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Phytochemical constituents and IL-6 inhibitory Activities of Artemisia selengensis TURCZ

조선대학교 대학원

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물쑥(Artemisia selengensis TURCZ)의
화학성분과 IL-6 저해활성

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이 논문을 약학 석사학위신청 논문으로 제출함.

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국문 초록

물쑥(*Artemisia selengensis TURCZ*)의 화학성분과 IL-6 저해활성

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물쑥은 국화과에 속하는 다년생초본으로서, 주로 습지나 물가에서 자라며, 옛부터 주로 황달, 간염, 간암, 간경변증, 간디스토마증, 구토, 설사 등에 사용되었습니다. 이전에 보고된 물쑥에 대한 화학성분 조사는 주로 sesquiterpenoids 만의 분리를 보고하였습니다. 그러나 이 식물에 대한 항 염증 활성 (IL-6 저해활성) 은 아직 보고 된 바가 없습니다.

그래서 물쑥에 존재하는 식물 화학적 성분과 항 염증 활성 (IL-6 저해활성) 을 탐색하기 위하여 본 실험을 진행하였으며 다이클로로메테인(CH_2Cl_2)과 부탄올 (n-BuOH) 분획으로부터, 5개의 화합물 : artanomaloide(1), canin(2), eupatilin(3), Quercetin-3-O-glucoside-7-O-rhamnoside(4) and Isoquercitin(5) 을 분리, 동정하였습니다. 화합물들 (1-5) 의 구조는 ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}HCOSY, HSQC, HMBC 분광 분석 등의 1D 및 2D NMR에 기초하여 확인하였습니다. 이들 화합물들은 물쑥에서 처음으로 분리된 화합물들입니다.

이렇게 분리한 화합물들은, MG-63 cell에 TNF-α를 유도하여 IL-6 저해활성 (항 염

증 활성) 을 검사하였습니다. 분리한 화합물 중, artanomaloide(1)와 canin(2) 이 MG-63 cell에서 IL-6의 유리를 억제하는 것으로 확인되었습니다.

본 연구에서는 물쑥으로부터 분리된 화합물들에 대해 IL-6 저해 효과 여부를 처음으로 확인하였습니다.

키워드 : *Artemisia selengensis*; 국화과; guaianolide; IL-6

ABSTRACT

Phytochemical constituents and IL-6 inhibitory activities of *Artemisia selengensis TURCZ*

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Artemisia selengensis is a perennial herb belonging to the family Compositae, growing mainly wetlands, and waterside and is used traditionally as a jaundice, hepatitis, liver cancer, liver cirrhosis, clonorchiosis, vomiting, and diarrhea. Previous phytochemical investigation on this plant reported the isolation of principal sesquiterpenoids. However, the anti-inflammatory activity of this plant has not been reported yet. During our search for the phytochemical constituents of the dichloromethane (CH_2Cl_2) and *n*-butanol (BuOH) soluble fraction of this plant, five compounds : artanomaloide(**1**), canin(**2**), eupatilin(3), Quercetin-3-O-glucoside-7-O-rhamnoside(4) and Isoquercitin(5) were isolated from the aerial parts of *A.selengensis.* The structures of compounds(**1-5**) were identified based on 1D and 2D NMR, including ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}HCOSY, HSQC, HMBC spectroscopic analyses. These compounds were isolated from this plant for the first time. For these isolated compounds, the

inhibitory activity of IL-6 production in the TNF-α stimulated MG-63 cell was examined. Among these isolated compounds, artanomaloide(1), and canin(2) appeared to have potent inhibitory activity of IL-6 production in the TNF-α stimulated MG-63 cell. This is the first report on the anti-inflammatory effect of the components from *Artemisia selengensis TURCZ.*

Key Words : *Artemisia selengensis*; Compositae; guaianolide; IL-6

1. INTRODUCTION

Artemisia(Compositae) is a world wide genus of approximately 500 species, about 260 of which have been investigated in the fields of phytochemistry, biochemistry, pharmacology, etc (Hu 1996). *Artemisia selengensis Turcz*, "Hong-Chen-Ai" in Chinese, is a species of the well-known traditional Chinese medicine "Liu-Ji-Nu" (Zhang and Jiang 1992). It is growing wild in the southwestern part of China and used locally for anti-inflammation, hemostasis, invigorating the blood circulation and relieving dysmenorrhea. Several groups (Bohlmann and Rode 1967; Birnecker W et al., 1988; Jang and Lee, K.R. 1993) carried out chemical investigations of A. selengensis, and the anti-tumor and immunomodulating activities of its polysaccharide fractions have been reported (Koo et al., 1994). Although Artemisia genus is rich in normal sesquiterpenoids (Kelsey and Shafizadeh 1979), no sesquiterpenoid except for a bisabolene endoperoxide (Jang and Lee, K.R. 1993) has been reported in these previous works.

However, the anti-inflammatory activity of this plant has not been reported yet. Therefore we now report five compounds were isolated form the aerial parts of A. selengensis. For the isolated compounds, the inhibitory activity of IL-6 production in TNF-α stimulated MG-63 was examined. The results are showed in this paper.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts of *Artemisia selengensis TURCZ* were collected from the Herbarium at the College of Pharmacy, Chosun University, Korea, in December 2012. A voucher specimen was deposited in the Herbarium at the College of Pharmacy, Chosun University (CSU-1041-17).

2.2. General procedure

2.2.1. TLC and Column chromatography

TLC and Column chromatography were performed on precoated Si Gel F_{254} plates (Merck,art.5715), RP-18 F_{254} plates (Merck,art.15389) and silica gel 60 (40-63 and 63-200 μm, Merck), MCI gel CHP20P (75-150 μ, Mitsubishi Chemical Co.), as well as LiChroprep RP-18 (40-63 μm, Merck).

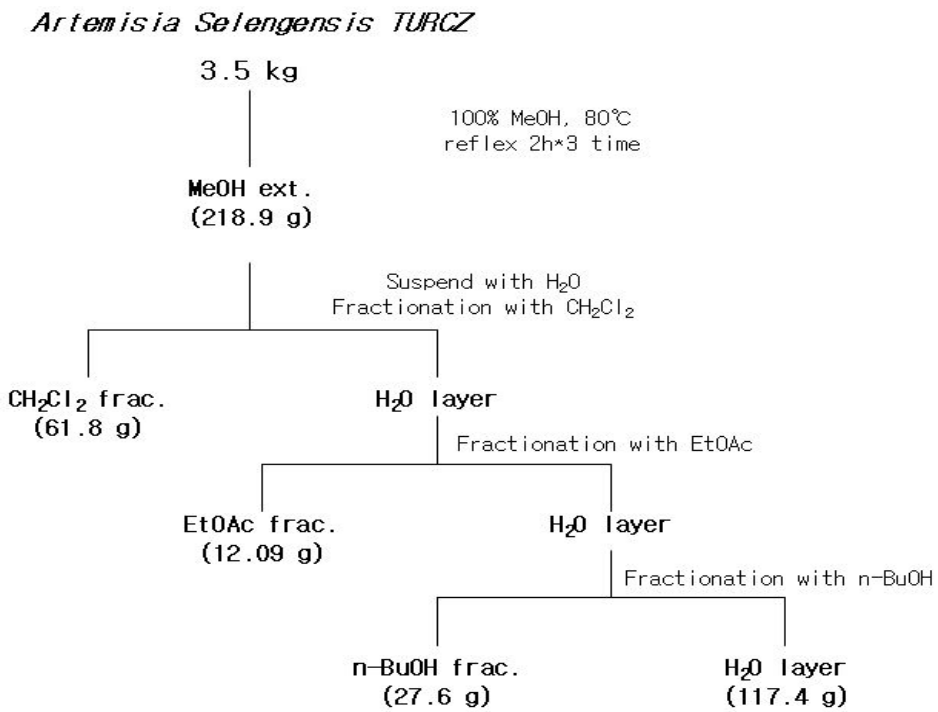
2.2.2. Equipment

IR : JASCO FT/IR-300E (JASCO Co., Japan)
UV : JASCO V-550 (JASCO Co., Japan)
^{1}H-NMR : Varian Unity Inova 600 MHz, 500 MHz (KBSI-Gwangju center)
^{13}C-NMR : Varian Unity Inova 150 MHz, 125 MHz (KBSI-Gwangju center)

2.3. Extraction and isolation

2.3.1. Extraction

The air-dried aerial parts of *Artemisia selengensis TURCZ* (3.5kg) were extracted three times with MeOH under refluxand 218.9g of residue were produced. The MeOH extract was suspended in water, which was then partitioned sequentially with equal volumes of dichloromethane (CH_2Cl_2), ethylacetate (EtOAc) and *n*-butanol (BuOH). Each fraction was evaporated *in vaccuo* to yield residues of CH_2Cl_2 (61.8g), EtOAc (12.1g), *n*-BuOH (27.6g), and water (117.4g) extract.

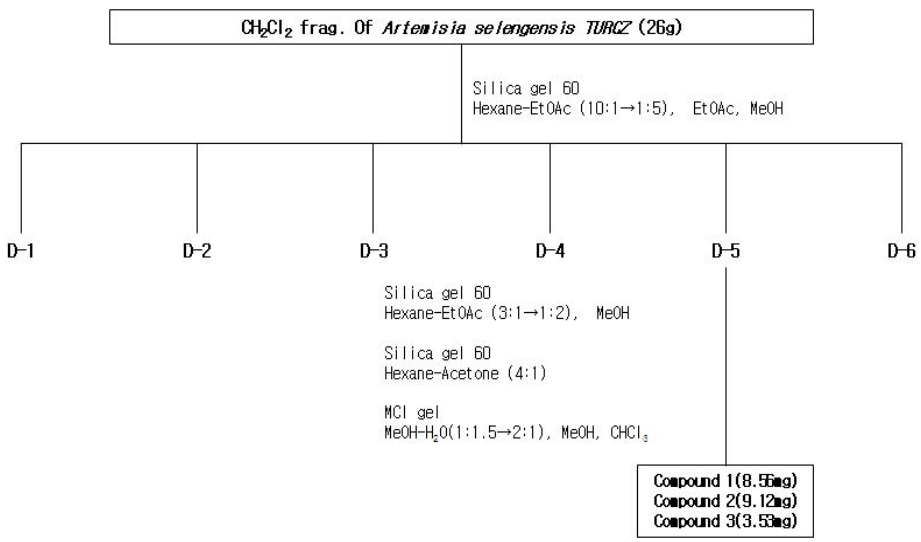


Scheme.1. Extraction and fractionation of the MeOH extract from *Artemisia selengensis TURCZ*

2.3.2. Isolation

Isolation procedure from CH_2Cl_2 soluble fraction of *Artemisia selengensis.*

The CH_2Cl_2 fraction (26.0g) was chromatographed over a silica gel column chromatography (CC), using a gradient solvent system of Hexane : Ethyl acetate (10:1→1:5), to give six subfractions (D1~D6).



Scheme.2. Isolation of compounds 1-3 from CH_2Cl_2 frag. of *Artemisia selengensis TURCZ*

2.3.2.1. Compound 1

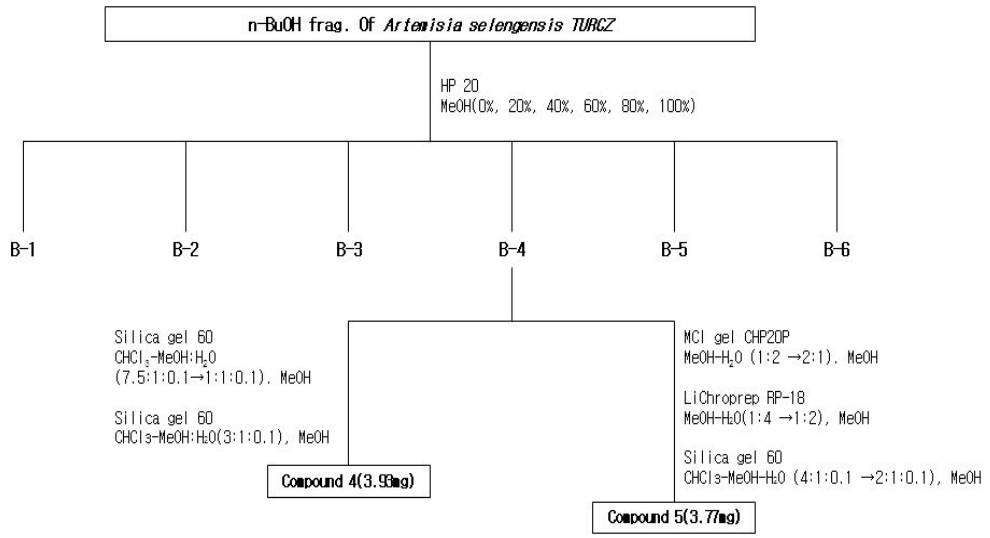
The subfraction D5 (1.75g) was subjected to a silica gel column chromatography (CC) eluting with a gradient solvent system of Hexane : Ethyl acetate (3:1→1:2) to yield nine subfractions (D5-1~D5-9). The subfraction D5-5 (210.81mg) was eluted with Hexane : Acetone (4:1) to yield seven subfractions (D5-5-1~D5-5-13). The subfraction D5-5-9 (41.84mg) was subjected to RP-18 column chromatography (CC) eluting with a gradient solvent system of MeOH : H_2O (1:1.5→2:1) to produce compound 1 (8.56mg).

