ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

GRADUATE THESIS (CHEM 750)

CHEMICAL STUDY OF "*JIMMA ENCHET*": WOOD SOLD IN MEDICINAL PLANT MARKETS

By: Hanna Kassaye

Advisor: Professor Ermias Dagne

A Graduate Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

October, 2014

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

CHEMICAL STUDY OF "*JIMMA ENCHET*": WOOD SOLD IN MEDICINAL PLANT MARKETS

By: Hanna Kassaye

Department of Chemistry

College of Natural Sciences

Approved by:

Prof. Ermias Dagne _____

Advisor

Prof. Wendimagegn Mammo _____

Examiner

Dr. Ashebir Fiseha _____

Examiner

DECLARATION

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

Name: **Hanna Kassaye**

Signature: _____

This MSc Thesis has been submitted for examination with my approval as a university advisor.

Name: **Professor Ermias Dagne**

Signature: _____

Place of Submission and Date: Department of Chemistry

Addis Ababa University

October, 2014

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my advisor Professor Ermias Dagne for the offer of scholarship and supervision. I am also deeply grateful to Professor Wendimagegn Mammo who contributed immensely to the isolation and structure elucidation of compounds presented in this Thesis. Thanks is also due to Dr. Tarekegn Gebreyesus for mentorship and guidance. I thank all members and co-workers of our Research Group, to all academic and technical staff of the Chemistry Department, to my relatives and friends, all of whom in many ways contributed to my training and research endeavors.

Table of Contents

List of Tables

List of Schemes

List of Figures

List of Abbreviations and Symbols

NMR: Nuclear Magnetic Resonance
DEPT: Distortionless enhancement by polarization transfer
COSY: Correlation Spectroscopy
HSQC: Hetronuclear Single Quantum Correlation
HMBC: Hetronuclear Multiple Bond Correlation
IR: Infrared
UV-Vis: Ultraviolet Visible
ppm: parts per million
δ: Chemical shift
J: Coupling constant
Hz: Hertz
s: singlet
d: doublet
t: triplet
dd: doublets of doublet
m: multiplet
λ_{max}: maximum absorption wavelength
TLC: thin-layer chromatography
EtOH: ethanol
MeOH: methanol
EtOAc: ethyl acetate

CHEMICAL STUDY OF "*JIMMA ENCHET*": WOOD SOLD IN MEDICINAL PLANT MARKETS

By: Hanna Kassaye

Advisor: Prof. Ermias Dagne

Abstract

Column chromatographic fractionation of the EtOH extract of the wood of "*Jimma Enchet*" afforded two secoiridoids (ligstroside and oleuropein), one lignan (olivil 4-O-β-D-Glucoside), and two sugars (sucrose and mannitol). The structures of these compounds were established using physical (melting point) and spectroscopic data such as NMR (1D and 2D), UV and IR. In addition some mixtures of aldehydes were also identified.

Key Words: *Jimma Enchet*, secoiridoids, lignan, sugars, NMR.

1. Introduction

1.1 Medicinal plants in Ethiopia

Medicinal plants have been identified and used for the treatment of many diseases throughout human history. The use of herbs to treat diseases arising from worms, fungi, virus and protozoa is almost universal among non-industrialized societies, and is often more affordable than purchasing modern pharmaceuticals [1]. The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care [1].

As elsewhere in Africa, the use of traditional medicine is still widespread in Ethiopia, and its acceptability, availability and popularity is of no doubt as about 90% of the population use it for health care needs [1]. Ways of using these traditional medicines depend on traditional knowledge and culture of the society. People use freshly harvested or dried plant parts such as root, bark, wood, leaf, fruit, seed and stem by squeezing, powdering, crushing, chewing, rinsing, cooking, pounding, tying, or smoking, etc.

Ethno-botanical studies of traditional medicinal plants used by indigenous people in the south, southwest, central, eastern, north and north-western parts of the country show that Ethiopia has a flora that is extremely rich in its diversity [1, 2, 3, 4, 5, 6]. It is therefore not surprising that some of these plants have chemical compounds of therapeutic value which may be used in the treatment of several diseases [1].

The society selects medicinal plants from traditional knowledge and use them by collecting from wild (garden), purchasing from market and directly getting from the healers. For scientific examination different scholars have developed sound and practical strategies for selecting medicinal plants. One of these strategies followed by groups involved in drug discovery programs is to use bioassay-guided fractionation to isolate and identify substances that have positive activity. Other approaches involve ethnomedicinal information as leads and more recently combinatorial chemistry have been employed as possible strategies for drug discovery programs in many countries [7]. Not all natural product chemists participate in drug discovery

programs but they play a great role in finding chemically enhancing compounds and selection of plants. To select the plants, some are guided by the existing belief that plants which have acquired the status of a marketed commodities have already been screened by traditional methods, and researchers can use these marketed medicinal plants for chemical study [7].

1.2. Marketed medicinal plants in Ethiopia

Marketed plants have a variety of uses including flavor, fragrance, poisons and insecticides. The most common ones among plants sold in market are medicinal plants which have widespread acceptance [7].

It is customary to find medicinal plants in markets where food items and spices are sold. Fresh and dried leaves, flowers, roots, bark, seeds, etc., of medicinal plants are displayed for sale in most markets in Ethiopia along with spices such as pepper, cardamom, ginger, etc. Some of the common uses of medicinal plants sold in markets include fumigation, vermifuge, pain relief, treating skin infections, etc. Plants with antimicrobial property are among the major medicinal plants that are commonly available in markets [8].

Some of commercially available medicinal plants in Ethiopia are *Hagenia abyssinica* (*kosso*), *Olea europaea (weyra), Lepidium sativum* (*feto*), *Piper capense (timize), Syzygium guineense (doqma), Glinus lotoides (mettere), Lupinus albus (gibto), Prunus Africana (tikur enchet), Terminalia brownii (weyba), Echinops kebericho* (*kebericho*), *Taverniera abyssinica* (*dengetegna*), etc. Some of these medicinal plants are available in Merkato, which is the biggest market in Addis Ababa, Ethiopia, which helps researchers to get a wide variety of plants collected from different parts of the country.

Figure 1: Some medicinal plants sold in Merkato (Picture by H. Kassaye)

The significance of some medicinal plants sold in Merkato were depicted in Table 1 with the information obtained from medicinal plant sellers.

Table 1: Medicinal importance of some plants sold in Merkato

Local name	**Scientific name**	**Plant part**	**Medicinal use**
Abalo	*Brucea antidysentrica*	seed	Leprosy, scabies and skin diseases
Weyba	*Terminalia brownii*	wood	Diarrhoea, hyperbilirubinemia
Jimma Enchet	-	wood	Skin diseases
Unsi	-	bark	Skin diseases
Feto	*Lepidium sativum*	seed	Anthrax, stomach pain, tonsillitis, diarrhea
Keshele	-	seed	tonsillitis
Gibto	*Lupinus albus*	seed	Hypertension

1.3. Chemistry of marketed medicinal plants

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Since the beginning of the 19th century, a large number of biologically active secondary metabolites of plant origin have been found to have commercial application as drugs. In 2001, researchers identified 122 compounds used in modern medicine which were derived from plant sources including morphine (analgesic), scopolamine atropine (anticholinergics), galantamine (Alzheimer's disease), quinine (antimalarial), paclitaxel, vincristine and vinblastine (anticancer drugs) [9].

Many researchers have done chemical study of marketed medicinal plants from many years ago till now. Different scholars have found several novel or known natural products from marketed plants. For example from *Hagenia abyssinica* (*kosso*) compounds such as quercetin-3-O-glucuronide (**1**), rutin (**2**), ellagic acid (**3**), protokossin (**4**), kossotoxin (**5**), quercetin-3-O-glucoside (**6**), etc. were reported [10]. Previously isolated compounds from *Taverniera abyssinica* (*dengetegna*) include formononetin (**7**), afrormosin (**8**), medicarpin (**9**), 3,4-Dihydroxy-9-methoxypterocarpan (**10**), etc [11]. Likewise oleuropein (**11**), olivin (**12**), olivil (**13**), cinchonidine (**14**), etc were reported from *Olea europaea* (*weyra*) [12, 13].

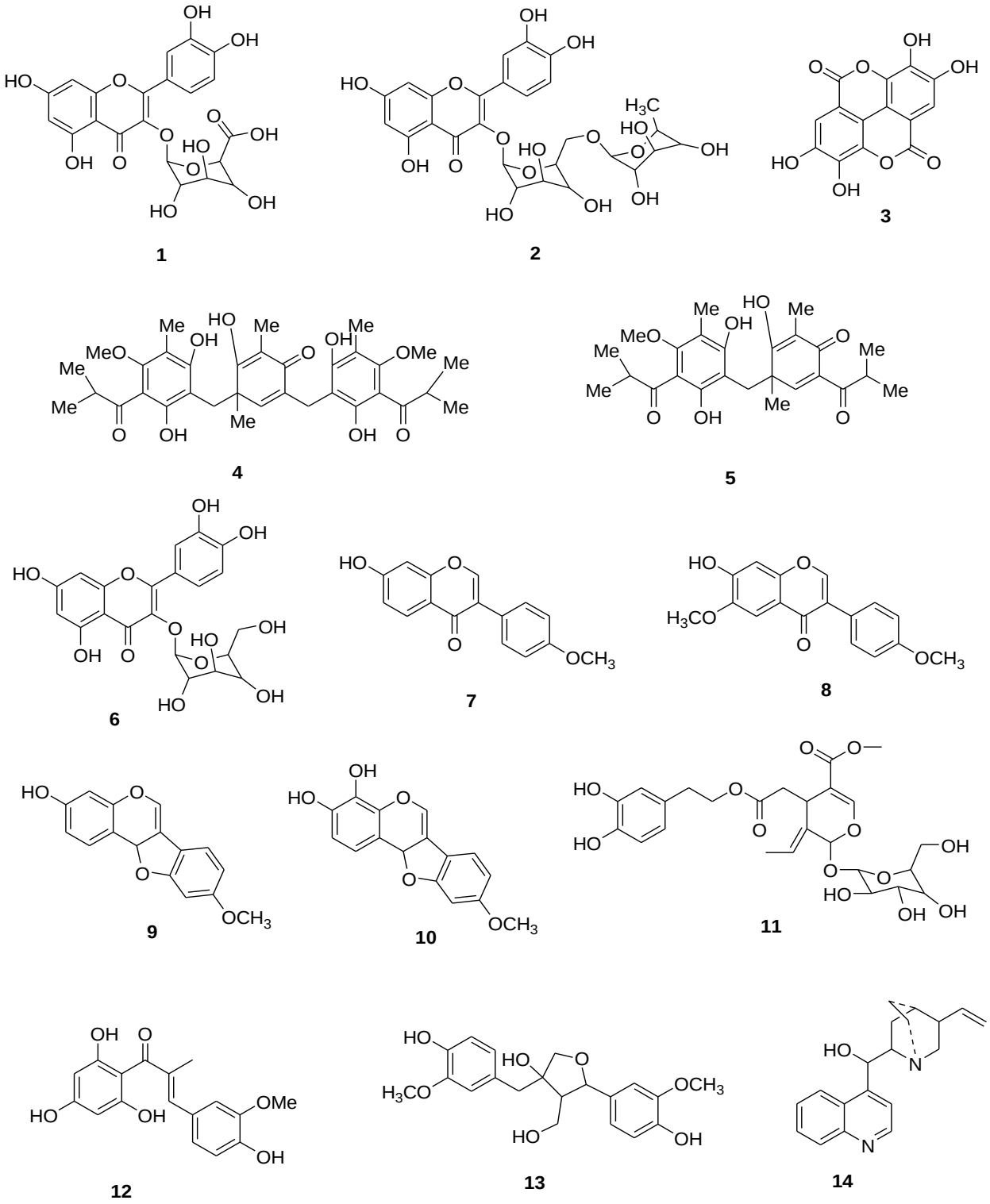


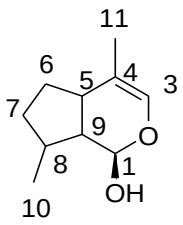
Figure 2: Some compounds isolated from different marketed medicinal plants

As observed above different classes of compounds such as coumarins, flavonoids, alkaloids and tannins were reported. In the course of this project terpenes and phenolic were isolated and characterized. Hence, the biosynthesis of these two classes of compounds leading to the isolated compounds were briefly described below.

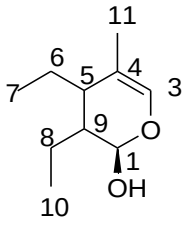
1.4. Terpenes

Terpenes are made from the union of two or more isopentyl (isoprene) units which are usually linked in a head-to-tail manner. They are classified by the number of carbon units they contain as: hemiterpenes (C5), monoterpenes (C10), sesquiterpens (C15), diterpenes (C20), sesterpenes (C25), triterpenes (C30) and tetratrepenes (C40) [14].

Iridoids and secoiridoids are produced from secondary metabolism of monoterpenes and usually found bound with glycoside [15]. Secoiridoids (**16**) are derived by opening of the cyclopentane ring of iridoid skeleton (**15**).



15



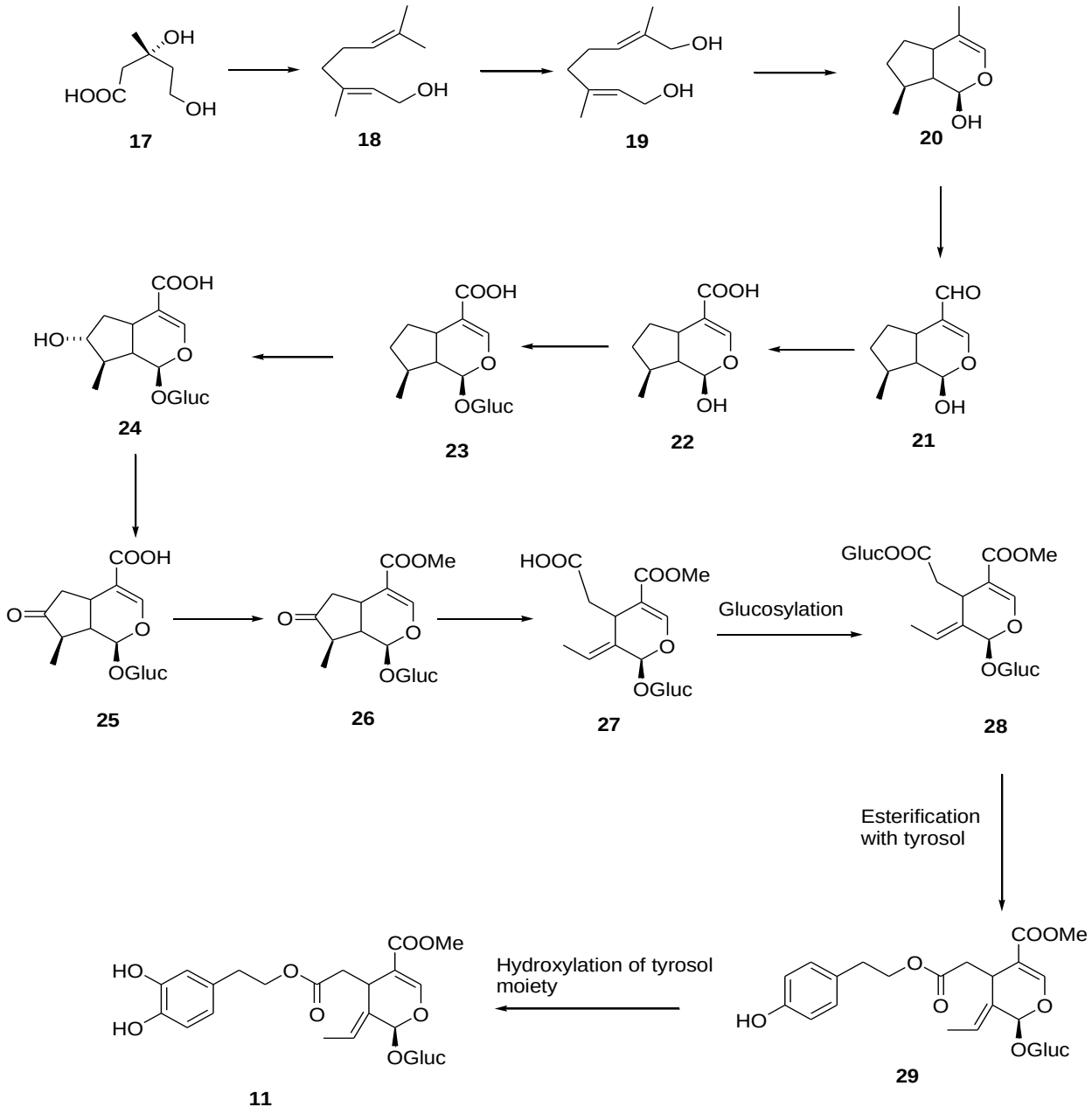
16

Ligstroside and Oleuropein belong to a very specific group of coumarin-like compounds called secoiridoids.

1.5. Biosynthesis of Ligstroside and oleuropein

The biosynthesis of oleuropein proceeds via branching in the mevalonic acid (**17**) pathway of secondary metabolism, resulting in the formation of oleosides which are the biosynthetic precursors for secoiridoids. The biosynthesis of oleosides is similar to that of secologanin in which the carbon skeleton is derived from mevalonic acid (**17**). Geraniol (**18**), 10-hydroxygeraniol (**19**) and iridoidal (**20**) are known precursors of loganin. Later, deoxyloganic acid (**23**), 7-epiloganic acid (**24**) and loganic acid are incorporated into ligstroside (**29**), a direct precursor of oleuropein (**11**), via a 7-ketologanic acid (**25**) intermediate. In *O. europaea*, both

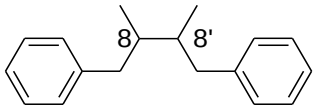
possible epoxides of secologanin and secoxyloganin can be precursors for oleuropein [14]. A plausible biosynthetic route from deoxyloganic acid (**23**), 7-epiloganic acid (**24**), 7-ketologanic acid (**25**), 7-ketologanin (**26**), oleoside 11-methyl ester (**27**), 7-β-1-D-glucopyranosyl 11-methyl oleoside (**28**) and ligstroside (**29**) to oleuropein (**11**) is shown in Scheme 1 [15, 16, 17].



Scheme 1: Biosynthetic route leading to the formation of ligstroside and oleuropein

1.6. Lignans

Lignans are a large group of naturally occurring compounds characterized by the coupling of two C6-C3 units and linked by a bond between positions 8 and 8' (**30**).



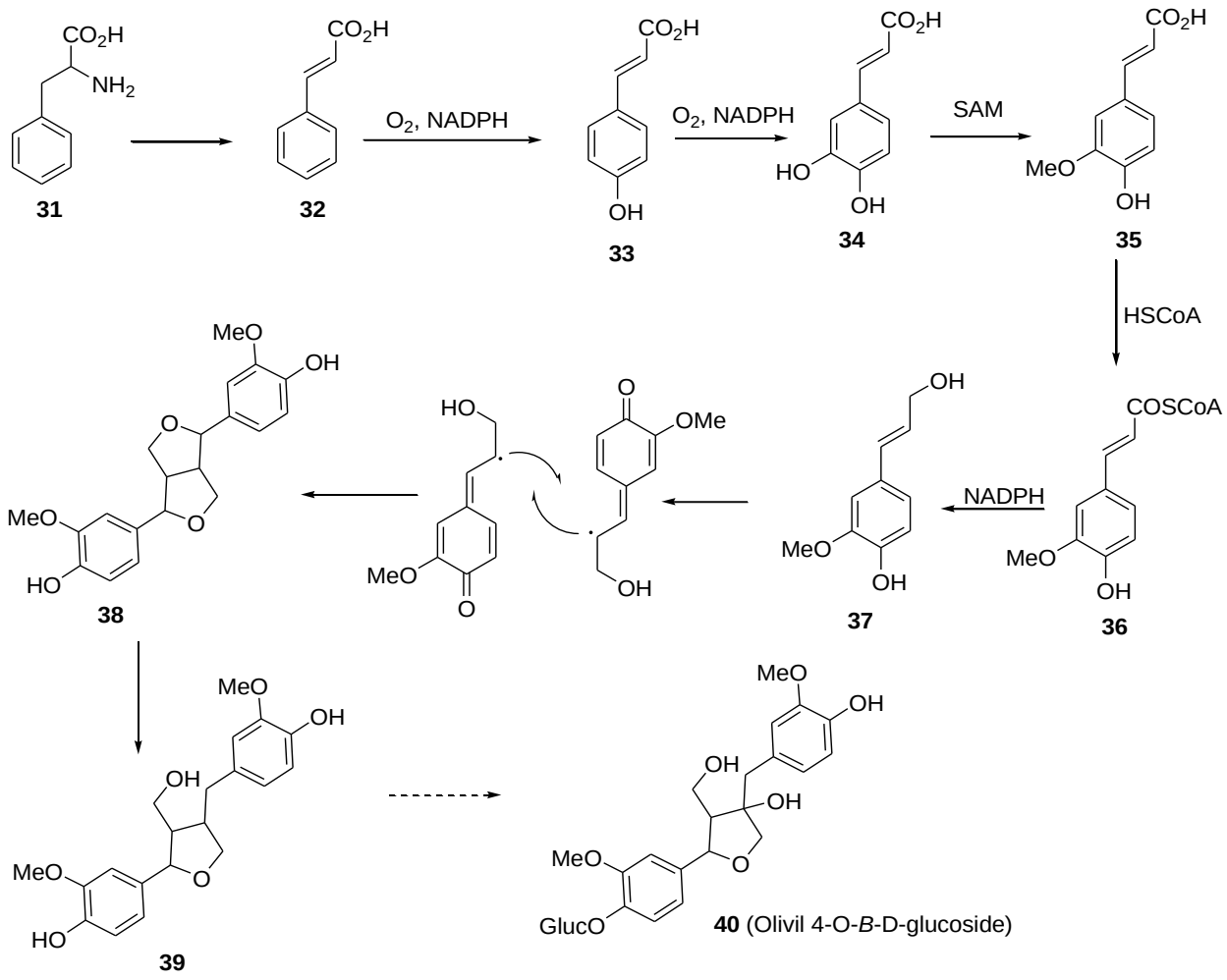
30

Upon the way in which oxygen is incorporated into the skeleton and the cyclization pattern, lignans are classified into the following eight subgroups: furofuran, furan, dibenzylbutan, dibenzylbutyrolactone, aryltetralin, arylnaphthalene, dibenzocyclooctadiene and dibenzylbutyrolactol. Lignans of each subgroup vary substantially in oxidation levels of both the aromatic rings and propyl side chains. Some lignans of furan, dibenzylbutane, and dibenzocyclooctadiene have no oxygen at C9 (C9'), while some lignans have extra hydroxyl groups at C7 (C7') or C8 (C8'). 3-methoxy-4-hydroxyphenyl (guaiacyl), 3,4-dimethoxyphenyl (veratryl), 3,4-methylenedioxyphenyl (piperonyl), 3,5-dimethoxy-4- hydroxyphenyl (syringyl) and 3,4,5-trimethoxyphenyl are the most occurring aromatic rings found in lignans [18]. Below is an overview of the biosynthesis of furan type lignans.

1.7. Biosynthesis of Olivil 4-O-β- D-glucoside

The biosynthesis of furan type lignans starts from aromatic amino acids such as L-phenylalanine (**31**) and L-tyrosine which are obtained from shikimic acid pathway, and then converted in a series of cinnamic acid derivatives. The reduction of these acids via coenzyme A of related esters and aldehydes forms three alcohols (*p*-coumaryl alcohol, coniferyl alcohol (**37**) and sinalpyl alcohol) that are the main precursors of all lignans. The peroxidase induces one-electron oxidation of the phenol group allowing the delocalization of the unpaired electron through resonance forms. In these hydroxycinamyl alcohols, conjugation allows the unpaired electron to be delocalized also into the side chain. The radicals pairs up to form product susceptible to nucleophilic attack from the hydroxyl groups, leading to a wide range of lignans. Among these subgroups, the biosynthesis of C9 (9´)-oxygen lignans is the most well-known. This type of lignan is formed through the enantioselective dimerization of two coniferyl alcohol (**37**) monomeric units into pinoresinol (**38**) via intermolecular 8,8´ oxidative coupling with the aid of

dirigent protein. Then sequencial stereoselective enzymatic reduction of pinosresinol involve by pinoresinol/lariciresinol reductase to generate lariciresinol [18].



