THE ISOLATION OF BIOACTIVE COMPOUNDS FROM THE BRANCHES OF *PLUMBAGO INDICA* L.

SIRAWIT MANYAEM

Graduate School Srinakharinwirot University

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THE ISOLATION OF BIOACTIVE COMPOUNDS FROM THE BRANCHES OF *PLUMBAGO INDICA* L.

SIRAWIT MANYAEM

A Thesis Submitted in Partial Fulfillment of the Requirements

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THE ISOLATION OF BIOACTIVE COMPOUNDS
FROM THE BRANCHES OF *PLUMBAGO INDICA* L.

BY

SIRAWIT MANYAEM

HAS BEEN APPROVED BY THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE
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(Assoc. Prof. Dr. Chatchai Ekpanyaskul, MD.)
Dean of Graduate School

ORAL DEFENSE COMMITTEE

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Title	THE ISOLATION OF BIOACTIVE COMPOUNDS FROM THE BRANCHES OF *PLUMBAGO INDICA* L.
Author	SIRAWIT MANYAEM
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Thesis Advisor	Associate Professor Siritron Samosorn
Co Advisor	Assistant Professor Maneekarn Namsa-aid

Plumbago indica L., known as Chettamuun Phloeng Daeng, is a medicinal plant belonging to the family Plumbaginaceae. This plant is the source of a naphthoquinone, and commercial interest for its wide range of pharmacological properties. The study aimed to isolate, separate and evaluate compounds' antifungal activities from the branches of *P. indica* L.. Six constituents, plumbagin (1), β-sitosterol (2), maritinone (3), 7,7´-biplumbagin (4), *cis*-isoshinanolone (5) and *trans*-isoshinanolone (6), were isolated from the branches of *P. indica* L..Plumbagin showed antifungal activity against *Aspergillus flavus* and *Talaromyces marneffei* with zones of inhibition of 40.0 and 70.0 mm (at 1.0 mg/disc). *Cis*-isoshinanolone showed antifungal activity against *A. flavus* and *T. marneffei* with zones of inhibition of 7.0 and 12.0 mm, respectively (at concentrations of 1.0 and 0.25 mg/disc), and *trans*-isoshinanolone showed activity against *A. flavus* and *T. marneffei* with zones of inhibition of 7.0 and 10.0 mm, respectively (at concentrations of 1.0 and 0.25 mg/disc). This study is the first time to report the isolation of compounds maritinone, 7,7´-biplumbagin and *trans*-isoshinanolone from *P. indica* L.

Keyword : Plumbago indica L.; Plumbaginaceae; Antifungal activity; Medicinal plants

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Finally, I would like to express my deepest gratitude to my family and my friends for their support and understanding. This thesis would have been quite incomplete without them. I would be very happy if my thesis could be of use for further studies.

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CHAPTER 1
INTRODUCTION

Background

In the past, humans have learned to take advantage of the plant used for healing human ailments because of a wide variety of plants exhibit phytochemical and pharmacological activities. As mentioned previously, researchers have attempted to isolate bioactive compounds from those plants or herbs. Some active compounds that are obtained from natural sources play an important role in maintaining human health (Farombi, 2003).

Plumbago is a genus of 15-20 species in the family Plumbaginaceae, a family of 24 genera and about 400 species (Singh et al., 2017). The family Plumbaginaceae was described by Antoine Laurent de Jussieu in 1789. This family include mainly herbs, shrubs and lianas. The presence of secretory glands is a family-specific feature (Wilson, 1890). The Plumbaginaceae species are highly pharmacologically important plants. The species of *Plumbago* which are spread in many parts of India, include 3 species, namely *Plumbago indica* L. (*P. rosea* L.), *P. capensis* Lam. (*P. auriculata* Lam.) and *P. zeylanica* L., which are spread in many parts of India (K et al., 1998).

Table 1. Species of *Plumbago*

Species (Singh et al., 2017)	Locality of *Plumbago*
P. amplexicaulis Oliv.	Tanzania
P. aphylla Bojer.	Madagascar, Aldabra Islands and Tanzania
P. auriculata Lam.	South Africa, Africa, America, Asia and India
P. ciliata Engl.	Tanzania
P. coerulea Kunth	Chile
P. dawei Rolfe	Ethiopia, Kenya and Uganda
P. europaea L.	Europe and Turkey
P. glandulicaulis Wilmot-Dear	Kenya and Tanzania
P. indica L.	Asia, Africa, India, Indonesia and Philippines
P. madagascariensis M. Peltier	Madagascar
P. montis-elgonis Bullock	Ethiopia, Kenya and Tanzania
P. pearsonii L. Bolus	Namibia
P. pulchella Boiss.	Mexico
P. stenophylla Wilmot-Dear	Kenya and Tanzania
P. scandens L.	Brazil
P. tristis Aiton	South Africa
P. wissii Friedr.	Namibia
P. zeylanica L.	Africa, Asia, Australia, Ethiopia, India and China

Source: Singh, K., Naidoo, Y., & Baijnath, H. (2017). A comprehensive review on the genus *Plumbago* with focus on *Plumbago auriculata* (Plumbaginaceae). African Journal of Traditional, Complementary and Alternative medicines, 15(1), 199-215.

Botanical description

P. indica L. previously known as P. rosea L. before, this plant is a perennial herb or flowering plant in the family Plumbaginaceae., that grows well in tropical climates, tropical Africa, tropical Asia and the Pacific region. It grows up to 1.2 m wide and 2.0 m tall. Its trunk is straight from the ground, its leaves are oblong. The red petals are divided into 5 lobes, while the leaf blade is narrowly oblong to elliptical-ovate in form and is 5–15 cm by 2–8 cm (Priyanjani et al., 2021).

From the book, Thai plant names, the tree species of *Plumbago* found in Thai land are *P. indica* L., *P. zeylanica* L. and *P. auriculata* Lam. (Tem, 2014).

A B C

Figure 1. A: *P. indica* L., B: *P. zeylanica* L. and C: *P. auriculata* Lam.

Source: Swamibhut. (2001). *Plumbago zeylanica* / Chitraka / Wild leadwort /Chitra. Retrieved October 28, 2020, https://naturalherbssite.wordpress./2019/12/05/ *Plumbago zeylanica*.

Ethnopharmacological Uses of *P. indica* L.

P. indica L. is a perennial herb or medicinal plant that was long used in several countries. In Indonesia and Malaysia, a poultice of the roots and leaves is used as a pill for toothache (as a counter-irritant), paralysis, rheumatism, leprosy, swollen glands and tumors. The root bark is used as a vesicant. Hence, the fresh roots are cut into thin strips which are masked on the skin for the treatment of skin diseases. Likewise, these slices may also be applied to the brow to prevent headaches (Priyanjani et al., 2021). In

Eastern Africa, it is used medically in the same way as traditionally used in India. Preferably, the roots have many uses, it is stimulant, acrid, digestive, vesicant, alterative and a powerful oral contraceptive and abortifacient. An infusion of the roots of *P. indica* L. is taken to retain cough, bronchitis, colic and dyspepsia. The roots are used to make a liniment by mixing with vegetable oil, it is used to retain headache and rheumatism. The roots and bark are astringent and carminative making them suitable for retaining dysentery, intestinal troubles, dyspepsia and diarrhea (Batugal et al., 2004). In Thailand, the dried roots stimulate or increase menstrual flow and help with appetite or assist digestion. In the Philippines, the roots of *P. indica* L. are used in poultices to retain a headache and the bark is used as a vesicant. The bark is also an antidyspeptic (Priyanjani et al., 2021).

Medicinal preparations from the roots of this plant are acrid and astringent and confirm to have carminative, anti-inflammatory, thermogenic, abortifacient, anthelmintic, antiperiodic, nerve stimulatory, digestive and rejuvenating properties (Joy, 1998). The crude extract from this plant has been reported to display a wide range of biological activities such as antibacterial (Paul, 2014), wound healing (Karthikeyan et al., 2013), antifertility (Sheeja et al., 2009a), hepatoprotective (Eldhose & Kuriakose, 2017), antioxidant (Karthikeyan et al., 2013), anti-*helicobacter pylori* and anticancer activities (Hiradeve et al., 2011; Saha & Paul, 2012).

Figure 2. *P. indica* L.: A-Shoot, B-Root, C-Flowers

Source: Padumadasa, C., AbeysekeraA, M., & MeedinSD, K. (2016). A preliminary investigation of the Shodhana (Detoxification) of roots of *Plumbago indica* L. in Ayurveda International Journal of Ayurveda and Pharma Research, 3.

Major Chemical Constituents

Plumbagin (5-hydroxy-2-methylnaphthalene-1,4-dione), a naphthoquinone derivative (1,4-naphthoquinone) that is a major bioactive compound of *P. indica* L. It possesses various pharmacology. This compound has been shown to support the therapy of various diseases such as cardiotonic (Itoigawa et al., 1991), abortifacient (Zubaid et al., 2007), antiarthritic (Aparanji & Athota, 2010), anticoagulant (Santhakumari et al., 1978), antifeedant (Tokunaga et al., 2004), antifungal (Shin et al., 2007), Anti-*helicobacter pylori* (Wang & Huang, 2005), antimalarial (Likhitwitayawuid et al., 1998), antimicrobial (Nadkarni & Nadkarni, 1955), antioxidant (Kumar et al., 2013), antiviral (Min et al., 2001), anticancer (Sinha et al., 2013), insecticidal (Kubo et al., 1983), leishmanicidal (Iwu et al., 1994), microfilaricidal (Mathew et al., 2002), prolongevity (Hunt et al., 2011), radiosensitizing (Nair et al., 2008), tumor angiogenesis inhibition (Lai et al., 2012), neuroprotective (Luo et al., 2010) and anti-inflammatory activities (Sheeja et al., 2006).

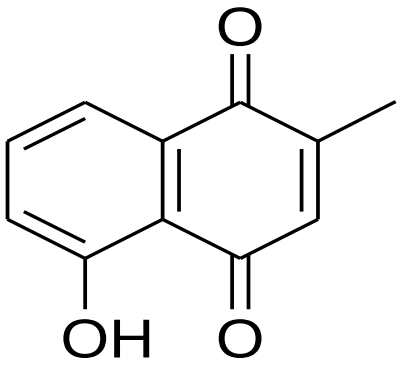

Figure 3. Structure of plumbagin

Objectives of this Study

1. To isolate and purify compounds from the branches of *P. indica* L. by chromatography techniques.

2. To elucidate chemical the structures of the isolated compounds from the branches of *P. indica* L. by spectroscopy techniques.

3. To evaluate the antifungal activity of the isolated compounds from the branches of *P. indica* L. by measuring zone of inhibition.

Scopes of this Study

1. Extraction of the crude extracts from the branches of *P. indica* L.

2. Isolation and purification of crude extracts by chromatography techniques.

3. Structure elucidation of the isolated compounds by spectroscopy techniques.

4. Antifungal activity evaluation of the isolated compounds.

CHAPTER 2
LITERATURE REVIEW

***Plumbago indica* L.**

P. indica L. is in the family Plumbaginaceae as well as *P. capensis* Lam. and *P. zeylanica* L., immensely called Lal Chitrak (Hindi), Chethikoduveli (Malayalam), Rose Leadwort (English) and Chetta mun phloeng daeng (Thai). This plant is an essential perennial herb or small shrub distributed in tropical Africa, Asia and the Pacific region. (Jose et al., 2014).

Figure 4. *P. indica* L.

Source: Jose, B., Dhanya B. P., Silja, P. K., Dhanya, B. P., & Krishnan, P. N. (2014). *Plumbago rosea* L. A Review on Tissue Culture and Pharmacological Research. International Journal of Pharmaceutical Sciences Review and Research, 25(4):246-256.

Table 2. Taxonomic Hierarchy: *P. indica* L.

Plant classification	Name of classification
Kingdom	Plantae – plants
Subkingdom	Viridiplantae – green plants
Infrakingdom	Streptophyta – land plants
Superdivition	Embryophyta
Division	Tracheophyta – vascular plants
Class	Magnoliopsida
Superorder	Caryophyllanae
Order	Caryophyllales
Family	Plumbaginaceae – leadwort
Genus	*Plumbago*
Species	*P. indica* L.

Source: ITIS Report. (2020) . *Plumbago indica* L. Retrieved October 31, 2020, https://www.itis.gov/servlet/SingleRpt/SingleRpt.

Geographical distribution

The Sikkim and Khasi highlands of India are where this species first appeared. This species is dispersed over tropical Asia, Africa and the Pacific. It is seen in Nagaland, Manipur, Assam, Meghalaya, Arunachal Pradesh, Odisha and West Bengal. Besides, it is widely cultivated in other parts of the world such as South India, Philippines, Kenya, Tanzania, Zimbabwe, Mozambique, Madagascar, Africa, Europe, Indonesia, China, Malaysia and the Arabian Peninsula for reaping its tuberous roots for medicinal (Sharma & Yadav, 2019).

Morphology of *P. indica* L.

This species is in the family Plumbaginaceae and it is a perennial herb or flowering plant, that grows well under warm tropical climates and grows up to 1.0 – 1.5 m in height. This plant was described as the morphology by Binoy Jose et al. The stem of P. indica L. is erect, trailing or climbing. The leaves of *P. indica* L. are alternate, simple and entire and are about 10 cm in length, ovate-elliptic in shape, simple, having alternate arrangement with entire margins and exstipulate. The petiole of *P. indica* L. is short and the auricles are absent. *P. indica* L. inflorescence is a multi-floral, elongated spike or raceme that is 10–30 cm long. The ovate-shaped bracts of *P. indica* L. are 2-3 mm in length. The peduncle of *P. indica* L. is 2–10 cm long. The flowers of *P. indica* L. are bisexual. The hairy calyx is a characteristic feature of this genus. The pedicel of *P. indica* L. is 0-1 mm long. The calyx of *P. indica* L. is tubular, 8-9 mm long and red in colour. The corolla tube of *P. indica* L. is 2.5-4.5 cm long. (Kurian & Sankar, 2020).

Table 3. Morphology of *P. indica* L.

Plant components	Figure	Reference
Stem		Boldsystems. (2014) . *Plumbago indica*. Retrieved November 16, 2020, from https://v3.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=648424.
Petiole and leaves		India Biodiversity. (2019). *Plumbago indica.* Retrieved November 16, 2020, from https://indiabiodiversity.org/group/plantsindia/observation/show/1823252?lang=en.
Inflorescence		Cfgphoto. (2019). *Plumbago indica.* Retrieved November 16, 2020, from https://www.cfgphoto.com/photo-70238.htm.

Table 3. (Continued)

Plant components	Figure	Reference
Peduncle, pedicel and corolla		Flower Song. (2010). Indian Leadwort *(Plumbago indica).* Retrieved November 16, 2020, from https://flowersong.in/flowers/indian-leadwort.
Roots		Lee. (2019). Changsha Gentcare Natural Ingredients Inc. Retrieved November 16, 2020, from https://www.tradesparq.com/products/476762/Plumbago-zeylanica-Root-Extract-Plumbago-indica.

Pharmacological activities of *Plumbago* spp.

The plant *Plumbago* spp. exhibits large numbers of medicinal properties and they were reported as follows.

Antibacterial activity

In 2014, the methanol extract of *P. indica* L. (leaves and stems) was assayed against gram-positive such as *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus* and *Staphylococcus aureusand* and gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysentariae*, *Shigella sonnei*, *Salmonella typhi*, *Vibrio cholera* and *Salmonella paratyphi* (Paul, 2014).

Table 4. Antibacterial activity of the methanol extract of *P. indica* L.

Tested bacteria	Diameter of zone of inhibition (mm)			
	Methanol extract of		Ciprofloxacin	Control
	250 (µg/disc)	500 (µg/disc)	500 (µg/disc)	
Gram Positive bacteria				
Bacillus subtilis	10	18	24	-
Bacillus megaterium	9	17	25	-
Bacillus cereus	14	15	28	-
Staphylococcus aureus	11.5	24	27.5	-
Gram Negative bacteria				
Pseudomonas aeruginosa	10	21	31	-
Escherichia coli	12	23	28	-
Shigella dysentariae	9.5	21	32	-
Shigella sonnei	7	18	27	-
Salmonella typhi	13	20	23.5	-
Vibrio cholera	7.5	19	24	-
Salmonella paratyphi	13.5	22	26	-

Source: Paul, S. (2014). Antibacterial activity of *Plumbago indica*. Turk J Pharm Sci, 11, 217-222

Antiviral activity

The roots of *P. zeylanica* L. were extracted with 80% methanol without defatting. The crude extract was evaluated against CVB3, influenza virus A and HSV-1 by using CPE inhibitory assay. In addition, the extracts of *P. zeylanica* L. inhibited CVB3 (Gebre-Mariam et al., 2006).

Table 5. IC_{50}(μg/ml) of the crude extracts

Extract	IC_{50} (μg/ml)		
	CVB3	Influenza A virus	HSV-1
Acokanthera schimperi	1.34	Inhibition <50%	Inhibition <50%
Euclea schimperi	33.6	6.22	67.5
Inula confertiflora	-	6.50	96.8
Melilotus elegans	-	Inhibition <50%	-
Plumbago zeylanica L.	82.1	Inhibition <50%	-

Source: Gebre-Mariam, T., Neubert, R., Schmidt, P., Wutzler, P., & Schmidtke, M. (2006). Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders. Journal of ethnopharmacology, 104, 182-187.

Antioxidant activity

In 2013, Mariam et al. extracted the roots of *P. indica* L. with hexane, chloroform and methanol. The antioxidant activities of different root extracts of *P. indica* L. were investigated, including DPPH, free radicals of superoxide anion and scavenging activity of hydrogen peroxide *in vitro* (Karthikeyan et al., 2013).

DPPH radical scavenging activity

When an antioxidant compound donates an electron to DPPH (2,2-Diphenyl-1-picrylhydrazyl radical), the DPPH is decolourized, which can be quantitatively measured from absorbance changes. From the experimental data of this work, the ethanol extract has more scavenging activity than the chloroform and hexane extracts.

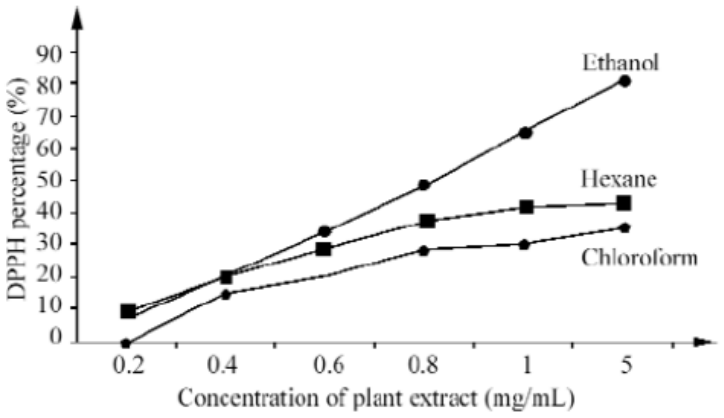

Figure 5. The scavenging effect of different the root extracts on DPPH.

Source: Kumar, S., Gautam, S., & Sharma, A. (2013). Antimutagenic and antioxidant properties of plumbagin and other naphthoquinones. Mutation research, 755.

Superoxide scavenging activity

Among the crude extracts of root extracts of *P. indica* L., the ethanolic extract was shown to be an efficient scavenger of superoxide radical in the riboflavin NBT light system.

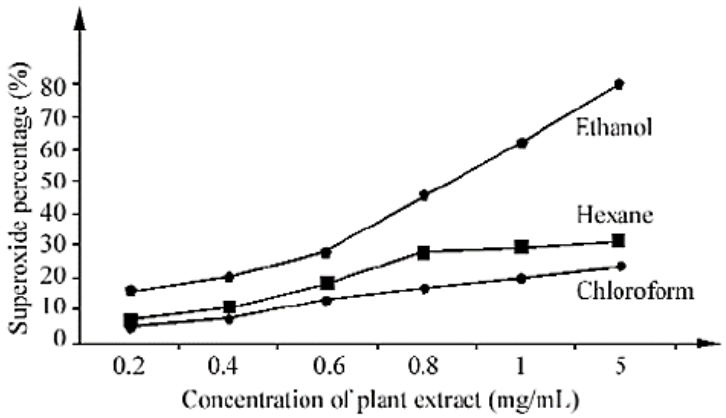

Figure 6. The scavenging effect of different the root extracts on superoxide radicals.

Source: Kumar, S., Gautam, S., & Sharma, A. (2013). Antimutagenic and antioxidant properties of plumbagin and other naphthoquinones. Mutation research, 755.

Hydroxyl radical scavenging assay

The scavenging ability of the crude extracts of *P. indica* L. with hydrogen peroxide (H_2O_2) is exhibited in figure 7. It was found that the ethanolic extract of *P. indica* L. displayed the most effectiveness in scavenging activity as with other DPPH and superoxide scavenging activity.

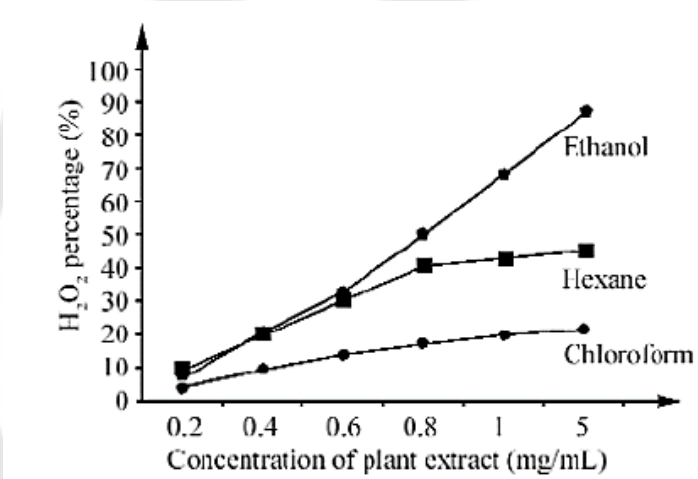

Figure 7. The scavenging effect of different the root extracts on hydroxyl radicals.

Source: Kumar, S., Gautam, S., & Sharma, A. (2013). Antimutagenic and antioxidant properties of plumbagin and other naphthoquinones. Mutation research, 755.

Wound healing activity

As reported by Kodati et al., The methanol extract of *P. zeylanica* L. displayed effective wound-healing activity in an excision wound model. Wound epithelization with methanol extract (10% w/w) ointment treated group was found to be earlier than the control group. In the group of ointment-treated rats (10% w/w of the

methanol extract), wound-treated rats healed in 16 ± 2 days, but in controlled animals more than 20 ± 2 days (Kodati et al., 2011).

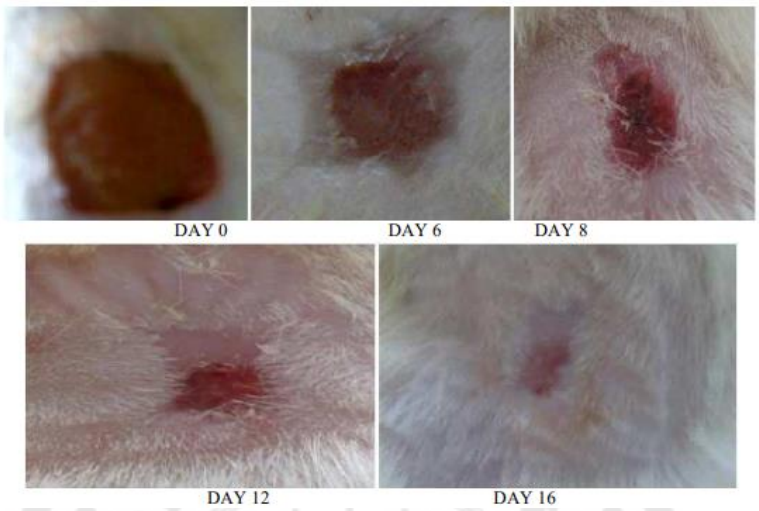

Figure 8. Photographical representation of contraction rate on different days in the treatment group

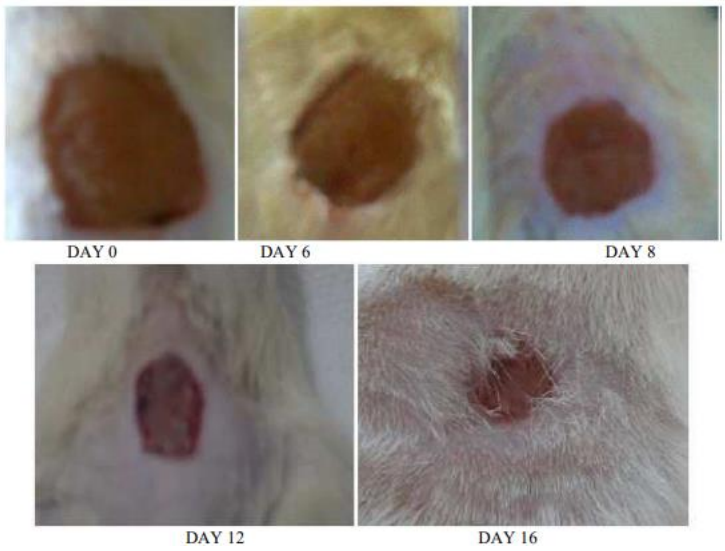

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Figure 9. Photographical representation of contraction rate on different days in the control group.

Source: Kodati, D.R. et al. (2011). Evaluation of Wound healing activity of methanolic root extract of *Plumbago zeylanica* L. in wistar albino rats. Pelagia Research Library, 3(3): 13-19.