2.3.2.2. Compound 2 and Compound 3

The subfraction D5 (1.75g) was subjected to a silica gel column chromatography (CC) eluting with a gradient solvent system of Hexane : Ethyl acetate (3:1→1:2) to yield nine subfractions (D5-1~D5-9). The subfraction D5-5 (210.81mg) was eluted with Hexane : Acetone (4:1) to yield seven subfractions (D5-5-1~D5-5-13). The subfraction D5-5-6 (35.48mg) was subjected to LiChroprep RP-18 column chromatography (CC) eluting with a gradient solvent system of MeOH : H_2O (1:1.5→2:1) to produce compound 2 (9.12mg) and compound 3 (3.53mg).

Isolation procedure from n-BuOH soluble fraction of *Artemisia selengensis.*

The *n*-BuOH fraction (12.5g) was chromatographed over a HP-20 column chromatography (CC), using a gradient solvent system of MeOH (0%→100%) to give six subfractions (B1~B6).



Scheme.3. Isolation of compounds 4-5 from *n*-BuOH frag. of *Artemisia selengensis TURCZ*

2.3.2.3. Compound 4

The first time *n*-BuOH fraction (12.5g) was chromatographed over a HP-20 column chromatography (CC), using a gradient solvent system of MeOH (0%→100%) to give six subfractions (1B1~1B6). The subfraction 1B4 (552.11mg) was subjected to a silica gel column chromatography (CC) eluting with a gradient solvent system of $CHCl_3$: MeOH : H_2O (7.5:1:0.1→1:1:0.1) to yield twenty-four subfractions (1B4-1~1B4-24). The subfraction 1B4-18,19,20 (61.09mg) was eluted with $CHCl_3$: MeOH : H_2O (3:1:0.1) to produce compound 4 (3.93mg).

2.3.2.4. Compound 5

The second time *n*-BuOH fraction (12.5g) was chromatographed over a HP-20 column chromatography (CC), using a gradient solvent system of MeOH (0%→100%) to give six subfractions (2B1~2B6). The subfraction 2B4 (2.5g) was subjected to MCI gel CHP20P column chromatography (CC) eluting with a gradient solvent system of MeOH : H_2O (1:2→2:1) to yield seventeen subfractions (2B4-1~2B4-17). The subfraction 2B4-9 (260.4mg) was subjected to RP-18 column chromatography (CC) eluting with a gradient solvent system of MeOH : H_2O (1:4 →1:2) to yield twenty-four subfractions (2B4-9-1~2B4-9-24). The subfraction 2B4-9-21,22 (39.24mg) was subjected to a silica gel column chromatography (CC) eluting with a gradient solvent system of $CHCl_3$: MeOH : H_2O (4:1:0.1→2:1:0.1) to produce compound 5 (3.77mg).

2.4. Bioassay of human interleukin (IL)-6

IL-6 bioassay was carried out using a slight modification of an already established method (Kim et al., 2003; Liu et al., 2006). Briefly, 500 mL of the MG-63 cells (3×10^4cells/mL) in DMEM, containing 10% FBS, were dispensed into a 24-well plate. Then, the culture was incubated for 24 h at 37 ° C, and 5mL of TNF-a and 5 mL of the DMSO with or without the compound were added. After

incubation at 37 ° C with 5 % CO_2 for 24 h, the medium was stored at -20 ° C until measurement. The medium was used to determine its IL-6 content with an ELISA procedure. 96-well plate were coated with 100mL of purified rat anti-human IL-6 monoclonal antibody in 0.1M $NaHCO_3$ (pH 9.6) by an overnight incubation at 4 ° C. Blocked with 200mL of 3% BSA in PBS for 2 h at RT, the wells were incubated with 100 mL of specific antibody for 2 h at room temperature (RT). A 100 mL of HRP conjugated rabbit anti-goat IgG (1:1000 dilution) was added to the well and incubated for 2 h at RT. A 100 mL of TMB (3,3',5,5'-tetramethyl-benzidine) substrate solution was added to the well and incubated for 10 min at RT. The color reaction was stopped with 50 mL of 0.4N HCl and the optical density was read at 450 nm using a Microplate Reader (Molecular Devices Co., Ltd., U.S.A.).

3. RESULTS AND DISCUSSIONS

3.1. Structures

The structures of compounds (**1-5**) were identified based on 1D and 2D NMR, including ^{1}H-^{1}H COSY, HSQC, HMBC spectroscopic analyses.

3.1.1. Compound 1 (Artanomaloide)

Colorless gum
Molecular formula : $C_{32}H_{36}O_8$
MP : 192 - 195 ℃,
IR $_{max}$ ($CHCl_3$) cm^{-1} : 3600 (OH), 1780, 1770 (γ-lactone), 1740 (OAc), 1695 (C=CC=O)
MS *m/z* (rel. int.) : 548.241 $[M]^+$

^{1}H-NMR (500 MHz, $CDCl_3$) δ :
1.23 (3H, s, H-14′), 1.51 (1H, d, *J* = 12.0 Hz, H-13a), 1.54 (3H, s, H-15′), 1.83 (2H, m, H-9′), 1.84 (1H, m, H-8′ a), 1.98 (1H, d, *J* = 9.8 Hz, H-5′), 2.04 (3H, s, OAc), 2.10 (1H, m, H-8′ b), 2.31 (3H, br s, H-14), 2.36 (1H, d, *J* = 2.5 Hz, H-9a), 2.37 (3H, s, H-15), 2.43 (1H, d, *J* = 12.0 Hz, H-13b), 2.90 (1H, dd, *J* = 10.5, 13.0 Hz, H-9b), 3.06 (1H, m, H-7), 3.15 (1H, m, H-7′), 3.78 (1H,br d, H-6), 3.79 (1H, br d, H-5), 4.22 (1H, t, *J* = 9.5 Hz, H-6′), 5.15 (1H, ddd. *J* = 2.5, 10.5 Hz, H-8), 5.47 (1H, d, *J* = 3.0 Hz, H-13′ a), 5.88 (1H, d, *J* = 5.5 Hz, H-3′), 6.04 (1H, d, *J* = 3.5 Hz, H-13′ b), 6.17 (1H, s, H-3), 6.28 (1H, d, *J* = 5.5 Hz, H-2′);

^{13}C-NMR (125 MHz, $CDCl_3$) δ :
136.0 (C-1), 197.3 (C-2), 136.6 (C-3), 174.6 (C-4), 51.2 (C-5), 81.0 (C-6),

57.6 (C-7), 68.1 (C-8), 44.1 (C-9), 145.9 (C-10), 61.5 (C-11), 178.5 (C-12), 38.0 (C-13), 20.5 (C-14), 20.2 (C-15), 65.15 (C-1′), 143.3 (C-2′), 134.2 (C-3′), 58.2 (C-4′), 67.9 (C-5′), 81.9 (C-6′), 44.9 (C-7′), 22.1 (C-8′), 35.7 (C-9′), 73.1 (C-10′), 142.8 (C-11′), 172.7 (C-12′), 119.9 (C-13′), 29.6 (C-14′), 15.2 (C-15′), 25.0, 172.7 (OAc).

Compound 1 was obtained as colorless gums, and the molecular formula was determined as $C_{32}H_{36}O_8$ by HFRAB-MS at *m/z* 548.241 $[M]^+$ indicating fifteen double-bond equivalents in the molecule. The ^{13}C-NMR and HSQC spectrum of compound 1 exhibited 32 carbons, which revealed carbon signals for five methyls, five methylenes, ten methines, eight quaternary carbons, and four carbonyl groups. The structure of compound 1 was determined by the cross-peaks in a COSY spectrum and HMBC correlations. Based on the above results, the structure of compound 1 showed very similar chemical shifts of 8-acetylarteminolide [6]. However, analysis of ^1H-NMR of them showed a quite different chemical shifts at H-5′ (δ_H 3.08 for 8-acetylarteminolide and 1.98 for compound 1, respectively). Accordingly, based on the spectral analysis and reported data, the structure of compound 1 was identified as atranomaloide.

3.1.2. Compound 2 (Canin)

Colorless needle crystal
Molecular formula : $C_{15}H_{18}O_5$
MP : 242 - 244 ℃
IR $_{max}$ (KBr) cm^{-1} : 3500, 1755, 1655
HRFAB-MS : *m/z* 279.1236 $[M + H]^+$

^1H-NMR (500 MHz, $CDCl_3$) δ :
1.27 (3H, s, H-14), 1.57 (3H, s, H-15), 1.84 (1H, m, H-9a), 2.05 (1H, m, H-9b), 2.10 (1H, m, H-8a), 2.33 (1H, m, H-8b), 2.63 (1H, d, *J* = 11.5 Hz, H-5), 3.45

(1H, m, H-7), 3.70 (1H, br s, H-2), 4.07 (1H, br s, H-3), 4.34 (1H, dd, *J* = 9.5, 11.5 Hz, H-6), 5.49 (1H, d, *J* = 3.0 Hz, H-13a), 6.21 (1H, d, *J* = 3.5 Hz, H-13b);

^{13}C-NMR (125 MHz, $CDCl_3$) δ:
73.3 (C-1), 64.6 (C-2), 64.3 (C-3), 83.3 (C-4), 57.8 (C-5), 79.8 (C-6), 45.0 (C-7), 23.5 (C-8), 35.0 (C-9), 72.2 (C-10), 139.3 (C-11), 169.4 (C-12), 120.0 (C-13), 26.5 (C-14), 22.1 (C-15).

Compound 2 was obtained as colorless needle crystals, and the molecular formula was determined as $C_{15}H_{18}O_5$ by HRFAB-MS at *m/z* 279.1236 $[M + H]^+$ (calcd *m/z* 279.1232) indicating seven double-bond equivalents in the molecule. The ^{1}H-NMR spectrum of compound 2 showed and *exo*-methylene signals [δ_H 5.49 (1H, d, *J* = 3.0 Hz, H-13a), and 6.21 (1H, d, *J* = 3.5 Hz, H-13b)], three oxygenated proton signals [δ_H 3.70 (1H, br s, H-2), 4.07 (1H, br s, H-3), and 4.34 (1H, dd, *J* = 9.5, 11.5 Hz, H-6)], and two methyl groups [δ_H 1.27 (3H, s, H-14) and 1.57 (3H, s, H-15)]. The ^{13}C-NMR spectrum of compound 2 revealed the presence of 15 carbon signals, including two methyls, two methylenes, as exo-methylene, five methines, and five quaternary carbons. These observations suggested that compound 2 was a 1,2,;3,4-diepoxyguaianolide sesquiterpene lactone with two tertiary methyls and a hydroxyl group [14,19]. The two methyl groups were located at C-4 and C10 on the basis of HMBC correlations from CH_3-15 (δ_H 1.57) to C-3 (δ_C 64.3), C-4 (δ_C 83.3), and C-5 (δ_C 57.8) and from CH_3-14 (δ_H 1.27) to C-1 (δ_C 73.3), C-9 (δ_C 35.0), and C-10 (δ_C 72.2), respectively.). Based on the above results, the structure of compound 2 was identified as canin.

3.1.3. Compound 3 (Eupatilin)

Yellow amorphous powder
Molecular formula : $C_{18}H_{16}O_7$

MP : 228 - 230 ℃

UV (MeOH) λ_{max} nm : 274, 340

IR $_{max}$ (KBr) cm^{-1}: 3390, 3265, 1655, 1620, 1584, 1460, and 1425

ESI-MS: *m/z* 344 [M^+]

^{1}H-NMR (500 MHz, $CDCl_3$) δ:

3.94 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 6.61 (1H, s, H-3), 6.56 (1H, s, H-8), 6.98 (1H, d, *J* = 9.0 Hz, H-5′), 7.34 (1H, d, *J* = 2.0 Hz, H-2′), 7.53(1H, dd, *J* = 2.0, 8.5 Hz, H-6′);

^{13}C-NMR (125 MHz, $CDCl_3$) δ:

163.9 (C-2), 104.5 (C-3), 182.6 (C-4), 153.2 (C-5), 132.6 (C-6), 158.7 (C-7), 90.6 (C-8), 153.1 (C-9), 106.1 (C-10), 123.8 (C-1′), 108.7 (C-2′), 149.3 (C-3′), 152.2 (C-4′), 111.1 (C-5′), 120.1 (C-6′), 60.86 (OCH_3), 56.33 (OCH_3), 56.11 (OCH_3).

Compound 3 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^+] indicating eleven double-bond equivalents in the molecule. The ^{13}C-NMR and HSQC spectrum of compound 1 exhibited 32 carbons, which revealed carbon signals for three methyls, five methines, nine quaternary carbons, and one carbonyl groups. 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 [12]. Accordingly, compound 3 was identified as eupatilin.