Scheme 2: Biosynthesis of Olivil 4-O-β- D-glucoside

1.8. Objective of the study

The main objective of this project work is to isolate and characterize the constituents from the wood of *Jimma Enchet.* The plant was selected for the study because the society use this plant as a traditional medicine and sold in medicinal plant markets.

2. Results and Discussion

2.1. Characterization of compounds from *Jimma Enchet*

The dried wood of *Jimma Enchet* (50 g) was successively extracted with $CHCl_3$ and EtOH. The ethanol extract (8 g, 16%) was subjected to further fractionation using column silica gel chromatography to afford five compounds (89-204fr10 (15 mg), 89-204fr13 (35 mg), 89-204fr20 (20 mg), 89-206fr5 (3 mg) and 89-204B (30 mg)). The characterizations of these compounds using physical and spectroscopic methods are briefly described as follows.

2.1.1. 89-204fr10

Compound 89-204fr10 was obtained as white solid and mp. 85-87^{o}C. The TLC Rf value of the compound using EtOAc:MeOH (4:1) as eluent was 0.7. The compound give a purple color when the TLC was sprayed with vanillin: sulfuric acid mixture. The UV-Vis spectrum (in EtOH) showed maximum absorption bands at 226 and 276 nm, which indicating the presence of $\Pi \rightarrow \Pi^*$ transition of C=C double bond and $n \rightarrow \Pi^*$ transition of C=O bond.

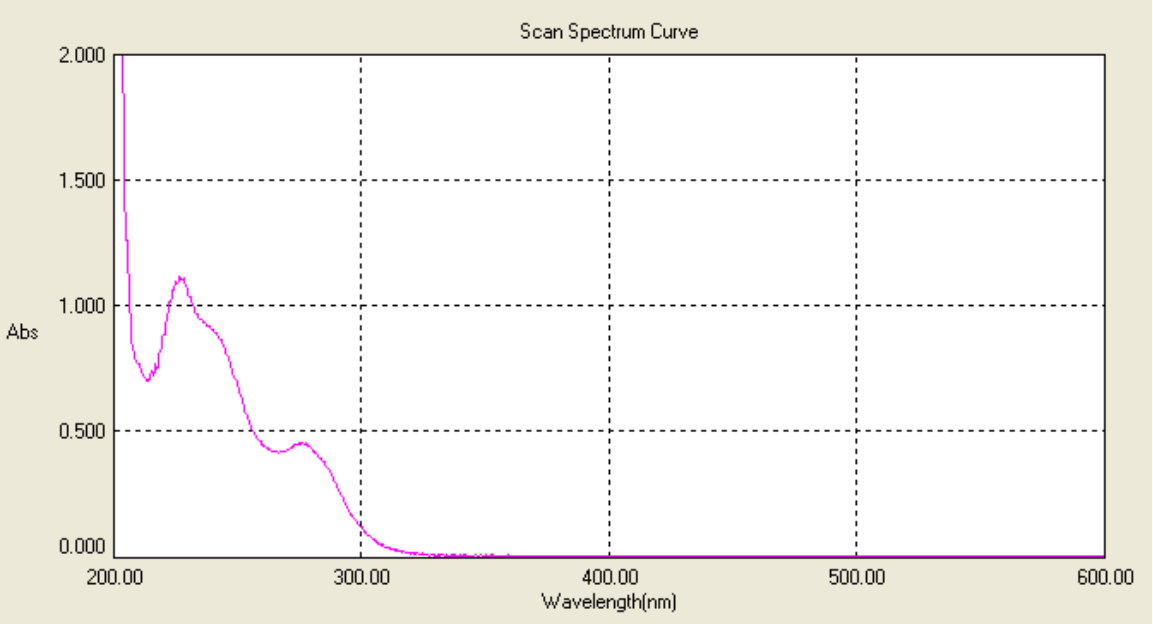


Figure 3: UV-Vis Spectrum of compound 89-204fr10

The IR spectrum of 89-204fr10 (Appendix 1) showed strong and fairly sharp absorption band at 3420 cm^{-1} (O-H stretching) indicating the presence of OH in the compound. The medium and sharp band at 2922 cm^{-1} is due to C-H stretching, the strong sharp band at 1709 and 1631 cm^{-1} indicates the compound has carbonyl functional group (C=O stretching). The strong and sharp bands at 1441 and 1518 cm^{-1} indicates aromatic C=C bond. Moreover, the strong band at 1076 cm^{-1} is due to C-O stretching.

The proton NMR spectrum (Appendix 2) of 89-204fr10 suggested the presence of one methyl protons in the aliphatic region appearing at δ 1.68 (3H, *dd*, *J* = 6.8, 1.2 Hz). The compound exhibited one methoxy signal appearing at δ 3.75. The proton NMR spectrum also showed four methylene protons. The first methylene protons appeared at δ 2.43 (1H, *dd*, *J* = 14.4, 9.6 Hz) and 2.75 (1H, *dd*, *J* = 14.4, 6.8 Hz). These protons are diastereotopic as observed from its HSQC spectrum. The second methylene protons appeared at δ 2.85 (2H, *t*, *J* = 7 Hz). Another diastereotopic methylene protons also appeared at δ 3.75 (1H) and 3.87 (1H, *d*, *J* = 7.2 Hz). These protons are typical methylene protons of the glucose moiety. The fourth diastereotopic methylene protons appeared at δ 4.10 (1H, m) and 4.23 (1H, m). The proton NMR spectrum also exhibited four methine protons at δ 3.37, 3.40, 3.41 and 3.48 (1H, *t*, *J* = 8.7 Hz) which evidence the presence of only one sugar moiety in the structure of the compound. This is confirmed by the appearance of a signal at δ 4.85 (1H, *d*, *J* = 7.6 Hz) which is characteristic of an anomeric proton. The compound also showed one methine proton at δ 3.98 (1H, *dd*, *J* = 9.6, 4 Hz). The one hydrogen singlet at δ 5.94 is ascribed to an acetal proton of the iridoid moiety in the structure of the compound. The proton NMR spectrum further revealed the presence of two olefinic protons at δ 6.04 (1H, *q*) and 7.50 (1H, *s*). Two doublet signal appearing at δ 6.79 (2H, *d*, *J* = 8 Hz) and δ 7.10 (2H, *d*, *J* = 8 Hz) are typical of symmetrically situated protons on unsymmetrically *para* substituted aromatic ring.

The proton decoupled ^{13}C NMR spectrum (Appendix 3, Table 2) of 89-204fr10 analyzed with the aid of DEPT-135 spectrum (Appendix 3), showed six quaternary carbon signals. Among these the carbon resonances appearing at δ 167.5 (C-11) and 172.0 (C-7) are due to carbonyl carbons. The remaining four quaternary carbon signals appearing at δ 109.5 (C-4), 129.9 (C-3'), 130.8 (C-9) and 157.3 (C-6') are accounted for carbon α to carbonyl, aromatic carbon bearing

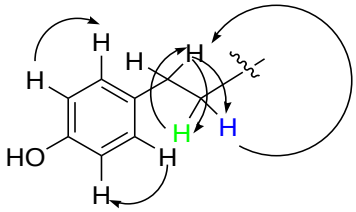
alkyl group, olefinic carbon, and oxygenated aromatic carbon, respectively. Two methine carbon signals appeared in the aromatic region at δ 116.4 (C-5', 7') and 131.1 (C-4', 8') due to symmetrically placed carbons on unsymmetrically *para* substituted aromatic ring. Two olefinic carbon signals appeared at δ 124.5 (C-8) and 154.5 (C-3). The down field shift of the olefinic carbon signal at δ 154.5 is most likely due to the fact that the carbon is situated β to a carbonyl carbon. And also two carbon signals at δ 94.9 (C-1) and 100.9 (C-1'') were assigned to acetal carbon of the iridoid and anomeric carbon of the sugar moiety, respectively. This was in agreement with the proton NMR spectrum of the compound. The other carbon signals of the sugar moiety appeared at δ 71.9 (C-4''), 75.0 (C-5''), 78.2 (C-3'') and 78.4 (C-2''). Furthermore the signals appearing at δ 35.1 (C-2'), 41.1 (C-6), 63.3 (C-6'') and 66.5 (C-1') are due to methylene carbons. Among these the one appearing at δ 35.1 is typical of a methylene carbon attached to an aromatic ring. The spectrum showed one methoxy carbon signal at δ 51.8 which suggests the presence of a methyl ester in the structure of the compound. Furthermore, the compound exhibited signals attributable to a terminal methyl group at δ 13.9 (C-10) and methine carbon at δ 31.8 (C-5).

Table 2: Proton decoupled ^{13}C NMR data for compound 89-204fr10

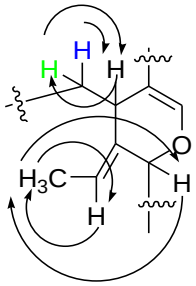
Carbon No	**δ**	**Remark**	**Carbon No**	**δ**	**Remark**
C-10	13.9	CH_3	C-1''	100.9	CH
C-5	31.8	CH	C-4	109.5	Quaternary
C-2'	35.1	CH_2	C-5', 7'	116.4	2CH
C-6	41.1	CH_2	C-8	124.5	CH
	51.8	OCH_3	C-3'	129.9	Quaternary
C-6''	63.3	CH_2	C-9	130.8	Quaternary
C-1'	66.5	CH_2	C-4', 8'	131.1	2CH
C-4''	71.9	CH	C-3	154.5	CH
C-5''	75.0	CH	C-6'	157.3	Quaternary
C-3''	78.2	CH	C-11	167.5	Quaternary
C-2''	78.4	CH	C-7	172.0	Quaternary
C-1	94.9	CH			

The structure of compound 89-204fr10 was further deduced by extensive 2D experiments. The ^{1}H-^{1}H coupling networks were determined by the COSY spectrum. The one-bond C-H chemical shift correlations were established by the HSQC experiment, while long range ^{1}H-^{13}C correlations were made by using HMBC experiment. The HMBC experiment also helped to connect the structural fragments together.

The H-H COSY spectrum (Appendix 4) of compound 89-204fr10 revealed correlation between the proton signal at δ 2.85 with proton signals at δ 4.10 and 4.23. The proton signals at δ 4.10 and 4.23 (found on carbon at δ 66.5) are due to diastereotopic protons as observed from the HSQC spectrum (Appendix 5). Another coupling was observed between the proton resonance at δ 6.79 and the signal at δ 7.10. These protons are most likely situated *ortho* to one another on a benzene ring as revealed by their coupling constant. Diastereotopic proton signals appearing at δ 2.43 and 2.75 are correlated with the proton signal at δ 3.98. Long range coupling was observed between the proton signal appearing at δ 1.68 with proton signals appearing at δ 5.94 and 6.04. The partial structures **41** and **42** were observed from the COSY in conjunction with proton and HSQC spectra of compound 89-204fr10.



41



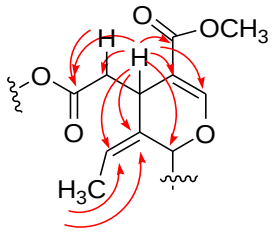
42

Correlations between protons attached on carbon were measured using HSQC and the whole correlation are shown in Table 3.

Table 3: HSQC ($^{1}H \rightarrow ^{13}C$) data for compound 89-204fr10

Position of carbon	δ	Position of proton	δ	HSQC($^{1}H \rightarrow ^{13}C$)	Remark
C-10	13.9	H-10	1.68	H-10,→C-10	CH_3
C-5	31.8	H-5	3.98	H-5→C-5	CH
C-2’	35.1	H-2’	2.85	H-2’ →C-2’	CH_2
C-6	41.1	H-6a, H-6b	2.43 and 2.75	H-6a,6b →C-4	CH_2
	51.8		3.75		OCH_3
C-6’’	63.3	H-6’’a, H-6’’b	3.75 and 3.87	H-6’’a,6’’b →C-6	CH_2
C-1’	66.5	H-1’a, H-1’b	4.10 and 4.23	H-1’a,1’b →C-7	CH_2
C-4’’	71.9	H-4’’	3.40	H- 4’’→C-4’’	CH
C-5’’	75.0	H-5’’	3.37	H-5’’ →C-5’’	CH
C-3’’	78.2	H-3’’	3.48	H- 3’’→C-3’’	CH
C-2’’	78.4	H-2’’	3.41	H-2’’ →C-2’’	CH
C-1	94.9	H-1	5.94	H-1→C-1	CH
C-1’’	100.9	H-1’’	4.85	H-1’’→C-1’’	CH
C-5’, 7’	116.4	H-5’, 7’	6.79	H-5’, 7’→C-5’, 7’	2CH
C-8	124.5	H-8	6.04	H-8→C-8	CH
C-4’, 8’	131.1	H-4’, 8’	7.10	H-4’, 8’→C-4’, 8’	2CH
C-3	154.5	H-3	7.50	H-3→C-3	CH

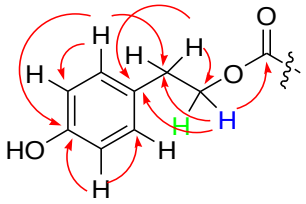
The HMBC spectral data (Appendix 6) of 89-204fr10 revealed that the methyl proton signal at δ 1.68 correlated with the carbon signals at δ 124.5 (C-8) and 130.8 (C-9). This indicates that this methyl carbon is attached to the olefinic carbon C-8 at (δ 124.5). Further correlation was observed between the diastereotopic hydrogen signals at δ 2.43 and 2.75 with the carbon signal at δ 31.8 (C-5), the quaternary carbon signals at δ 109.5 (C-4), 130.8 (C-9) and the carbonyl carbon signal at δ 172.0 (C-7). The proton signal at δ 3.98 showed correlations with eight carbon signals δ 41.1 (C-6), 94.9 (C-1), 109.5 (C-4), 124.5 (C-8), 130.8 (C-9), 154.5 (C-3), 167.5 (C-11) and 172.0 (C-7). These correlations are indicative of the presence of an iridoid moiety in the structure. The above HMBC correlations allowed the establishment of the partial structure **43**.



43

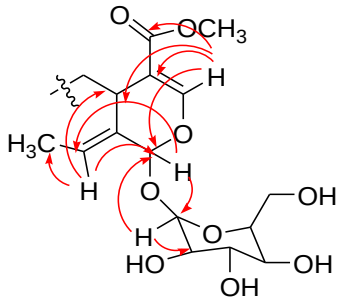
Further HMBC correlations were observed between the proton signal at δ 2.85 and the carbon signals at δ 66.5 (C-1') and 129.9 (C-3'). The diastereotopic proton signals at δ 4.10 and 4.23 correlated with the carbon signals at δ 35.1 (C-2'), 129.9 (C-3') and 172.0 (C-7). This indicates that carbon bearing these diastereotopic protons bridges the benzylic carbon with the carbonyl functional group of the iridoid moiety.

The aromatic proton signals appearing at δ 6.79 correlated with the carbon signals at δ 131.1 (C-4', 8') and 157.3 (C-6') and the proton signal at δ 7.10 correlated with the carbon signals at δ 35.1 (C-2'), 116.4 (C-5', 7') and 157.3 (C-6'). The combination of the correlations observed from COSY, HSQC and HMBC spectra of compound 89-204fr10 allowed the assignment of the partial structure **44**.



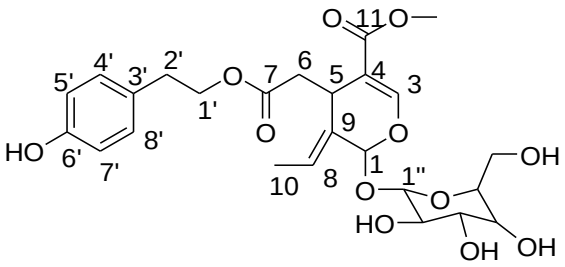
44

Additional HMBC correlations occurred between the anomeric proton signal of the glucose moiety at δ 4.85 with the carbon signals at δ 78.4 (C-2'') and 94.9 (C-1). Furthermore, the proton signal at δ 5.94 correlated with the carbon signals at δ 100.9 (C-1'') and 124.5 (C-8). The carbon signals at δ 13.9 (C-10), 31.8 (C-5) and 94.9 (C-1) correlated with the oleifinc proton signal at δ 6.04. Proton resonance situated at δ 7.50 correlated with the carbon signals at δ 31.8 (C-5), 94.9 (C-1), 109.5 (C-4) and 167.5 (C-11). These correlations allowed the assignment of partial structure **45**.



45

Based on the above spectroscopic data, compound 89-204fr10 was identified to be **29**. Comparison of the experimental data with those reported for Ligstroside in the literature [19] revealed a very close agreement. Ligstroside was previously obtained from *Ligustrum obtusifolium, Fraxinus griffithi, Fraxinus excelsior and Olea europaea*. This compound was reported to have antiinflammatory property [20].



29

Table 4: Comparison of the ^{1}H and ^{13}C NMR data of 89-204fr10 with those reported in the literature for ligstroside

Carbon	**89-204fr10**		**Ligstroside**		**Remark**
No	**δ ^{1}H NMR**	**δ ^{13}C NMR**	**δ ^{1}H NMR**	**δ ^{13}C NMR**	
C-10	1.68	13.9	1.62	13.6	CH_3
C-5	3.98	31.8	3.95	31.7	CH
C-2’	2.85	35.1	2.80	35.1	CH_2
C-6	2.43; 2.75	41.1	2.41; 2.69	41.2	CH_2
	3.75	51.8	3.69	51.9	OCH_3
C-6’’	3.75; 3.87	63.3	3.63; 3.88	62.6	CH_2

C-1’	4.10; 4.23	66.5	4.09; 4.20	66.8	CH_2
C-4’’	3.40	71.9		71.3	CH
C-5’’	3.37	75.0		78.3	CH
C-3’’	3.48	78.2		77.8	CH
C-2’’	3.41	78.4		74.6	CH
C-1	5.94	94.9	5.90	95.0	CH
C-1’’	4.85	100.9	4.80	100.6	CH
C-4		109.5		109.2	Quaternary
C-5’, 7’	6.79	116.4	6.71	116.1	CH
C-8	6.04	124.5	6.06	124.7	CH
C-3’		129.9		130.2	Quaternary
C-9		130.8		129.8	Quaternary
C-4’, 8’	7.10	131.1	7.03	130.8	CH
C-3	7.50	154.5	7.50	154.9	CH
C-6’		157.3		156.8	Quaternary
C-11		167.5		168.4	Quaternary
C-7		172.0		172.9	Quaternary

2.1.2. 89-204fr13

Compound 89-204fr13 was obtained as a white solid. It is optically active with an optical rotation of $[\alpha]^{20}$ = -178^{o} (C, 0.50 in EtOH) and has melting point 90-92^{o}C (Lit. 89-91 ^{o}C) [20]. The TLC Rf value of the compound using EtOAc:MeOH (4:1) as eluent was 0.68. The compound give a purple color when the TLC was sprayed with vanillin: sulfuric acid mixture. The UV-Vis spectrum (in EtOH) displayed two absorption bands at 230 and 281 nm indicating the presence of $\Pi \rightarrow \Pi^{*}$ and $n \rightarrow \Pi^{*}$ transition, which showed the molecule has conjugation.

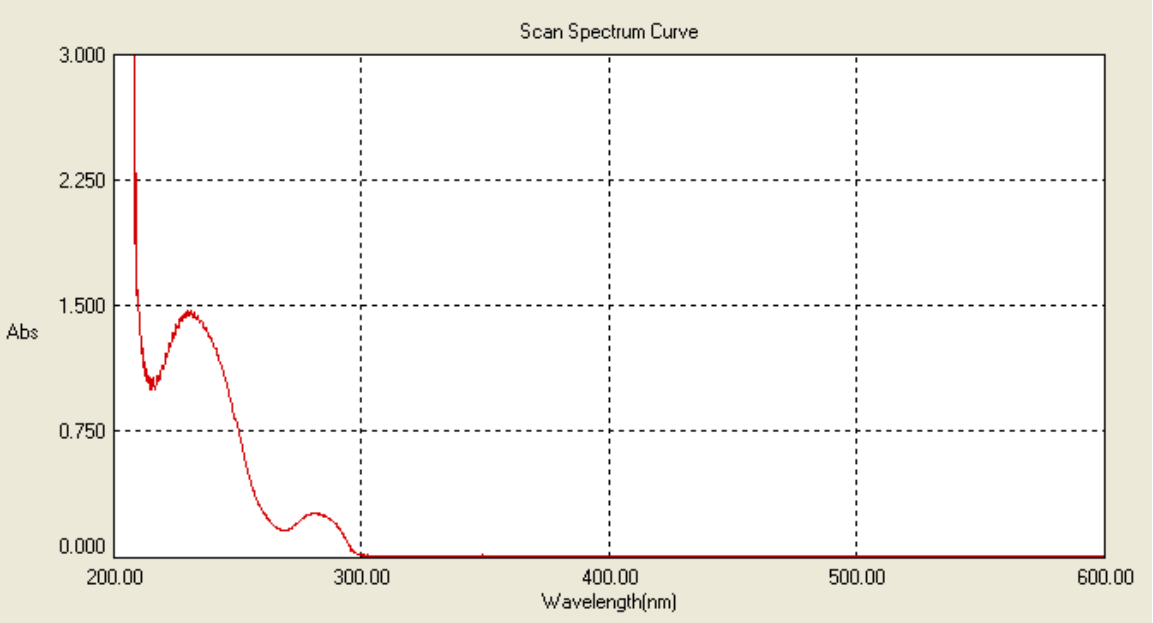


Figure 4: UV-Vis Spectrum of compound 89-204fr13

The presence of hydroxyl group was clearly observed from IR spectrum (Appendix 7) by having absorption band at 3409 cm^{-1} (O-H stretching). Bands at 2922, 1708, and 1443cm^{-1} is due to C-H stretching, C=O stretching of ester carbonyl and aromatic C=C bond stretching respectively. Band at 1286 cm^{-1} and 1076 cm^{-1} is due to C-O stretching.

The proton NMR spectrum (Appendix 8) of 89-204fr13 suggested the presence of one methyl protons in the aliphatic regions. These methyl protons appeared at δ 1.68 (3H, *dd*, *J* = 7.2, 1.2 Hz). The proton NMR spectrum also showed four methylene protons. The first methylene protons appearing at δ 2.41 (1H, *dd*, *J* = 14.4, 9.6 Hz) and 2.72 (1H, *dd*, J = 14, 4 Hz). These protons are diastereotopic as observed from HSQC spectrum. The second methylene protons appeared at δ 2.76 (2H, *t*, *J* = 7 Hz). The third methylene protons appeared at δ 3.75 (1H) and 3.90 (1H, *d*, *J* = 12 Hz). The diastereotopic proton at δ 3.75 overlapped with the methoxy protons. The spectrum also displayed another diastereotopic methylene protons at δ 4.10 (1H, *m*) and 4.22 (1H, *m*). The proton NMR spectrum further exhibited four methine protons in the sugar moiety at δ 3.39 (1H), 3.44 (2H) and 3.55 (1H, *t*, *J* = 8.6 Hz). The compound also showed one methine proton at δ 3.98 (1H, *dd*, *J* = 9.6, 4 Hz). The doublet signal appearing at δ 4.87 (1H, *d*, *J* = 7.6 Hz) and singlet signal at δ 5.95 (1H, *s*) indicate the presence of two anomeric protons. The

proton NMR spectrum revealed the presence of two oleifinc protons at δ 6.03 (1H, *q*) and 7.50 (1H, *s*). The signals appearing at δ 6.58 (1H, *dd, J* = 8, 2 Hz), δ 6.75 (1H, *d*, *J* = 2 Hz) and δ 6.76 (1H, *d, J* = 8 H) is due to protons on a tri substituted aromatic ring.

