Master's Thesis

Isolation and Identification of Anticancer Substances from *Peucedanum japonicum* Thunb. Roots

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ABSTRACT

Peucedanum japonicum Thunb. (Umbelliferae) is a wild plant of white flower blooming from June and August at the coast in Korea. This plant is well known to have antifungal, stamina, antifood poisoning and antiphlegm, anticancer agent. This study was conducted to isolate anticancer substance from *P. japonicum* roots and to evaluate its anticancer activity. Roots of *P. japonicum* were air-dried, chopped and extracted with 100% MeOH. The MeOH extract has been partitioned with $CHCl_3$ and BuOH. $CHCl_3$ layer were purified by repeated column chromatography and recrystallization. As results, two compounds isolated from $CHCl_3$ layer were identified as (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol (Comp. I) and anomalin (Comp. II) by NMR spectroscopic analysis. Quantitative analysis using UPLC (Ultra Performance Liquid Chromatography) revealed that roots of *P. japonicum* contain 0.015 (Comp. I) and 1.69 mg/g (Comp. II).

In vitro cytotoxic activity of Comp. I and Comp. II was evaluated against human cancer cell lines; HeLa, HepG2, SNU-16 and AGS by MTT assay. Comp. I showed the most potent cytotoxic activity against HepG2 cell (IC_{50} = 6.04 ㎍/mL), and Comp. II showed the most potent cytotoxic activity against SNU-16 cell (IC_{50} = 18.24 ㎍/mL) among the tested human cancer cell lines. However, no significant cell death was observed in CCD-25Lu (Human normal lung fibroblast cell).

Thus, these results indicated that the (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol and anomalin may serve as potential phytochemicals with anticancer activity.

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1. Introduction

Traditionally natural products have played an important role in drug discovery. Nature products is an attractive source of new therapeutic candidate compounds. Also, a tremendous chemical diversity have been found in millions of species of plants, animals, marine organisms and micro organisms. Natural products have been invaluable as tools for deciphering the logic of biosynthesis and as platforms for developing front-line drugs (Newman et al., 2000). For example, between 1981 and 2002, 5% of the 1,031 new chemical entities approved as drugs by the US Food and Drug Administration (FDA) were natural products, and another 23% were natural-product-derived molecules. Vincristine, irinotecan, etoposide and paclitaxel are examples of plant-derived compounds that are being employed in cancer treatment (Newman et al., 2003).

Today, millions of people are living with cancer or cancer patients. Cancer is the second leading cause of death in the United States (Hemminike et al., 2002). In korea, the incidence of cancers is increasing by western diet and low physical activity. Although the disease has therefore existed for at least several thousand years, its prevalence has been steadily increasing. In just the past 50 years, a person`s chance of developing cancer within his or her lifetime has doubled, and doctors are now examining more cases of the disease than ever before. If we live until average life expectancy, the probability of cancer occurrence is 26.1% (Kushi et al., 2006).

The oldest description of cancer was discovered in Egypt and dates back to approximately 1600 B.C. The term cancer, which meanes `crab` in Latin, was coined by Hippocrates. Cancer develops when cells in a part of the body begin to grow out of control. Even though there are many kinds of cancer, they all start because of abnormal

cells that grow out of control. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide more rapidly until the person becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries. Because cancer cells continue to grow and divide, they are different from normal cells. Cancer cells often travel to other parts of the body where they begin to grow and replace normal tissue (AICA., 2005). The potential of using natural products as anticancer agents was recognized in the 1950s by the U.S. National Cancer Institute (NCI) and has made major contributions to the discovery of new naturally occurring anticancer agents.

Peucedanum japonicum Thunb. is a perennial herb distributed in Korea, Japan, the Philippines, China, and Taiwan. *Peucedanum japonicum* Thunb. (*P. japonicum*) is a perennial plant up to 60 ~ 100 cm in height. Which is commonly known as Getgirumnamul in Korea and Botanbofu in Japan (Fig. 1). The leaves are frequently served as a vegetable or a garnish for raw fish in Jeju, Korea. The root was used for cough, cold, headache, and as an anodyne (Ikesshiro et al., 1992). The chemical constituents of *P. japonicum* have been studied to some extent and the khellactone coumarins were shown to be the characteristic components. Examination of the *P. japonicum* roots led to the isolation of four new khellactones esters and 17 compounds known as isoimperatorin, psoralen, bergapten, xanthotoxol, eugenin, cnidilin, (-)-selinidin, (-)-deltoin, (+)-pteryxin, (+)-peucedanocoumarin III, xanthotoxin, imperatorin, (-)-hamaudol, (+)-visamminol, (+)-marmesin, (+)-oxypeucedanin hydrate and (+)-peucedanol (Chen et al., 1995).

Figure 1. *Peucedanum japonicum* Thunb.

Some of the coumarins isolated from *P. japonicum* are reported to have antiplatelet (Chen et al., 1996; Hsiao et al., 1998; Jong et al., 1992), antiallergic (Takeuchi et al., 1991), antagonistic, and spasmolytic (Aida et al., 1998) activity. And *P. japonicum* leaf extract are reported to have strong antioxidant activity (Hisamoto et al., 2002). Also, hyuganin C isolated from stem of *P. japonicum* was reported to have anticancer activity (Jang et al., 2008) and it exhibit the most HL-60 cell (IC_{50} = 13.2 μg/mL), A549 cell (IC_{50} =18.1 μg/mL).

But, identification of anticancer substance has never been reported from *P. japonicum* roots. Thus, Isolation, identification, measurement, and anticancer activity have been studied in this paper.

2. Materials and Methods

2. 1. Plant material.

Roots of *P. japonicum* were collected from a wild population growing in Jeju, Korea during July, 2007.

2. 2. Reagents

RPMI 1640 medium, Dulbecco's modified Eagle's medium (DMEM), trypsin/EDTA, fetal bovine serum (FBS), penicillin, streptomycin were purchased from Invitrogen Life Technologies Inc. (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO), MTT were purchased from Sigma Chemical Co. (St. Louis, MO, USA). UPLC-grade water and acetonitrile were from EMD Chemicals (Darmstadt, Germany).

2. 3. Instruments.

UV spectra were measured on a Varian Cary100 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR at 500 MHz were obtained on a Bruker AM 500 spectrometer in $CDCl_3$. EIMS was obtained on a JEOLJMS-700 mass spectrometer. TLC was conducted on precoated Kieselgel 60F_{254} plates (Art. 5715; Merck) and the spots were detected either by examining the plates under a UV lamp or treating the plates with a 10% ethanolic solution of phosphomolybdic acid (Wako Pure Chemical Industries) followed by heating at 110°C. UPLC was performed using a Waters US/ACQUITY UPLC Module, Waters US/ACQUITY UPLC Photodiode PDA Detector and Bondapak^{TM} C_{18} column (1.7 μM 2.1 × 150 mm) (Waters, Ireland).

2. 4. Cell culture

HeLa (Human cervix cancer cells), HepG2 (Human hepatoblastoma cancer cells) and CCD-25Lu (Human normal lung fibroblast cell) cells were cultured at 37 ℃ in a humidified atmosphere under 5% CO_2 in DMEM containing 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. SNU-16, AGS (Human carcinoma cancer cell) cells were cultured at 37℃ in a humidified atmosphere under 5% CO_2 in RPMI 1640 containing 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. Exponentially growing cells were treated with various concentrations of the compound I and II as indicated.

2. 5. Solvent extraction and fractionation of *P. japonicum* roots

Ten kilograms of roots of *P. japonicum* were air-dried, chopped and extracted three times with 100% MeOH (72 L) for 14 days at room temperature. The combined extract was evaporated to dryness under reduced pressure at a temperature below 40℃. After filtration and concentration, the resultant extract (326 g) was suspended in H_2O (2 × 300 mL) and partitioned with organic solvents ($CHCl_3$, BuOH) of the different polarities to afford soluble-chloroform ($CHCl_3$, 84 g), soluble-butanol (BuOH, 168 g), and soluble-water (H_2O, 74 g) extracts, respectively (Fig. 2). The $CHCl_3$ extracts was subjected to column chromatography using silica gel with Methylene chloride (Mc) : ether (Et_2O) gradient.

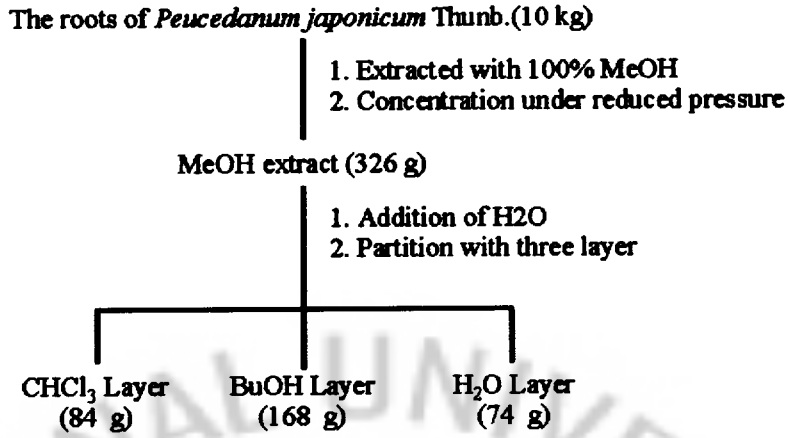

Figure 2. Solvent extraction scheme of *P. japonicum* roots.

2. 6. Column chromatography of $CHCl_3$ layer

The $CHCl_3$ layer (84 g) was subjected to column chromatography (glass column 10 x 80 cm) over silica gel (500 g; 70 - 230 mesh; Merk), eluted with gradient mixtures of Mc : Et_2O, of increasing polarity (200 : 1 → 1 : 10), and finally with MeOH. eight pooled fractions (Fraction. I - Fraction. VIII) were obtained after combining fractions with similar TLC profiles from this initial column chromatography.

2. 7. Column chromatography of Compound I

Fraction. VII (2.9 g) and silica gel (6.0 g, 70 - 230 mesh) was dissolved in a minimum volume of acetone and concentrated under vacuum. And these precoated samples were loaded onto the top of a glass column containing silica gel (300 g, 230 - 400 mesh) in hexane. Elution was carried out using mixtures of hexane and aceton to the ratio of 5 : 1→1 : 2 with increasing polarity. All together, 12 fractions of 35 mL each were collected and combined to give five subfractions (Fraction. VII. 1 – Fraction. VII. 5) based on the comparison of TLC profile using hexane : aceton (1 : 1) after

examination by shortwave UV light (254 nm) and by spraying with anisaldehyde in ethanol. From subfraction Fraction. VII. 3 was pooled and further purified using a small chromatographic column containing siliga gel (7 g, 230 - 400 mesh), eluting with hexane: ethyl acetoacetate (1 : 1) to afford compound I (24 mg).

2. 8. Column chromatography of Compound II

Fraction. IV (4.0 g) and silica gel (6.0 g, 70 - 230 mesh) was dissolved in a minimum volume of acetone and concentrated under vacuum. And these precoated samples are loaded onto the top of a glass column containing silica gel (300 g, 230 - 400 mesh) in hexane. Elution was carried out using mixtures of hexane and ethyl acetoacetate (EtoAc) to the ratio of 15 : 1 → 1 : 4 with increasing polarity. All together, 35 fractions of 100 ml each were collected and combined to give four subfractions (Fraction. IV. 1 – Fraction. IV. 4) based on the comparison of TLC profile using hexane:EtoAc (2 : 1) after examination by shortwave UV light (254 nm) and by spraying with anisaldehyde in ethanol. Fraction. IV. 2 from subfraction was pooled and further purified using a small chromatographic column containing siliga gel (7 g, 230 - 400 mesh), eluting with hexane: ethyl acetoacetate (2 : 1) to afford compound II (67 mg).

2. 9. UPLC apparatus and measurements.