3.1.4. Compound 4 (Quercetin-3-O-glucoside-7-O-rhamnoside)

Yellow powder

Molecular formula : $C_{27}H_{30}O_{16}$

MP : 190 - 192 ℃

IR $_{max}$ (MeOH) cm^{-1} : 3450-3290 (OH), 1660 (C=O), 1618, 1575, 1520 (C=C), 1100-1000 (glycoside)
EI-MS *m/z* : 611.1614 $[M+H]^+$

^{1}H-NMR (500 MHz, CD_3OD) δ :
1.12 (3H, d, *J* = 6.0 Hz, H-6‴), 3.08 (1H, m, H-4″), 3.24 (1H, d, *J* = 7.5 Hz, H-3″), 3.24 (1H, d, *J* = 7.5 Hz, H-2″), 3.29 (1H, d, *J* = 9.0 Hz, H-4‴), 3.31 (2H, m, H-6″), 3.40 (1H, m, H-5‴), 3.60 (1H, m, H-5″), 3.63 (1H, dd, *J* = 2.9, 9.5 Hz, H-3‴), 3.83 (1H, dd, *J* = 2.0, 2.9 Hz, H-2‴), 4.52 (d, *J* = 1.2 Hz, H-1‴), 5.11 (1H, d, *J* = 7.5 Hz, H-1″), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.87 (1H, d, *J* = 8.5 Hz, H-5′), 7.63 (1H, dd, *J* = 2.0, 8.5 Hz, H-6′), 7.67 (1H, d, *J* = 2.5 Hz, H-2′);

^{13}C-NMR (125 MHz, CD_3OD) δ :
159.4 (C-2), 135.7 (C-3), 179.6 (C-4), 163.1 (C-5), 100.2 (C-6), 166.5 (C-7), 95.1 (C-8), 158.7 (C-9), 104.9 (C-10), 123.7 (C-1′), 117.8 (C-2′), 146.0 (C-3′), 150.0 (C-4′), 116.2 (C-5′), 123.2 (C-6′), 105.7 (C-1″), 75.9 (C-2″), 76.4 (C-3″), 71.5 (C-4″), 78.3 (C-5″), 68.7 (C-6″), 102.6 (C-1‴), 74.1 (C-4‴), 71.4 (C-5‴), 72.3 (C-3‴), 69.9 (C-2‴), 18.0 (C-6‴).

Compound 4 was obtained as yellow powder, and the molecular formula was determined as $C_{27}H_{30}O_{16}$ by ESI-MS at *m/z* 611.1614 $[M + H^+]$ indicating thirteen double-bond equivalents in the molecule. In ^{13}C-NMR 12 carbon resonances assigned for glucose and rhamnose among which the most downfield signals at δ_C 105.7 and 102.6 assigned for the two anomeric carbons C-1″ and C-1‴, respectively and a signal at δ_C 18.0 for C-6‴. The ^{1}H-NMR spectrum of compound 4 showed the proton signals of quercetin. H-2′ appeared as a doublet at δ_H 7.67 and H-6′ as a doublet at δ_H 7.62 due to orthocoupling with H-5′ which appeared at δ_H 6.87. The two aromatic protons of ring appeared at δ_H 6.40 and 6.21 assigned to H-8 and H-6, respectively. The glucose moeity at C-3 was

evidenced by the downfield location of aromatic proton at δ_H 5.11 (1H, d, J = 7.5 Hz), while the anomeric proton of rhamnose appeared at δ_H 4.52 (d, J = 1.2 Hz). Based on the above results, the structure of compound 4 was identified as quercetin-3-O-glucoside-7-O-rhamnoside.

3.1.5. Compound 5 (Isoquercitin)

Yellow powder
Molecular formula : $C_{21}H_{20}O_{12}$
MP : 235 ℃
IR $_{max}$ (MeOH) cm^{-1} : 3350 (OH), 1655 (C=O), 1600 (C=C), 1100-1000 (glycoside)
EI-MS m/z : 465 $[M]^+$

^{1}H-NMR (500 MHz, CD_3OD) δ :
3.71-3.35 (6H, m, H-2″-6″), 5.25 (1H, d, J = 8.0 Hz, H-1″), 6.20 (1H, d, J = 2.0 Hz, H-6), 6.39 (1H, d, J = 2.0 Hz, H-8), 6.87 (1H, d, J = 8.5 Hz, H-5′), 7.59 (1H, dd, J = 2.0, 8.5 Hz, H-6′), 7.78 (1H, d, J = 2.0 Hz, H-2′);

^{13}C-NMR (125 MHz, CD_3OD) δ :
159.1 (C-2), 135.7 (C-3), 179.6 (C-4), 163.2 (C-5), 100.1 (C-6), 166.5 (C-7), 94.9 (C-8), 158.6 (C-9), 104.4 (C-10), 123.2 (C-1′), 117.7 (C-2′), 146.1 (C-3′), 150.0 (C-4′), 116.1 (C-5′), 123.3 (C-6′), 105.7 (C-1″), 75.9 (C-2″), 78.5 (C-3″), 71.3 (C-4″), 78.3 (C-5″), 62.7 (C-6″).

Compound 5 was obtained as Yellow powder, and the molecular formula was determined as $C_{21}H_{20}O_{12}$ by ESI-MS at m/z 465 [M^+] indicating twelve double-bond equivalents in the molecule. The ^{1}H-NMR and ^{13}C-NMR showed characteristic signals of 4, while no signals of rhamnose could be detected. Accordingly, based on the above results, the structure of compound 5 was identified as Isoquercetin.

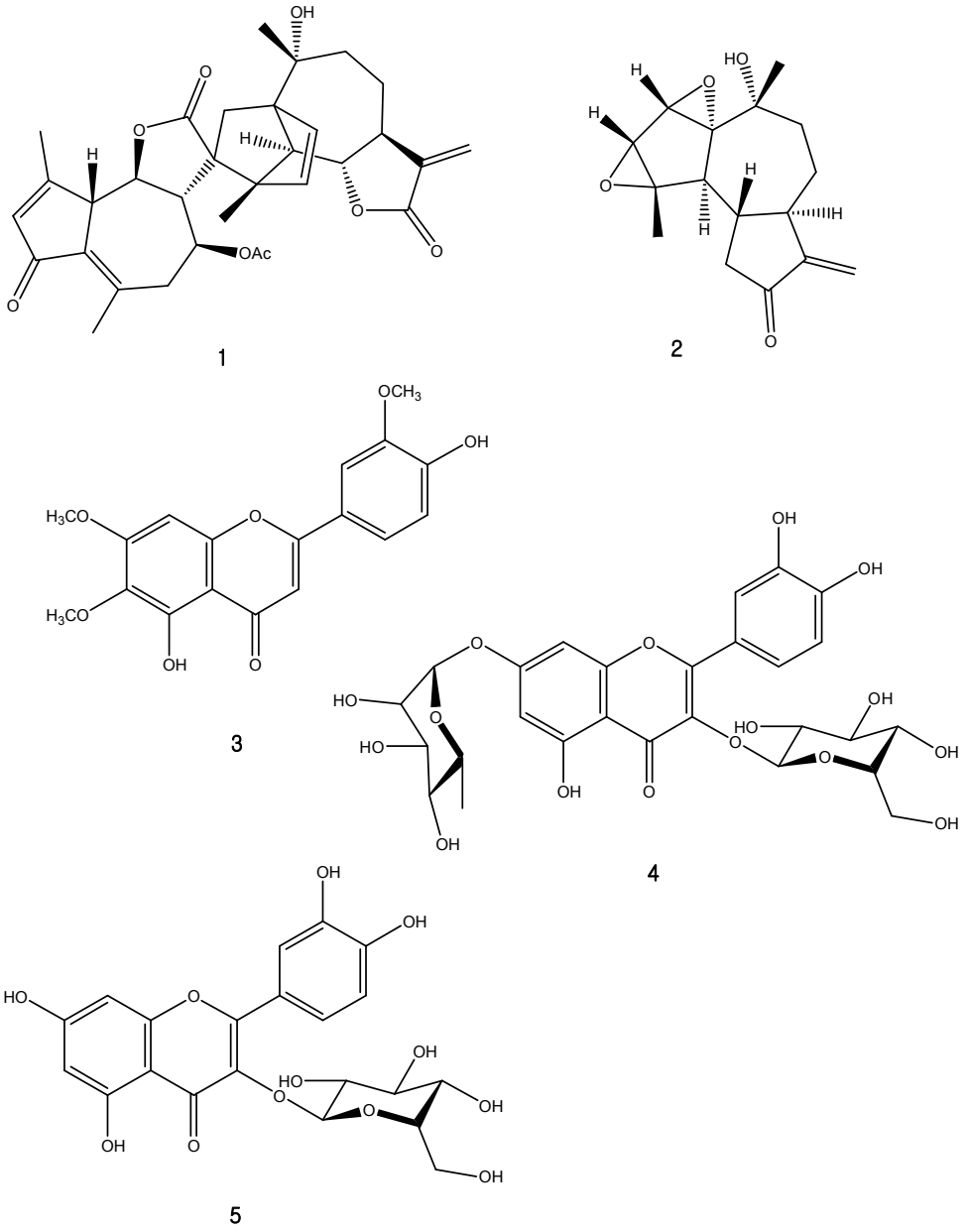


Fig. 1. Structures of compounds 1-5 isolated from *Artemisia selengensis TURCZ*

3.2. IL-6 inhibitory activities

Inhibitory effect of compounds 1-3 on IL-6 production in TNF-α-sitimulated MG-63cell. MG-63 (3×10^4 cells/well) was incubated for 24h. Culture were incubated with or without compounds and them stimulated with TNF-α for 24 h. IL-6 in the supernatant was measured by ELISA. Results were expressed as the as the mean±S.E. from three different experiments. *p<0.05 or **p<0.01 compared with TNF-α treated values.

Among the isolated compounds, artanomaloide(1), and canin(2) showed potent inhibitory effect on IL-6 production in TNF-α stimulated MG-63.

Treatment	IL-6 (pg/mL)	Inhibition (%)
None	37.6±0.81	-
TNF-α	250.6±5.05	-
Compound 1	5.0±1.71**	97.7**
Compound 2	12.5±1.52**	94.7**
Compound 3	205.5±10.54**	17.6**

Table. 1. Inhibition effect of compound 1-3 on IL-6 production in TNF-α stimulated MG-63 cell

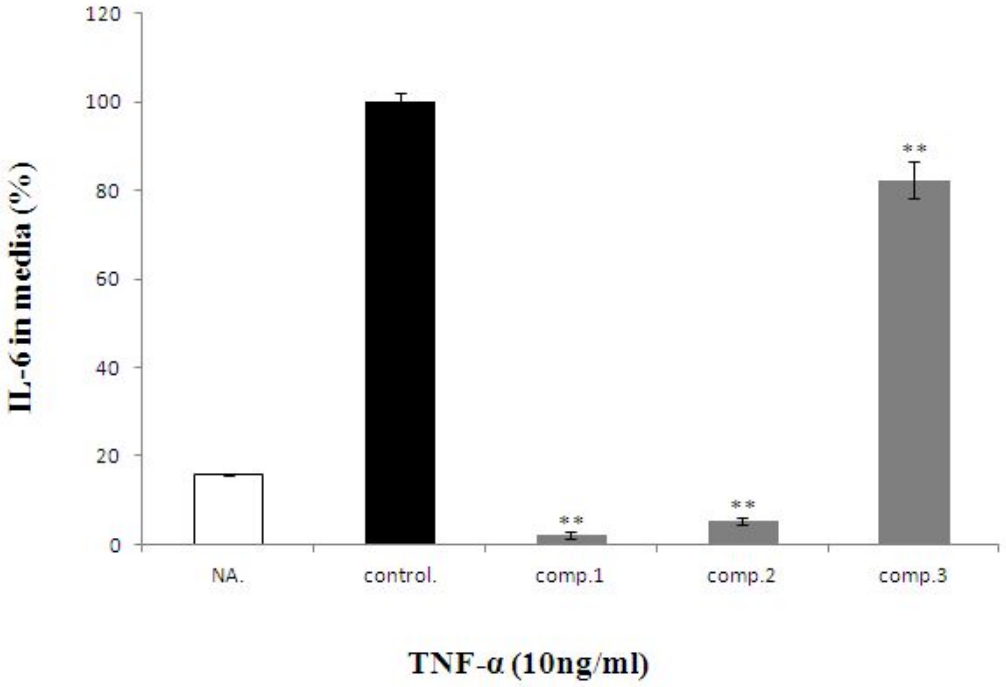


Fig. 2. Inhibition effect of compound 1-3 on IL-6 production in TNF-α stimulated MG-63 cell

4. CONCLUSIONS

Repeated, during our search for the phytochemical constituents of the dichloromethane (CH_2Cl_2) and *n*-butanol (BuOH) soluble fraction of this plant, two guaianolides, artanomaloide(**1**) and canin(**2**) and three flavonoids, eupatilin(**3**), Quercetin-3-O-glucoside-7-O-rhamnoside(**4**) and Isoquercitin(**5**) were isolated from the aerial parts of *Artemisia selengensis TURCZ.* The structures of compounds (**1-5**) were identified based on 1D and 2D NMR, including ^1H-^1H COSY, HSQC, HMBC spectroscopic analyses. These compounds were isolated from this plant for the first time. For the isolated compounds, the inhibitory activity of IL-6 production in TNF-α stimulated MG-63 was examined. Among the isolated compounds, artanomaloide(**1**), and canin(**2**) showed potent inhibitory effect on IL-6 production in TNF-α stimulated MG-63 cell. This is the first report on the anti-inflammatory effect of the components from *Artemisia selengensis TURCZ.*