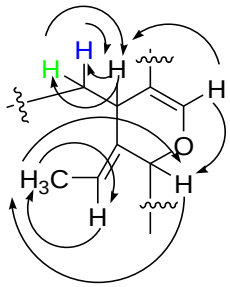
The proton decoupled ^{13}C NMR spectrum (Appendix 9, Table 5) of 89-204fr13 analyzed with the aid of DEPT-135 spectrum (Appendix 9), showed seven quaternary carbon signals. Among these the two signals appearing at δ 166.4 (C-11) and 170.9 (C-7) are accounted for two carbonyl carbons. Three carbons at δ 129.4 (C-3'), 143.6 (C-6') and 144.8 (C-5') are due to aromatic quaternary carbons. The remaining two quaternary carbons appearing at δ 108.2 (C-4) and 129.5 (C-9) are due to carbon α to carbonyl and olefinic carbon in the compound, respectively. Three methine carbon signals appeared in the aromatic region at δ 115.2 (C-4'), 116.0 (C-7') and 120.2 (C-8'). The compound also showed signals of two olefinic carbons at δ 123.3 (C-8) and 153.4 (C-3) and also two anomeric carbons at δ 93.8 (C-1) and 99.7 (C-1''). Also observed carbon resonances at δ 70.5 (C-4''), 73.6 (C-5''), 76.8 (C-3'') and 76.9 (C-2'') is due to methine carbons in the glucose moiety. Additional methine carbon observed at δ 30.5 (C-5). Furthermore the signal that appeared at δ 34.1 (C-2'), 39.8 (C-6), 61.9 (C-6'') and 65.3 (C-1') are the methylene carbons. The presence of methoxy and methyl signals were observed at δ 50.7 and δ 12.7 (C-10) respectively.

Table 5: Proton decoupled ^{13}CNMR data for compound 89-204fr13

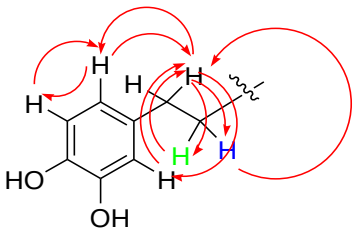
Carbon No	**δ**	**Remark**	**Carbon No**	**δ**	**Remark**
C-10	12.7	CH_3	C-4	108.2	Quaternary
C-5	30.5	CH	C-4'	115.2	CH
C-2'	34.1	CH_2	C-7'	116.0	CH
C-6	39.8	CH_2	C-8'	120.2	CH
	50.7	OCH_3	C-8	123.3	CH
C-6''	61.9	CH_2	C-3'	129.4	Quaternary
C-1'	65.3	CH_2	C-9	129.5	Quaternary
C-4''	70.5	CH	C-6'	143.6	Quaternary
C-5''	73.6	CH	C-5'	144.8	Quaternary

C-3''	76.8	CH	C-3	153.4	CH
C-2''	76.9	CH	C-11	166.4	Quaternary
C-1	93.8	CH	C-7	170.9	Quaternary
C-1''	99.7	CH			

2D NMR was used to further deduce the structure of compound 89-204fr13. In this regard, the ^{1}H-H COSY spectrum (Appendix 10) of compound 89-204fr13 revealed correlation between proton signal at δ 2.76 with proton signals at δ 6.58, 6.75, 4.10 and 4.22 . The proton signals at δ 4.10 and δ 4.22 are due to diastereotopic protons as witnessed from its HSQC spectrum (Appendix 11). Additionally, proton signals at δ 6.58 and 6.76 couples each other. Diastereotopic proton signals at δ 2.41 and 2.71 are correlated with the proton signal at δ 3.98. Long range coupling was observed between the proton signal appearing at δ 1.68 with proton signals at δ 5.95 and δ 6.03. Another long range coupling was also observed between the proton signal appearing at δ 7.50 with proton signals at δ 3.98 and 5.95. The partial structures **42** and **46** observed from the COSY in conjunction with proton and HSQC spectra of compound 89-204fr13.



42



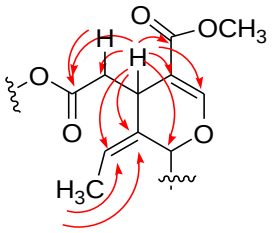
46

Correlations between protons attached on carbon were measured using HSQC and the whole correlation are shown in Table 6.

Table 6: HSQC ($^{1}H \rightarrow ^{13}C$) data for compound 89-204fr13

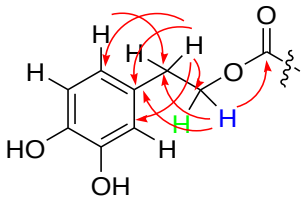
Position of carbon	δ	Position of proton	δ	HSQC($^{1}H \rightarrow ^{13}C$)	Remark
C-10	12.7	H-10	1.68	H-10→C-10	CH_3
C-5	30.5	H-5	3.98	H-5→C5	CH
C-2’	34.1	H-2’	2.76	H-2’→C-2’	CH_2
C-6	39.8	H-6a, H-6b	2.41 and 2.71	H-6a,6b →C-6	CH_2
	50.7		3.75		OCH_3
C-6’’	61.9	H-6’’a, H-6’’b	3.75 and 3.90	H-6’’a,6’’b →C-6’’	CH_2
C-1’	65.3	H-1’a, H-1’b	4.10 and 4.22	H-1’a,1’b →C-1’	CH_2
C-4’’	70.5	H-4’’	3.44	H-4’’ →C-4’’	CH
C-5’’	73.6	H-5’’	3.39	H-5’’ →C-5’’	CH
C-3’’	76.8	H-3’’	3.55	H- 3’’→C-3’’	CH
C-2’’	76.9	H-2’’	3.40	H-2’’ →C-2’’	CH
C-1	93.8	H-1	5.95	H-1→C-1	CH
C-1’’	99.7	H-1’’	4.87	H-1’’→C-1’’	CH
C-4’	115.2	H-4’	6.75	H-4’→C-4’	CH
C-7’	116.0	H-7’	6.76	H-7’→C-7’	CH
C-8’	120.2	H-8’	6.58	H-8’→C-8’	CH
C-8	123.3	H-8	6.03	H-8→C-8	CH
C-3	153.4	H-3	7.50	H-3→C-3	CH

The HMBC spectral data (Appendix 12) of 89-204fr13 revealed that diastereotopic proton signals at δ 2.41 and 2.72 correlated with the carbon signals at δ 30.5 (C-5), 108.2 (C-4), 129.5 (C-9) and 170.9 (C-7). Proton found at δ 3.98 coupled with eight carbons signals at δ 39.8 (C-6), 93.8 (C-1), 108.2 (C-4), 123.3 (C-8), 129.5 (C-9), 153.4 (C-3), 166.4 (C-11) and 170.9 (C-7). These correlations are indicative of the presence of an iridoid moiety in the structure. Further correlations was observed between proton signal appearing at δ 1.68 with a carbon signals appearing at 93.8 (C-1), 123.3 (C-8) and 129.5 (C-9). The above HMBC correlations allowed the establishment of the partial structure **43**.



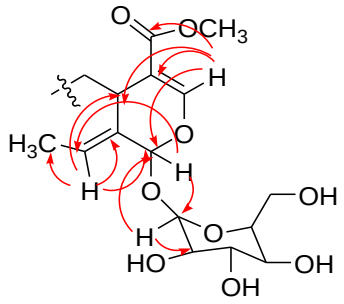
43

Another HMBC correlations were observed between the proton signal at δ 2.76 and the carbon signals at δ 65.3 (C-1'), 115.2 (C-4'), 120.2 (C-8') and 129.4 (C-3'). This indicates that the carbon bearing proton signal at δ 2.76 links aromatic ring with methylene bearing oxygen in the compound. The diastereotopic proton signals at δ 4.10 and 4.22 coupled with carbon appearing at δ 34.1 (C-2'), 129.4 (C-3') and 170.9 (C-7). The partial structure of the compound is most likely be represented as **47**.



47

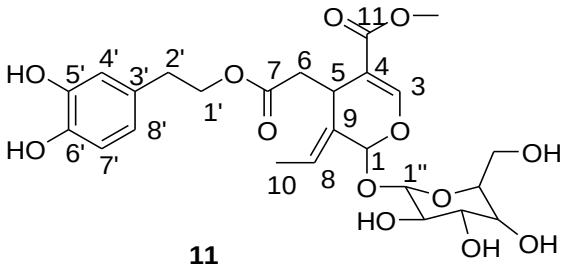
Additional correlation occurred between anomeric protons of glucose moiety appearing at δ 4.87 with a carbon appearing at δ 76.9 (C-2'') and 93.8 (C-1). Furthermore a proton found at δ 5.95 correlates with carbon appearing at δ 99.7 (C-1'') and 123.3 (C-8). The carbon appearing at δ 12.7 (C-10), 30.5 (C-5), 93.8 (C-1) and 129.5 (C-9) correlates with the olefinic proton at δ 6.05. Protons situated at δ 7.50 correlates with a carbon appearing at δ 30.5 (C-5), 93.8 (C-1), 108.2 (C-4) and 166.4 (C-11). The partial fragment observed was depicted as **45**.



45

Based on the above information and full spectral data, compound 89-204fr13 was assigned to structure **11**. Comparison of the experimental data with those reported for oleuropein in the literature [21] revealed a very close agreement. Oleuropein (11) was previously reported from *Olea genus*, but also occurs in many other genera belonging to the Oleaceae family and has been previously described in *Fraxinus excelsior*, *F. angustifolia*, *F. chinensis*, *Syringa josikaea*, *S. vulgaris*, *Philyrea latifolia*, *Ligustrum ovalifolium*, *L. vulgare*, and many others.

Oleuropein has several pharmacological properties including antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer, antimicrobial, and antiviral, and for these reasons, it is commercially available as food supplement in Mediterranean countries. In addition, oleuropein has been shown to be cardioprotective against acute adriamycin cardiotoxicity and has been shown to exhibit anti-ischemic and hypolipidemic activities [16].



11

Table 7: Comparison of the ^{1}H and ^{13}C NMR data of 89-204fr13 with those reported in the literature for oleuropein

Carbon	89-204fr13		Oleuropein		Remark
No	δ ^{1}H NMR	δ ^{13}C NMR	δ ^{1}H NMR	δ ^{13}C NMR	
C-10	1.68	12.7	1.65	13.6	CH_3
C-5	3.98	30.5	3.96	31.8	CH
C-2’	2.76	34.1	2.75	35.3	CH_2
C-6	2.41; 2.72	39.8	2.43; 2.70	41.2	CH_2
	3.75	50.7	3.70	51.9	OCH_3
C-6’’	3.75; 3.90	61.9	3.67; 3.88	62.7	CH_2
C-1’	4.10; 4.22	65.3	4.1; 4.2	66.9	CH_2
C-4’’	3.44	70.5	3.35	71.4	CH
C-5’’	3.39	73.6	3.44	77.9	CH
C-3’’	3.55	76.8	3.36	78.3	CH
C-2’’	3.44	76.9	3.32	74.7	CH
C-1	5.95	93.8	5.90	95.2	CH
C-1’’	4.87	99.7	4.82	100.8	CH
C-4		108.2		109.3	Quaternary
C-4’	6.77	115.2	6.70	116.4	CH
C-7’	6.76	116.0	6.70	117.0	CH
C-8’	6.58	120.2	6.55	121.3	CH
C-8	6.03	123.3	6.07	124.9	CH
C-3’		129.4		130.4	Quaternary
C-9		129.5		130.7	Quaternary
C-6’		143.6		144.8	Quaternary
C-5’		144.8		146.2	Quaternary
C-3	7.50	153.4	7.50	155.2	CH
C-11		166.4		168.7	Quaternary
C-7		170.9		173.2	Quaternary

2.1.3. 89-204fr20

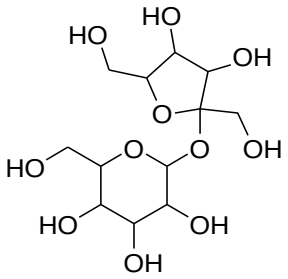
Compound 89-204fr20 was obtained as solid material. The UV-Vis spectrum (in EtOH) showed no absorption band indicating the absence of chromphore in the compound. The IR spectrum of 89-204fr20 (Appendix 13) showed strong and fairly sharp absorption band at 3412 cm^{-1} (O-H stretching) indicating the presence of OH in the compound. Medium and sharp absorption band at 2924 cm^{-1} is due to C-H stretching. Additionally the IR spectrum of 89-204fr20 displayed strong and sharp absorption band at 1070 cm^{-1} due to C-O stretching.

The proton NMR spectrum (Appendix 14) of 89-204fr20 suggested the presence of anomeric signal at 5.41 (1H, *d*, *J* = 4 Hz). The remaining signals appearing in the region between 3.32 to 4.60 are assigned for protons of sucrose moiety. The proton decoupled ^{13}C NMR spectrum (Appendix 15) of 89-204fr20 analyzed with the aid of DEPT-135 spectrum (Appendix 15) showed one quaternary carbon appearing at δ 103.9. Eight methine carbon signals appeared at δ 69.9, 71.7, 72.9, 73.2, 74.3, 77.9, 82.3 and 92.2. Furthermore the signals appearing at δ 60.8, 62.0 and 62.6 are indicative for the presence of three oxygenated methylene carbons in the compound.

Table 8: Proton decoupled ^{13}C NMR data for compound 89-204fr20

δ	**Remark**
60.8	CH_2
62.0	CH_2
62.6	CH_2
69.9	CH
71.7	CH
72.9	CH
73.2	CH
74.3	CH
77.9	CH
82.3	CH
92.2	CH
103.9	Quaternary

Based on the above information and full spectral data of UV, IR, ^{1}H NMR, ^{13}C NMR and DEPT-135 compound 89-204fr20 was found to be the same as sucrose (**48**).



48

2.1.4. 89-206fr5

Compound 89-206fr5 was obtained as amorphous solid. The TLC Rf value of the compound using EtOAc:MeOH (4:1) as eluent was 0.5. The compound give a green color when the TLC was sprayed with vanillin: sulfuric acid mixture. The UV-Vis spectrum (in EtOH) showed maximum absorption bands at 227 and 279 nm indicating the presence of conjugation in the molecule.

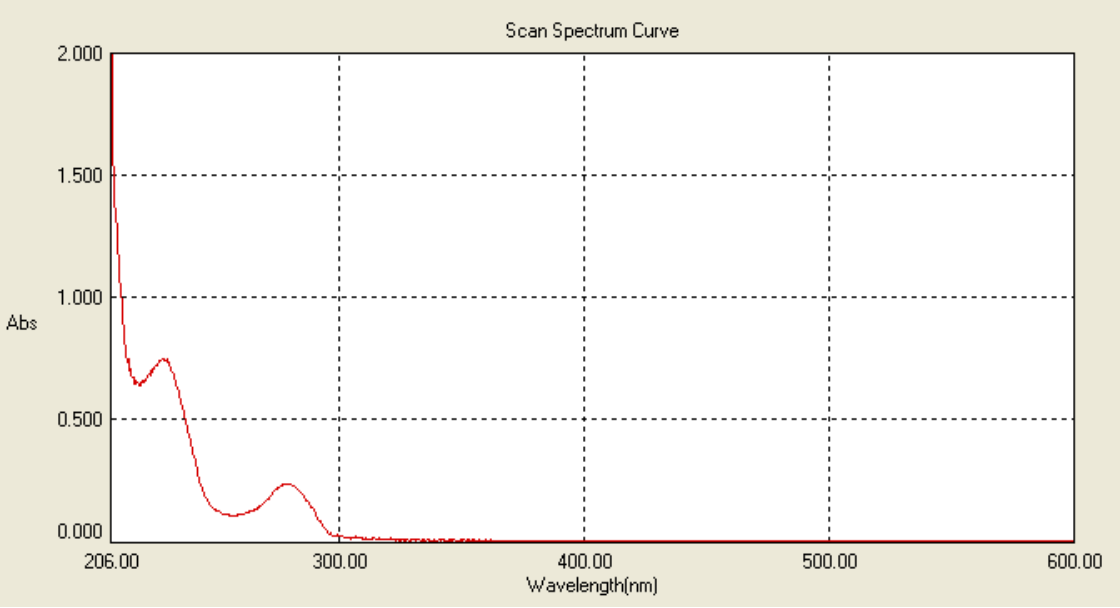


Figure 5: UV-Vis spectrum of compound 89-206fr5

The IR spectrum of 89-206fr5 (Appendix 16) showed strong and fairly sharp absorption band at 3401 cm^{-1} (O-H stretching) indicating the presence of OH in the compound.

Weak and sharp band at 2923 cm^{-1} is due to C-H stretching. The absence of strong and sharp absorption band at 1800-1650 cm^{-1} indicate the absence of carbonyl functional group in the compound, strong and sharp band at 1516 cm^{-1} indicate aromatic C=C bond. Peak at 1270 cm^{-1} and 1076 cm^{-1} due to C-O stretching.

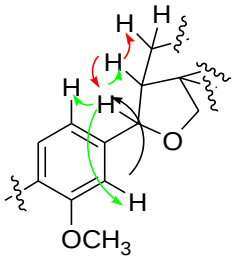
The proton NMR spectrum (Appendix 17) of 89-206fr5 suggested the presence of one methine proton appearing at δ 2.28 (1H, *q*).The compound exhibited four methylene protons signals at δ 2.95 (2H, *q*), 3.79 (1H, *m*); 3.88 (1H, *m*), 3.72 (1H, *m*); 3.89 (1H, *m*) and 3.65 (1H, *m*); 3.87 (1H, *m*). All methylene protons are diastereotopic as observed from its HSQC spectrum. The proton NMR spectrum also showed two methoxy signal appearing at δ 3.87 (6H). The proton NMR spectrum also exhibited four methine proton signals in the region δ 3.40 – 3.64 which indicates the presence of sugar moiety in the structure of the compound. This is confirmed by the presence of one proton at δ 4.88 (1H, *d*, *J* = 7.2 Hz) which is characteristics of the anomeric proton. The compound also showed one methine proton at δ 4.82 (1H, *d*, *J* = 6.8 Hz). The proton NMR spectrum revealed six proton signals at δ 6.73 (2H, unresolved doublet), 6.90 (1H, *br. s*), 6.99 (1H, *dd*, *J* = 1.6, 8.4 Hz), 7.13 (1H, *d*, *J* = 8.4 Hz) and 7.24 (1H, *d*, *J* = 1.6 Hz) which indicates the presence of two tri substituted aromatic group.

The proton decoupled ^{13}C NMR spectrum (Appendix 18, Table 9) of 89-206fr5 analyzed with the aid of DEPT-135 spectrum (Appendix 18) showed seven quaternary carbon signals. Among these the carbon resonating at δ 128.9 (C-1'), 137.7 (C-1), 144.7 (C-4'), 145.9 (C-4), 147.1 (C-3') and 149.3 (C-3) are due to aromatic quaternary carbons. The remaining quaternary carbon appeared at δ 81.2 (C-8'). The spectrum showed two methoxy carbon signals at δ 54.9 and 55.2. Furthermore the signals at δ 39.1 (C-7'), 59.4 (C-9), 61.0 (C-6'') and 76.6 (C-9') are due to methylene carbons. And also carbon signal appearing at δ 101.5 (C-1'') are assigned to anomeric carbon of the sugar moiety. The other carbon signals of the sugar moiety were situated at δ 69.9, 73.5, 76.4 and 76.7. Furthermore the signals appearing at δ 84.1 (C-7), 110.8 (C-2), 113.8 (C-2'), 114.3 (5'), 116.2 (C-5), 119.1 (C-6) and 122.4 (C-6') are the methine carbons. Among these the one appearing at δ 84.1 is typical of methine carbon attached to aromatic ring. The remaining six signals are ascribed to aromatic methine.

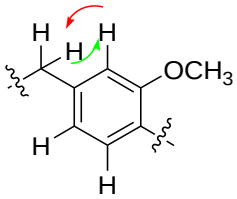
Table 9: Proton decoupled ^{13}CNMR data for compound 89-206fr5

Carbon No	δ	Remark	Carbon No	δ	Remark
C-7’	39.1	CH_2	C-1’’	101.5	CH
	54.9	OCH_3	C-2	110.8	CH
	55.2	OCH_3	C-2’	113.8	CH
C-9	59.4	CH_2	C-5’	114.3	CH
C-8	60.2	CH	C-5	116.2	CH
C-6’’	61.0	CH_2	C-6	119.1	CH
C-4’’	69.9	CH	C-6’	122.4	CH
C-2’’	73.5	CH	C-1’	128.9	Quaternary
C-5’’	76.4	CH	C-1	137.7	Quaternary
C-9’	76.6	CH_2	C-4’	144.7	Quaternary
C-3’’	76.7	CH	C-4	145.9	Quaternary
C-8’	81.2	Quaternary	C-3’	147.1	Quaternary
C-7	84.1	CH	C-3	149.3	Quaternary

The H-H COSY spectrum (Appendix 19) of compound 89-206fr5 revealed correlation between proton signal appearing at δ 2.28 with a proton signals at δ 3.79, 3.88 and 4.82. The proton signals at δ 3.79 and 3.88 (found on carbon at δ 59.4) are due to diastereotopic protons as observed from the HSQC spectrum (Appendix 20). Another coupling was observed between the proton signal at δ 4.82 with a proton signals appearing at δ 6.99 and 7.24. Proton resonance at δ 2.95 couples with proton signal appearing at δ 6.90. The fragments observed from the COSY in conjunction with proton and HSQC spectrum of compound 89-206fr5 were shown below.



49



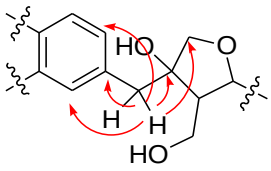
50

The HSQC spectrum (Appendix 20) showed correlations between carbon with attached protons and the whole correlation is tabulated in Table 10.

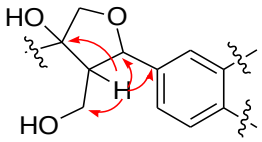
Table 10: HSQC ($^{1}H \rightarrow ^{13}C$) data for compound 89-206fr5

Position of carbon	δ	Position of proton	δ	HSQC($^{1}H \rightarrow ^{13}C$)	Remark
C-7’	39.1	H-7’	2.95	H-7’,→C-7’	CH_2
	54.9		3.87		OCH_3
	55.2		3.87		OCH_3
C-9	59.4	H-9a, H-9b	3.79 and 3.88	H-9a,9b →C-9	CH_2
C-8	60.2	H-8	2.28	H-8→C-8	CH
C-6’’	61.0	H-6’’a, H-6’’b	3.72 and 3.89	H-6’’a,6’’b→C-6’’	CH_2
C-9’	76.6	H-9’a, H-9’b	3.65 and 3.87	H-9’a,9’b →C-9’	CH_2
C-7	84.1	H-7	4.83	H-7→C-7	CH
C-1’’	101.5	H-1’’	4.88	H-1’’→C-1’’	CH
C-2	110.8	H-2	7.24	H-2→C-2	CH
C-2’	113.8	H-2’	6.90	H-2’→C-2’	CH
C-5’	114.3	H-5’	6.73	H-5’→C-5’	CH
C-5	116.2	H-5	7.13	H-5→C-5	CH
C-6	119.1	H-6	6.99	H-6→C-6	CH
C-6’	122.4	H-6’	6.73	H-6’→C-6’	CH

The HMBC spectral data (Appendix 21) of 89-206fr5 revealed that the methylene proton signal at δ 2.95 correlates with the carbon signals at δ 76.6 (C-9’), 81.2 (C-8), 113.6 (C-2’), 122.4 (C-6’) and 128 (C-1’). Further correlation was observed between the hydrogen signal appearing at δ 2.28 with a carbon signals at 59.4 (C-9), 81.2 (C-8), 84.1 (C-7) and 137.7 (C-1). The above HMBC correlations allowed the establishment of the partial structures **51** and **52**.

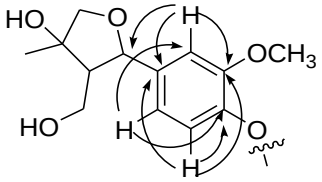


51



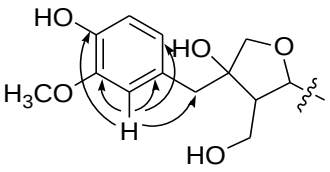
52

Further correlation observed in the HMBC spectrum was found between a proton appearing δ 7.24 with a carbon appearing at δ 84.1 (C-7), 119.1 (C-6), 137.7 (C-1), 145.9 (C-4) and 149.3 (C-3). Another aromatic proton appearing at δ 7.13 correlates with a carbon appearing at δ 137.7 (C-1), 145.9 (C-4) and 149.3 (C-3). The Carbon appearing at δ 110 (C-2) and 145.9 (C-4) correlates with proton appearing at δ 6.99. The partial structure of the compound is most likely represented as **53**.

