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Phytochemical studies on the chemical constituents of *Artemisia absinthium* L.

조선대학교 대학원

약 학 과

고 해 주

Phytochemical studies on the chemical constituents of *Artemisia absinthium* L.

쓴쑥(*Artemisia absinthium* L.)의
화학 성분

2015년 2월 25일

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고 해 주

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이 논문을 약학 석사학위신청 논문으로 제출함.

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국문 초록

쓴쑥(*Artemisia absinthium* L.)의 화학성분

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일반적으로 쑥으로 알려진 쓴쑥(국화과)은(는) 털이와 골이 줄기를 가진 관목 식물이다. 쓴쑥(*Aretemisia absinthium* L.)은 2,100m의 고도까지 카슈미르에서 발견된다. 전통적으로 간질, 위장, 비장, 비뇨기 질환을 치료하는데 사용하고, 상처에도 치유된다. 쓴쑥(*Aretemisia absinthium* L.)에 대한 이전의 연구는 sesquiterpenes, dimeric guaianolides, flavones, lignans , and volatile oils 등 다양한 성분으로 분리 되었다.[1] 쓴쑥(*Aretemisia absinthium* L.)의 메탄올 추출물에 대해 조사하였다. 쓴쑥(*Aretemisia absinthium* L.)의 지상부로부터 실리카겔, RP-18, MCI-gel, MPLC 컬럼크로마토그래피를 반복적으로 수행하여 여섯 개의 화합물 eupatilin(1), dammaradienyl acetate(2), glutinol acetate(3), 3β-acetoxyoleanan-12-one(4), taraxasterol(5), quercetin-3,4′ -dimethyl(6)을 분리했다. 이 화합물 (1-6)의 화학구조는 ^{1} H-^{1} H COSY, HSQC, HMBC 포함한 1D and 2D NMR에 기초하여 규명했다. 이 화합물 중 네 개의 triterpene 화합물들은 쓴쑥(*Aretemisia absinthium* L.) 식물에서 처음 분리되었다.

쓴쑥(*Aretemisia absinthium* L.)에서 triterpene 화합물들이 아직 보고되지 않았다. 이에 관한 분리 및 화합물의 구조적 특성을 보고하고자 한다.

키워드 : 쓴쑥(*Aretemisia absinthium* L.) ; 쑥 ; 화학성분

ABSTRACT

Phytochemical studies on the chemical constituents of *Artemisia absintium* L.

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Artemisia absinthium L.(*Asteraceae*), commonly known as wormwood, is a shrubby plant having hairy and ribbed stem. It is found in Kashmir, up to an altitude of 2100m. Traditionally it is used to treat epilepsy, gastric problems, enlargement of the spleen, urinary disorders, and for wound healing. Previous studies on *A.absinthium* resulted in the isolation of diverse components, including sesquiterpenes, dimeric guaianolides, flavones, lignans , and volatile oils.[1] In an ongoing investigation from this plant, the methanol extract of *A. absinthium* was investigated. By means of repeated column chromatography using silica gel, and LiChroprep RP-18, MCI-gel and MPLC, six compounds eupatilin(1), dammaradienyl acetate(2), glutinol acetate(3), 3β-acetoxyoleanan-12-one(4), taraxasterol(5), quercetin-3,4′ -dimethyl(6) were isolated from the aerial parts of *A.absinthium*. The chemical structures of compounds(1-6) were identified based on 1D and 2D NMR, including ^{1}H-^{1}H COSY, HSQC, HMBC spectroscopic analyses.

Among these compounds four triterpene were isolated from this plant for the first time. The triterpene type compounds of *A. absinthium* L. has not been reported yet. This treatise reports the isolation and structural characterization of these compounds.

keywords : *Artemisia absinthium* L.; *Asteraceae* ; Chemical constituents.

Ⅰ. INTRODUCTION

Artemisia spp. is a large, diverse genus of plants with between 200 and 400 species belonging to the daisy family *Asteraceae*. Common names for various species in the genus include mugwort, wormwood, and sagebrush. *Artemisia spp* comprises hardy herbaceous plants and shrubs, which are known for the powerful chemical constituents in their essential oils. *Artemisia* species grow in temperate climates of both hemispheres, usually in dry or semiarid habitats. Notable species include *A. vulgaris* (common mugwort), *A. tridentata* (big sagebrush), *A. annua* (sagewort), *A. absinthium* (wormwood), *A. dracunculus* (tarragon), and *A. abrotanum* (southernwood). The leaves of many species are covered with white hairs.[2]

Artemisia absinthium L., commonly known as wormwood. Wormwood is native to Europe, although it can now be found in many other parts of the world, especially North America. It is a perennial plant that flowers year after year growing between 30 to 90cm(12 to 36in) tall and has small, yellowish flower heads. It is a yellow-flowering, perennial plant distributed throughout various parts of Europe and siberia and is used for antiparasitic effects and to treat anorexia and indigestion. Traditionally it is used to treat epilepsy, gastric

problems, enlargement of the spleen, urinary disorders, and for wound healing. The flowers are prescribed to cure stomach diseases and as a vermifuge. The plant is reported to have antimicrobial, antioxidant, hepatoprotective, anthelminitic, and neuroprotective activities.[3].

Previous studies on *A. absinthium* L. resulted in the isolation of diverse components. This experiment was conducted to explore the chemical components in the plant *Artemisia absinthium* L. Therefore we now report six compounds were isolated from CH_2Cl_2 fraction of *A. absinthium* L. The chemical structures of compounds(1-6) were identified based on 1D and 2D NMR, including ^1H-^1H COSY, HSQC, HMBC spectroscopic analyses.

Ⅱ. MATERIALS AND METHODS

1. Plant material

The aerial parts of *Artemisia absinthium* L. were collected from the Herbarium at the College of Pharmacy, Chosun University, Korea, in 2012. A voucher specimen was deposited in the Herbarium at the College of Pharmacy, Chosun University (CSU-1040-17).

2. General procedure

2.1. TLC and Column chromatography

TLC and column chromatography were performed on precoated Si Gel F_{254} plates (Merck,art.5715), RP-18F_{254} plates (Merck, art.15389) and silica gel 60(40-63 and 63-200 mm, Merck), MCI gel CHP20P (75-150m, Mitsubishi Chemical Co.), as well as LiChroprep RP-18 (40-63 ㎛, Merck) and MPLC (Grace, USA, Reveleris flash Chromatography system , Part No. 5148513).

2.2. Equipment

IR : JASCO FT/IR-300E (JASCO Co., Japan)

UV : JASCO V-550 (JASCO Co., Japan)

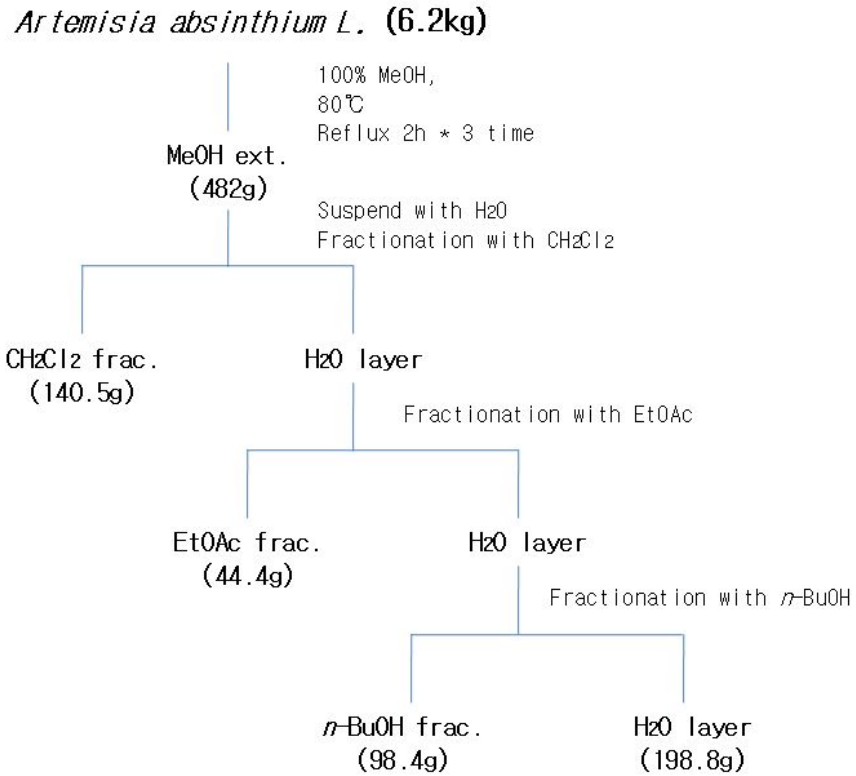
^{1}H-NMR : Varian Unity Inova 600MHz, 500MHz (KBSI-Gwangju center)

^{13}C-NMR : Varian Unity Inova 150MHz, 125MHz (KBSI-Gwangju center)

3. Extraction and isolation

3.1. Extraction

The air-dried aerial parts of *Artemisia absinthium* L. (6.2kg) were extracted three times with MeOH under reflux and 482g of residue were produced. The MeOH extract was suspended in water, which was then partitioned sequentially with equal volumes of dichloromethane(CH_2Cl_2), ethyl acetate(EtOAc), and n-butanol(BuOH). Each fraction was evaporated *in vaccuo* to yield the residues of CH_2Cl_2(140.5g), EtOAc(44.4g), n-BuOH(98.4g), and water(198.8g) extract. (Scheme.1.)

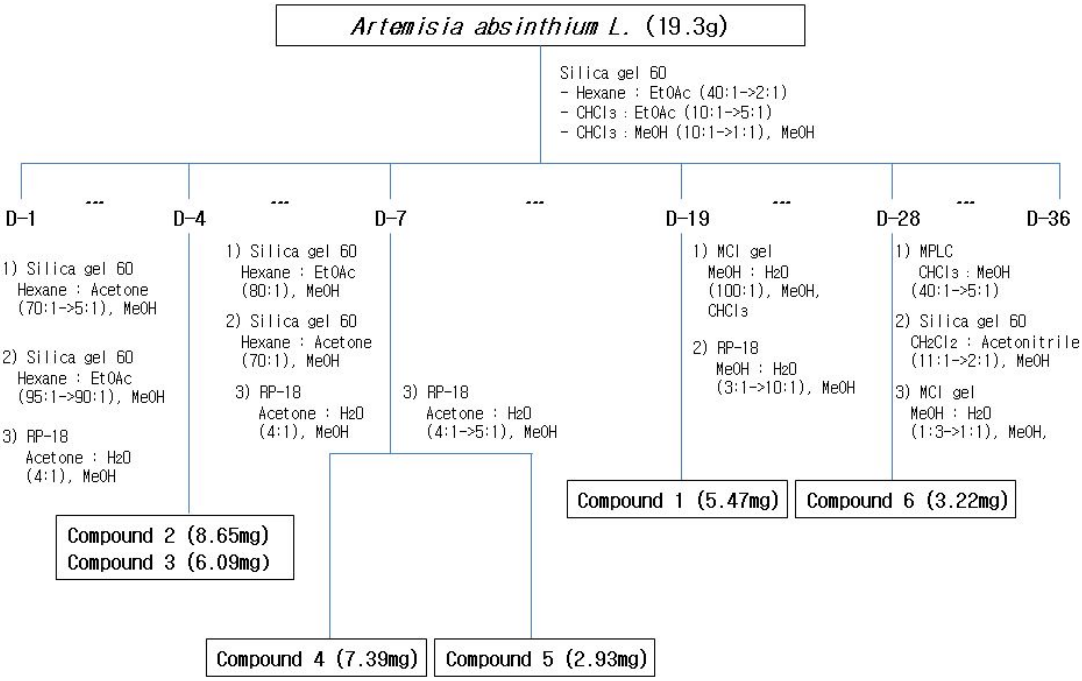


Scheme.1. Extraction and fractionation of MeOH extract from *Artemisia absinthium* L.

3.2. Isolation

Isolation procedure from CH_2Cl_2 soluble fraction of Artemisia absinthium.

The CH_2Cl_2 fraction (19.3g) was chromatographed over a silica gel column chromatography (CC), using a gradient solvent system of Hexane : Ethyl acetate(40:1->2:1), and Chloroform : Ethyl acetate (10:1->5:1), Chloroform : Methanol (10:1->1:1), the last MeOH, to give thirty-six subfractions. (scheme.2.)



Scheme.2. Isolation of compound 1-6 from CH_2Cl_2 frac. of *Artemisia absinthium* L.

3.2.1. Compound 1

The subfraction D19(1.3g) was subjected to a MCl-gel column chromatography (CC) eluting with a isocratic solvent system of MeOH : H_2O (100:1) to yield six subfractions(D19-1~D19-6). The subfraction D19-2(98.4mg) was subjected to RP-18 open Column chromatography eluting with a gradient solvent system of MeOH : H_2O (3:1->10:1) to produce compound 1 (5.47mg).

3.2.2 Compound 2

The subfraction D4(271.0mg) was subjected to a silica gel column chromatography (CC) eluting with a gradient solvent system of Hexane : Acetone (70:1->5:1) to yield fourteen subfractions(D4-1~D4-14). The subfraction D4-2(106.76mg) was subjected once more to silica gel column chromatography (CC) eluting with a gradient solvent system of Hexane : Ethyl acetate(95:1->90:1) to yield eight subfractions(D4-2-1~D4-2-8). The subfraction D4-2-4(64.8mg) was subjected to RP-18 open column chromatography (CC) eluting with a isocratic solvent system of Acetone : H_2O (4:1) to produce compound 2(8.65mg)

3.2.3 Compound 3

The subfraction D4(271.0mg) was subjected to a silica gel column chromatography (CC) eluting with a gradient solvent system of Hexane : Acetone(70:1->5:1) to yield fourteen subfractions(D4-1~D4-14). The subfraction D4-2(106.76mg) was subjected once more to silica gel column chromatography (CC) eluting with a gradient solvent system of Hexane : Ethyl acetate(95:1->90:1) to yield eight subfractions(D4-2-1~D4-2-8). The subfraction D4-2-4(64.8mg) was subjected to RP-18 open column chromatography (CC) eluting with a isocratic solvent system of Acetone : H_2O (4:1) to produce compound 3(6.09mg)

3.2.4 Compound 4

The subfraction D7(410.9mg) was subjected to a silica gel column chromatography (CC) eluting with a isocratic solvent system of Hexane : Ethyl

acetate(80:1) to yield subfractions(D7-1~D7-26). The subfraction D7-21(119.3mg) was subjected once more to silica gel column chromatography (CC) eluting with a isocratic solvent system of Hexane : Acetone (70:1)to yield fourteen subfractions(D7-21-1~D7-21-14). The subfraction D7-21-6(21.78mg) was subjected to RP-18 open column chromatography (CC) eluting with a isocratic solvent system of Acetone : H_2O (4:1) to produce compound 4(7.39mg)

3.2.5 Compound 5

The subfraction D7(410.9mg) was subjected to a silica gel column chromatography (CC) eluting with a isocratic solvent system of Hexane : Ethyl acetate(80:1) to yield subfractions(D7-1~D7-26). The subfraction D7-21(119.3mg) was subjected once more to silica gel column chromatography (CC) eluting with a isocratic solvent system of Hexane : Acetone (70:1) to yield fourteen subfractions(D7-21-1~D7-21-14). The subfraction D7-21-7(45.28mg) was subjected to RP-18 open column chromatography (CC) eluting with a gradient solvent system of Acetone : H_2O (4:1->5:1) to produce compound 5(2.93mg)

3.2.6 Compound 6

The subfraction D28(1.7g) was subjected to a MPLC (CC) eluting with a gradient solvent system of $CHCl_3$: MeOH(40:1->5:1) to yield fifteen subfractions(D28-1~D28-15). The subfraction D28-4(106.9mg) was subjected once more to silica gel column chromatography (CC) eluting with a gradient solvent system of CH_2Cl_2 : Acetonitrile (11:1->2:1) to yield twenty-three subfractions(D28-4-1~D28-4-23). The subfraction D28-4-7(20.2mg) was subjected to MCI-gel column chromatography (CC) eluting with a gradient solvent system of MeOH : H_2O (1:3->1:1) to produce compound 6(3.22mg)

III. RESULTS AND DISCUSSIONS

3.1. Structures

The structures of compounds (1-6) were identified based on 1D and 2D NMR, including ^{1}H-^{1}H COSY, HSQC, HMBC spectroscopic analyses.