5. REFERENCE

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APPENDIX

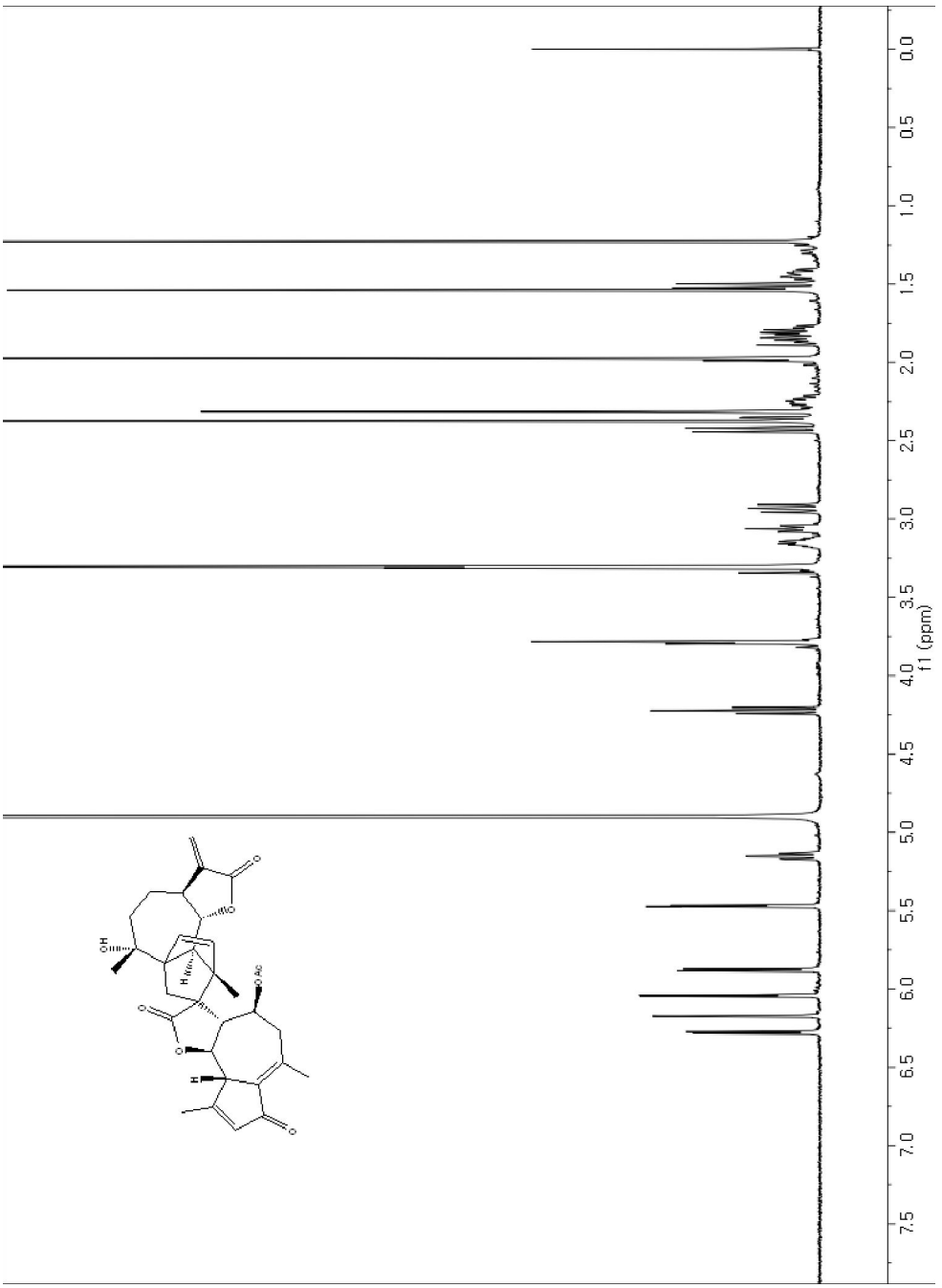

Fig. 3. ^{1}H-NMR spectrum of compound 1 (500MHz, $CDCl_3$)

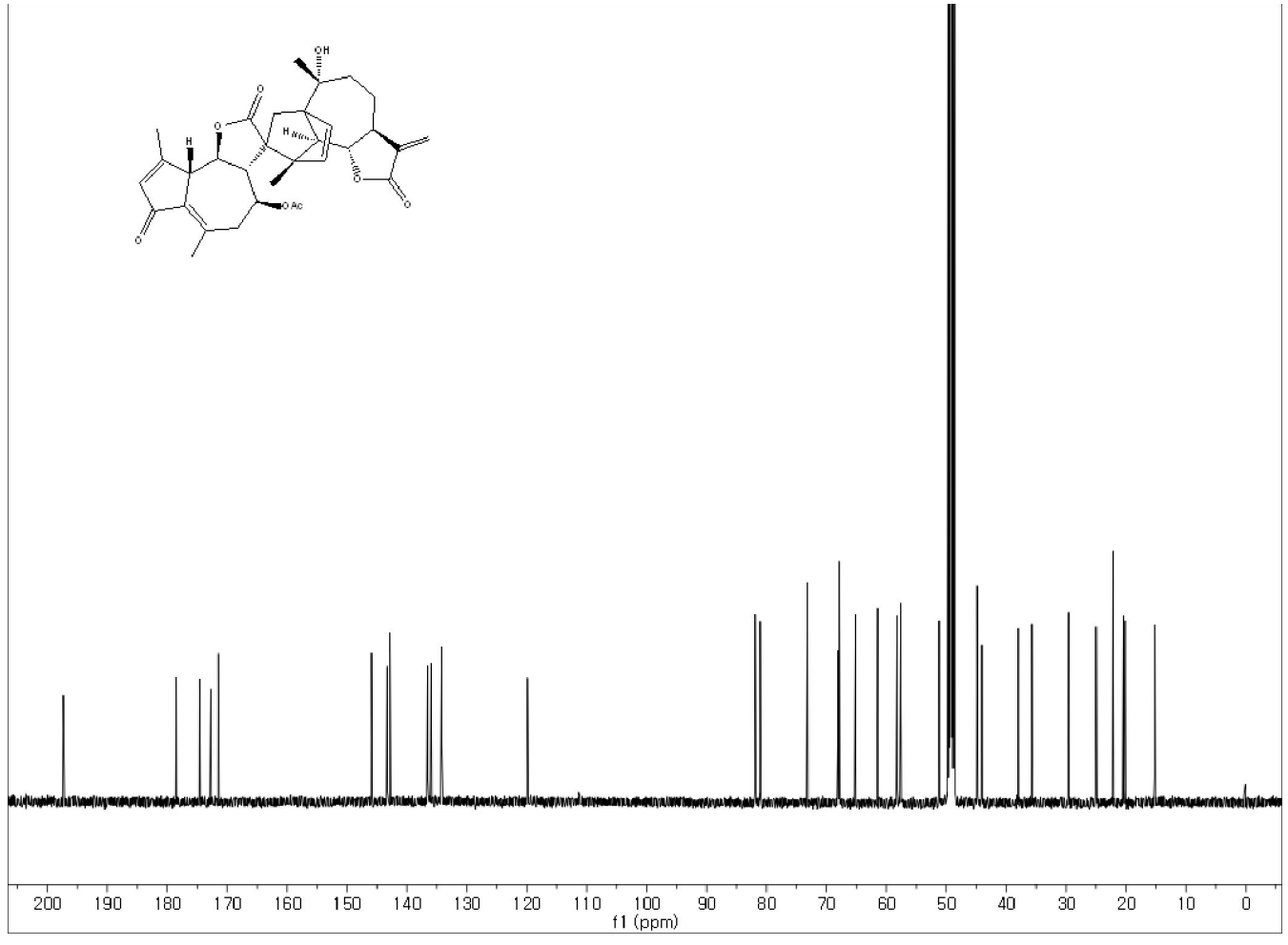

Fig. 4. ^{13}C-NMR spectrum of compound 1 (125MHz, $CDCl_3$)

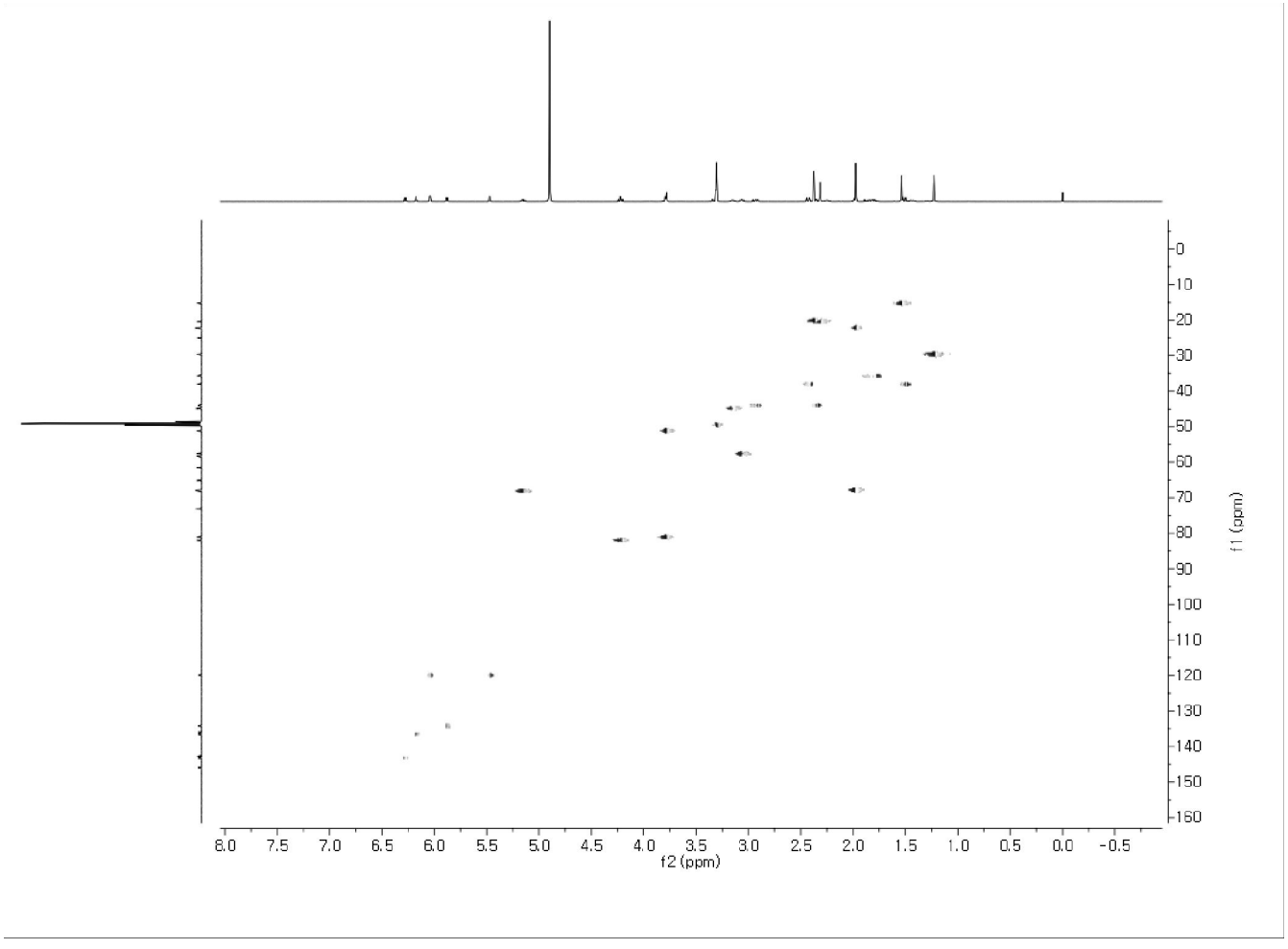

Fig. 5. HSQC spectrum of compound 1 (500MHz, $CDCl_3$)

