EGE UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

(MASTER OF SCIENCE THESIS)

ISOLATION AND STRUCTURAL IDENTIFICATION OF COMPOUNDS FROM *NOTOBASIS SYRIACA L. cass*

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Sayın **Onur ÖZCAN** tarafından **Yüksek Lisans tezi** olarak sunulan ***"Notobasis Syriaca L. cass* Bitkisinin Bileşenlerinin İzolasyonu Ve Yapılarının Aydınlatılması"** başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 04.09.2009 tarihinde yapılan tez savunma sınavında aday oybirliği / oyçokluğu ile başarılı bulunmuştur.

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ÖZET

NOTOBASIS SYRIACA L. cass BİTKİSİNİN BİLEŞENLERİNİN İZOLASYONU VE YAPILARININ AYDINLATILMASI

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Bu çalışmada *Notobasis Syriaca L. cass* bitkisinin bileşenlerinin izolasyonu ve yapılarının aydınlatılması çalışılmıştır. İzolasyon ve saflandırma işlemleri sonucunda beş bileşik izole edilmiştir. İzole edilen bileşiklerin yapıları çeşitli spektroskopik (^{1}H NMR, $^{!3}$C NMR, COSY, HMQC, HMBC) yöntemler kullanılarak aydınlatılmaya çalışılmış olmasına ragmen , izole edilen bileşiklerden üç tanesinin kesin yapısı kampeferol, β-sitosterol ve urs-20(30)-en-3-yl asetat ve olean-12-en-3-yl-asetat karışımı olarak belirlenmiştir. Diğer bileşiklerin yapı aydınlatma çalışmaları devam etmektedir.

Anahtar Kelimeler: Notobasis Syriaca., flavanoid, terpen

ABSTRACT

ISOLATION AND STRUCTURAL IDENTIFICATION OF COMPOUNDS FROM *NOTOBASIS SYRIACA L. cass*

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In this study, the isolation and structure characterization of some components from *Notobasis Syriaca L.* cass have been carried out. After isolation from the flowers of *Notobasis Syriaca L cass* five compounds were obtained. Although the structrual determinations of pure compounds were carried out using different spectroscopic (^{1}H NMR, ^{13}C NMR, COSY or HMQC, HMBC) methods, all isolated compound were not identified. Isolated three compounds were identified as Kampeferol , β-sitosterol and mixture of urs-20(30)-en-3-yl acetate and olean-12-en-3-yl-acetate.

Key Words: Notobasis Syariaca, flavanoid, terpene

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LIST OF ABBREVIATION

Lt	Liter
Kg	Kilogram
mg	Miligram
EtOAc	Ethylacetate
CH_2Cl_2	Methylene Chloride
CC	Column Chromatography
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
^{1}H-NMR	Proton Nuclear Magnetic Resonans
^{13}C-NMR	Carbon Nuclear Magnetic Resonans
1D	One Dimension
2D	Two dimension
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coharence
COSY	Correlation Spectroscopy

1. INTRODUCTION

1.1 General Information of Natural Products

Products of natural origins can be called ‘‘natural products’’. Natural products include: an entire organism (e.g. a plant, an animal, or a microorganism) that has not been subjected to any kind of processing or treatment other than a simple process of preservation (e.g., drying), part of an organism (e.g., leaves or flowers of a plant, an isolated animal organ), an extract of an organism or part of an organism, and exudates, and pure compounds (e.g., alkaloids, coumarins, flavonoids, glycosides, lignans, steroids, sugars, terpenoids, etc.) isolated from plants, animals, or microorganisms. However, in most cases the term natural products refers to secondary metabolites, small molecules (mol wt <2000 amu) produced by an organism that are not strictly necessary for the survival of the organism. Concepts of secondary metabolism include products of overflow metabolism as a result of nutrient limitation, shunt metabolism produced during idiophase, defense mechanism regulator molecules, etc. Natural products can be from any terrestrial or marine source: plants (e.g., paclitaxel [Taxol] from Taxus brevifolia), animals (e.g., vitamins A and D from cod liver oil), or microorganisms (e.g., doxorubicin from Streptomyces peucetius).

Strategies for research in the area of natural products have evolved quite significantly over the last few decades. These can be broadly divided into two categories:

Older Strategies;

- Focus on chemistry of compounds from natural sources, but not on activity.
- Straightforward isolation and identification of compounds from natural sources followed by biological activity testing (mainly in vivo).
- Chemotaxonomic investigation.
- Selection of organisms primarily based on ethno pharmacological information, folkloric reputations, or traditional uses.

Modern strategies:

- Bioassay-guided (mainly in vitro) isolation and identification of active ‘‘lead’’ compounds from natural sources.
- Production of natural products libraries.
- Production of active compounds in cell or tissue culture, genetic manipulation, natural combinatorial chemistry, and so on.
- More focused on bioactivity.
- Introduction of the concepts of dereplication, chemical fingerprinting, and metabolomics.
- Selection of organisms based on ethno pharmacological information, folkloric
- Reputations, or traditional uses, and also those randomly selected. (Sarker et al, 2005)

1.1.1. Historical Perspective

The use of natural products, especially plants, for healing is as ancient and universal as medicine itself. The therapeutic use of plants certainly goes back to the Sumerian civilization, and 400 years before the Common Era, it has been recorded that Hippocrates used approximately 400 different plant species for medicinal purposes. Natural products played a prominent role in ancient traditional medicine systems, such as Chinese, Ayurveda, and Egyptian, which are still in common use today. According to the World Health Organization (WHO), 75% of people still rely on plant-based traditional medicines for primary health care globally. (Sarker et al, 2005).

1.1.2. Present and Future

Nature has been a source of therapeutic agents for thousands of years, and an impressive number of modern drugs have been derived from natural sources, many based on their use in traditional medicine. Over the last century, a number of top selling drugs have been developed from natural products (vincristine from Vinca rosea, morphine from Papaver somniferum, Taxol_ from T. brevifolia, etc.). In recent years, a significant revival of interest in natural products as a potential source for new medicines has been observed among academia as well as pharmaceutical companies. Several modern drugs (~40% of the modern drugs in use) have been developed from natural products. More precisely,

according to Cragg et al. 39% of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60–80% of antibacterial and anticancer drugs were from natural origins. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancers had natural origins. In 2001, eight (simvastatin, pravastatin, amoxycillin, clavulanic acid, azithromycin, ceftriaxone, cyclosporin, and paclitaxel) of the 30 top-selling medicines were natural products or their derivatives, and these eight drugs together totaled US $16 billion in sales.

Apart from natural product-derived modern medicine, natural products are also used directly in the ‘‘natural’’ pharmaceutical industry, which is growing rapidly in Europe and North America, as well as in traditional medicine programs being incorporated into the primary health care systems of Mexico, the People’s Republic of China, Nigeria, and other developing countries. The use of herbal drugs is once again becoming more popular in the form of food supplements, nutraceuticals, and complementary and alternative medicine.

Natural products can contribute to the search for new drugs in three different ways:

- by acting as new drugs that can be used in an unmodified state (e.g., vincristine from Catharanthus roseus).
- by providing chemical ‘‘building blocks’’ used to synthesize more complex molecules (e.g., diosgenin from Dioscorea floribunda for the synthesis of oral contraceptives).
- by indicating new modes of pharmacological action that allow

complete synthesis of novel analogs (e.g., synthetic analogs of penicillin from Penicillium notatum).

Natural products will certainly continue to be considered as one of the major sources of new drugs in the years to come because

- they offer incomparable structural diversity.
- many of them are relatively small (<2000 Da).
- they have ‘‘drug-like’’ properties (i.e., they can be absorbed and metabolized).

Only a small fraction of the world’s biodiversity has been explored for bioactivity to date. For example, there are at least 250,000 species of higher plants that exist on this planet, but merely 5–10% of these have been investigated so far. In addition, reinvestigation of previously studied plants has continued to produce new bioactive compounds that have drug potential. Much less is known about marine organisms than other sources of natural products. However, research up to now has shown that they represent a valuable source for novel bioactive compounds. With the development of new molecular targets, there is an increasing demand for novel molecular diversity for screening. Natural products certainly play a crucial role in meeting this demand through the continued investigation of the world’s biodiversity, much of which remains unexplored. With less than 1% of the microbial world currently known, advances in technologies for microbial cultivation and the extraction of nucleic acids from environmental samples from soil and marine habitats will offer

access to an untapped reservoir of genetic and metabolic diversity. This is also true for nucleic acids isolated from symbiotic and entophytic microbes associated with terrestrial and marine macro organisms.

Advent, introduction, and development of several new and highly specific in vitro bioassay techniques, chromatographic methods, and spectroscopic techniques, especially nuclear magnetic resonance (NMR), have made it much easier to screen, isolate, and identify potential drug lead compounds quickly and precisely. Automation of these methods now makes natural products viable for high-throughput screening (HTS). (Sarker et al, 2005)

1.2. General Information of *Notobasis Syriaca L. cass.*

Notobasis Syriaca L. cass is known as Syrian Creeping Thistle in Turkey. *Cirsium Syriacum* and *Circus Syriacus* are synonym names of the *Notobasis Syriaca L .cass* in Latin language. It is an annual plant material and it grows in arid geography where dominate by Mediterranean climate. In generally, it grows between 30 cm and 100 cm, it has a flower until 2 cm hoe. Violet color flowers enclose the body spirally and it has extremely circular white veins. There are a lot of sharp thistles on the top of the flowers.

1.3 Previous Study on *Notobasis Syriaca L. cass.*

Apigenin, Luteolin, Chrysoeriol, Kaempferol, Isorhamnetin, Apigenin-7-Glucoside, Kaempferol-7-Glucoside, Schaftoside and Isoschaftoside were isolated from *Notobasis Syriaca L .cass* on previous study. (Meriçli and Dallamonica, 1983).