The roots of *P. japonicum* (10 g) were extracted with 100 mL MeOH overnight in a vortex mixer at room temperature to form the final extract which was then centrifuged. The extracts used for UPLC analysis passed through a 0.20 μm filter (Advantec MFS, Inc. CA, USA) before injected into a reverse phase μBondapak^{TM} C_{18} and a 20 μL

portion of these solution was injected into the UPLC system. The mobile phase was water containing 0.1% formic acid (A) and acetonitrile (B). The linearly gradient of A - B (0 min 80 : 20, 2.5 min 80 : 20, 6 min 40 : 60, 8 min 0 : 100, 8.50 min 0 : 100, 9 min 80 : 20 v/v). The flow rate was adjusted to 0.4 mL/min and the wavelength of detection was set at 310 nm while the temperature was held constant at 30℃.

2. 10. Standard solution and calibration curves.

An external standard method was utilized for quantification. About 5 – 10 mg of a standard was dissolved in a 10 mL volumetric flask with MeOH to obtain the stock solution, and stored in a freezer. The working standard solutions were diluted to a series of concentrations with MeOH. The mean areas generated from the standard solutions were plotted against the concentration to establish calibration equations.

2. 11. Cell viability assay

The effect of the roots of *P. japonicum* on the viability of various cancer cell lines was determined by an MTT-based assay (Hansan et al., 1989). Briefly, exponential-phase cells were collected and transferred to a microtiter plate. The cells were then incubated for 72 hours in the presence of various concentrations of the roots of *P. japonicum*. After incubation, 5 mg/mL of MTT solution (Sigma, MO, USA) was added to each well and the cells were incubated at 37°C for 4 hours. The plates were then centrifuged at 2,500 rpm for 20 min at room temperature and the medium was carefully removed. DMSO (150 μL) was added to each well to dissolve the formazan crystals. The plates were read immediately at 570 nm on a Sunrise microplate reader (Sunrise, Tecan, Salzburg, Austria).

3. Results and Discussion

3. 1. Purification of anticancer substances from *P. japonicum* roots

$CHCl_3$ and BuOH layer were evaluated *in vitro* cytotoxic activity against human cervix cancer cell (HeLa) and human hepatoblastoma cancer cells (HepG2) by MTT assay. $CHCl_3$ layer had a potent cytotoxic activity. The $CHCl_3$ layer was subjected to column chromatography over silica gel eluted with gradient mixtures of Mc : Et_2O of increasing polarity. Eight pooled fractions were obtained after combining fractions with similar TLC profiles from this initial column chromatography (Fig. 3). All isolated fractions were evaluated *in vitro* cytotoxic activity against human cervix cancer cell (HeLa) and human hepatoblastoma cancer cells (HepG2) by MTT assay. The activity of isolated fractions (I - VIII) was evaluated at 400 ppm, fractions IV (Rf = 0.6) and VII (Rf = 0.4) had a potent cytotoxic activity. As results, two anticancer substances was purified from the fractions IV and VII, respectively (Fig. 4, 5).

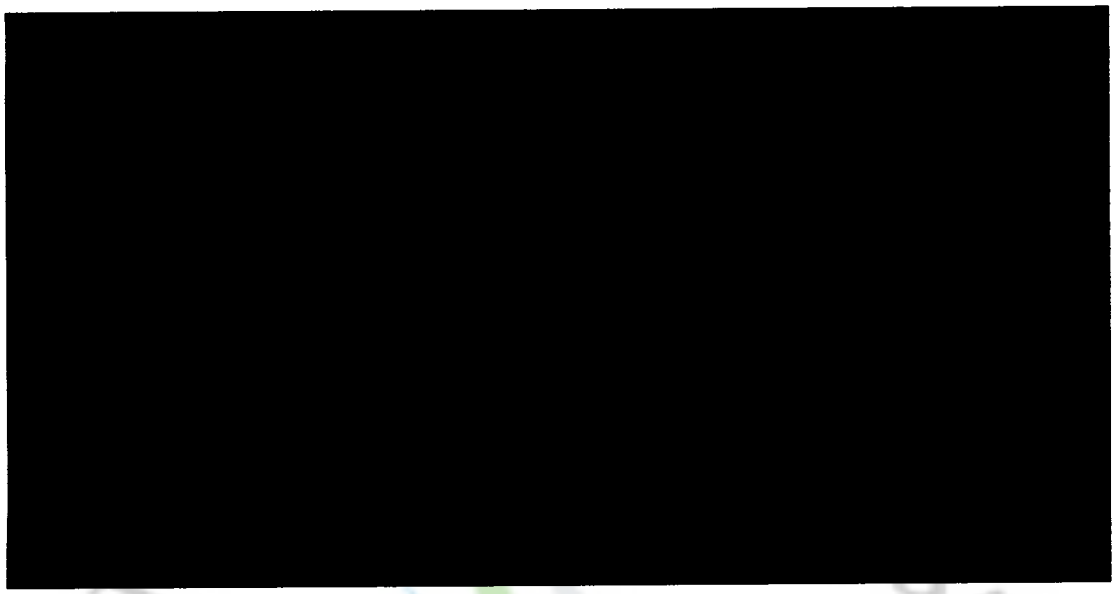

Figure 3. TLC of $CHCl_3$ layer.

Figure 4. TLC of compound I purified from fractions VII (Hexane : EtoAc = 1 : 1).

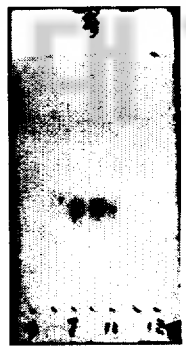

Figure 5. TLC of compound II purified from fractions IV (Hexane : EtoAc = 2 : 1).

3. 2. Identification of compound I and II isolated from *P. japonicum* roots

1) Structure elucidation of Compound I

The exact structure of compound I was inferred from a detailed analysis of ^{1}H and ^{13}C-NMR data, together with 2D-NMR experiments. The ^{1}H and ^{13}C-NMR data with DEPT experiments showed the presence of seventeen carbon atoms as one sp^2 methylene (δ_C 117.6, C-1) five sp^3 methylenes [δ_C (32.5, C-12), (29.1, C-13), (29.0, C-14), (31.9, C-15), (22.8, C-16)], one methyl carbons (δ_C 14.3, C-17), three methins [δ_C (63.7, C-3), (136.8, C-10), (126.9, C-11)] and four quaternary carbons [δ_C (78.2, C-4), (78.1, C-7), (70.3, C-5), (70.4, C-6)]. The ^{1}H-NMR data showed the evidence for five methylene protons [δ_H 5.46 (2H, m, H-12), 1.36 (2H, m, H-13), 1.25 (6H, m H-14, 15, 16)], one methyl groups δ_H 0.85 (3H, m, H-17), and five olefinic protons [δ_H 5.92 (1H, m, H-2), 5.85(1H, m, H-10), 5.48(1H, d, J = 6.5 Hz, H-11), 5.43(1H, m, H-1b), 5.23(1H, d, J = 10.0 Hz, H-1a)]. Compound I was obtained as amorphous yellow powder having the molecular formula of $C_{17}H_{24}O_3$ and a molecular ion peak at m/z 276. Thus, based on all the above obtained spectral data, the compound I was identified as (10E) 1,10-heptadecadiene-4,6-diyne-3,8,9-triol (Fig. 13). (10E) 1,10-heptadecadiene-4,6-diyne-3,8,9-triol were isolated from *Glehnia littoralis* (Matsuura et al., 1996). This compound was isolated from the roots of *P. japonicum* for the first time.

Table 1. NMR data of compound I (500 MHz, $CDCl_3$)[a]

Position	^1H	^{13}C [b]
1		117.6 (s)
2	5.92 (1H, m)	135.9 (d)
3	4.90 (1H, m)	63.7 (d)
4		78.2 (s)
5		70.3 (s)
6		70.4 (s)
7		78.1 (s)
8	4.23 (1H, d)	66.7 (d)
9	4.10 (1H, dd)	75.7 (d)
10	5.85 (1H, m)	136.8 (d)
11	5.48 (1H, d, J = 6.5 Hz)	126.9 (d)
12	5.46 (2H, m)	32.5 (t)
13	1.36 (2H, m)	29.1 (t)
14	1.25 (2H, m)	29.0 (t)
15	1.25 (2H, m)	31.9 (t)
16	1.25 (2H, m)	28.8 (t)
17	0.85 (3H, m)	14.3 (q)
1a	5.23 (1H, d, J = 10.0 Hz)	
1b	5.43 (1H, m)	

[a] Assignments were made by ^1H-^1H COSY, HMQC, and HMBC data. [b] Multiplicity was established from DEPT data.

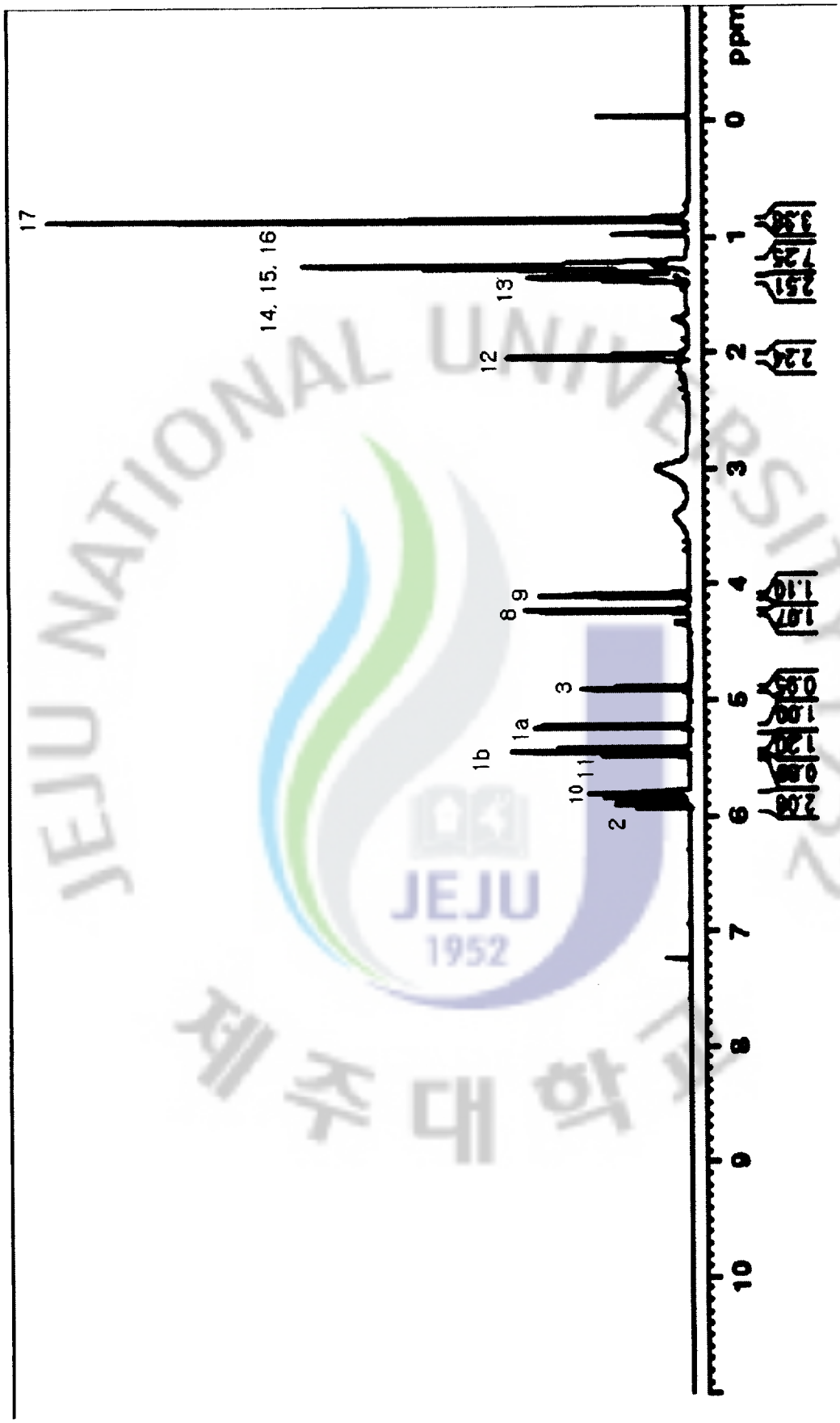

Figure 6. ^{1}H spectrums of compound I (500 MHz, $CDCl_3$).

