br t, H-3), 3.11 (1H, d, H-5), 7,19(1H, d, H-6), 2.36(9α-H,d) &2.32(9β-H, d), 1.53(3H, s, H-12), 1.49(3H, s, H-13), 1.03(3H, s, H-14),1.61(3H,s, H-16), 3.07(1H, q, H_3'), 1.34(3H, d, H-4'), 1.56(3H, s, H_5') and 2.095(3H,s, H-9').

5. Conclusions

Laggera tomentosa (known locally as “Keskese”) is a perennial fragrant bushy herb and endemic to Ethiopia. Locally, *Laggera tomentosa* is used in traditional medicine. The juice of the crushed plant is ingested as a treatment for stomachache, and is used against migraine[14]. Phytochemical investigation on pet-ether extract of the aerial parts of *Laggera tomentosa* afforded two eudesmane derivatives, LTP-F4 (4-acetoxy-3-angeloyloxy-7,11-dehydroeudesman-8-one) and LTP-F6 (4-acetoxy-3-(2’,3’-epoxy-2’-methylbutyrate)-11-hydroxy-6,7-dehydroeudesman-8-one). LTP-F4 was isolated before from *Bluma alata*[21] and Laggera tomentosa[11]. LTP-F6 was isolated before from *Pluchea odorata*[23].

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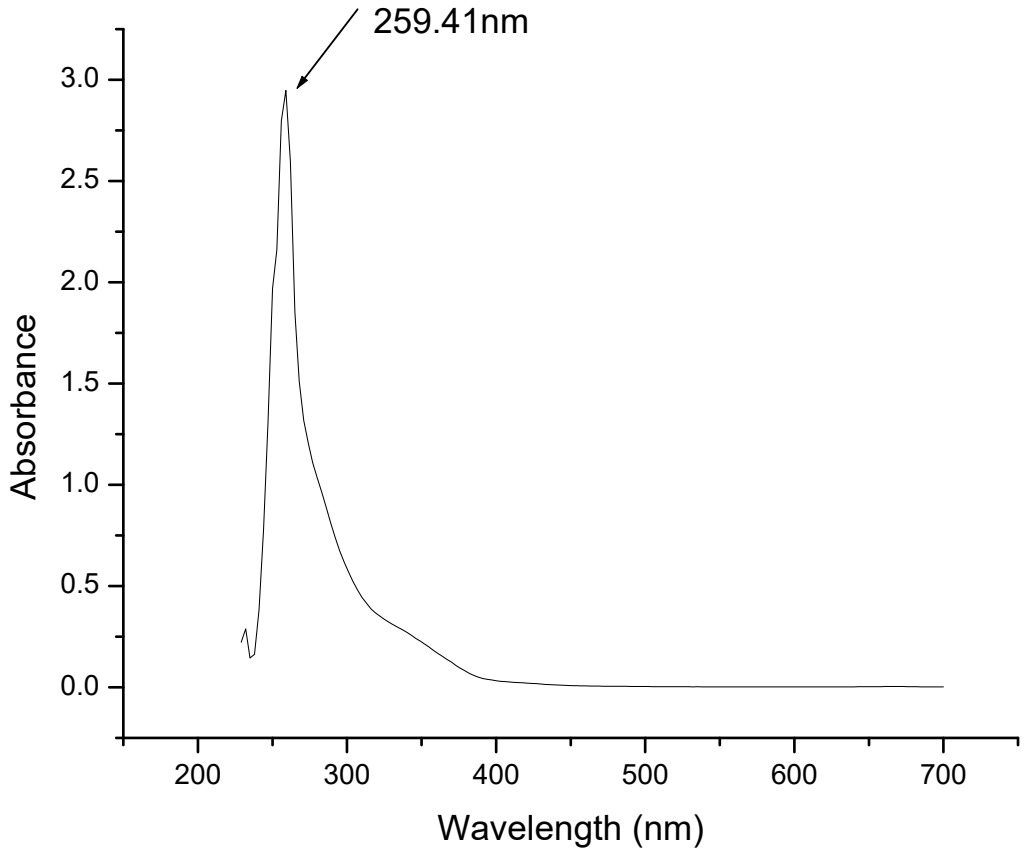
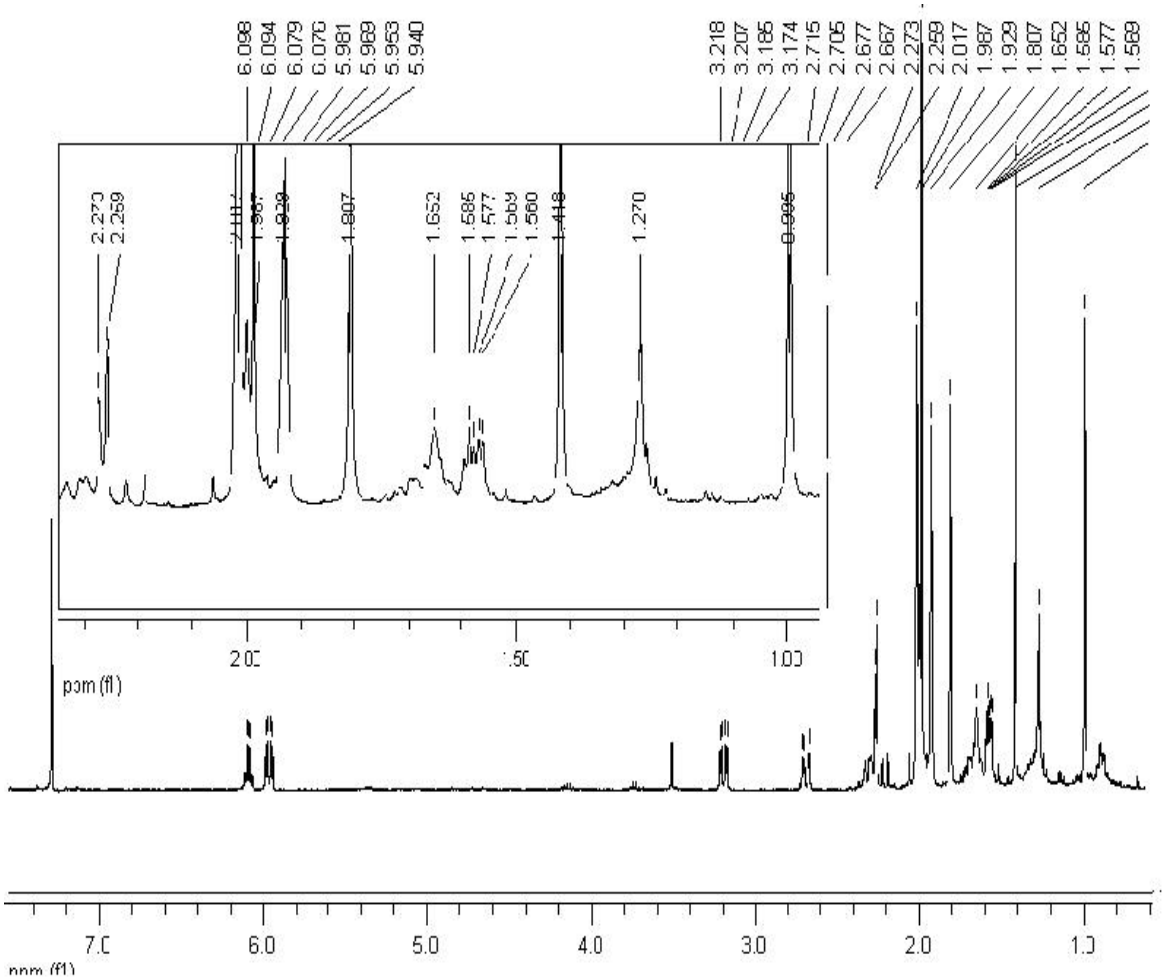
8. Appendices