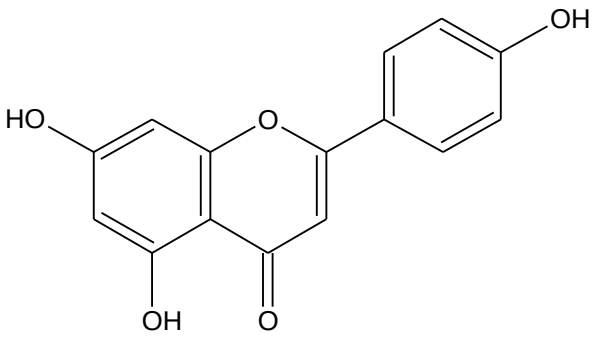


Figure 1: Apigenin

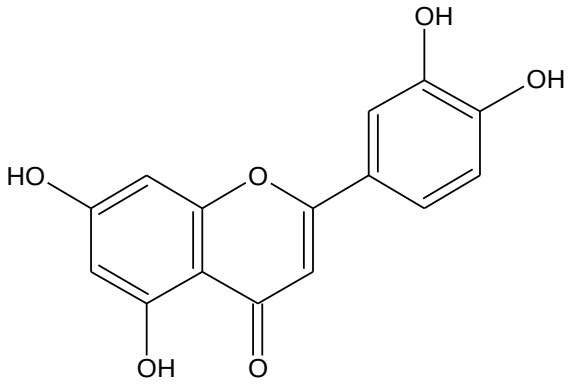


Figure 2: Luteolin

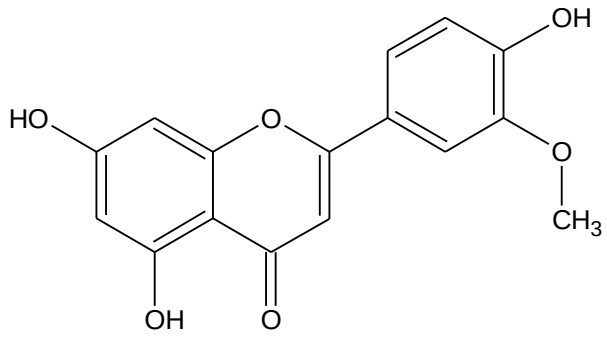


Figure 3: Chrysoeriol

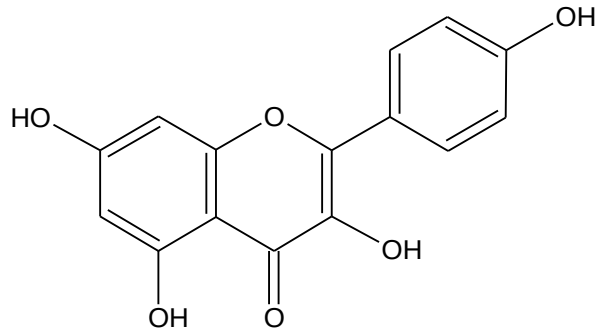


Figure 4: Kaempferol

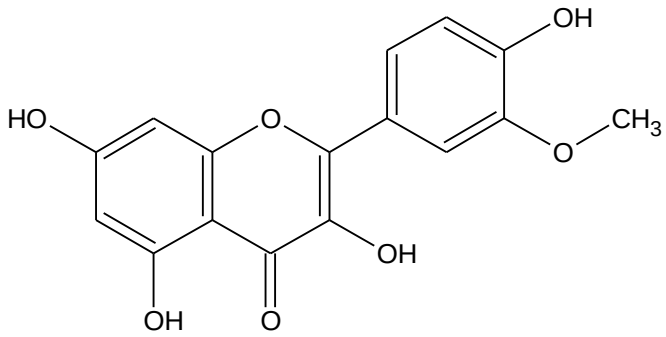


Figure 5: Isorhamnetin

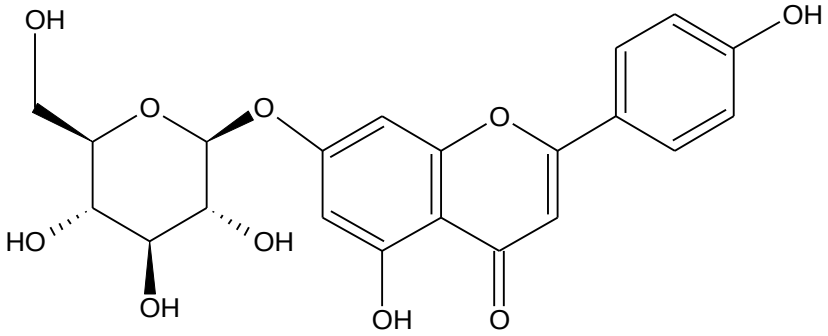


Figure 6: Apigenin-7-O-Glucoside

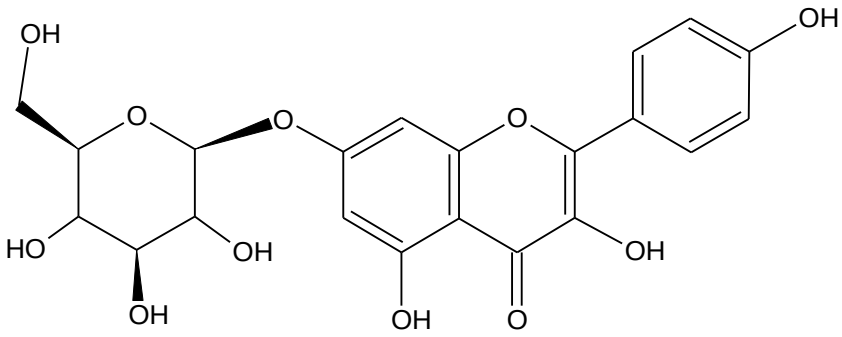


Figure 7: Kaempferol-7-Glucoside

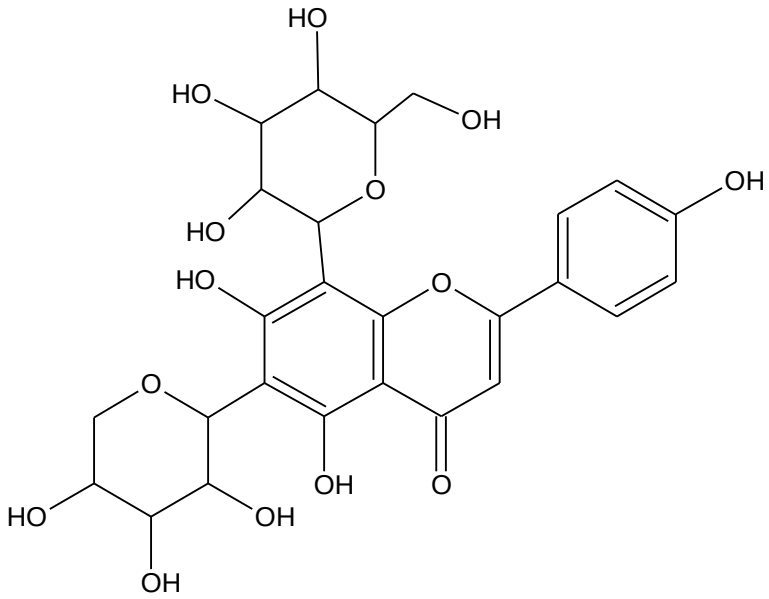


Figure 8: Isoschaftoside

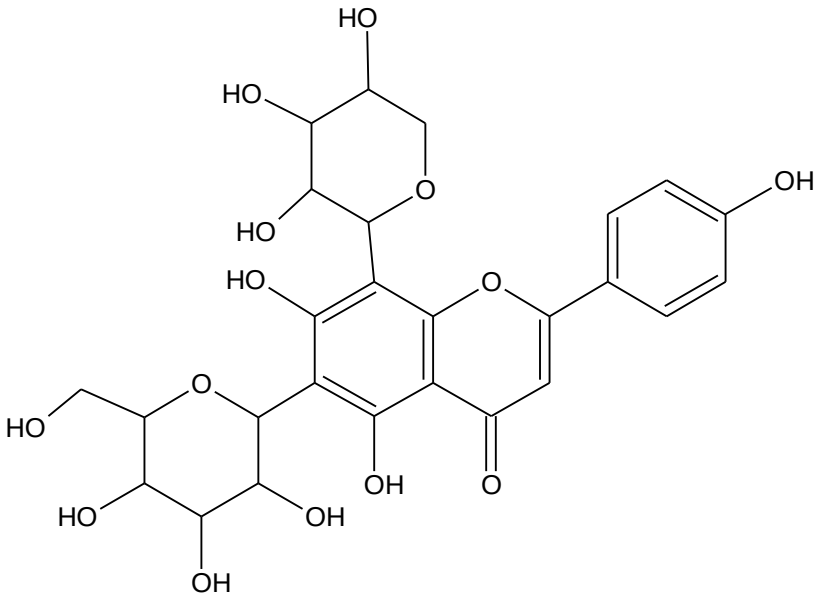


Figure 9: Schaftoside

2. MATERIAL AND METHODS

2.1. General Information

The NMR spectra were measured in $CDCl_3$, MeOD and DMSO at 400 MHz for ^{1}H and 100 MHz for ^{13}C-NMR on a Varian-ASW spectrometer. CC and PTLC were carried out on Sigel (7734 Merck) and preparative silica (7747 Merck). TLC was carried out on Alugram (Sil G/UV 254, Merck) using %20 sulfuric acid.

2.1.1. Short Explanation of the Methods Used for the Separations in This Project

2.1.1.1. Column Chromatography (CC)

Column Chromatography is generally the first separation step carried out after determination of the polarity of the extract components. Non-polar compounds are usually chromatographed on silica gel stationary phases. Although reverse phase packings can also be used for natural products, these were not used in this work because our compounds were not sufficiently polar to require this type of paking subsequent to column chromatography. Further purification was carried out by PTLC or CPTLC.

2.1.1.2. Preparative TLC (PTLC)

Preparative TLC (PTLC) has long been a popular method of isolation because of its simplicity and availability to researchers working in natural products chemistry. Separation can be effected rapidly and the amount of material isolated generally falls into the 1 mg to 1g range, which is certainly sufficient for structure elucidation purposes.

Although separations depend on the level of complexity of an extract, PTLC is nearly used as a final purification step in an isolation procedure and was used for this purpose in this work.

2.2. Plant Material

Notobasis Syriaca L. cass had been collected in June 2008 from Sasalı National Park (IZMIR-TURKEY). A voucher specimen (EGE. HEB. 40780) is preserved by Dr. Serdar ŞENOL in the Herbarium Center of Ege University.

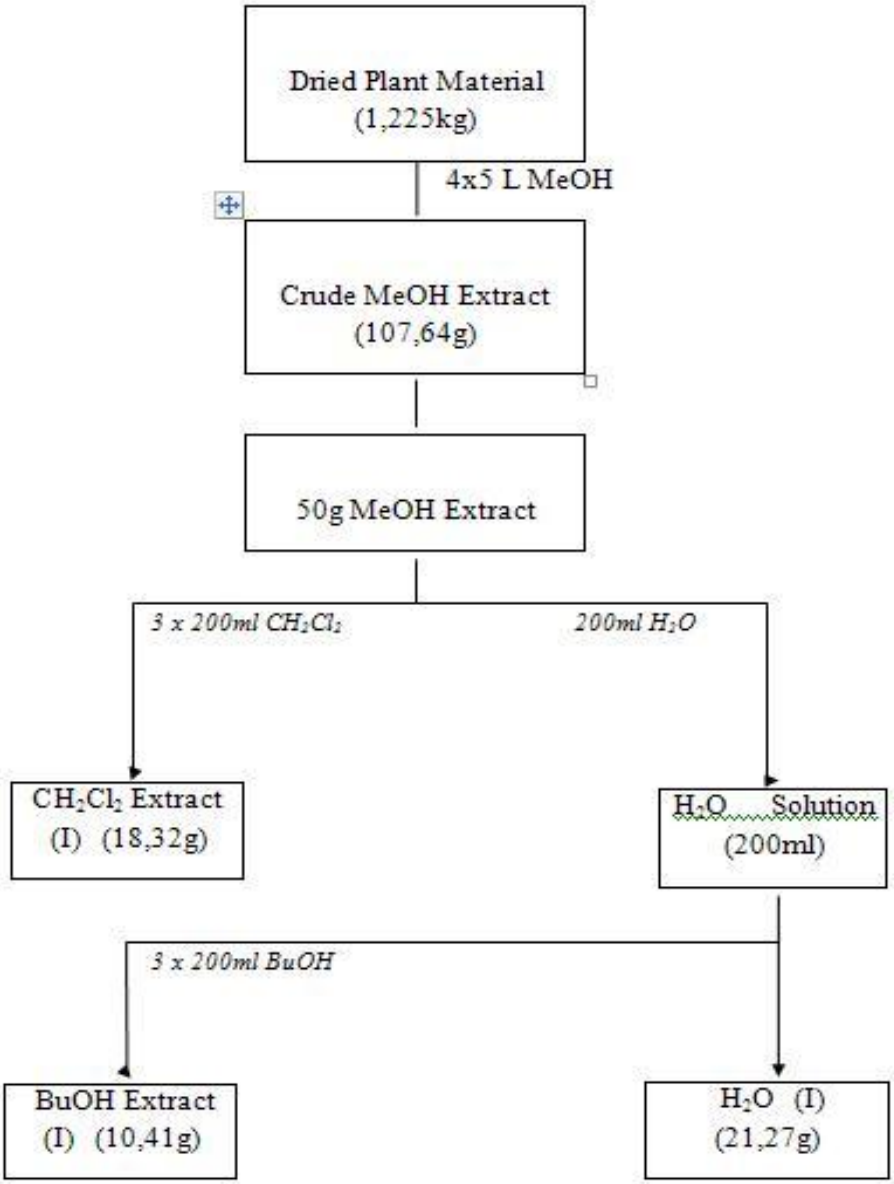
2.3. Extraction, Isolation and Purification

Shade dried flowers of *Notobasis Syriaca L.cass* (1.225kg) was pulverized using grinding machine at room temperature. After pulverizing, the flowers residue was exhaustively extracted with MeOH (4x5L) using emulsifier at room temperature. Each extraction was carried out leaving the plant material in the solvent for a night and than filtrated.

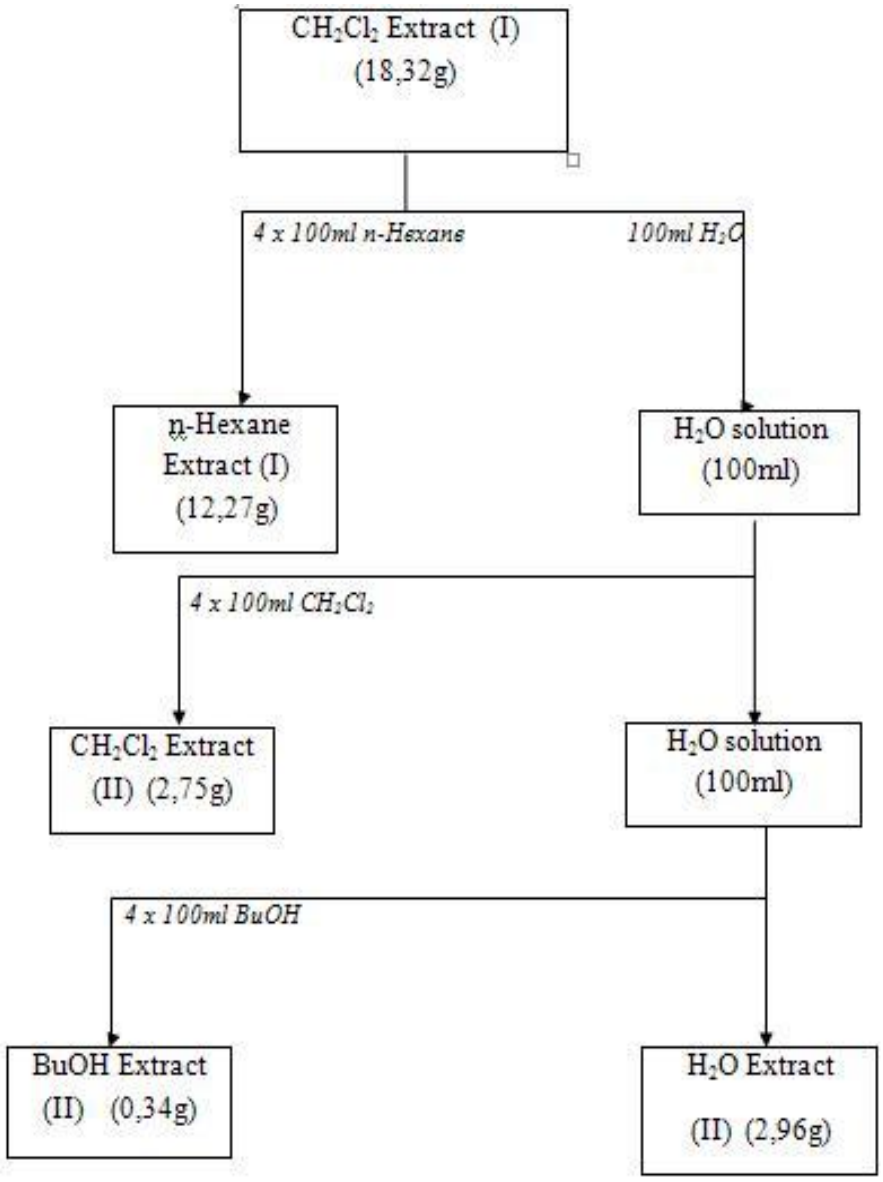
After that, removing the solvent under vacuum at low pressure at ~40^{0}C by rotary evaporator to give 107,64g extract. 50g of extract was dissolved in H_2O (200ml), and than was extracted with CH_2Cl_2 (3x200ml), BuOH (3x200ml) respectively. H_2O was used to capture the most polar compounds because of its high polarity. 18.32g CH_2Cl_2 extract and 10,41g BuOH extract was obtained.