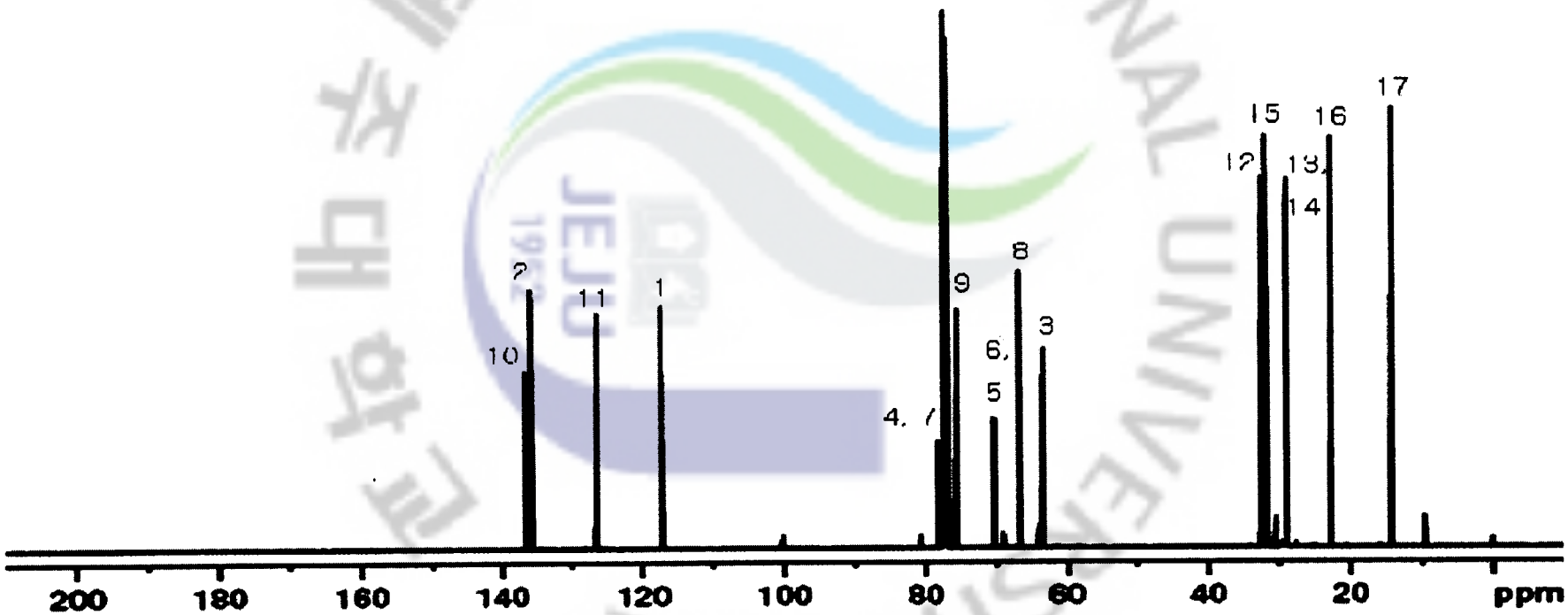

Figure 7. ^{13}C spectrums of compound I (500 MHz, $CDCl_3$).

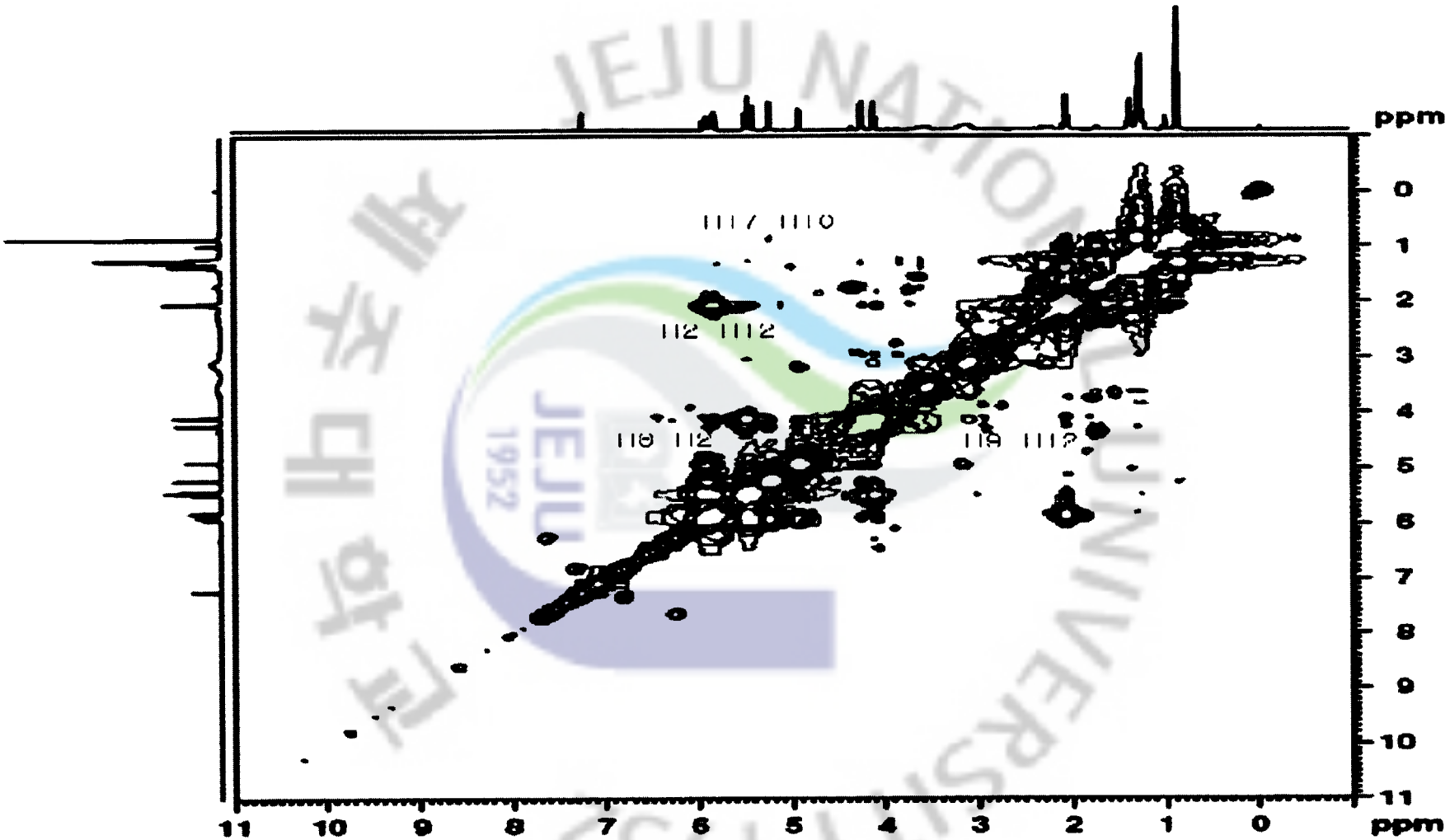

Figure 8. COSY spectrums of compound I (500 MHz, $CDCl_3$).

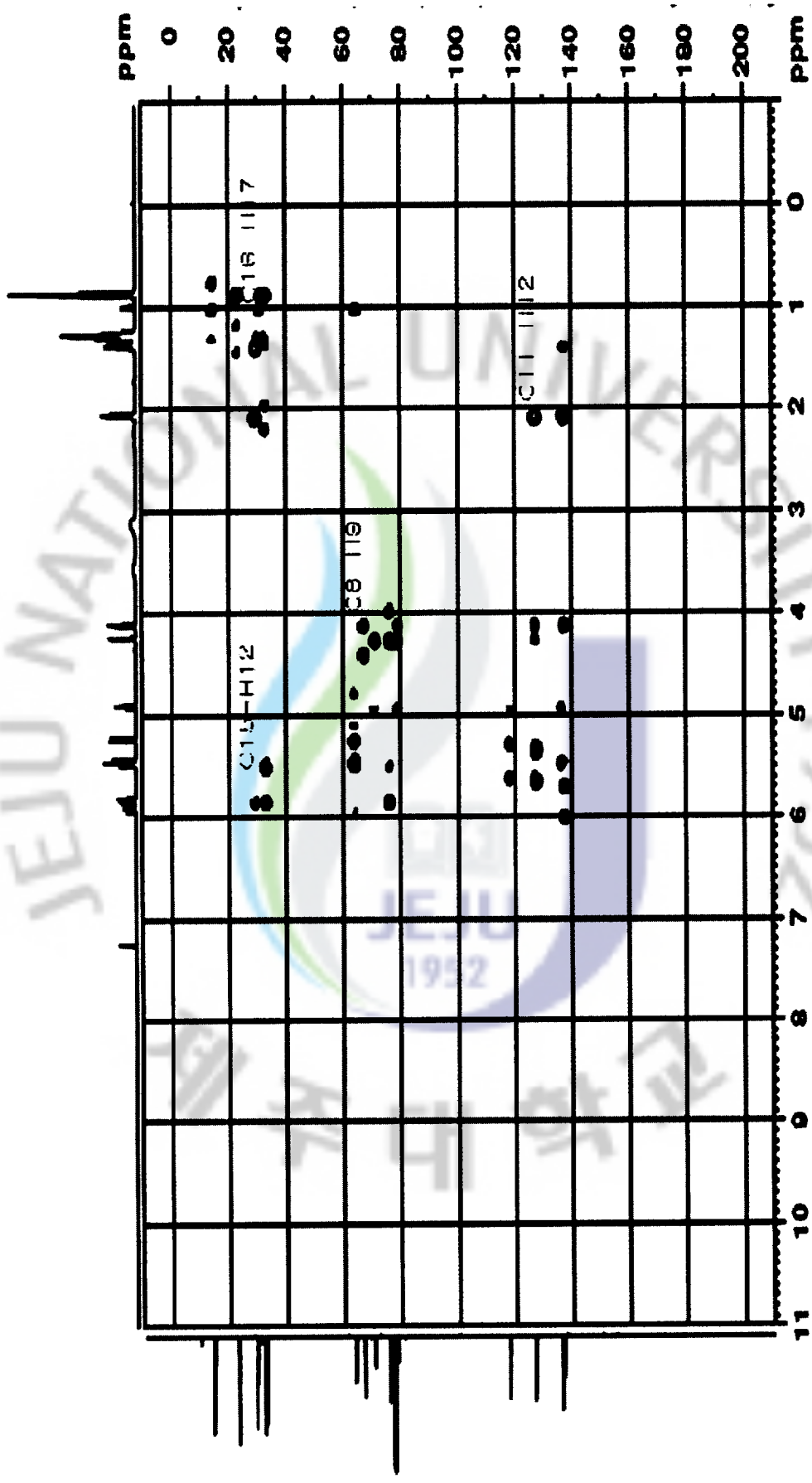

Figure 9. HMBC spectrums of compound I (500 MHz, $CDCl_3$).