3.1.1 Compound 1 (Eupatilin)

Yellow amorphous powder
Molecular formula : $C_{18}H_{16}O_7$
ESI-MS : *m/z* 344 $[M]^+$
MP : 228-230 ℃

^{1}H-NMR(500MHz,$CDCl_3$) δ:
7.52 (1H, d, *J* =8.5Hz, H-6′), 7.33 (1H, s, H-2′), 6.98 (1H, d, *J* =8.5Hz, H-5′), 6.61 (1H, s, H-3), 6.58(1H, s, H-8), 4.05 (3H, s, OCH_3), 3.96(6H, d, *J* = 6.5Hz, OCH_3)

^{13}C-NMR(125MHZ, $CDCl_3$) δ:
164.0(C-1), 104.0(C-3), 182.8(C-4), 153.1(C-5), 130.3(C-6), 155.0(C-7), 93.3(C-8), 152.2(C-9), 105.6(C-10), 123.7(C-1′), 108.7(C-2′), 149.2(C-3′), 152.0(C-4′), 111.1(C-5′), 120.0(C-6′), 60.8(OCH_3), 56.0(OCH_3)

Compound 1 was obtained as Yellow amorphous powder, and the molecular formula was determined as $C_{18}H_{16}O_7$ by ESI-MS at *m/z* 344 $[M]^+$. The ^{13}C-NMR and HSQC spectrum of compound 1 exhibited 18 carbon, which revealed carbon signals for two methyls at δc 60.8 and δc 56.0. But the ^{1}H-NMR data was revealed singnal

at δ_H 3.96(6H, d, J = 6.5Hz, OCH_3) in one of the two methyl singnals peak. And compound 1 known three methoxyl groups and two methines of flavnone ring at δ_H 6.61 (C-3), 6.58 (C-8) and three methines at δ_H 7.52 (C-6′), δ_H 7.33 (H-2′), δ_H 6.98 (C-5′). Based no the above results, the structure of compound 1 was identified by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literatures.[**4**] Accordingly compound 1 was identified as eupatilin.

3.1.2. Compound 2 (Dammaradienyl acetate)

Needles

Molecular formula : $C_{32}H_{52}O_2$

EI-MS : m/z 468$[M]^+$

MP : 148-149 ℃

^{1}H-NMR(500MHz,$CDCl_3$) δ :

5.13 (1H, tt, J =1.5, 6.5Hz, H-24), 4.74(1H, br s, H-21a), 4.70(1H, d, J =1.5Hz, H-21b), 4.50(1H, dd, J =5.5, 10.5Hz, H-3), 2.04(3H, s, -COC$\underline{H}_3$), 1.69(3H, s, H-26), 1.62(3H, s, H-27), 0.97(3H, s, H-18), 0.87(3H, s, H-19), 0.86(6H, d, H-28,30), 0.85(3H, s, H-29).

^{13}C-NMR(125MHZ, $CDCl_3$) δ :

34.1(C-1), 27.0(C-2), 80.9(C-3), 37.8(C-4), 55.9(C-5), 18.1(C-6), 35.3(C-7), 40.4(C-8), 50.8(C-9), 37.1(C-10), 21.37(C-11), (C-12), 45.2(C-13), 49.4(C-14), 31.3(C-15), 24.9(C-16), 47.7(C-17), 15.8(C-18), 16.2(C-19), 152.7(C-20), 107.4(C-21), 38.7(C-22), 28.8(C-23), 124.4(C-24), 131.4(C-25), 25.7(C-26), 17.7(C-27), 27.9(C-28), 15.6(C-29), 16.4(C-30), 170.9(-$\underline{C}$OCH$_3$), 21.32(-CO$\underline{C}$H$_3$).

Compound 2 was obtained as needles, and the molecular formula was determined as

$C_{32}H_{52}O_2$ by EI-MS at *m/z* 468$[M]^+$.

The ^{1}H-NMR and HSQC spectrum of compound 2 exhibited seven methly groups, and among them is five methyl group of signals at δ_H 0.97(3H, s, H-18), δ_H 0.87(3H, s, H-19), δ_H 0.86(6H, d, H-28,30), δ_H 0.85(3H, s, H-29) and two secondary methyl groups at δ_H 1.69(3H, s, H-26), δ_H 1.62(3H, s, H-27). And compound 2 was expected triterpene type compounds from 32 carbon signals. The ^{1}H-NMR data showed one exo-methylene at δ_H 4.74(1H, br s, H-21a), δ_H 4.70(1H, d, *J* =1.5Hz, H-21b) and one olefinic proton signals at δ_H 5.13 (1H, tt, *J* =1.5, 6.5Hz, H-24). The ^{13}C-NMR data were showed one acetyl group signals at δ_C 170.9, δ_C 21.32. Based on the above results, the structure of compound 2 was identified by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literatures.[5] Accordingly compound 2 was identified as dammaradienyl acetate.

3.1.3. Compound 3 (Glutinol acetate)

Colourless needles

Molecular formula : $C_{32}H_{52}O_2$

EI-MS : *m/z* 468.3 $[M+H]^+$

MP : 189-190 ℃

^{1}H-NMR(500MHz,$CDCl_3$) δ:

5.56(1H, d, *J* =6Hz, H-6), 4.70(1H, t, *J* =3.5Hz, H-3), 2.01(3H, s, -OCO$\underline{CH_3}$), 1.16(3H, s, H-28), 1.10(3H, s, H-27), 1.07(3H, s, H-23), 1.04(3H, s, H-24), 1.01(3H, s, H-26), 0.99(3H, s, H-30), 0.95(3H, s, H-29), 0.85(3H, s, H-25)

^{13}C-NMR(125MHZ, $CDCl_3$) δ:

23.4(C-1), 18.8(C-2), 78.5(C-3), 39.0(C-4), 142.0(C-5), 119.8(C-6), 23.4(C-7), 43.0(C-8), 39.2(C-9), 49.8(C-10), 34.58(C-11), 30.3(C-12), 37.8(C-13), 39.2(C-14), 33.0(C-15), 35.9(C-16), 30.0(C-17), 47.3(C-18), 35.1(C-19),

28.2(C-20), 34.51(C-21), 38.9(C-22), 29.0(C-23), 25.0(C-24), 16.0(C-25), 18.4(C-26), 19.5(C-27), 32.0(C-28), 34.7(C-19), 32.3(C-30), 170.8(-O$\underline{C}$OCH$_3$), 21.2(-OCO$\underline{C}$H$_3$)

Compound 3 was obtained colourless needles, and the molecular formula was determined as $C_{32}H_{52}O_2$ by EI-MS at *m/z* 468.3 $[M]^+$.
The ^{13}C-NMR spectrum of compound 3 showed at 32 carbon signals and was expected triterpene type compound. Among them were confirmed as eight methyl group at δc 29.0(C-23), δc 25.0(C-24), δc 16.0(C-25), δc 18.4(C-26), δc 19.5(C-27), δc 32.0(C-28), δc 34.7(C-19), δc 32.3(C-30) and one olefinic proton signal at δc 119.8(C-6) and one acetyl group signals between δc 170.8 and δc 21.2. The HMBC showed correlation from δ_H 1.07(3H, s, H-23) to C-3 [δc 78.5], C-24 [δc 25.0]. Based on the above results, the structure of compound 3 was identified by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literatures[6-7]. Also, EI-MS analysis was confirmed. Accordingly, compound 3 was identified as glutinol acetate.

3.1.4. Compound 4 (3β-acetoxyoleanan-12-one)

White needles
Molecular formula :$C_{32}H_{52}O_3$
EI-MS : 484*m/z* $[M]^+$

^{1}H-NMR(500MHz,$CDCl_3$) δ :
4.56(1H, dd, *J* = 5,11Hz, H-3), 1.10(3H, s, H-26), 1.09(3H, s, H-30), 0.95(3H, s, H-25), 0.90(3H, s, H-28), 0.89(3H, s, H-23),0.87(3H, s, H-27), 0.86(3H, s, H-24), 0.85(3H, s, H-29).

^{13}C-NMR(125MHZ, $CDCl_3$) δ :

35.4(C-1), 20.8(C-2), 80.6(C-3), 40.7(C-4), 47.8(C-5), 17.9(C-6), 29.7(C-7), 48.7(C-8), 47.1(C-9), 39.4(C-10), 32.8(C-11), 215.5(C-12), 52.1(C-13), 45.2(C-14), 25.3(C-15), 26.4(C-15), 26.4(C-16), 28.0(C-17), 35.7(C-18), 37.5(C-19), 26.7(C-20), 30.1(C-21), 31.5(C-22), 15.1(C-23), 25.7(C-24), 18.0(C-25), 18.2(C-26), 20.0(C-27), 19.2(C-28), 25.9(C-29), 18.3(C-30), 170.9(-COCH3), 21.3(-COCH3)

Compound 4 was obtained as white needles, and the molecular formula was determined as $C_{32}H_{52}O_3$ by EI-MS at 484*m/z* $[M]^+$.
These the same compound 3 was exhibited signal of 32 carbons. The compound 4 was expected to triterpene type compounds. The ^{1}H-NMR and HSQC spectrum of compound 4 showed eight methyl groups at δ_H 0.89(3H, s, H-23), δ_H 0.86(3H, s, H-24), δ_H 0.95(3H, s, H-25), δ_H 1.10(3H, s, H-26), δ_H 0.87(3H, s, H-27), δ_H 0.90(3H, s, H-28), δ_H 0.85(3H, s, H-29), δ_H 1.09(3H, s, H-30). The ^{13}C-NMR spectrum of compound 4 was confirmed one carbonyl group at δ_c 215.5(C-12) and acetyl group signals at δ_c 170.9, δ_c 21.3. The acetyl group was confirmed at C-3 and C-23, C-24 on the basis of HMBC correlation from δ_H 0.86 to δ_c 80.6(C-3), δ_c 40.7(C-4). Based on the above results, the structure of compound 4 was identified by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literatures.[8] Accordingly, compound 4 was identified as 3β-acetoxyoleanan-12-one.

3.1.5. Compound 5 (Taraxasterol)

White needle crystal
Molecular formula : $C_{30}H_{50}O$
EI-MS : *m/z* 426.7 $[M]^+$
MP : 224-226 ℃

^{1}H-NMR(500MHz,$CDCl_3$) δ:
4.75(1H, br s, H-30), 4.66(1H, br s, H-30), 3.28(1H, dd, *J* = 5,11 Hz, H-3), 1.03(3H, d, *J* = 7Hz, H-29), 1.02(3H, s, H-28), 0.97(3H, s, H-23), 0.97(3H, s, H-24), 0.90(3H, s, H-25), 0.89(3H, s, H-26), 0.81(3H, s, H-27)

^{13}C-NMR(125MHZ, $CDCl_3$) δ:
35.5(C-1), 28.1(C-2), 78.8(C-3), 33.8(C-4), 52.2(C-5), 19.9(C-6), 29.8(C-7), 45.3(C-8), 48.7(C-9), 31.2(C-10), 21.1(C-11), 26.05(C-12), 36.1(C-13), 47.1(C-14), 26.5(C-15), 31.9(C-16), 30.3(C-17), 47.9(C-18), 40.5(C-19), 157.1(C-20), 26.0(C-21), 34.9(C-22), 25.4(C-23), 18.0(C-24), 19.3(C-25), 18.3(C-26), 13.9(C-27), 21.8(C-28), 21.9(C-29), 105.9(C-30)

Compound 5 was obtained as white needle crystal and the molecular formula was determined as $C_{30}H_{50}O$ by EI-MS at *m/z* 426.7 $[M]^+$.
The ^{13}C-NMR spectral data showed 30 carbon signals and was expected triterpene type compounds. The same spectrum data showed one hydroxyl group at δ_C 78.8. Also ^{1}H-NMR data confirmed for the presence of one hydroxyl group at δ_H 3.28(1H, dd, *J* = 5,11 Hz) and one methylene protons at δ_H 4.75(1H, br s, H-30a) and 4.66(1H, br s, H-30b) as two singlet. The ^{1}H-NMR and HSQC spectrum of compound 5 was exhibited seven methyl groups at δ_H 1.03(3H, d, *J* = 7Hz, H-29), δ_H 1.02(3H, s, H-28), δ_H 0.97(3H, s, H-23), δ_H 0.97(3H, s, H-24), δ_H 0.90(3H, s, H-25), δ_H 0.89(3H, s, H-26), δ_H 0.81(3H, s, H-27). And one hydroxyl group was located at C-3 on the basis of HMBC correlation from H-24[δ_H 0.97(3H, s, H-24)] to C-3 (δ_C 78.8), C-4(δ_C 33.8), C-23(δ_C 25.4). Based on the above results, the structure of compound 5 was identified by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literatures[9-11]. Accordingly, compound 5 was identified as taraxasterol.

3.1.6. Compound 6 (Quercetin-3,4′-dimethyl ether)

Yellow powder

Molecular formula : $C_{17}H_{14}O_7$

EI-MS : *m/z* 330$[M]^+$

MP : 235-236 ℃

^{1}H-NMR(500MHz,CD_3OD) δ :

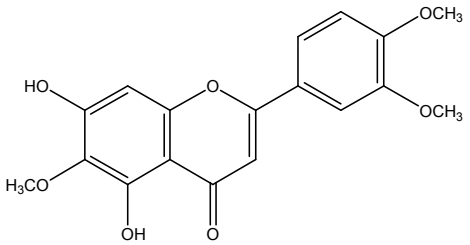
7.46(1H, d, *J* =9Hz, H-6′), 7.44(1H, s, H-2′), 6.92(1H, d, *J* =8.5Hz, H-5′), 6.58(1H, br s, H-6), 6.51(1H, br s, H-8), 3.95(3H, br s, OCH3), 3.87(3H, br s, OCH3)

^{13}C-NMR(125MHZ, CD_3OD) δ :

158.6(C-2), 131.7(C-3), 182.6(C-4), 158.6(C-5), 102.2(C-6), 164.6(C-7), 94.2(C-8), 153.4(C-9), 103.9(C-10), 122.2(C-1′), 109.0(C-2′), 150.7(C-3′), 148.0(C-4′), 115.3(C-5′), 120.2(C-6′), 59.4(OCH3), 55.2(OCH3).