Antifertility activity

The crude extracts (Petroleum ether, chloroform, acetone and methanol extracts) of *P. rosea* L. were tested for antifertility activity. The studies indicated that the acetone extract of the stems of *P. rosea* L. had the function of antifertility, while all the remaining sections were inactive when compared to the control , 2009b). This activity may be due to the presence of naphthoquinones and flavonoids in the acetone extract of *P. rosea* L.

Table 6. The estrogenic and anti-estrogenic activity of acetone extract

Groups	Dose (mg/kg bw)	Uterine weight (mg/100 g bw)
Ethinyl estradiol	0.02	142.00 ± 4.83
Acetone extract	200	68.13 ± 4.97
Acetone extract	400	74.50 ± 2.73
Ethinyl estradiol + acetone extract	0.02 + 200	138.53 ± 4. 07
Ethinyl estradiol + acetone extract	0.02 + 400	126.23 ± 4.23
Control	Tween-80	45.50 ± 2.27

Source: Sheeja, E., Joshi, B., & Jain, D.C. et al. (2009). Antifertility Activity of Stems of *Plumbago rosea* in Female Albino Rats. Pharmaceutical Biology, 920–927).

Hepatoprotective activity

Eldhose et al. planned research to assess the hepatoprotective effects on albino Wistar rats. Different dosages of *P. Indica* L. (PLBM) methanol root extracts (100 and 200 mg/kg bw) were tested against thioacetamide-induced liver damage. Thioacetamide (100 mg/kg bw) was used to cause liver damage, which was then measured by monitoring the activity of enzymes that indicate liver function and lipid

peroxidation. The presence of histological alterations in the liver sections was assessed. The root extracts from *P. indica* L. in methanol substantially ($p \leq 0.05$) reversed the increase in hepatic antioxidant indicators and blood-liver enzymes. The therapeutic activity of *P. indica* L. was proven by histopathological research in a dose-dependent way. As a result, this research offers evidence-based justification for employing *P. indica* L. roots as a potential treatment for a variety of liver problems (Eldhose & Kuriakose, 2017).

Table 7. Curative Effects of *P. Indica* L. methanol root extracts against the levels of lipid peroxidation and conjugated dienes in the liver of control and experimental rats.

Groups	TBARS (mmol/mg tissue)	CD (mmol/mg tissue)
Normal control	0.54 ± 0.04	51.08 ± 6.21
TAA (100mg/kg, sc.)	0.83 ± 0.05 †	74. 12 ± 7.72 †
Silymarin (100mg/kg bw) + TAA	0.62 ± 0.03 *	59.2 ± 3.46 *
Methanol extracts (100mg/kg bw) + TAA	0.56 ± 0.05*	52.83 ± 6.81 *
Methanol extracts (200mg/kg bw) + TAA	0.6 ± 0.07*	52.83 ± 6.81*

Source: Eldhose, B., & Kuriakose, J. (2017). Curative effect of *Plumbago indica* root extract on thioacetamide induced hepatotoxicity in experimental rats. International journal of phytomedicine, 9, 138-143.

Larvicidal activity

In 2009, as reported by Maniafu et al., tree *Plumbago* spp. (*P. zeylanica* L., *P. stenophylla* Wilmot-Dear and *P. dawei* Rolfe) had been tested for mosquito larvicidal activity. The larvicidal activity of the organic extracts was found to be species-dependent. It was found that the crude extracts from *P. zeylanica* Linn, *P. stenophylla* Bull, and *P. dawei* Rolfe with the best larvicidal activity against *Anopheles gambiae*

were hexane (LC_{50} = 6.4 g/mL), chloroform (LC_{50} = 6.7 ug/mL), and ethyl acetate (LC_{50} = 4.1 g/mL). These LC_{50} values were within 95% confidence limits (Maniafu et al., 2009).

Table 8. Lethal concentrations (LC) of most active extracts of *Plumbago* spp.

Species of *Plumbago*	Lethal concentrations (95% confidence limits)				
	Extract	LC_{25}	LC_{50}	LC_{75}	LC_{90}
P. dawei Rolfe	EtOAc	2.5	4.1	6.7	10.6
P. zeylanica L.	C_6H_{14}	3.1	6.4	13.4	26.2
P. stenophylla Wilmot-Dear	$CHCl_3$	4.3	6.7	10.5	15.6

Source: Maniafu, M., Wilber, L., Ndiege, I., Wanjala, C., & Akenga, T. (2009). Larvicidal activity of extracts from three *Plumbago* spp against Anopheles gambiae. Memórias do Instituto Oswaldo Cruz, 104, 813-817.

Anticancer activity

It was observed that the *P. zeylanica* L. plant has an anticancer effect against different types of cancer cell lines. There have been many reports of the anticancer activity of the *P. zeylanica* L. plant (Jain et al., 2014).

In an animal model, Hiradeve et al. (2011) conducted a preliminary phytochemical screening and anticancer assessment of an ethanol extract of *P. zeylanica* L. leaves against Ehrlich Ascites Carcinoma cells. hey found that the ethanol extract of *P. zeylanica* L. showed considerable anticancer action and also decreased the raised level of lipid peroxidation because of the higher presence of terpenoids and flavonoids (100 mg/kg and 200 mg/kg).

Table 9. Result of phytoconstituent identification tests of ethanol extract of *P. zeylanica* L.

Phytoconstituent	Phytosterol	Flavonoids	Triterpenoids	Saponin
Ethanol extract	+	+	+	+

Table 10. Effect of different doses of ethanol extract of *P. zeylanica* L. on different biochemical parameter in EAC-bearing mice.

Parameter	Normal saline 0.5 ml/kg	EAC control 2 X 106 cells / mice	EAC + EEAV 100 mg/kg	EAC + EEAV 200 mg / kg
Lipid peroxidation n mole MDA/gm of tissue	0.92±0.02	1.36±0.09	1.27±0.04	1.13±0.02
Catalase (units /mg tissues)	2.51±0.72	1.71±0.15	1.75±0.13	2.34±0.23
Protein content (gm / 100 ml)	12.66±0.69	17.25±0.76	16.50±0.70	16.10±0.55
Superoxide dismutase	4.37±0.41	3.20±0.71	2.30±0.48	2.65±0.02

Source: Hiradeve, S. et al. (2011). Evaluation of anticancer activity of *Plumbago zeylanica* Linn. leaf extract. International Journal of Biomedical Research, 1-9)

Antimalarial activity

Using a SYBR Green I-based test, the *in vitro* antimalarial activity of plumbagin against K1 and 3D7 *Plasmodium falciparum* clones was evaluated. Utilizing a mouse model infected with *Plasmodium berghei*, *in vivo* antimalarial activity was studied. Plumbagin showed excellent antimalarial activity, with i*n vitro* IC_{50} values of 580 (270-640) nM for 3D7 chloroquine-sensitive *P. falciparum* and 370 (270-490) nM for K1 chloroquine-resistant *P. falciparum* clones, respectively. (Sumsakul et al., 2014)

Table 11. *In vitro* antimalarial activity of plumbagin, chloroquine and artesunate.

Compounds	IC_{50} against 3D7 chloroquine-sensitive *P. falciparum* clones (nM)	IC_{50} against K1 chloroquine resistant *P. falciparum* clones (nM)
Plumbagin	580	370
Chloroquine	10.5	128.7
Artesunate	2.1	1.91

Source: Sumsakul. W. et al. (2014). Antimalarial activity of plumbagin *in vitro* and in animal models. BMC complementary and alternative medicine, 14, 15-15.

Anti-inflammatory activity

In 2008, the phosphate-buffered saline extract of the roots of *P. zeylanica L.* (pH 7.2) was investigated for anti-inflammatory activity in Wistar rats. The extract of *P. zeylanica* L. stabilized red blood cells subjected to both hypotonic-induced lyses and heat. In the liver homogenates of formaldehyde-induced arthritic rats, the enzymatic activities of both acid phosphatases and alkaline were lowered while the activity of adenosine triphosphatase in the liver was increased. It has been hypothesized that *P. zeylanica* L. extract may have an anti-inflammatory effect (Oyedapo, 2008).

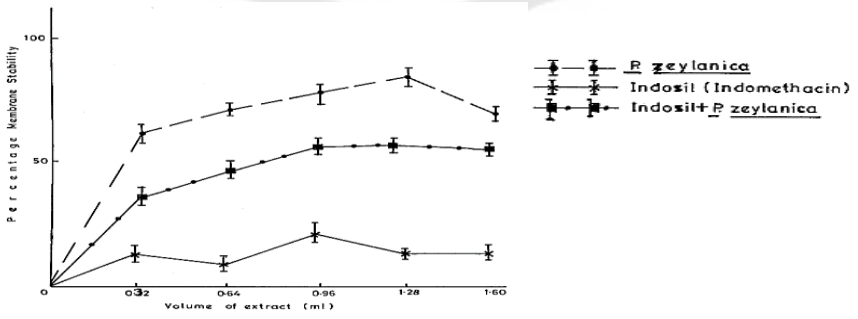


Figure 10. Membrane stabilizing activity of PBS extract of *P. zeylanica* L. on bovine red blood cell (BRBC) subjected to heat and hypotonic induced lyses.

Source: Oyedapo, O.O. (2008). Studies on Bioactivity of the Root Extract of *Plumbago zeylanica*. International Journal of Pharmacognosy, 365–369)

Table 12. Effect of *P. zeylanica* L. root extract on the activities of AlkPase, AcPase and ATPase on the liver homogenates of arthritic rats.

Groups	Dose (mg/kg bwt)	Alkaline phosphatase (p-nitrophenol/mg protein/15min at 37 ^{O}C	Acid phosphatase (p-nitrophenol/mg protein/15min at 37 ^{O}C	Adenosine triphosphatase (inorganic phosphate/mg protein/15min at 37 ^{O}C
Group I: Control rats	-	33.65±2.35 umoles	21.7±4.6 mmoles	0.75±0.11 umoles
Group II: arthritic rats (formaldehyde induced arthritis)	-	49.00±1.50 umoles	43.9±3.3 mmoles	0.31±0.07 umoles
Group III: arthritic rats + extract of *P. zeylanica* L.	200	34.85±3.90 umoles	25.1±3.2 mmoles	0.51±0.16 umoles
Group IV: rats + extract of *P. zeylanica* L.	200	33.46±3.62 umoles	21.7±2.2 mmoles	0.72±0.24 umoles

Source: Oyedapo, O.O. (2008). Studies on Bioactivity of the Root Extract of *Plumbago zeylanica*. International Journal of Pharmacognosy, 365–369)

Anti-sickling activity

A point mutation in the B-globin gene causes sickle cell anemia, a hereditary blood condition where there aren't enough healthy red blood cells and the

valine amino acid replaces the glutamic acid residue at position 6 of the B-chain of hemoglobin. Rounded red blood cells often flow fast through blood arteries. The crimson blood of sickle cell anemia is shaped like sickles or crescent moons.

In 2010, the crude methanol extract and aqueous extract of *P. zeylanica* L. (root) were evaluated for anti-sickling activity using p-hydroxybenzoic acid and normal saline positive and negative controls. The anti-sickling activity of *P. zeylanica* L. extracts/fractions was considerably greater ($p < 0.05$) at the tested doses of 10.0, 1.0, and 0.1 mg/ml. (Adejumo et al., 2010).

Table 13. Anti-sickling effect (% inhibition of sickling) of methanol and aqueous extract of the root of *P. zeylanica* L.

Time of incubation (min)	Normal saline	p-Hydroxy benzoic acid (PHBA)	Methanol extract concentration (mg/ml)			Aqueous extract concentration (mg/ml)		
			10	1	0.1	10	1	0.1
0	-(0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
30	-(2)	48 (55)	64 (48)	56 (40)	16 (33)	68 (50)	60 (40)	20 (32)
60	-(4)	50 (59)	73 (51)	66 (41)	35 (34)	77 (52)	69 (44)	39 (40)
90	-(6)	53 (62)	64 (54)	58 (44)	17 (38)	69 (55)	61 (51)	23 (46)
120	-(8)	55 (63)	45 (60)	39 (57)	9 (51)	51 (58)	45 (55)	18 (51)

Source: Adejumo, O.E. et al. (2010). *In vitro* anti-sickling activities and phytochemical evaluation of *Plumbago zeylanica* and *Uvaria chamae*. African Journal of Biotechnology, 9032–9036).

Cytotoxic activity

In 2012, Saha and Paul investigated the crude methanol extract of *P. indica* L. The LC_{50} and LC_{90} values of *P. indica* L. were measured in order to assess its potential cytotoxic activity utilizing the brine shrimp lethality bioassay technique. The determined LC_{50} and LC_{90} values for the extract against brine shrimp nauplii were 5.0 and 12 mg/ml, respectively (Saha & Paul, 2012).

Table 14. Determination of LC_{50} and LC_{90} Methanolic extract of *P. indica* L.

Test groups	Conc. (mg/ml)	Log (Conc.)	No. of alive shrimp			Mean alive	% mortality	LC_{50}(mg /ml)	LC_{90} (m g/ml)
			t1	t2	t3				
MEPI	20	1.30	7	7	8	7.33	26.66	5.0	12.0
	40	1.60	6	5	6	5.66	43.33		
	60	1.78	4	3	4	3.66	63.33		
	80	1.90	3	4	3	3.33	66.66		
	100	2	2	2	2	2	80		
	120	2.07	1	2	0	1	90		
	140	2.14	0	0	0	0	100		
Control	20	1.30	10	10	10	10	0		
	40	1.60	10	10	10	10	0		
	60	1.77	10	10	10	10	0		
	80	1.90	10	10	10	10	0		
	100	2	10	10	10	10	0		
	120	2.07	9	10	9	9.33	6.7		
	140	2.14	8	8	8	8.33	16.7		

TC = Test Column, LC = Lethal Concentration, MEAC=Methanolic Extract of *P. indica* L.

Source: Saha, d. & Paul, S. (2012). Cytotoxic Activity of Methanolic Extract of *Plumbago indica* L. Asian Journal of Pharmacy and Technology, 59-61).

Phytochemical constituents

Table 15. Phytochemicals isolated from different parts of *P. indica* L.

Plant part	Name of compounds	Reference
Root	Plumbagin and roseanone	(Dinda, Das, & Hajra, 2010)
	6-hydroxy plumbagin, droserone and elliptinone	(Jose et al., 2014)
	Plumbagic acid lactone, ayanin, myricyl palmitate, palmitic acid and azaleatin	(Dinda, Das, Hajra, et al., 2010)
	Arachidyl alcohol, α-naphthylamine and β-sitosterol	Dinda, Das and Hajra (2010)
	Myricetin 3,7,3',5'-tetramethyl ether and ampelopsin-3',4',5',7-tetramethyl ether	Ariyanathan et al. (2010)
Aerial part	Campesterol, β-sitosterol, stigmasterol, plumbagin and 6-hydroxyplumbagin	Dinda and Chel (1992)
	Plumbaginol	Dinda et al. (1994)
Flower	Pelargonidin, cyanidin, delphinidin and kaempferol	Harborne (1962)
	Galloylglucose	Harborne (1967)

Table 16. Structures of phytoconstituents.

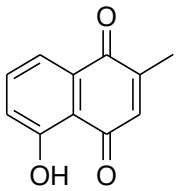
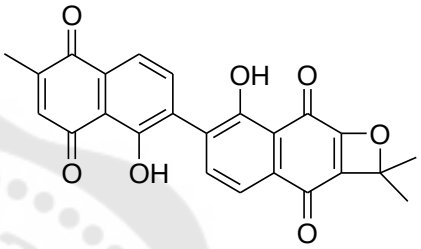
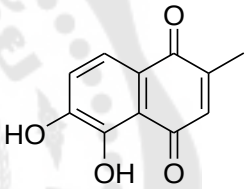
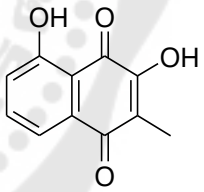
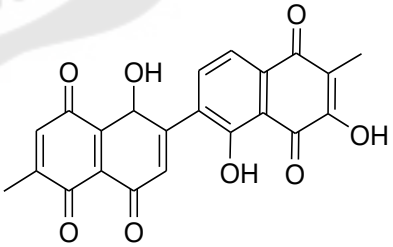
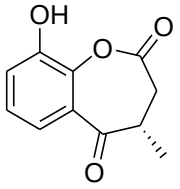
Name of compounds	Structures
Plumbagin	
Roseanone	
6-Hydroxy plumbagin	
Droserone	
Elliptinone	
Plumbagic acid lactone	

Table 16. (Continued)

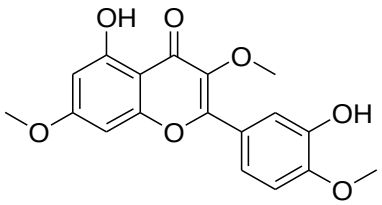
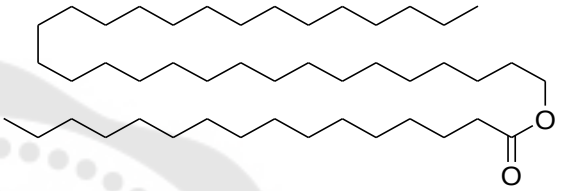
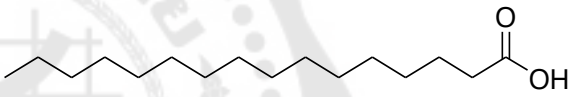
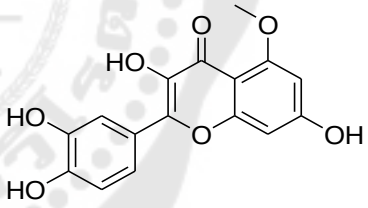
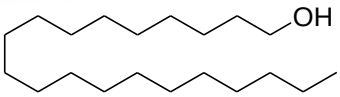
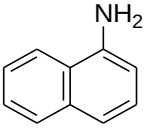
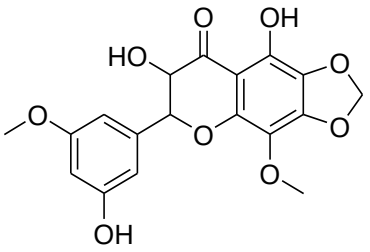
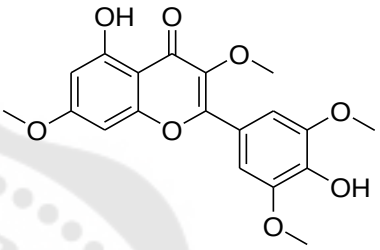
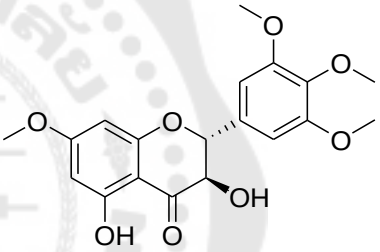
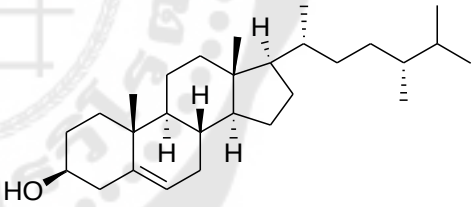
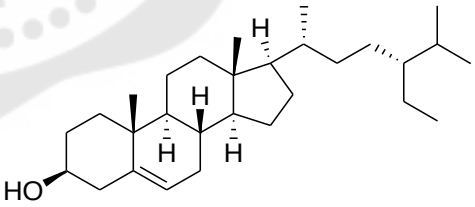
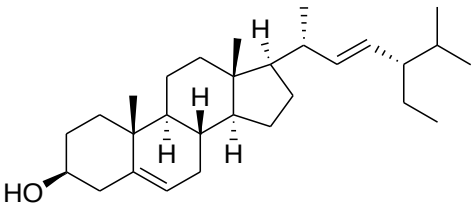
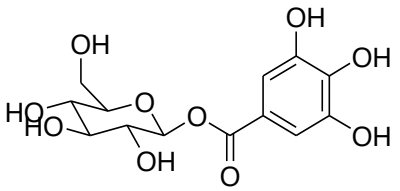
Name of compounds	Structures
Ayanin	
Myricyl palmitate	
Palmitic acid	
Azaleatin	
Arachidyl alcohol	
α-Aaphthylamine	

Table 16. (Continued)

Name of compounds	Structures
Plumbaginol	
Myricetin 3,7,3',5'-tetramethyl ether	
Ampelopsin-3',4',5',7-tetramethyl ether	
Campesterol	
β-Sitosterol	
Stigmasterol	
Galloyl-glucose	

CHAPTER 3
RESEARCH METHODOLOGY

Plant materials

The branches of *P. indica* L. (dried) were purchased from an herbal pharmacy (TPC-HERB), Bangkok, in 2017. A voucher specimen has been deposited at the Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand.

Fungal materials

Aspergillus flavus (TISTR 3041) was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. *Talaromyces marneffei (*ATCC18224) was purchased from the Microbiology Department, Faculty of Medicine, Chiang Mai University, Thailand. *A. flavus* produces aflatoxin and poses a significant risk of liver cancer while *T. marneffei* is considered to commonly cause infection in individuals with human immunodeficiency virus (HIV) infection.

General techniques

1. Thin-layer chromatography (TLC) : One-dimensional technique (Merck, 1.05554).

1.1 Detection: Fluorescence analysis and chemical reagent.

1.1.1 Using a fluorescence analysis cabinet (Spectroline, MODEL CM-10) at wavelengths 254 and 365 nm, spots on TLC were seen under ultraviolet light.

1.1.2 Anisaldehyde-sulphuric reagent 0.50% (v/v), which contains 4.98% v/v sulphuric acid and 9.95% v/v glacial acetic acid. Anisaldehyde in absolute methanol as the developing agent. With this reagent, the spots of organic compounds will produce distinct colours after the TLC plate has been warmed on a hot plate for a few seconds.

2. Column chromatography (CC)

2.1 Adsorbent: Silica gel 60

2.1.1 Particle size < 0.063 mm (Merck, 1.07729)

2.1.2 Particle size 0.040-0.063 mm (Merck, 1.09385)

2.1.3 Particle size 0.063-0.200 mm (Merck, 1.07734)

2. 2 Packing strategy: Slurry packing for silica gel 60 particles that are between 0.040 and 0.063 millimeters in size.

2.3 Sample loading:

2.3.1 A appropriate solvent system was used to dissolve the sample in a little amount. Using the thin-layer chromatography (TLC) method to characterize the solvent system.

2. 3. 2 The sample is not dissolved in the solvent system: In a small volume of an appropriate organic solvent, the sample was dissolved. The solution of the mixture will include silica gel particles that are 0.063-0.200 mm in size. Using a rotary evaporator (BUCHI, Thailand), the sample will be evaporated at a reduced pressure before being added to the top of the column.

2. 4 Elution: Following the loading of the sample onto the column, the appropriate solvent solution will be utilized as the mobile phase. In the gradient systems, beginning with the least polar solvent system, elute the sample, then, progressively increase the polarity of the solvent system and in the isocratic systems, the composition of the mobile phase remains constant throughout the isolation.

3. Size-Exclusion column chromatography (SEC)

3.1 Absorbent: Sephadex LH-20, slurry packing method

3.2 Sample loading: A less amount of methanol was used to dissolve the sample before it was placed on the column's top.

3.3 Elution: Methanol was used to elute the sample.

Spectroscopy techniques

1. Nuclear Magnetic Resonance (NMR) spectra: 1D-NMR and 2D-NMR spectra were recorded on a Bruker AVANCE III HD system (Billerica, MA, USA) (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR) and Bruker AVANCE III (500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR).

2. Mass spectra: The HR TOF mass spectra were obtained using a micrOTOF Bruker spectrometer.

Extraction and Isolation

Preparation of the crude extracts from *P. indica* L.

Figure 11. The branches of *P. indica* L. (dried)

The dried branches of *P. indica* L. were extracted with dichloromethane, ethyl acetate and 20% H_2O: methanol 5 times (each 2 days). The plant material was successively extracted with different organic solvents in the increasing polarity order at room temperature. Firstly, the dried branches of *P. indica* L. (1 kg) were extracted with dichloromethane (5L) for 5 times (each 2 days). The resulting residue (Marc) was extracted with ethyl acetate and methanol, respectively with the same procedure as the dichloromethane extraction. Evaporation of the filtrate under reduced pressure (about 45

°C) gave CH_2Cl_2 extract, EtOAc extract and 20% H_2O: MeOH extract. The extraction process is shown in figure 12.