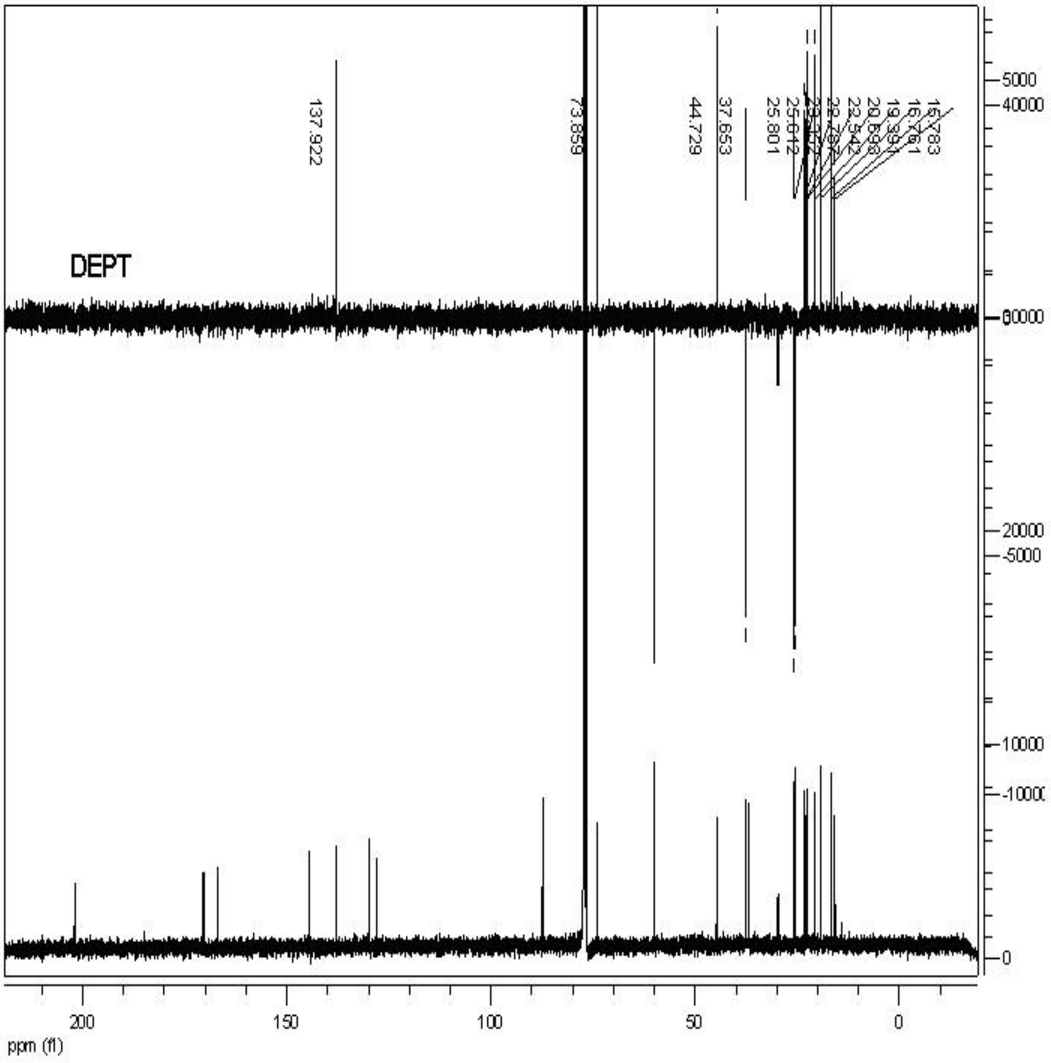
Appendix 1. UV spectrum of LTP-F4



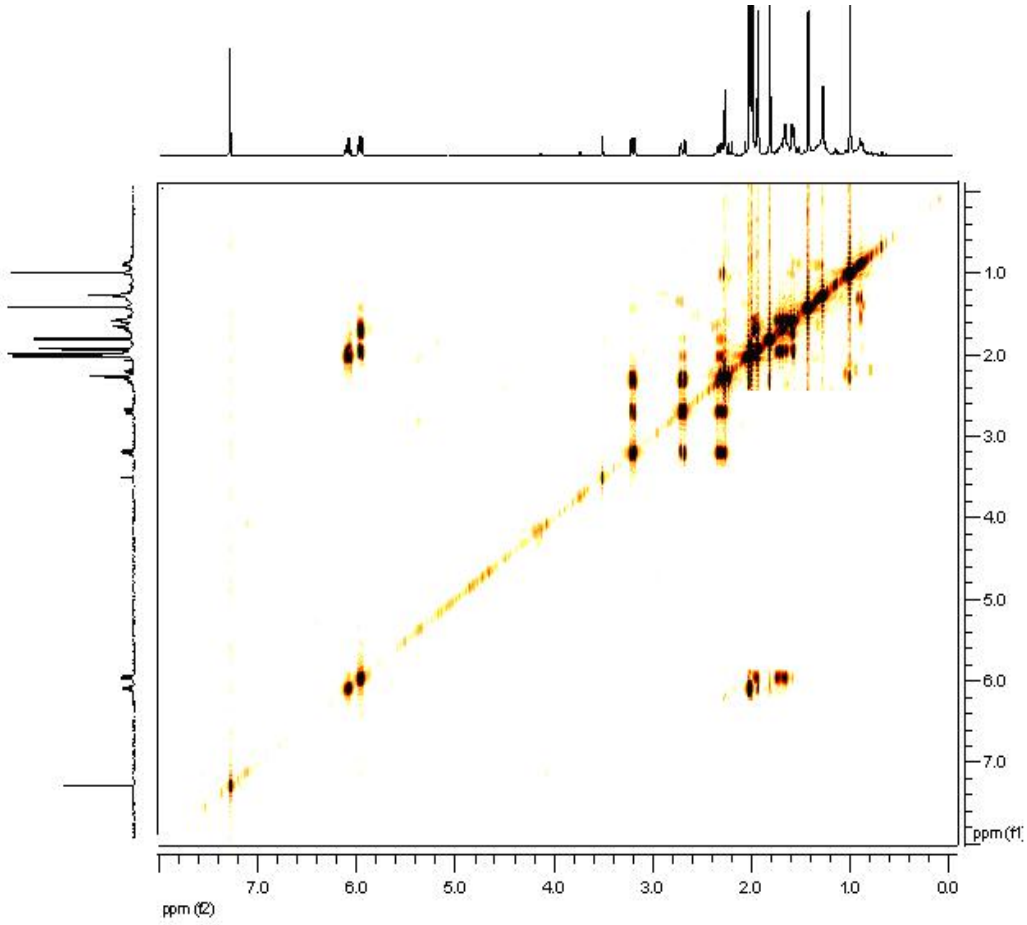
Appendix 2: ^{1}H NMR spectrum of LTP-F4