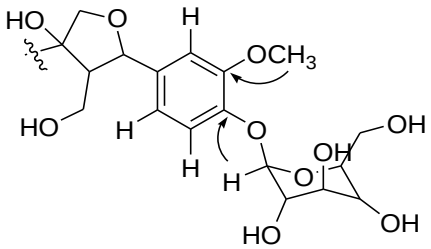


53

Additional correlation occurred between anomeric protons of glucose moiety appearing at δ 4.88 with a carbon appearing at δ 145.9 (C-4). Furthermore a proton appearing at δ 6.90 correlates with carbon appearing at δ 39.1 (C-7'), 122.4 (C-6'), 128.9 (1'), 144.7 (4') and 147.1 (C-3'). The two methoxy protons correlate with carbon appearing at δ 147.1 (C-3') and 149.3 (C-3). The partial fragment observed was depicted as **54** and **55**.

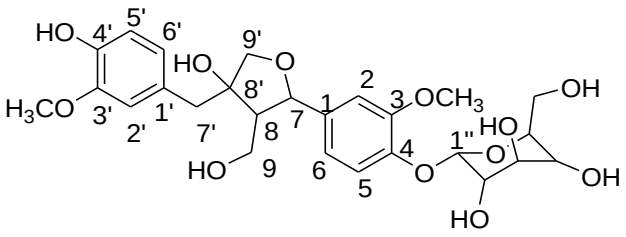


54



55

Based on the above spectroscopic data, compound 89-206fr5 was assigned to structure **40**. Comparison of the experimental data with those reported for olivil 4-O-β-D-glucoside in the literature [22] revealed a very close agreement. Olivil 4-O-β-D-glucoside is a furan type lignan previously reported from *Olea europaea*, *Cerbera* sp. and *Eucommia ulmoides* [13, 20].



40

Table 11: Comparison of the ^{13}C NMR data of 89-206fr5 with those reported in the literature for olivil 4-O-β-D-glucoside

Carbon No	**89-206fr5 δ ^{13}C NMR (methanol-d_4)**	**olivil 4-O-β-D-Glucoside δ ^{13}C NMR (pyridine-d_5)**	**Remark**
C-7’	39.1	40.5	CH_2
	54.9	55.8	OCH_3
	55.2	55.8	OCH_3
C-9	59.4	60.4	CH_2
C-8	60.2	62.0	CH
C-6’’	61.0	62.3	CH_2
C-4’’	69.9	71.2	CH
C-2’’	73.5	74.9	CH
C-5’’	76.4	78.5	CH
C-9’	76.6	78.0	CH_2
C-3’’	76.7	78.7	CH
C-8’	81.2	81.9	Quaternary
C-7	84.1	84.5	CH
C-1’’	101.5	102.5	CH
C-2	110.8	112.1	CH

C-2’	113.8	115.4	CH
C-5’	114.3	116.1	CH
C-5	116.2	116.1	CH
C-6	119.1	119.7	CH
C-6’	122.4	123.8	CH
C-1’	128.9	130.0	Quaternary
C-1	137.7	138.8	Quaternary
C-4’	144.7	146.7	Quaternary
C-4	145.9	149.3	Quaternary
C-3’	147.1	148.2	Quaternary
C-3	149.3	147.2	Quaternary

2.1.5. 89-204B

Compound 89-204B was obtained as white solid. Mp. 162-163 ^{o}C (Lit. 166 ^{o}C,) [23]. The absence of chromphore was observed from its UV-Vis spectral analysis. The IR spectrum of 89-204B (Appendix 22) showed strong and fairly sharp absorption band at 3273 cm^{-1} (O-H stretching) indicating the presence of OH in the compound. Another absorption band at 2943 and 1090 cm^{-1} is due to the presence of C-H and C-O stretching respectively.

The proton NMR spectrum (Appendix 23) of 89-204B suggested the presence of one methylene protons appearing at δ 3.52 (2H, *dd*, *J* = 11.6, 6 Hz) and 3.72 (2H, *dd*, *J* = 11.6, 2.4 Hz). This indicates that these protons are diastereotopic as confirmed from its HSQC spectrum (Appendix 25). The proton NMR spectrum also exhibited two methine proton signals appearing at δ 3.61 (2H, *ddd*) and 3.65 (2H).

Table 12: ^{1}H NMR data of compound 89-204B

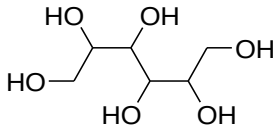
Proton chemical shift (δ in , multiplicity and J in Hz)	No of hydrogen	Remark
3.52 (2H, dd, J = 11.6, 6 Hz) ; 3.72 (2H, dd, J = 11.6, 2.4 Hz)	4H	$2CH_2$
3.61 (2H, ddd)	2H	2CH
3.65 (2H)	2H	2CH

The proton decoupled ^{13}C NMR spectrum (Appendix 24, Table13) of 89-204B analyzed with the aid of DEPT-135 spectrum (Appendix 24) showed one methylene and two methine carbons. The methylene carbon appeared at δ 63.1 while the two methine carbon appearing at δ 69.1 and 70.6. As observed from the proton and carbon NMR spectrum, the compound is open chain and symmetrical.

Table 13: Proton decoupled ^{13}CNMR data for compound 89-204B

δ	Remark
63.1	$2CH_2$
69.1	2CH
70.6	2CH

^{1}H-^{1}H COSY, HSQC and HMBC were recorded for compound 89-204B. The spectral data obtained along with UV-Vis, IR, ^{1}H NMR, ^{13}C NMR and DEPT-135 confirms that compound 89-204B is mannitol (**56**).

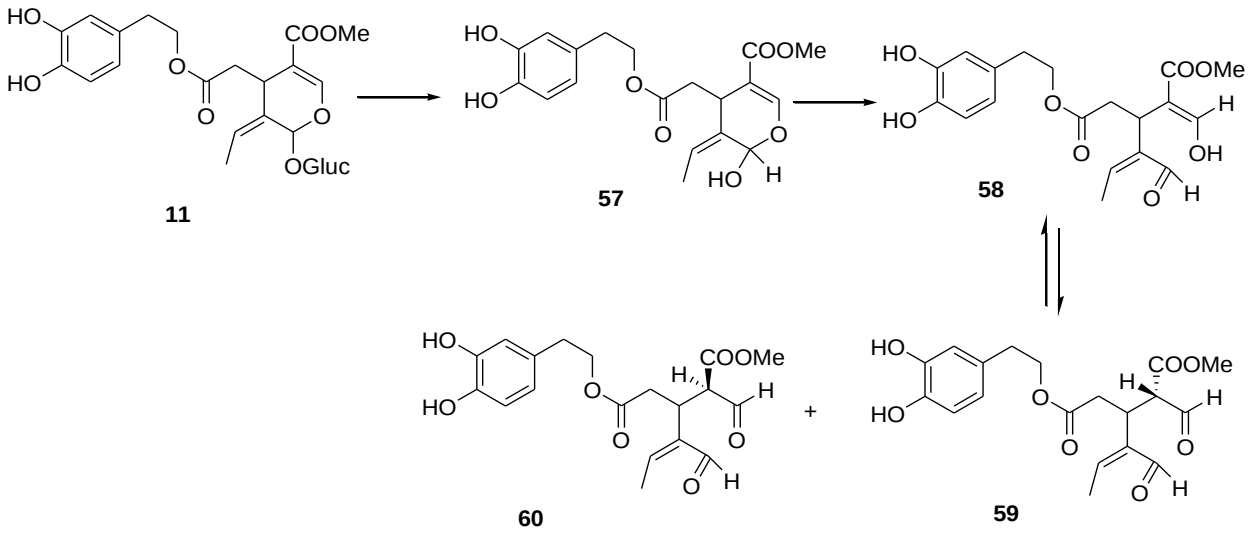


56

2.2. Analysis of 89-204fr2

In the course of this project work, column fractions eluted using 100% EtOAc (89-204fr2) were also analyzed using NMR (Appendix 26). The spectral analysis showed the presence of proton resonances appearing at δ 9.20, 9.50 and 9.61 which are typical of an aldehydes. This is also evident from ^{13}C NMR spectra, as three carbon resonances were clearly observed at δ 195.4, 200.1 and 200.8. Hence an attempt was made to separate these aldehydes using column chromatography and Sephadex LH 20.

The NMR result of these subfractions again ends up in a mixtures of aldehyde exhibiting same NMR pattern as the previous one. Similar attempts were made to separate these aldehydes by different scholars. Literature report showed that the enzymatic hydrolysis of oleuropein results in such kind of mixtures [15, 23] as shown below (Scheme 3).

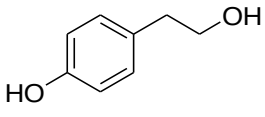


Scheme 3: Enzymatic hydrolysis of oleuropein

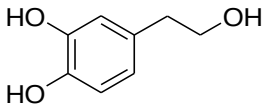
The NMR spectrum of these mixture of aldehydes (89-204fr2) was compared with the literature report. The result obtained was in good agreement with the literature report of **58**, **59** and **60** [23].

2.3. Acid hydrolysis of 89-204fr12

Another attempt made was hydrolysis of 89-204fr12 (containing ligstroside as a minor and oleuropein as a major constituent) using 1N H_2SO_4. The hydrolysis resulted in a mixture of products (89-207A, 30 mg) as observed from TLC and NMR. Hence separation of the mixture was attempted using Sephadex LH 20 eluted with $CHCl_3$:MeOH (1:1) to afford ten fractions. Among these the sixth fraction showed two spots on TLC. The ^{1}H NMR spectrum (Appendix 27) showed a signal at δ 7.05 (1H, *d*, *J* = 8 Hz), 6.75 (1H, *d*, *J* = 8 Hz), 6.72 (2H, *m*), 6.55 (1H, *m*), 3.36 (1H, *m*), 3.62 (2H, *m*), 2.67 (1H, *m*) and 2.65 (2H, *m*). This indicated that fraction six contained tyrosol (**61**) and hydroxytyrosol (**62**) in 1:2 ratio, respectively.



61



62

3. Conclusion and recommendations

In this project work, the EtOH extract of *Jimma Enchet* gave five compounds (Ligstroside, Oleuropein, olivil 4-O-β-D-Glucoside, sucrose and mannitol), which were isolated and characterized using spectroscopic techniques. Oleuropein was found to be the major compound.

Acid hydrolysis of oleuropein containing small amount of ligstroside gave mixtures of two phenolic alcohols (tyrosol and hydroxytyrosol).

Further recommendations made are to

- identify the plant material
- scaling up of the EtOH extract and isolate additional compounds
- quantify the amount of oleuropein.
- isolate and characterize the aldehydes found in *Jimma Enchet*.

4. Experimental

4.1. General

Column chromatography was performed using silica gel 60 (70-230 mesh) Merck. Samples were applied on the top of the column by adsorbing on silica gel. Analytical TLC was run on a 0.25 mm thick layer of silica gel GF254 (Merck) on aluminum plate. Spots were detected by observation under UV light (254 nm) and spraying with vanillin in H_2SO_4 and by heating with hot air gun.

1D and 2D NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400.13 and 100.6 MHz. The ultraviolet and visible (UV-Vis) spectra were taken on T60 UV-Visible spectrphotometer in the range 190-900 cm^{-1}. Infrared (IR) absorptions were measured as KBr pellets on Perkin-Elmer spectrum 65 FT-IR spectrometer in the range 4000-400 cm^{-1}. Melting points were recorded using Mettler-Toledo FP82HT hot stage with FP90 central processor and LEICA GALEN^{TM} III microscope apparatus and uncorrected. Optical rotation was measured on autopol IV automatic polarimeter.

4.2. Plant material

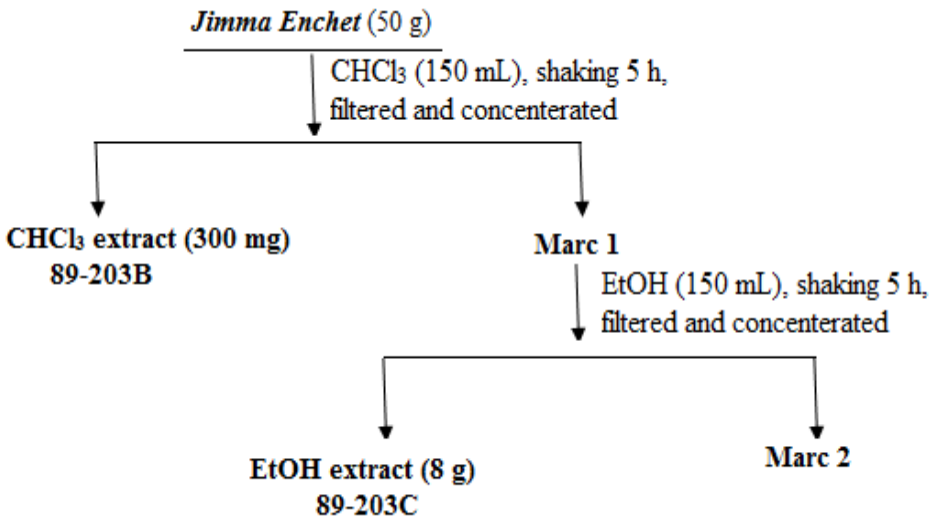
The plant material used in this study were purchased in May, 2014 from Merkato market Addis Ababa Ethiopia. Local name of the plant is *Jimma Enchet.*

4.3. Coding system

89 stands for laboratory note book, **202, 204, 206 and 208** stand for page numbers of the note book.

4.4. Extraction of *Jimma Enchet*

The pulverized wood of *Jimma Enchet* (50 g) was soaked in chloroform (150 mL) and extracted by shaking for 5 h at room temperature. It was filtered and concentrated under reduced pressure at 45^0C to yield 300 mg (0.6 %) of brown gummy extract. The marc was extracted further with EtOH (150 mL) by using shaker for 5 h then filtered and concentrated under reduced pressure at 45^0C to give 8 g (16 %) of brown gummy extract. The flow sheet diagram of the extraction procedure is shown in Scheme 4.



Scheme 4: Flow sheet diagram showing extraction of *Jimma Enchet*

4.5. Compound Isolation

EtOH extract of *Jimma Enchet* (5 g) was adsorbed on 3 g of silica gel and charged on to column packed with 70 g silica gel using hexane. In the course the column was eluted with different solvents to afford eight fractions as depicted in Table 14 below.

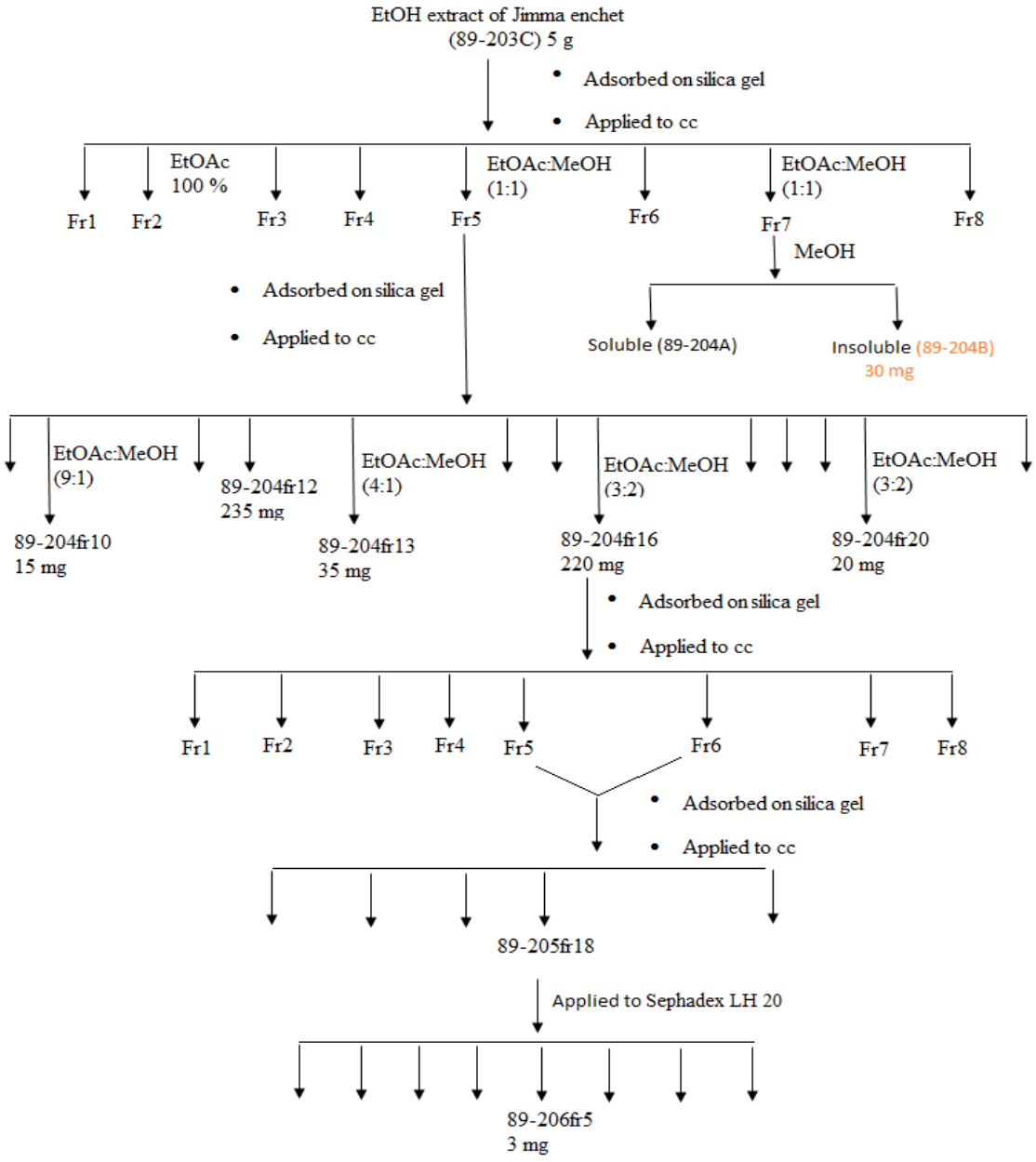
Table 14: Column chromatographic fractionation of EtOH extract of *Jimma Enchet*

Fraction	Eluted solvent	Ratio	Volume collected	Amount obt.
1	hexane:EtOAc	1:1	40 mL	4 mg
2	EtOAc	100%	"	130 mg
3	EtOAc:MeOH	1:1	"	1.4 g
4	"	"	"	320 mg
5	"	"	"	1.9 g
6	"	"	"	90 mg
7	"	"	"	100 mg
8	MeOH	100%	"	200 mg

Among these subfractions the TLC profile of fraction 5 and fraction 7 was found good. An attempt was made to dissolve fraction 7 (89-204fr7) using methanol. The insoluble portion was separated by decantation to afford white solid 89-204B (30 mg).

F5 (1.9 g) was subjected to column chromatography using hexane for packing. The column was eluted using EtOAc:MeOH of increasing polarities to afford thirteen fractions. Among these, the fraction eluted using EtOAc:MeOH (9:1, 40 mL) 15 mg, EtOAc:MeOH (4:1, 40 mL) 235 mg, EtOAc:MeOH (4:1, 40 mL) 35 mg, EtOAc:MeOH (3:2, 40 mL) 220 mg and EtOAc:MeOH (3:2, 40 mL) 20 mg were labeled as 89-204fr10, 89-204fr12, 89-204fr13, 89-204fr16 and 89-204fr20 respectively. Three fractions 89-204fr10, 89-204fr13 and 89-204fr20 showed only one spot on TLC.

89-204fr16 (220 mg) was subjected to column chromatography on silica gel. Elution was done using EtOAc:MeOH of increasing polarities to afford eight fractions. Among these fr5 and fr6 were combined and rechromatographed over silica gel which was eluted using EtOAc:MeOH of increasing polarities to afford five fractions. Further purification of fr4 (89-205fr18) was achieved using Sephadex LH 20 with MeOH:$CHCl_3$ (1:1) as eluent to afford eight fractions. The TLC of the fifth fraction (89-206fr5, 3 mg) showed one spot. All fractionation procedures are schematically shown in Scheme 5.



Scheme 5: Isolation of compounds from EtOH extract of *Jimma enchet*

4.6. Acid hydrolysis of 89-204fr12

50 mg of 89-204fr12 was hydrolyzed in 10 mL of 1N H_2SO_4 for 1h at 100 ^{o} C. Then cooled at room temperature and extract with EtOAC. The organic layer was concentrated and afford 30 mg

of oily product. The product was applied on Sephadex LH 20 and 10 fractions were collect by using $CHCl_3$: MeOH (1:1) solvent system.

4.7. Spectral data

Ligstroside (89-204fr10): white solid, m.p 85-87 ^oC, UV-Vis λmax (EtOH) nm: 226 and 276. IR υ_{max} (KBr) cm^{-1}: 3420, 2922, 1709, 1441, 1518, 1076. ^{1}H-NMR (acetone-d_6) δ: 1.68 (3H, *dd*, H-10), 3.98 (1H, *dd*, H-5), 2.85 (2H, *t*, H-2'), 2.43 (1H, *dd*, H-6a); 2.75 (1H, *dd*, H-6b), 3.75 (OCH_3), 3.75 (1H, H-6''a); 3.87 (1H, *d*, H-6''b), 4.10 (1H, *m*, H-1'a); 4.23 (1H, *m*, H-1'b), 3.40 (1H, H-4''), 3.37 (1H, H-5''), 3.48 (1H, *t*, H-3''), 3.41 (1H, H-2''), 5.94 (1H, *s*, H-1), 4.85 (1H, *d*, H-1''), 6.79 (2H, *d*, H-5', 7'), 6.04 (1H, *q*, H-8), 7.10 (2H, *d*, H-4', 8'), 7.50 (1H, *s*, H-3). ^{13}C-NMR (acetone-d_6) δ: 13.9 (C-10), 31.8 (C-5), 35.1 (C-2'), 41.1 (C-6), 51.8 (OCH_3), 63.3 (C-6''), 66.5 (C-1'), 71.9 (C-4''), 75.0 (C-5''), 78.2 (C-3''), 78.4 (C-2''), 94.9 (C-1), 100.9 (C-1''), 109.5 (C-4), 116.4 (C-5', 7'), 124.5 (C-8), 129.9 (C-3'), 130.8 (C-9), 131.1 (C-4', 8'), 154.5 (C-3), 157.3 (C-6'), 167.5 (C-11), 172.0 (C-7).

Oleuropein (89-204 fr13): white solid, m.p 90-92 ^oC, optical rotation of $[\alpha]^{20}$ = -178 (C, 0.50 in EtOH), UV-Vis λmax (EtOH) nm: 230 and 281. IR υ_{max} (KBr) cm^{-1}: 3409, 2922, 1708, 1443, 1286, 1076. ^{1}H-NMR (acetone-d_6) δ: 1.68 (3H, *dd*, H-10), 3.98 (1H, *dd*, H-5), 2.76 (2H, *t*, H-2'), 2.41 (1H, *dd*, H-6a); 2.72 (1H, *dd*, H-6b), 3.75 (OCH_3), 3.75 (1H, H-6''a); 3.90 (1H, *d*, H-6''b), 4.10 (1H, *m*, H-1'a), 4.22 (1H, *m*, H-1'b), 3.44 (2H, H-2'', 4''), 3.39 (1H, H-5''), 3.55 (1H, *t*, H-3''), 5.95 (1H, *s*, H-1), 4.87 (1H, *d*, H-1''), 6.75 (1H, *d*, H-4'), 6.76 (1H, *d*, H-7'), 6.58 (1H,*dd*, H-8'), 6.03 (1H, *q*, H-8), 7.50 (1H, *s*, H-3). ^{13}C-NMR (acetone-d_6) δ: 12.3 (C-10), 30.5 (C-5), 34.1 (C-2'), 39.8 (C-6), 50.7 (OCH_3), 61.9 (C-6''), 65.3 (C-1'), 70.5 (C-4''), 73.6 (C-5''), 76.8 (C-3''), 76.9 (C-2''), 93.8 (C-1), 99.7 (C-1''), 108.2 (C-4), 115.2 (C-4'), 116.0 (C-7'), 120.2 (C-8'), 123.3 (C-8), 129.4 (C-3'), 129.5 (C-9), 143.6 (C-6'), 144.8 (C-5'), 153.4 (C-3), 166.4 (C-11), 170.9 (C-7).