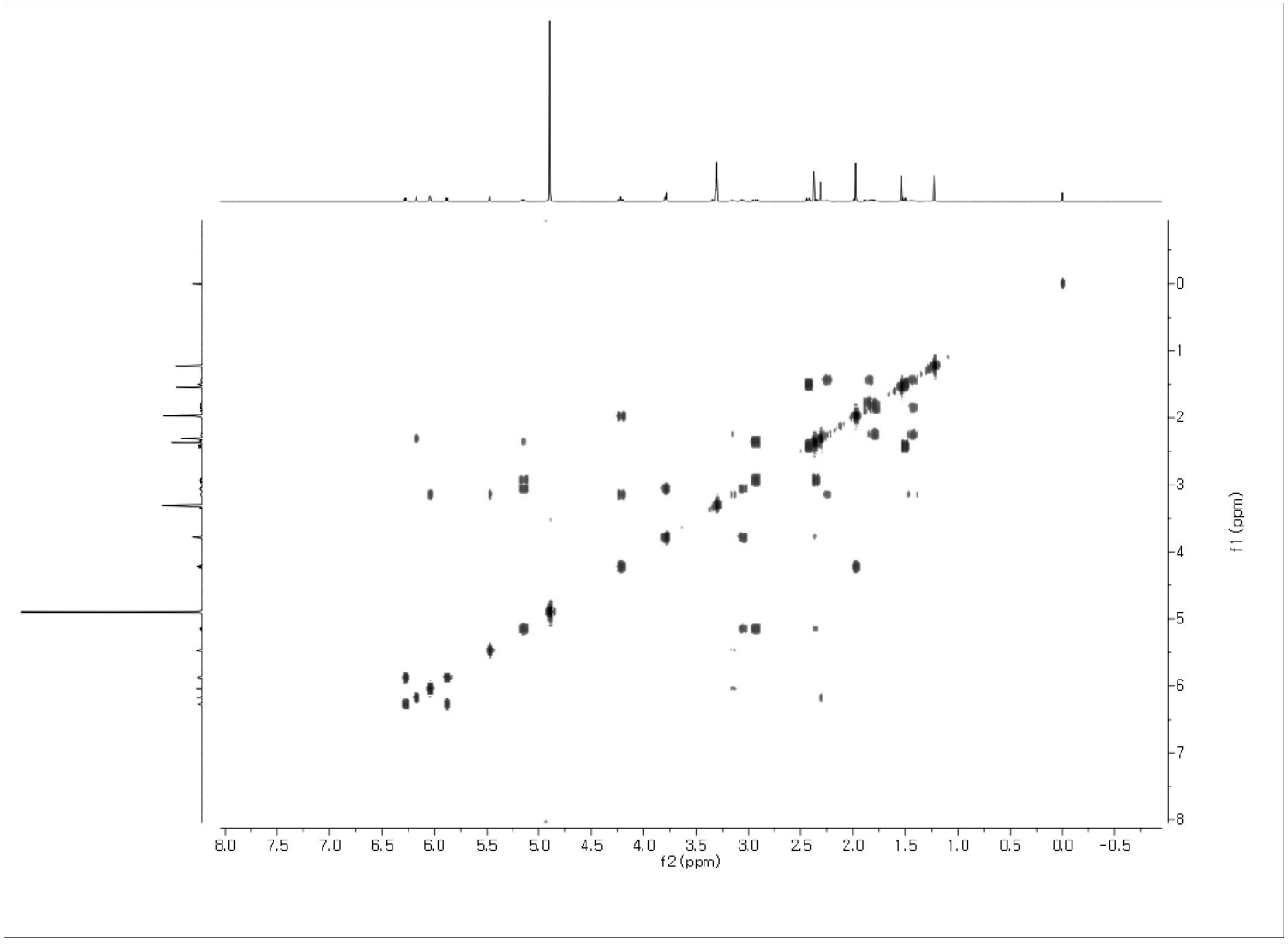

Fig. 6. COSY spectrum of compound 1 (500MHz, $CDCl_3$)

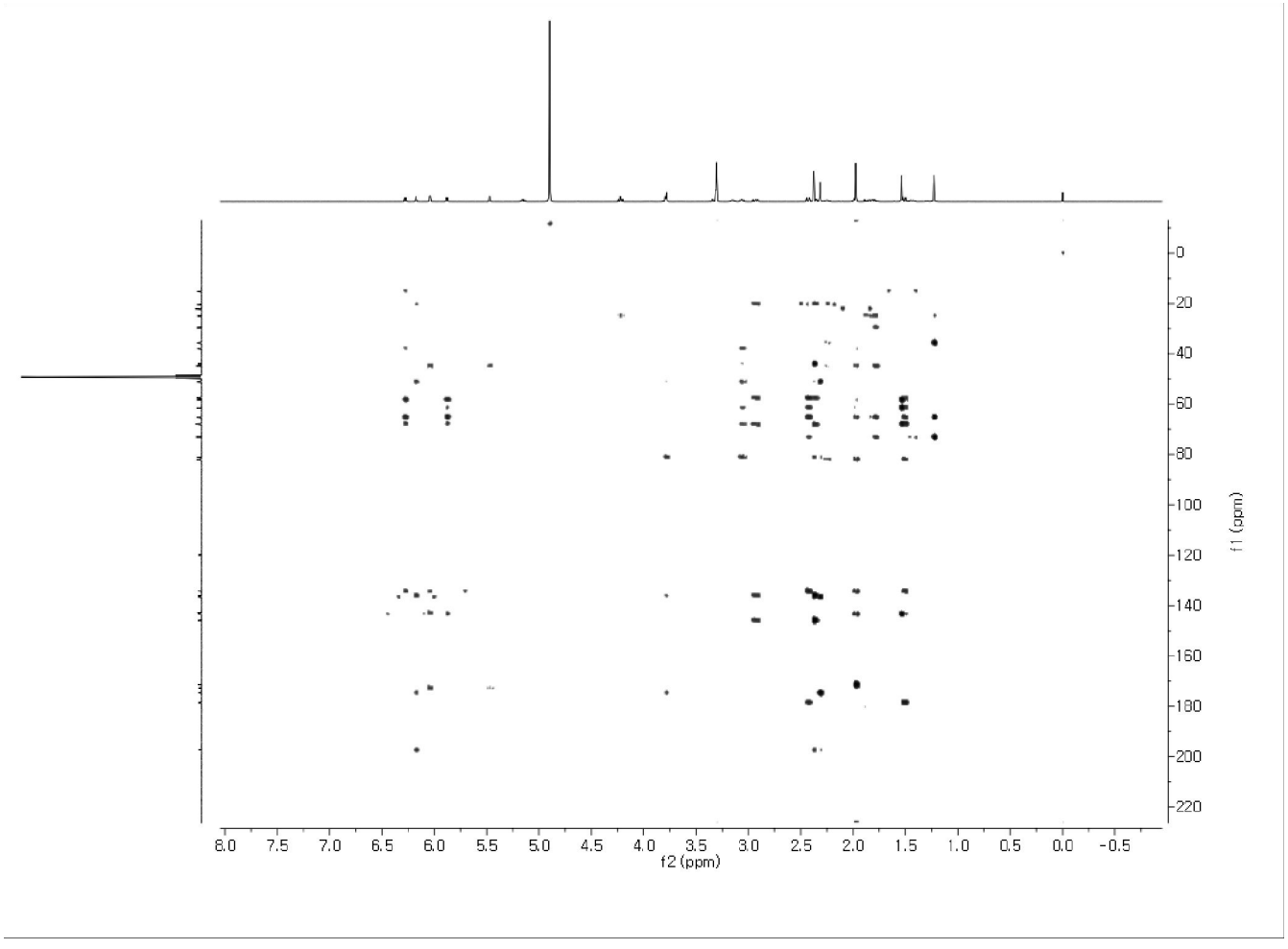

Fig. 7. HMBC spectrum of compound 1 (500MHz, $CDCl_3$)

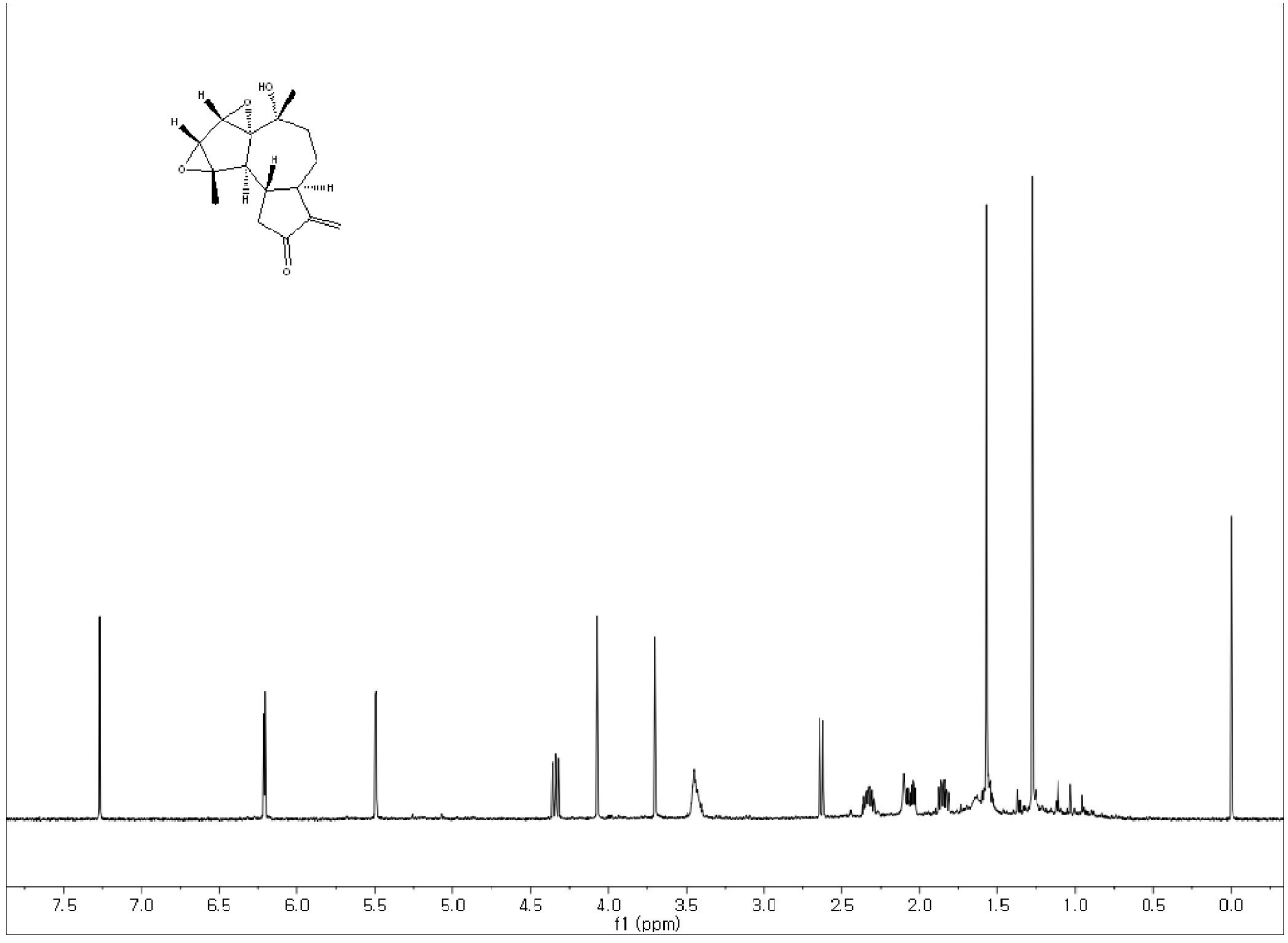

Fig. 8. ^{1}H-NMR spectrum of compound 2 (500MHz, $CDCl_3$)

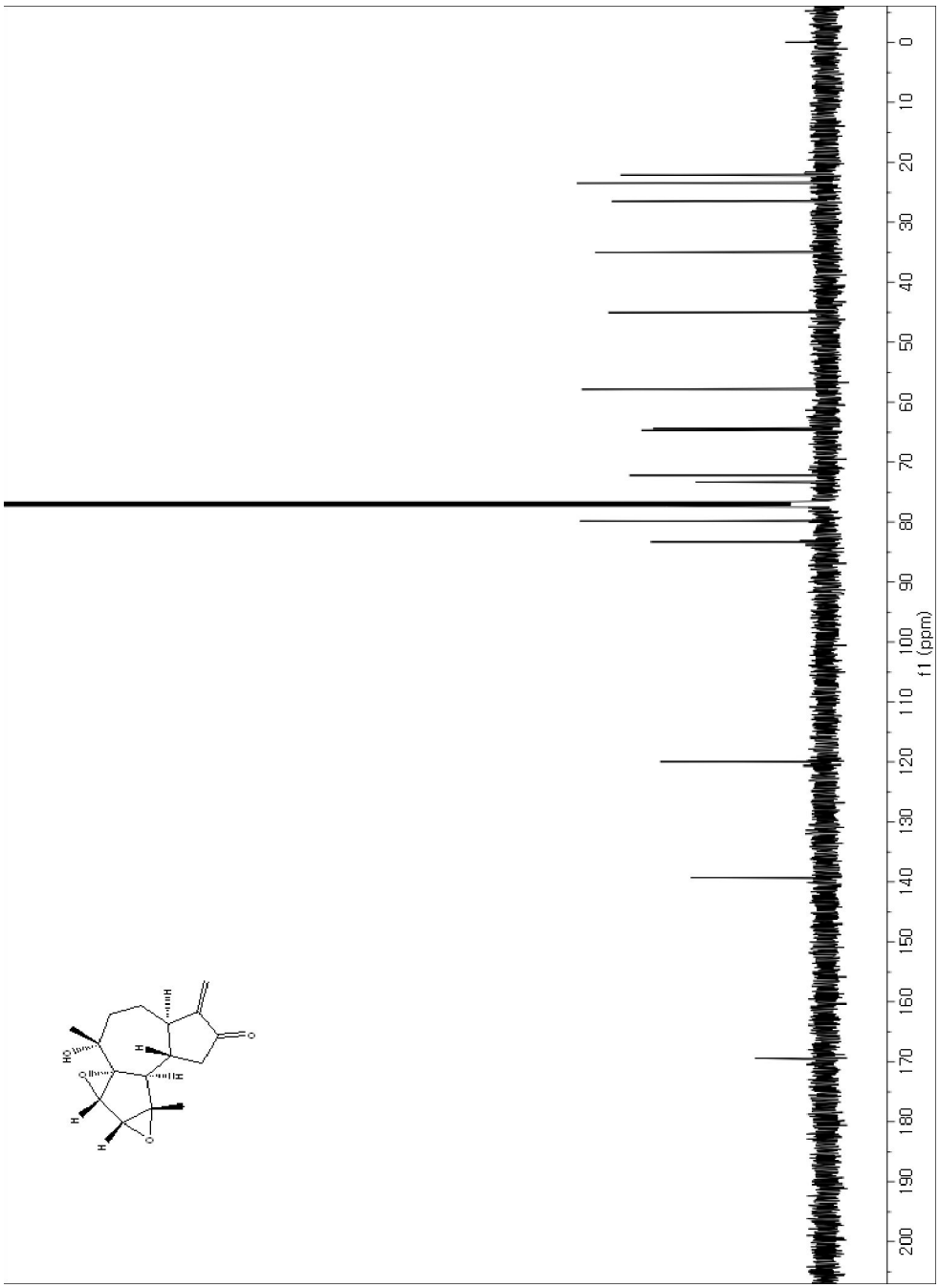

Fig. 9. ^{13}C-NMR spectrum of compound 2 (125MHz, $CDCl_3$)