Compound 6 was obtained as yellow powder and the molecular formula was determined as $C_{17}H_{14}O_7$ by EI-MS at *m/z* 330$[M]^+$.

The ^{1}H-NMR spectrum of compound 6 exhibited signals due to the H-6 and H-8 position of the flavone ring at δ6.58(1H, br s, H-6), δ6.51(1H, br s, H-8). The same spectrum data showed two methoxyl groups at δ3.95(3H, br s, OCH3, H-4′), 3.87(3H, br s, OCH3,H-3). The HMBC showed correlation signals from H-2′ [δ7.44(1H, s, H-2′)]to C-2′(δ109.0) and C-3′(δ150.7) , also showed from H-5′[δ6.92(1H, d, *J* =8.5Hz, H-5′)] to C-5′(δ115.3) and C-4′(δ148.0) and C-6′(δ120.2). And compound 6 was known two methoxyl groups and two methine groups at flavone ring and three methine groups of C ring. Based on the above results, the structure of compound 6 was identified by comparing ^{1}H-NMR, ^{13}C-NMR data with those reported in the literatures[12]. Accordingly, compound 6 was identified as quercetin-3,4′-dimethyl ether.



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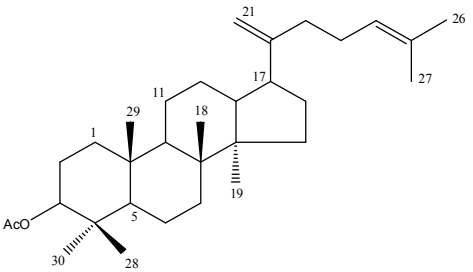

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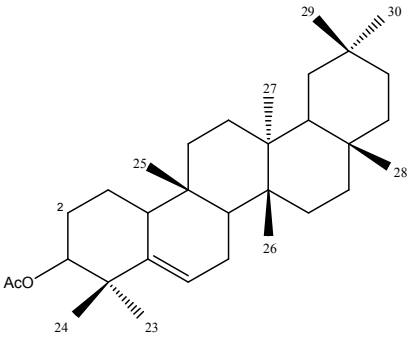

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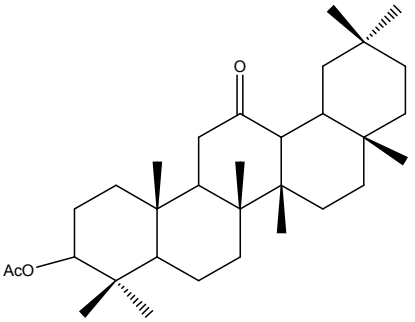

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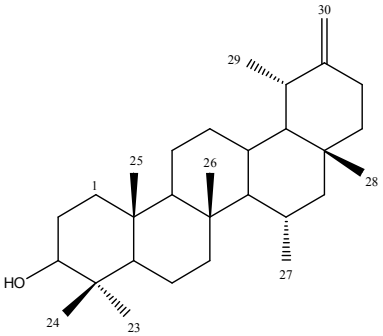

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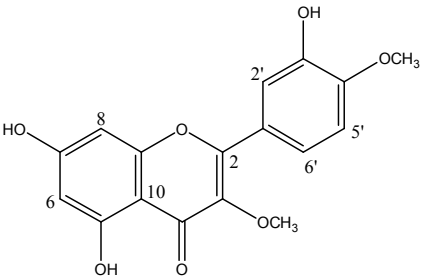

6

Fig.1. Structures of compounds 1-6 isolated from *Artemisia absinthium* L.

Ⅳ. CONCLUSIONS

In conclusion, phytochemical constituents of *Artemisia absinthium* L. was confirmed for six compounds from dichloromethane (CH_2Cl_2) fraction.

Among the six compounds, eupatilin (1) and quercetin-3,4'-dimethyl ether (6) are flavonoids and, the remaining four compounds are, dammaradienyl acetate(2), glutinol acetate(3), 3β-acetoxyoleanan-12-one(4) and Taraxasterol(5) triterpenes. The structures of these compounds (1-6) were identified based on 1D and 2D NMR, including ^{1}H-^{1}H COSY, HSQC, HMBC spectroscopic analyses. These compounds(2-5) did not report before that is first time isolated.

Ⅴ. REFERENCE

1) Colin. W.; *Artemisia, Artemisia Medicinal and Aromatic Plants - Industrial Profiles* And *Chemical Rubber Company (CRC),* p344 (2002)

2) Aberham, A.; Cicek, S.S.; Schneider, P.; Stuppner, H.;Analysis of sesquiterpene Lactones, Lignans, and Flavonoids in Wormwood(*Artemisia absinthium* L.) Using High-Performance Liquid Chromatography(HPLC)-Mass Spectrometry, Reversed Phase HPLC, and HPLC-Solid Phase Extraction-Nuclear Magnetic Resonance. *J.Agric.Food Chem.*, 58, 10817-10823 (2010).

3) Ahamad, J.; Naquvi, K.J.; Ali, M.; Mir, S.R.; Isoflavone glycosides from aerial parts of *Artemisia absinthium*. *Chemistry of Natural Compounds*. 49(6), 996-1000 (*January*,2014)

4) 김아련, [(석사논문) Phytochemical constituents and IL-6 inhibitory Activities of *Artemisia selengensis* TURCZ], 조선대학교 대학원, p12, (2013).

5) Liu, Rui.; Wang, Xiao-Bing.; Kong, Ling-Yi.; Dammaradienyl acetate. *Acta Cryst*. E62, o3544-o3546, (2006)

6) EL-SEEDI, Hesham Rushdey.; Antimicrobial triterpenes from *Poulsenia Armata* MIQ.STANDL, *Natural Product Reserch,* 19(2), 197-202, (February 2005)

7) Fingolo, Catharina E.; Santos, Thabata de S.; Vianna, Filho Marcelo D.M. ; Kaplan, Maria Auxiliadora C.; Triterpene Esters: Natural products from *Dorstenia arifolia* ,*Molecules*, 18, 4247-4256, (2013).

8) Gaydou, Emile M.; Faure, Robert.; Wollenweber, Eckhard.; β-Amyrin Acetate epoxide from *Canarina canariensis*., *Phytochemistry*, 42(4), 1115-1118, (1996)

9) Yekta, Marzieh Matin.; Alavi, Seyed Hamid Reza.; New Triterpenoids from *Peucedanum ruthemicum, Iranian Journal of Pharmaceutical Sciences*,

4(4), 289-294, (2008).

10) Carvalho, M.G.; Velandia, J.R.; Beserra, L.O.F.B.; Triterpenos isolated from Eschweilera longipes MIERS (LECYTHIDACEAE), *QUiMICA NOVA*, 21(6), 740-743 (1998)

11) AKIHISA, L.JOHN GOAD TOSHIHIRO, 『Analysis of sterols』, BLACKIE ACADEMIC & PROFESSIONAL.

12) Kwon, Yong Soo.; Kim, Chang Min.; Antioxidant constituents from the stem of Sorghum bicolor, *Arch Pherm Res*, 26(7), 535-539, (2003).

APPENDIX

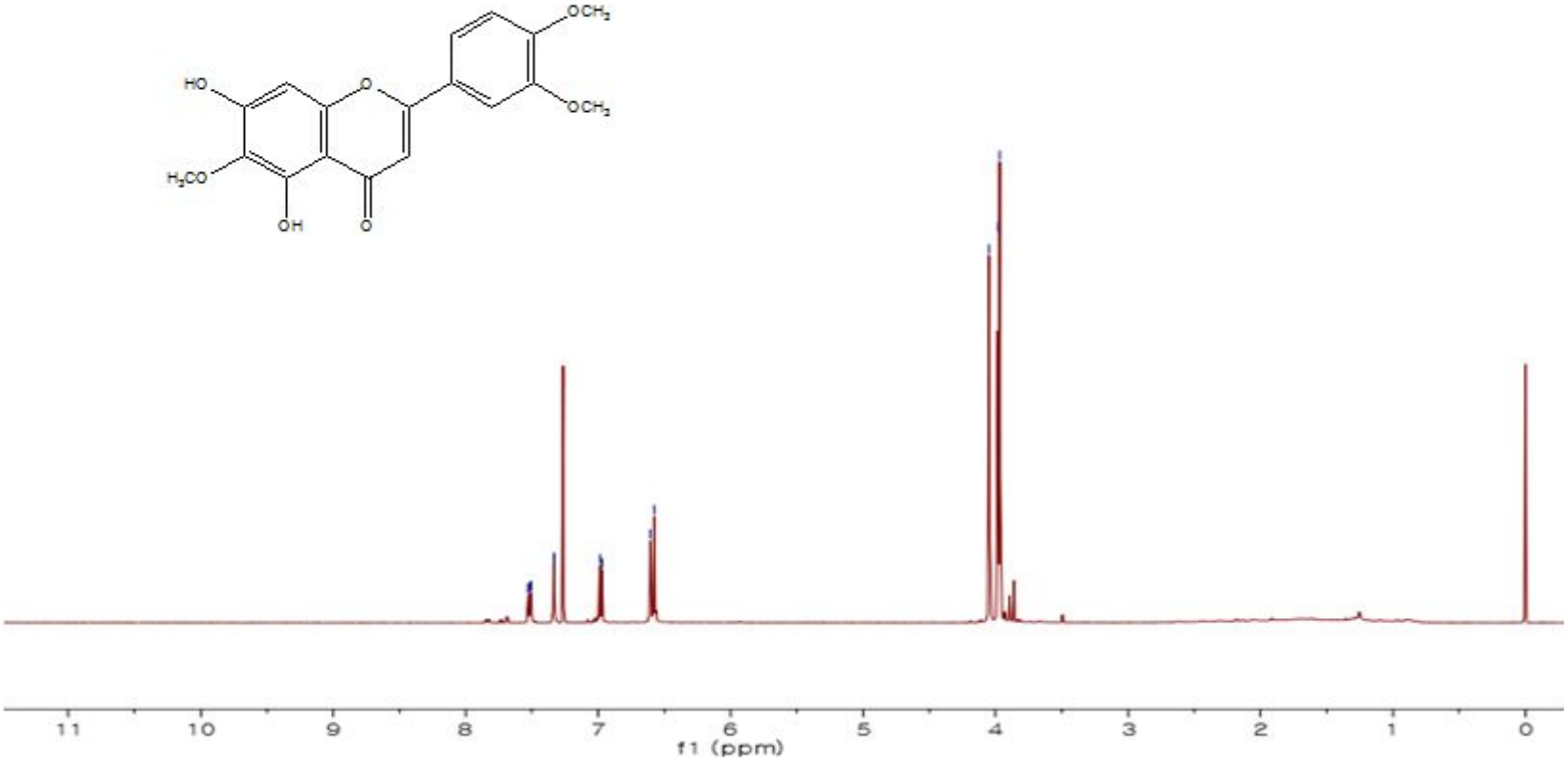

Fig.2. ^{1}H-NMR spectrum of compound 1 (500MHz, $CDCl_3$)

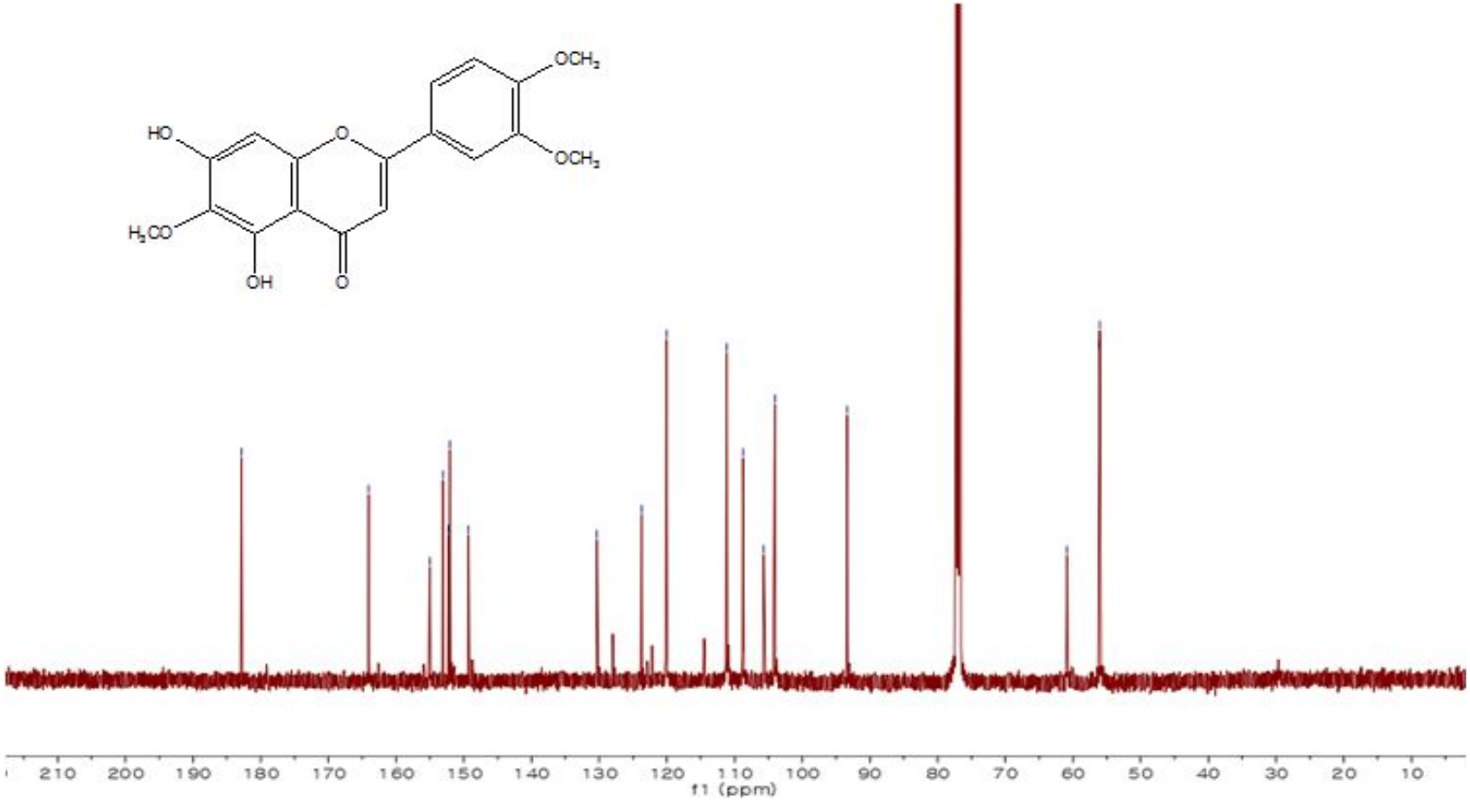

Fig.3. ^{13}C-NMR spectrum of compound 1 (125MHz, $CDCl_3$)