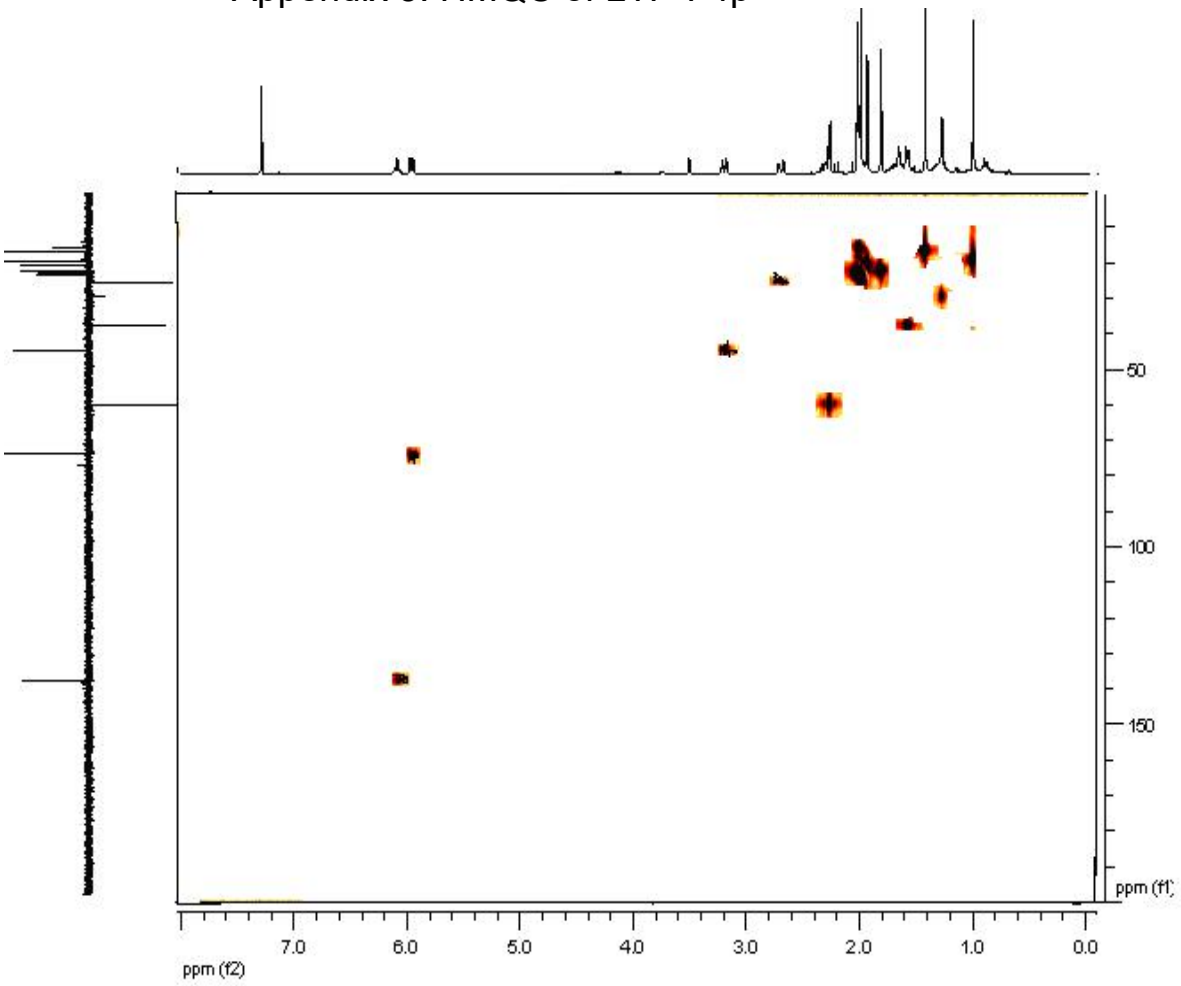
Appendix 3: ^{13}C NMR spectrum of LTP-F4p