The BuOH extract was chromatographed on silica gel column (600g, 6cmx90cm), by using CH_2Cl_2: MeOH: H_2O (90:10:0, 90:10:1, 80:20:2, 70:30.3) as eluting solvent. Further purification was achieved with the PTLC.

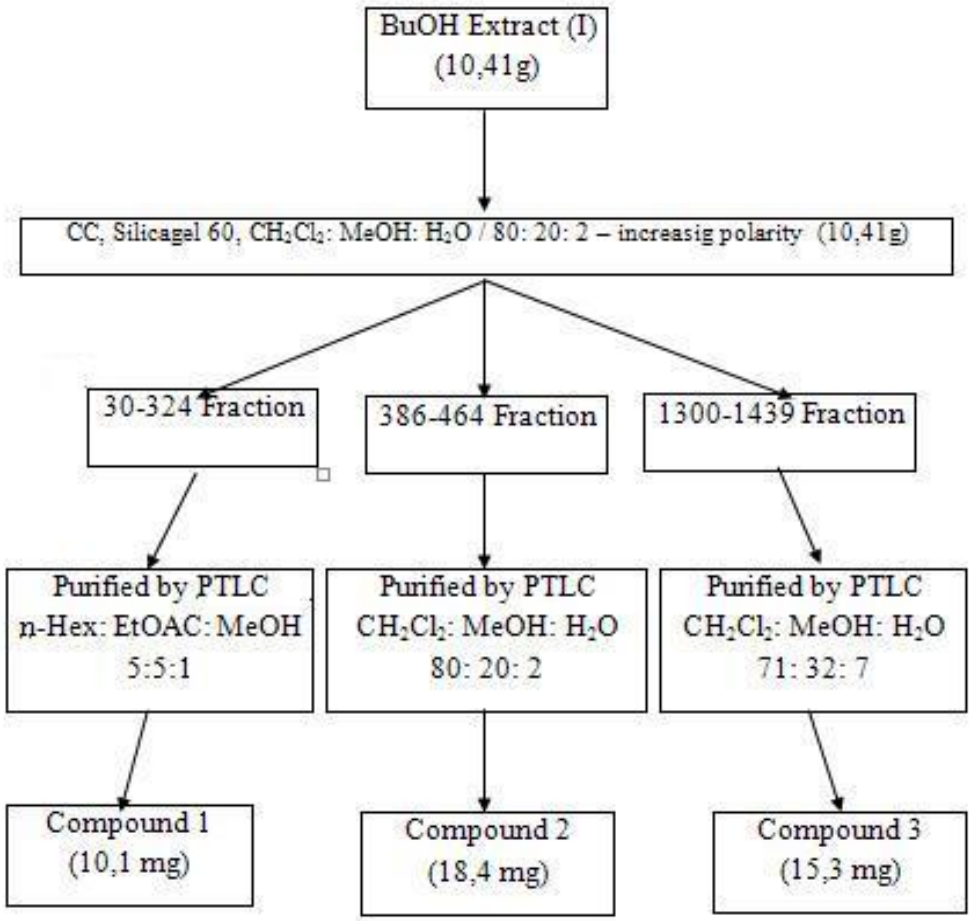
The CH_2Cl_2 extract was extracted with n-Hexane (4x100ml), CH_2Cl_2 (4x100ml) and BuOH (4x100ml) respectively. After the extracrion, the CH_2Cl_2 extract was chromatographed on silica gel column (250g, 4x90cm) by using CH_2Cl_2: MeOH (100:0, 95:5, 90:10) increasing polarity as eluting solvent.



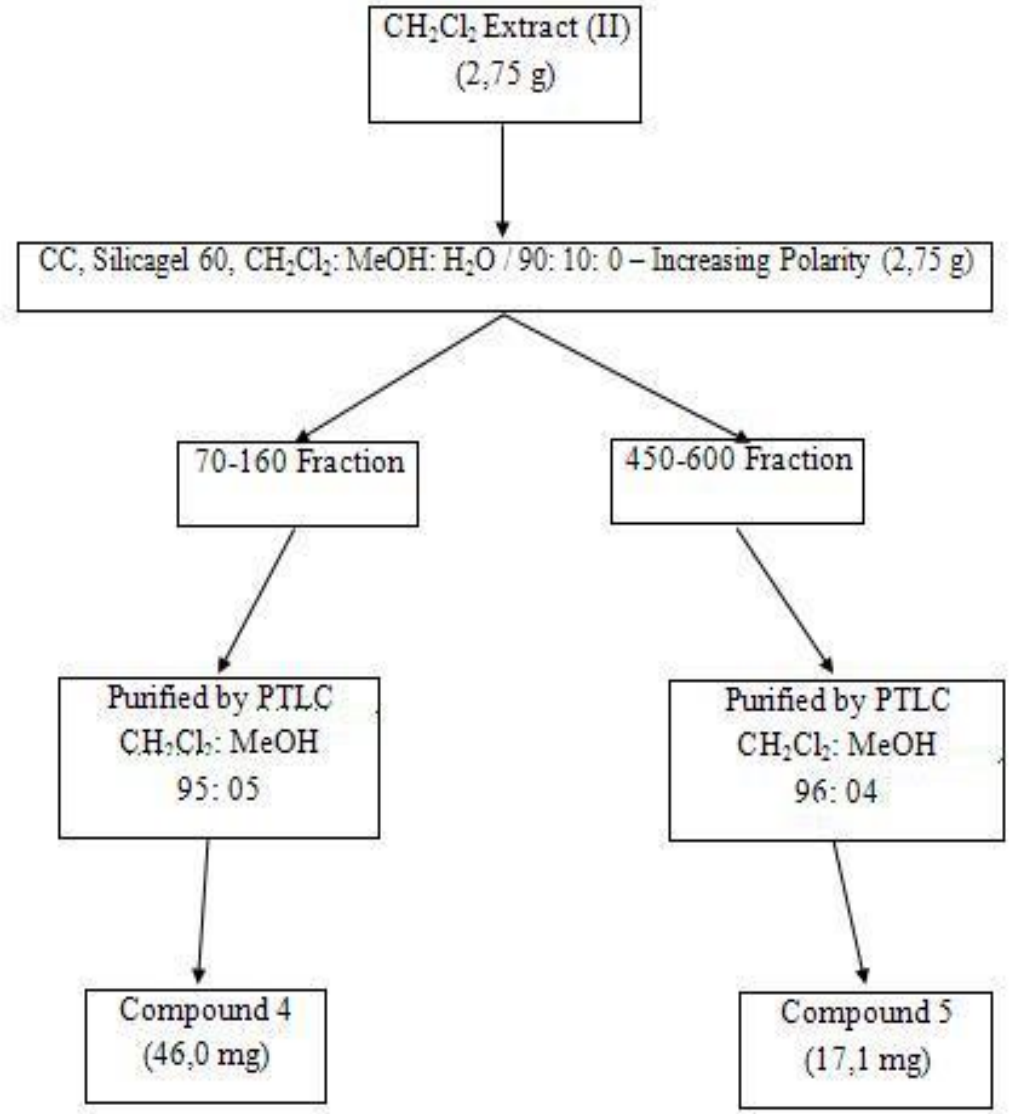
Scheme 1: Extraction of dried plant material



Scheme 2: Extraction of CH_2Cl_2 (I) extract



Scheme 3: Isolation and purification of compound 1, compound 2 and compound 3



Scheme 4: Isolation and purification of compound 4 and compound 5

2.4. Acetylation of Compound 3

Compound 3 (15.3 mg) was acetylated using Pyridine (3 ml) with Acetic Anhydride (3 ml) at room temperature for one day.

^{13}C NMR δ: 170-169 (11 carbonyl), 103 (anomeric carbon), 90 (anomeric carbon), 78-62 (12 CH and CH_2), 21-20 (11 CH_3)

3. RESULT AND DISCUSSION

3.1. Compound 1

Compound 1 was isolated as a yellow amorphous solid (10,1 mg). The ^{1}H NMR spectrum (CD_3OD) showed kaempferol's characteristic proton signals. (Table 1) at 7.57 (d, 2H, J=8.8 Hz, H-2′, H-6′), 6.84 (d,2H, J=8.8Hz, H-3′, H-5′), 6.37 (s, 1H, H8), and 6.18 (s, 1H, H6) (Yu Bo Wang et al, 2003). So compound 1 was identified as kaempferol (Figure 10).

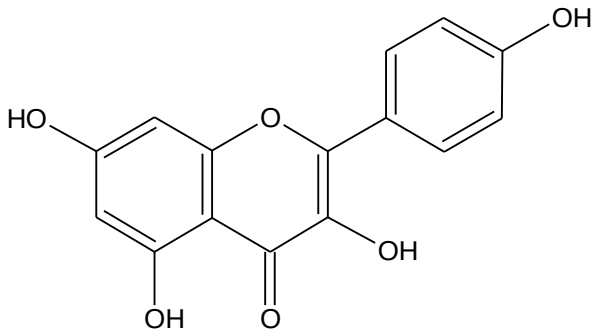


Figure 10: Kaempferol

No	δppm
H-6	6.18 (s, 1H, H6)
H-8	6.37 (s, 1H, H8)
H-2’	7.57 (d, 2H, J=8.8 Hz, H-2′)
H-3’	6.84 (d,2H, J=8.8Hz, H-3′),
H-5’	6.84 (d, 2H, J=8.8 Hz, H-5′)
H-6’	7.57 (d,2H, J=8.8Hz, H-6′),

Table 1: The assignment of ^{1}H- NMR signals of compound 1 (CD_3OD, 400MHz)