Sucrose (89-204fr20): IR υ_{max} (KBr) cm^{-1}: 3412, 2924 and 1074. ^{1}H-NMR (methanol-d_4) δ: 5.41 (1H, *d*), 3.32 to 4.60 (14H). ^{13}C-NMR δ: 60.8, 62.0, 62.6, 69.9, 71.7, 72.9, 73.2, 74.3, 77.9, 82.3 and 92.2.

Olivil 4-O-β-D-Glucoside (89-206fr5): UV-Vis λmax (EtOH) nm: 227 and 279. IR υ_{max} (KBr) cm^{-1}: 3401, 2923, 1516, 1270 and 1070.^{1}H-NMR (methanol-d_4) δ: 2.95(2H, *q*, H-7'), 3.87 (OCH_3), 3.87 (OCH_3), 3.79 (1H, *m*, H-9a); 3.88 (1H, *m*, H-9b), 2.28 (1H, *q*, H-8), 3.72 (1H, *m*, H-6''a); 3.89 (1H, *m*, H-6''b), 3.65 (1H, *m*, H-9'a); 3.87 (1H, *m*, H-9'b), 4.83 (1H, *d*, H-7), 4.88 (1H, *d*, H-7), 7.24 (1H, *d*, H-2), 6.90 (1H, *br. s*, H-2'), 6.73 (1H, *d*, H-5'), 7.13 (1H, *d*, H-5), 6.99 (1H, *dd*, H-6) and 6.73 (1H, *d*, H-6').^{13}C-NMR (methanol-d_4) δ: 39.1 (C-7'), 54.9 (OCH_3), 55.2 (OCH_3), 59.4 (C-9), 60.6 (C-8), 61.0 (C-6''), 69.9, 73.5, 76.4, 76.6 (C-9'), 76.7, 81.2 (C-8'), 84.1 (C-7), 101.5 (C-1''), 110.8 (C-2), 113.8 (C-2'), 114.3 (C-5'), 116.2 (C-5), 119.1 (C-6), 122.4 (C-6'), 128.9 (C-1'), 137.7 (C-1), 144.7 (C-4'), 145.9 (C-4), 147.1 (C-3'), 149.3 (C-3).

Mannitol (89-204B): Mp. 162-163 ºC. IR υ_{max} (KBr) cm^{-1}: 3273, 2943 and 1090. ^{1}H-NMR (D_2O) δ: 3.52 (2H, *dd*), 3.72 (2H, *dd*), 3.61 (2H, *ddd*) and 3.65 (2H). ^{13}C-NMR δ: 63.1, 69.1 and 70.6.

Tyrosol: ^{1}H-NMR (methanol-d_4) δ: 7.05 (1H, *d*, *J* = 8 Hz), 6.75 (1H, *d*, *J* = 8 Hz), 3.36 (1H, *m*) and 2.67 (1H, *m*)

Hydroxy**tyrosol:** ^{1}H-NMR (methanol-d_4) δ: 6.72 (2H, *m*), 6.55 (1H, *m*), 3.62 (2H, *m*), and 2.65 (2H, *m*)

5. References

[1]. Yirga, G. (**2010**). Use of Traditional Medicinal Plants by Indigenous People in Mekele Town, Capital City of Tigray Regional State of Ethiopia. *Journal of Medicinal Plants Research*, **4**(17), 1799-1804.

[2]. Abera, B. (**2014**). Medicinal Plants Used in Traditional Medicine by Oromo People, Gimbi District, Southwest Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **10**, 40-54.

[3]. Giday, M. and Teklehaymanot, T. (**2013**). Ethnobotanical Study of Plants Used in Management of Livestock Health Problems by Afar People of Ada'ar District, Afar Regional State, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **9,** 8-17.

[4]. Lulekal, E., Asfaw, Z., Kelbessa, E. and Damme, P. V. (**2013**). Ethnomedicinal Study of Plants Used for Human Ailments in Ankober District, North Shewa Zone, Amhara Region, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **9**, 63-75.

[5]. Zenebe, G., Zerihun, M. and Solomon, Z. (**2012**). An Ethnobotanical Study of Medicinal Plants in Asgede Tsimbila District, Northwestern Tigray, Northern Ethiopia. *Ethnobotany Research and Applications*, **10**, 305-320.

[6]. Ragunathan, M. and Solomon, M. (**2009**). The Study of Spiritual Remedies in Orthodox Rural Churches and Traditional Medicinal Practice in Gondar Zuria District, Northwestern Ethiopia. *PHCOGJ*, **1**(3), 178-183.

[7]. Abegaz, B., Ngadjui, B.T., Bezabih, M. and Mdee L.K. (**1999**). Novel Natural Products from Marketed Plants of Eastern and Southern Africa. *Pure Appl. Chem*., **71**, 919-926.

[8]. Tadeg, H. (**2004**). Phytopharmaceutical Study of some selected medicinal plants locally used in the treatment of skin disorders. MSc Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

[9]. Canuto, K.M., Silveira, E.R., Bezerra, A.M.E., Leal, L.K.A.M. and Viana, G.S.B. (). Phytochemistry, Pharmacology and Agronomy of Medicinal Plants: Amburana cearensis, an Interdisciplinary Study. Phytochemicals – A Global Perspective of Their Role in Nutrition and Health. **17**, 353-374.

[10]. Thomsen, H., Reider, K., Franke, K., Wessjohann, L.A., Keiser, J., Dagne, E. and Arnold, N. (**2012**). Characterization of Constituents and Anthelmintic Properties of *Hagenia abyssinica. Sci Pharm*.

[11]. Duddeck, H., Yenesew, A. and Dagne, E. (**1987**). Isoflavonoids from Taverniera Abyssinica. *Bull. Chem. Soc. Ethiopi*., **1**(1): 36-41.

[12]. Kadowaki, E., Yoshida, Y., Nitoda, T., Baba, N. and Nakajima, S. (**2003**). (－)-Olivil and (＋)-1-Acetoxypinoresinol from the Olive Tree (Olea europaea LINNE; Oleaceae) as Feeding Stimulants of the Olive Weevil (Dyscerus perforatus). *Biosci. Biotechnol. Biochem.*, **67** (2), 415–419.

[13]. Bonilla, M. P., Salido, S., Beek, T. A. V. and Altarejos, J. (**2014**). Radical-Scavenging Compounds from Olive Tree (Olea europaea L.) Wood. *J. Agric. Food Chem.*, **62**, 144−151.

[14]. Ndlovu, T. (**2007**). Isolation and Characterization of Some of the Major Compounds from Pentanisia Prunelloides. MSc Thesis, Faculty of science, University of Johannesburg.

[15]. Obied, H.K., Prenzler, P.D., Ryan, D., Servili, M., Taticchi, A., Espostob, S. and Robards, K. (**2008**). Biosynthesis and Biotransformations of Phenol-conjugated Oleosidic Secoiridoids from Olea europaea L. *Natural Product Reports*, **25**, 1167–1179.

[16]. OMAR, S.H. (**2010**). Oleuropein in Olive and its Pharmacological Effects. *Sci Pharm.*, **78**, 133–154.

[17]. Rosales, F. G., Romero, M. P., Casanovas, M., Motilva, M. J. and Mosquera, M. I. M. (**2010**). Metabolites Involved in Oleuropein Accumulation and Degradation in Fruits of Olea europaea L.: Hojiblanca and Arbequina Varieties. *J. Agric. Food Chem.*, **58**, 12924–12933.

[18]. Cunha, W.R., Silva, M.L.A., Veneziani, R.C.S., Ambrósio, S.R. and Bastos, J.K. (**2012**). Lignans: Chemical and Biological Properties. Phytochemicals – A Global Perspective of Their Role in Nutrition and Health. **10**, 213-233.

[19]. Lee, D.G., Choi, J.S., Yeon, S.W., Cui, E.J., Park, H.J., Yoo, J.S., Chung, I.S. and Baek, N.I. (**2010**). Secoiridoid Glycoside from the Flowers of Osmanthus fragrans var.aurantiacus Makino Inhibited the Activity of β-Secretase. *J. Korean Soc. Appl. Biol. Chem*., **53**(3): 371-374.

[20]. Dictionary of Natural Product on DVD (**2014**).

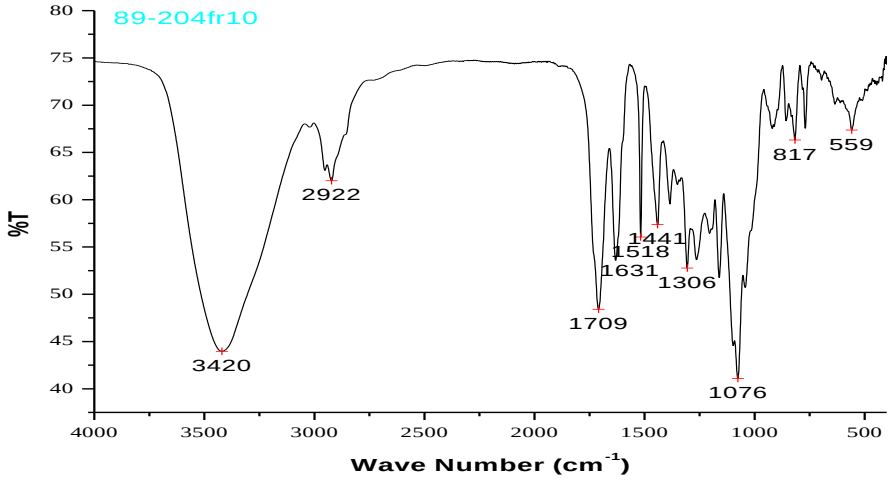
[21]. Kadowaki, E., Yoshida, Y., Baba, N. and Nakajima, S. (**2003**). Feeding Stimulative Activity of Steroidal and Secoiridoid Glucosides and Their Hydrolysed Derivatives toward the Olive Weevil (Dyscerus perforatus). *Z. Naturforsch*, **58**c, 441-445.

[22]. Abe, F., Yamauchi, T. and Wan, A.S.C. (**1988**). Lignans Related to Olivil from Genus Cerbera (Cerbera VI). *Chem. Pharm. Bull*., *36* (2), 795-799.

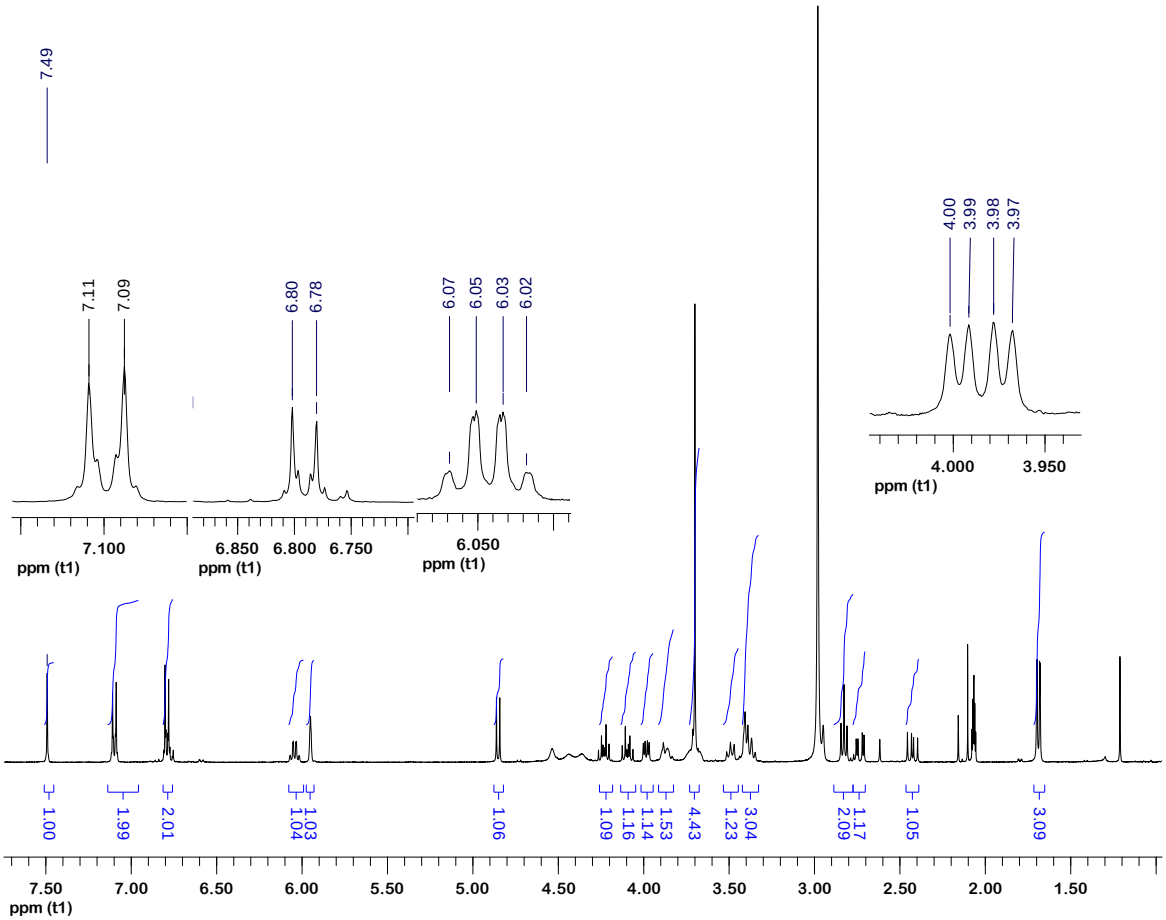
[23]. Bianco, A.D., Muzzalupo, I., Piperno, A., Romeo, G. and Uccella, N. (**1999**). Bioactive Derivatives of Oleuropein from Olive Fruits. *J. Agric. Food Chem.*, **47**, 3531-3534.

6. Appendix

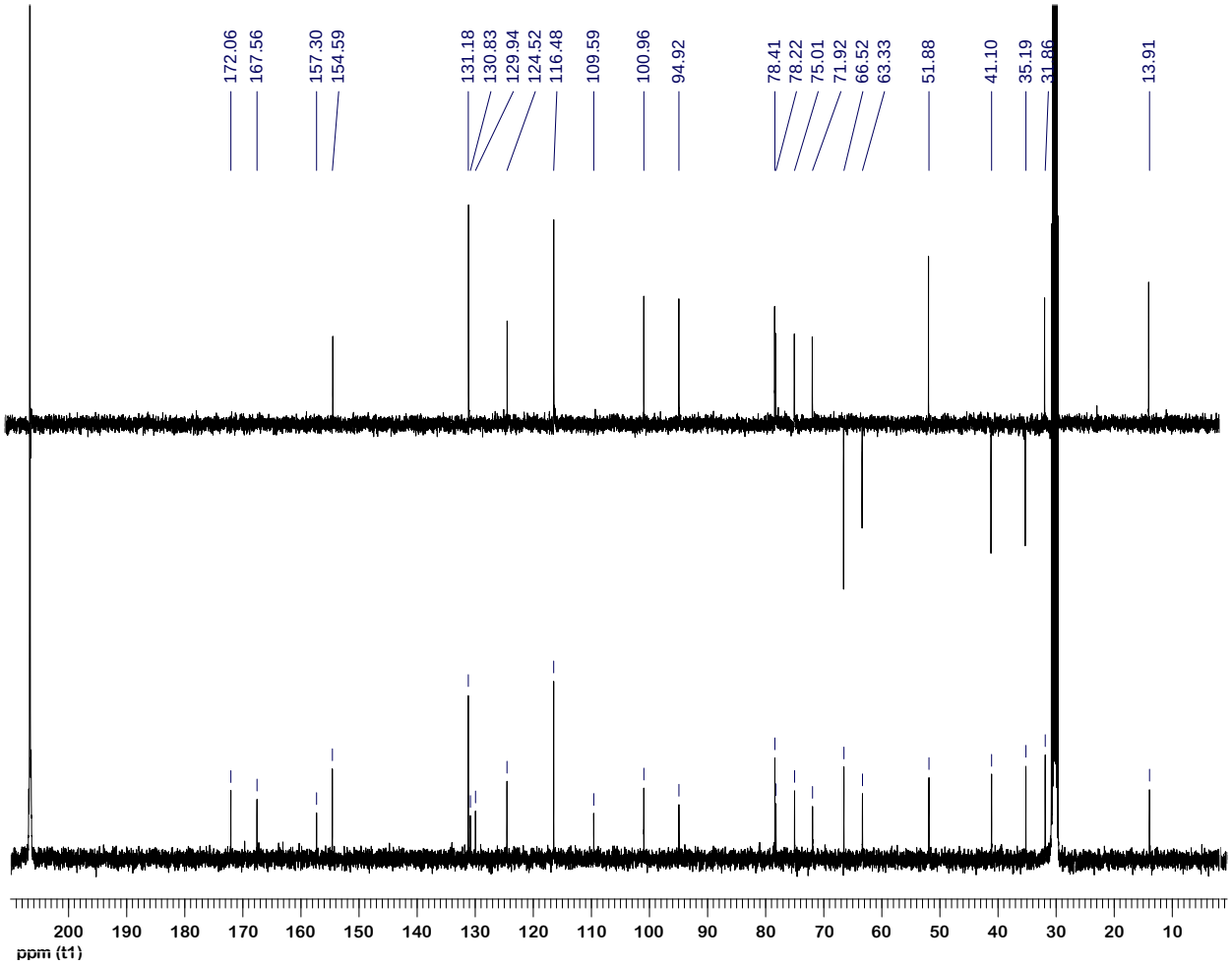
Appendix 1: IR spectrum of 89-204fr10 (Ligstroside)



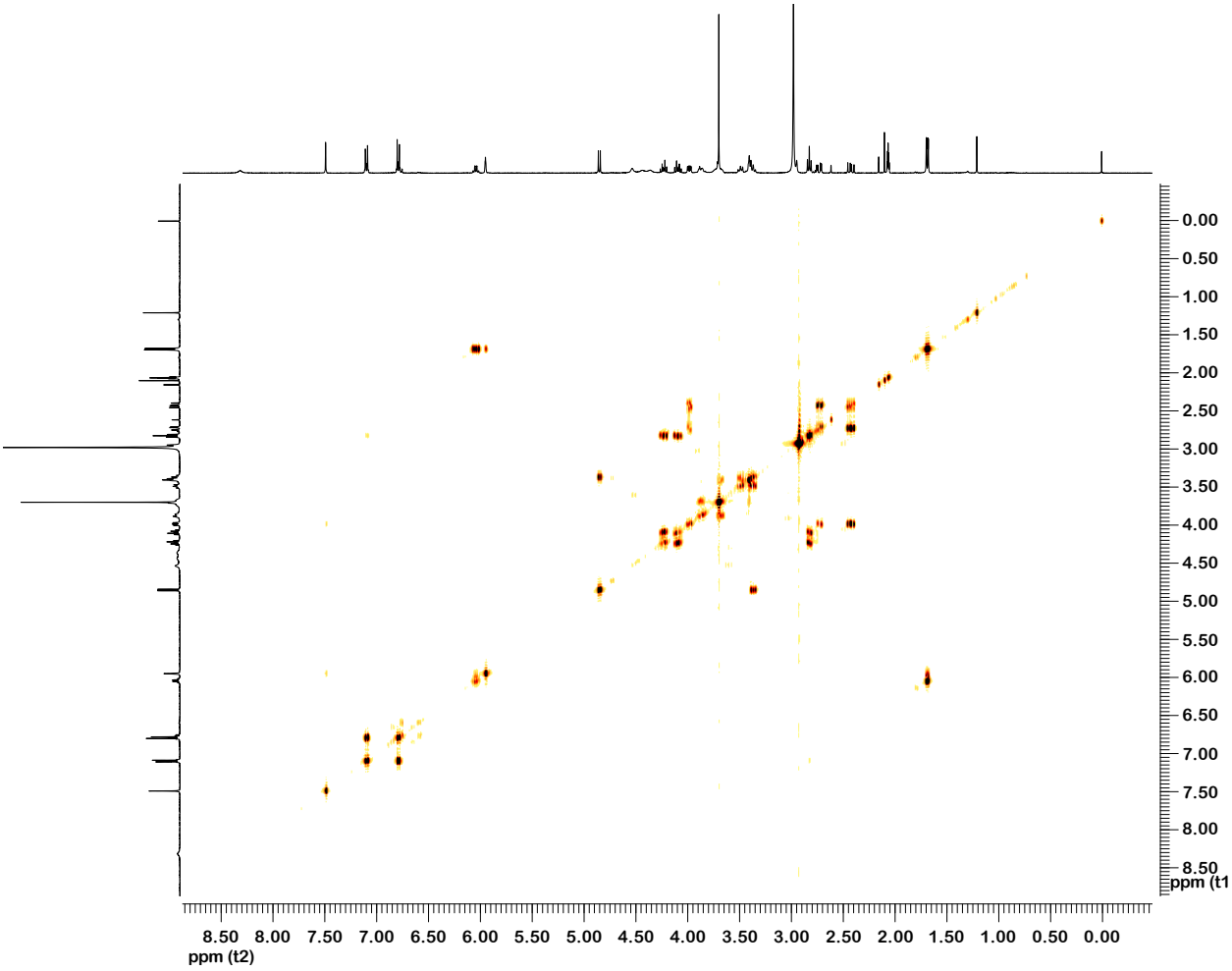
Appendix 2: ^{1}H NMR spectrum of 89-204fr10 (Ligstroside)



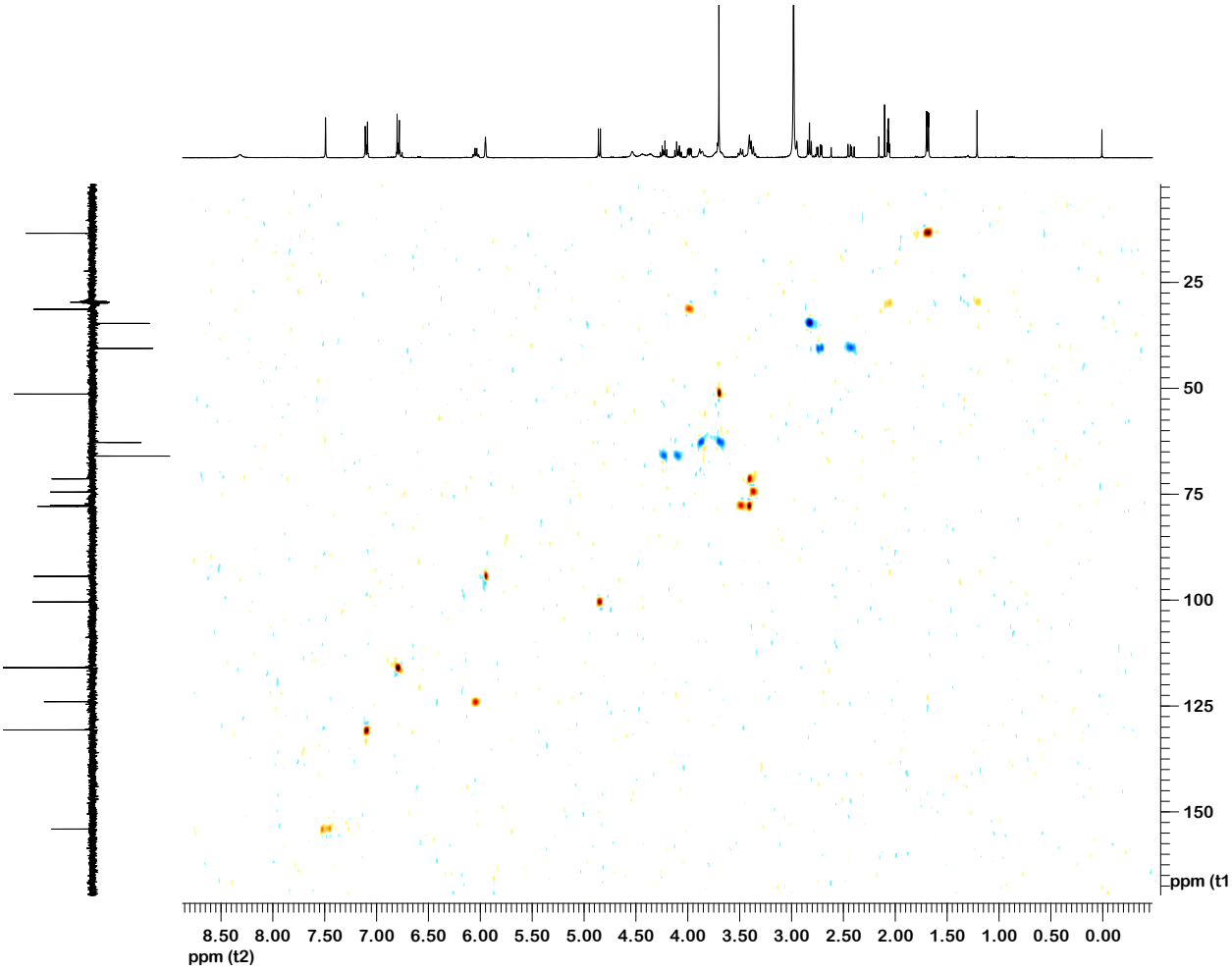
Appendix 3: ^{13}C and DEPT-135 spectrum of 89-204fr10 (Ligstroside)