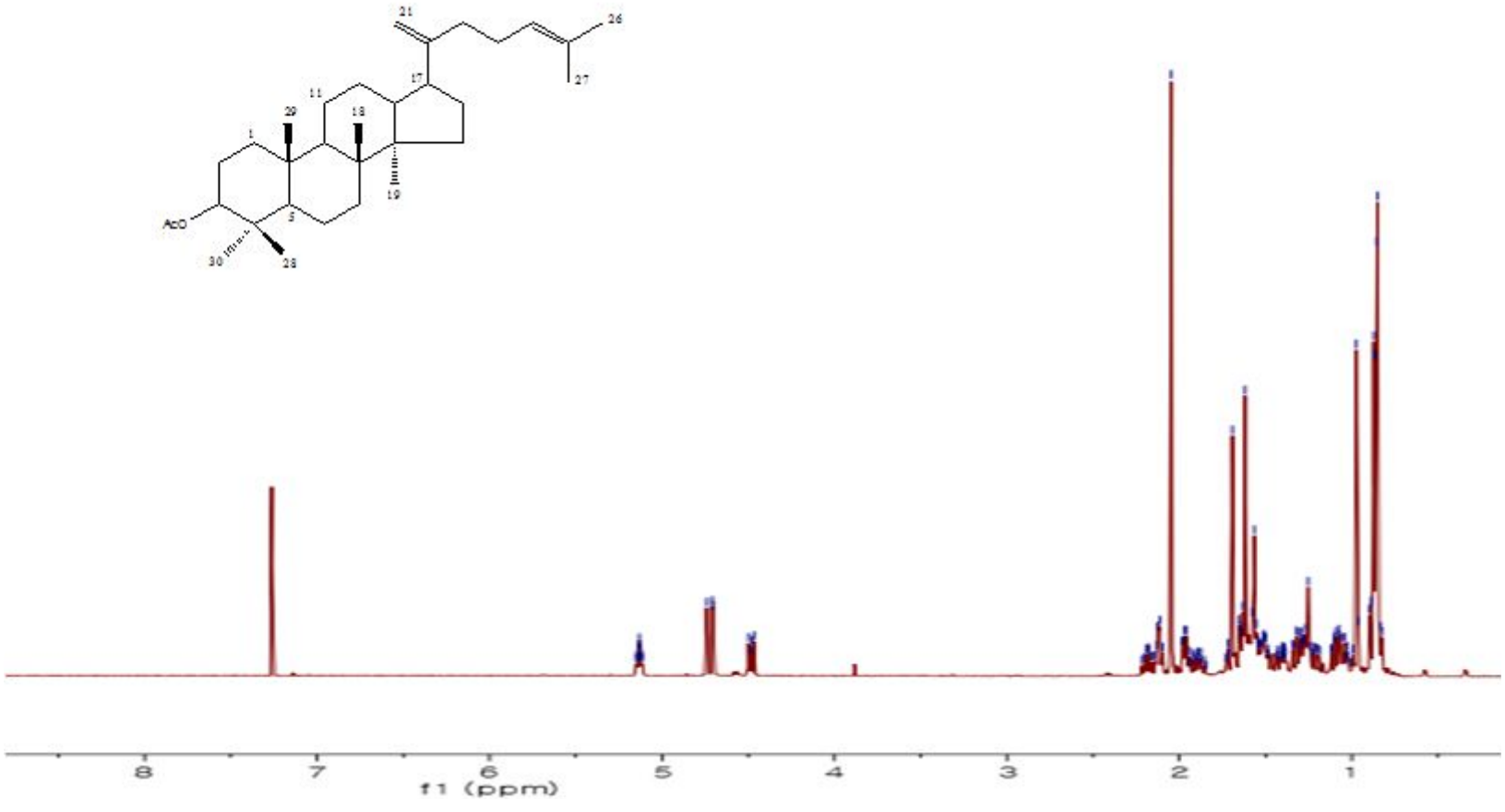

Fig.4. ^{1}H-NMR spectrum of compound 2 (500MHz, $CDCl_3$)

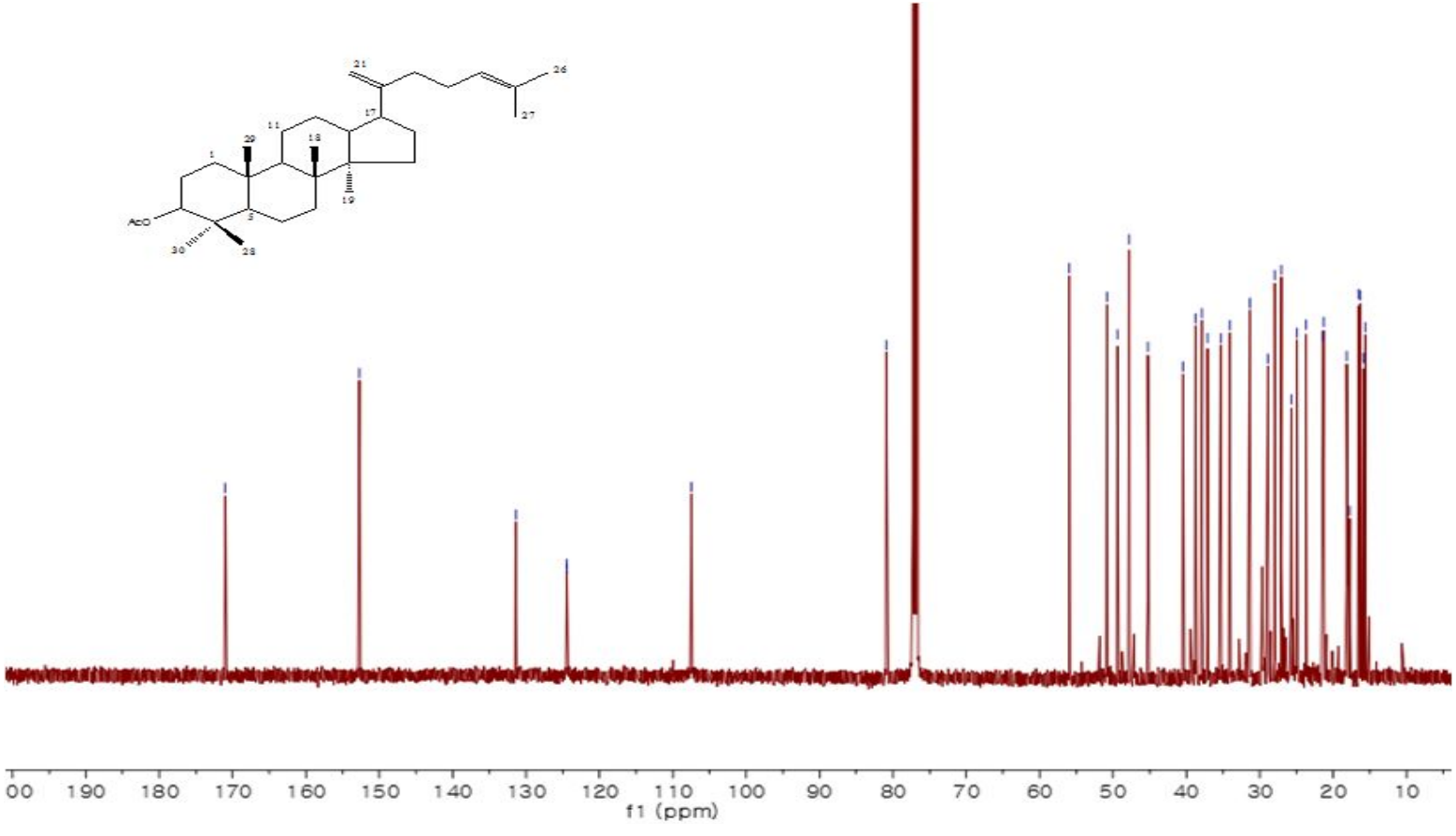

Fig.5. ^{13}C-NMR spectrum of compound 2 (125MHz, $CDCl_3$)