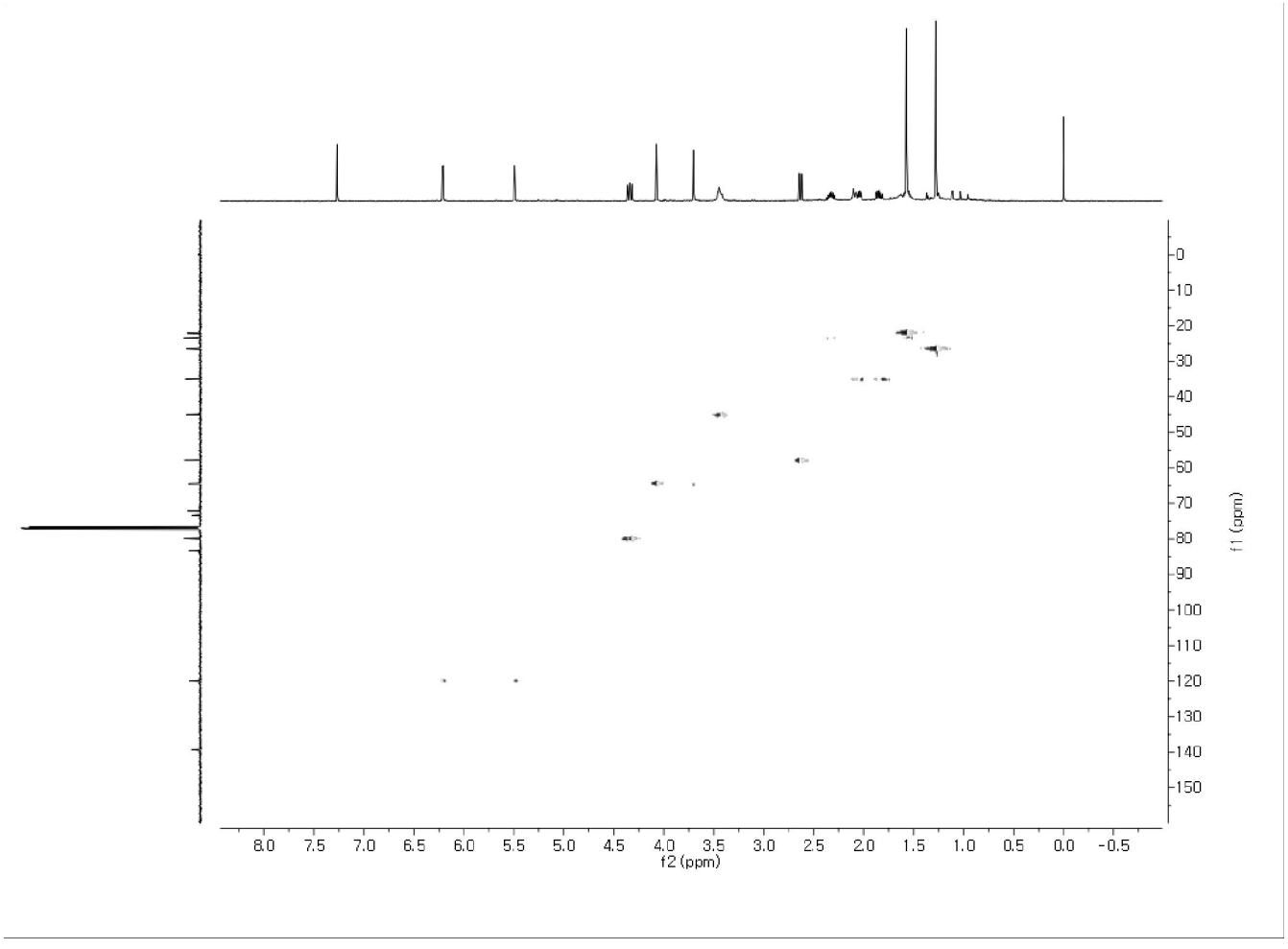

Fig. 10. HSQC spectrum of compound 2 (500MHz, $CDCl_3$)

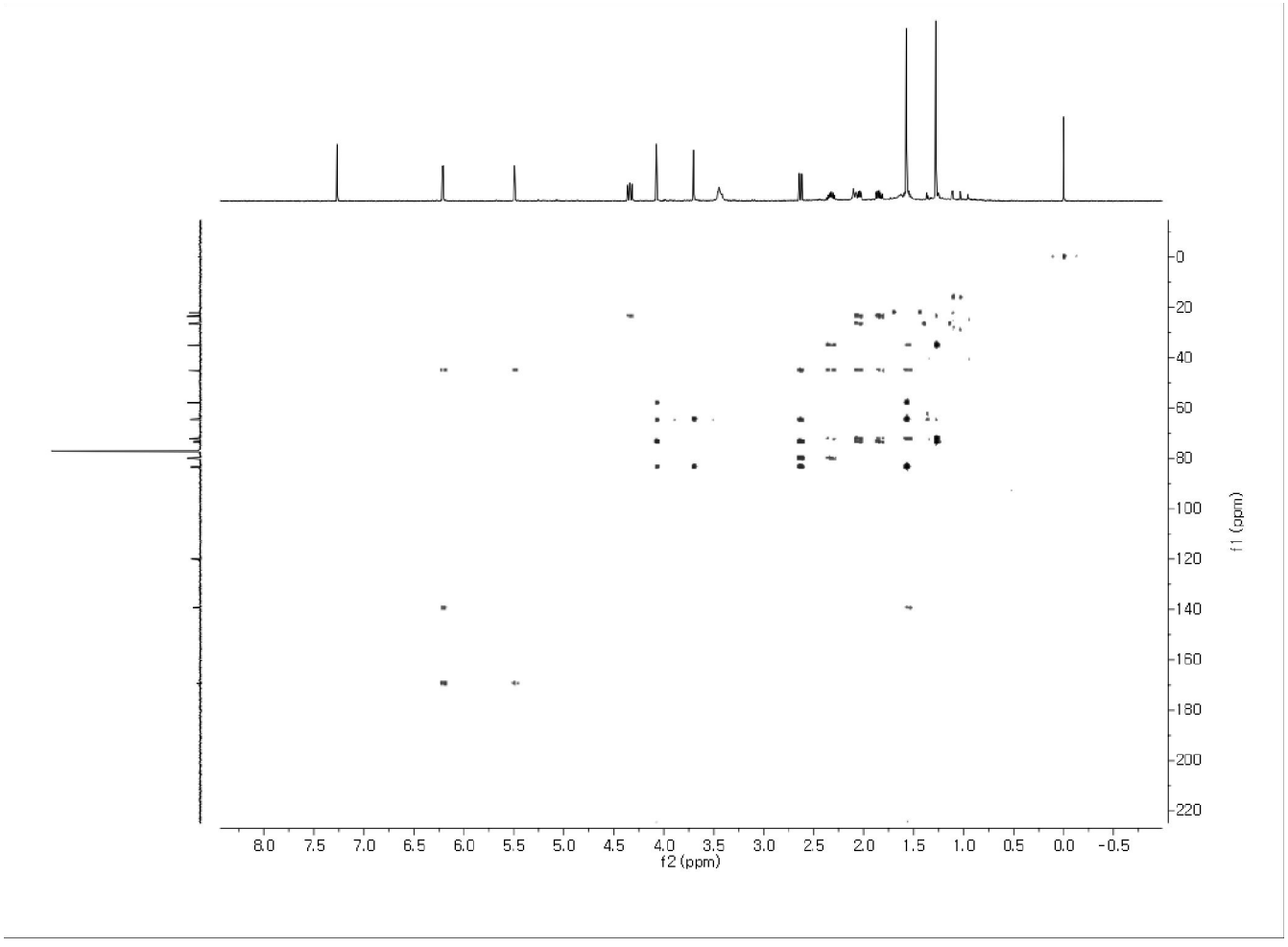

Fig. 11. HMBC spectrum of compound 2 (500MHz, $CDCl_3$)

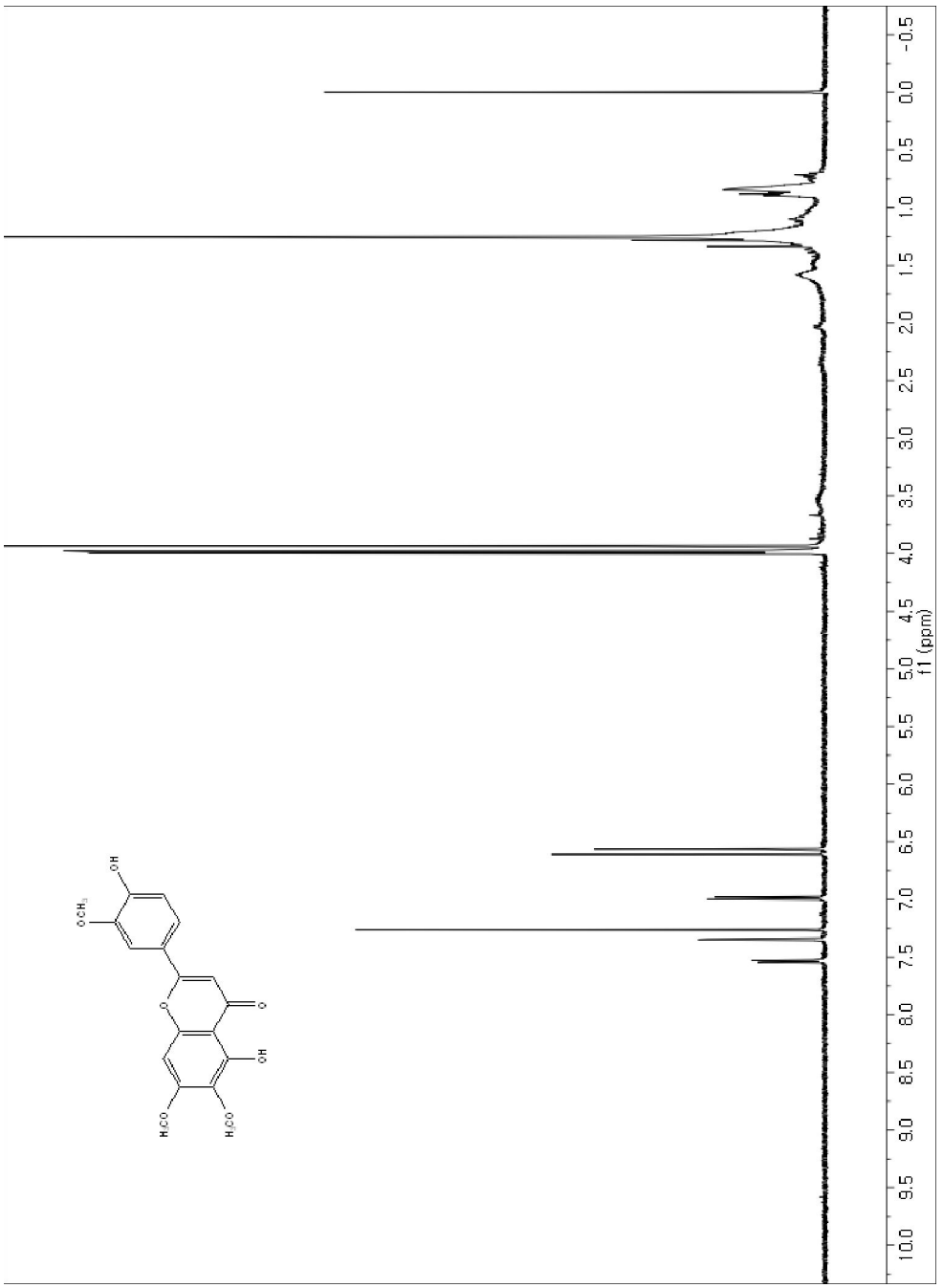

Fig. 12. ^{1}H-NMR spectrum of compound 3 (500MHz, $CDCl_3$)