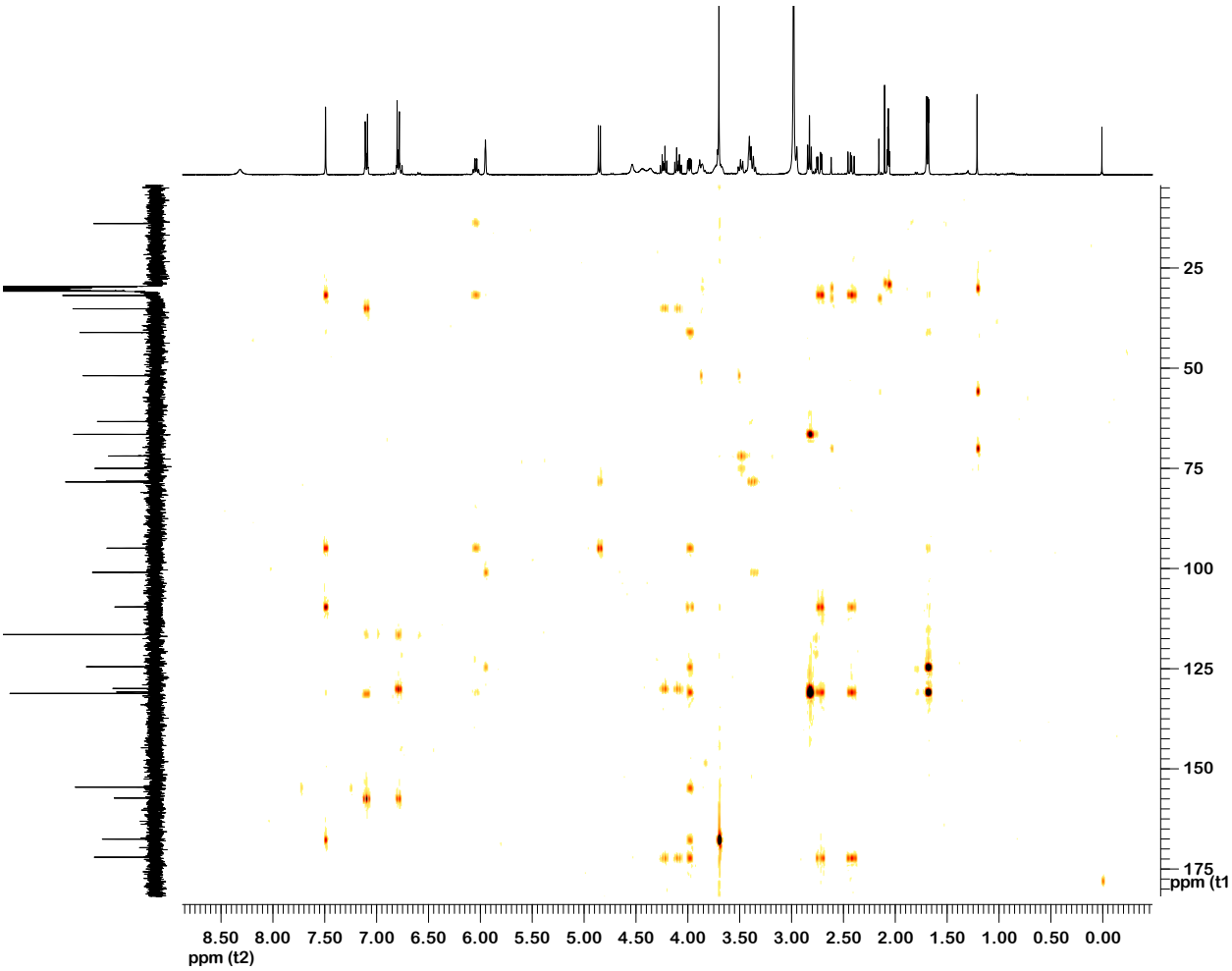
Appendix 4: COSY spectrum of 89-204fr10 (Ligstroside)



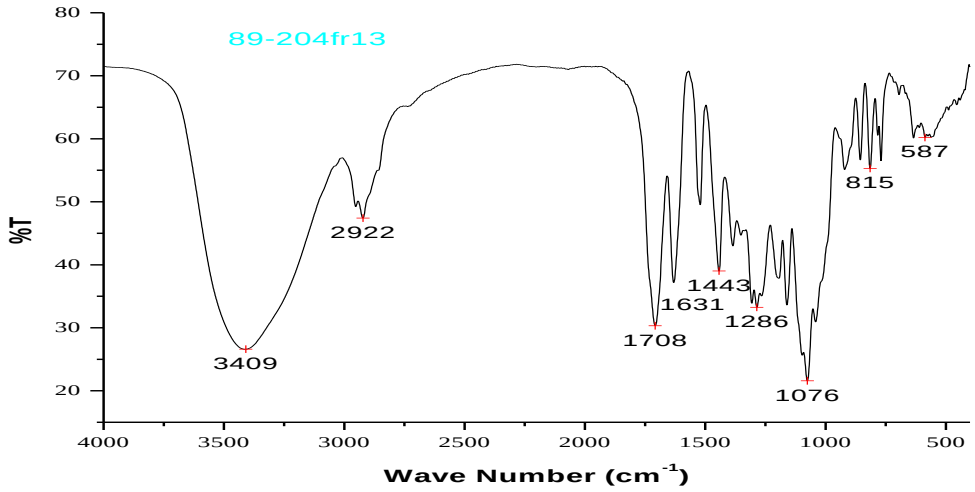
Appendix 5: HSQC spectrum of 89-204fr10 (Ligstroside)



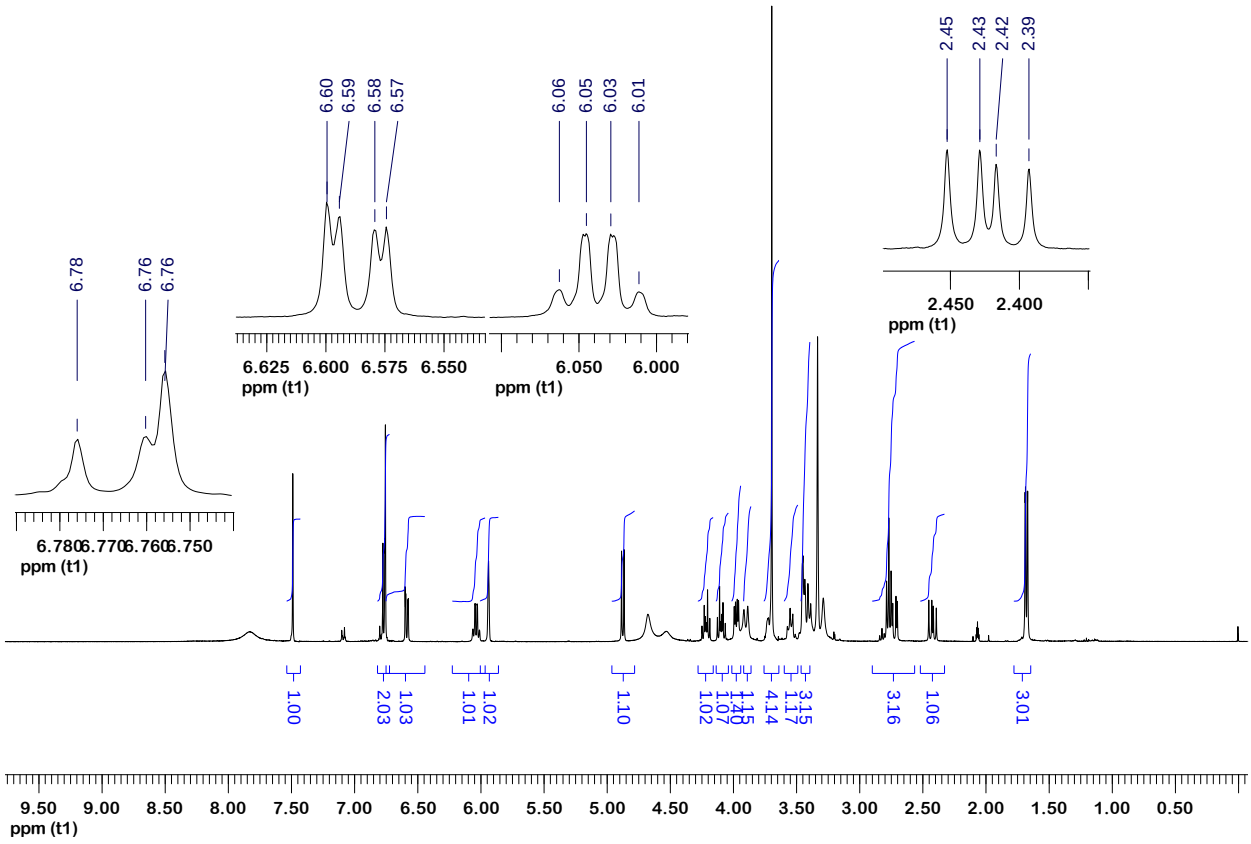
Appendix 6: HMBC spectrum of 89-204fr10 (Ligstroside)



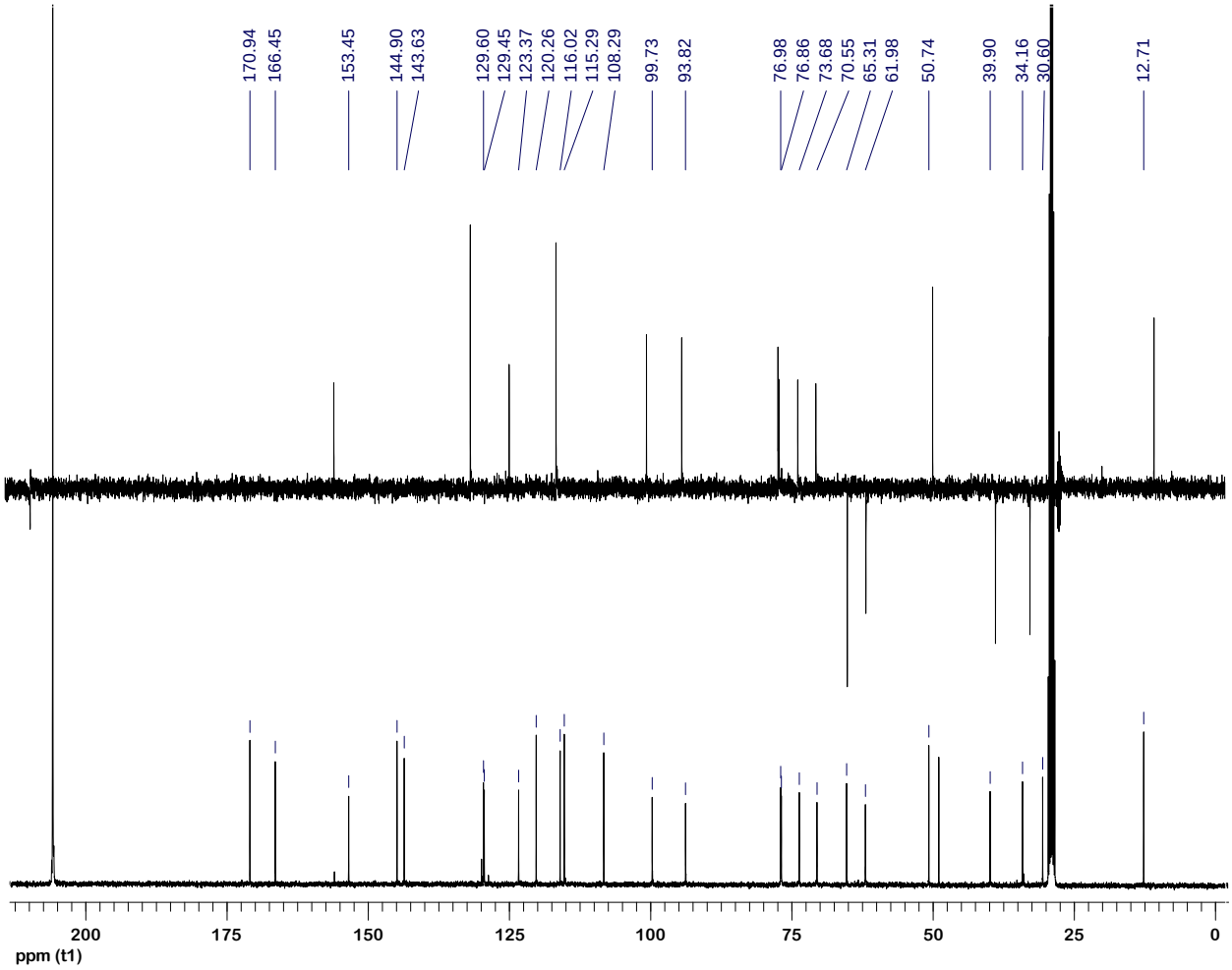
Appendix 7: IR spectrum of 89-204fr13 (Oleuropein)



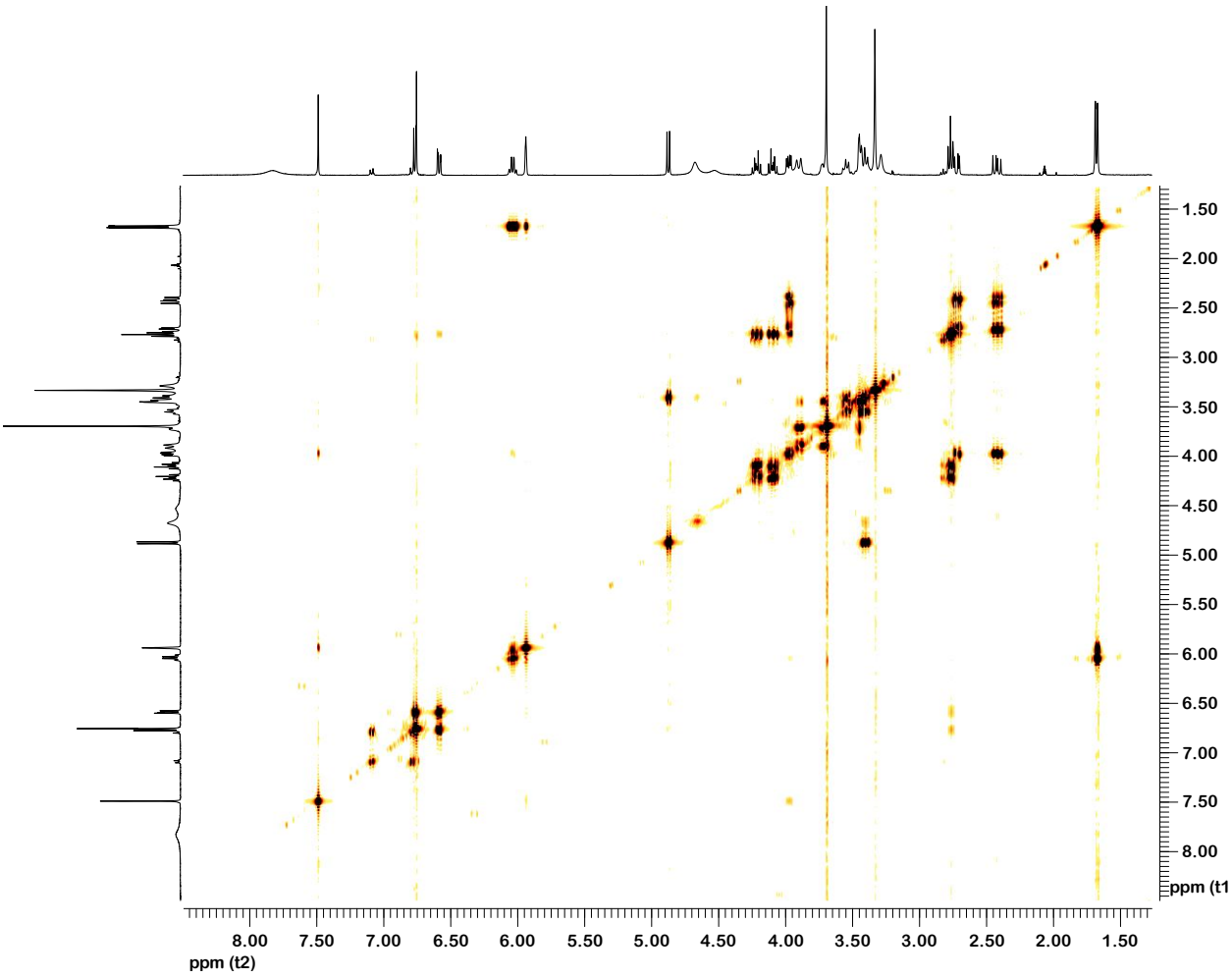
Appendix 8: ^{1}H NMR spectrum of 89-204fr13 (Oleuropein)



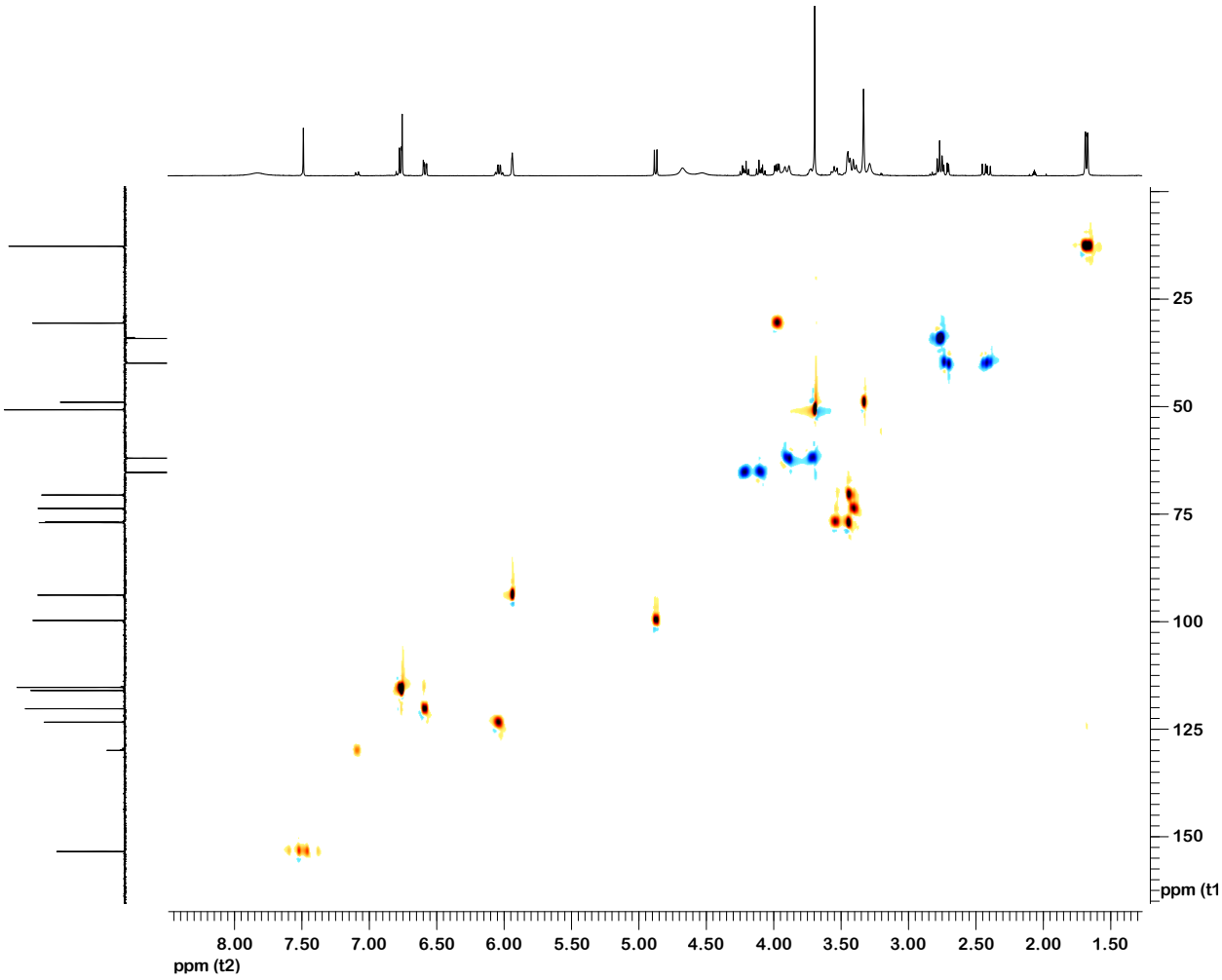
Appendix 9: ^{13}C and DEPT-135 spectrum of 89-204fr13 (Oleuropein)



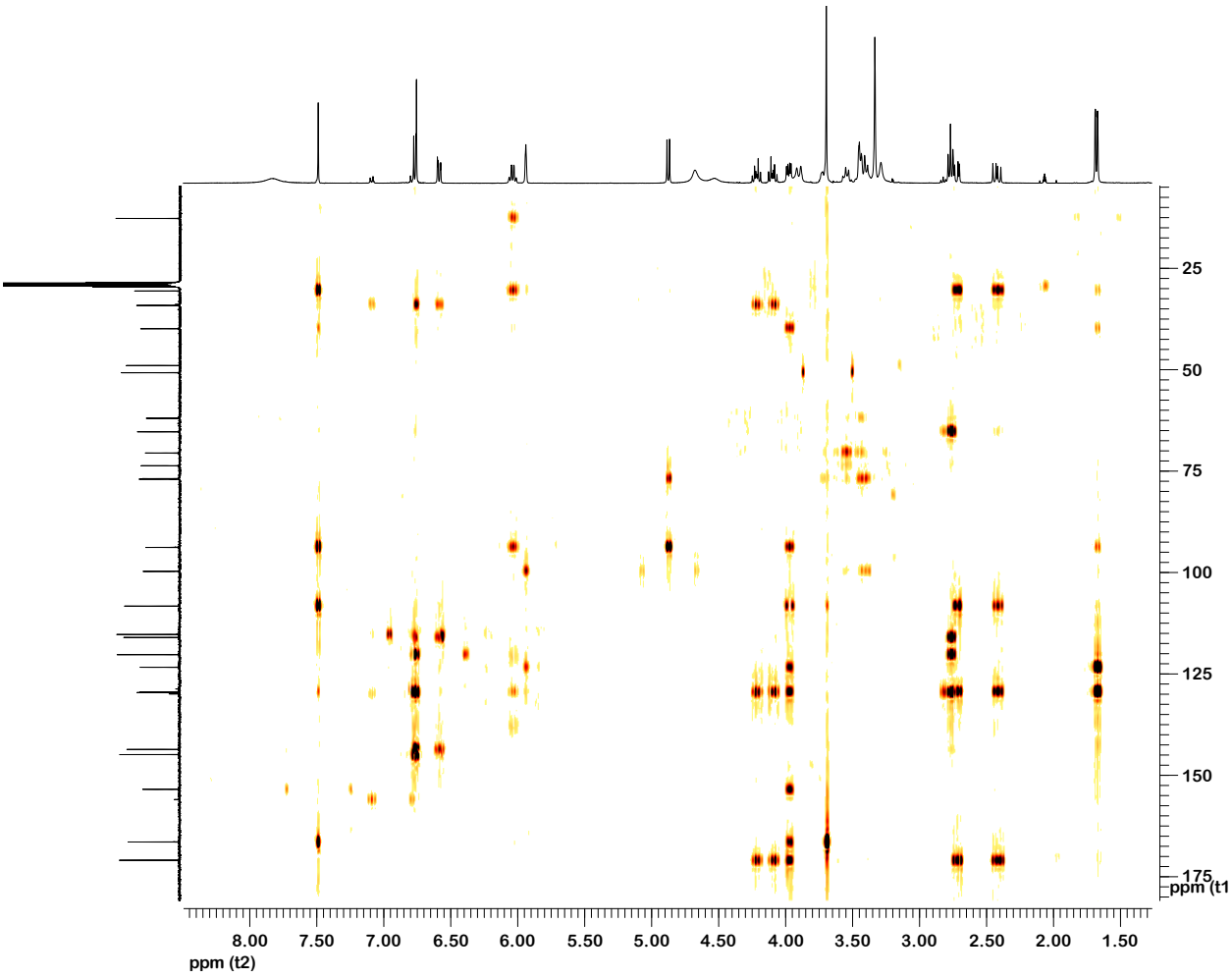
Appendix 10: COSY spectrum of 89-204fr13 (Oleuropein)