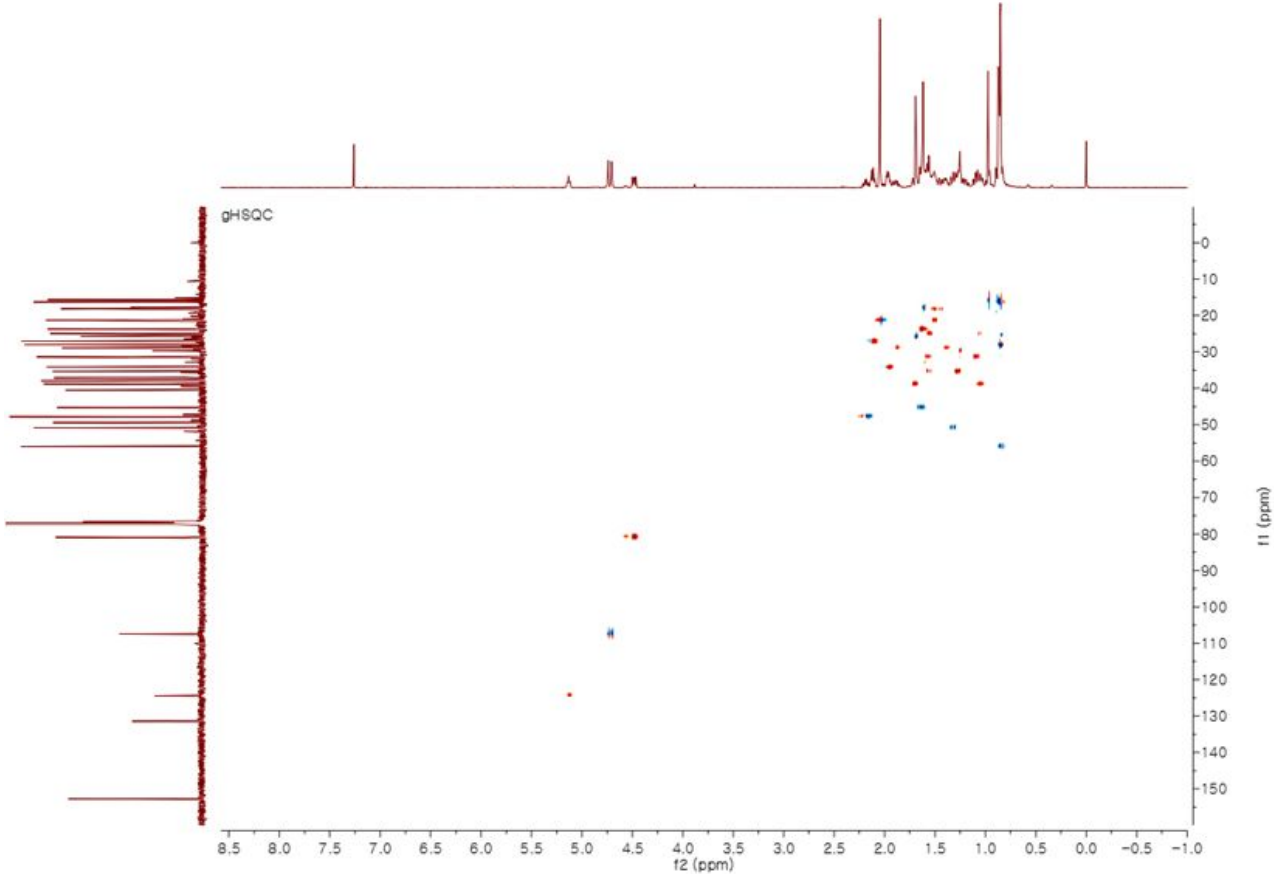

Fig.6. HSQC spectrum of compound 2

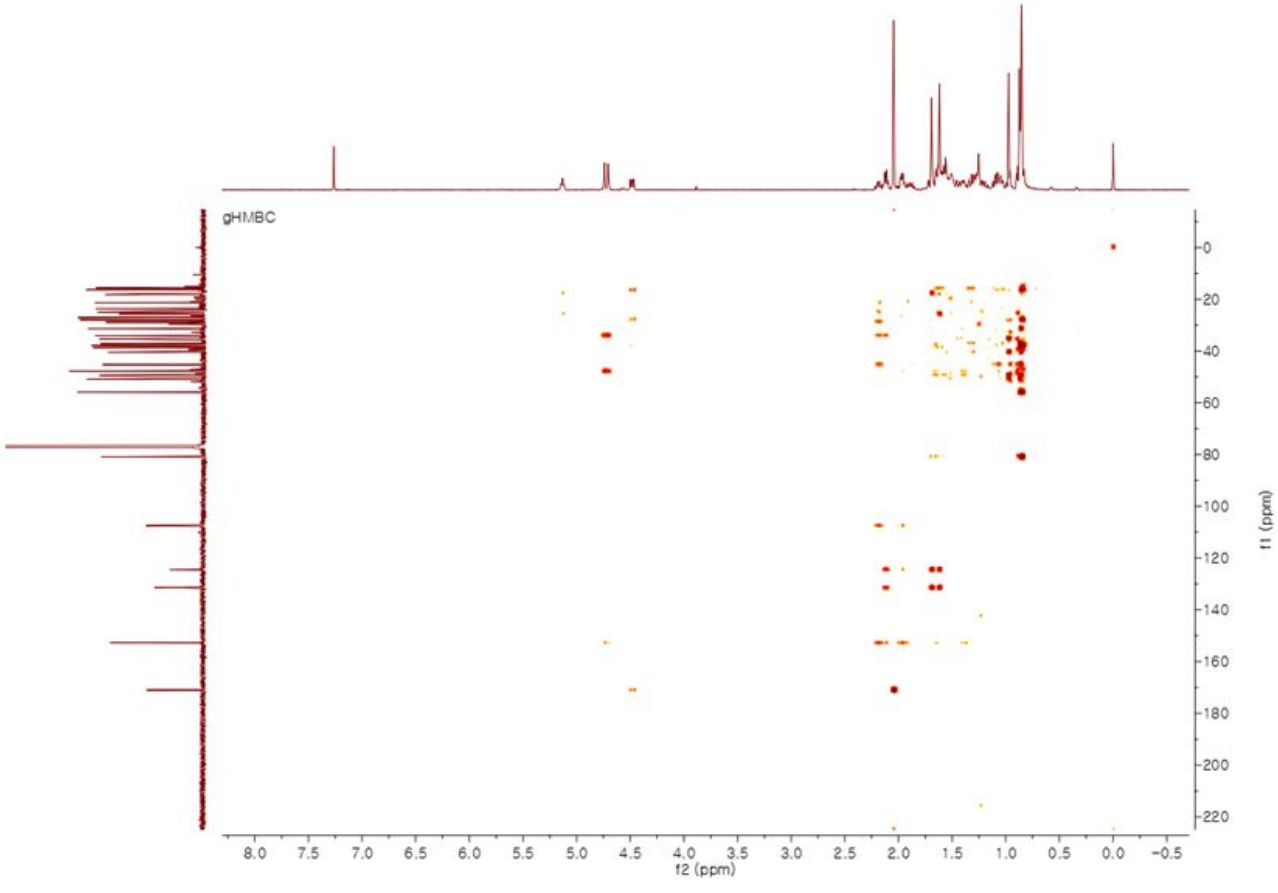

Fig.7. HMBC spectrum of compound 2

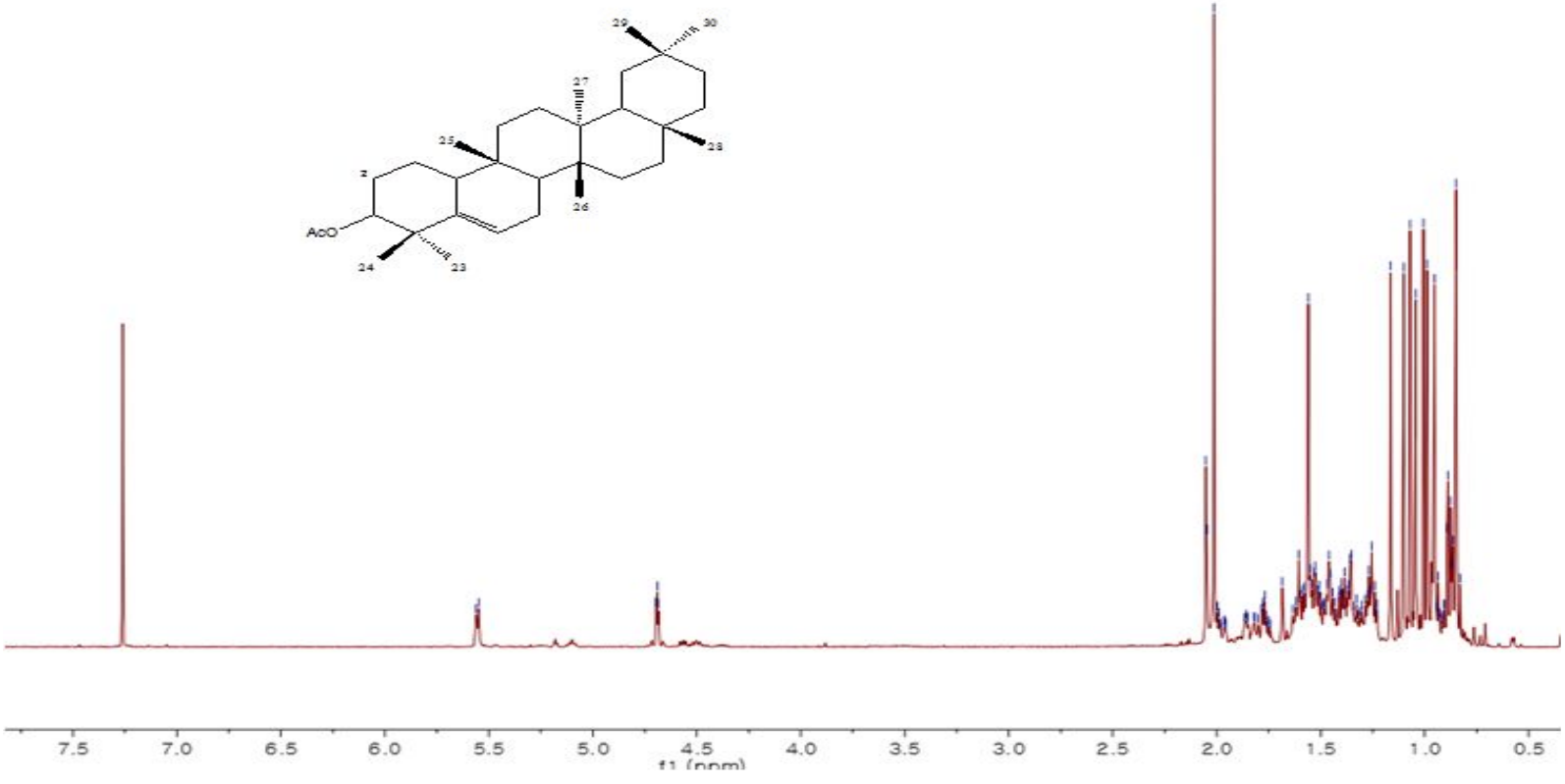

Fig.8. ^{1}H-NMR spectrum of compound 3 (500MHz, $CDCl_3$)

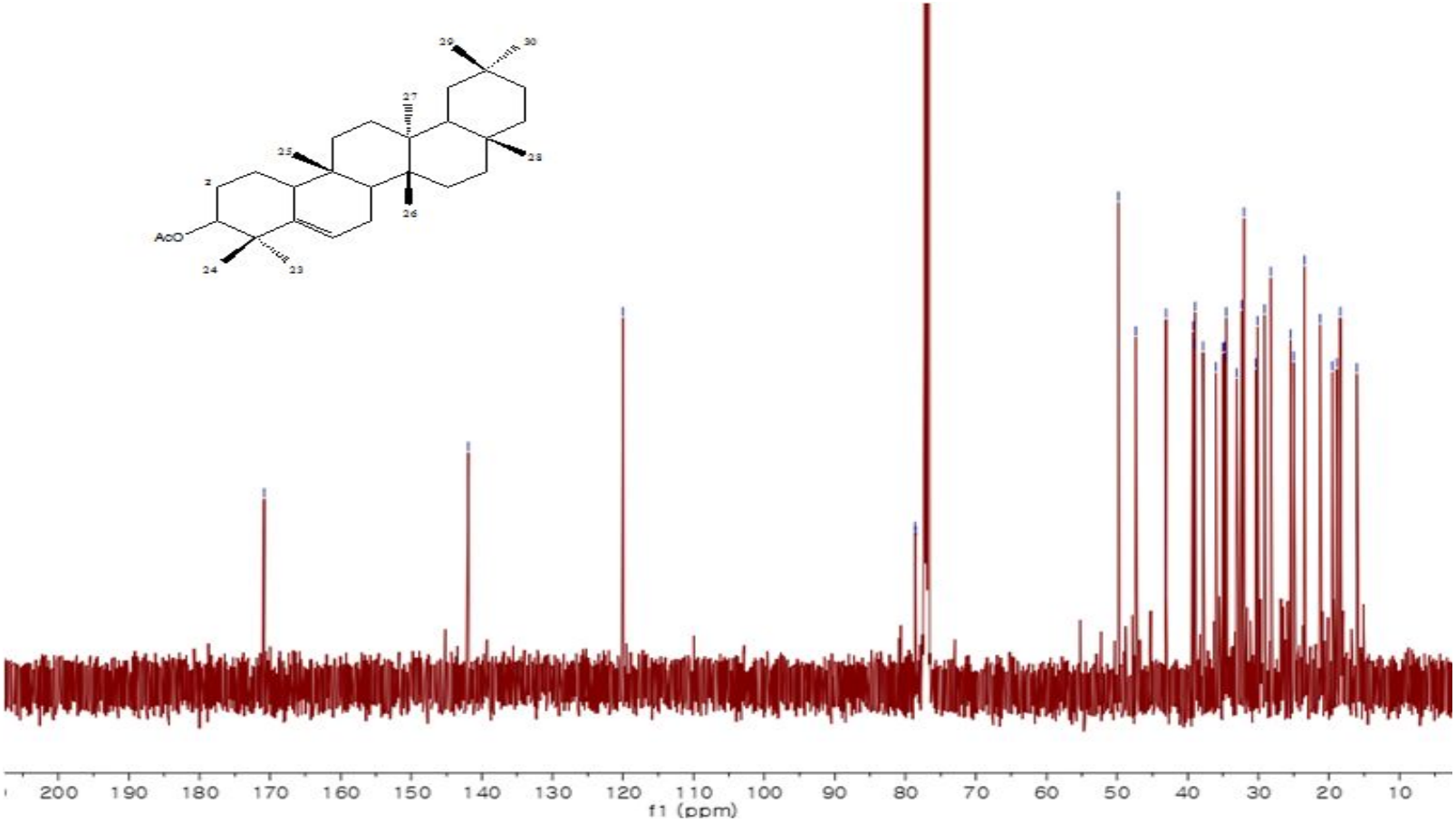

Fig.9. ^{13}C-NMR spectrum of compound 3 (125MHz, $CDCl_3$)