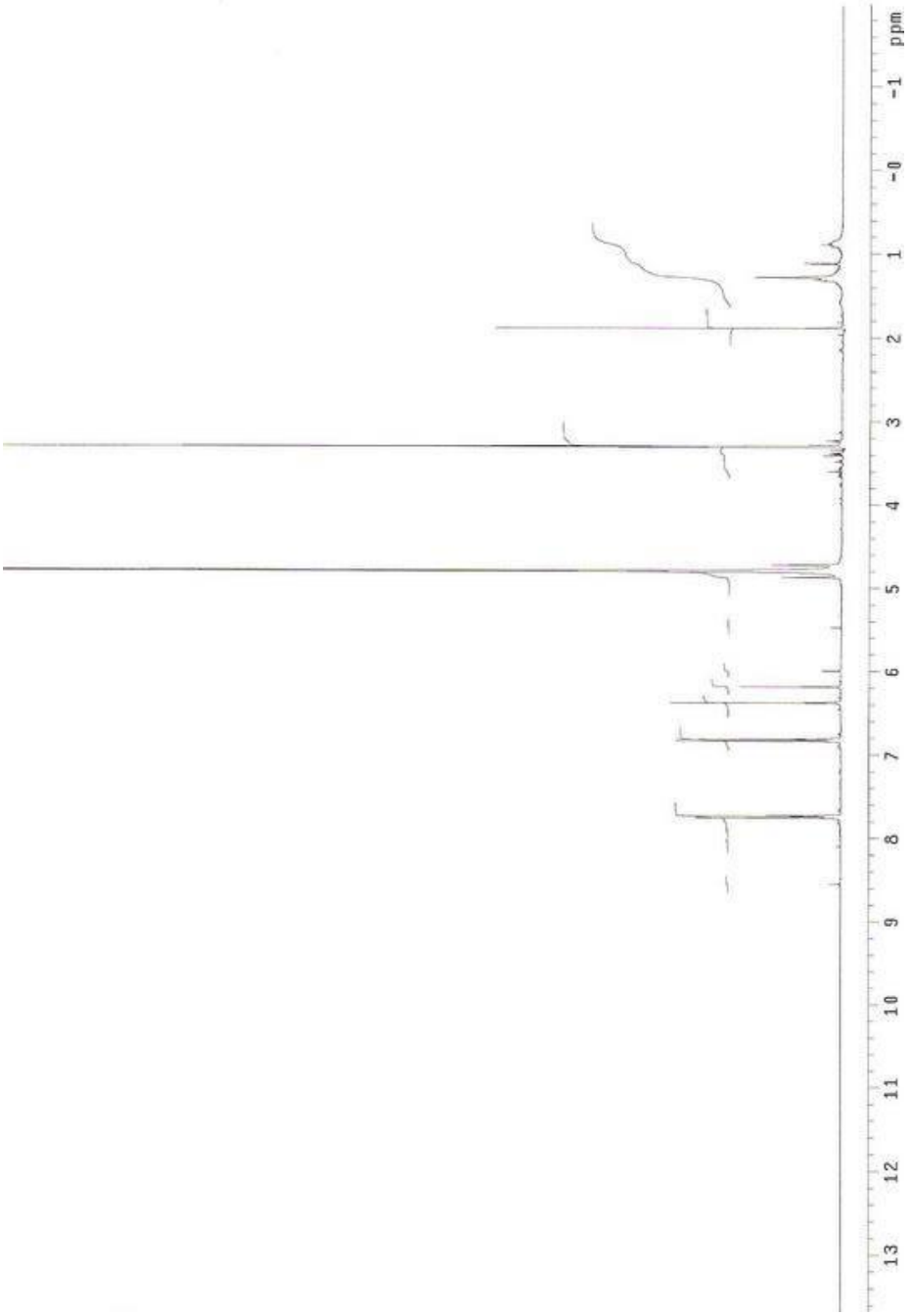


Figure 11: ^{1}H NMR spectrum of compound 1

3.2. Compound 2

Compound 2 was isolated as an amorphous yellow solid (18.1 mg). The ^{1}H NMR spectrum of the compound showed due to aromatic protons (δ 8.09, 1H, s; 7.71 2H, d;, 6.88, 2H, d; and 6.10, 1H, d), sugar proton (δ 5.32 (anomeric), 4.28-3.30) are belong to galactose or glucose (Table 2). ^{1}H-^{1}H COSY spectrum showed correlation between δ 7.71 and δ 6.87 (Figure 14).

The ^{13}C NMR spectrum analyis confirmed presence of 21 carbon atom in the molecule, 15 of which belonged to the non sugar part (Table 3). A comparation of ^{1}H and ^{13}C NMR spectra with the literature data (Cornelius et al., 1999, Gokdil et al., 1997, Hatano et. al, 1998, Leong et al, 2009, Lu et. al, 2000, Marchart et. al, 2003, Materska et. al, 2003, Zahid et. al, 2002) point to its great similarity to kaempherol glucoside. However, there were some differences. Although ^{1}H and ^{13}C NMR spectra data, structural identification of compound was not established.

δ_{1H}
8.09 (1H, s)
7.71 (2H, d, J=7.2 Hz)
6.88 (2H, d, J=7.2Hz)
6.10(1H, d, J=1.2 Hz)
5.32 (1H, d, J=1.6Hz)
4.21 (1H, dd, J=1.6 and 1.6 Hz)
3.71 (1H, dd, J=3.2 and 3.6 Hz)
3.37-3.28 (4H, m)

Table 2: ^{1}H NMR of compound 2 (CD_3OD, 400MHz)

δ_C [13]
177.29
175.24
162.09
161.32
158.12
157.01
134.15
130.51
129.45
129.31
120.88
115.93
102.28
101.79
78.41
72.17
71.02
70.79
63.13

Table 3: ^{13}C NMR of compound 2 (CD_3OD, 100MHz)

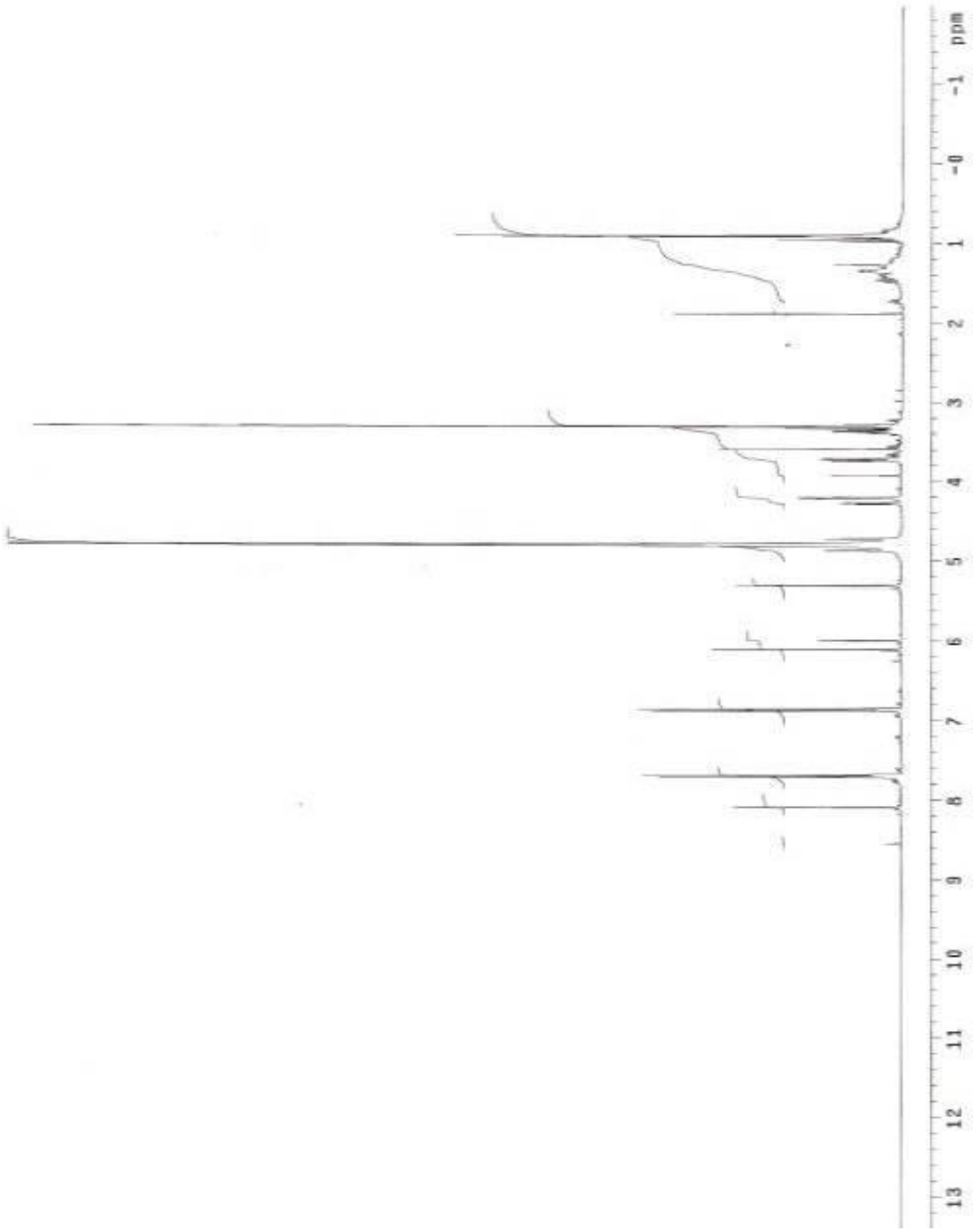


Figure: 12: ^{1}H NMR spectrum of compound 2

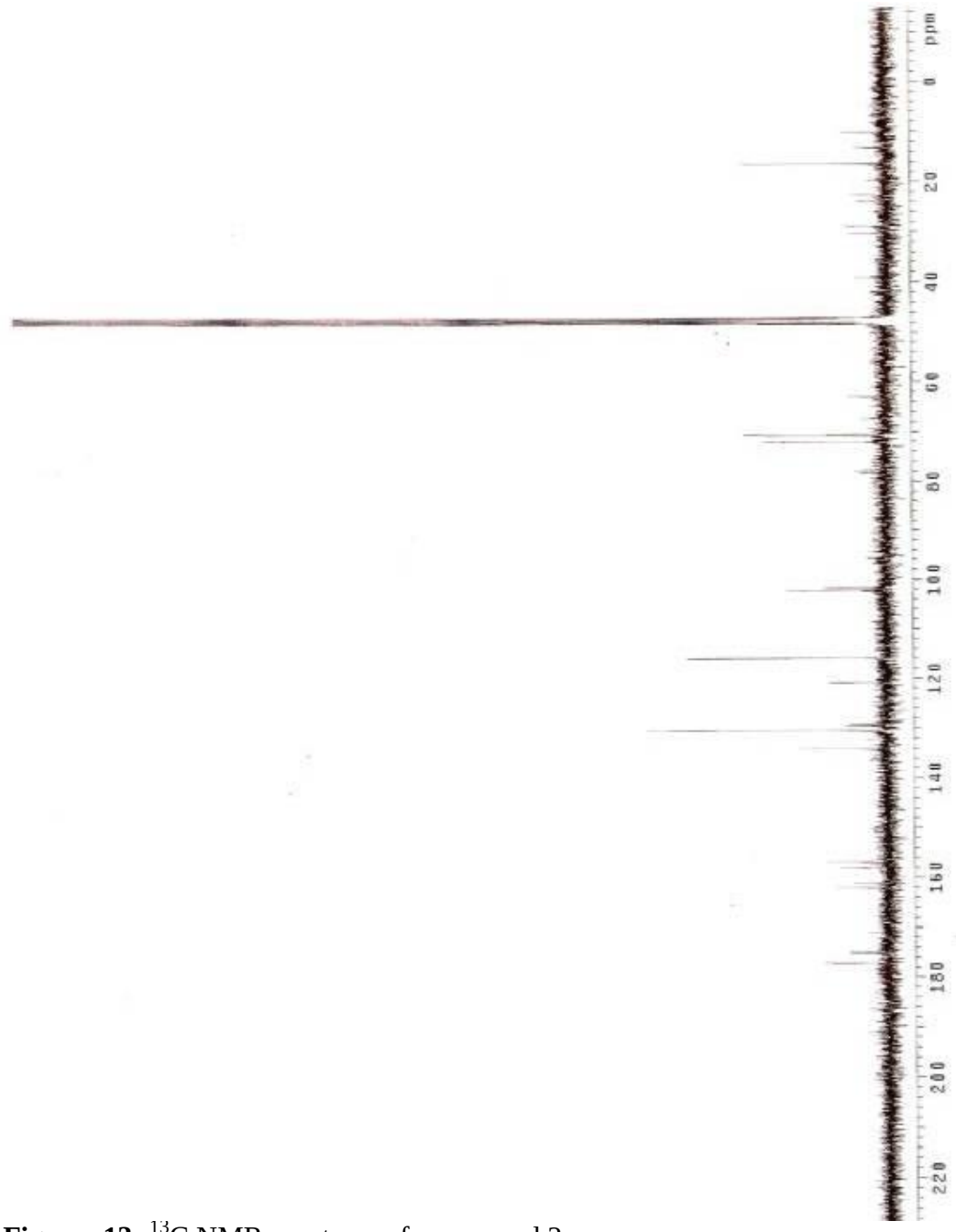


Figure: 13: ^{13}C NMR spectrum of compound 2

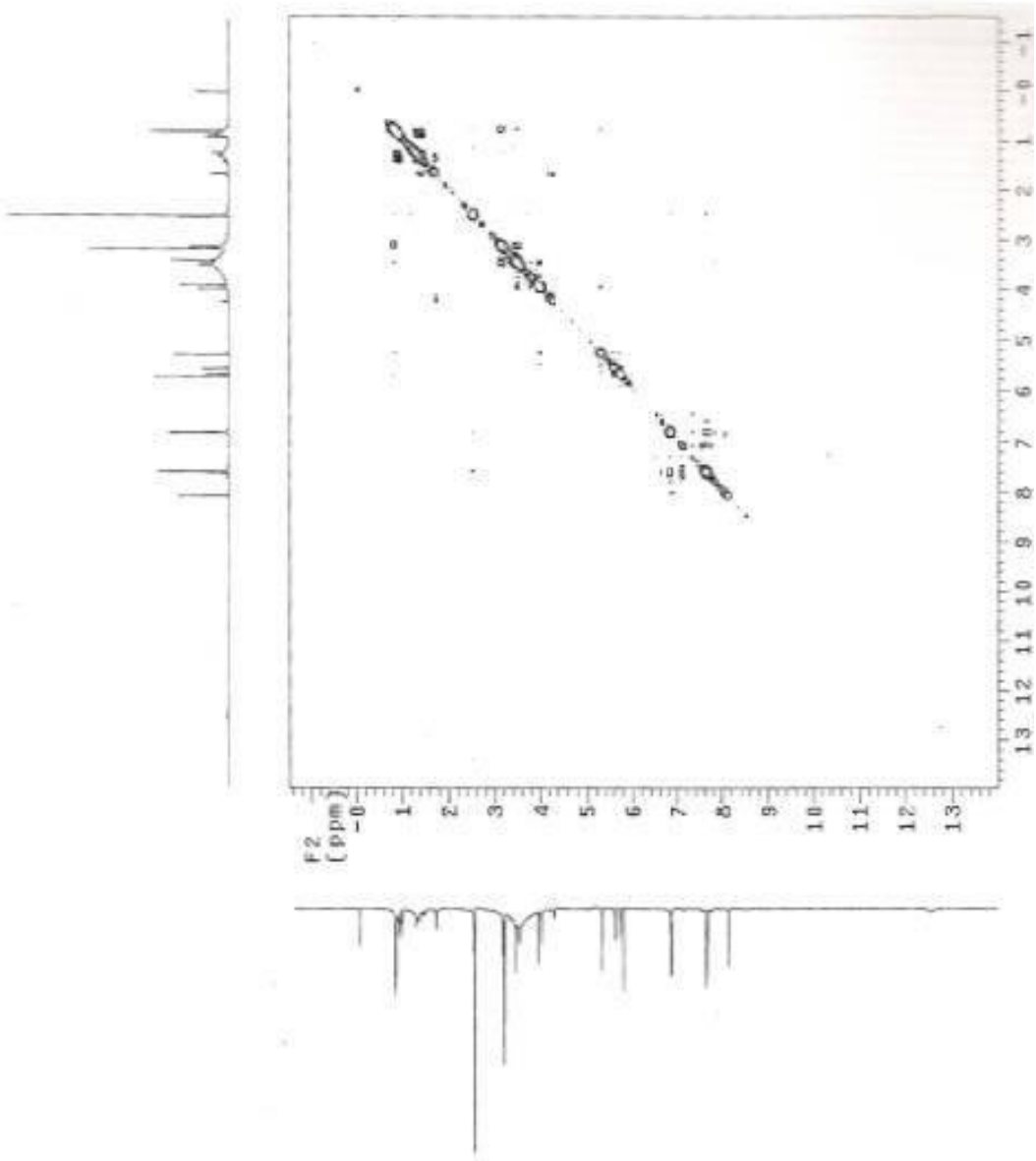


Figure: 14: ^{1}H-^{1}H COSY spectrum of compound 2

3.3. Compound 3

Compound 3 was isolated as an white solid (15.3mg). Although ^{1}H and ^{13}C spectras showed that the compound was diglucosides, structure of compound was not identified.