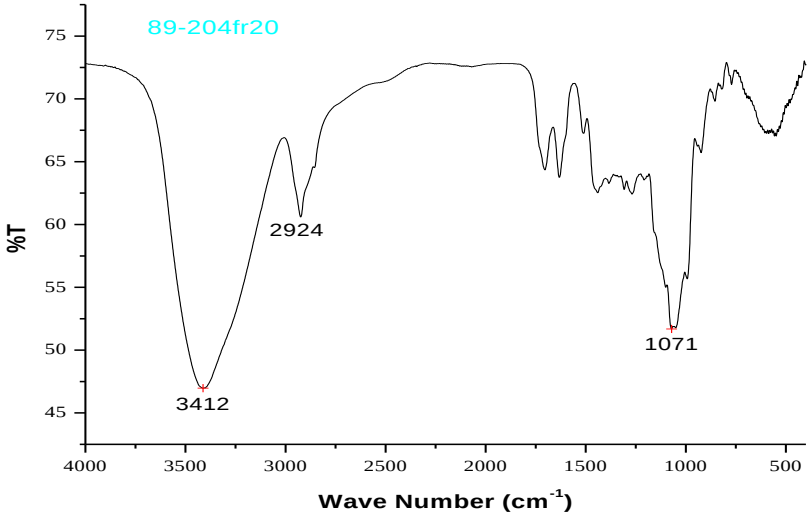
Appendix 11: HSQC spectrum of 89-204fr13 (Oleuropein)



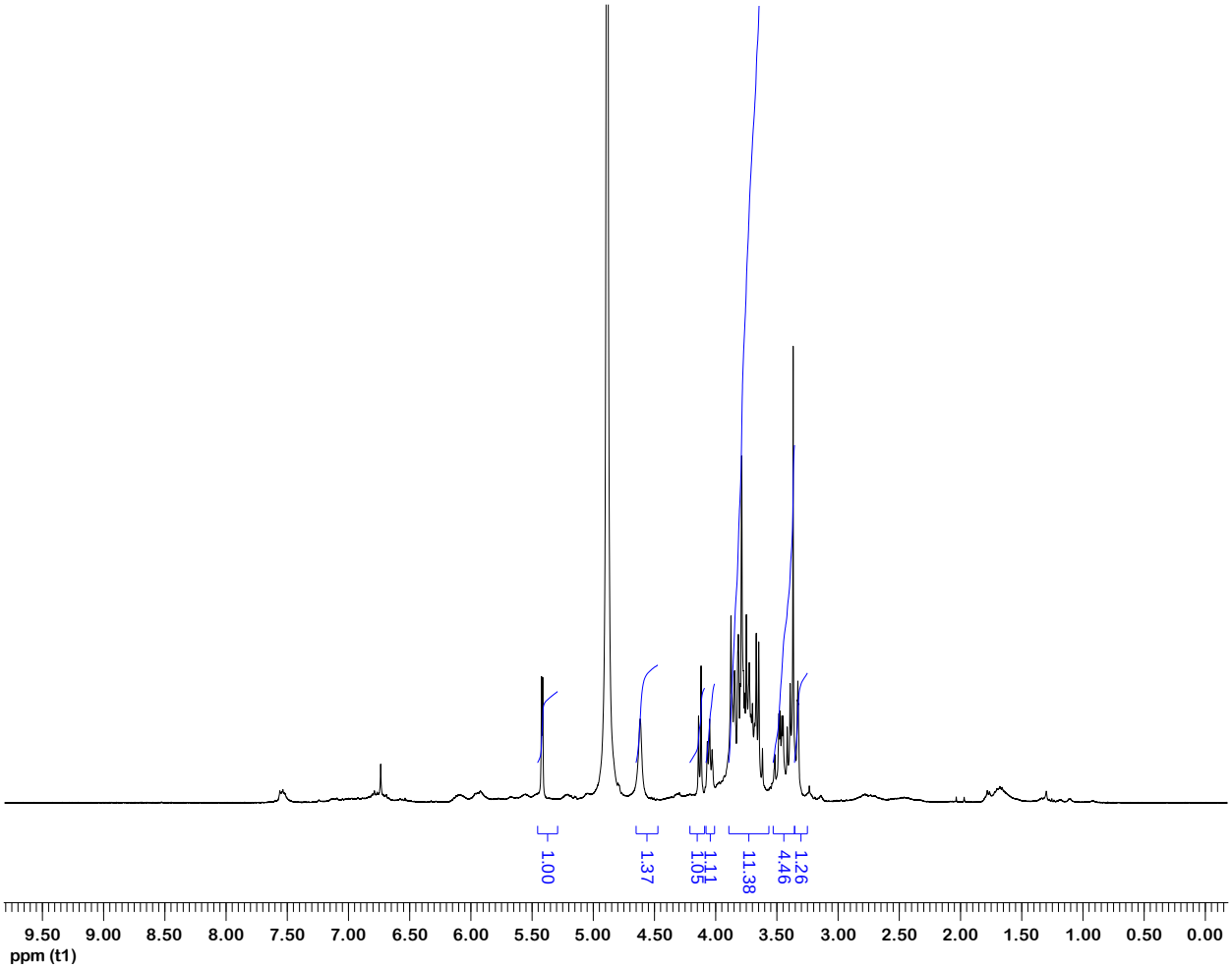
Appendix 12: HMBC spectrum of 89-204fr13 (Oleuropein)



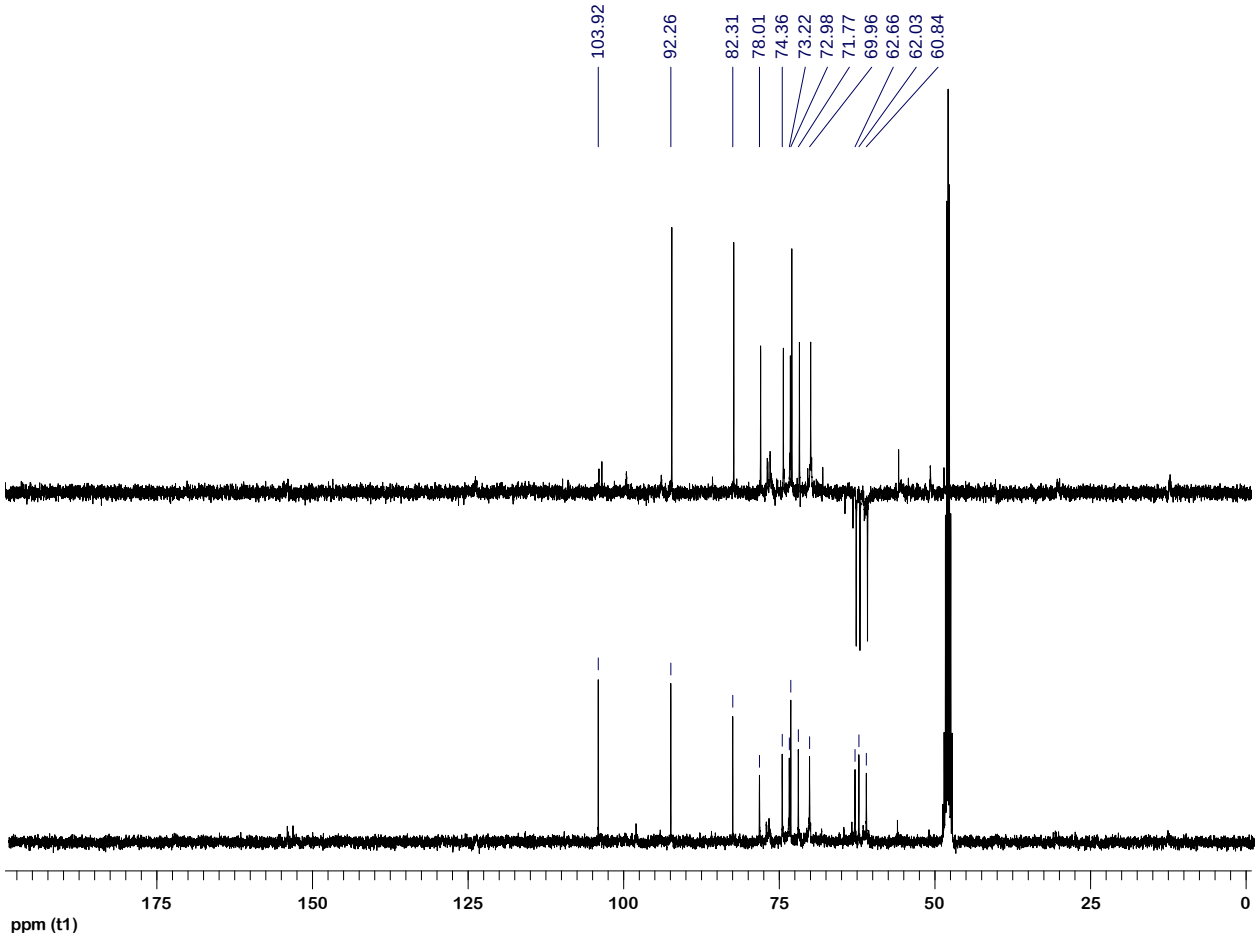
Appendix 13: IR spectrum of 89-204fr20 (Sucrose)



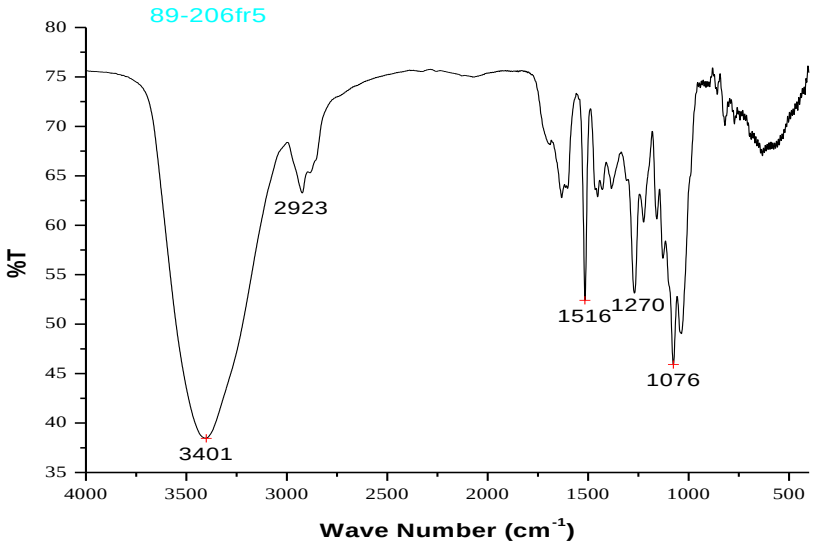
Appendix 14: ^{1}H NMR spectrum of 89-204fr20 (Sucrose)



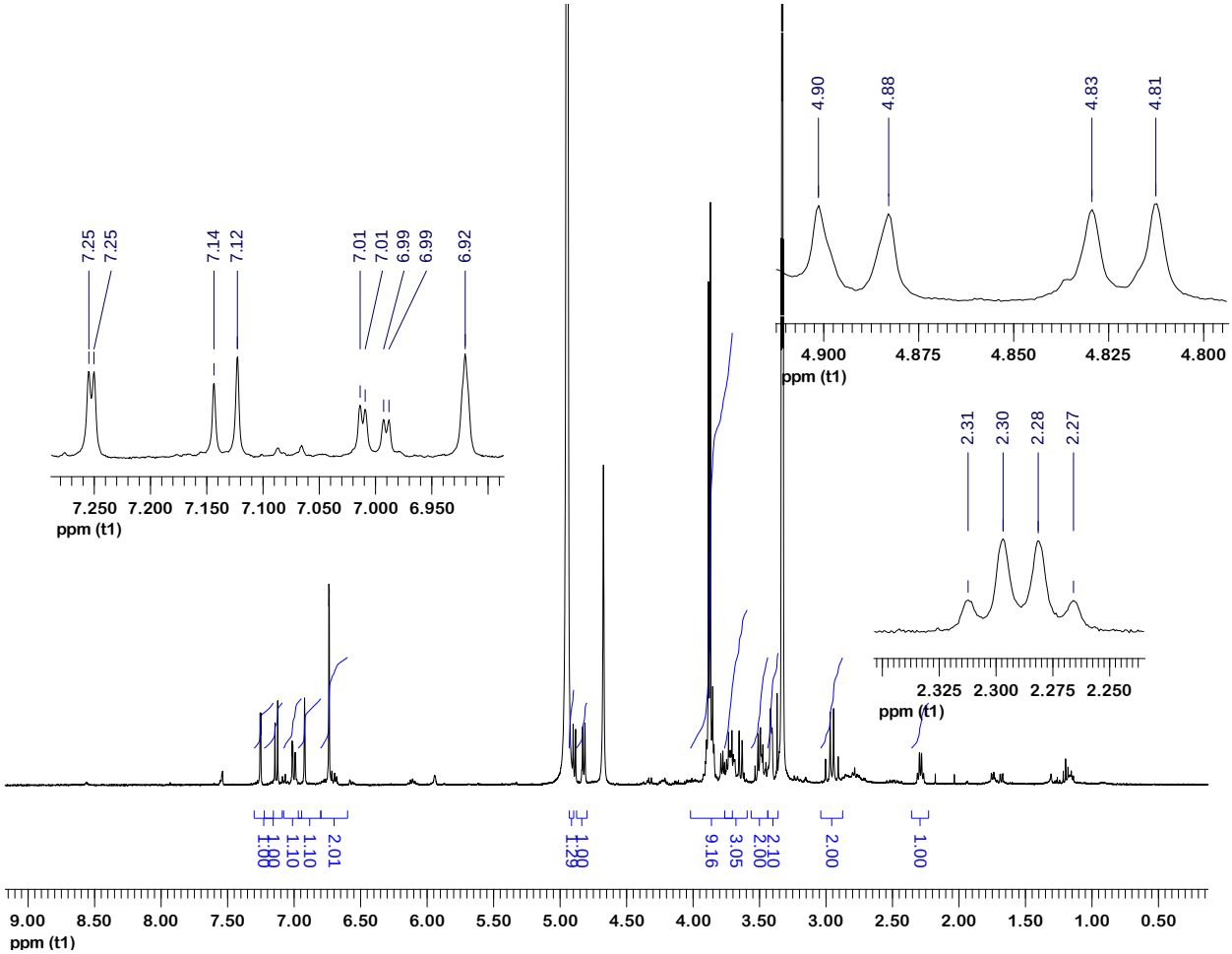
Appendix 15: ^{13}C and DEPT-135 spectrum of 89-204fr20 (Sucrose)



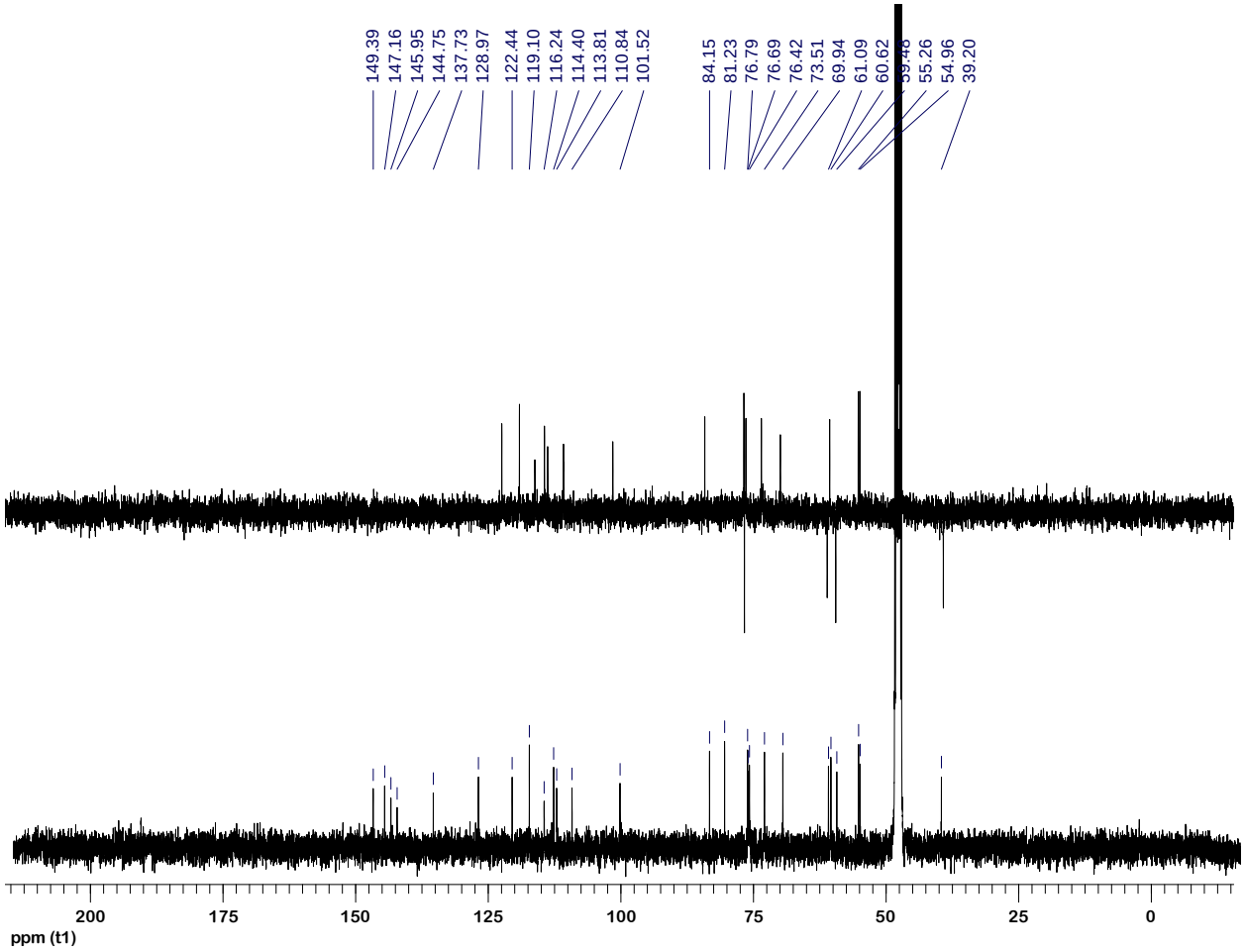
Appendix 16: IR spectrum of 89-206fr5 (Olivil 4-O-β-D-Glucoside)



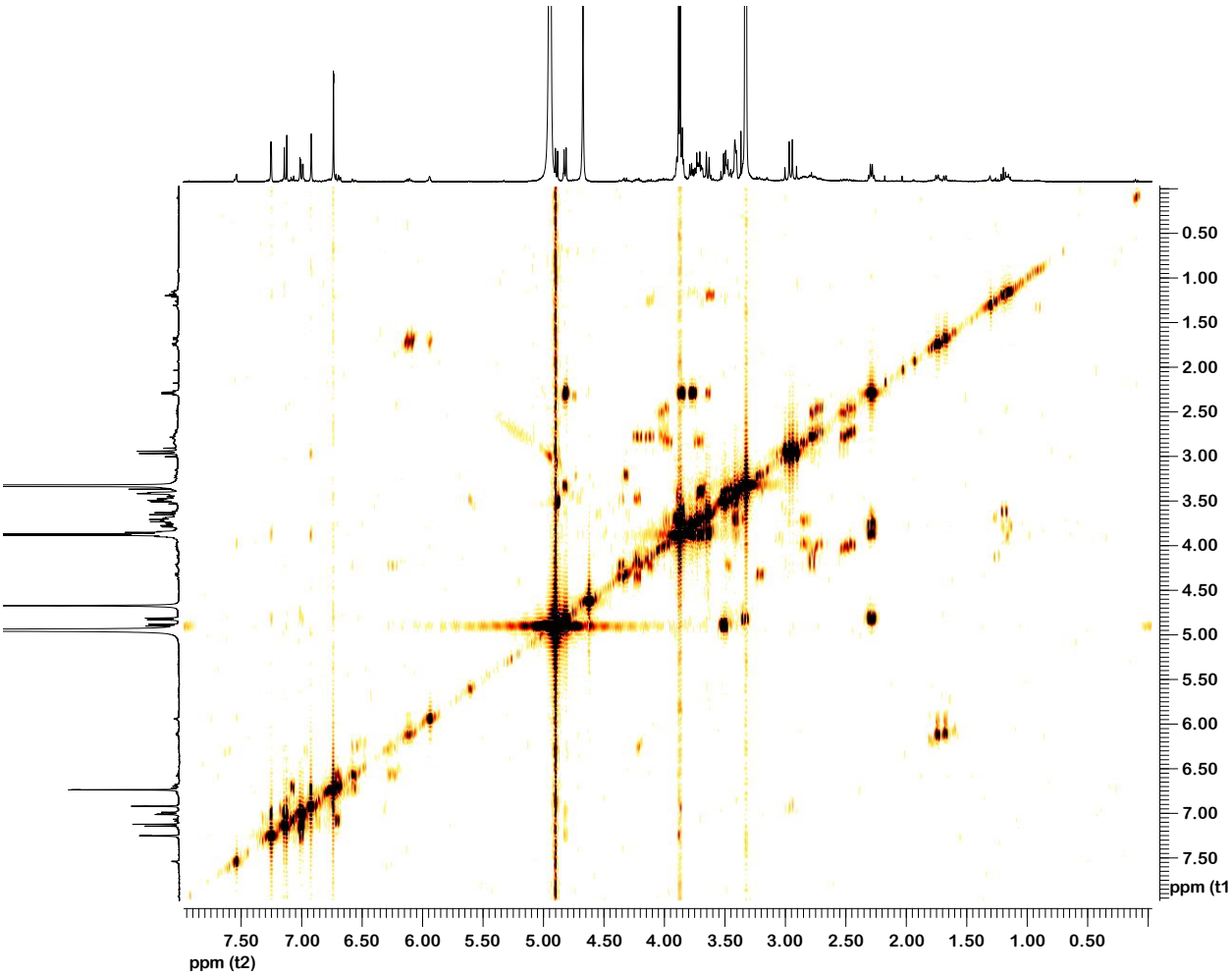
Appendix 17: ^{1}H NMR spectrum of 89-206fr5 (Olivil 4-O-β-D-Glucoside)