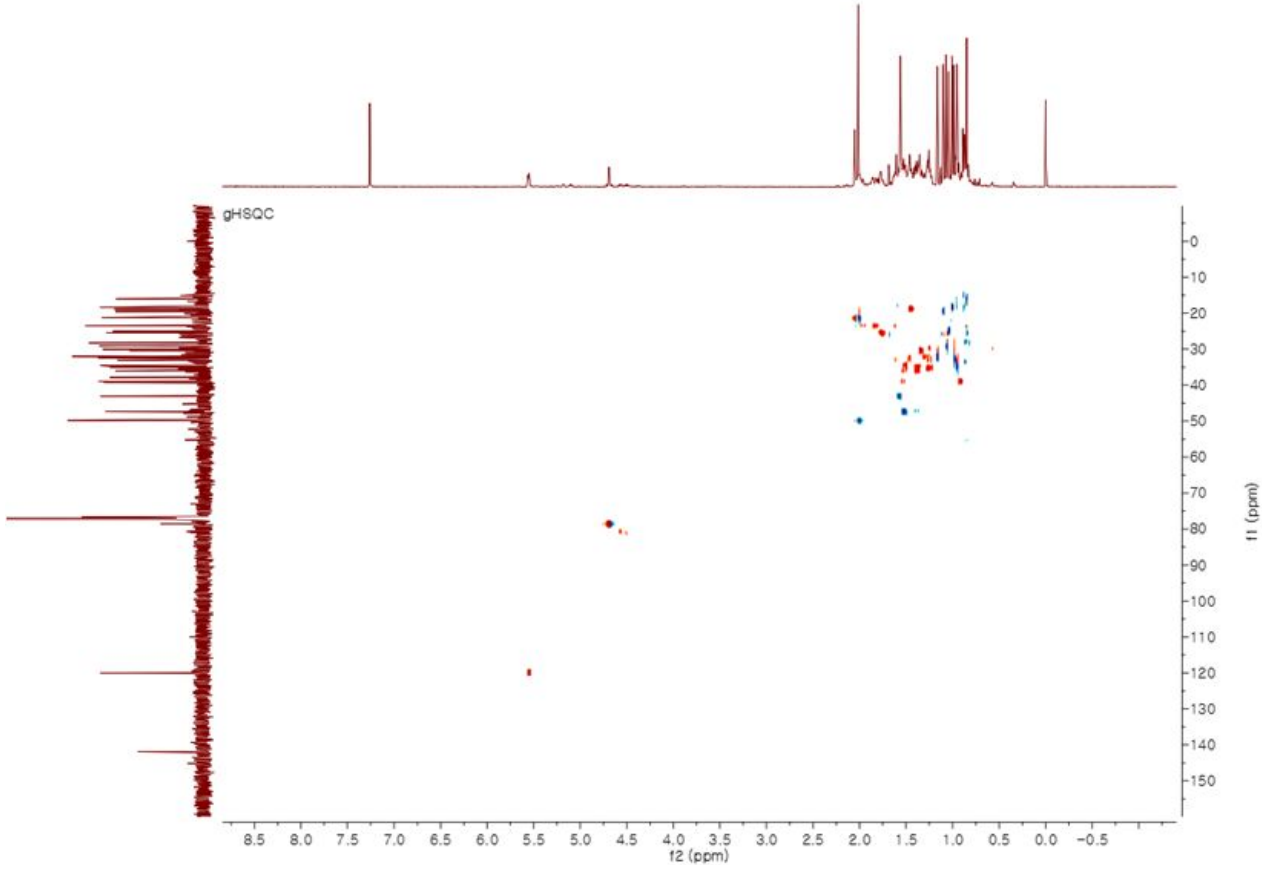

Fig.10. HSQC spectrum of compound 3

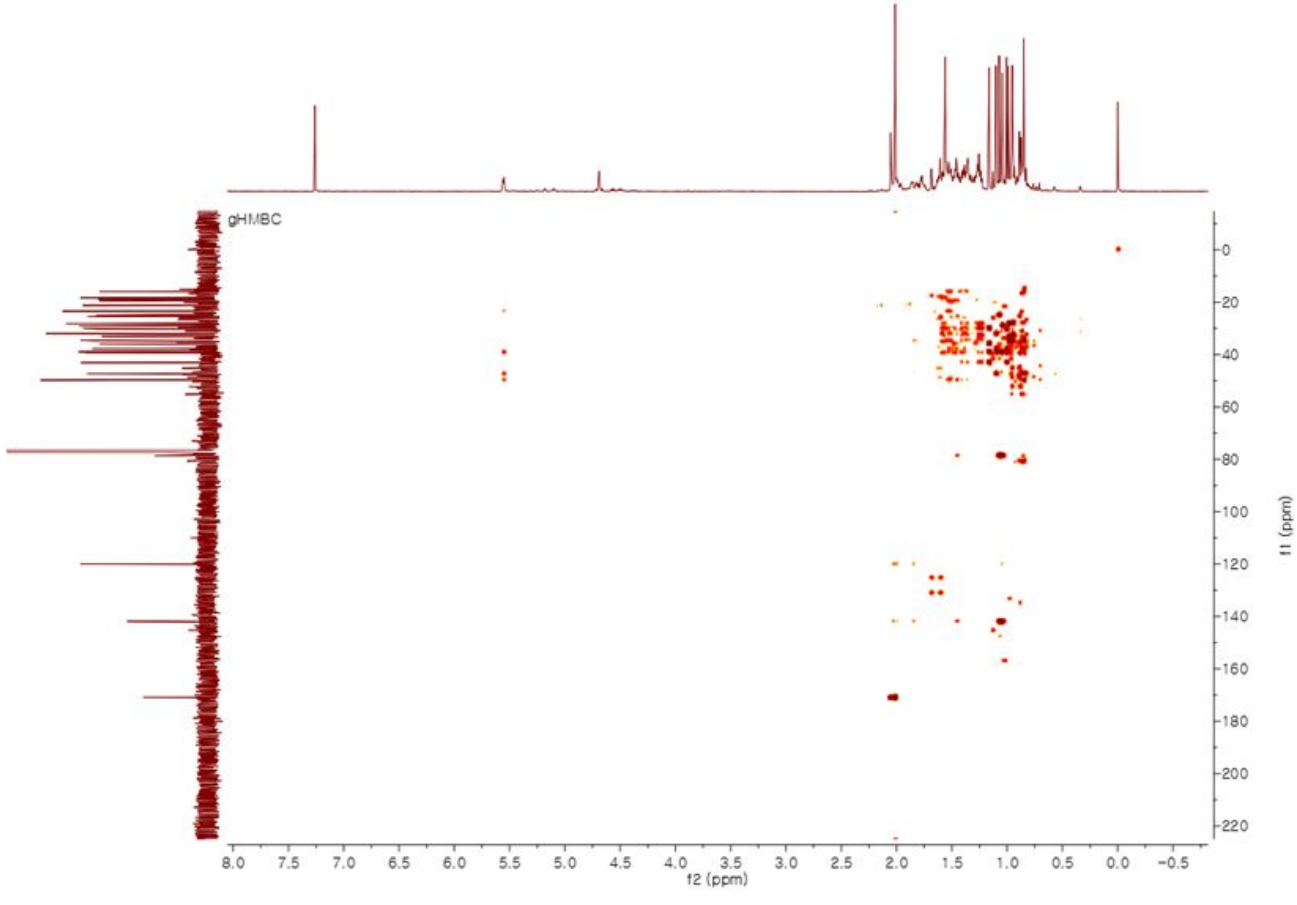

Fig.11. HMBC spectrum of compound 3

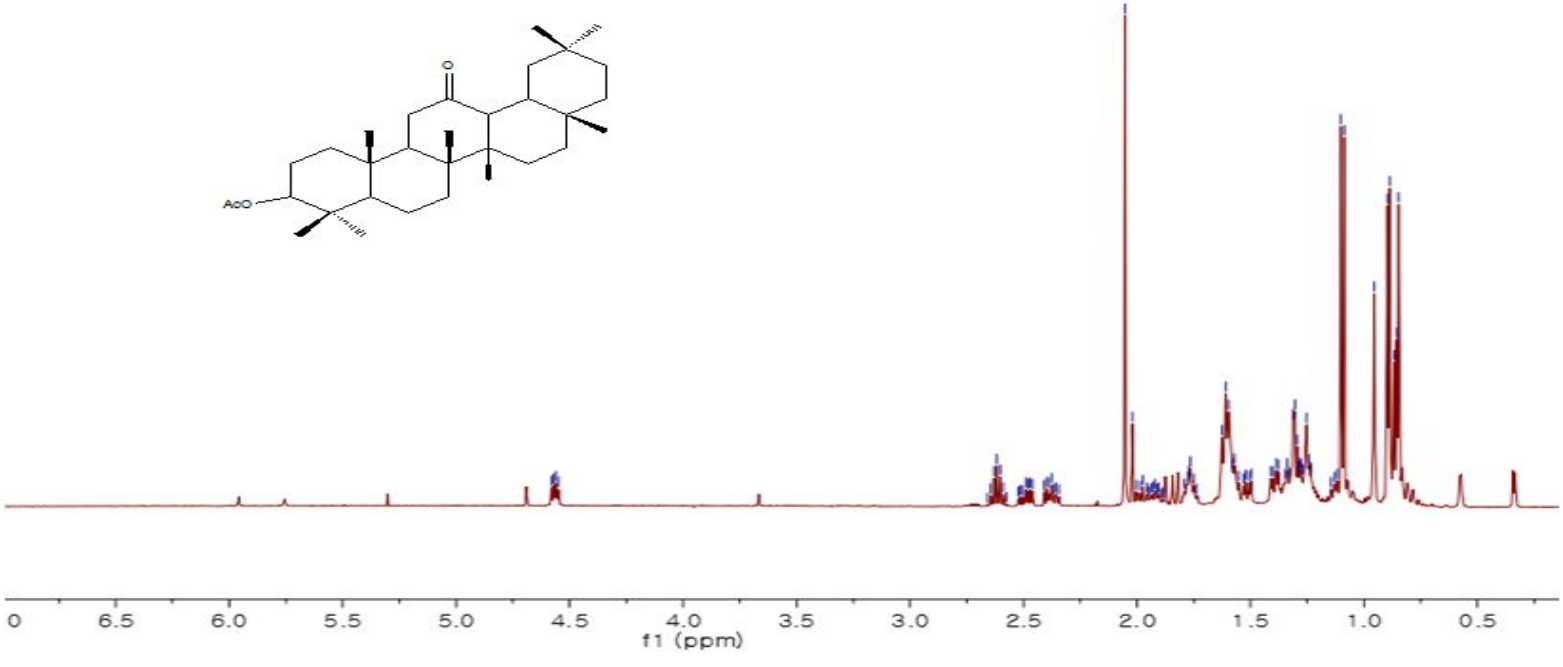

Fig.12. ^{1}H-NMR spectrum of compound 4 (500MHz, $CDCl_3$)

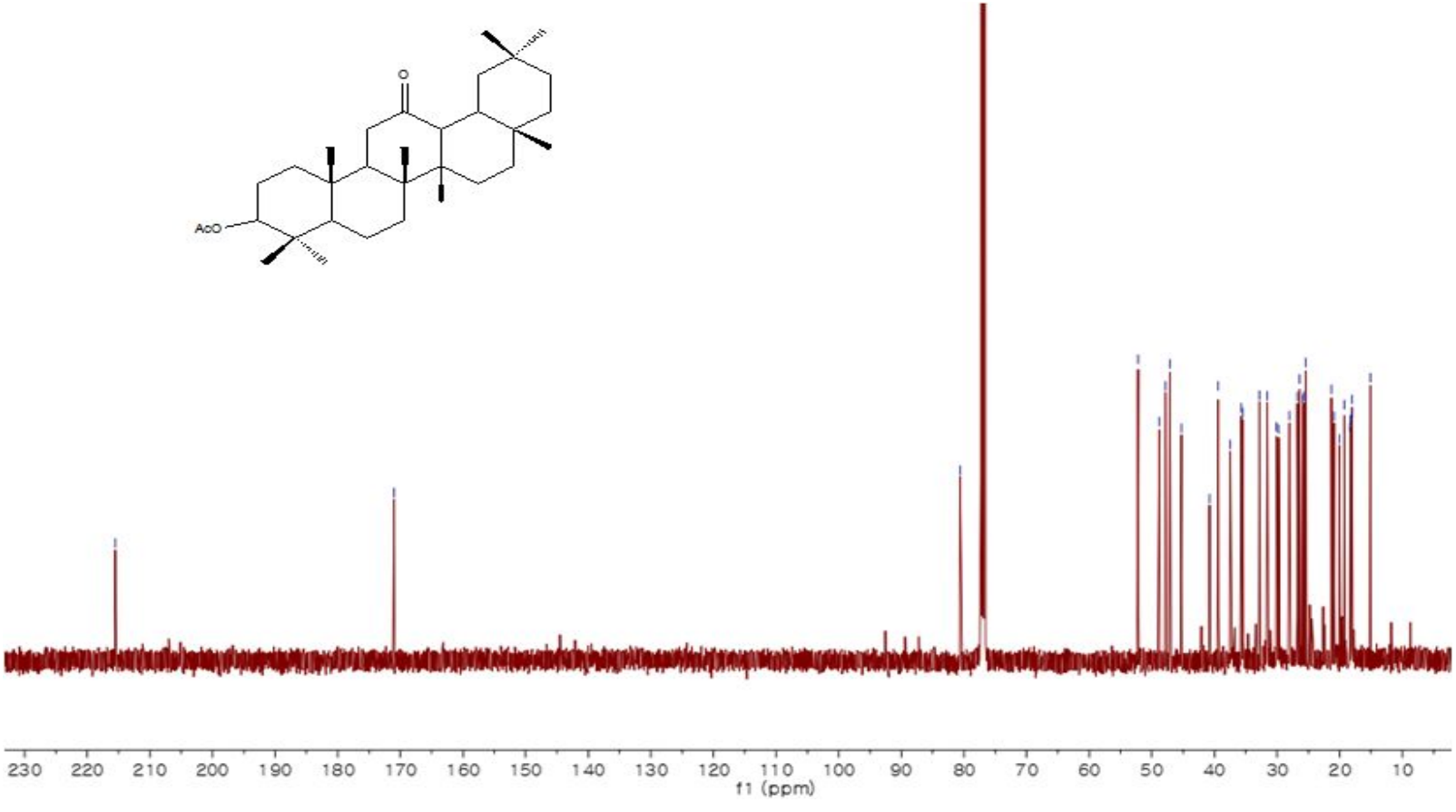

Fig.13. ^{13}C-NMR spectrum of compound 4 (125MHz, $CDCl_3$)