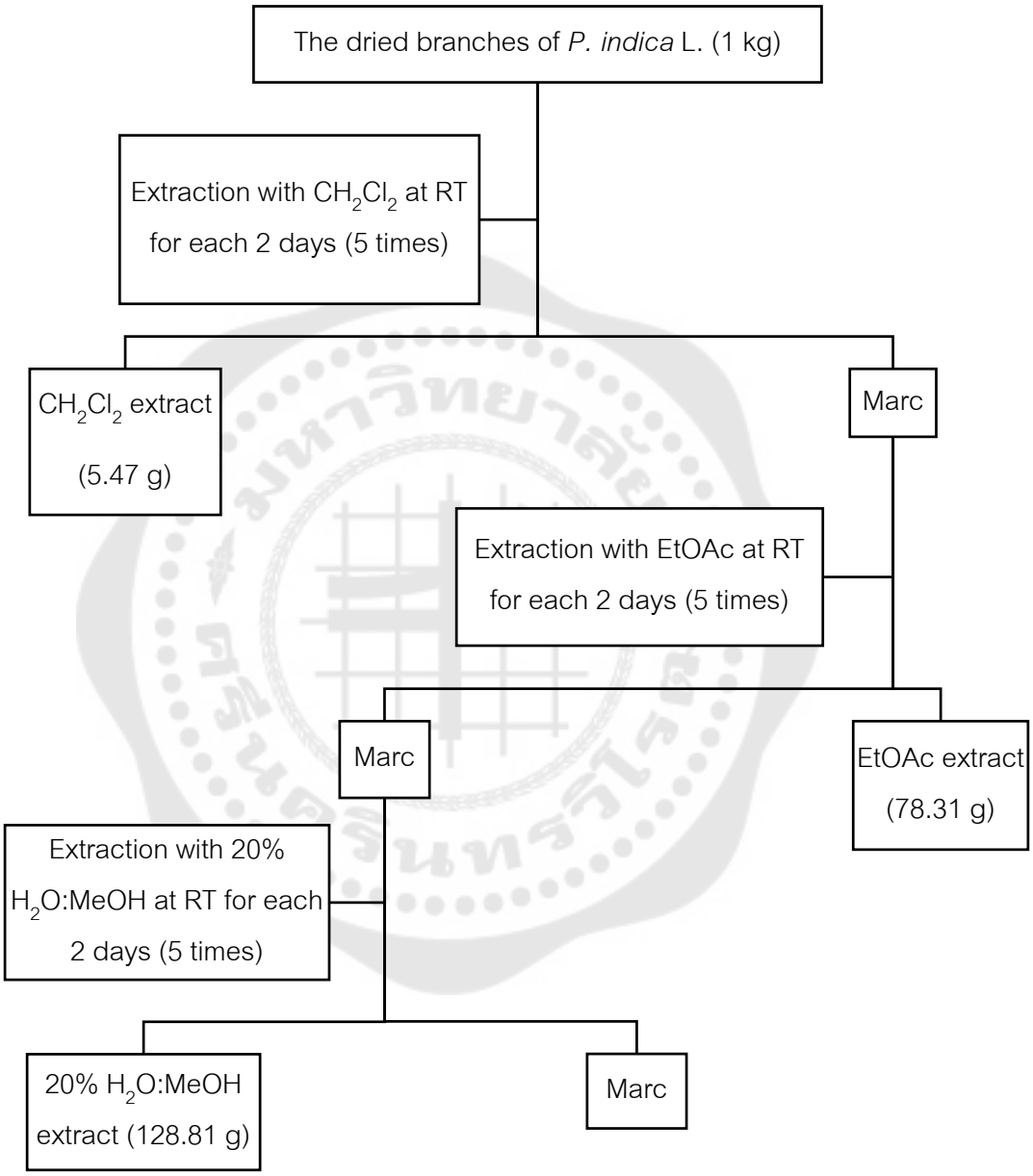


Figure 12. The extraction process of the dried branches of *P. indica* L.

Isolation of compounds from the branches of *P. indica* L.

The crude extracts of the branches of *P. indica* L. will be purified by using column chromatography (CC) techniques. The CH_2Cl_2 extract was subjected to silica gel column chromatography (CC) using a gradient of hexane-EtOAc (10:0 to 3:7) to obtain 10 fractions (C_1-C_{10}). Fraction C_2 (581.5 mg) was purified by silica gel CC eluting with a gradient of hexane-CH_2Cl_2 (9:1 to 0:10) to yield compound **1** (156.5 mg). Fraction C_4 (398.5 mg) was separated by silica gel CC eluting with hexane-EtOAc (9.8:0.2 to 0:10), yielding 19 fractions ($C_{4,1}$-$C_{4,19}$). Fraction $C_{4,5}$ (184.2 mg) was crystallized from CH_2Cl_2-MeOH to obtain compound **2** (70.0 mg). Fraction $C_{4,6}$ (81.6 mg) was further purified by silica gel CC eluting with hexane-EtOAc (9.6:0.4) to yield compound **3** (17.0 mg). Fraction C_5 (900.3 mg) was subjected to silica gel CC eluting with hexane-CH_2Cl_2 (6:4 to 0:10) to give 11 fractions ($C_{5,1}$-$C_{5,11}$). Fraction $C_{5,4}$, $C_{5,5}$ and $C_{5,6}$ (64.2 mg) were combined then fractionated by silica gel CC with hexane-CH_2Cl_2 (1:1 to 0:10) to provide compound **4** (1.5 mg). The EtOAc extract (78.31 g) was subjected to silica gel CC eluting with CH_2Cl_2-MeOH (10:0 to 1:1) to obtain 14 fractions (E_1-E_{14}). Fraction E_8 (2.2509 g) was separated by silica gel CC with hexane-EtOAc (9.5:0.5 to 7:3), yielding 15 fractions ($E_{8,1}$-$E_{8,15}$). Fraction $E_{8,11}$ (242.6 mg) was further purified by silica gel CC eluting with hexane-CH_2Cl_2 (2:8) to give 15 fractions ($E_{8,11,1}$- $E_{8,11,15}$). Fraction $E_{8,11,4}$ (156.0 mg) was further purified by size exclusion CC on Sephadex LH-20 eluting with CH_2Cl_2-MeOH (3:7) to provide 4 fractions ($E_{8,11,4,1}$-$E_{8,11,4,4}$). Fraction $E_{8,11,4,3}$ (142.2 mg) was fractionated by silica gel CC with hexane-EtOAc (1:9) to yield compounds **5** (15.7mg) and **6** (14.8 mg).

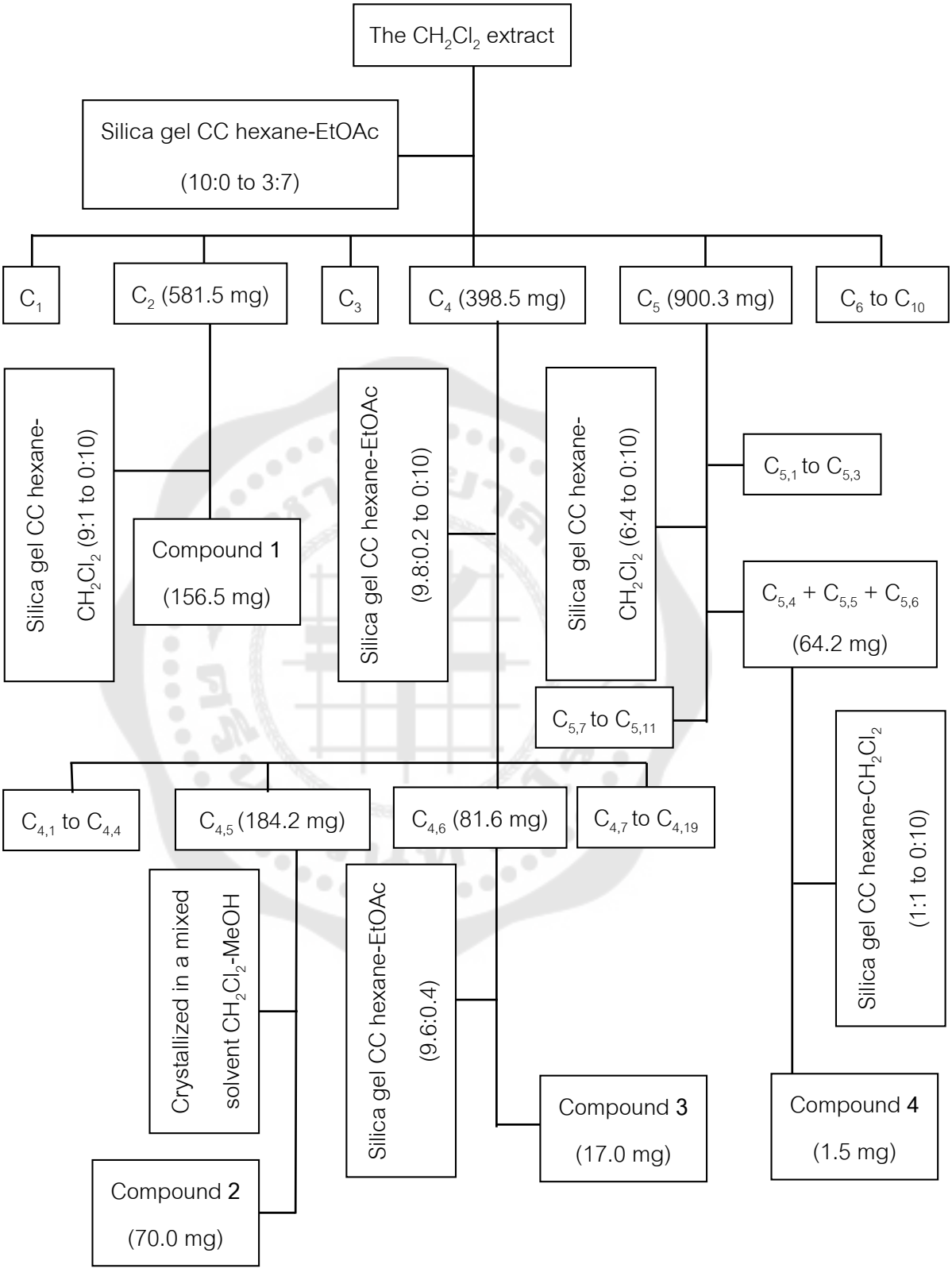


Figure 13. The isolation process of the CH_2Cl_2 extract

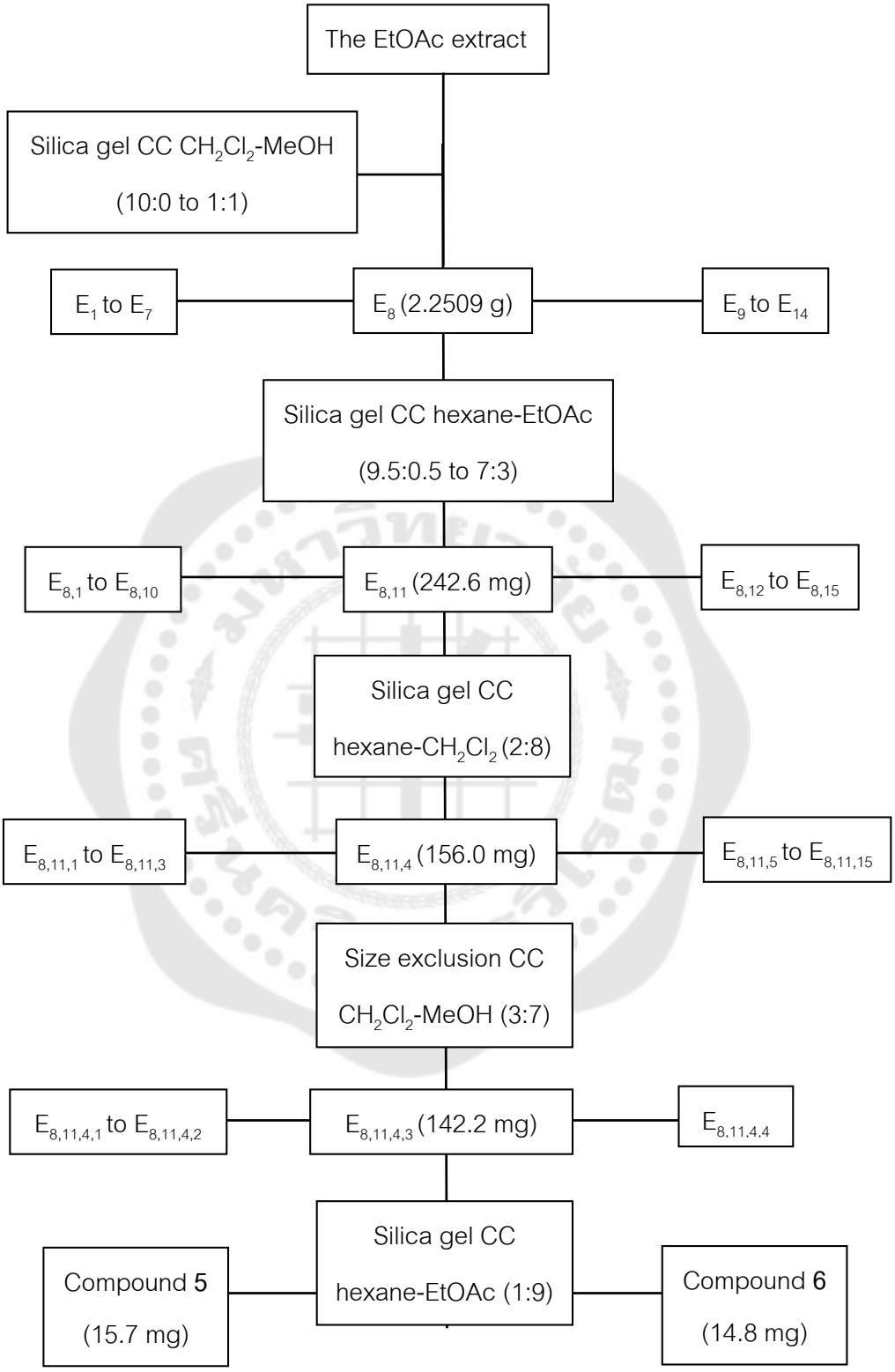


Figure 14. The isolation process of the EtOAc extract

Confirmation of the isolated compounds

The isolated compounds will be elucidated structure by spectroscopic techniques such as Nuclear Magnetic Resonance (NMR) Spectroscopy and Mass Spectrometry.

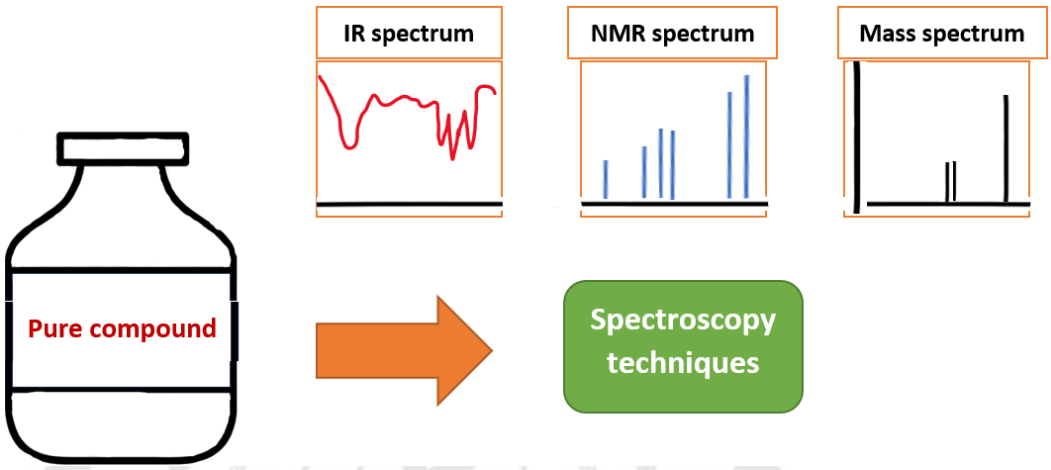


Figure 15. Confirmation of the isolated compounds by spectroscopy techniques

Antifungal assay

The antifungal assay used a modified disc diffusion method as previously described (Bansod & Rai, 2008). *A. flavus* (TISTR 3041) was grown on Potato Dextrose Agar (HiMedia, Mumbai, India) at 28 °C for 7 days, while *T. marneffei* (ATCC) was cultivated on Malt Extract Agar (HiMedia) at 28 °C for 14 days. Briefly, the fungi were harvested in 0.01 % Tween 80 by scraping with a sterile cotton swab and filtered through cotton wool. The fungal conidia were counted and diluted to 1 x 10^5 conidia/mL. One hundred µL of 1 x 10^4 conidia was used for the pour agar plate assay. The isolated compounds were diluted to obtain 20 µL of 0.25, 0.5 and 1 mg and then spotted on a paper disc placed on a plate containing the target fungus. The control disc was spotted with 20 µL of DMSO. The antifungal activity of each extract on the fungus was measured after incubation for 3 days at 28 °C and presented as the diameter of the inhibition zone.

CHAPTER 4
RESULTS AND DISCUSSION

The extraction of *P. indica* L.

The branches of *P. indica* L. (1 kg) were extracted with 3 different solvents to give CH_2Cl_2 extract (5.47 g, 0.547%), EtOAc extract (78.31g, 7.831%) and 20%H_2O:MeOH extract (128.81, 12.81%).

Structure identification

Identification of compound 1

Compound **1** (156.5 mg, yield 2.86%) was isolated as an orange solid and the molecular formula was deduced to be $C_{11}H_7O_3$ based on HR-ESI-TOFMS data (*m/z* 187.0405 $[M-H]^-$, calcd 187.0401). The NMR spectroscopic data (Table 17) represented signals for one hydroxyl proton signal at δ_H 11.97 (1H, s, 5-OH), three sets of aromatic protons signal of the benzene ring at δ_H 7.60 (1H, dd, *J* = 8.1, 7.5 Hz, H-7), 7.63 (1H, dd, *J* = 8.1, 1.4 Hz, H-8) and 7.25 (1H, dd, *J* = 7.5, 1.4 Hz, H-6), one aromatic proton signal of *quinone ring at* δ_H 6.80 (1H, q, *J* = 1.5 Hz, H-3) and one methyl group at δ_H 2.19 (3H, d, *J* = 1.5 Hz, 11-CH_3). A methyl group at δ_H 2.19 coupling with olefinic protons at δ_H 6.80. The ^{13}C NMR and DEPT spectral data revealed the presence of 11 carbon signals, which were characterized as four methine at δ_C 135.4 (C-3), 124.1 (C-6), 136.1 (C-7) and 119.2 (C-8), one methyl carbon at δ_C 16.5 (11- CH_3) and six quaternary carbon signals at δ_C 190.2 (C-4), 149.6 (C-2), 184.7 (C-1), 161.1 (C-5), 132.0 (C-9) and 115.1 (C-10). Compound **1** was therefore identified as plumbagin (Figure 16) when comparing its spectral data with those previously reported by Raj et al. (2011).

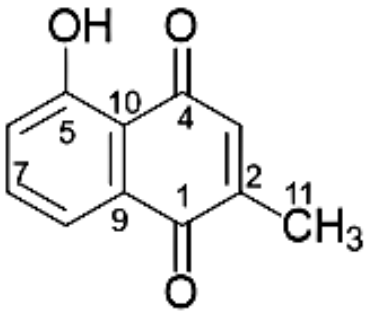


Figure 16. Chemical structure of plumbagin (**1**)

Table 17. ^{1}H and ^{13}C-NMR spectroscopic data of compounds **1** ($CDCl_3$; 500 MHz).

Position	Compound 1		Plumbagin (Raj et al., 2011)	
	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)	δ_C
1	-	184.7	-	184.75
2	-	149.6	-	149.54
3	6.80 (q; 1.5)	135.4	6.8 (s)	136.07
4	-	190.2	-	190.25
5-OH	11.97 (s)	161.1	11.95 (s)	161.19
6	7.25 (dd; 7.5, 1.4)	124.1	7.25 (m)	124.14
7	7.60 (dd; 8.1, 7.5)	136.1	7.63 (m)	135.44
8	7.63 (dd; 8.1, 1.4)	119.2	7.63 (m)	119.26
9	-	132.0	-	132.08
10	-	115.1	-	115.14
11-CH_3	2.19 (d; 1.5)	16.5	2.19 (s)	16.47