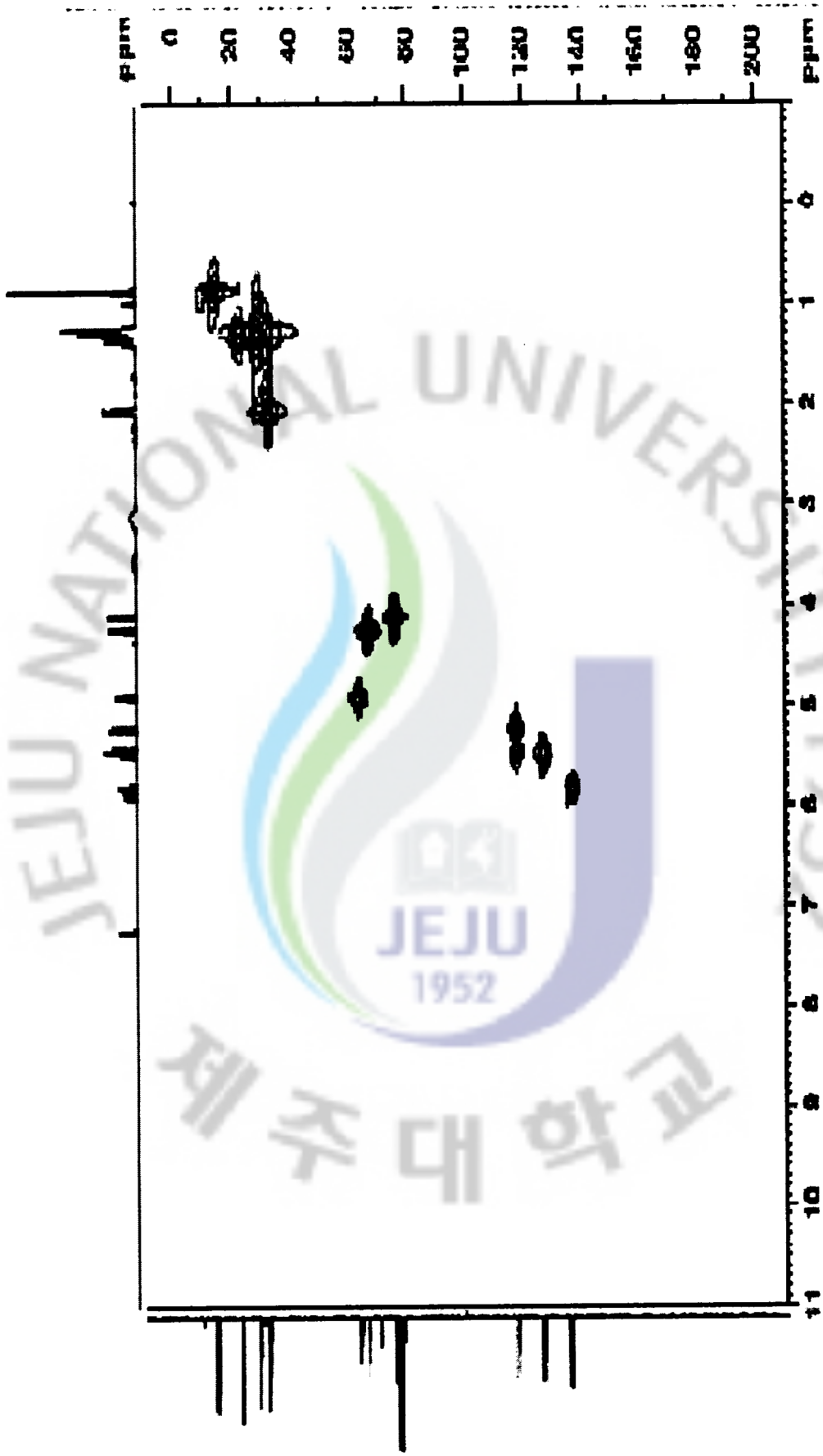

Figure 10. HMQC spectrums of compound I (500 MHz, $CDCl_3$).

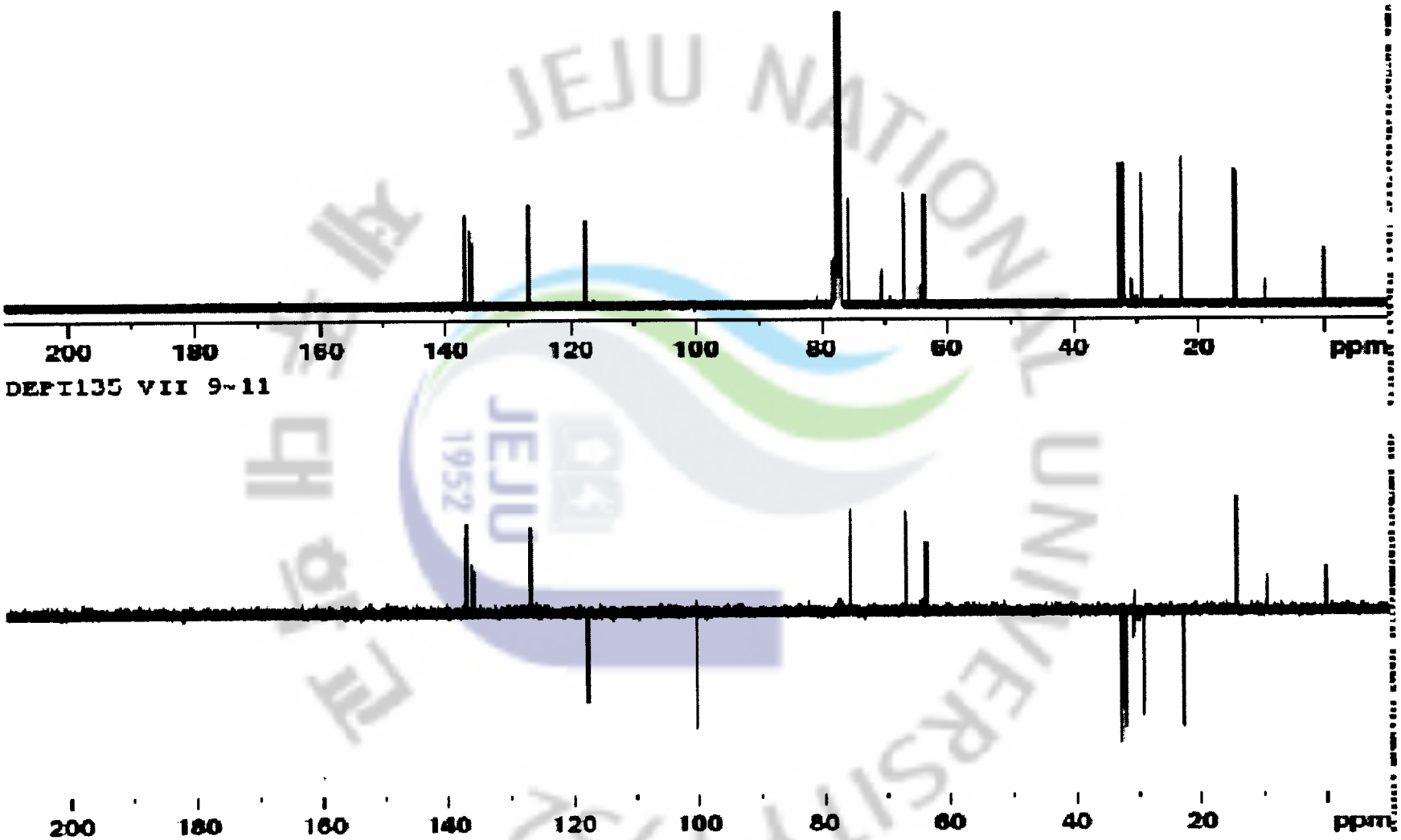

Figure 11. DEPT spectrums of compound I (500 MHz, $CDCl_3$)

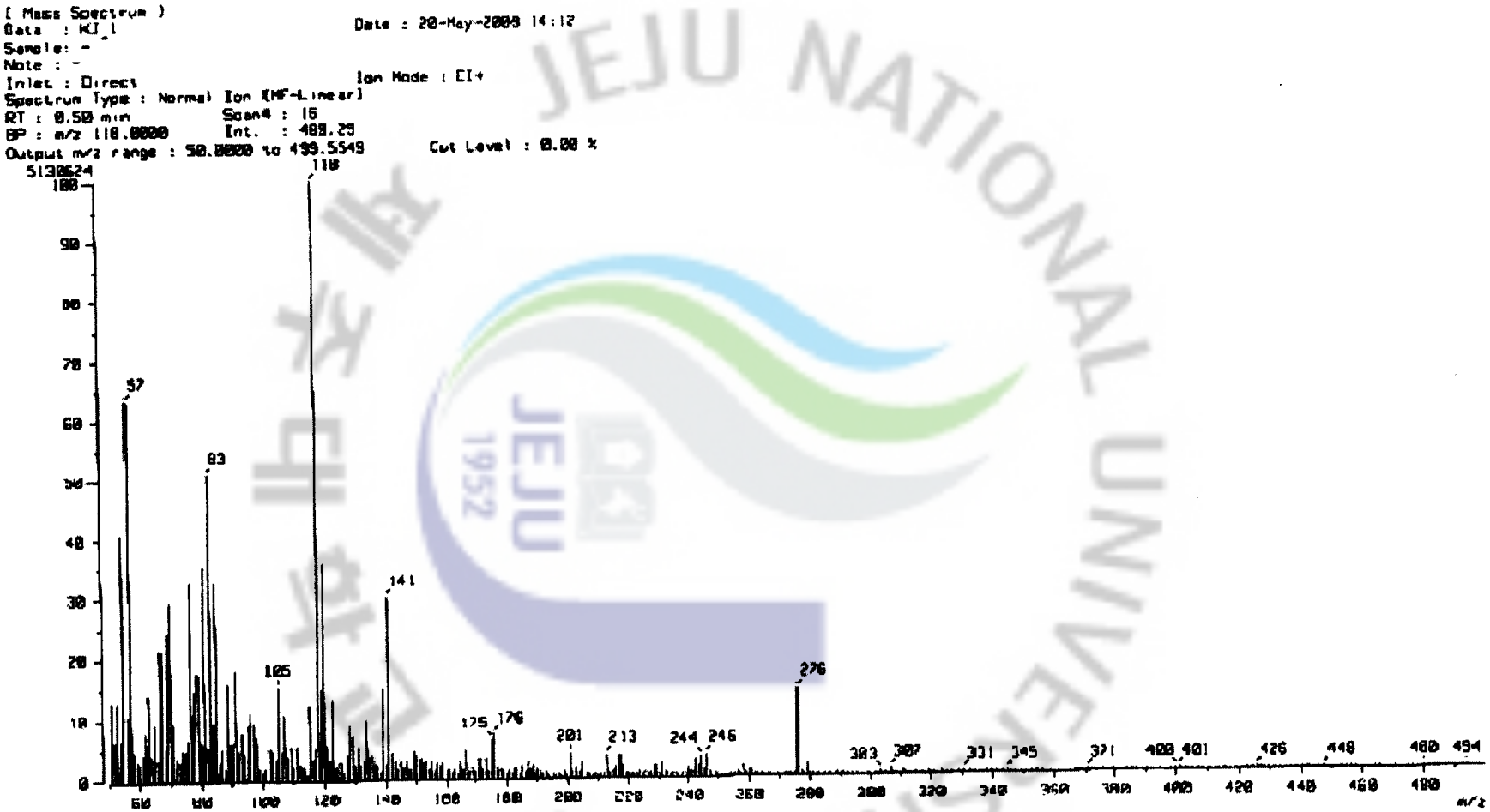

Figure 12. Mass spectrums of compound I.

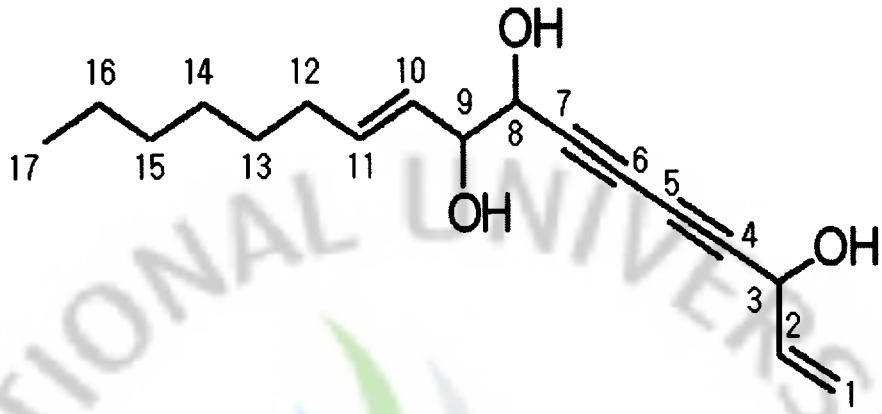

Figure 13. Structure of (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol.