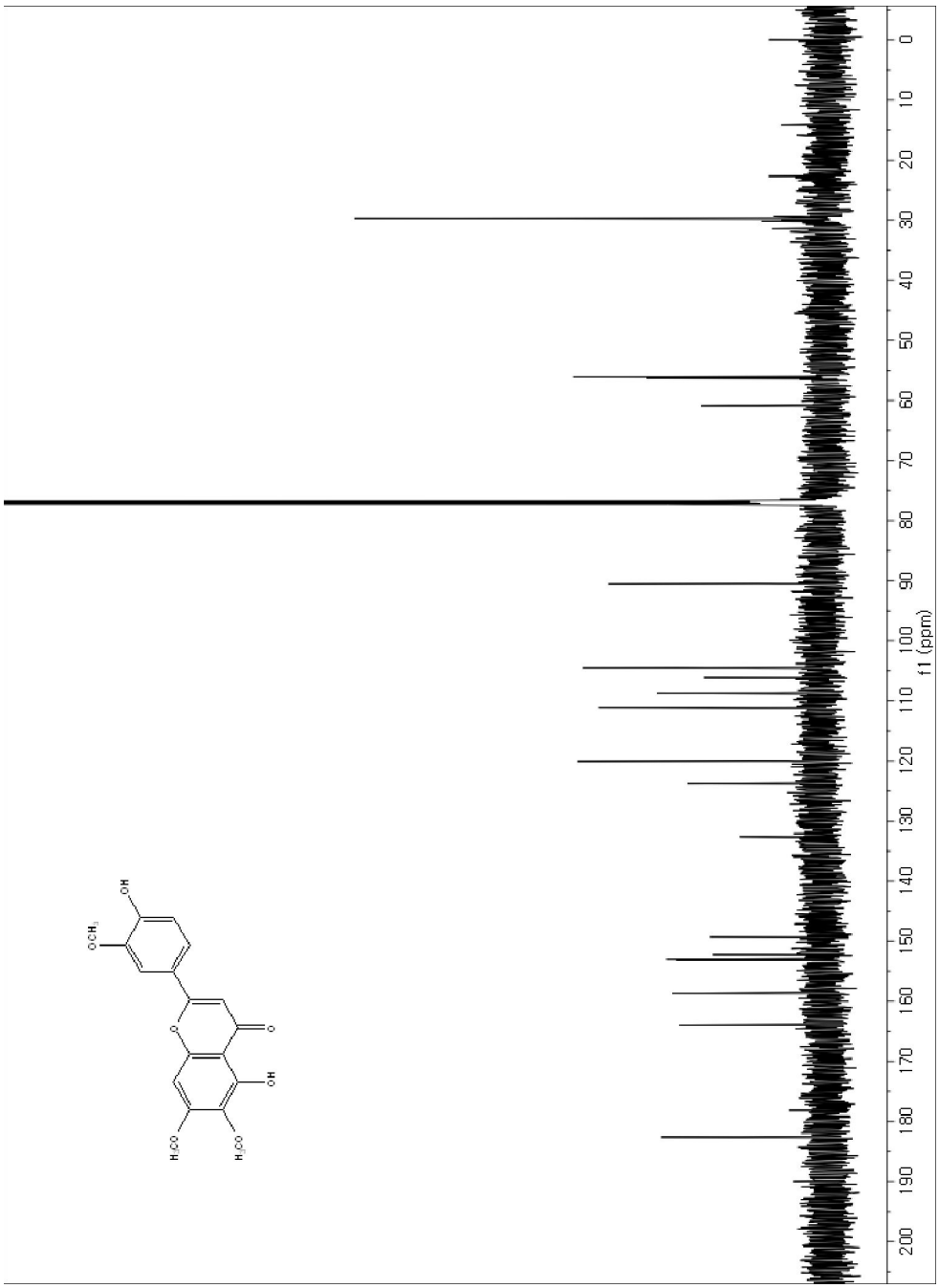

Fig. 13. ^{13}C-NMR spectrum of compound 3 (125MHz, $CDCl_3$)

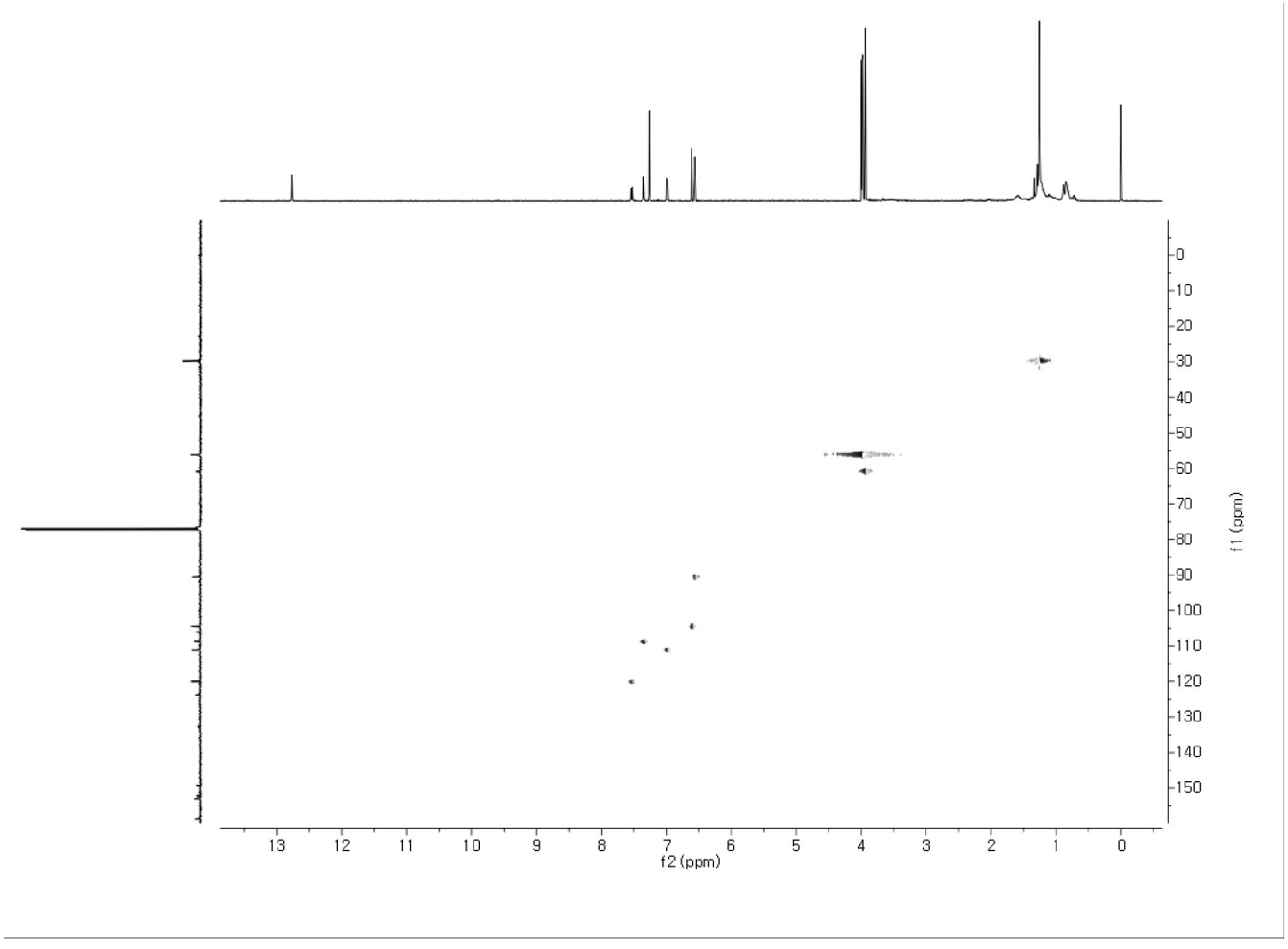

Fig. 14. HSQC spectrum of compound 3 (500MHz, $CDCl_3$)

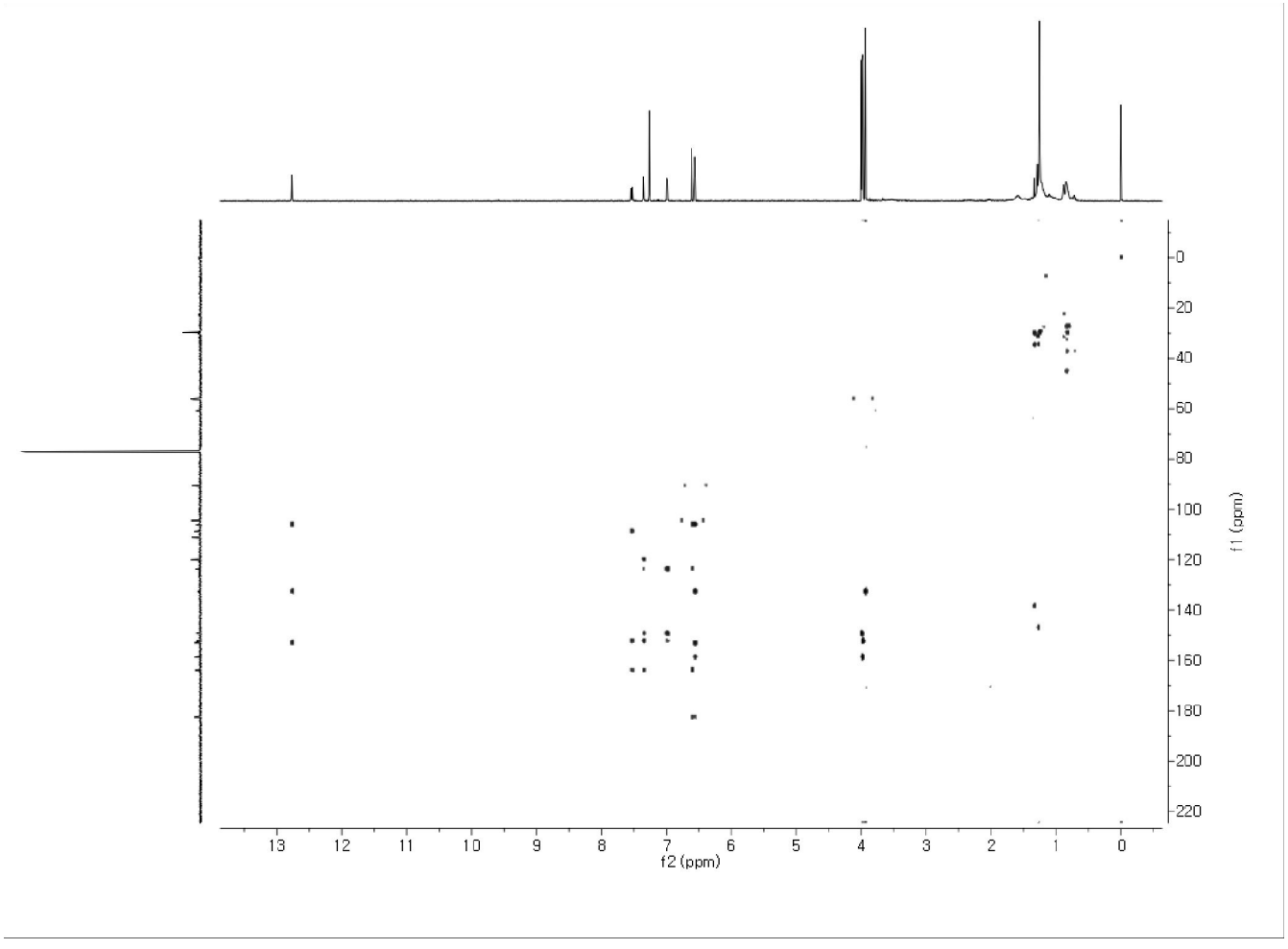

Fig. 15. HMBC spectrum of compound 3 (500MHz, $CDCl_3$)

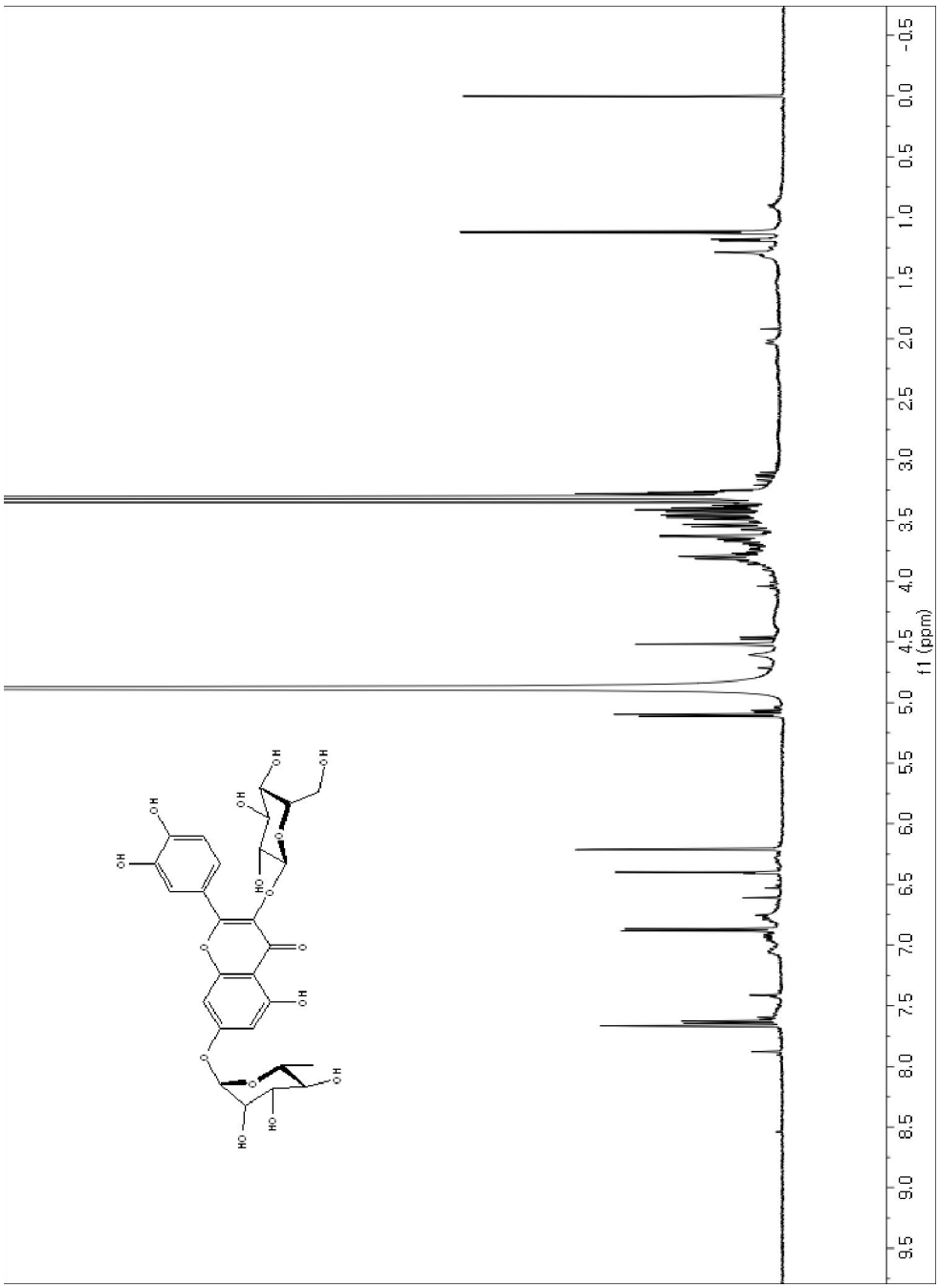

Fig. 16. ^{1}H-NMR spectrum of compound 4 (500MHz, CD_3OD)