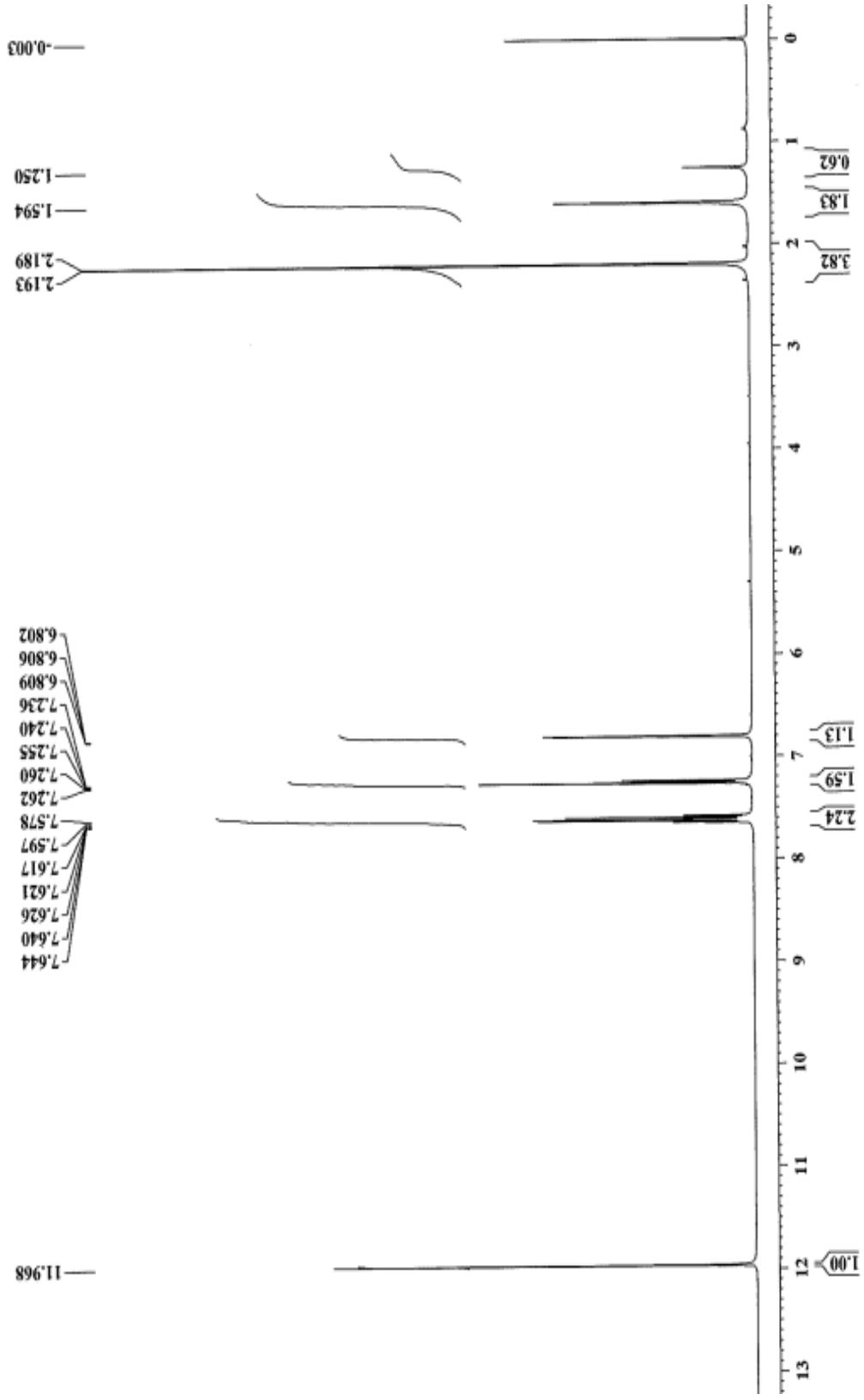


Figure 17. ^{1}H-NMR ($CDCl_3$; 500 MHz) of compound **1**

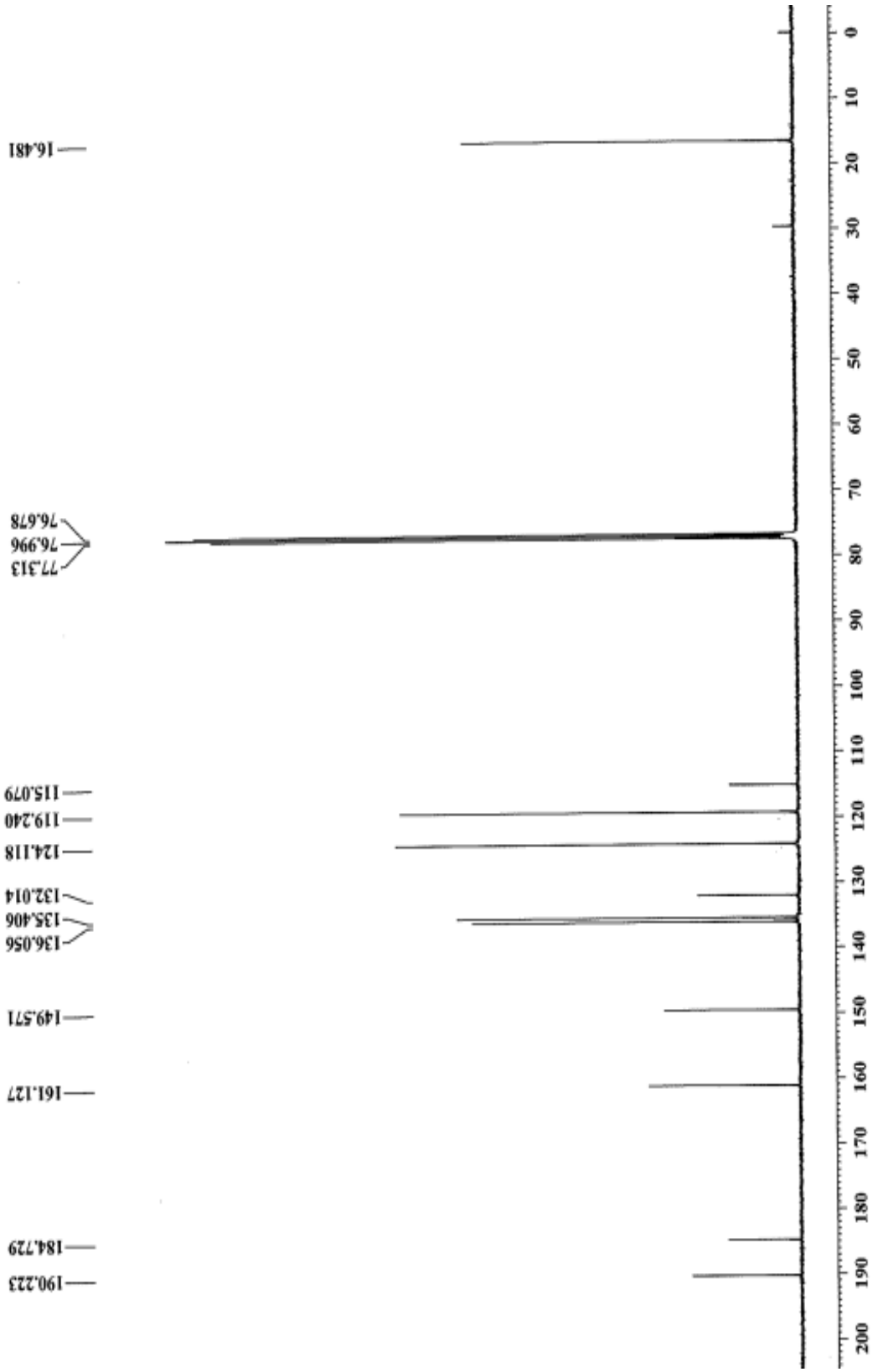


Figure 18. ^{13}C-NMR of compound **1**

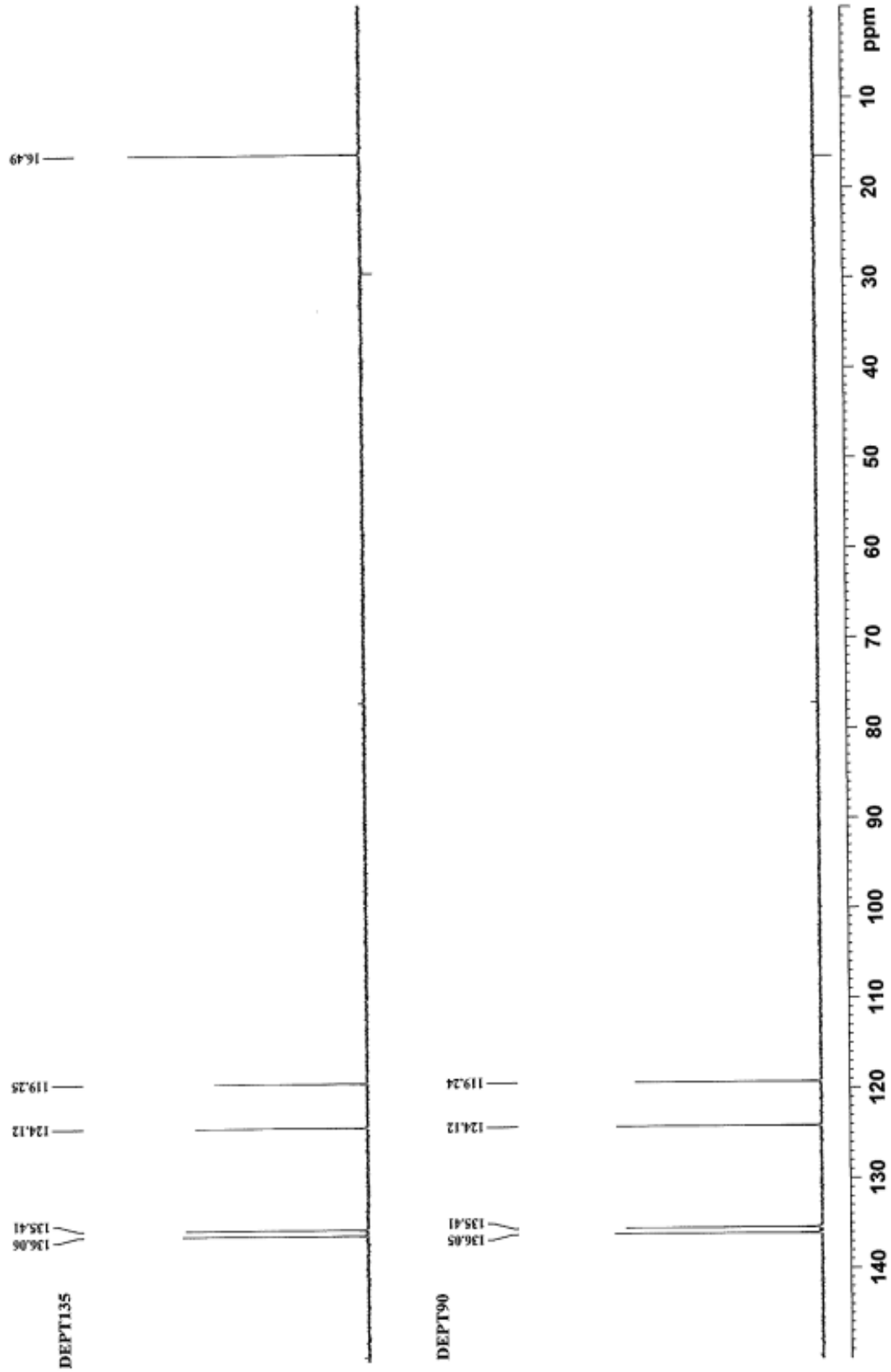


Figure 19. DEPT spectra of compound **1**

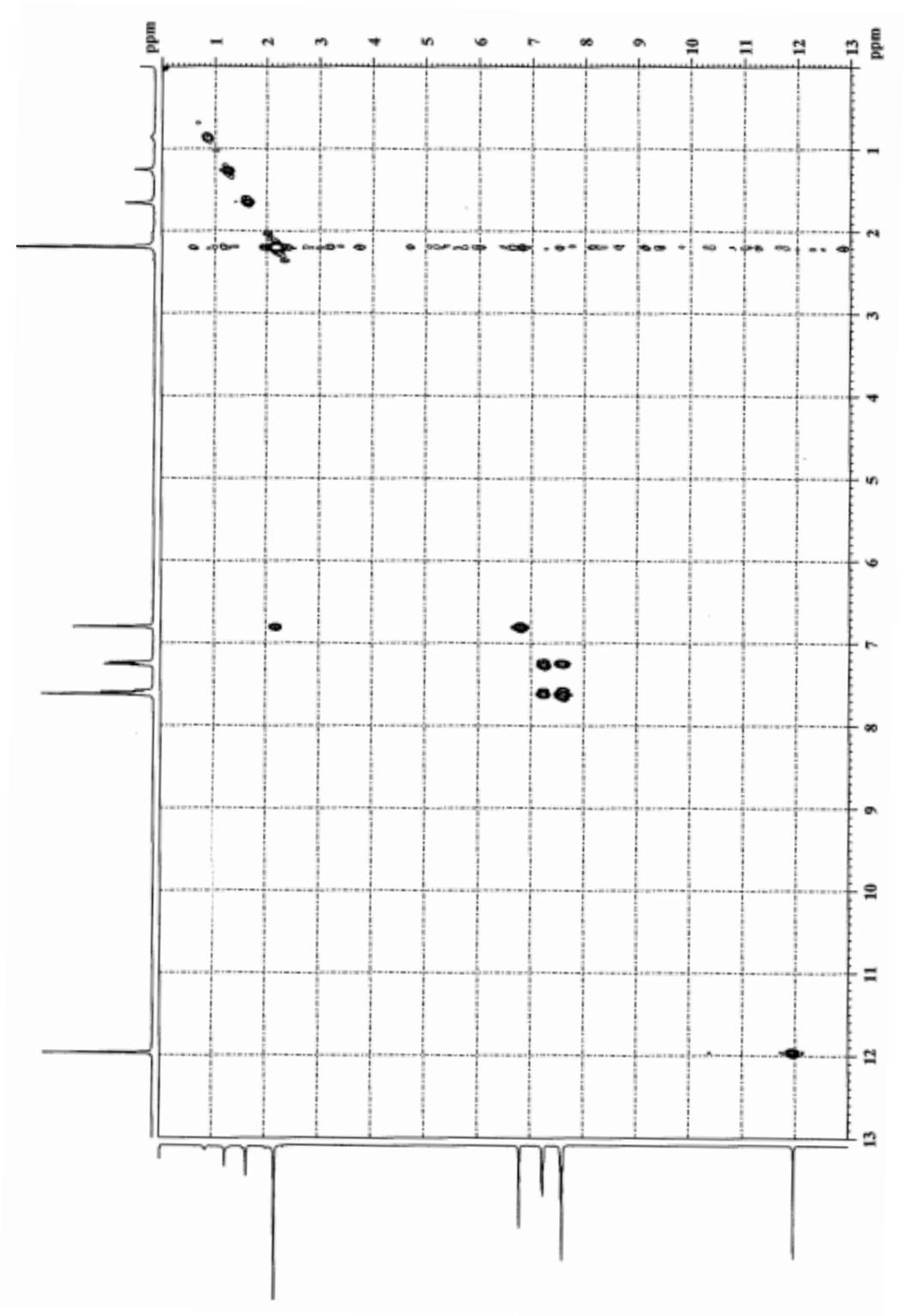


Figure 20. ^{1}H-^{1}H COSY spectra of compound **1**

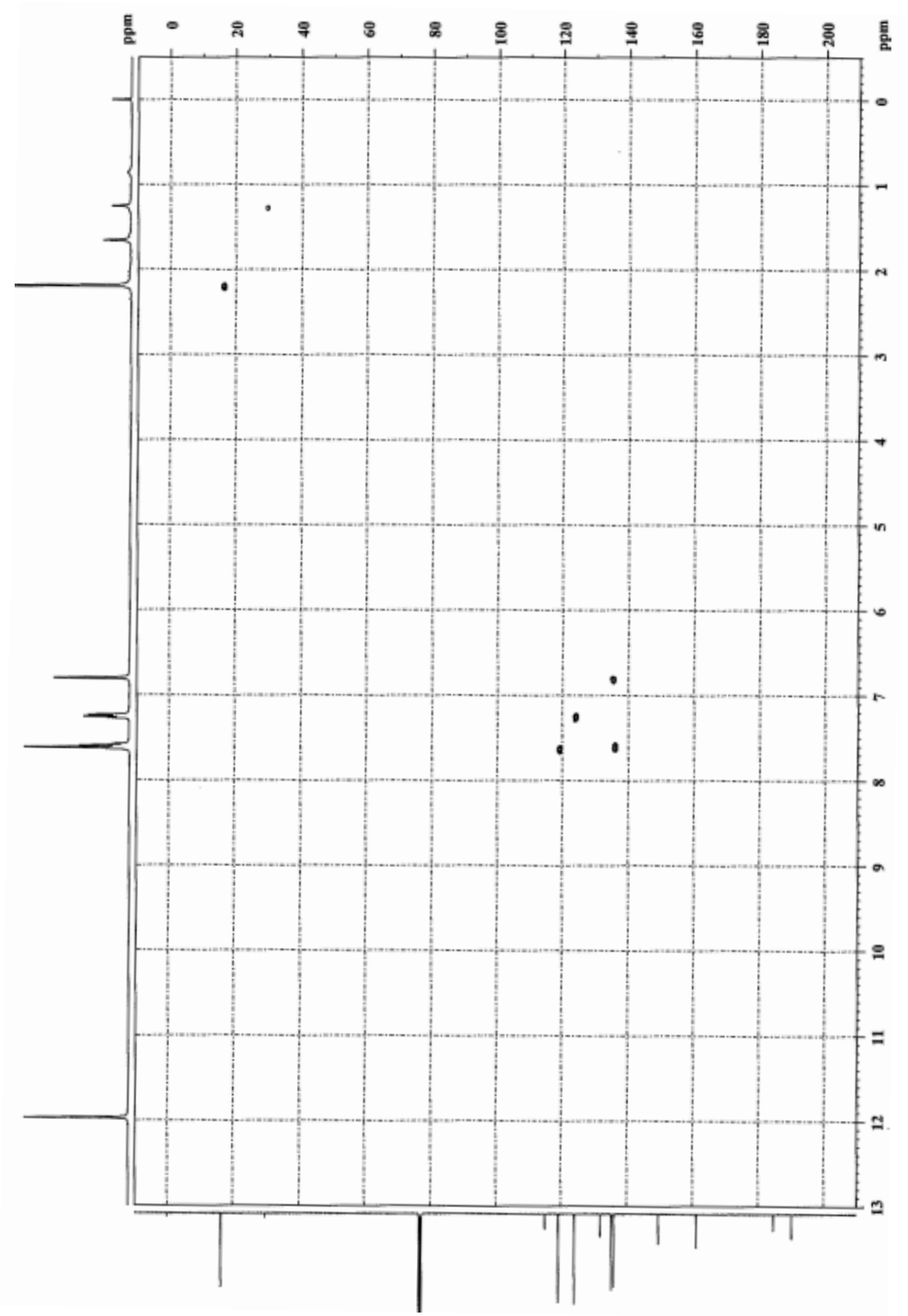


Figure 21. HSQC spectra of compound **1**

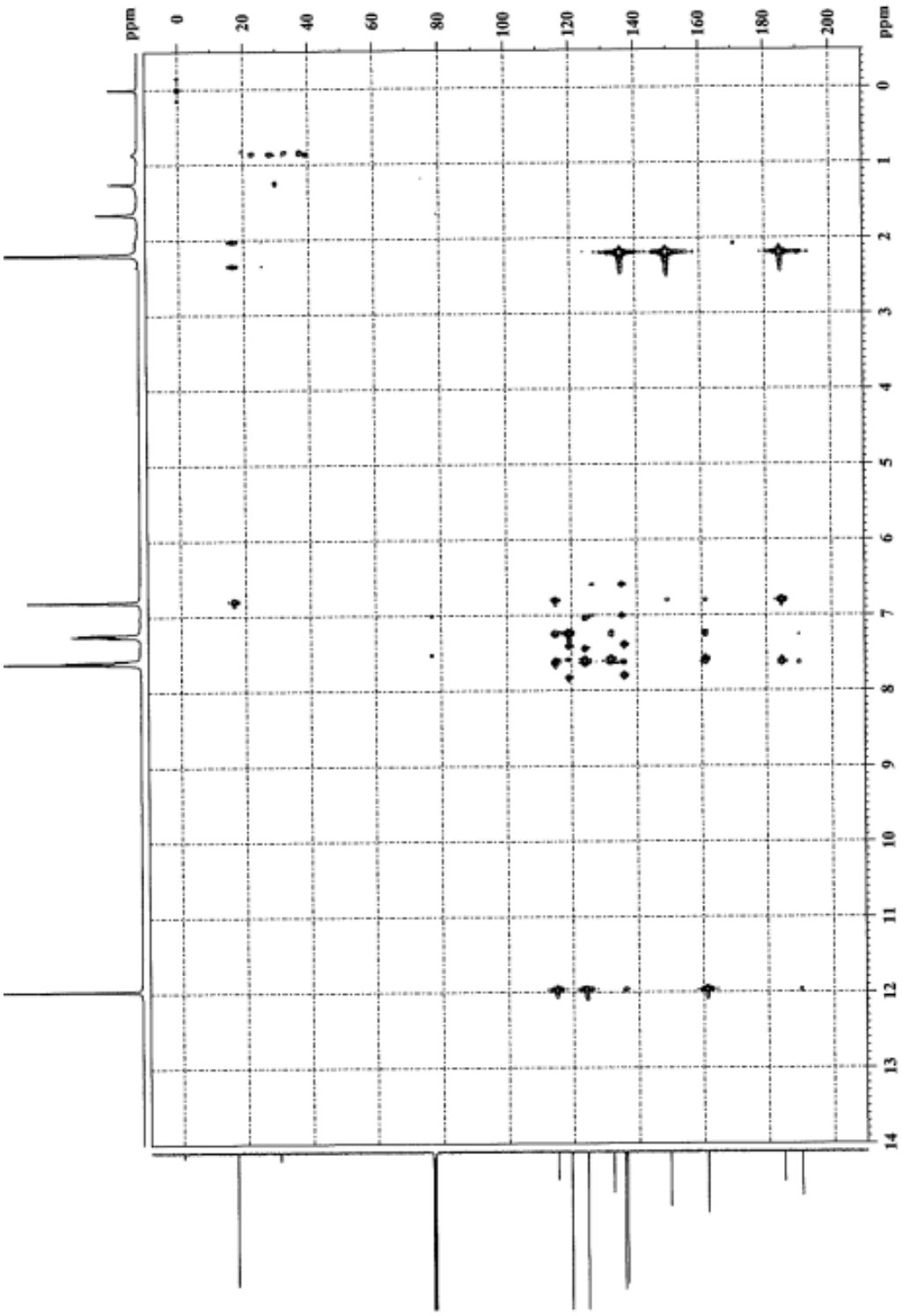


Figure 22. HMBC spectra of compound **1**

High resolution report

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Sample Name	JR001 neg	Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

Acquisition Parameter

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Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

Spectrum View

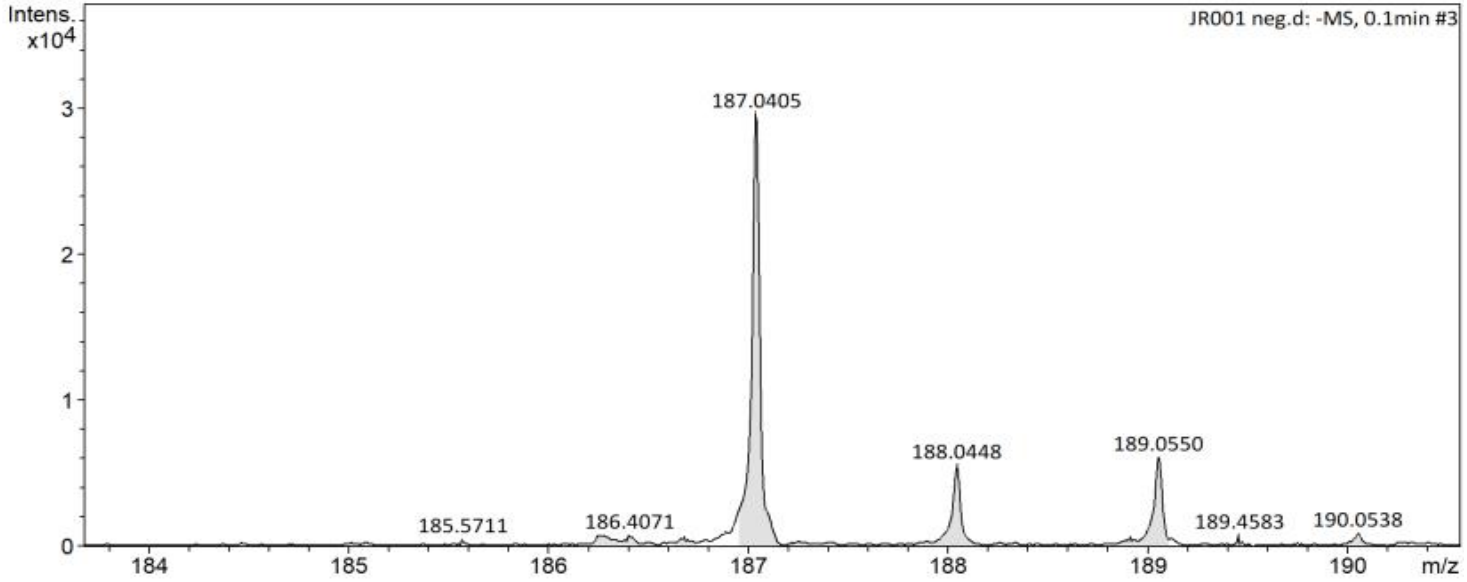

Figure 23. Mass spectroscopy of compound 1

Identification of compound 2

Compound **2** (70.0 mg, yield 1.28%) was isolated as a white solid and the molecular formula was deduced to be $C_{29}H_{50}ONa$ bases on HR-ESI-TOFMS data (*m/z* 437.3750 $[M+Na]^+$, calcd 437.3754). The ^{1}H-NMR spectrum of Compound **2** (Table 18) it was seen that H-3 proton appeared at δ_H 3.52 as a multiplet, H-6 olefinic proton showed a triplet at δ_H 5.35 with a *J* value of 5.3 Hz and the presence of six methyl signals that appeared as two methyl singlets at δ_H 0.68 and 1.00; three methyl doublets that appeared at δ_H 0.93 and 0.82 (H-26 and H-27) and one methyl triplet at δ_H 0.84. Thus, the structure of **2** was identified as a steroid by comparing its spectroscopic data (^{1}H and ^{13}C-NMR) with those reported in the literature (Chaturvedula, 2012) and was named β-sitosterol.

Table 18. ^{1}H and ^{13}C-NMR spectroscopic data of compounds **2** ($CDCl_3$; 300 MHz).

Position	Compound 2		β-sitosterol (Chaturvedula, 2012)	
	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)	δ_C
1		37.3		37.5
2		31.7		31.9
3	3.52 (m)	71.8	3.53 (tdd; 4.5, 4.2, 3.8)	72.0
4		42.3		42.5
5	-	140.8	-	140.9
6	5.35 (t; 5.3)	121.7	5.36 (t; 6.4)	121.9
7		31.9		32.1
8		31.9		32.1
9		50.1		50.3
10	-	36.1	-	36.7
11		21.1		21.3
12		39.8		39.9
13	-	42.3	-	42.6

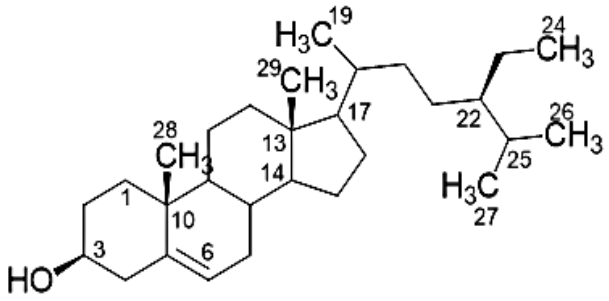

Figure 24. Chemical structure of β-sitosterol (2)

Table 18. (Continued)

Position	Compound 2		β-sitosterol (Chaturvedula, 2012)	
	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)	δ_C
14		56.8		56.9
15		24.3		26.3
16		28.2		28.5
17		56.1		56.3
18		36.5		36.3
19	0.93 (d; 6.5)	19.0	0.93 (d; 6.5)	19.2
20		34.0		34.2
21		26.1		26.3
22		45.9		46.1
23		23.1		23.3
24	0.84 (t; 7.0)	11.9	0.84 (t; 7.2)	12.2
25		29.2		29.4
26	0.82 (d; 6.5)	18.8	0.83 (d; 6.5)	20.1
27	0.82 (d; 6.5)	19.4	0.81 (d; 6.5)	19.6
28	0.68 (s)	19.8	0.68 (s)	19.0
29	1.00 (s)	12.0	1.01 (s)	12.0