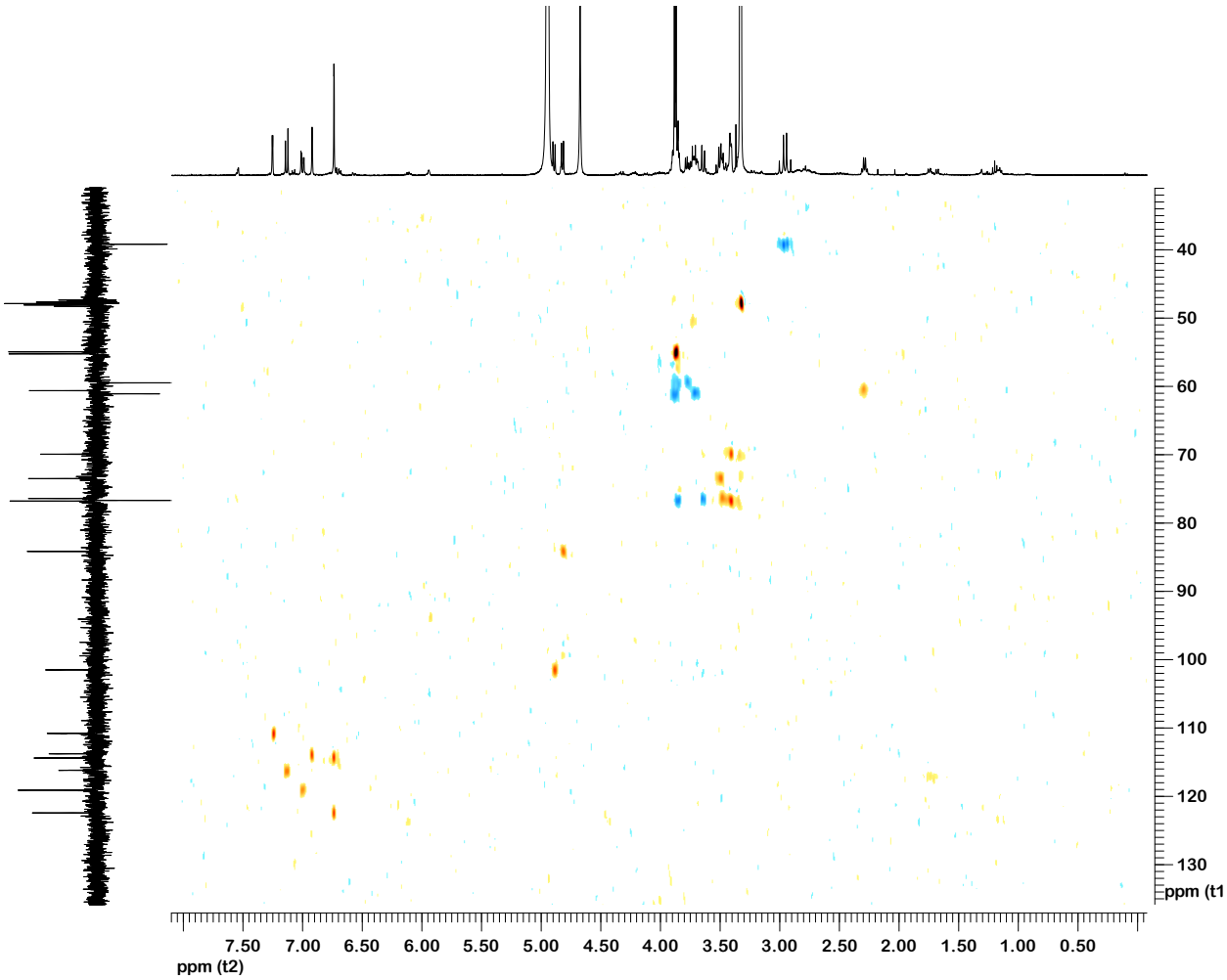
Appendix 18: ^{13}C and DEPT-135 spectrum of 89-206fr5 (Olivil 4-O-β-D-Glucoside)



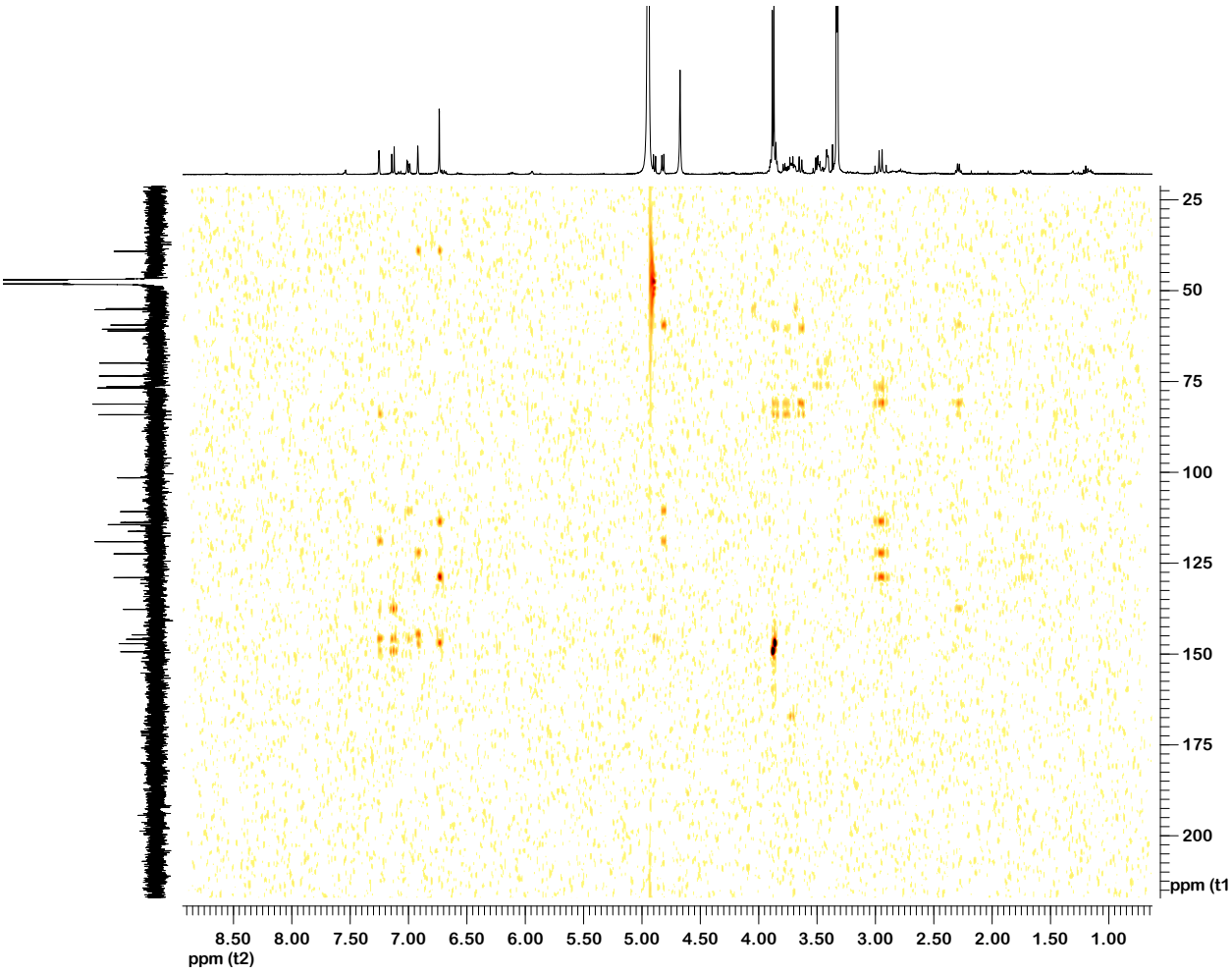
Appendix 19: COSY spectrum of 89-206fr5 (Olivil 4-O-β-D-Glucoside)



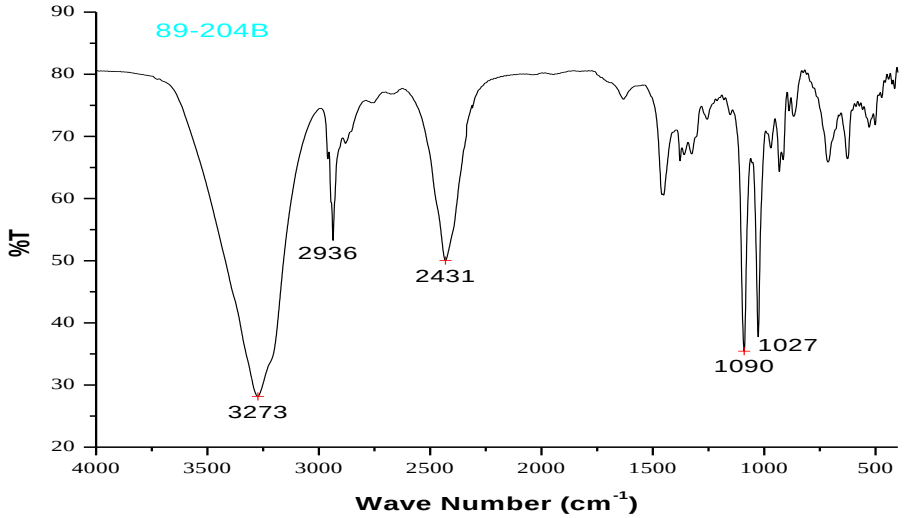
Appendix 20: HSQC spectrum of 89-206fr5 (Olivil 4-O-β-D-Glucoside)



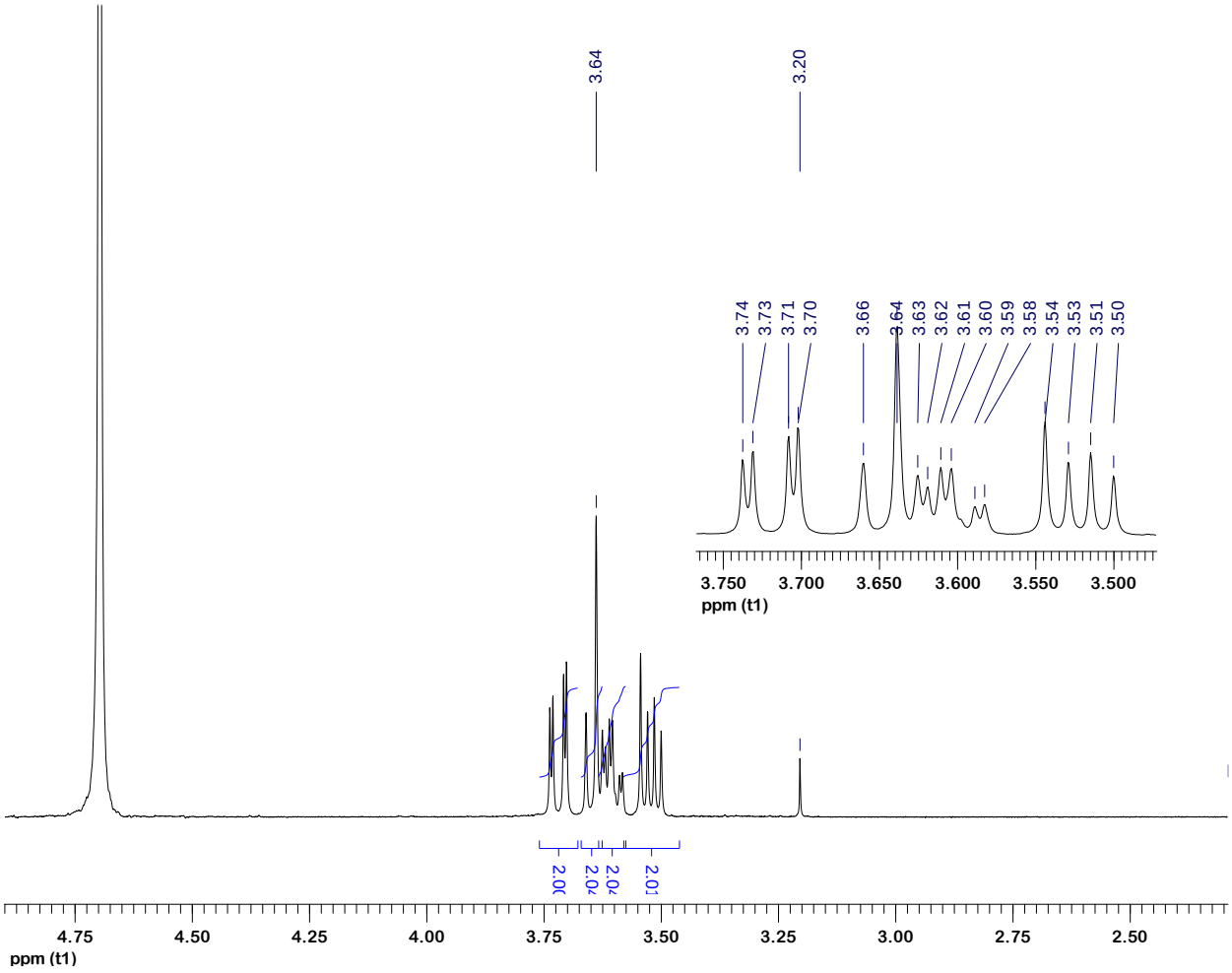
Appendix 21: HMBC spectrum of 89-206fr5 (Olivil 4-O-β-D-Glucoside)



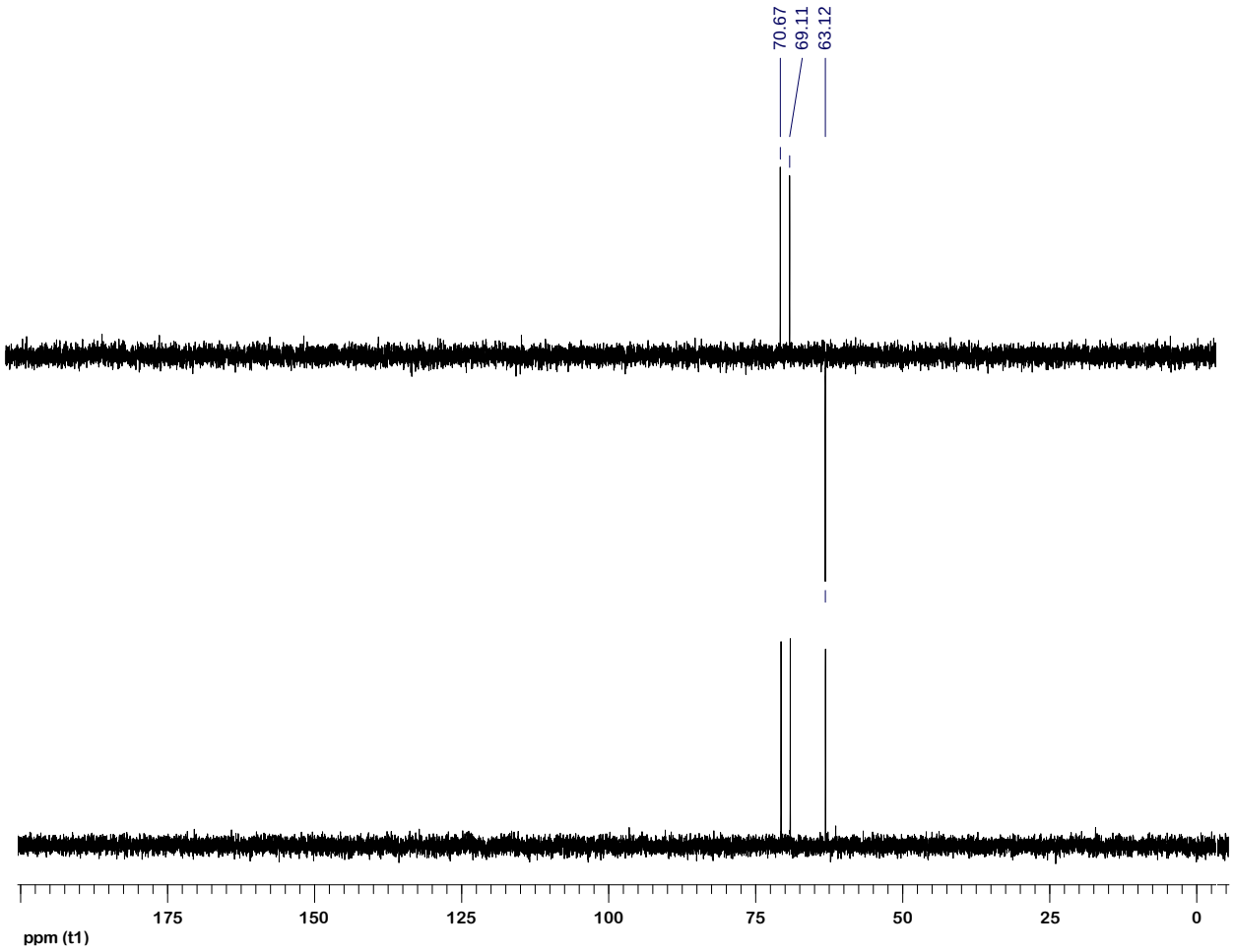
Appendix 22: IR spectrum of 89-204B (Mannitol)



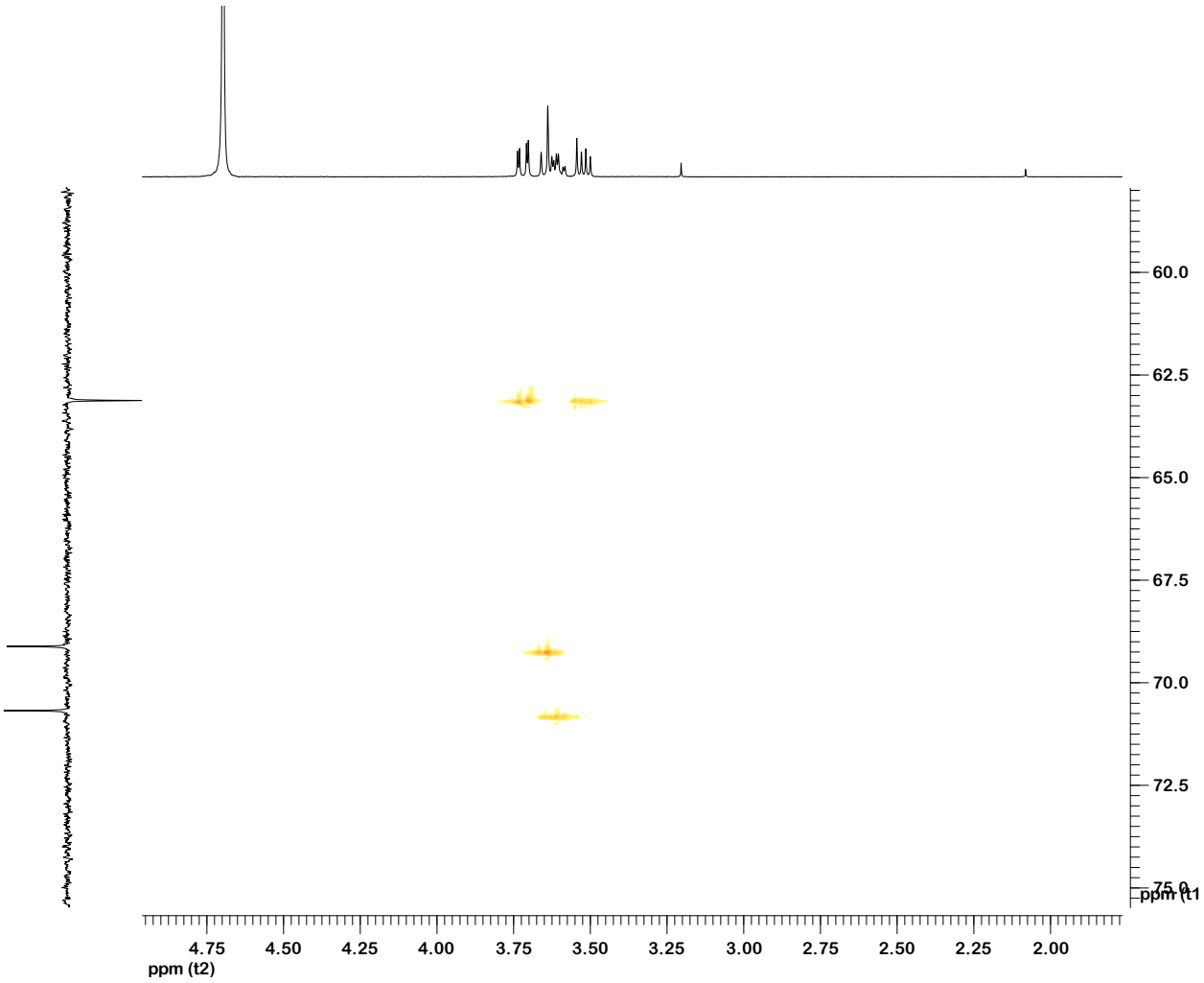
Appendix 23: ^{1}H NMR spectrum of 89-204B (Mannitol)



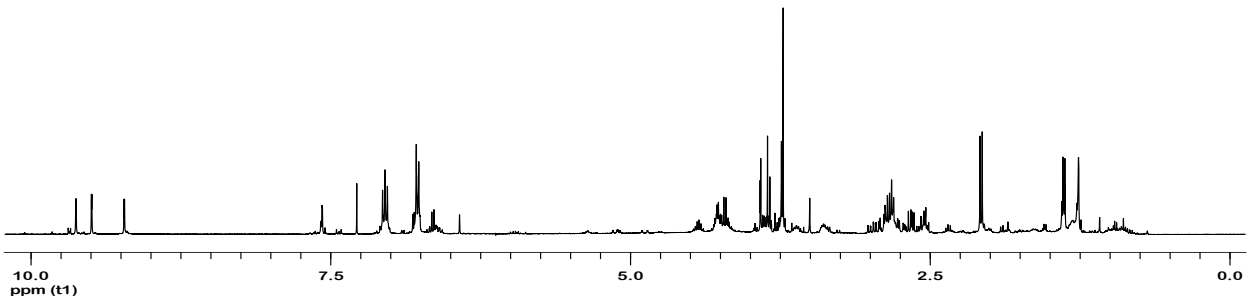
Appendix 24: ^{13}C and DEPT-135 spectrum of 89-204B (Mannitol)

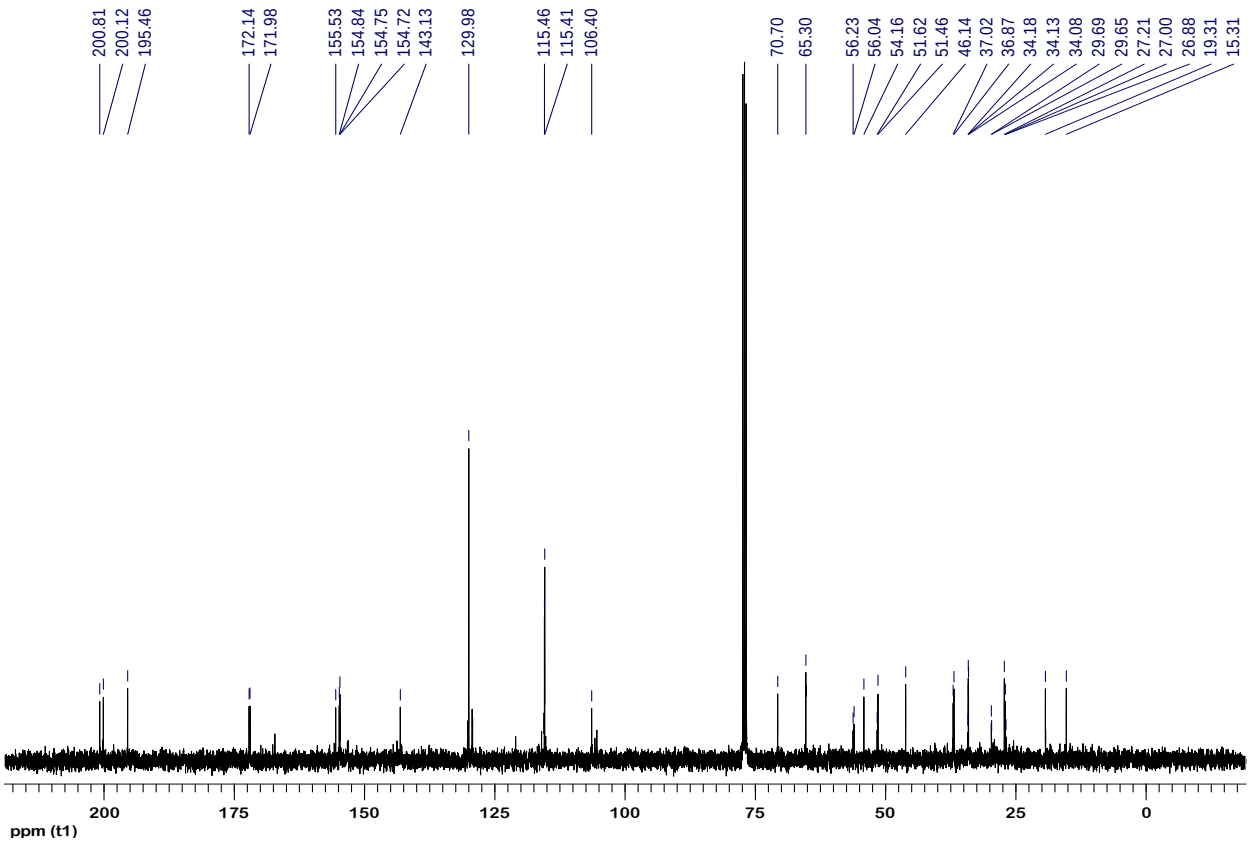


Appendix 25: HSQC spectrum of 89-204B (Mannitol)



Appendix 26: ^{1}H and ^{13}C NMR spectrum of 89-204fr2 (aldehydes)





Appendix 27:^{1}H NMR spectrum of 89-207fr6 (tyrosol and hydroxy tyrosol)