2) Structure elucidation of Compound II

The exact structure of compound II was inferred from a detailed analysis of ^{1}H and ^{13}C-NMR data, together with 2D-NMR experiments. The ^{1}H and ^{13}C-NMR data with DEPT experiments showed the presence of twenty seven carbon atoms as four sp^3 methylene [δ_C (27.6, gem-CH_3), (25.2, gem-CH_3), (22.8, angeloyl-CH_3), (20.5, angeloyl-CH_3)], four methins [δ_C (143.3, C-4), (129.1, C-5), (114.5, C-6), (113.4, C-3)] and four quaternary carbons [δ_C (157.0, C-7), (154.3, C-9), (112.7, C-10), (107.8, C-8)]. The ^{1}H-NMR data showed the evidence for five methylene protons [δ_H 1.43 (3H, s, gem-CH_3), 1.39 (3H, s, gem-CH_3), 2.14 (6H, m, CH_3), 1.85 (6H, angeloyl - CH_3)], four aromatic protons [δ_H 7.55 (1H, d, J = 9.5 Hz, H-4), 7.31 (1H, d, J = 9.0 Hz, H-5), 6.77 (1H, d, J = 8.5 Hz, H-6), 6.17 (1H, d, J = 9.5 Hz, H-3)]. Compound II was obtained as amorphous yellow powder having the molecular formula of $C_{24}H_{26}O_7$. Thus, based on all the above obtained spectral data, the compound II was identified as anomalin (Fig. 20). It has been reported that anomalin was isolated from *Saposhnikovia divaricata* Schischk, *flaccida Kommarov* and *P. japonicum* (Kim, 2008; Woo et. al., 1988).

Table 2. NMR data of compound II (500 MHz, $CDCl_3$)[a]

Position	^1H	^{13}C [b]
2		160.1 (s)
3	6.17 (1H, d, J = 9.5 Hz)	113.4 (d)
4	7.55 (1H, d, J = 9.5 Hz)	143.3 (d)
5	7.31 (1H, d, J = 9.0 Hz)	129.1 (d)
6	6.77 (1H, d, J = 8.5 Hz)	114.5 (d)
7		157.0 (s)
8		107.8 (s)
9		154.3 (s)
10		112.7 (s)
2`		77.9 (s)
3`	5.33 (1H, d)	69.6 (d)
4`	6.59 (1H, d)	60.0 (d)

[a] Assignments were made by ^1H-^1H COSY, HMQC, and HMBC data. [b] Multiplicity was established from DEPT data.

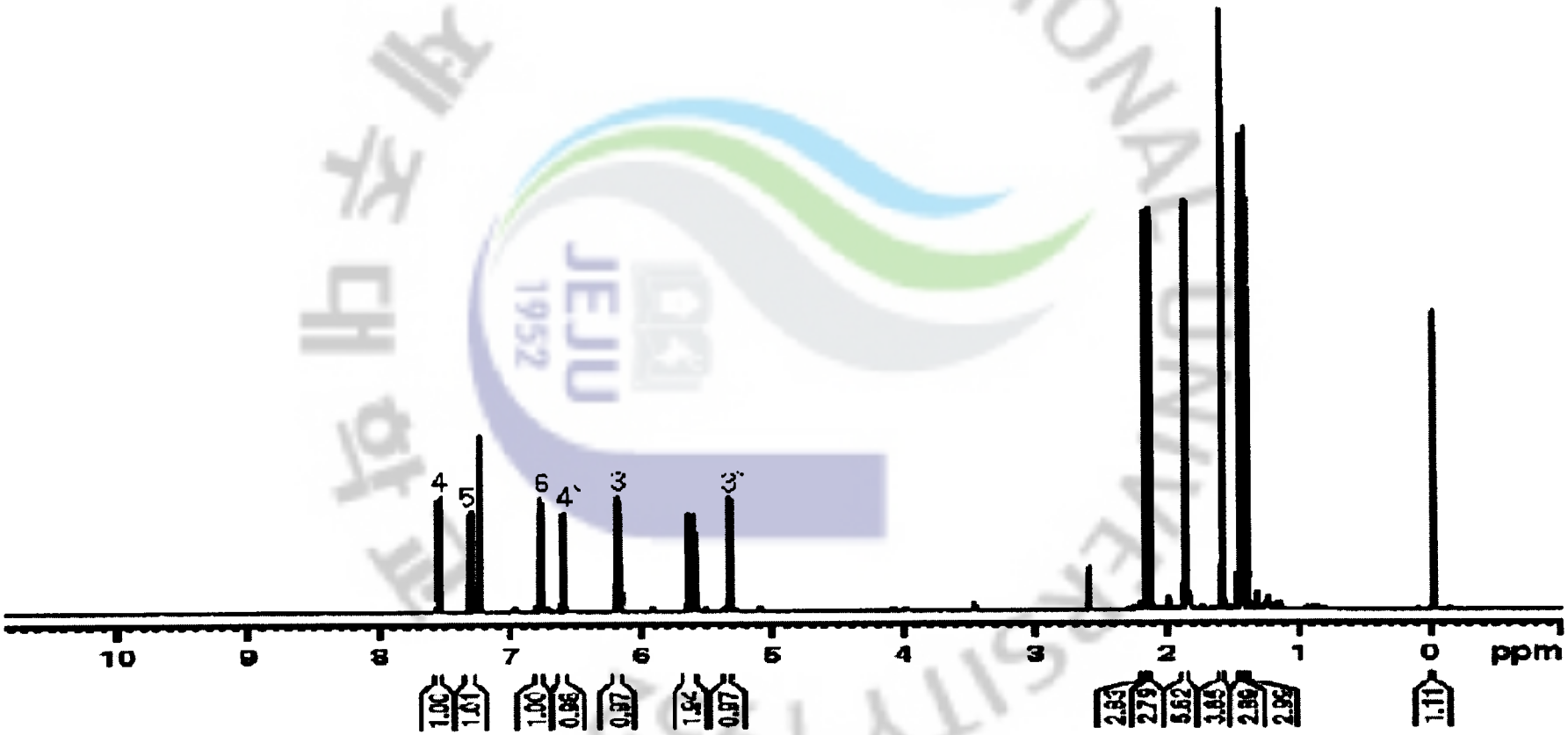

Figure 14. ^{1}H spectrums of compound II (500 MHz, $CDCl_3$).

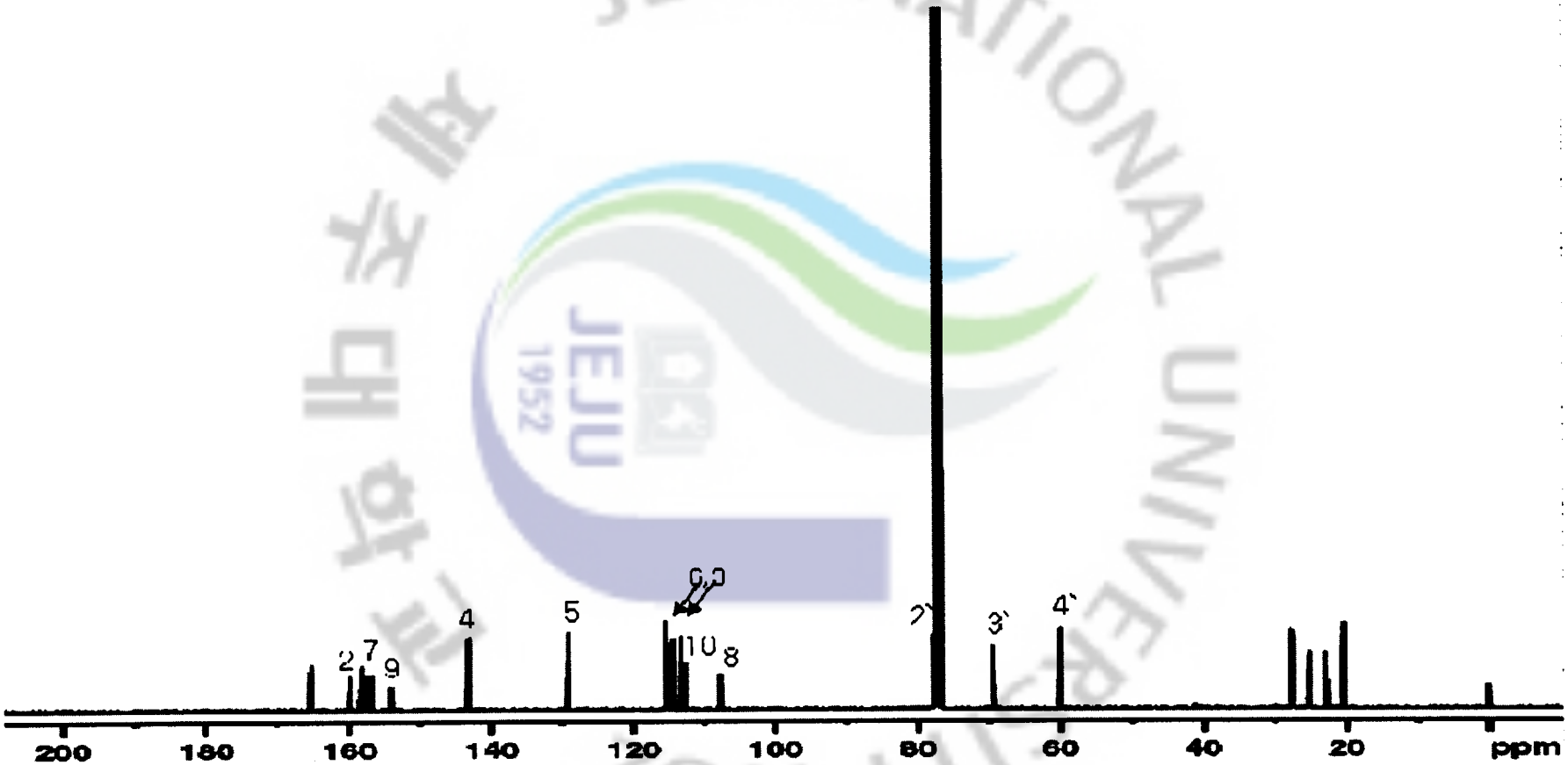

Figure 15. ^{13}C spectrums of compound II (500 MHz, $CDCl_3$).