δ ^{13}C
104.75
92.46
83.26
79.85
77.83
75.04
73.85
73.57
73.51
72.88
72.34
71.45
70.59
62.83
61.23

Table 4: ^{13}C NMR of compound 3 (CD_3OD, 100MHz)

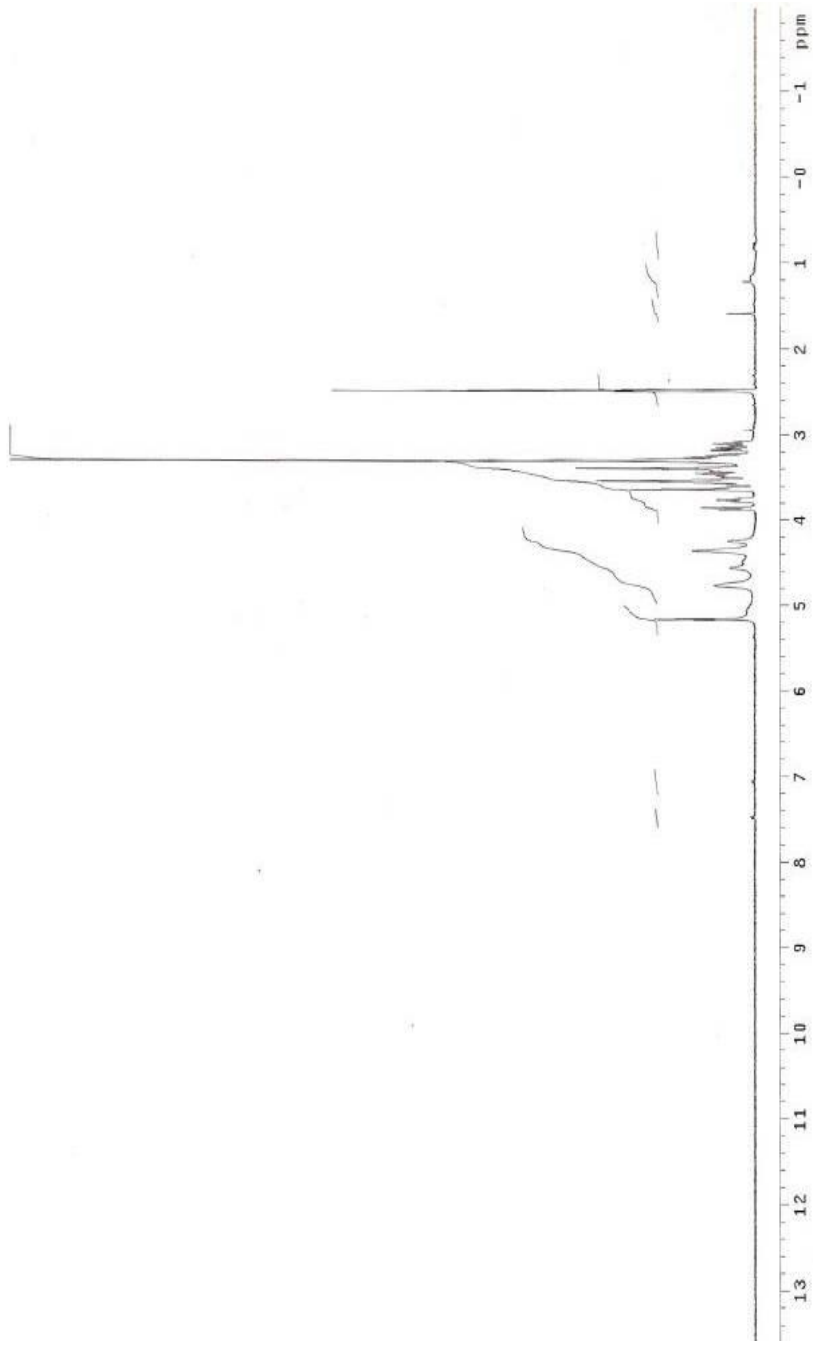


Figure 15: ^{1}H NMR spectrum of compound 3

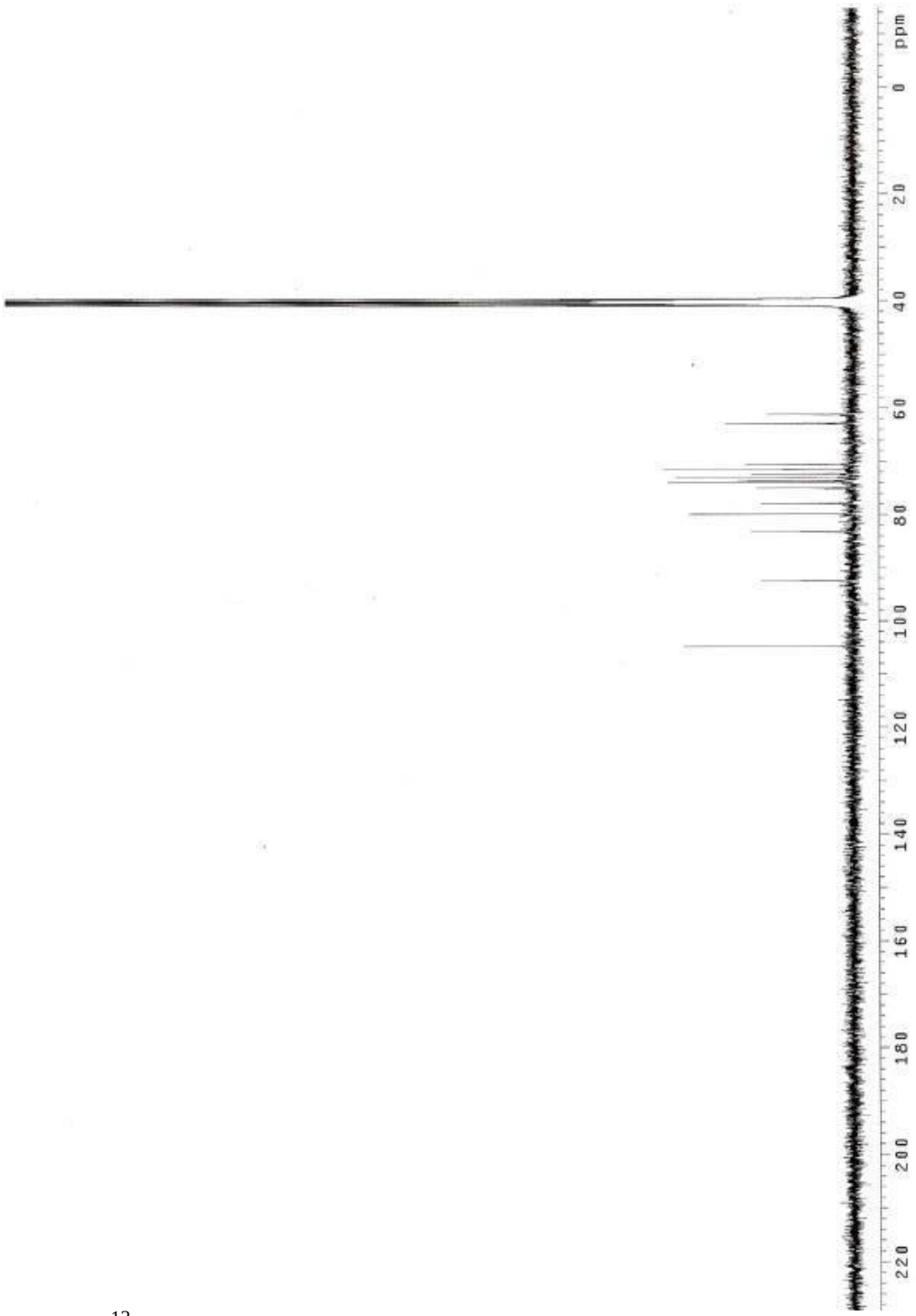


Figure 16: ^{13}C NMR spectrum of compound 3

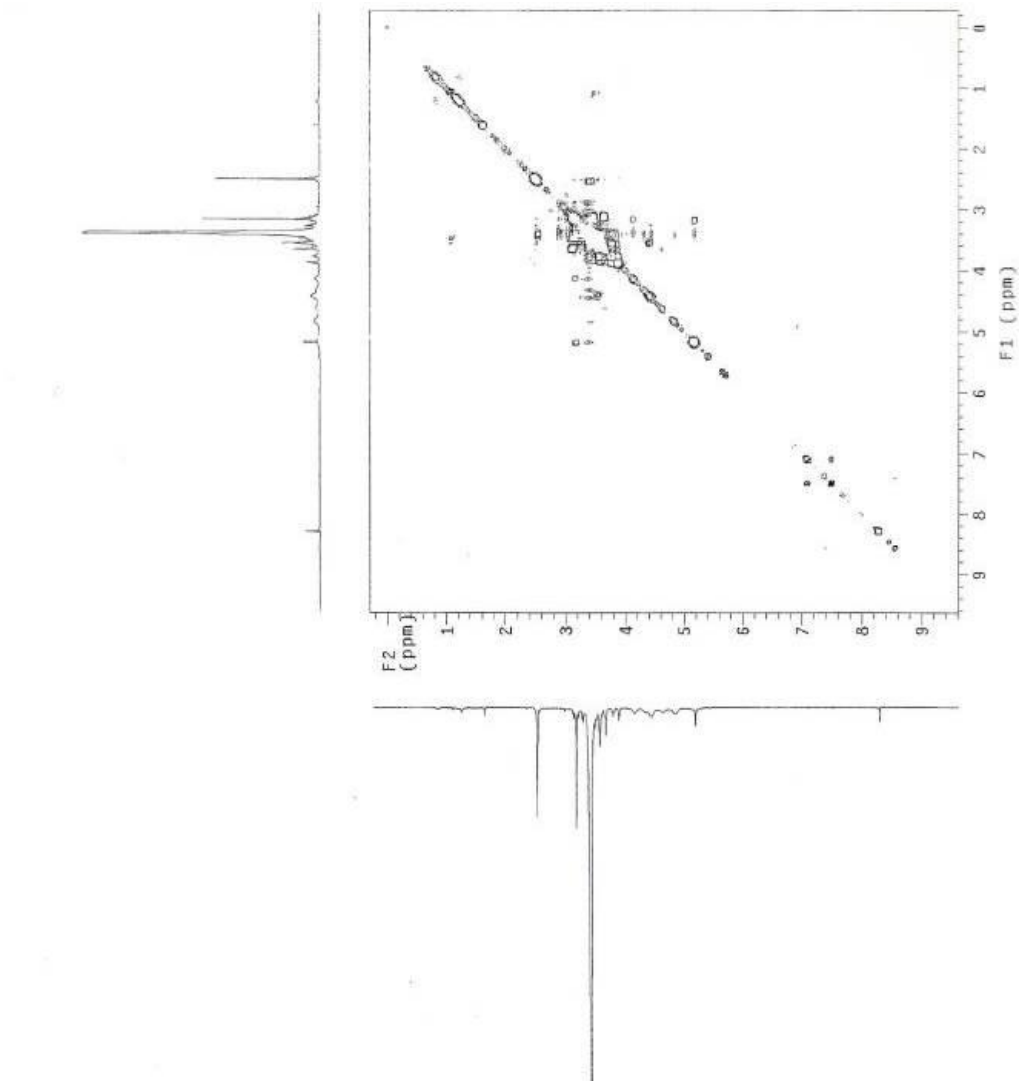


Figure 17: ^{1}H-^{1}H COSY spectrum of compound 3

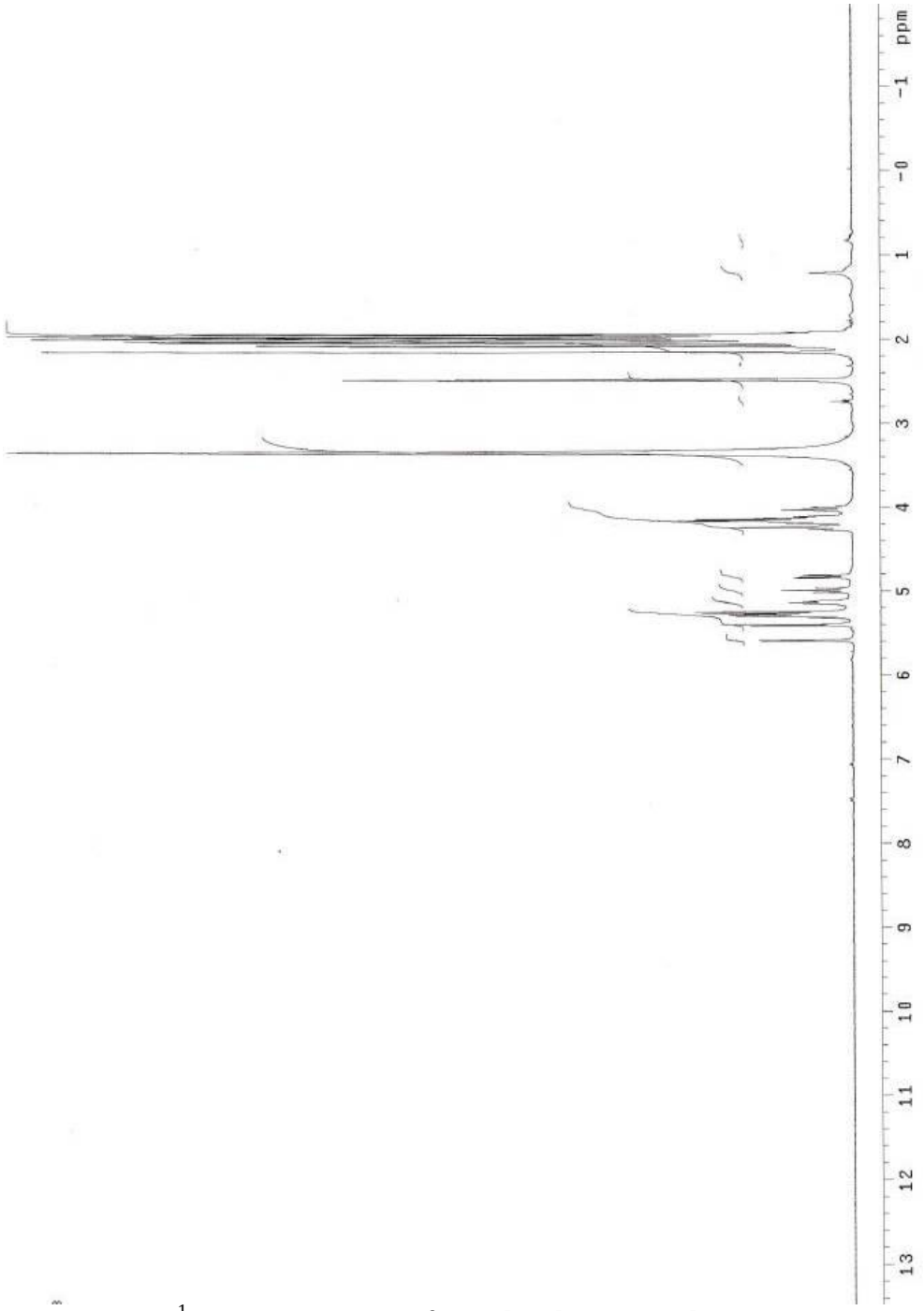


Figure 18: ^{1}H NMR spectrum of acetylated compound 3

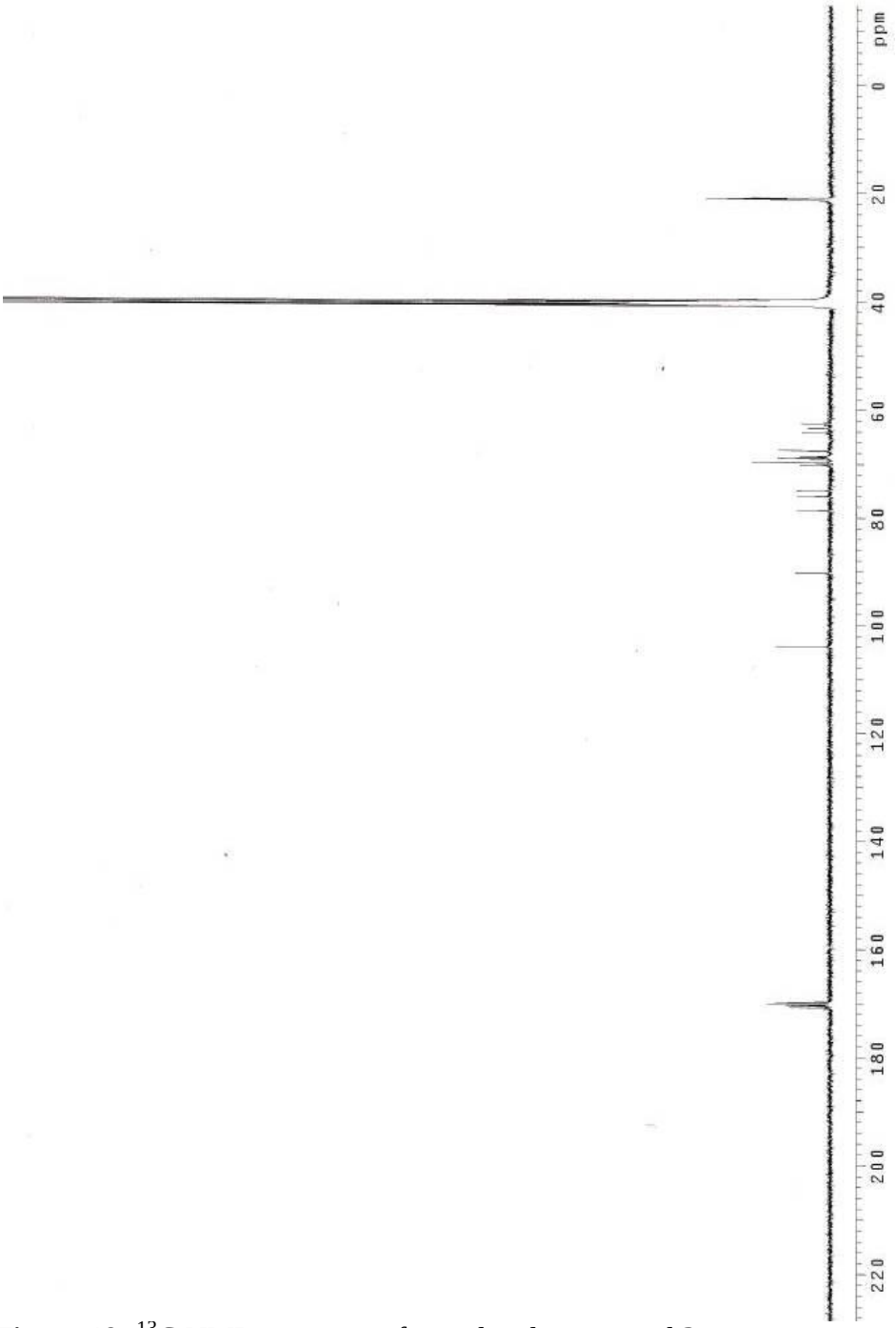


Figure 19: ^{13}C NMR spectrum of acetylated compound 3

3.4. Compound 4