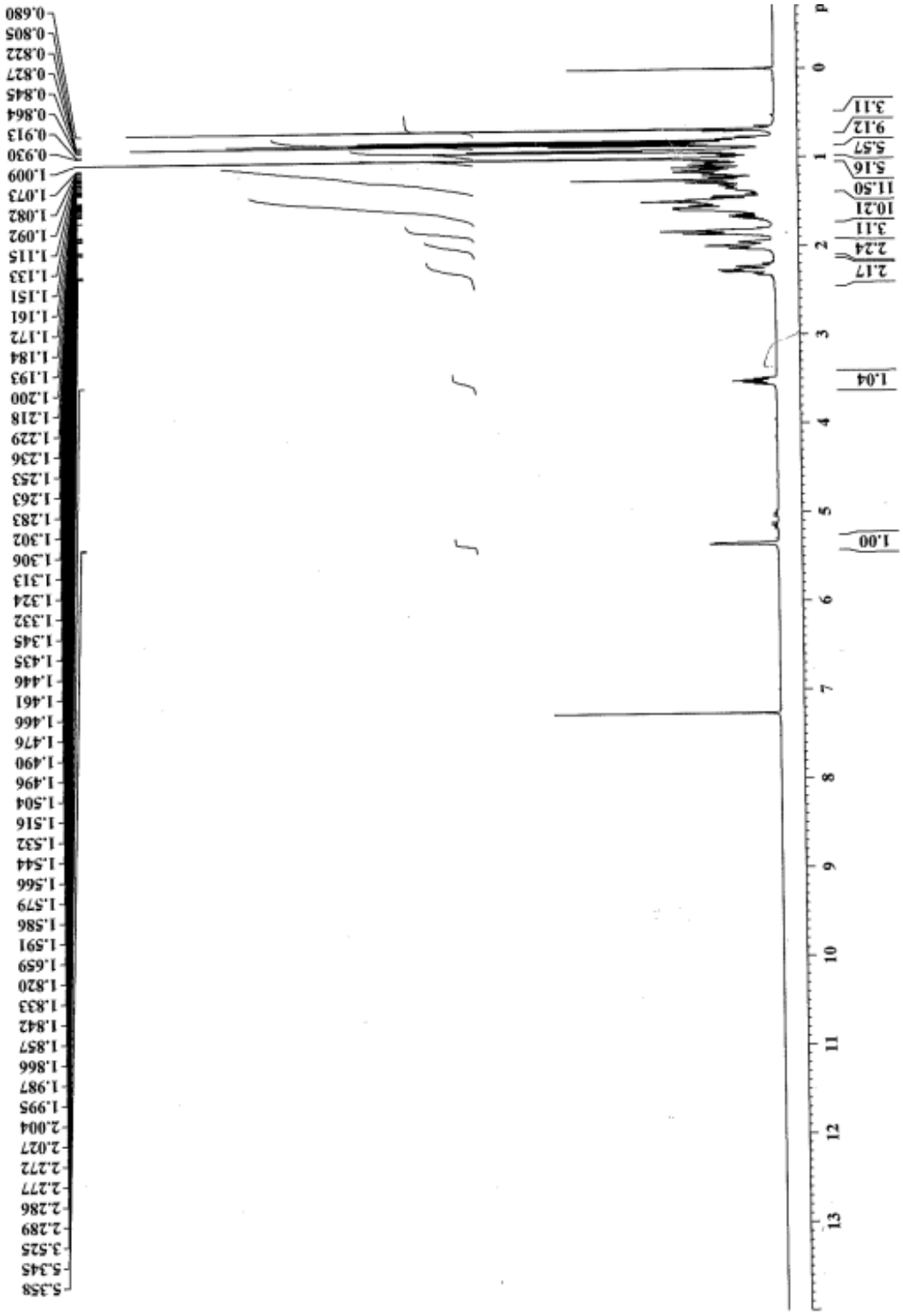

Figure 25. 1H-NMR (CDCl3; 300 MHz) of compound 2

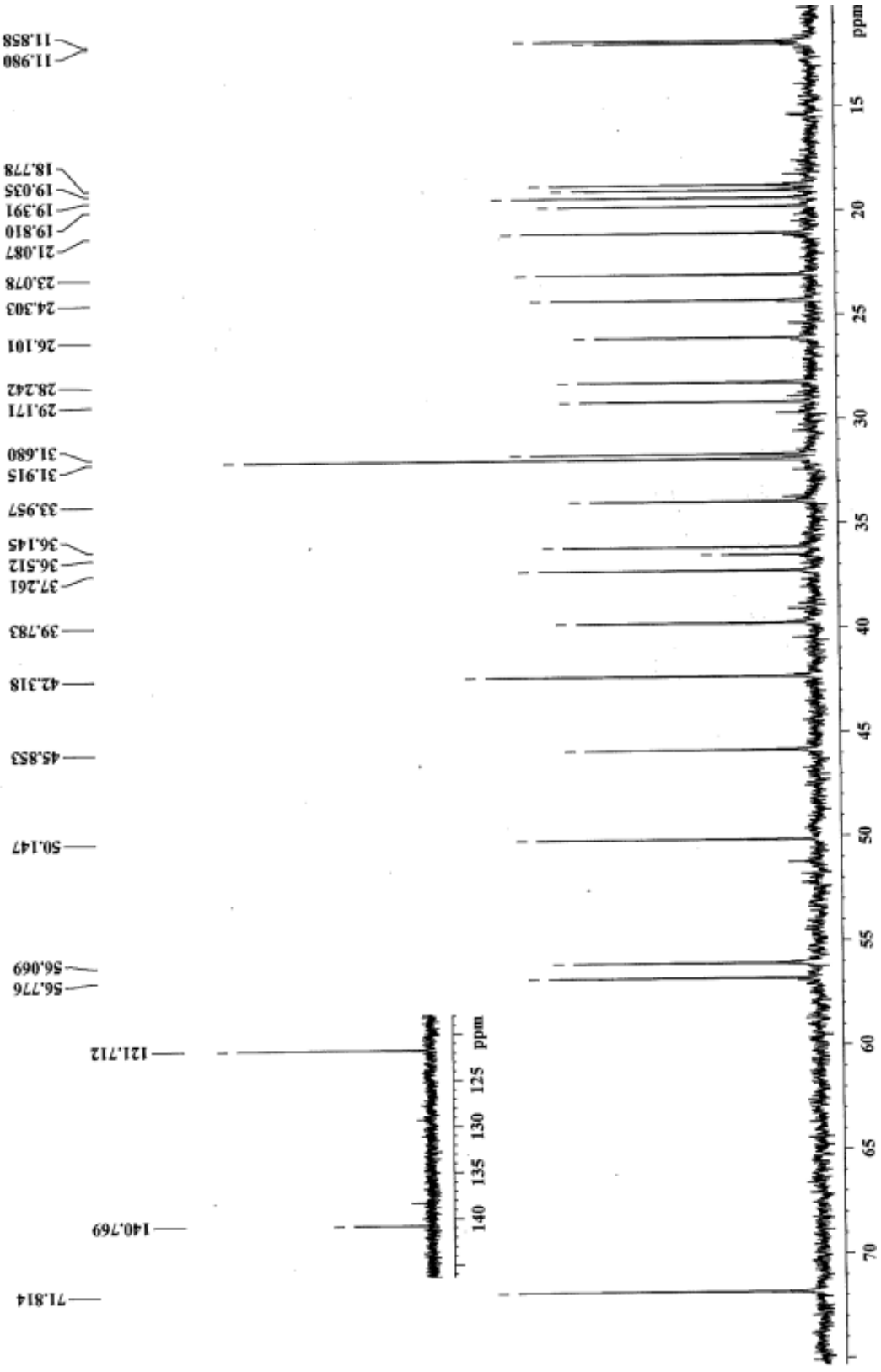

Figure 26. ^{13}C-NMR of compound **2**

High resolution report

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		Calibrate by	Sodium Formate

Acquisition Parameter

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Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

Spectrum View

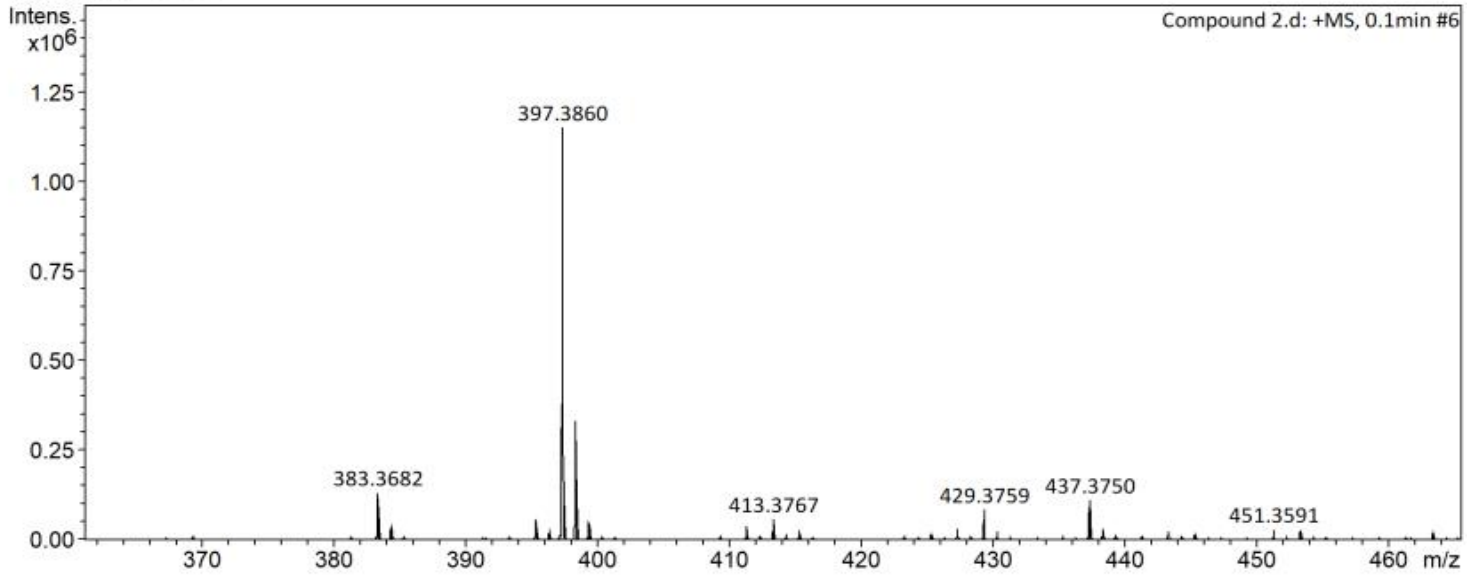

Figure 27. Mass spectroscopy of compound 2

Identification of compound 3

Compound **3** (17.0 mg, yield 0.31%) was isolated as an orange amorphous solid and the molecular formula was deduced to be $C_{22}H_{14}O_6Na$ based on HR-ESI-TOFMS data (*m/z* 397.0677 $[M+Na]^+$, calcd 397.0683). The structure of compound **3** was characterized by analyses of the ^1H, ^{13}C, DEPT, ^1H-^1H COSY, HSQC and HMBC spectroscopic data. The ^1H-NMR spectrum of compound **3** (Table 19) exhibited a hydroxyl group at δ_H 12.58 (1H, s, 5-OH), three sets of aromatic protons signal at δ_H 7.29 (1H, dd, *J* = 8.7 Hz, H-6), 7.20 (1H, d, *J* = 8.7 Hz, H-7) and 6.81 (1H, q, *J* = 1.4 Hz, H-3) and methyl proton signal at δ_H 2.01 (3H, d, *J* = 1.4 Hz, 11-CH_3). Analyses of the ^{13}C and DEPT of compound **3** revealed the presence of three methine (δ_C 138.0, 134.9 and 124.3), one methyl (δ_C 16.6) and seven quaternary carbons (δ_C 190.5, 185.2, 161.3, 150.0, 135.6, 128.2 and 115.5). The HSQC spectrum helped to assign the attached protons to their corresponding carbon atoms, while the ^1H-^1H COSY spectrum demonstrated the partial structure from H-6 to H-7. The HMBC spectra showed the correlations of the OH-5 to C-5, C-6 and C-10 and from the methyl protons H-11 to C-1, C-2 and C-3, thus establishing that the OH group was attached to C-5 and the methyl groups H-11 to C-2, respectively.

A comparison of NMR spectral data between compounds **1** and **3** revealed similarities, but the splitting pattern for H-6 and H-7 was simplified due to the absence of H-8, which established the dimer linkage at C-8 to C-8′, suggesting that compound **3** was a dimer of compound **1** and was further supported by mass spectral data of compound **3**. The structure of compound **3** was identified as 8,8'-biplumbagin by spectroscopic methods (1D and 2D-NMR) including a comparison with data reported in the literature (Gu et al., 2004) and was named maritinone.

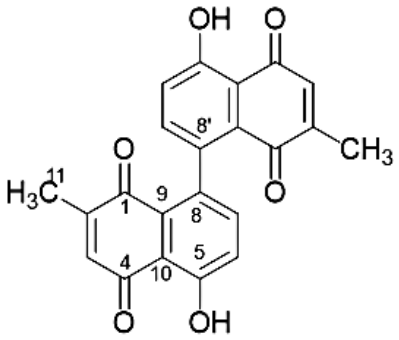

Figure 28. Chemical structure of maritinone (**3**)

Table 19. ^{1}H,^{13}C-NMR and HMBC data of compounds **3** ($CDCl_3$; 500 MHz).

Position	Compound 3			Maritinone (Gu et al., 2004)	
	δ_H (mult.; *J* in Hz)	δ_C	HMBC	δ_H (mult.; *J* in Hz)	δ_C
1, 1′	-	185.2	-	-	185.1
2, 2′	-	150.0	-	-	150.0
3, 3′	6.81 (q; 1.4)	134.9	C-1, C-10, C-11	6.81 (q; 1.5)	134.9
4, 4′	-	190.5	-	-	190.5
5-OH, 5-OH′	12.58 (s)	161.3	C-5, C-6, C-10	12.57 (s)	161.3
6, 6′	7.29 (d; 8.7)	124.3	C-5, C-9, C-10	7.29 (d; 8.7)	124.3
7, 7′	7.20 (d; 8.7)	138.0	C-5, C-8, C-9	7.21 (d; 8.7)	137.9
8, 8′	-	128.2	-	-	128.2
9, 9′	-	135.6	-	-	135.5
10, 10′	-	115.5	-	-	115.4
11-CH_3, 11-CH_3′	2.01 (d; 1.4)	16.6	C-1, C-2, C-3	2.01 (d; 1.5)	16.6