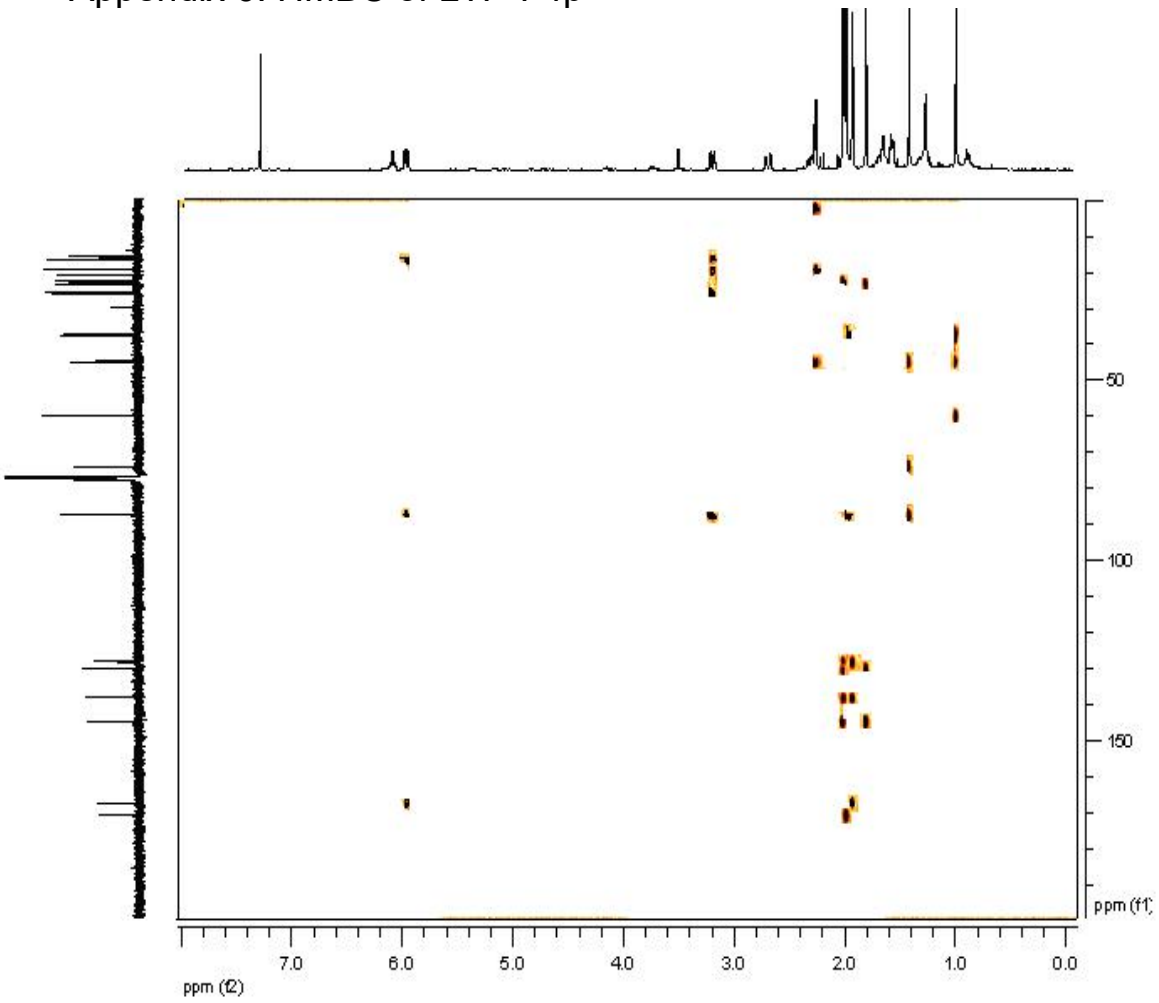
Appendix 4: ^{1}H-^{1}H COSY spectrum of LTP-F4p