Compound 4 was isolated as a white amorphous solid (17,1 mg). The analysis of its ^{1}H and ^{13}C NMR spectra (Table 5) and literature data clearly indicated that compound 4 was the β- sitosterol. (Dupont et al.,1997).

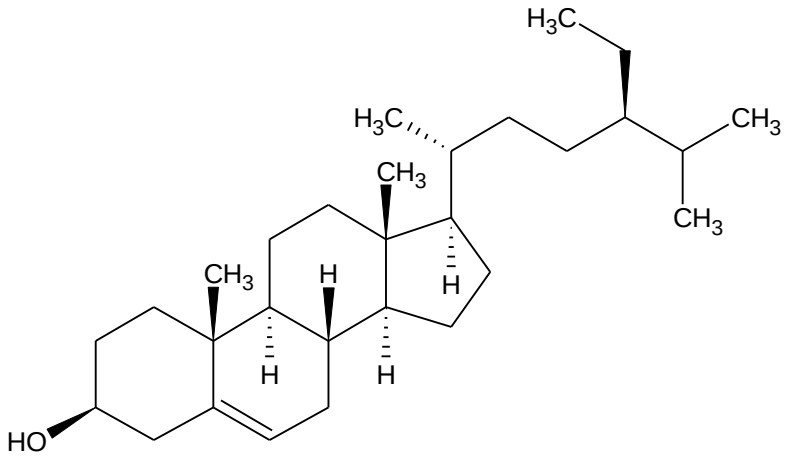


Figure 20: β-Sitosterol

δ_C ^{13}		δ_H ^{1}
140.99		5.34 (1H, C=CH)
121.90		3.50 (1H, CH-OH)
72.00		2.31-1.03 (CH and CH_2)
57.00		1.00-0.67 (CH_3)
56.32		
50.38		
46.08		
42.52		
40.68		
39.92		
37.50		
36.73		
36.37		
34.19		
32.14		
31.88		
29.42		
29.12		
28.46		
26.36		
24.53		
23.31		
20.03		
19.61		
19.27		
19.00		
12.20		
12.07		

Table 5: The assignment of ^{13}C NMR and ^{1}H of compound 4 ($CDCl_3$, 400 and 100 MHz)