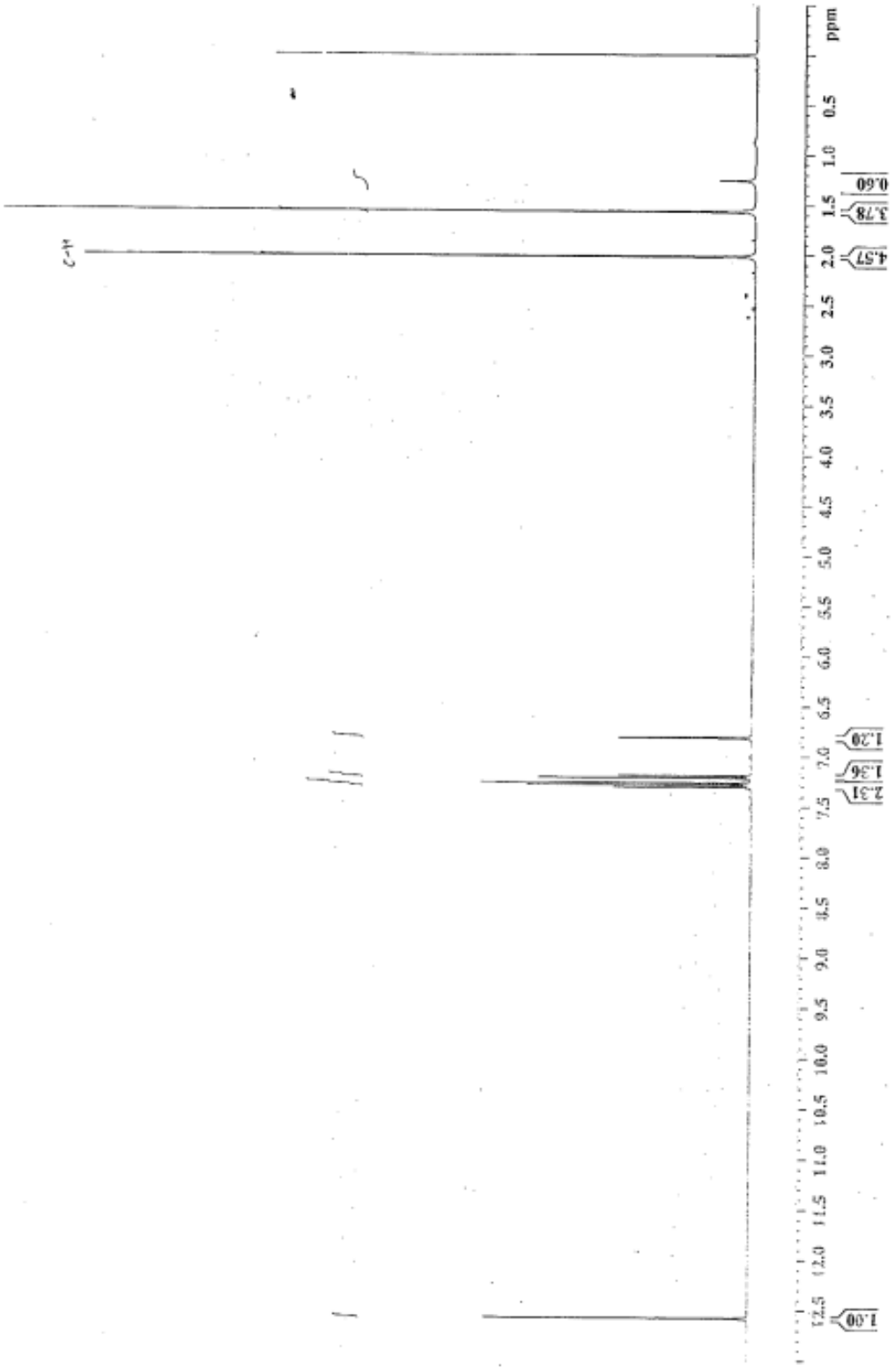

Figure 29. ^{1}H-NMR ($CDCl_3$; 500 MHz) of compound **3**

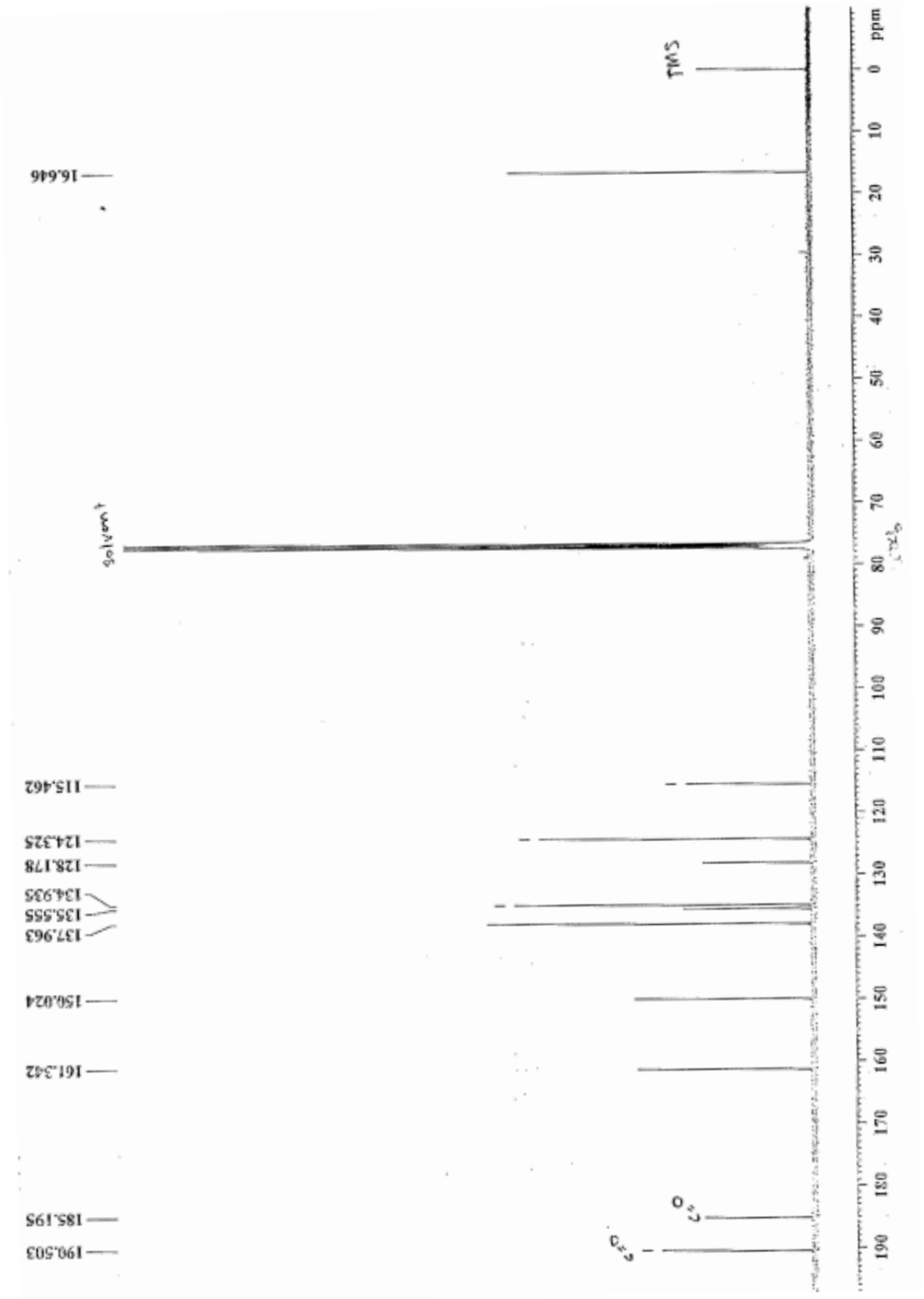

Figure 30. ^{13}C-NMR of compound **3**

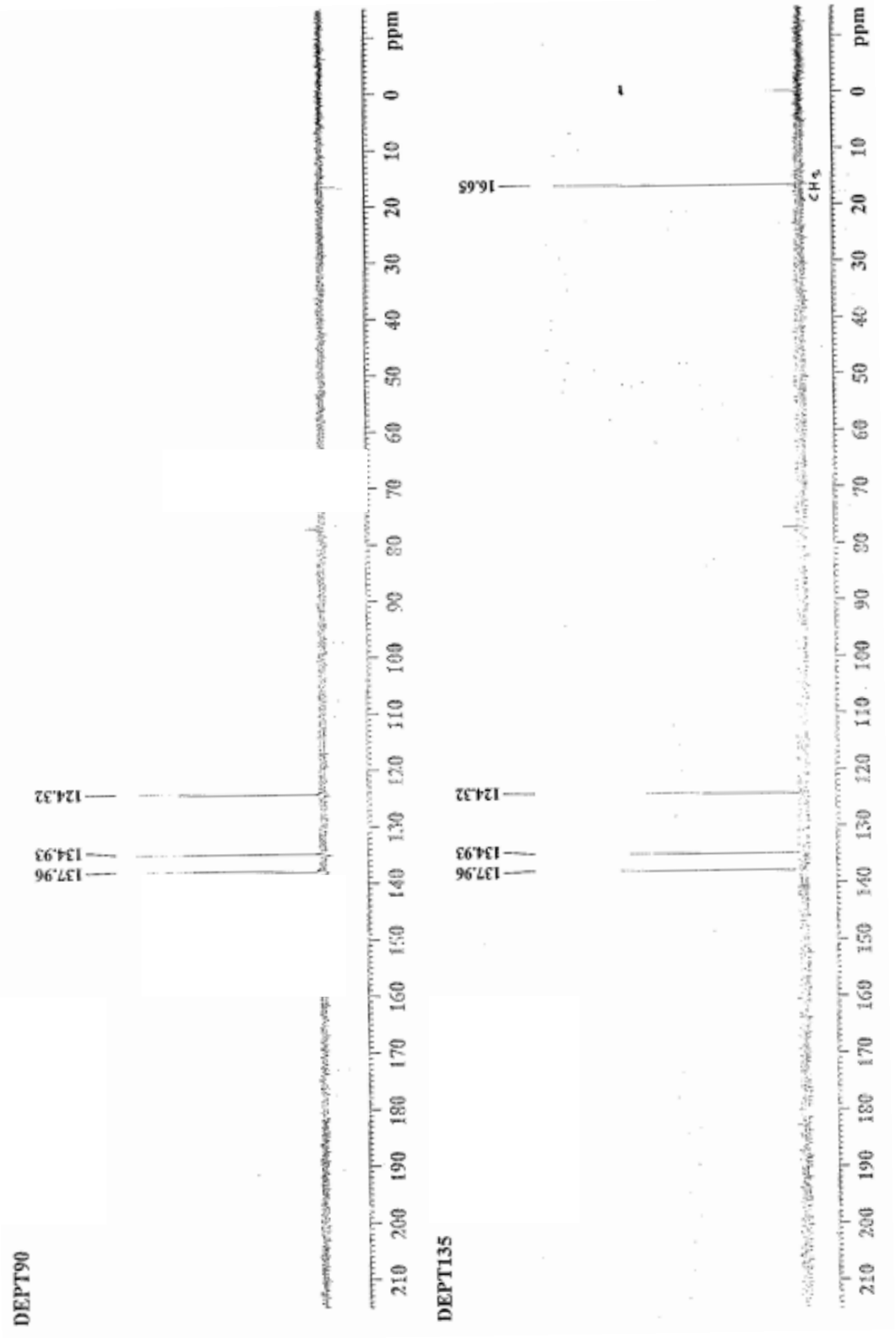

Figure 31. DEPT spectra of compound **3**

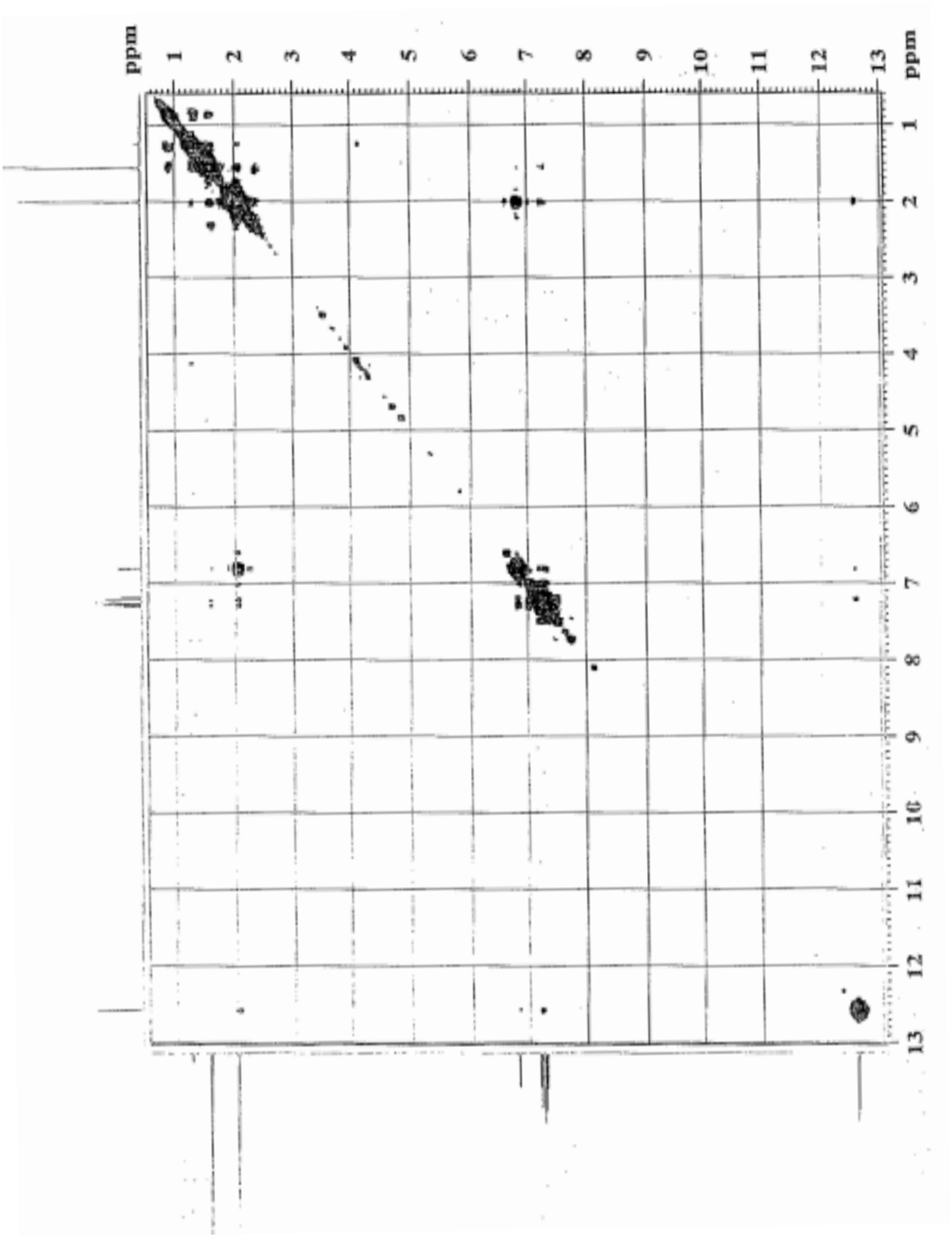

Figure 32. ^{1}H-^{1}H COSY spectra of compound **3**

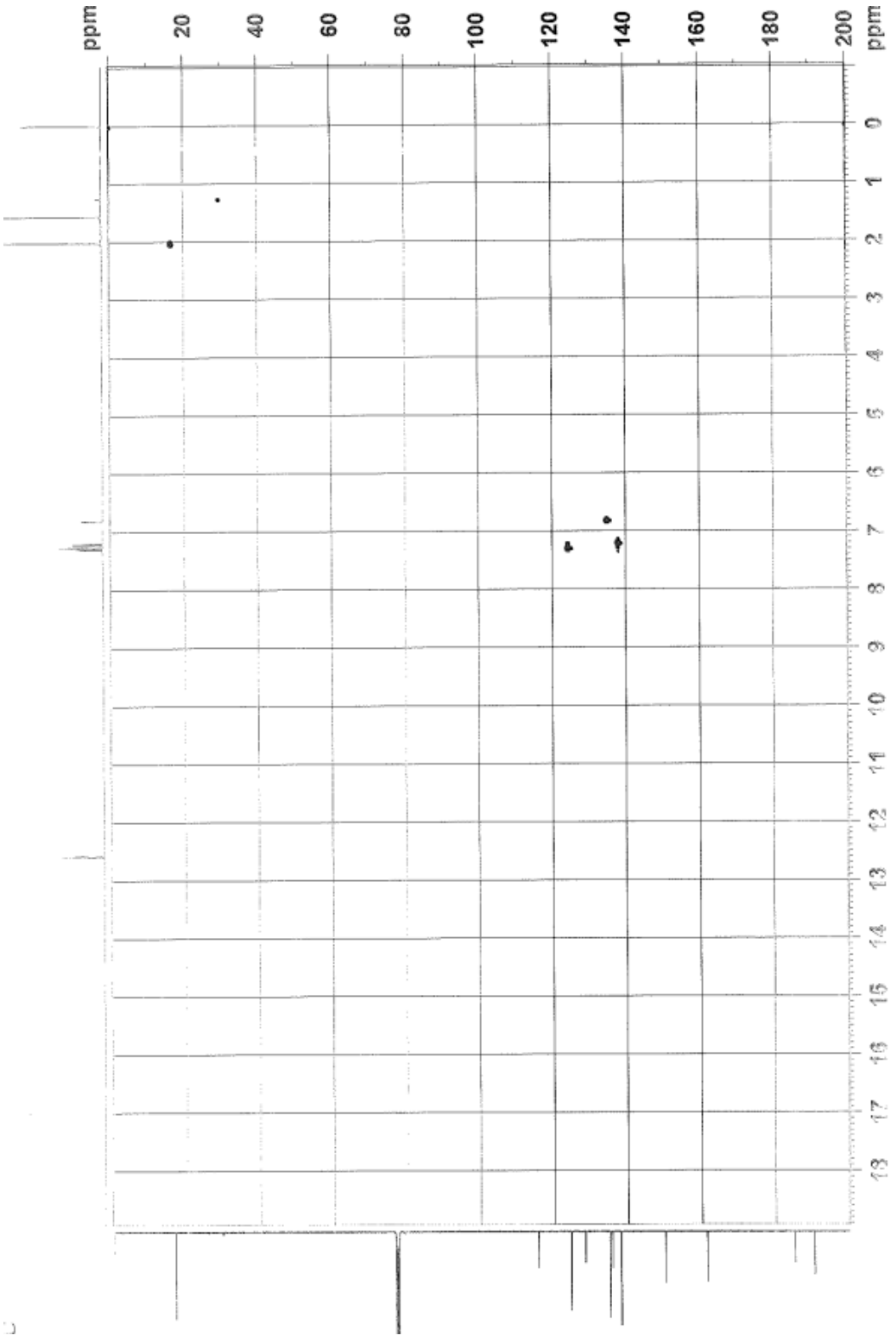

Figure 33. HSQC spectra of compound **3**

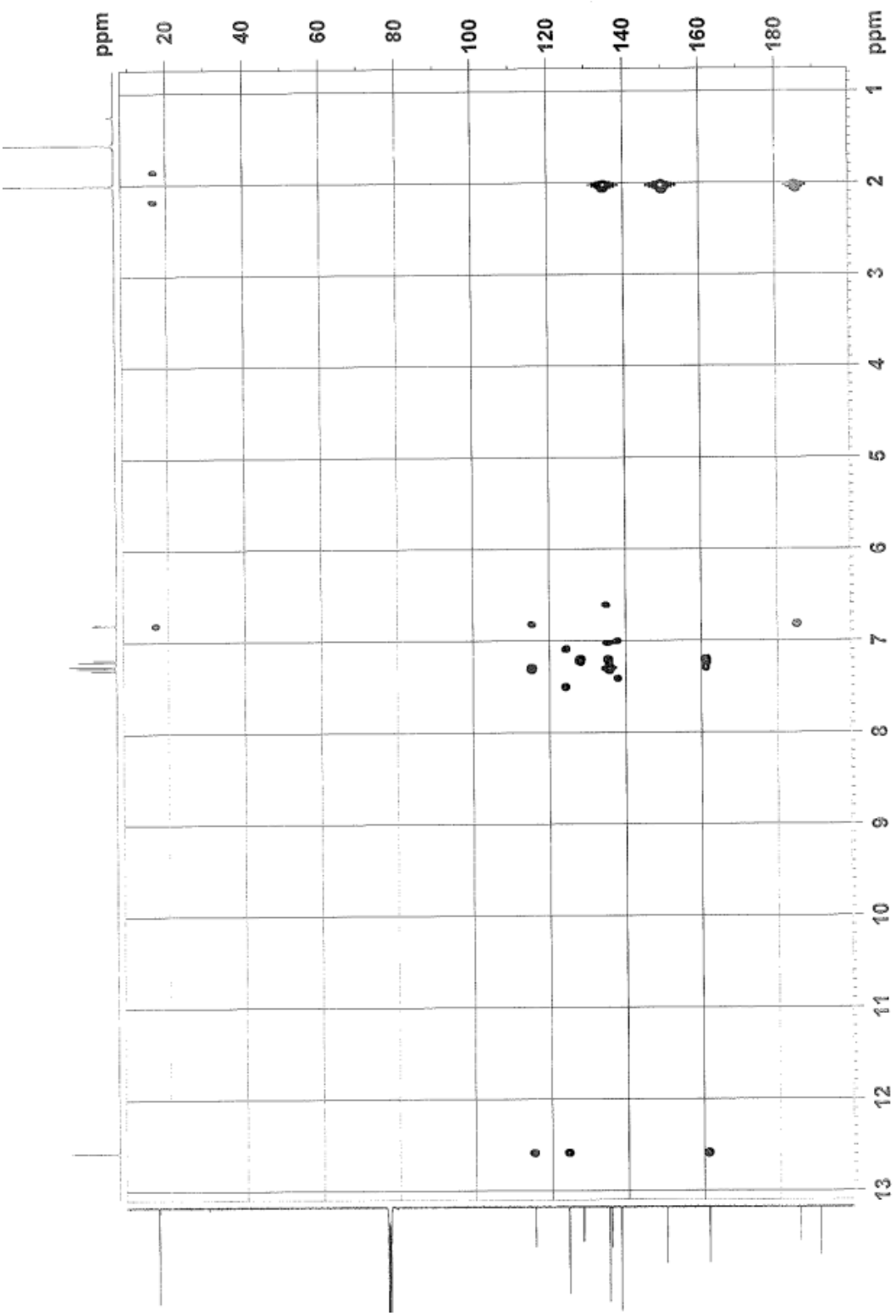

Figure 34. HMBC spectra of compound **3**

High resolution report

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		Calibrate by	Sodium Formate

Acquisition Parameter

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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

Spectrum View

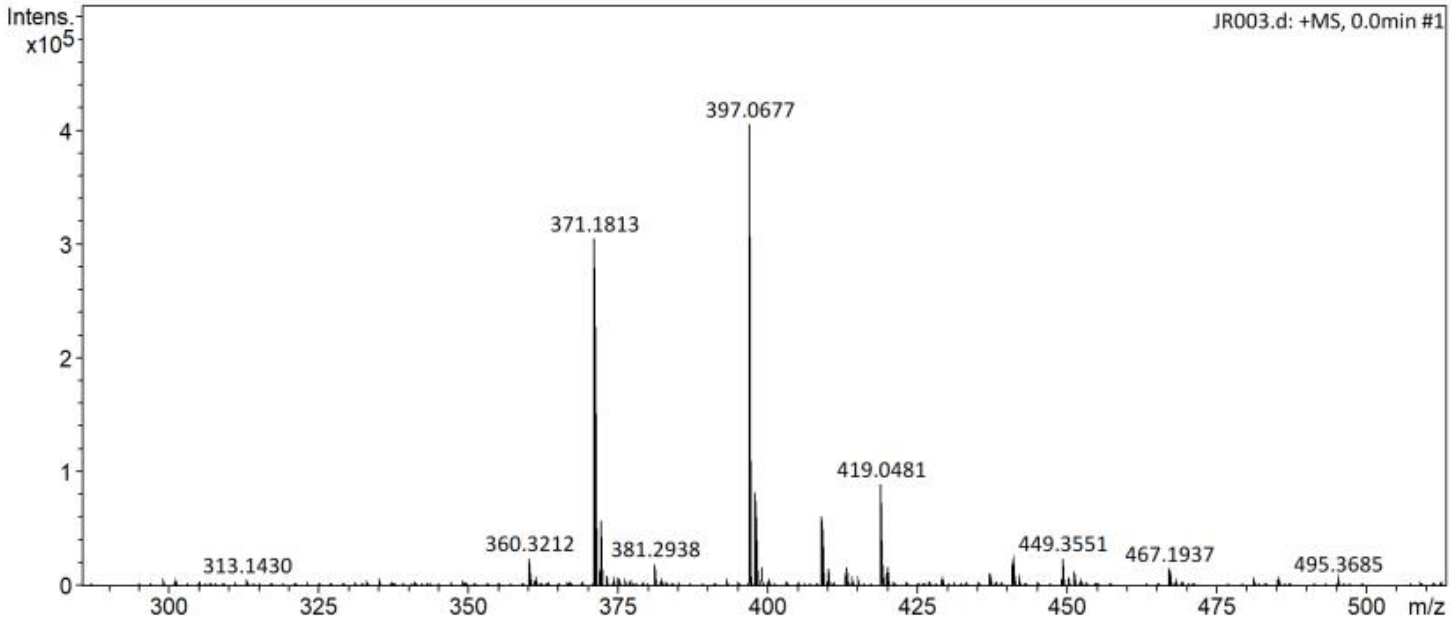

Figure 35. Mass spectroscopy of compound **3**

Identification of compound 4

Compound **4** (1.5 mg, 0.03%) was isolated as an orange amorphous solid and the molecular formula was deduced to be $C_{22}H_{15}O_6$ based on HR-ESI-TOFMS data (*m/z* 375.0861 $[M+H]^+$, calcd 375.0855). The ^{1}H-NMR spectrum of compound **4** (Table 20) exhibited a hydroxyl group at δ_H 12.50 (1H, s, 5-OH), three aromatic protons signal at δ_H 7.73 (2H, s, H-6 and H-8) and 6.84 (1H, q, *J* = 1.3 Hz, H-3) and methyl proton signal at δ_H 2.22 (1H, d, *J* = 1.3 Hz, 11-CH_3). The ^{1}H and ^{13}C-NMR spectral data showed just 11 carbon signals and proton signals corresponding to a plumbagin (**1**). However, the mass spectrum and the molecular formula both showed 22 carbons, indicating that compound **4** is a symmetrical dimer of plumbagin. The NMR comparison of compound **4** with compound **1** revealed that an aromatic proton, H-7, in compound **1** (δ_H 7.60) was absent. The doublet of doublet proton signals at H-6 and H-8 in compound **1** were replaced by singlet protons at H-6 and H-8 in compound **4**. Therefore, compound **4** was a dimer of plumbagin that showed a linkage between the 7 and 7′ positions. Analyses of ^{1}H-^{1}H COSY and HMBC spectra of compound **4**, in combination with a comparison of data from the literature (Salae et al., 2010), led to the assignment of the proton and carbon resonances in compound **4**. The key HMBC correlations were as follows: H-3 to C-1, C-2, C-10 and C-11; OH-5 to C-5, C-6 and C-10; H-6 to C-5, C-7, C-8 and C-10; H-8 to C-1, C-6, C-7 and C-10; and H-11 to C-1, C-2 and C-3. The structure of compound **4** has thus been established in the present study as 7,7′-biplumbagin.

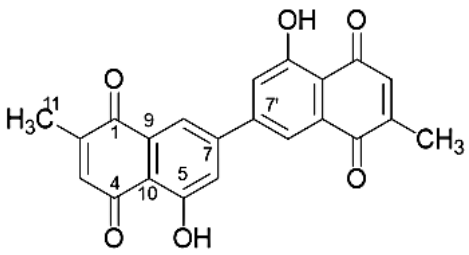

Figure 36. Chemical structure of 7,7′-biplumbagin (**4**)

Table 20. ^{1}H,^{13}C-NMR and HMBC data of compounds **4** ($CDCl_3$; 500 MHz).

Position	Compound 4			7,7′-biplumbagin (Salae et al., 2010)	
	δ_H (mult.; *J* in Hz)	δ_C	HMBC	δ_H (mult.; *J* in Hz)	δ_C
1, 1′	-	184.5	-	-	183.1
2, 2′	-	149.8	-	-	149.8
3, 3′	6.85 (q; 1.3)	135.5	C-1, C-2, C-10, C-11	6.85 (q; 1.5)	135.5
4, 4′	-	190.5	-	-	190.4
5-OH, 5-OH′	12.50 (s)	158.9	C-5, C-6, C-10	12.50 (s)	158.9
6, 6′	7.73 (s)	118.7	C-5, C-7, C-8, C-10	7.73 (br s)	118.7
7, 7′	-	131.2	-	-	131.2
8, 8′	7.73 (s)	137.7	C-1, C-6, C-7, C-9, C-10	7.73 (br s)	137.6
9, 9′	-	131.9	-	-	131.9
10, 10′	-	115.3	-	-	115.3
11-CH_3, 11-CH_3′	2.22 (d; 1.3)	16.5	C-1, C-2, C-3	2.22 (d; 1.5)	16.4