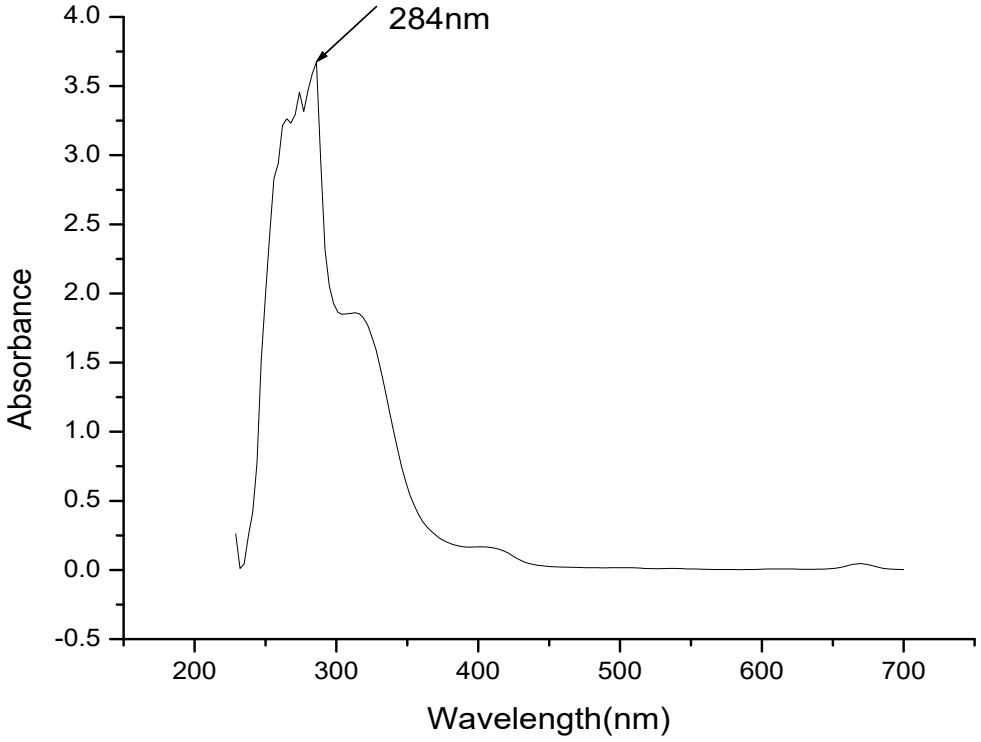
Appendix 5: HMQC of LTP-F4p



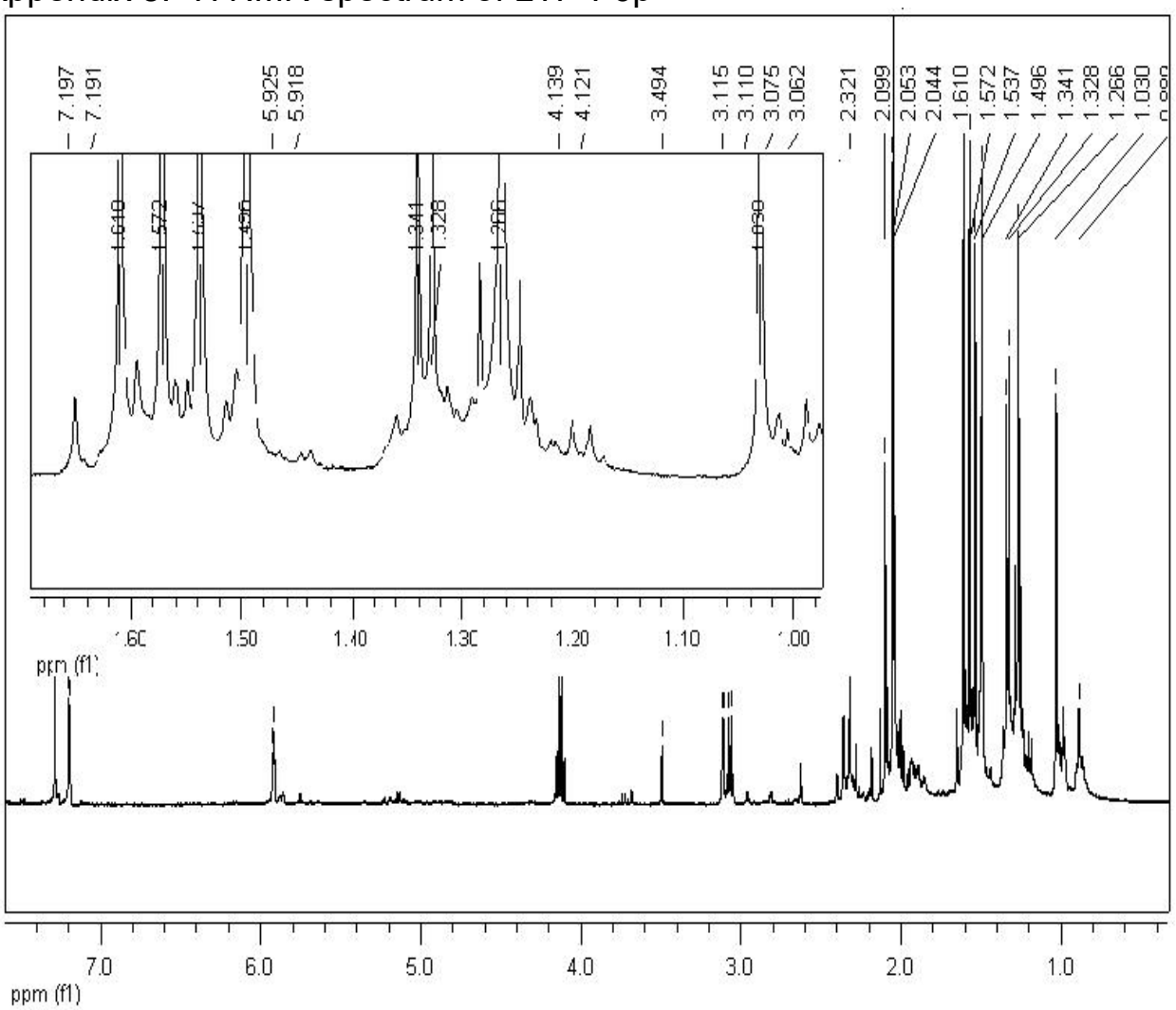
Appendix 6: HMBC of LTP-F4p