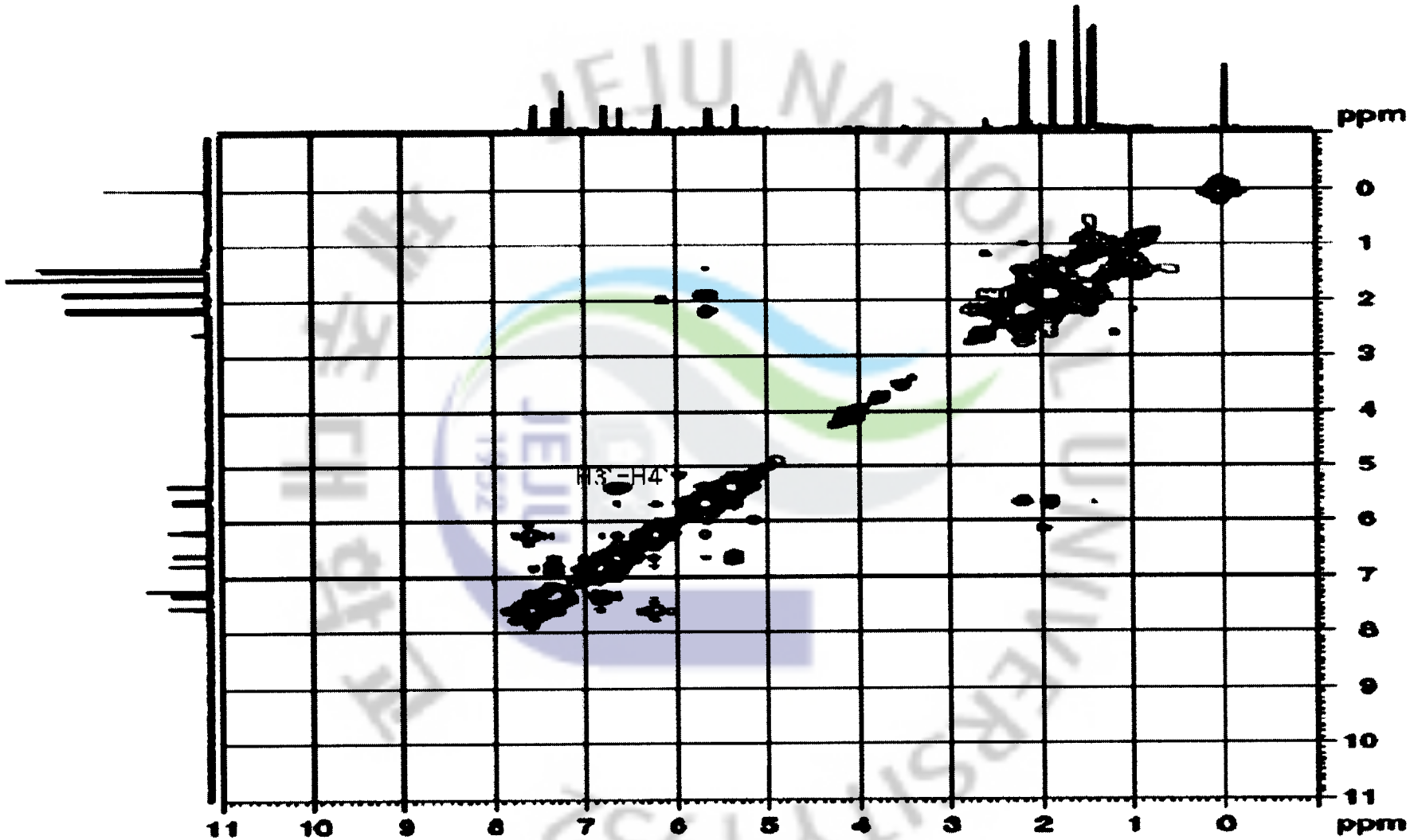

Figure 16. COSY spectrums of compound II (500 MHz, $CDCl_3$).

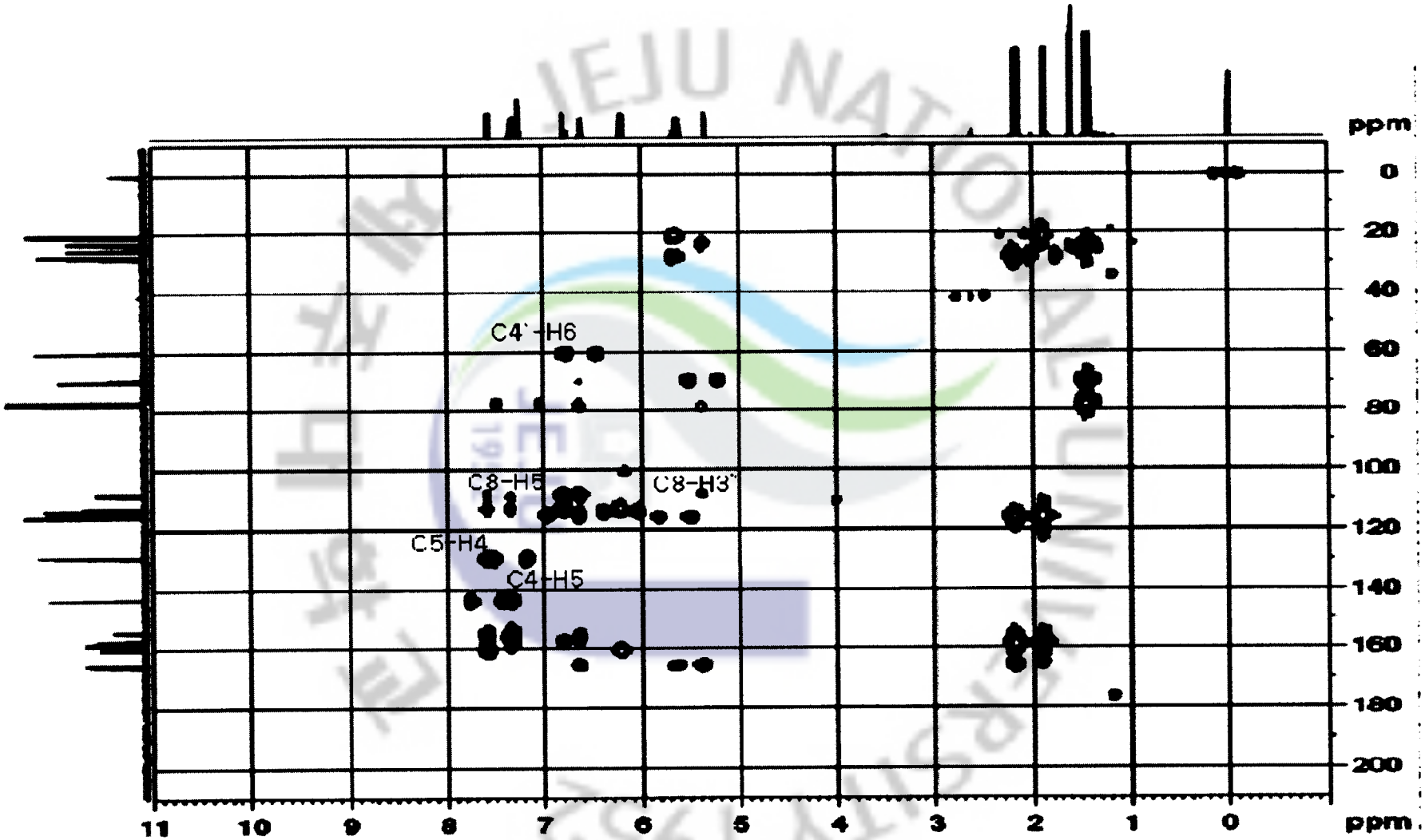

Figure 17. HMBC spectrums of compound II (500 MHz, $CDCl_3$).

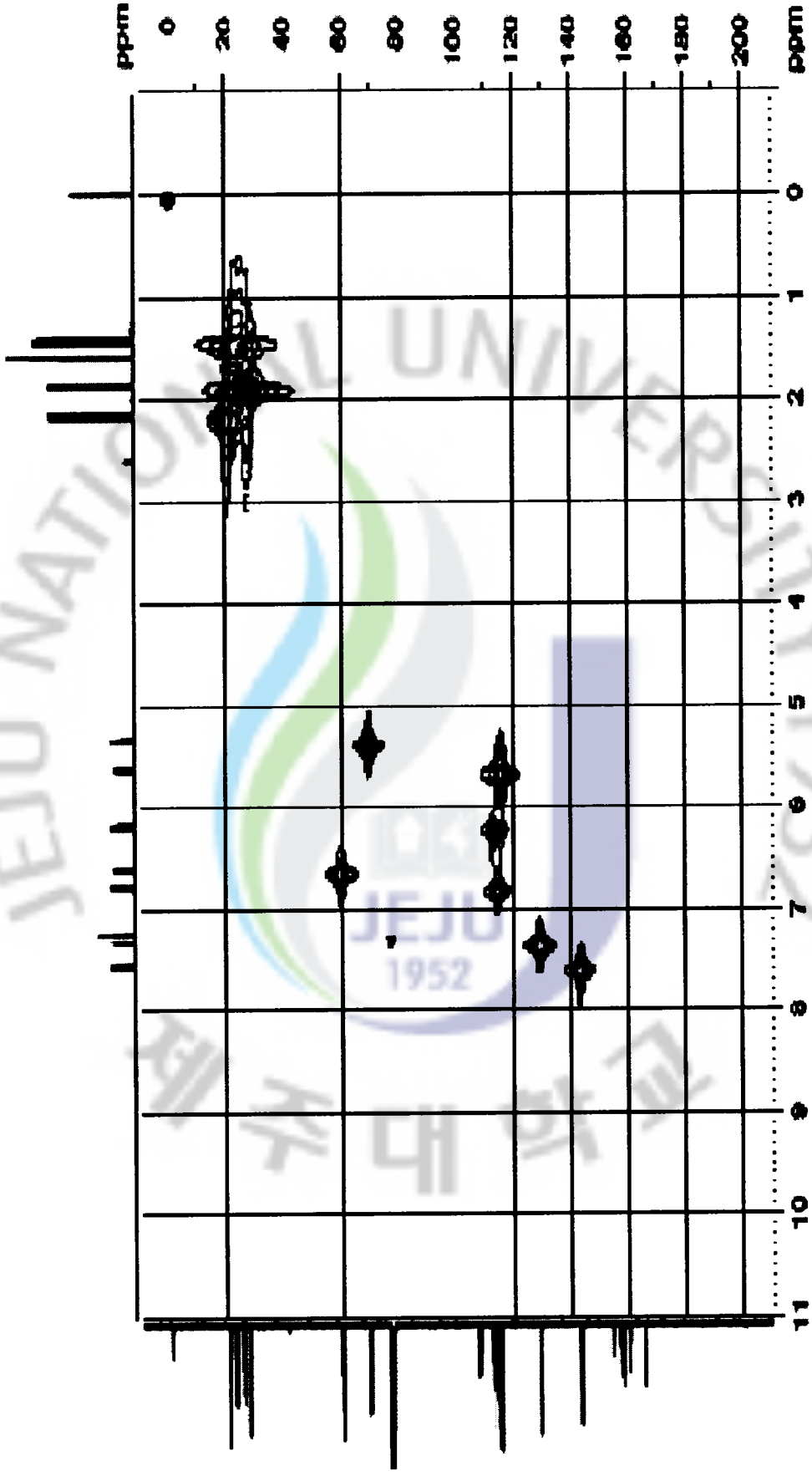

Figure 18. HMQC spectrums of compound II (500 MHz, $CDCl_3$).

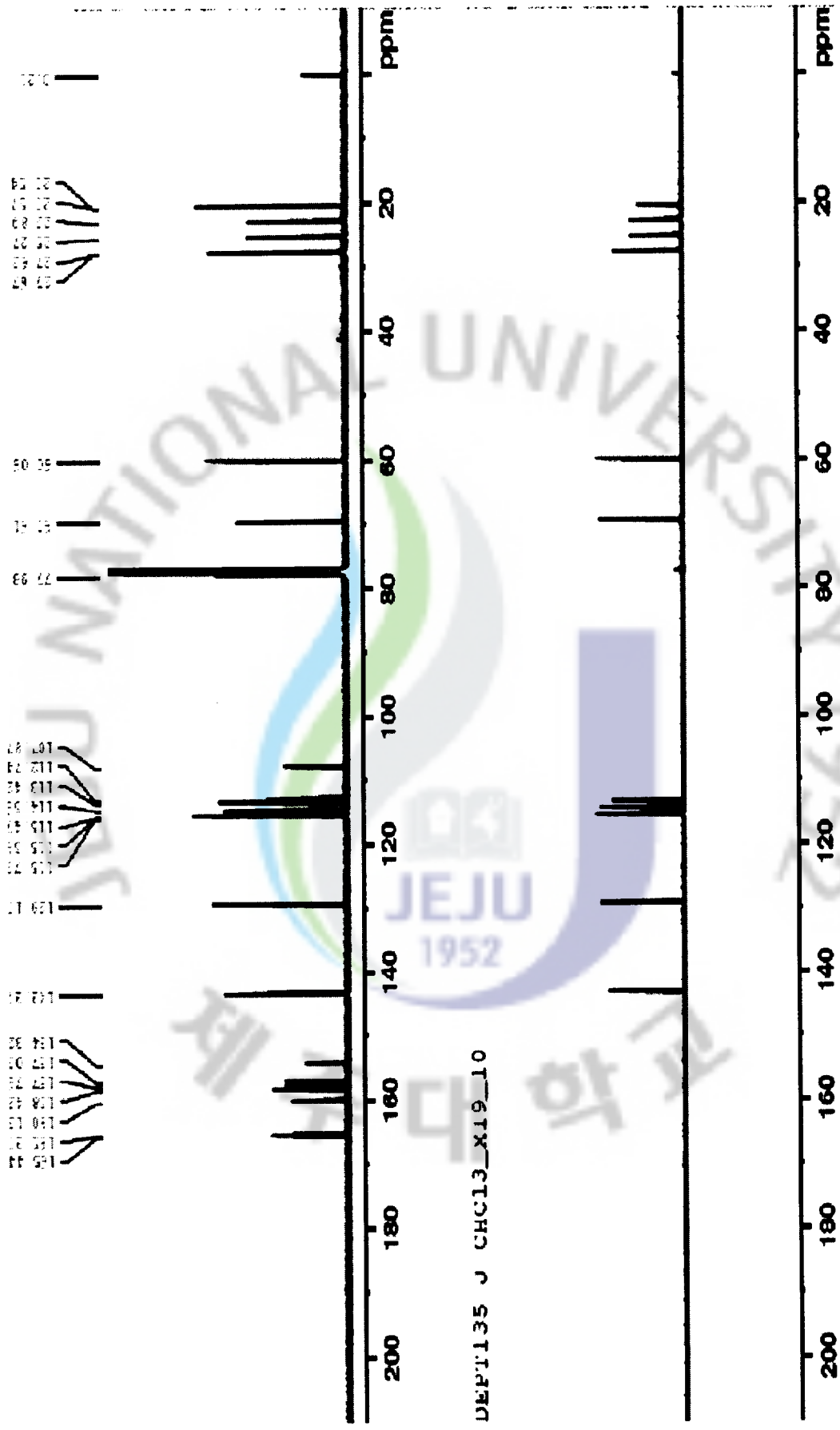

Figure 19. DEPT spectrums of compound II (500 MHz, $CDCl_3$).