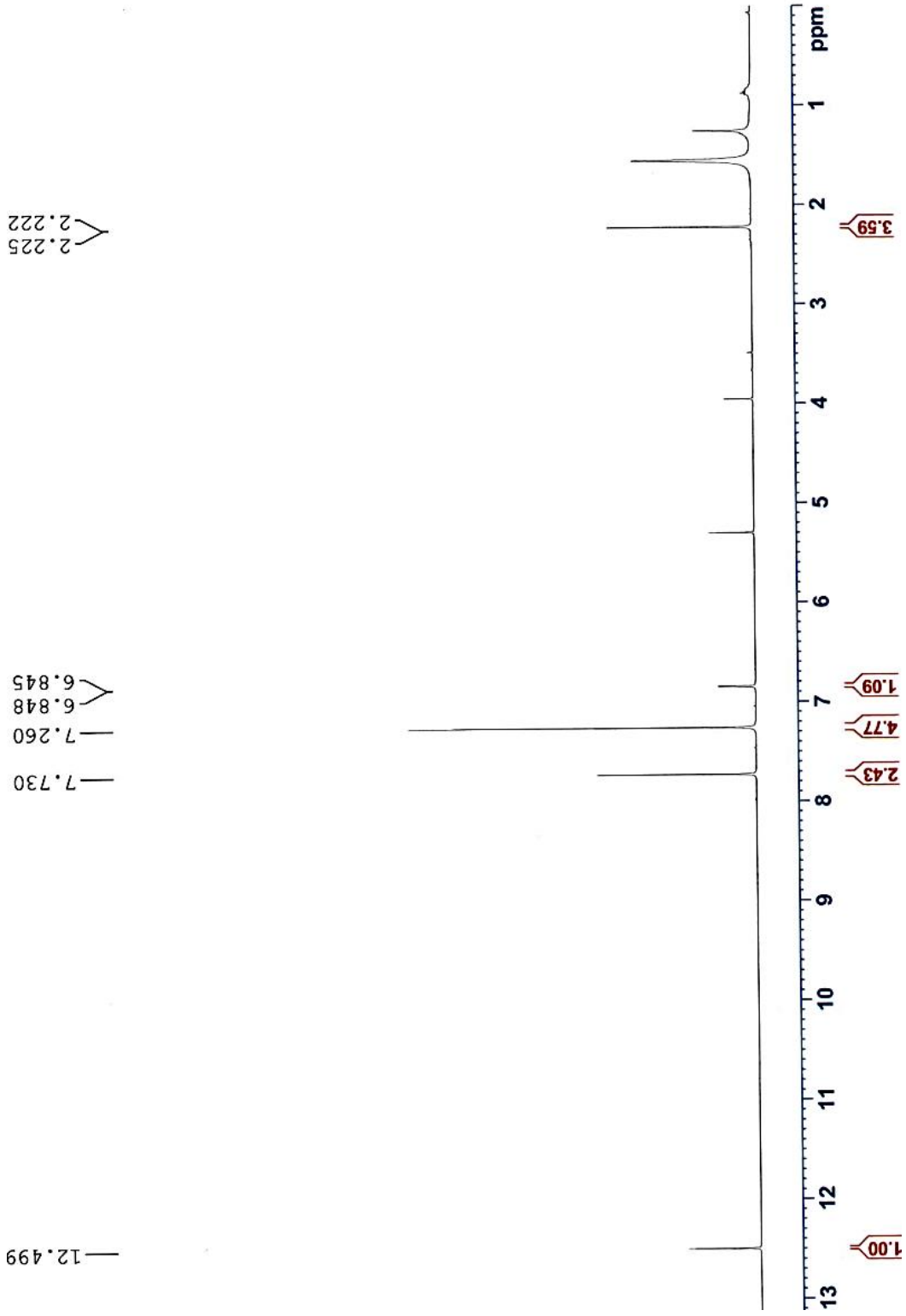

Figure 37. ^{1}H-NMR ($CDCl_3$; 500 MHz) of compound **4**

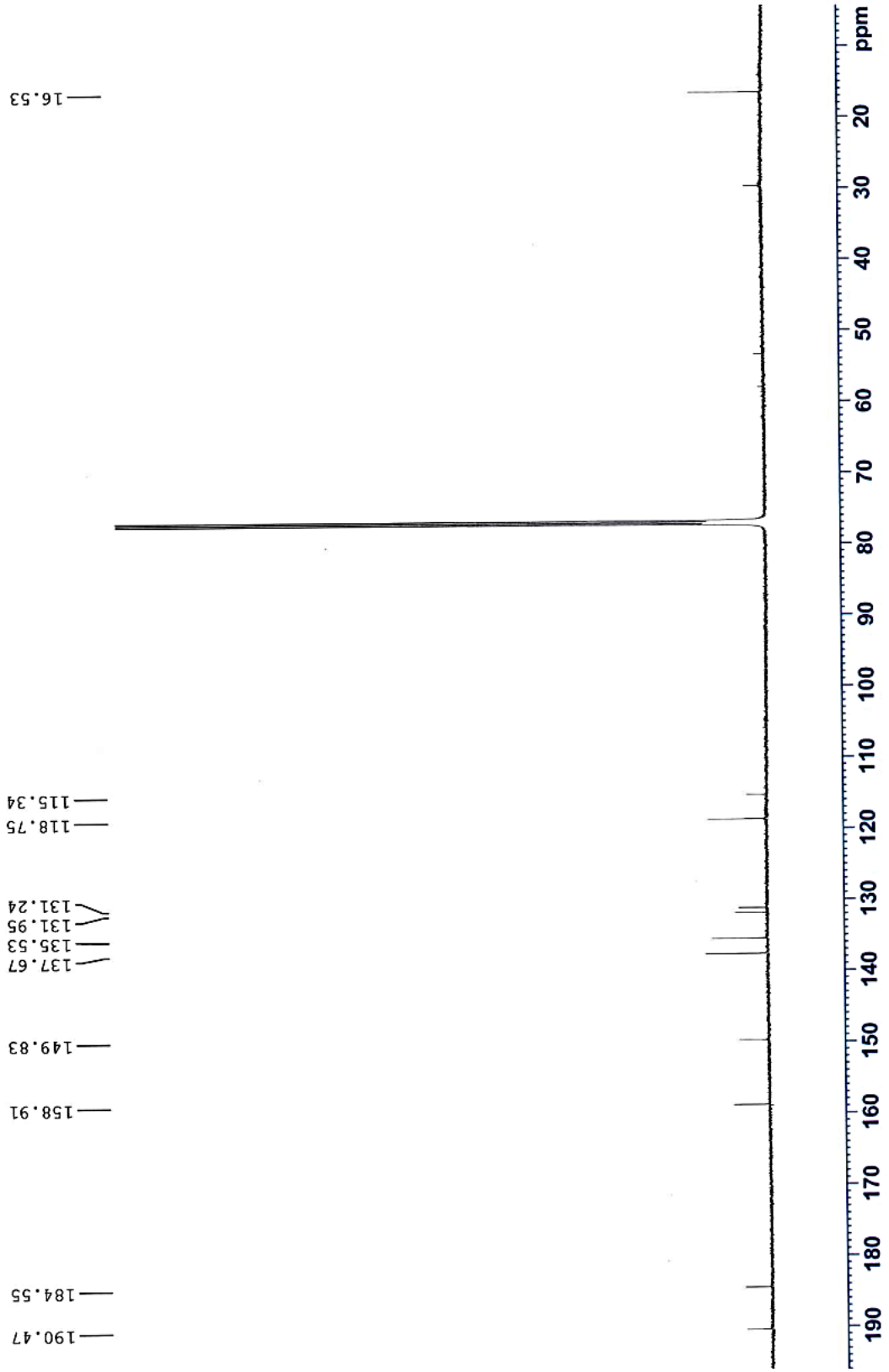

Figure 38. ^{13}C-NMR of compound **4**

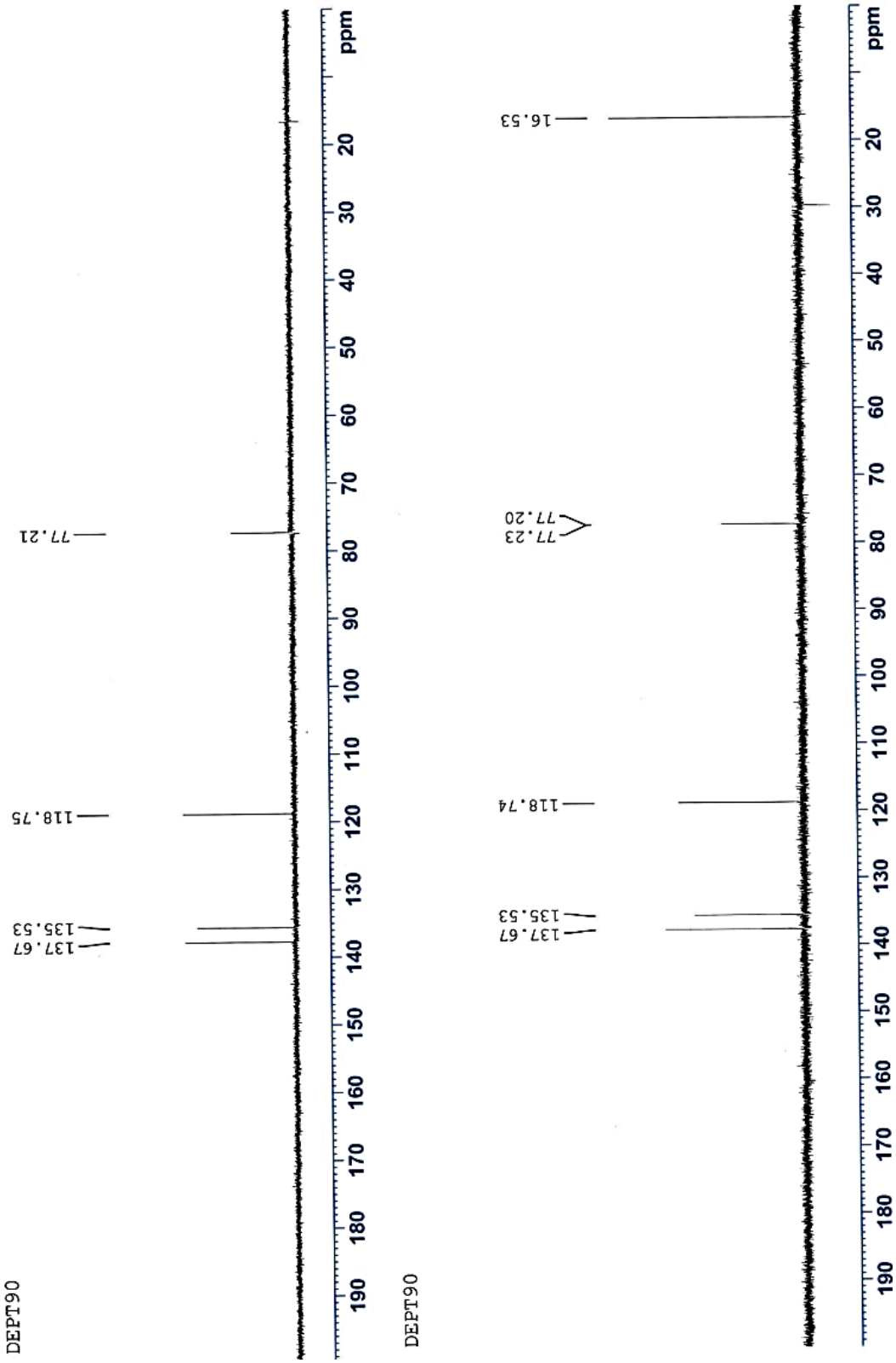

Figure 39. DEPT spectra of compound **4**

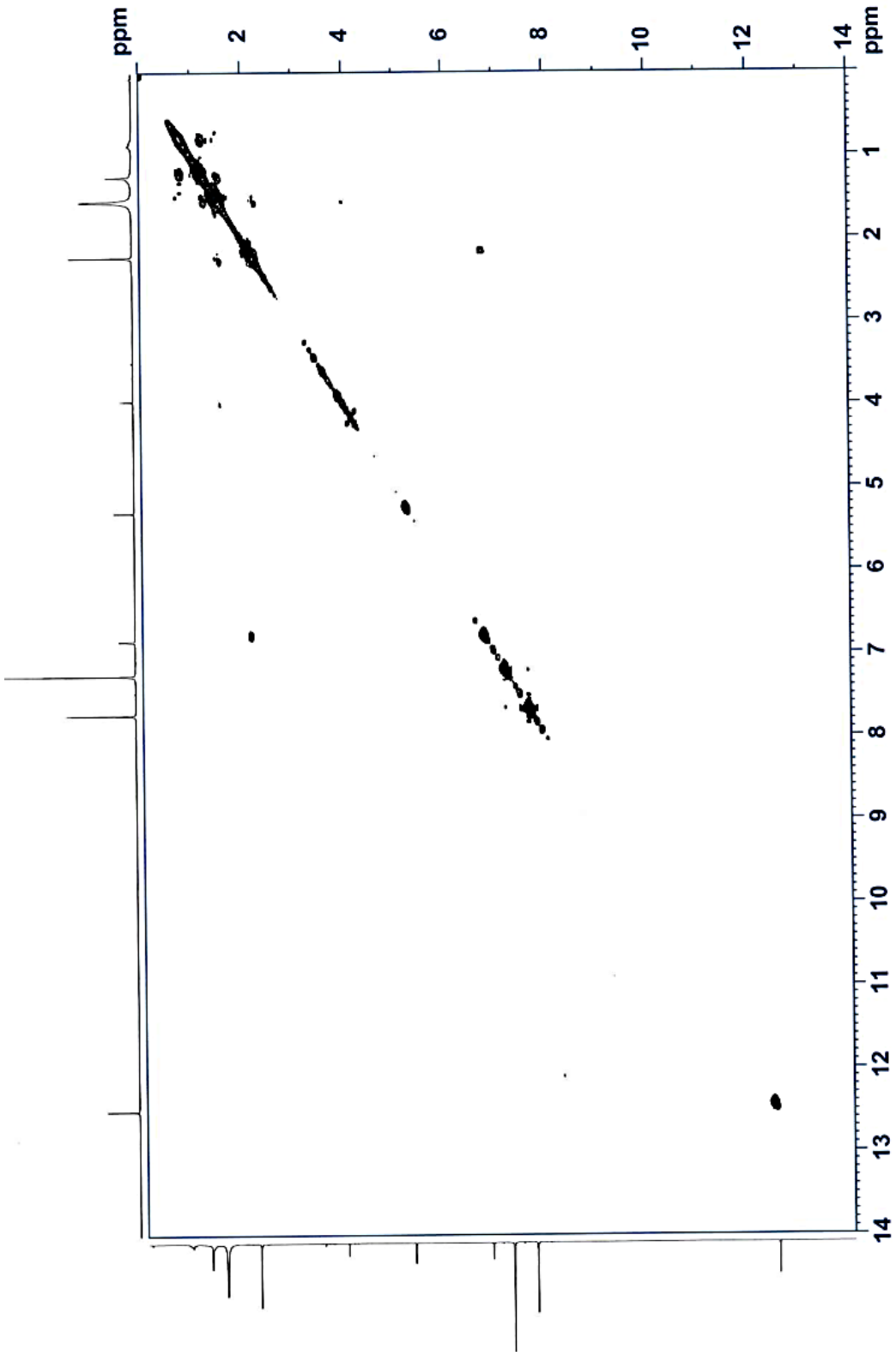

Figure 40. ^{1}H-^{1}H COSY spectra of compound **4**

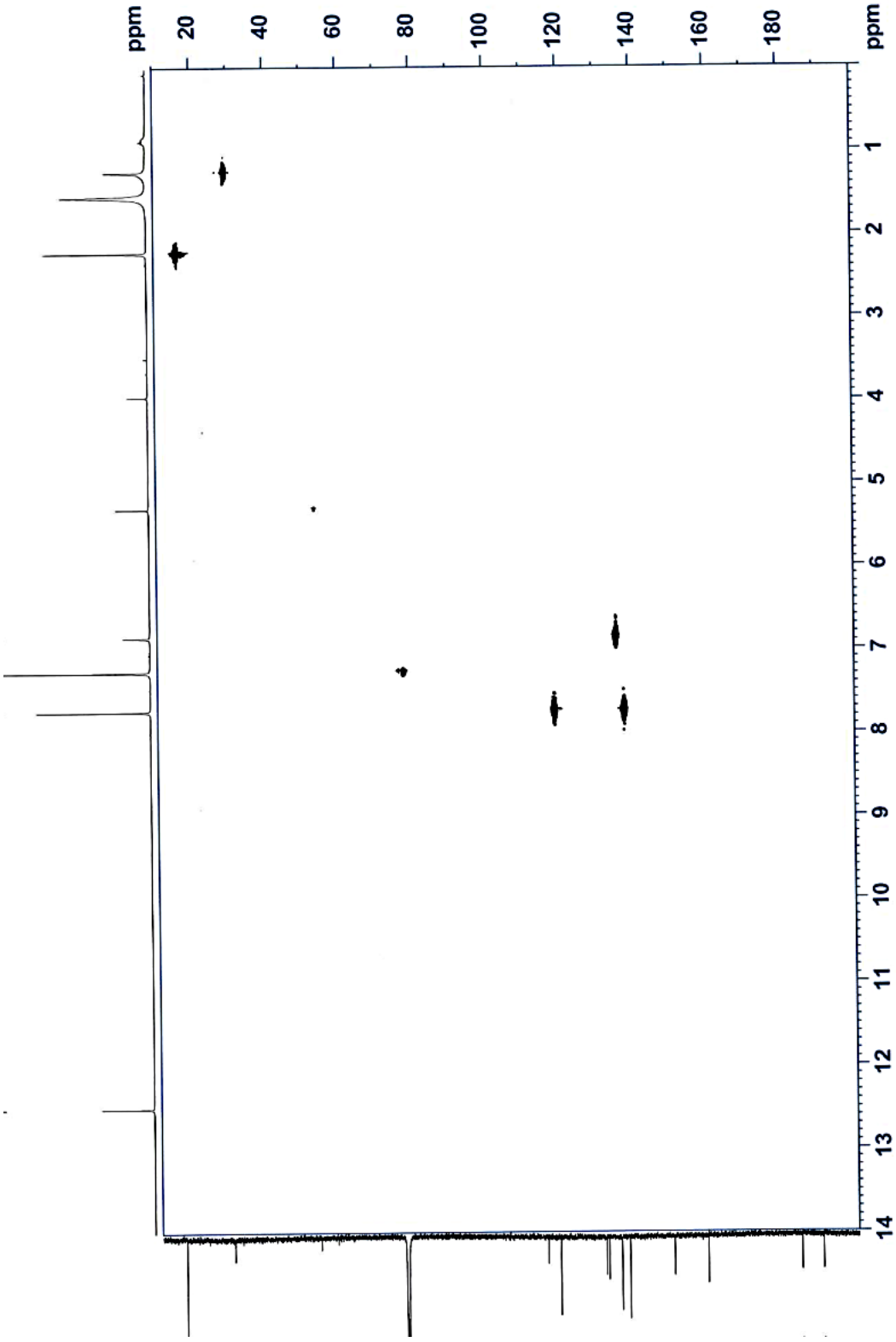

Figure 41. HSQC spectra of compound **4**

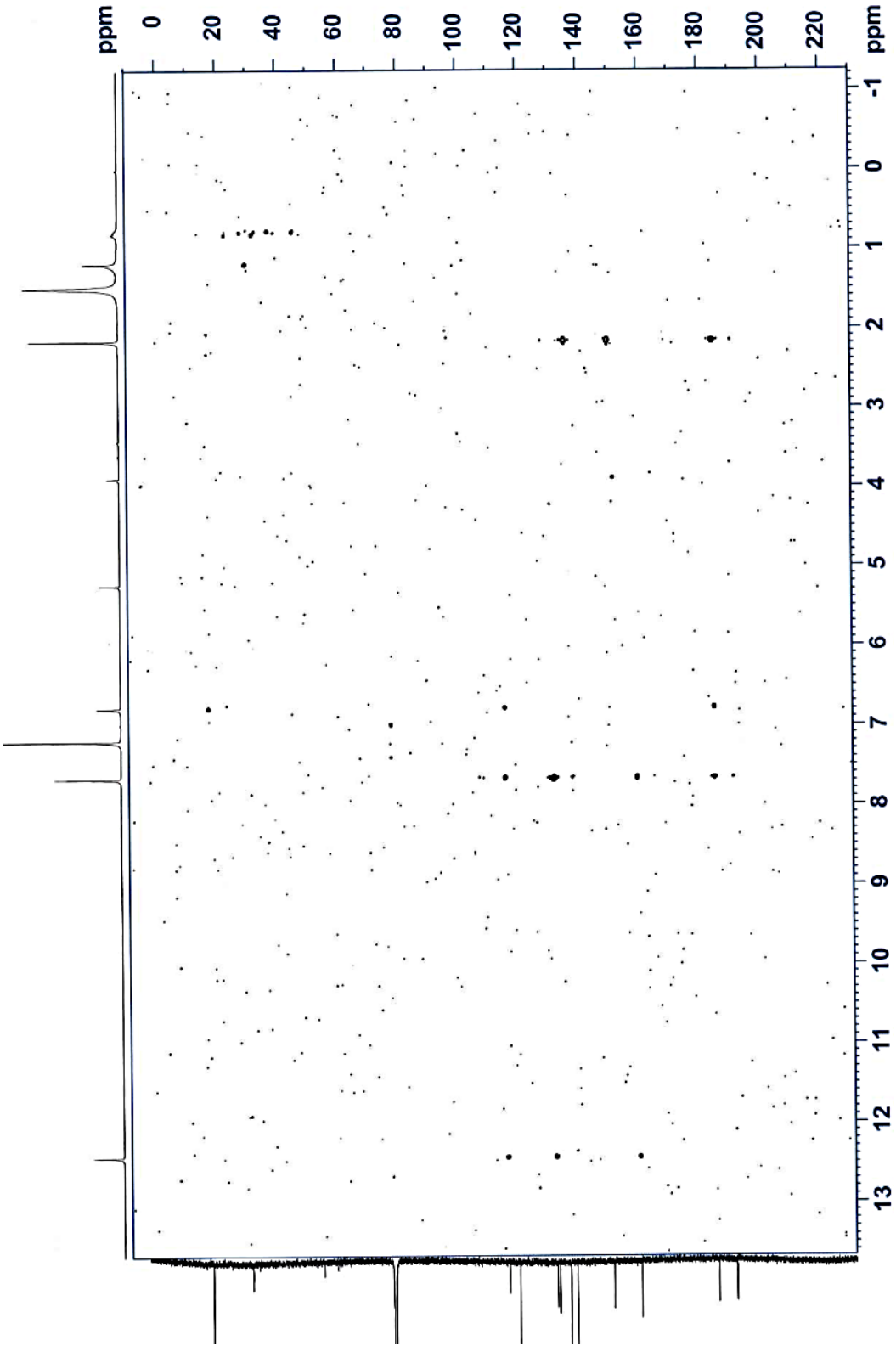

Figure 42. HMBC spectra of compound **4**

High resolution report

		Acquisition Date	16/6/2565 14:38:24
Analysis Name	\\10.222.72.169\Data\Taridaporn\JR0004 .d		
Method	NaFormate_pos low.m	Operator	Sutichai Ext: 3560
Sample Name	JR0004	Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	5.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

Compound Spectra

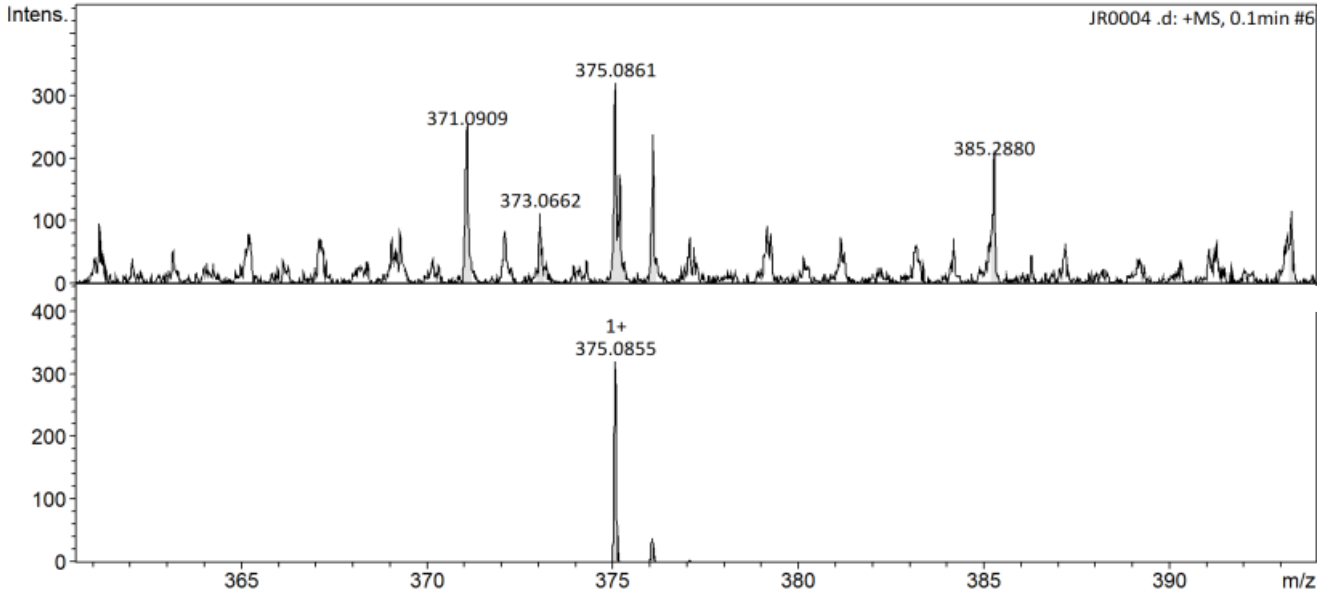

Figure 43. Mass spectroscopy of compound 4

Identification of compound 5

Compound **5** (15.7 mg, 0.29%) was isolated as a colourless oil and the molecular formula was deduced to be $C_{11}H_{11}O_3$ based on HR-ESI-TOFMS data (*m/z* 191.0711 $[M-H]^-$, calcd 191.0714). The ^{1}H-NMR spectrum of compound **5** (Table 21) showed ten resonances: two hydroxyl proton signals at δ_H 1.98 (1H, d, *J* = 3.0 Hz, OH-4) and 12.40 (1H, s, OH-8); three aromatic proton signals at δ_H 6.91 (1H, dd, *J* = 7.4 and 1.0 Hz, H-7, 7.47 (1H, dd, *J* = 8.3 and 7.5 Hz, H-6) and 6.92 (1H, dd, *J* = 8.4 and 1.0 Hz, H-7); two methine proton signals at δ_H 4.73 (1H, br d, *J* = 2.4 Hz, H-4) and 2.42 (1H, *m*, H-3); methylene proton signals at δ_H 2.85 (1H, dd, *J* = 17.6 and 11.0 Hz, H-2a) and 2.55 (1H, ddd, *J* = 17.6, 4.3 and 0.6 Hz, H-2b) and methyl proton signal δ_H 1.16 (3H, d, *J* = 6.9 Hz, 11-CH_3). The ^{13}C-NMR and DEPT spectral data revealed eleven carbon signals, characterized as: five methine carbons at δ_C 34.5 (C-3), 71.2 (C-4), 118.2 (C-7), 118.8 (C-5), 137.1 (C-6); a methylene carbon at δ_C 40.8 (C-2); a methyl carbon at δ_C 16.3 (CH_3-11) and four quaternary carbons at δ_C 204.9 (C-1), 162.8 (C-8), 145.2 (C-10) and 115.1 (C-9). The ^{1}H-^{1}H COSY spectrum revealed the proton-proton connectivity of H-2 to H-4, H-5 to H-7 and also the coupling between H-3 and H-11. The HMBC correlation established the connections of proton signals from H-2, H-7 and OH-8 to C-9; H-4, H-5, H-6 and OH-4 to C-10; and H-2 and H-3 to C-1, thus suggesting the naphthoquinone unit. The relative configurations at C-3 and C-4 can be proposed from the observed vicinal ^{1}H-^{1}H coupling constant (*J* = 2.4 Hz) between H-3 and H-4, indicating an axial-equatorial orientation of these protons, that supports a 3,4-*cis* configuration. Therefore, the structure of compound **5** was identified as *cis*-isoshinanolone by spectroscopic methods (1D and 2D-NMR) including a comparison with data reported in the literature (Bringmann et al., 1999).

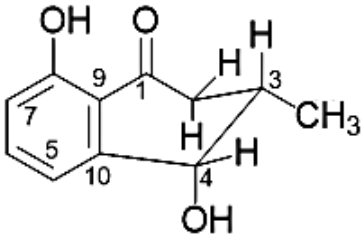

Figure 44. Chemical structure of *cis*-isoshinanolone (**5**)

Table 21. ^{1}H,^{13}C-NMR and HMBC data of compounds **5** ($CDCl_3$; 500 MHz).

Position	Compound 5			*cis*-isoshinanolone (Bringmann et al., 1999)	
	δ_H (mult.; *J* in Hz)	δ_C	HMBC	δ_H (mult.; *J* in Hz)	δ_C
1	-	204.9	-	-	204.7
2a	2.85 (dd; 11.0, 17.6)	40.8	C-1, C-3, C-4, C-9, C-11	2.87 (dd; 17.7, 11.0)	40.7
2b	2.55 (ddd; 17.6, 4.3, 0.6)	40.8	C-1, C-3, C-4, C-9, C-11	2.56 (ddd; 17.7, 4.3, 0.9)	40.7
3	2.42 (m)	34.5	C-1, C-2, C-11	2.44 (m)	34.4
4	4.73 (br d; 2.4)	71.2	C-2, C-3, C-5, C-9, C-10, C-11	4.75 (d; 2.5)	71.2
4-OH	1.98 (d; 3.0)	-	C-3, C-4, C-10	-	-
5	6.91 (dd; 7.5, 1.0)	118.8	C-4, C-6, C-7, C-9, C-10	6.92 (d; 7.3)	118.6
6	7.47 (dd; 7.5, 8.4)	137.1	C-5, C-7, C-8, C-10	7.48 (dd; 8.3, 7.3)	136.9
7	6.92 (dd; 8.4, 1.0)	118.2	C-5, C-6, C-8, C-9	6.94 (dd; 8.5, 1.2)	118.2
8-OH	12.40 (*s*)	162.8	C-7, C-8, C-9	12.42 (s)	162.7
9	-	115.1	-	-	114.9
10	-	145.2	-	-	145.0
11-CH_3	1.16 (d; 6.9)	16.3	C-2, C-3, C-4	1.19 (d; 6.7)	16.1