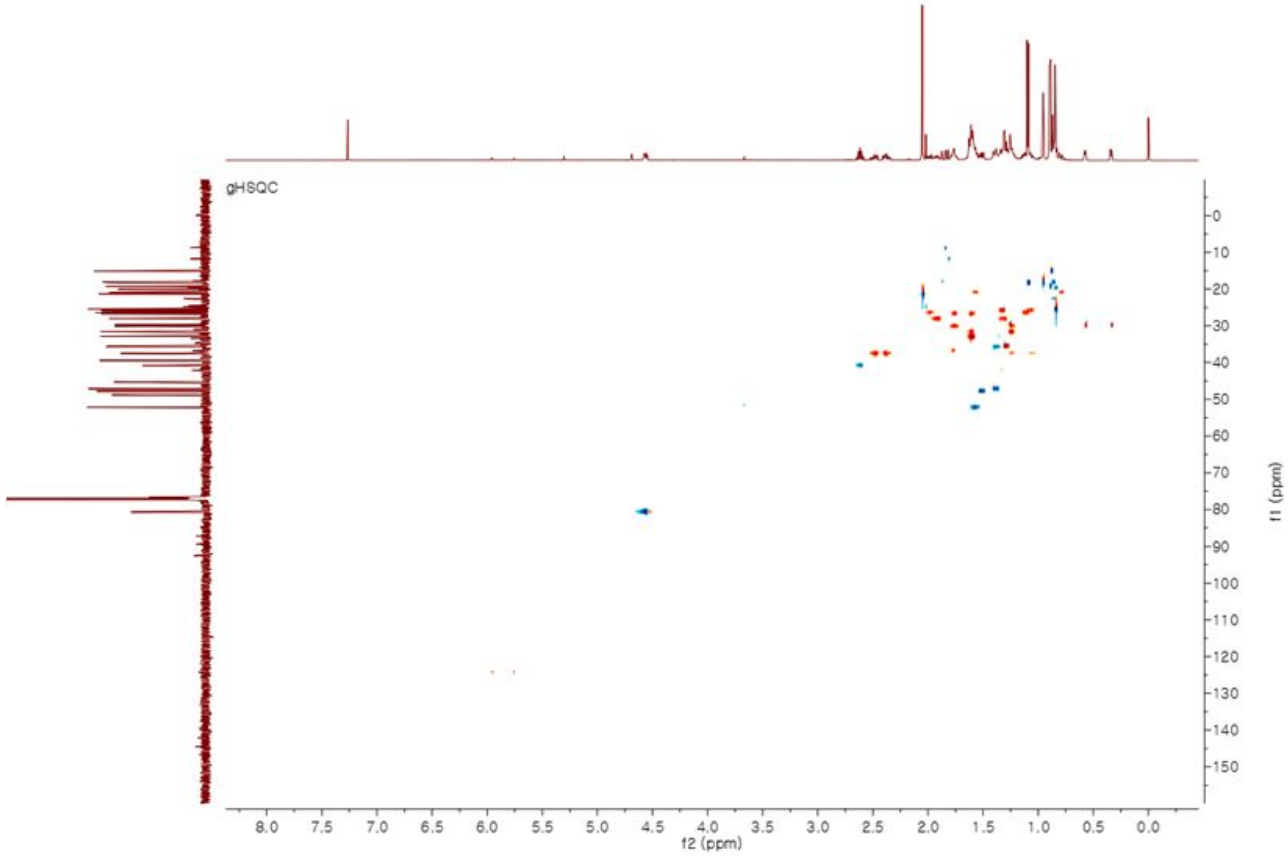

Fig.14. HSQC spectrum of compound 4

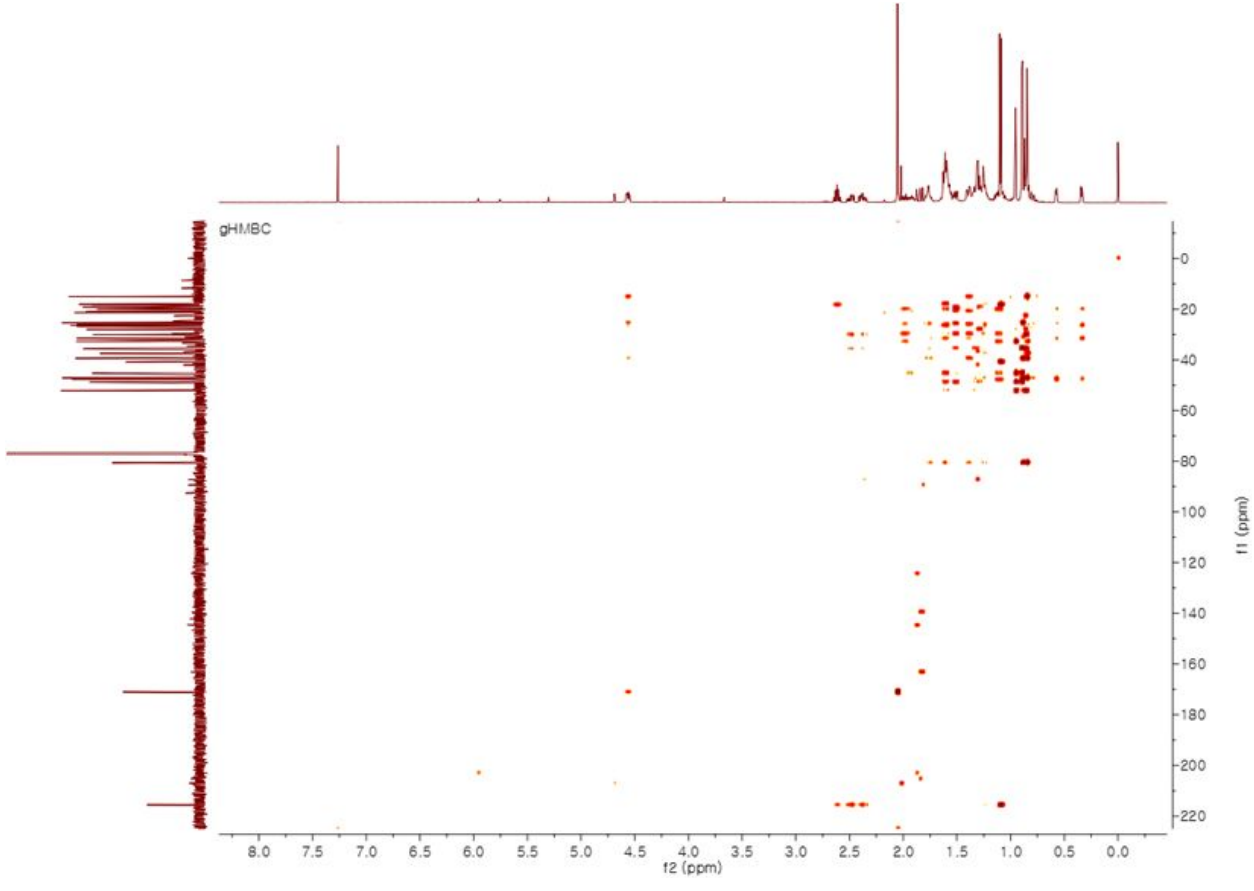

Fig.15. HMBC spectrum of compound 4

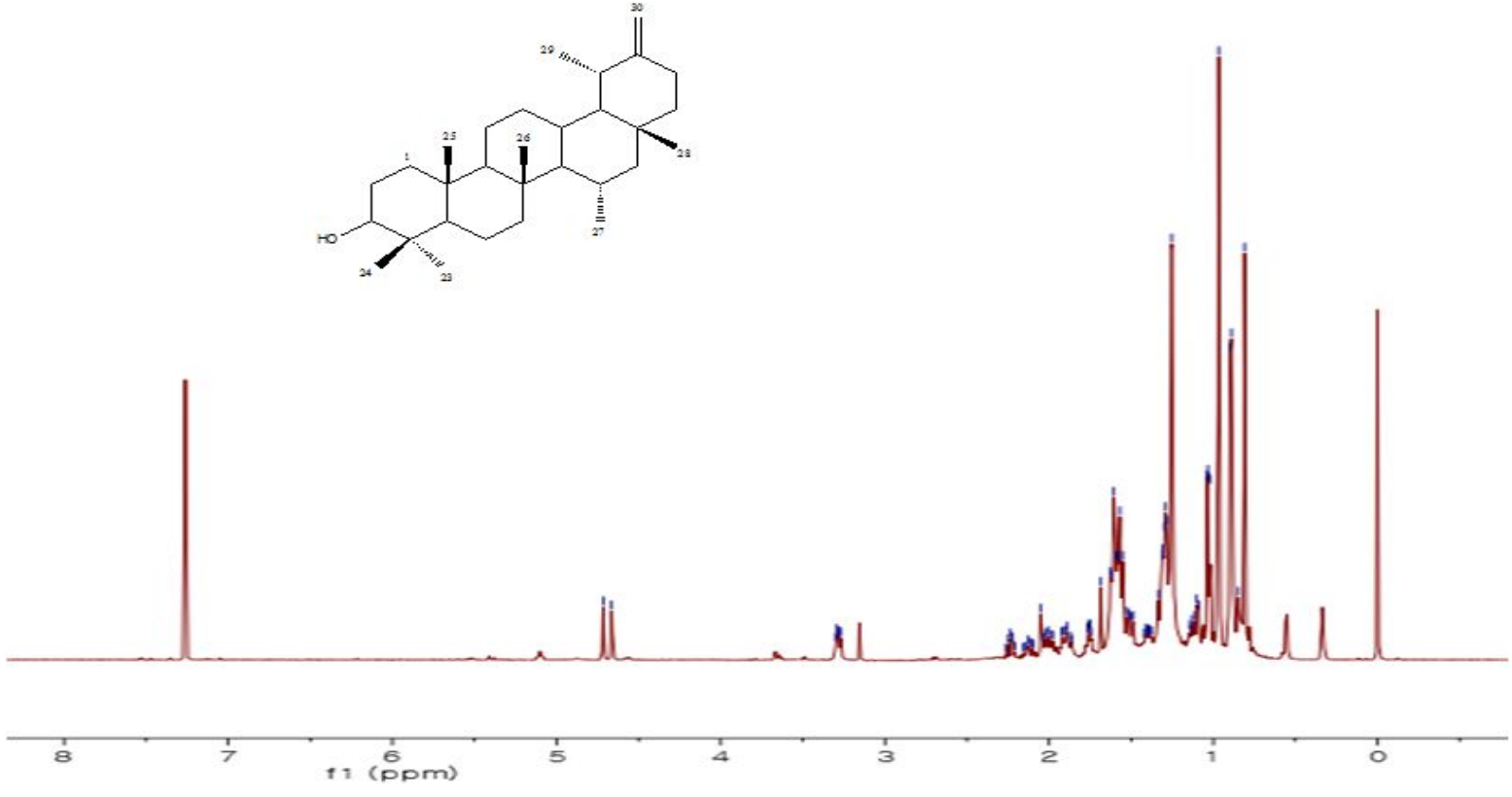

Fig.16. ^{1}H-NMR spectrum of compound 5 (500MHz, $CDCl_3$)

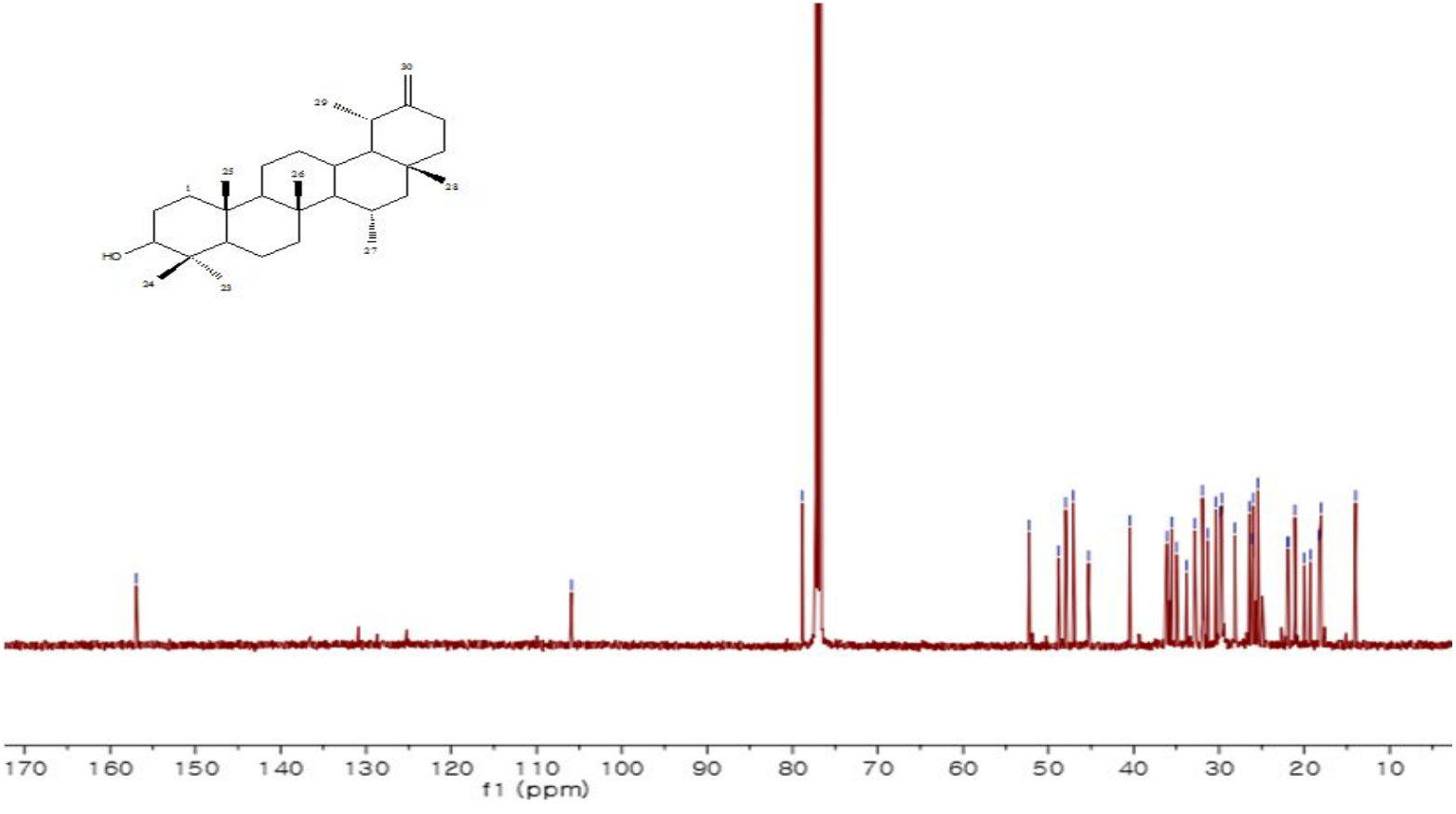

Fig.17. ^{13}C-NMR spectrum of compound 5 (125MHz, $CDCl_3$)