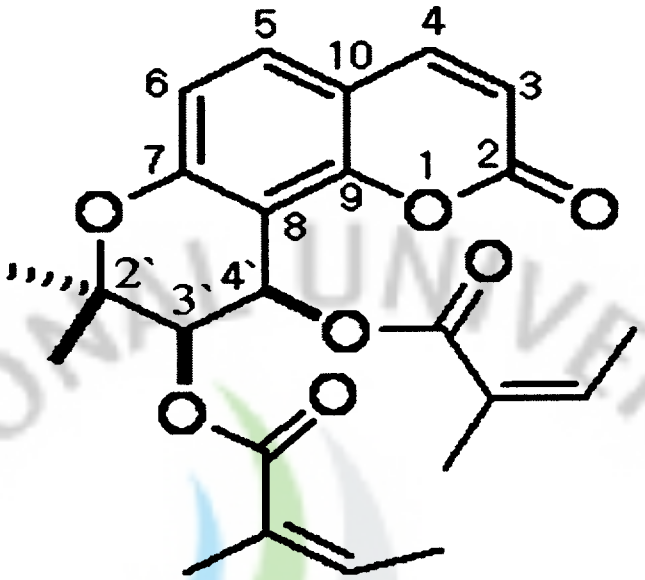

Figure 20. Structure of anomalin.

3. 3. Effect of the (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol and anomalin on the growth of canner cell

The effect of various concentrations of the (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol (Comp. I) and anomalin (Comp. II) on the growth of HeLa, HepG2, AGS and SNU-16 cells was examined by a MTT assay. The percent viabilities of all cells at 6.25, 12.5, 25, 50, and 100 μg/mL were decreased compared with the controls. The inhibition of cell growth was dose-dependent. Quercetin, chrysin and linoleic acid were reported to have anticancer activity in these cell lines. HeLa and HepG2 cell was inhibited by 58% and 60% treatment with 25 μg/mL of quercetin and chrysin, respectively (Zhang, 2004; Romos et. Al., 2008). AGS cell was inhibited by 78% treatment with 200 μg/mL of linoleic acid (Kwon, et. Al., 2008). Comp. I had the most potent cytotoxic activity against HeLa, HepG2, AGS and SNU-16 cell. Comp. I exhibited 69%, 92% and 78% inhibition at a concentration of 25 μg/mL on HeLa, HepG2 and SNU-16 cell, respectively. Also, Comp. I exhibited 82% inhibition at a concentration of 100 μg/mL on AGS cell (Fig. 21 – 24). Comp. II had also potent cytotoxic activity against HeLa and SNU-16 cell. Comp. II exhibited 63% and 71% inhibition at a concentration of 25 μg/mL on HeLa and SNU-16 cell, respectively (Fig. 21, 24). However, no significant cell death was observed in incubations of CCD25Lu (human normal lung fibroblast) with Comp I and II (Fig. 25). In these results, Comp. I and II showed the potent cytotoxic activity against several kinds of the human cancer cell lines. Although Comp. I and II were reported to have antibacterial and antifungal activity (Matsuura, 1996; Yasumasa, et. al., 1992), they have never been reported for anticancer activity.

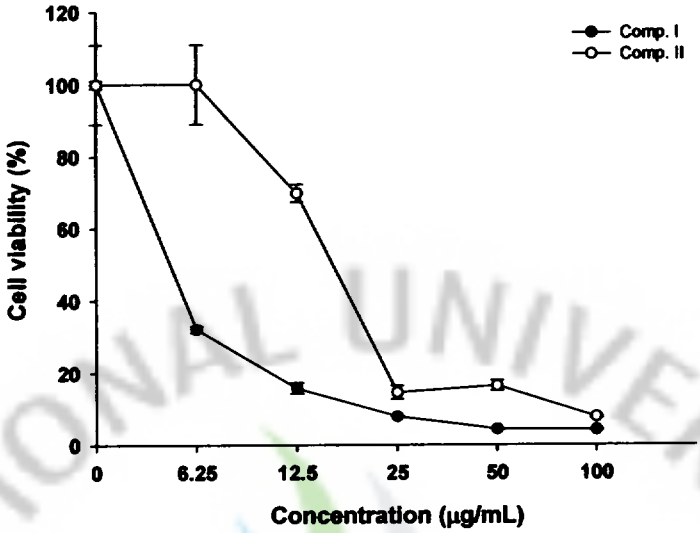

Figure 21. Growth inhibition of HeLa Human cervical cancer cell by Comp. I and II. Hela cells, plated at density of 1 x 10^4 cells/mL, were incubated with various concentration of Comp. I, II (6.25-100 μg/mL) for 48 hr. Viable cells were evaluated by MTT assay. Data show the only viable cells and are expressed as means S.D. ± (n = 4).

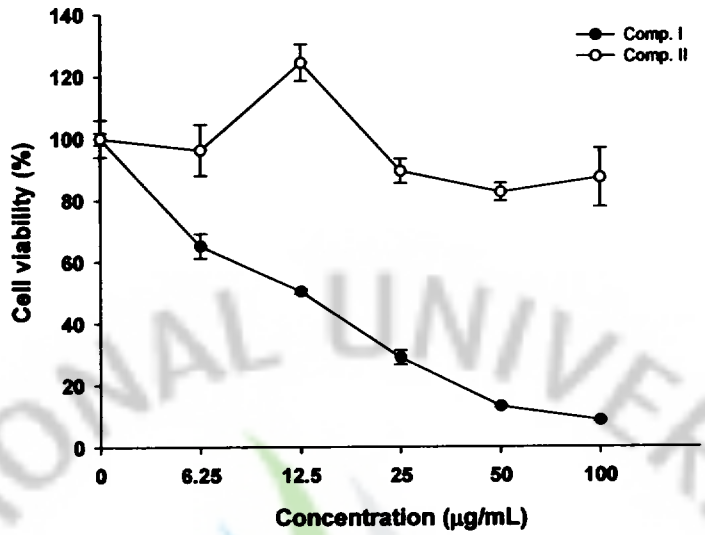

Figure 22. Growth inhibition of HepG2 Human hepatoblastoma cancer cell by Comp. I and II. HwpG2 cells, plated at density of 1 × 10^4 cells/mL, were incubated with various concentration of Comp. I, II (6.25-100 μg/mL) for 48 hr. Viable cells were evaluated by MTT assay. Data show the only viable cells and are expressed as means S.D. ± (n = 4).

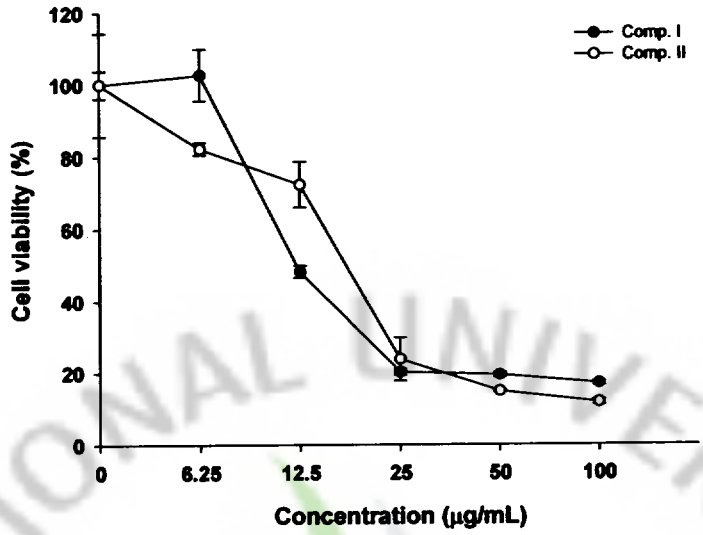

Figure 23. Growth inhibition of AGS Human carcinoma cancer cell by Comp. I and II. AGS cells, plated at density of 1 x 10^4 cells/mL, were incubated with various concentration of Comp. I, II (6.25-100 μg/mL) for 48 hr. Viable cells were evaluated by MTT assay. Data show the only viable cells and are expressed as means S.D. ± (n = 4).

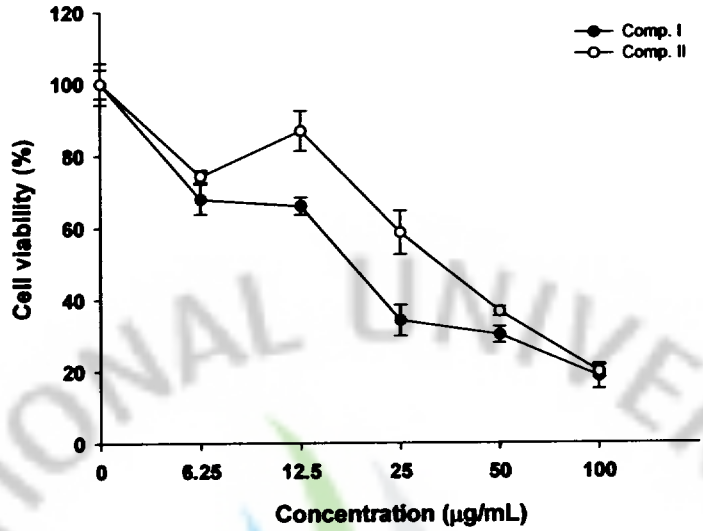

Figure 24. Growth inhibition of in SNU-16 Human carcinoma cancer cell by Comp. I and II. SNU-16 cells, plated at density of 1 x 10^4 cells/mL, were incubated with various concentration of Comp. I, II (6.25-100 μg/mL) for 48 hr. Viable cells were evaluated by MTT assay. Data show the only viable cells and are expressed as means S.D. ± (n = 4).

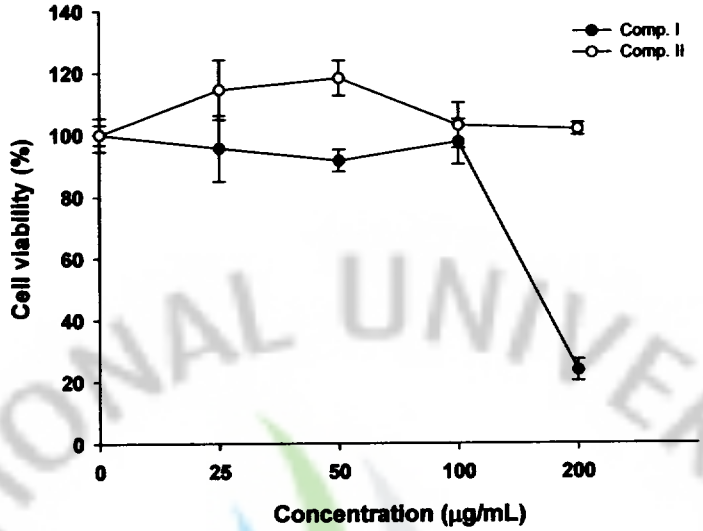

Figure 25. Growth inhibition of CCD-25Lu Human normal lung fibroblast cell by Comp. I and II. CCD-25Lu cells, plated at density of 1 x 10^4 cells/mL, were incubated with various concentration of Comp. I, II (25-200 μg/mL) for 48 hr. Viable cells were evaluated by MTT assay. Data show the only viable cells and are expressed as means S.D. ± (n = 4).