Appendix 7. UV spectrum of LTP-F6

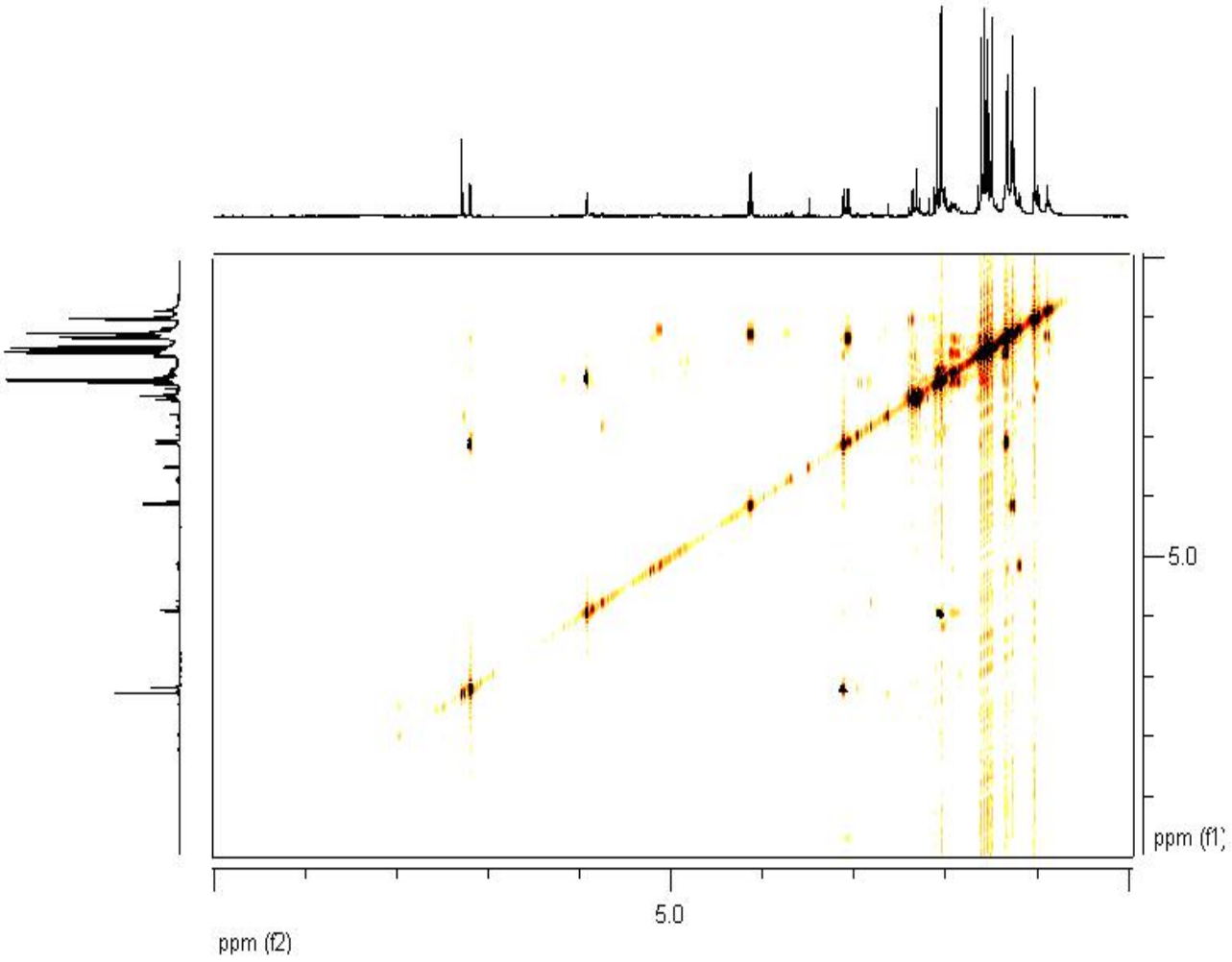


Appendix 8: ^{1}H NMR spectrum of LTP-F6p

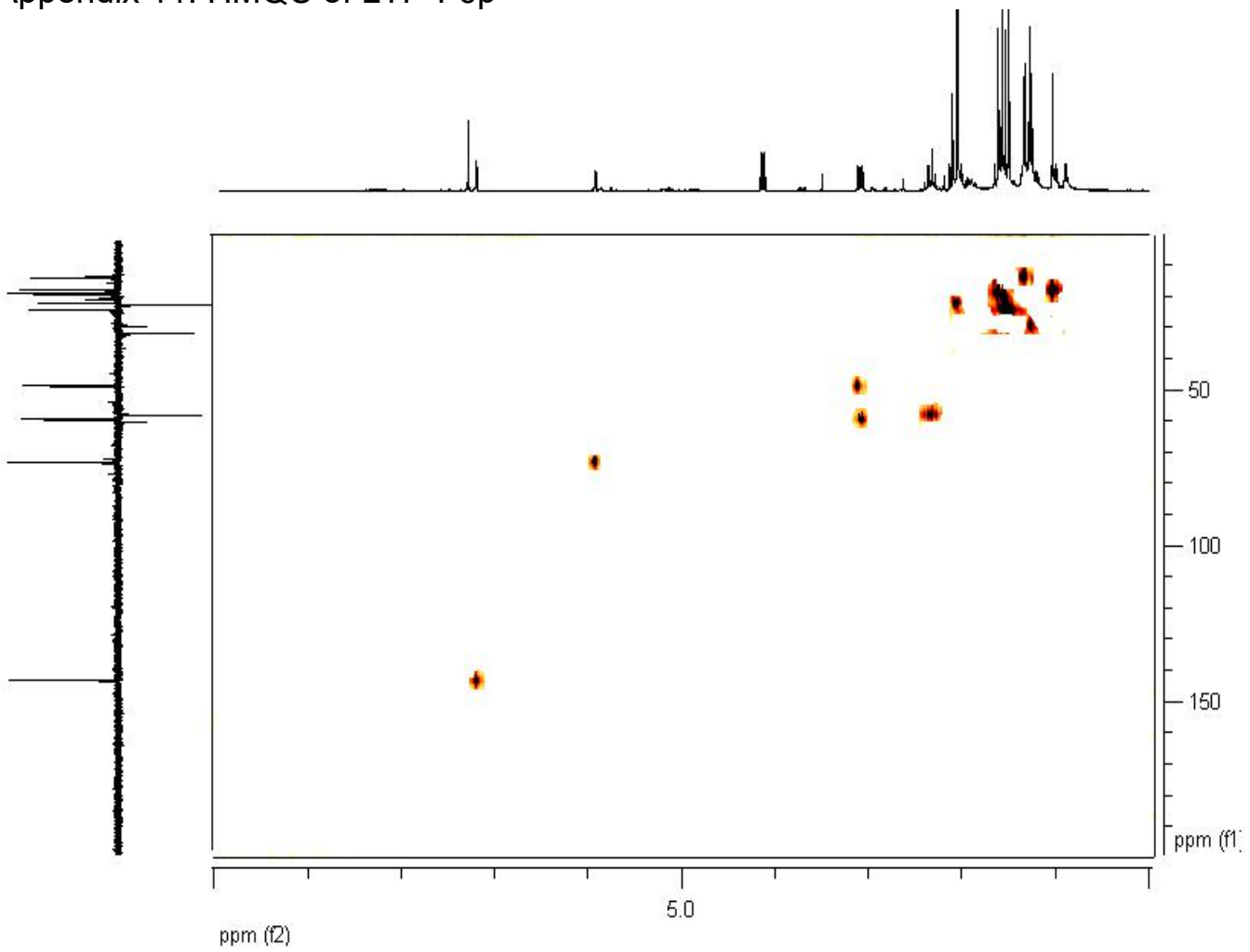


Appendix 9: ^{13}C NMR spectrum of LTP-F6p

Appendix 10:^{1}H-^{1}H COSY spectrum of LTP-F6p



Appendix 11: HMQC of LTP-F6p



Appendix 12: HMBC spectrum of LTP-F6p