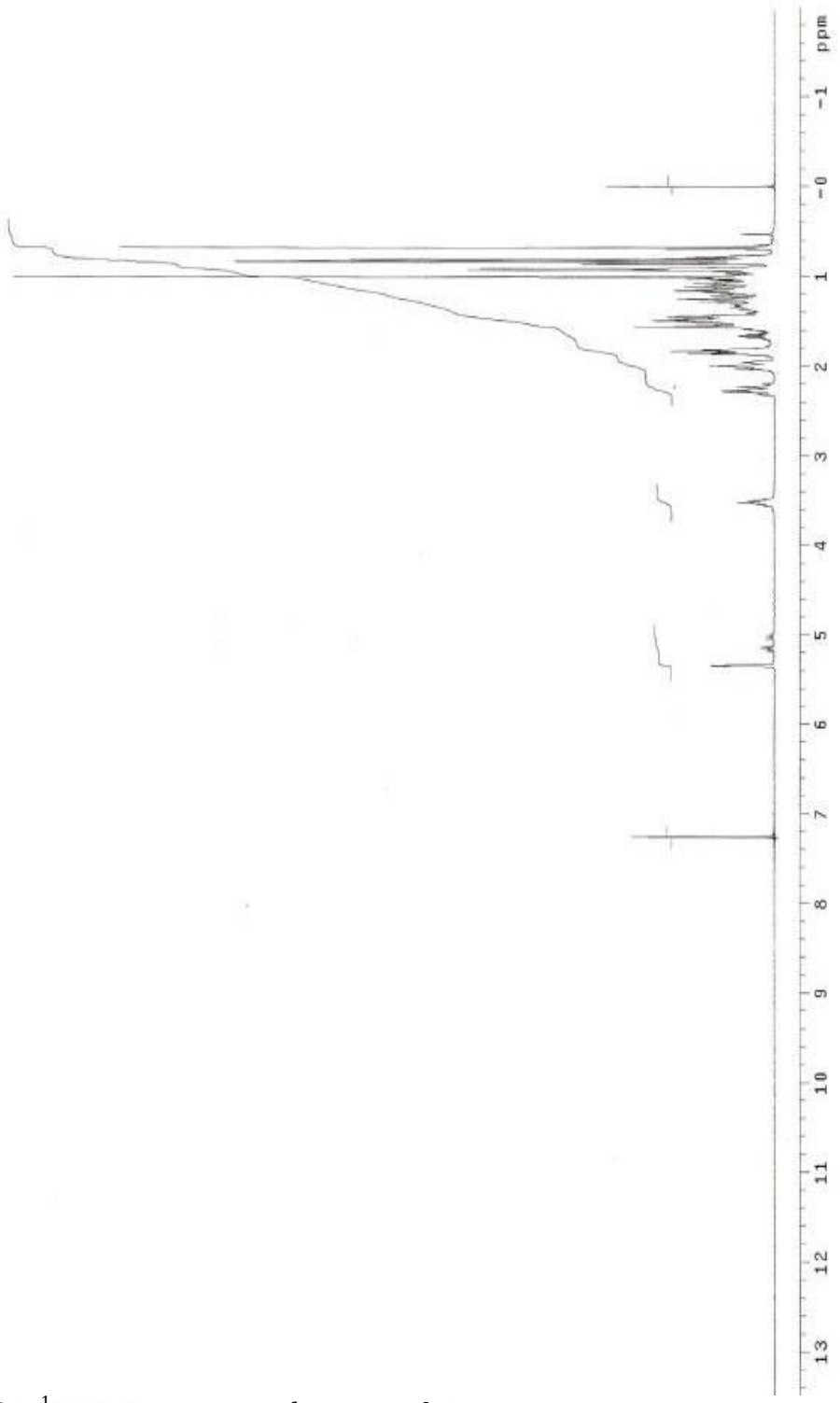


Figure 21: ^{1}H NMR spectrum of compound 4

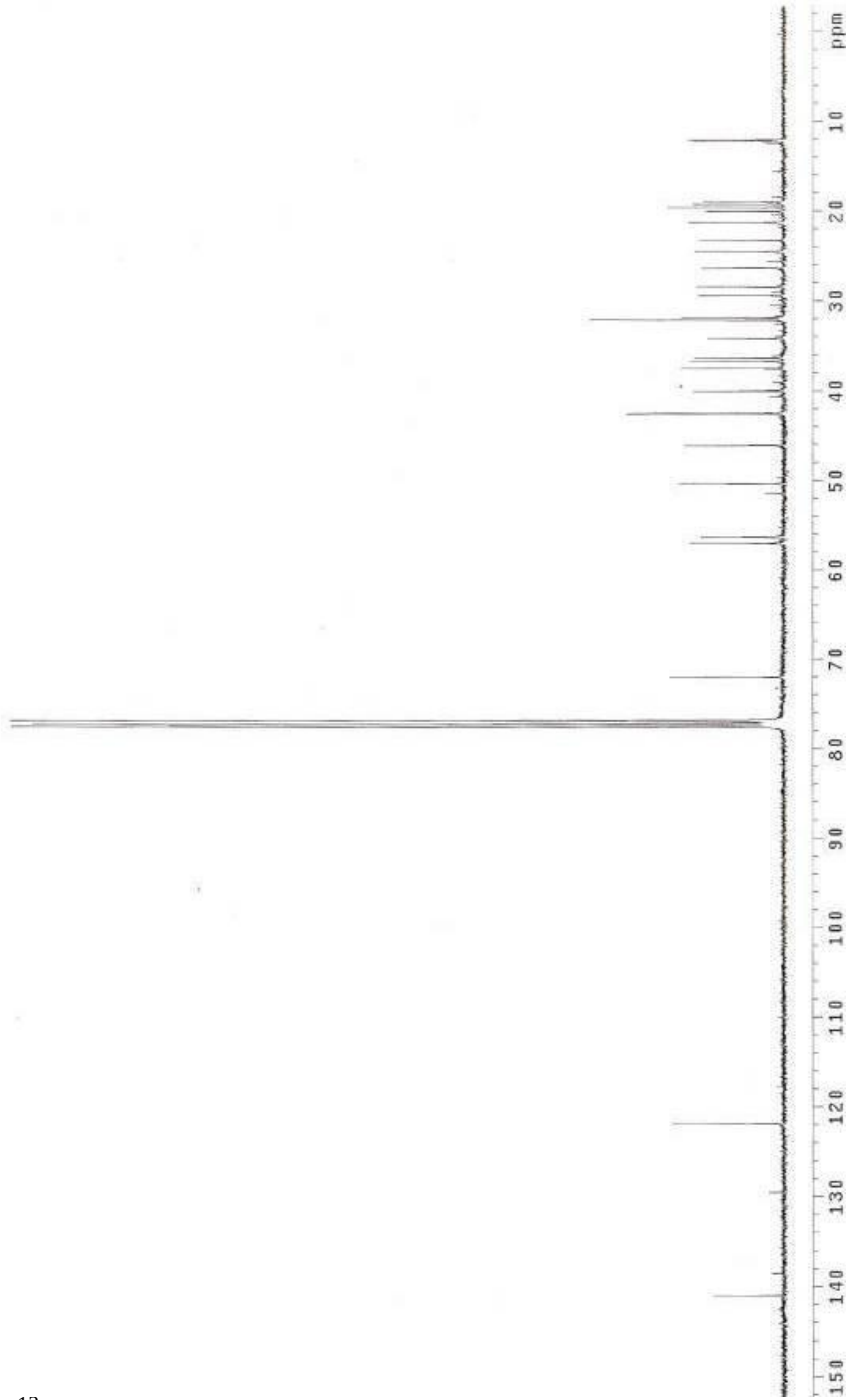


Figure 22: ^{13}C NMR spectrum of compound 4

3.5. Compound 5

Compound 5 was obtained as a colorless amorphous solid (46.0 mg). A comparation of ^{1}H and ^{13}C NMR spectra with the literature data point to mixture of to acetyleted triterpene as a mixture of urs-20(30)-en-3-yl acetate and olean-12-en-3-yl-acetate. (Thaden et. al, 2003). (Table 6 and Table 7).

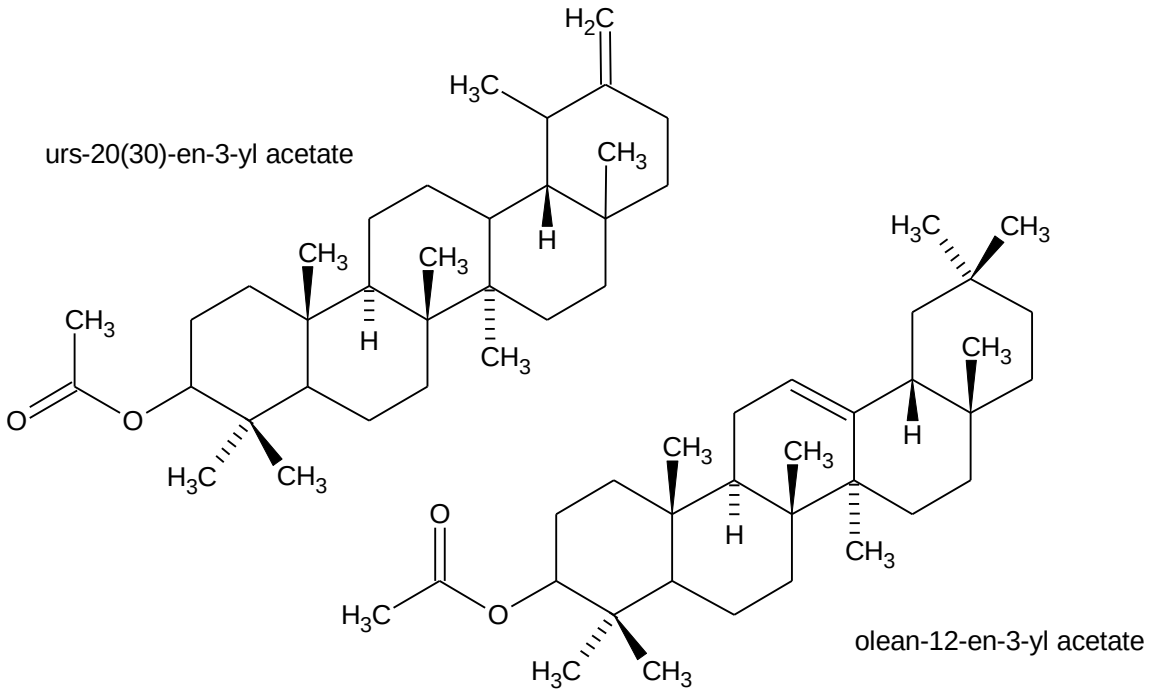


Figure 23: Mixture of urs-20(30)-en-3-yl acetate and olean-12-en-3-yl-acetate

δ_{1H}
4.62 (m, $=CH_2$)
4.45 (m, CH-OH)
2.08 (s, CH_3)
1.08-0.88 (CH, CH_2, CH_3)

Table 6: ^{1}H NMR of compound 5 (CD_3OD, 400MHz)