3. 4. Quantification of (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol and anomalin.

The isolated (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol and anomalin were identified by comparing its physical and spectroscopic data with those of the authentic standard. They were quantitative analysis using UPLC and the representative UPLC chromatogram is shown in (Fig. 26). A calibration curve was constructed at different concentrations of (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol and anomalin (6.25, 12.5, 25, 50, 100, and 200 μg /mL) as a standard. The linear regression equation of this curve and coefficient of determination (R^2) were calculated as y = 122.66x + 13692.0, R^2=0.999 and as y = 366.61x + 209.31, R^2=0.9998, respectively (Fig. 27, 28). The results revealed that *P. japonicum* contain 0.015 and 1.69 mg/g (dry weight) of (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol and anomalin, respectively in its root.

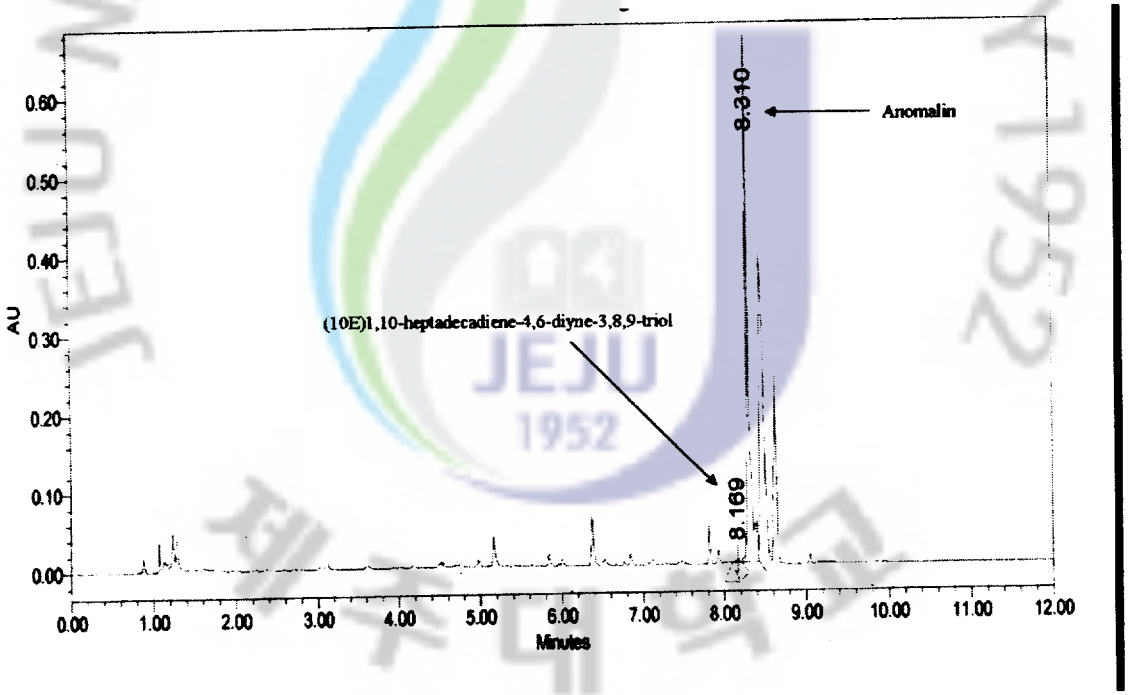

Figure 26. Characteristic UPLC chromatogram.

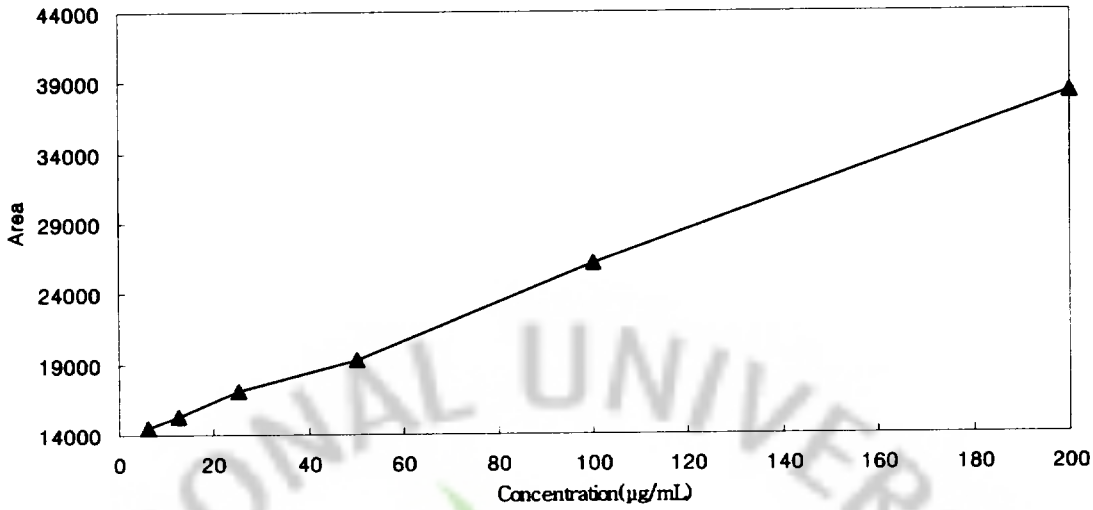

Figure 27. Calibration curve of (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol.

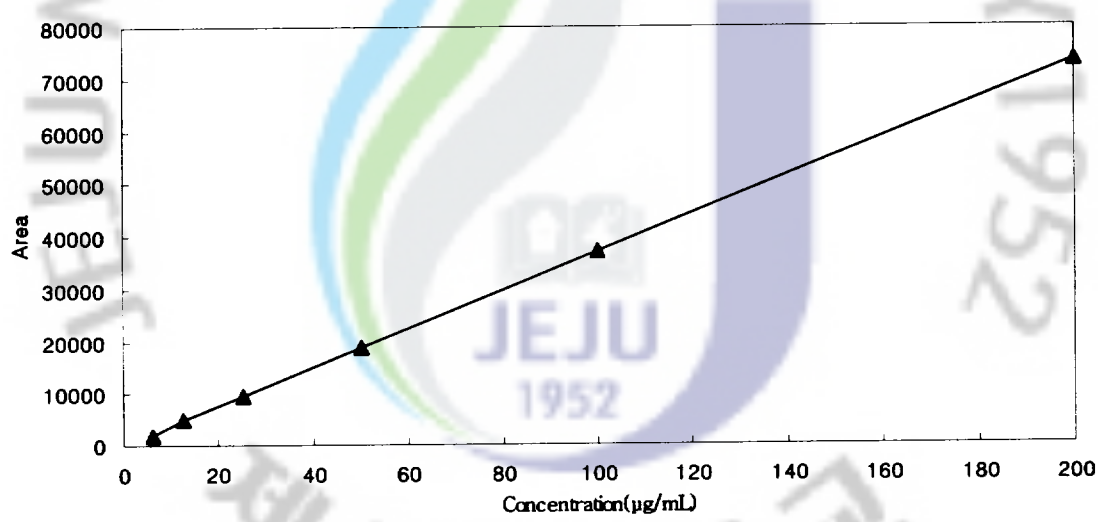

Figure 28. Calibration curve of anomalin.

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ABSTRACT IN KOREAN

갯기름나물은 백색 꽃의 야생식물로 한국에서는 6월에서 8월에 개화한다. 이 식물은 antifungal, stamina, antifood poisoning, antiphlegm, anticancer의 효과가 있는 것으로 알려져 있다. 본 연구는 갯기름나물 뿌리에서 항암물질을 분리, 동정하고 그 물질의 항암 활성을 측정하기 위하여 실시하였다. 갯기름나물 뿌리를 건조하고, 잘게 썬 뒤 100% 메탄올로 추출 하였다. 메탄올 추출물을 부탄올, 클로로포럼 층으로 분리 하였고, 클로로포럼층에 대하여 반복적인 컬럼크로그래피와 재결정화가 이루어 졌다. 그 결과 2개의 물질이 분리 되었고, 각각의 물질은 NMR 스펙트럼을 토대로 (10E)1,10-heptadecadiene-4,6-diyne-3,8,9-triol (Comp. I) 과 anomalin(Comp. II) 으로 밝혀졌다. 또한, 각각의 화합물을 UPLC를 이용하여 함량을 측정한 결과 0.015 (Comp. I), 1.69 (Comp. II) mg/g 으로 나타났다.

Comp. I 과 Comp. II에 대하여 HeLa, HepG2, AGS, SNU-16를 이용한 항암 활성을 측정해 본 결과 Comp. I은 모든 암세포에서 강한 세포성장 억제활성이 나타났고, Comp. II에서는 AGS, SNU-16세포에 대하여 강한 세포성장억제 활성을 나타내었다. 그러나 인간 정상 폐섬유 세포인 CCD-25Lu세포에 대해서는 세포성장억제 활성이 나타나지 않았다.

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4년전 학부생 3학년때 무작정 교수님 연구실 문을 처음으로 두드렸던 때가 생각이 납니다. 공부를 잘하는 학생도 아니었고, 그렇다고 실험을 잘하는 학생은 더더욱 아니었습니다. 주위의 만류도 있었고, 과연 내가 해낼 수 있을까 하는 두려움도 많았습니다. 논문을 마무리 하고 가만히 앉아서 지난날을 생각해 보니 아무것도 할 줄 모르던 제가 이렇게 졸업을 하게 된 것은 저 혼자 열심히 해서가 아닌 주위의 많은 분들의 도움이 있었기에 해낼 수 있었던 것 같습니다.

처음 실험실 생활을 하게 해주시고, 저의 말만 듣고 흔쾌히 아무것도 모르는 저의 지도교수님이 되어주신 김소미 교수님 진심으로 감사 드립니다. 제가 실험이란 것을 할 수 있도록 만들어주신, 항상 마음속으로 저의 스승이라 생각하고 있는 장기창 연구사님, 연구소 일에 바쁘신데도 불구하고 신경 써주시고 많은 도움주신 김성철 연구사님께도 감사 드립니다. 석사 학위논문 심사를 해주신 류기중 교수님께도 진심으로 감사 드립니다.

연구소에서 학연협동과정을 시작할 수 있도록 도움을 주신 성기철 박사님, 지금은 미국에 계시지만 많이 걱정해 주시고 많은 도움을 주신 이진수 연구사님 모르는 것이 있을 때 마다 많이 가르쳐준 김미선 박사님, 항상 열심히 하는 모습이 너무 보기 좋은 병돈이 처음 같이 실험실 생활 시작과 지금까지 나에게 너무나 많은 도움을 주고 있는 정용, 하나 지금은 육지에 있지만 항상 웃는 얼굴로 대해주던 진우형 열심히 하는 모습이 보기 좋은 윤정이 서울에서 열심히 공부하고 있을 정순이 같은 공감대를 가지고 있어 많은 조언과 이야기를 들어주는 경하 실험실 올 때마다 반갑게 인사해주는 실험실후배 현지, 덕현, 선이, 연우 집이 타지라 좀더 정이 많이 가는 종현이, 이비 항상 열심히 하고 많은 조언을 해주는 성원이 항상 힘들 때마다 힘내라고 격려 해주는 CRUSH 회원들 이렇게 저에게 힘이 되어준 모든 분들에게 감사의 말씀을 전합니다.

마지막으로 지금도 못난 아들을 위해 기도하고 계실 저희 어머니, 별 말씀 안 하시지만 항상 저를 믿고 지켜봐 주시는 저희 아버지 항상 감사 드리고 사랑합니다.