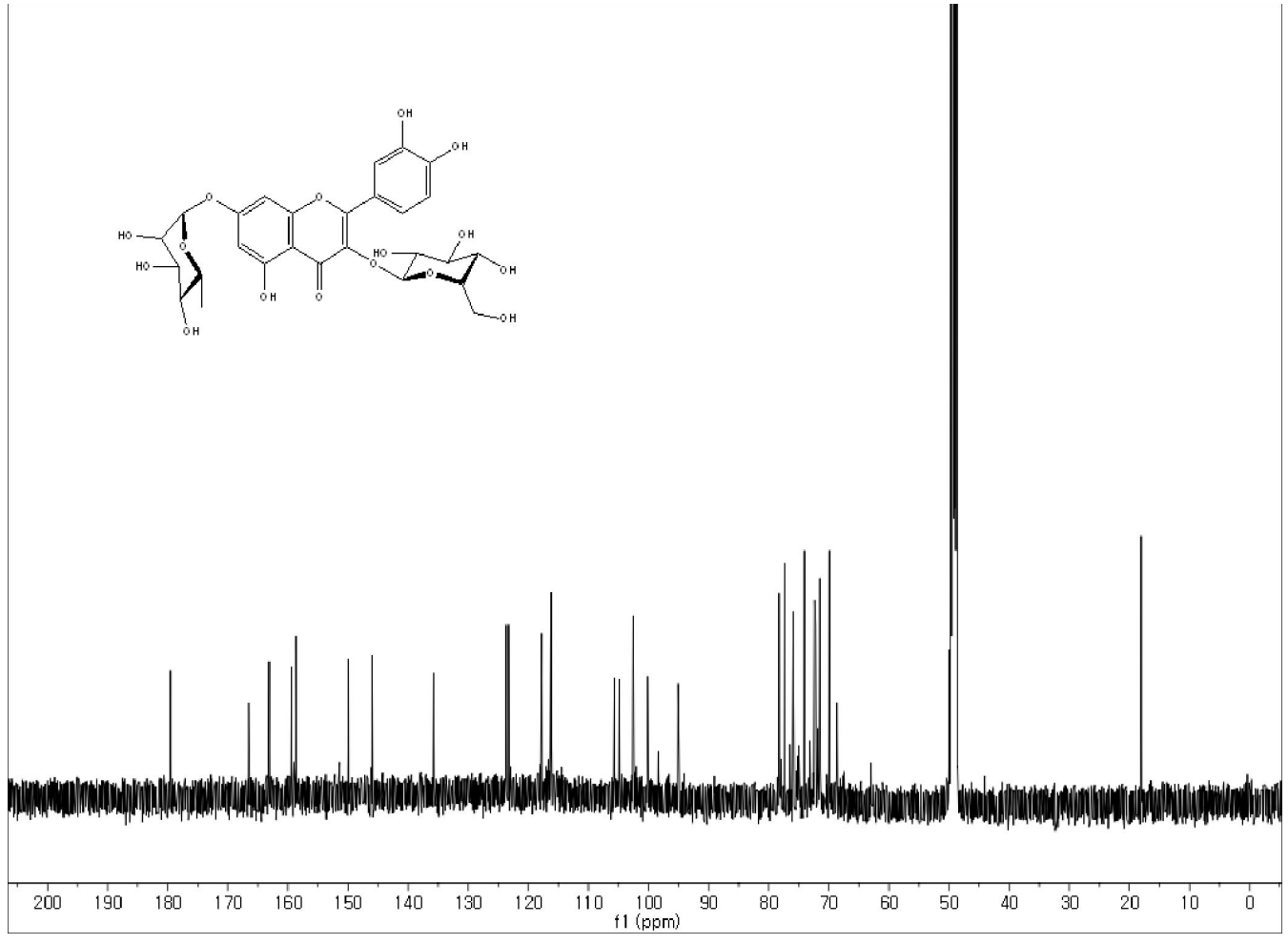

Fig. 17. ^{13}C-NMR spectrum of compound 4 (125MHz, CD_3OD)

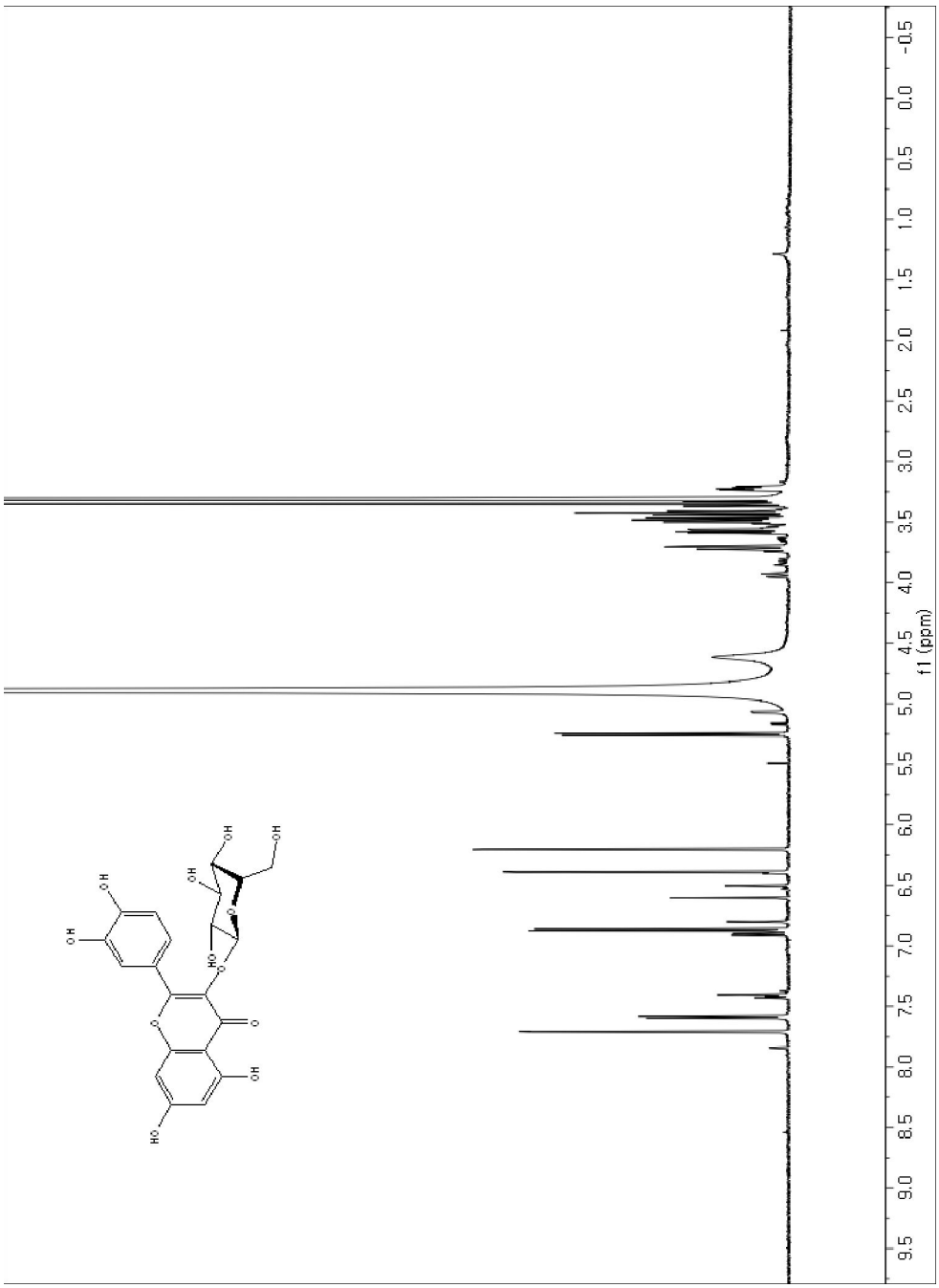

Fig. 18. ^{1}H-NMR spectrum of compound 5 (500MHz, CD_3OD)

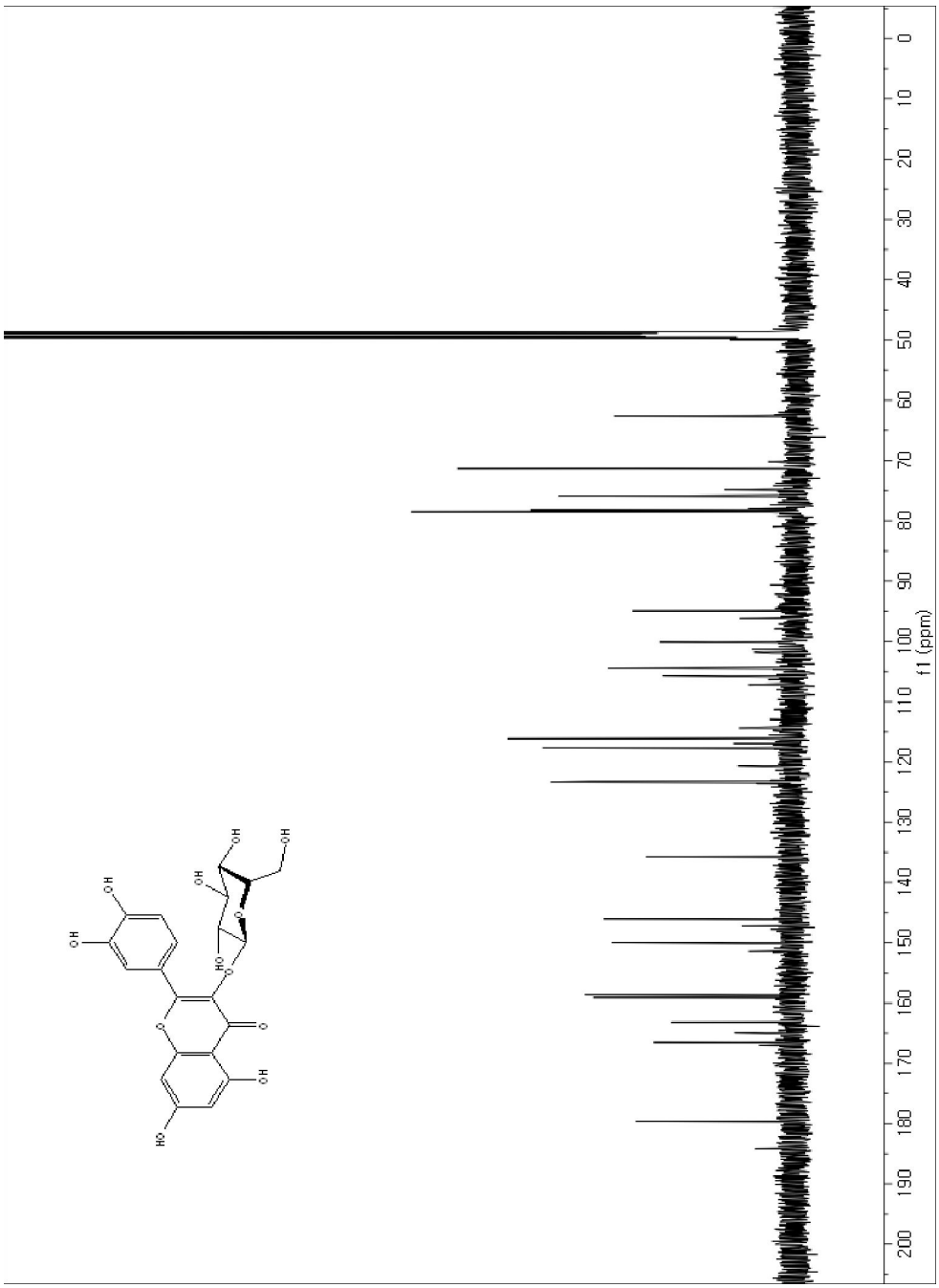

Fig. 19. ^{13}C-NMR spectrum of compound 5 (125MHz, CD_3OD)