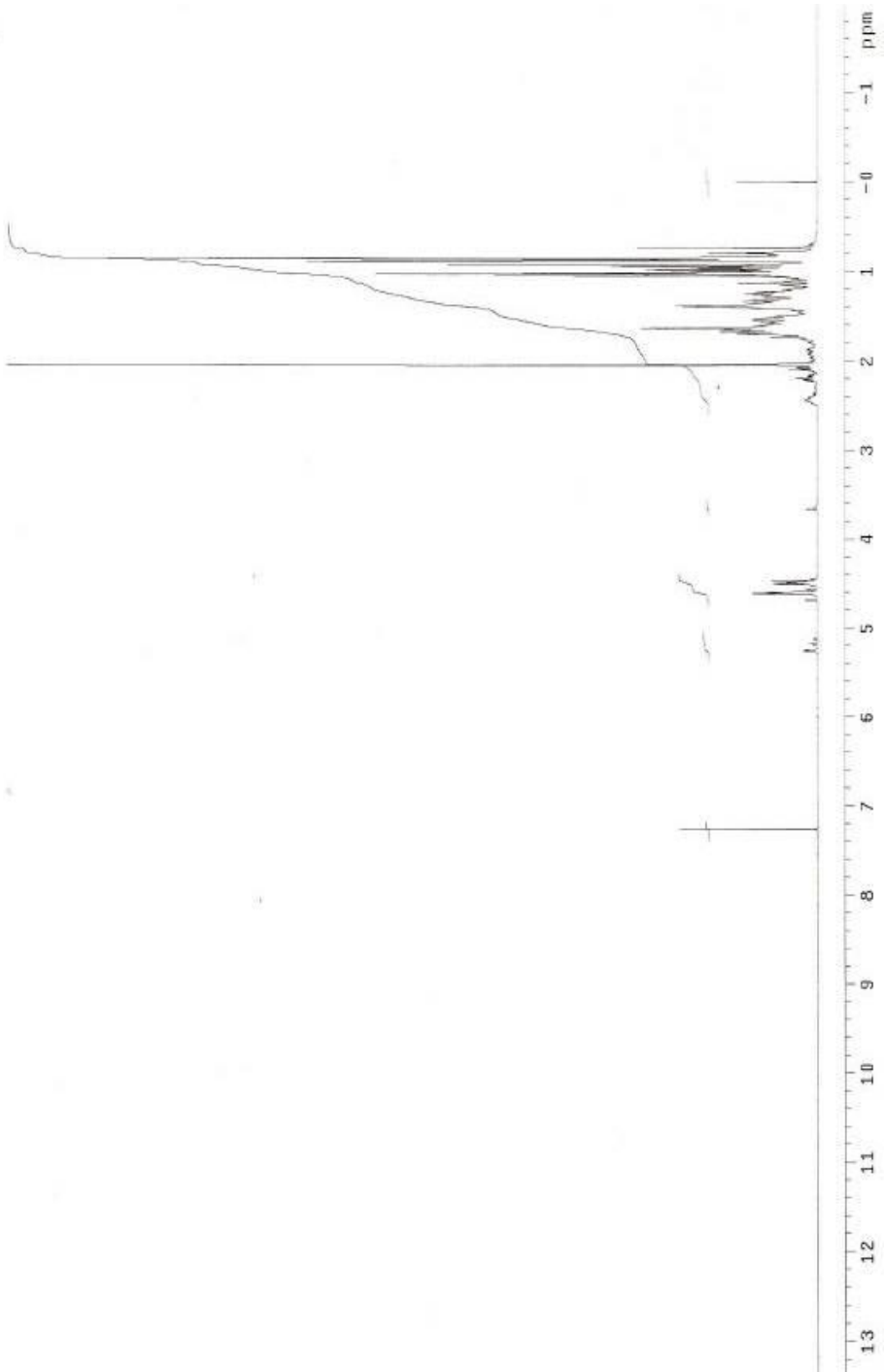


Figure 24: ^{1}H NMR spectrum of compound 5

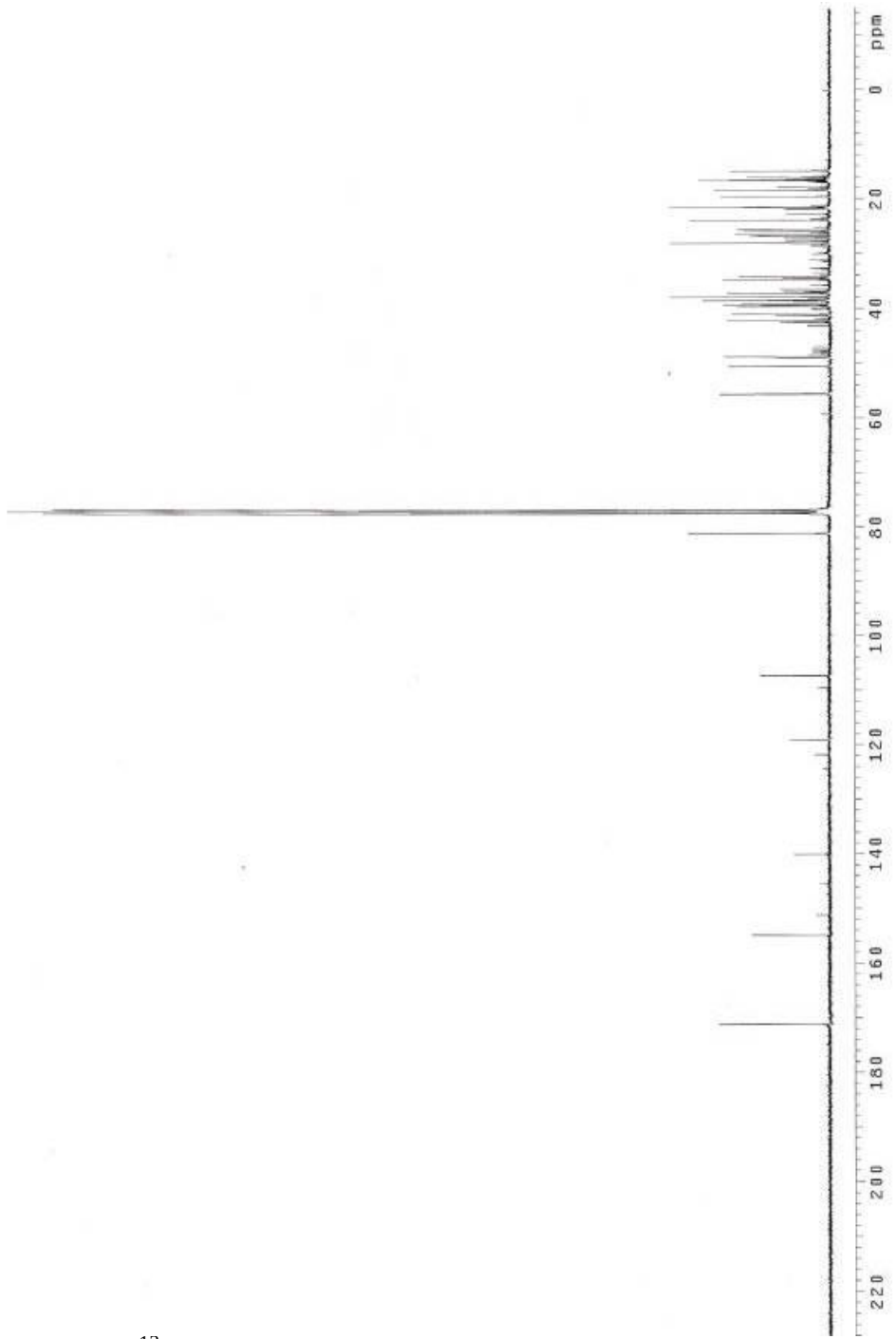


Figure 25: ^{13}C NMR spectrum of compound 5

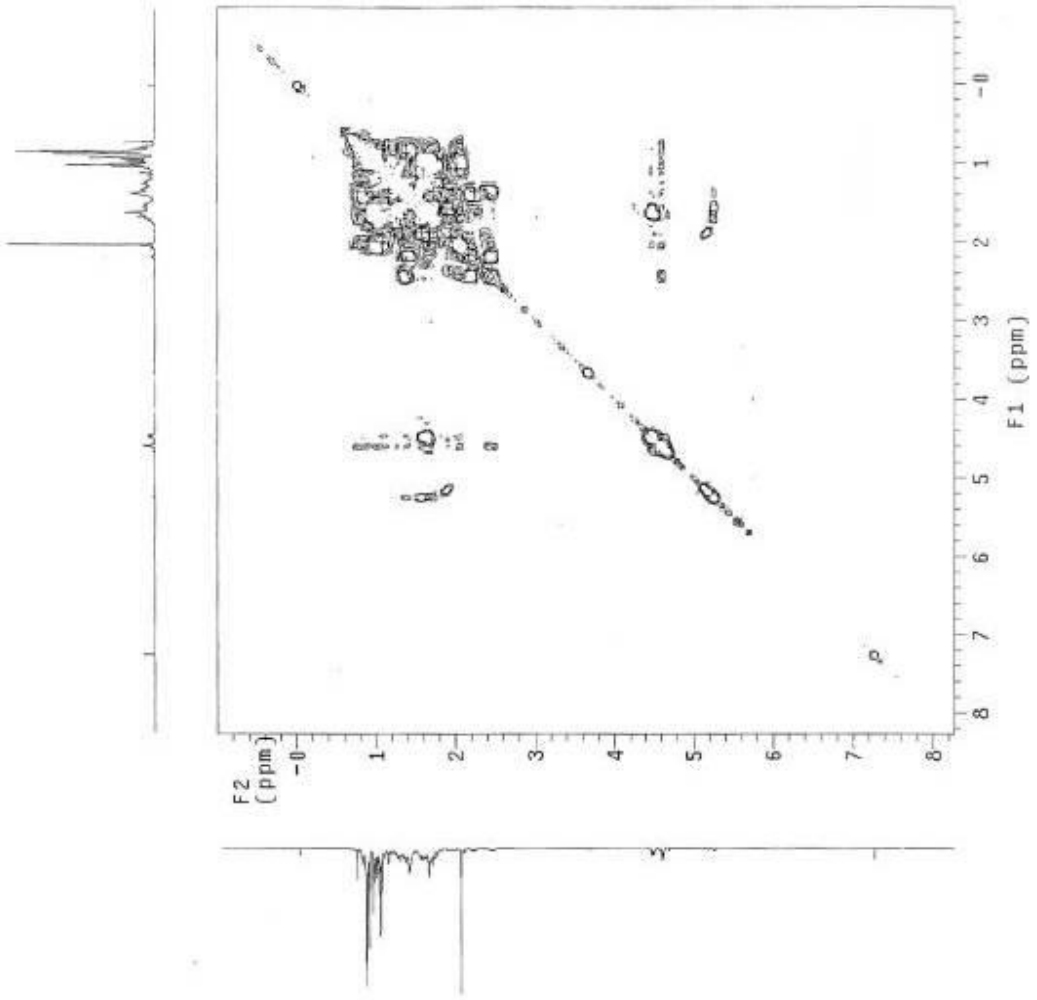


Figure 26: ^{1}H-^{1}H COSY spectrum of compound 5

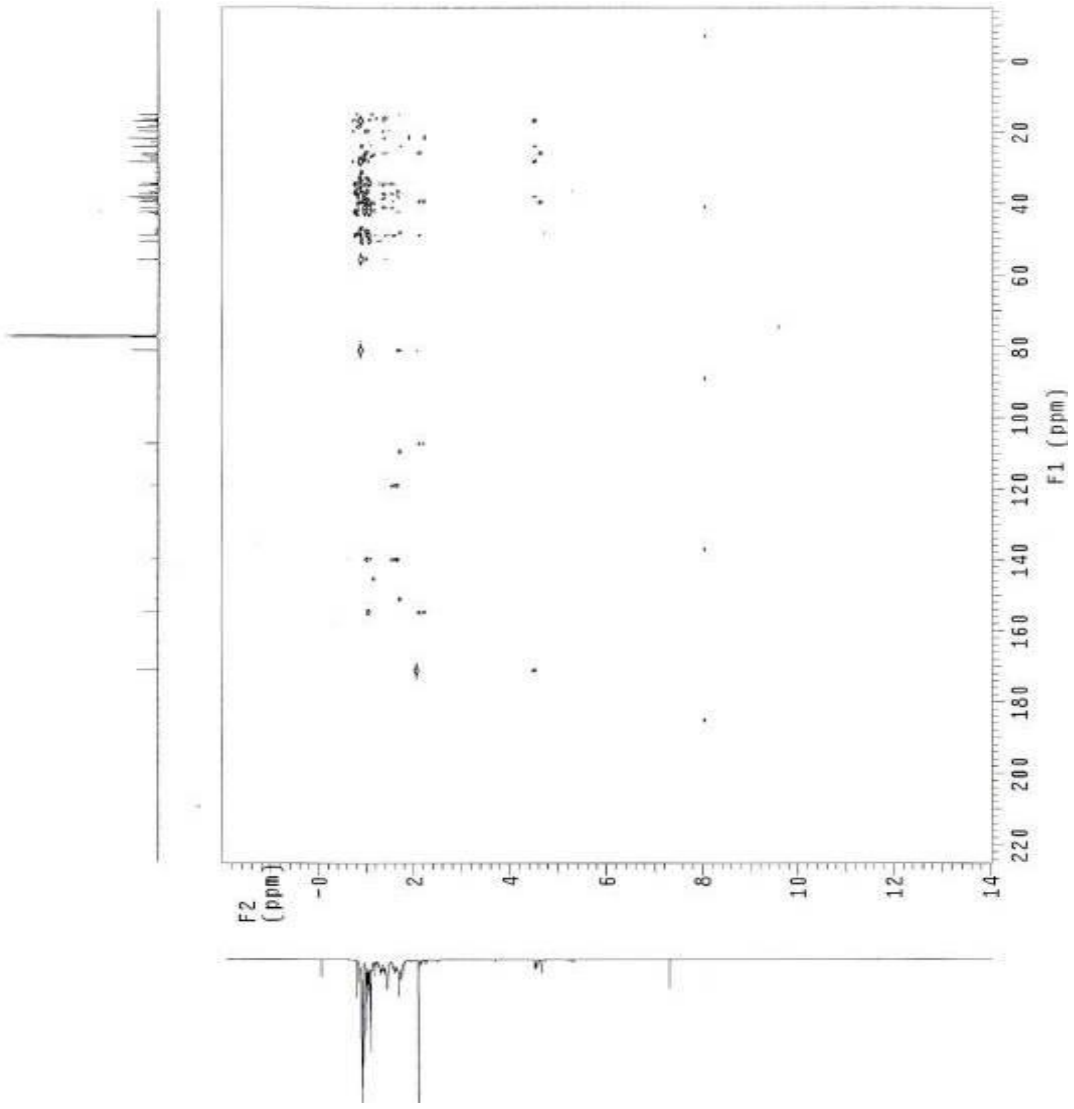


Figure 27: HMBC spectrum of compound 5

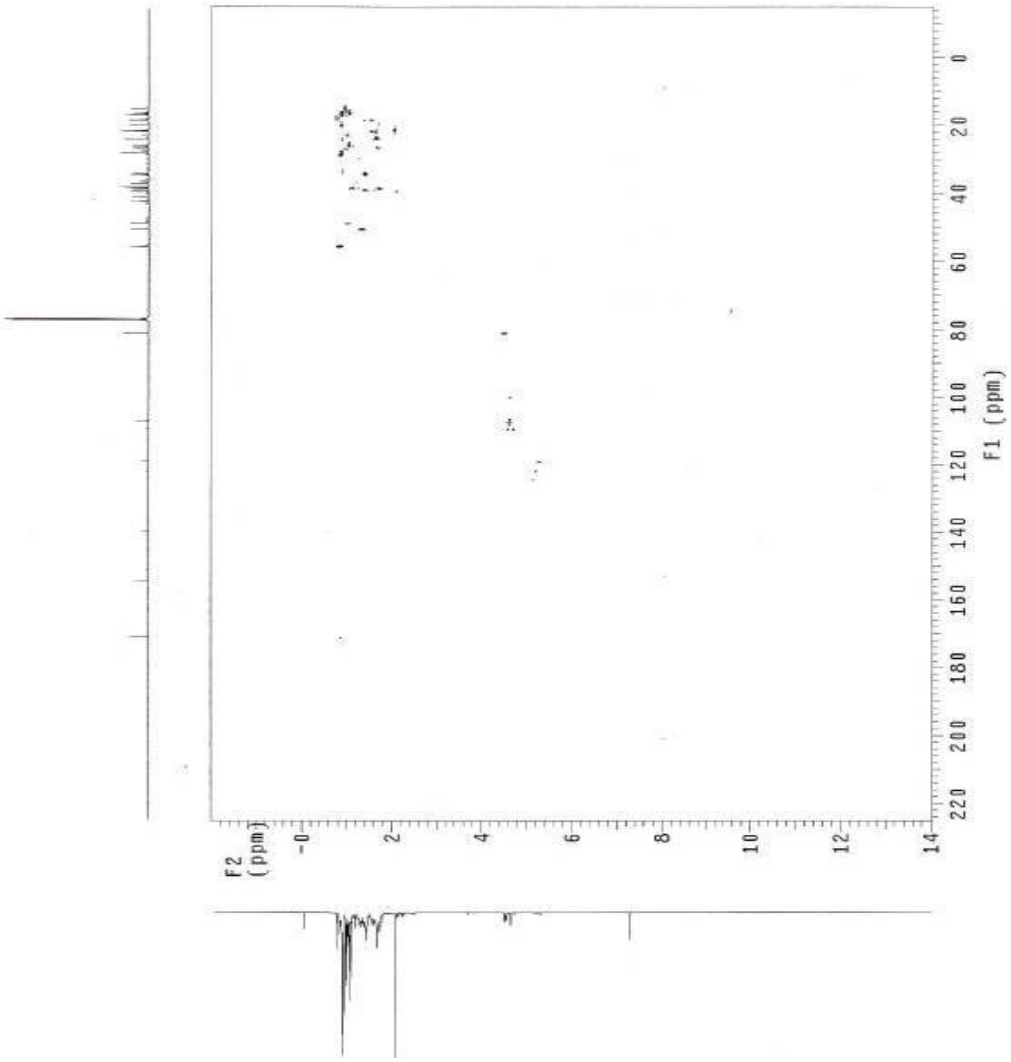


Figure 28: HMQC spectrum of compound 5

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