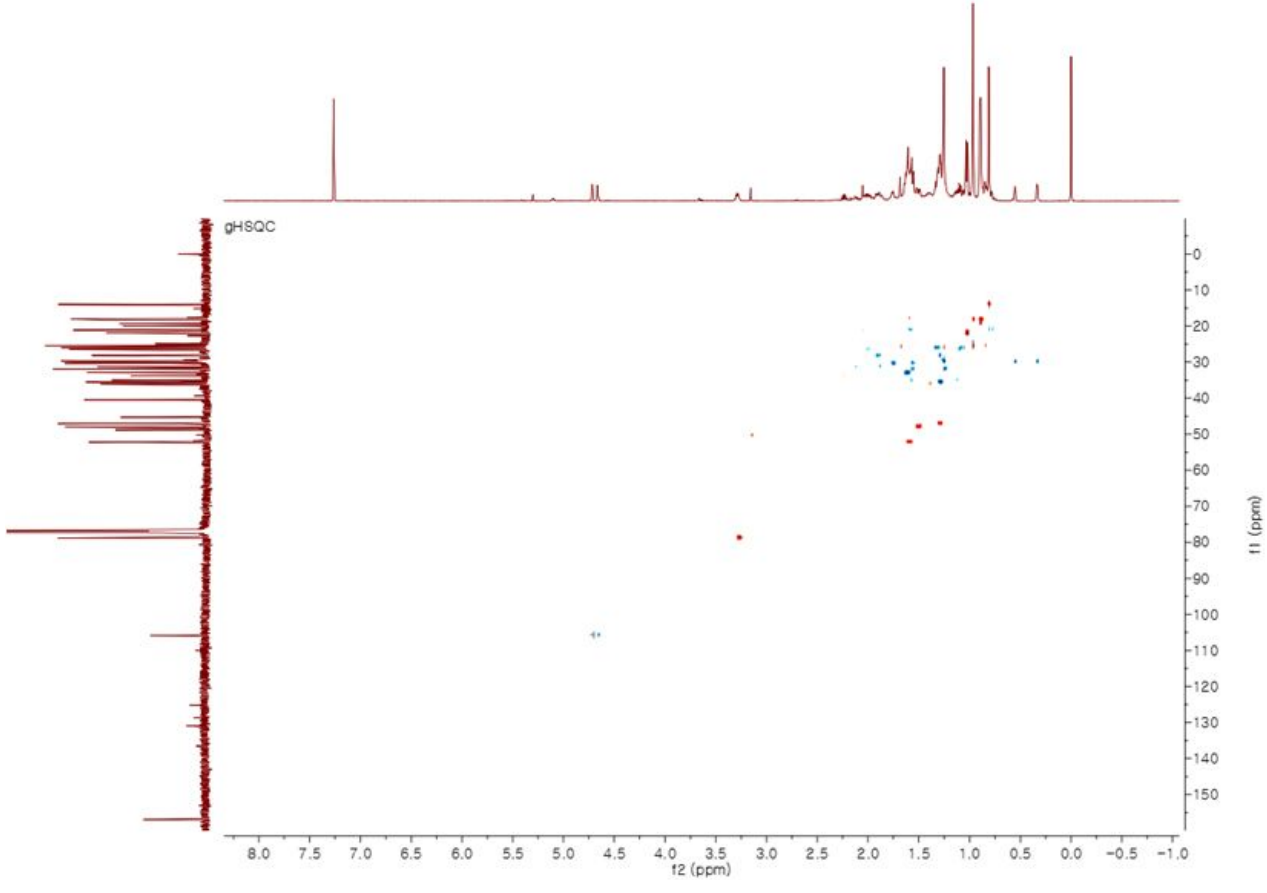

Fig.18. HSQC spectrum of compound 5

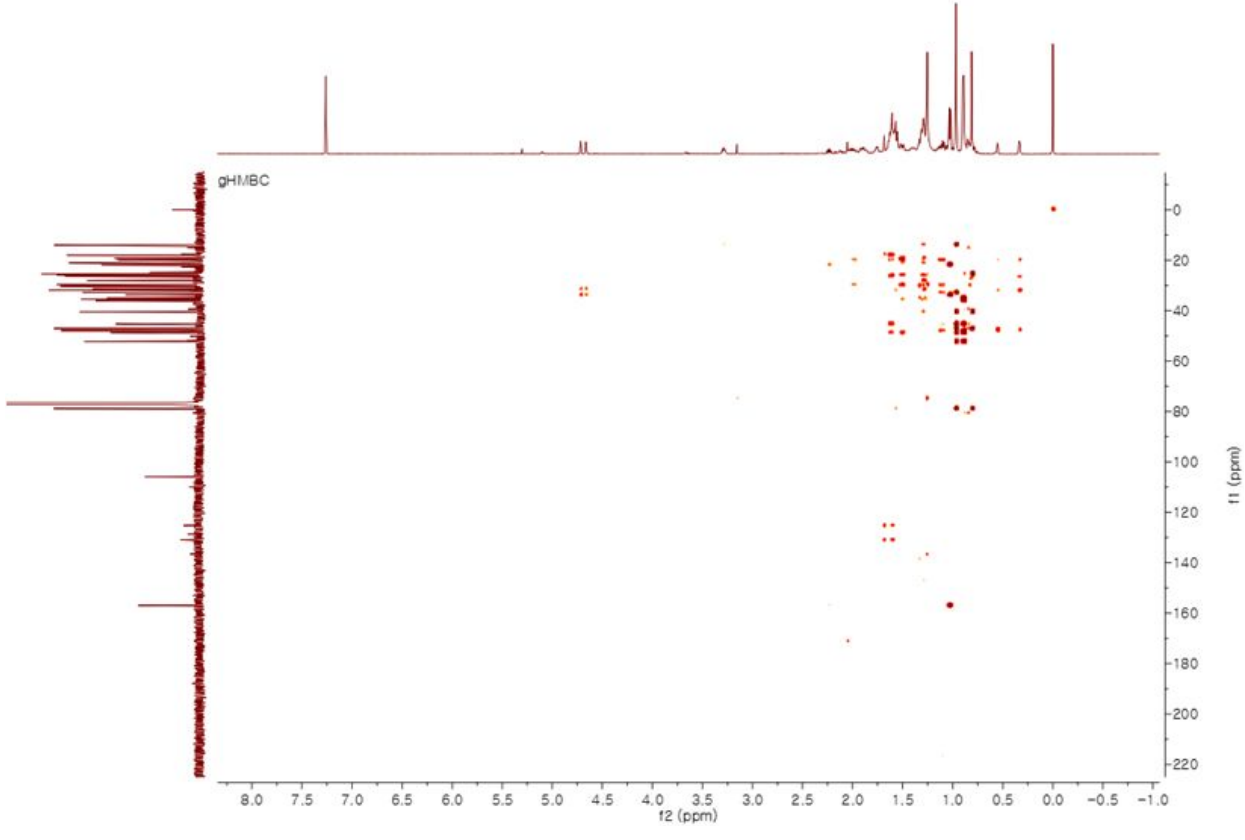

Fig.19. HMBC spectrum of compound 5

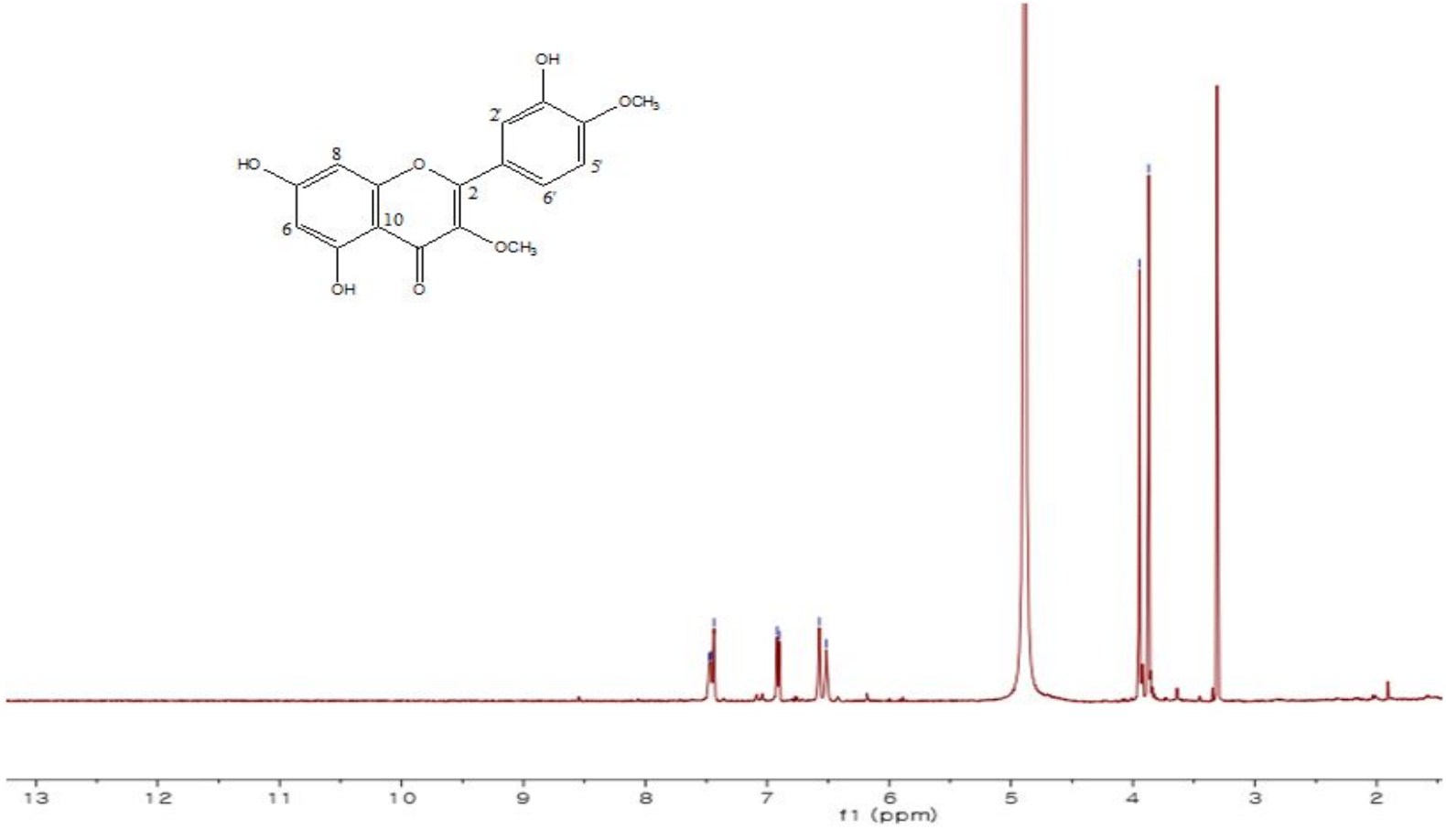

Fig.20. ^{1}H-NMR spectrum of compound 6 (500MHz, CD_3OD)

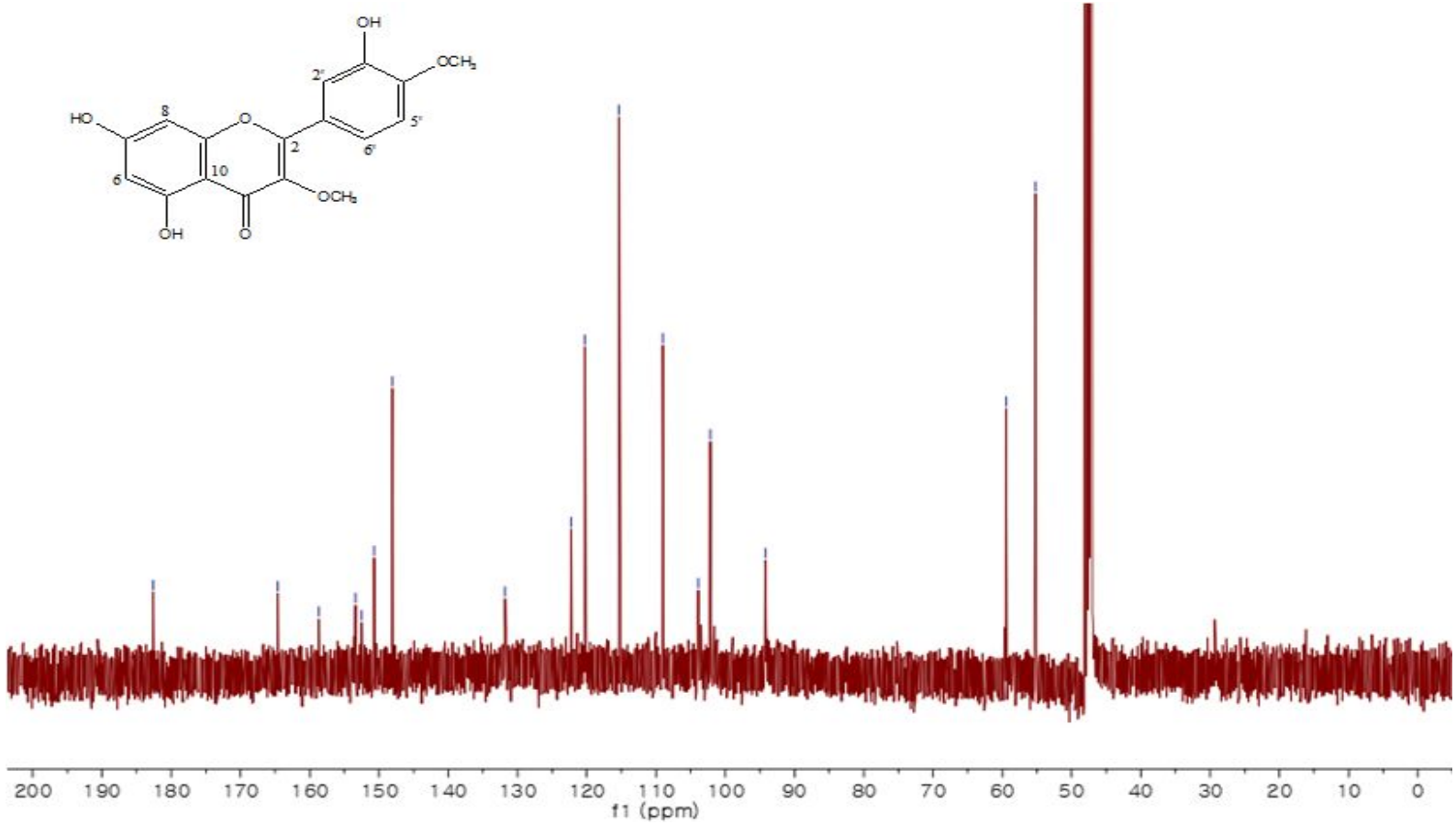

Fig.21. ^{13}C-NMR spectrum of compound 6 (125MHz, CD_3OD)

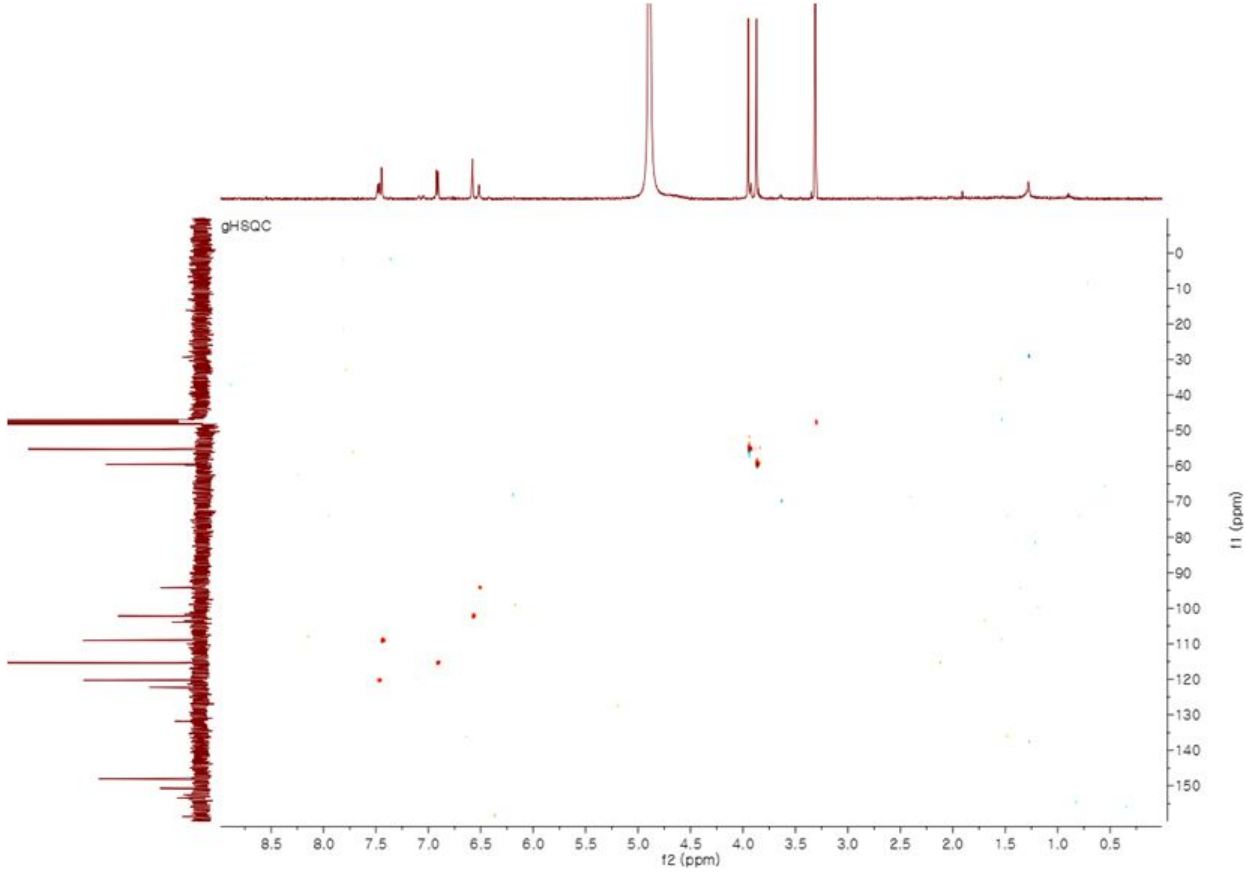

Fig.22. HSQC spectrum of compound 6

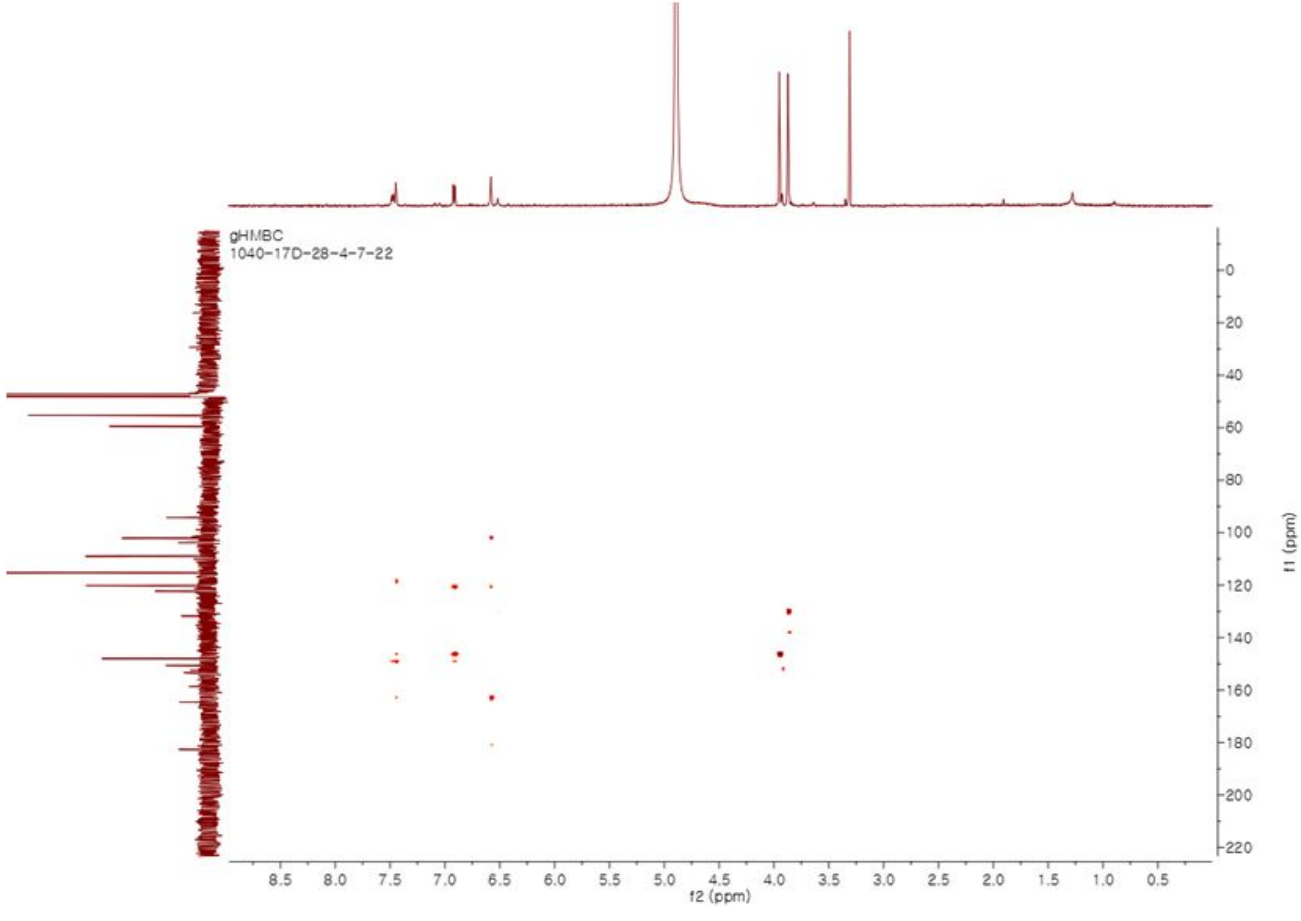

Fig.23. HMBC spectrum of compound 6