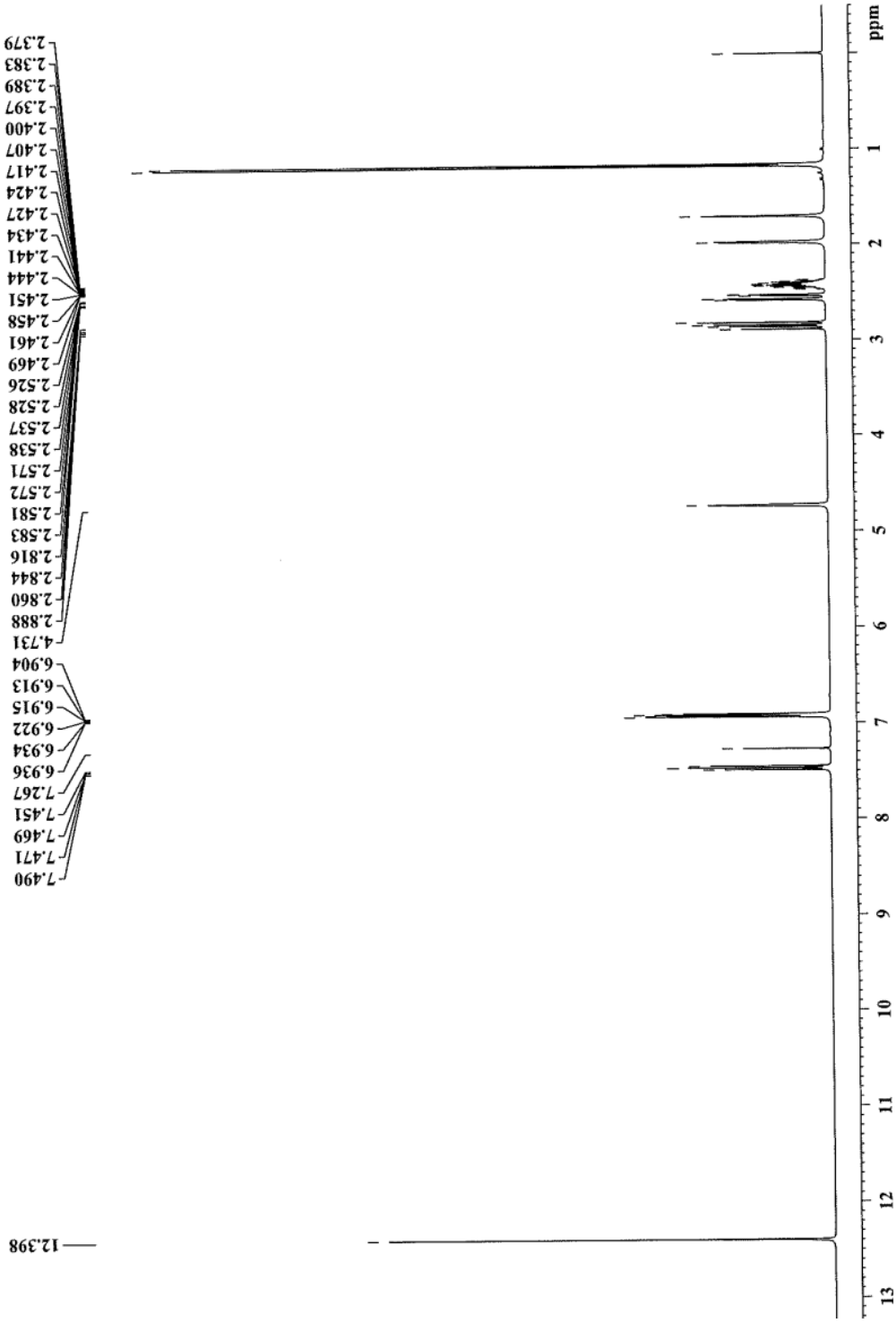

Figure 45. ^{1}H-NMR ($CDCl_3$; 500 MHz) of compound **5**

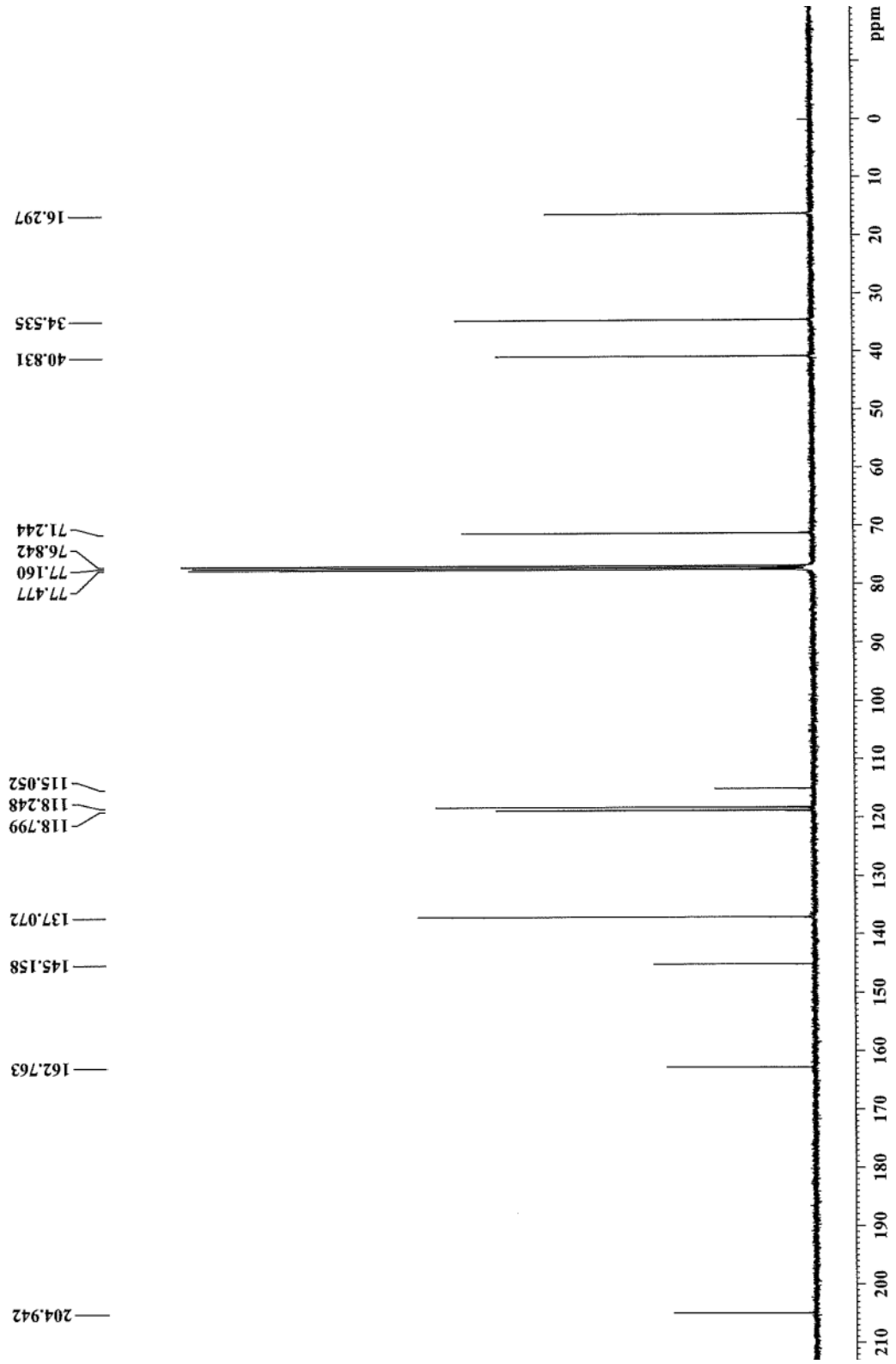

Figure 46. ^{13}C-NMR of compound **5**

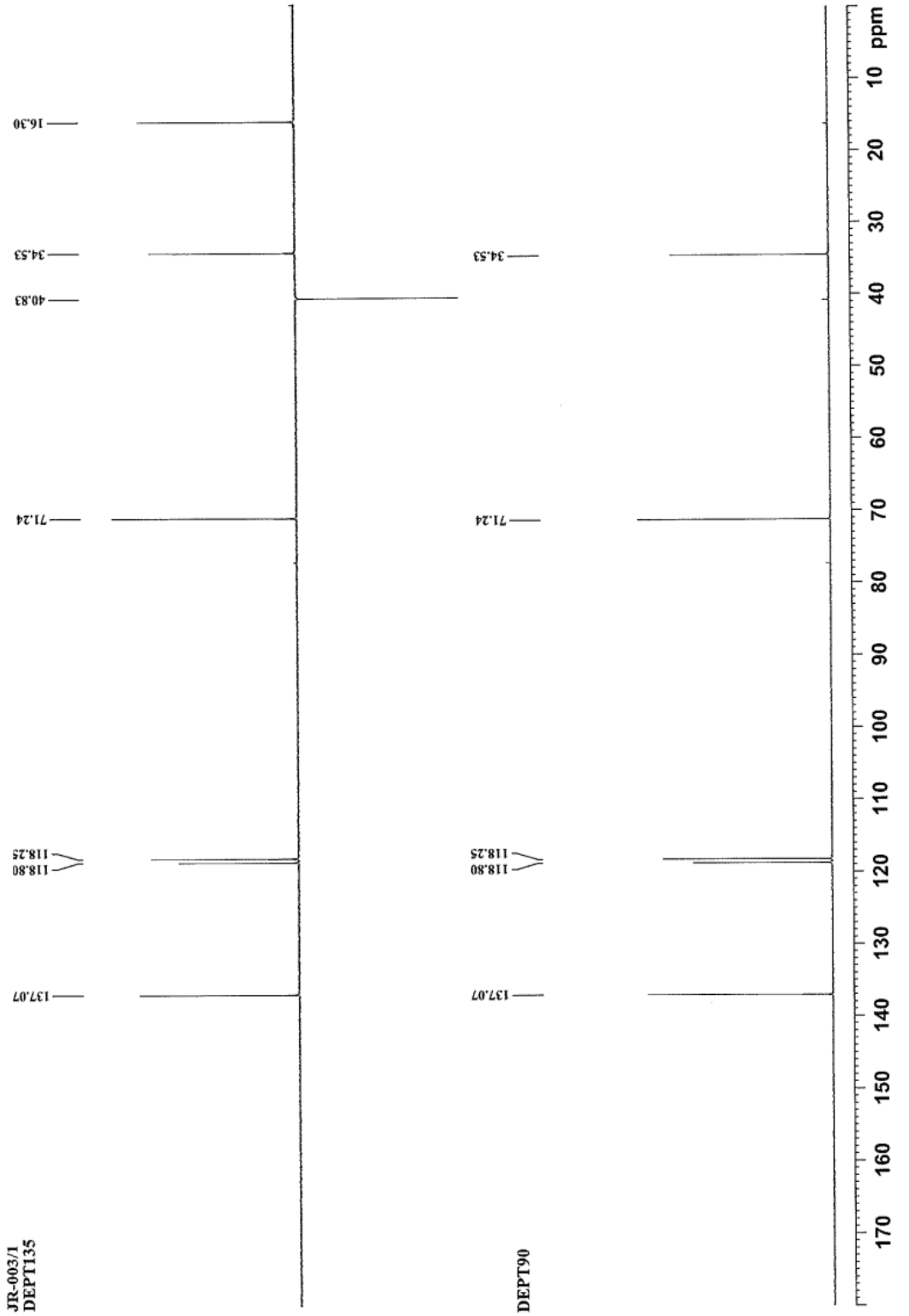

Figure 47. DEPT spectra of compound **5**

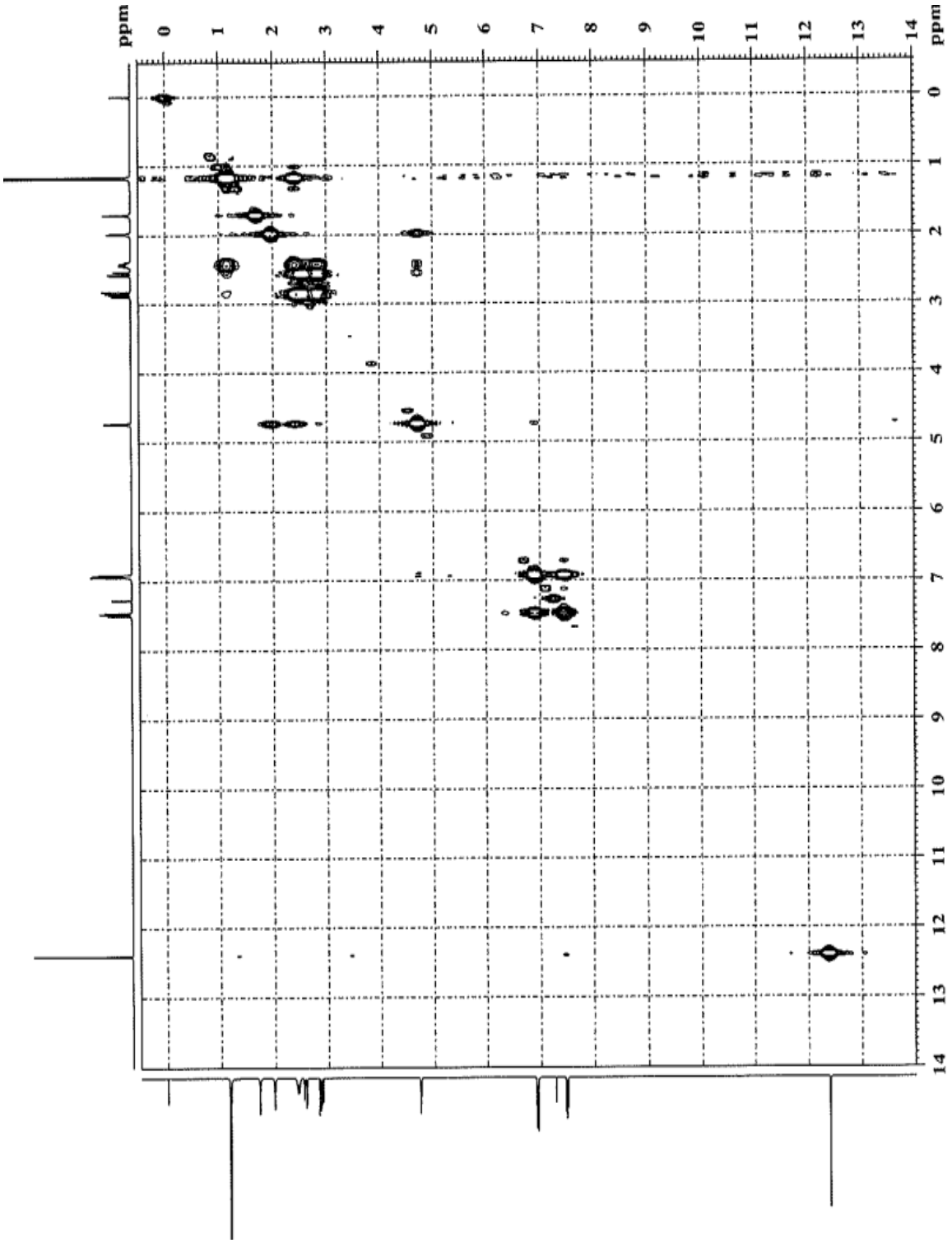

Figure 48. ^{1}H-^{1}H COSY spectra of compound **5**

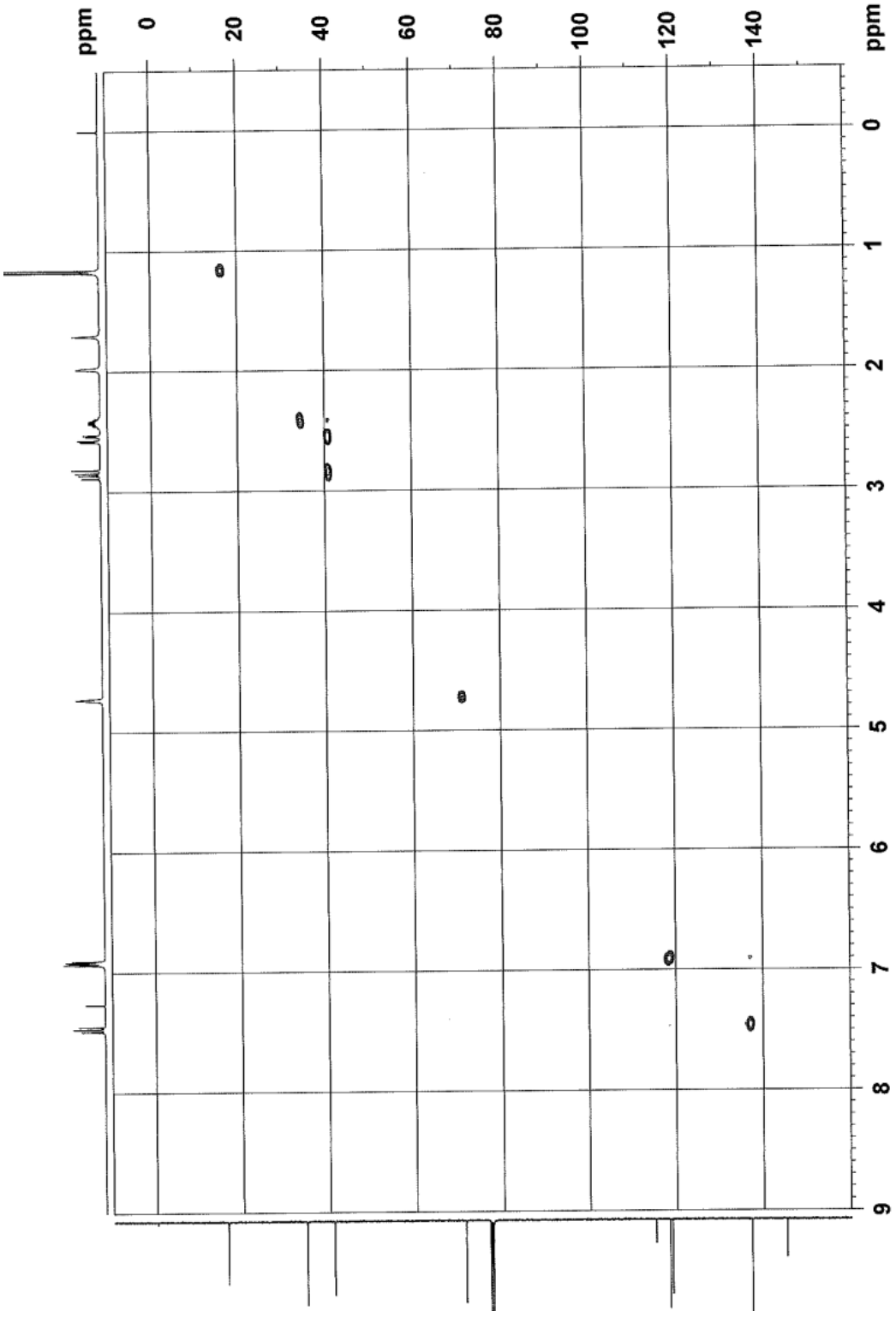

Figure 49. HSQC spectra of compound **5**

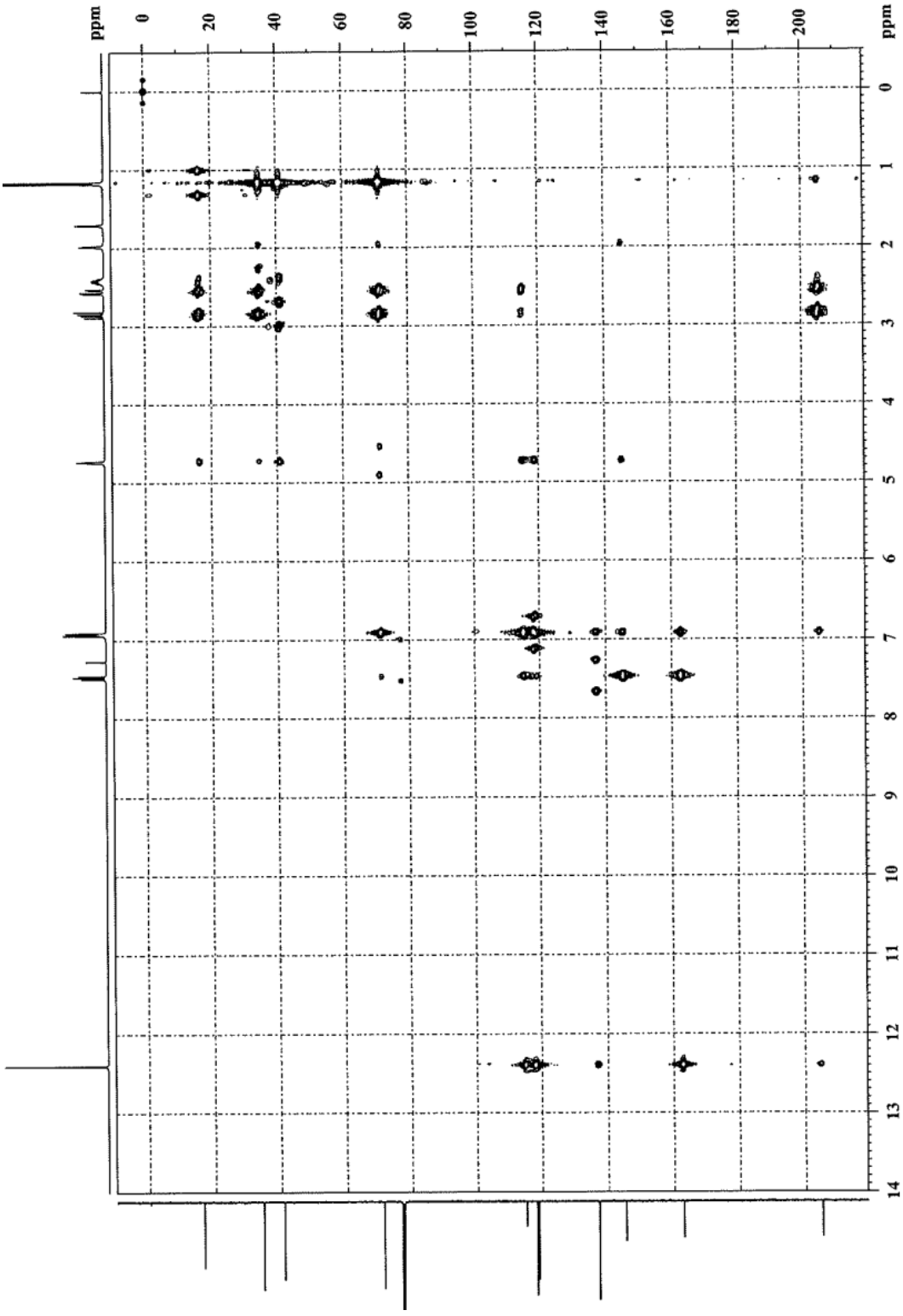

Figure 50. HMBC spectra of compound **5**

High resolution report

		Acquisition Date	27/5/2565 15:24:59
Analysis Name	\\10.222.72.169\Data\Taridaporn\JR005 neg.d		
Method	NaFormate_neg.m	Operator	Sutichai Ext: 3560
Sample Name	JR005 neg	Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

Spectrum View

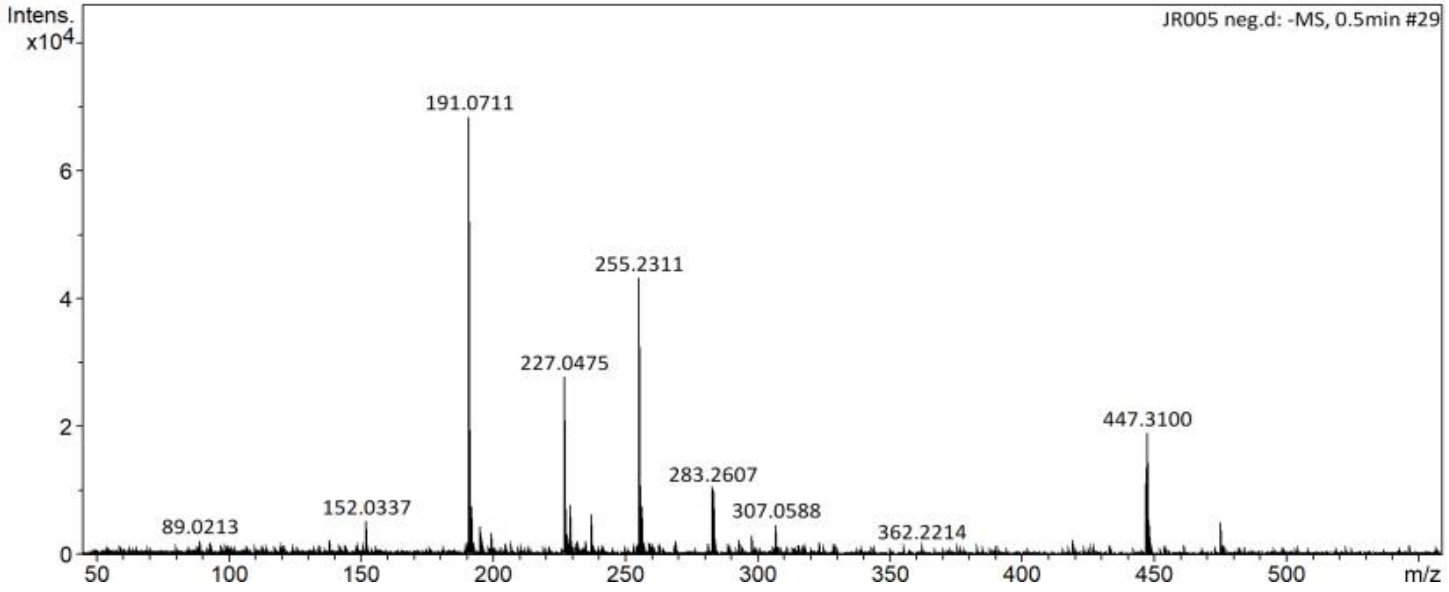

Figure 51. Mass spectroscopy of compound 5

Identification of compound 6

Compound **6** (14.8 mg, yield 0.27%) was isolated as a white solid and the molecular formula was deduced to be $C_{11}H_{11}O_3$ based on HR-ESI-TOFMS data (*m/z* 191.0715 $[M-H]^-$, calcd 191.0714). The ^{1}H-NMR spectrum of compound **6** (Table 22) exhibited ten resonances: two hydroxyl proton signals at δ_H 2.18 (1H, d, *J* = 6.5 Hz, OH-4) and 12.36 (1H, s, OH-8); three aromatic proton signals at δ_H 7.11 (1H, d, *J* = 7.5 Hz, H-5), 7.50 (1H, t, *J* = 8.0 Hz, H-6) and 6.90 (1H, d, *J* = 8.3 Hz, H-7); two methine proton signals at δ_H 4.50 (1H, br d, *J* = 7.4 Hz, H-4) and 2.27 (1H, *m*, H-3); methylene proton signals at δ_H 2.91 (1H, dd, *J* = 17.4 and 4.1 Hz, H-2a) and 2.43 (1H, dd, *J* = 17.4 and 10.2 Hz, H-2b) and methyl proton signal δ_H 1.18 (3H, d, *J* = 6.4 Hz, 11-CH_3). The ^{13}C-NMR and DEPT spectral data revealed eleven carbon signals, characterized as: five methine carbons at δ_C 37.6 (C-3), 73.8 (C-4), 117.3 (C-7), 117.4 (C-5), 137.2 (C-6); a methylene carbon at δ_C 43.5 (C-2); a methyl carbon at δ_C 17.9 (CH_3-11) and four quaternary carbons at δ_C 203.9 (C-1), 162.6 (C-8), 146.0 (C-10) and 115.4 (C-9).

Comparison of the ^{1}H and ^{13}C NMR spectroscopic data with that of compound **5** revealed that compound **6** had a similar pattern except for the coupling constant (*J* = 2.4 Hz) between H-3 and H-4 in compound **5**. The large coupling constant (*J* = 7.4 Hz) between H-3 and H-4 of compound **6** indicated 3,4-*trans* configuration. Therefore, the structure of compound **6** was identified as *trans*-isoshinanolone by spectroscopic methods (1D and 2D-NMR) including a comparison with data reported in the literature (Bringmann et al., 1999).

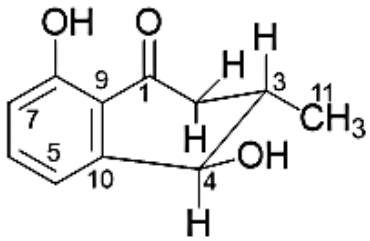

Figure 52. Chemical structure of *trans*-isoshinanolone (**6**)

Table 22. ^{1}H,^{13}C-NMR and HMBC data of compounds **6** ($CDCl_3$; 500 MHz).

Position	Compound 6			*trans*-isoshinanolone (Bringmann et al., 1999)	
	δ_H (mult.; *J* in Hz)	δ_C	HMBC	δ_H (mult.; *J* in Hz)	δ_C
1	-	203.9	-	-	203.6
2a	2.91 (dd; 4.1, 17.4)	43.5	C-1, C-3, C-4, C-9, C-11	2.91 (dd; 16.9, 3.8)	43.4
2b	2.43 (dd; 17.4, 10.2)	43.5	C-1, C-3, C-4, C-9, C-11	2.43 (dd; 17.0, 10.1)	43.4
3	2.27 (m)	37.6	C-1, C-2, C-4, C-10, C-11	2.25 (m)	37.4
4	4.50 (br d; 7.4)	73.8	C-2, C-3, C-5, C-9, C-10, C-11	4.50 (d; 8.0)	73.7
4-OH	2.18 (d; 6.5)	-	C-3, C-4, C-10	-	-
5	7.11 (d; 7.5)	117.4	C-4, C-6, C-7, C-9, C-10	6.90 (ddd; 8.4, 1.1, 0.6)	117.2
6	7.50 (t; 8.0)	137.2	C-5, C-7, C-8, C-10	7.49 (t; 8.0)	137.0
7	6.90 (d; 8.3)	117.3	C-5, C-8, C-9	7.10 (dd; 7.6, 1.0)	117.3
8-OH	12.36 (s)	162.6	C-7, C-8, C-9	11.74 (s)	162.5
9	-	115.4	-	-	115.3
10	-	146.0	-	-	145.9
11-CH_3	1.18 (d; 6.4)	17.9	C-2, C-3, C-4	1.19 (d; 6.5)	17.8

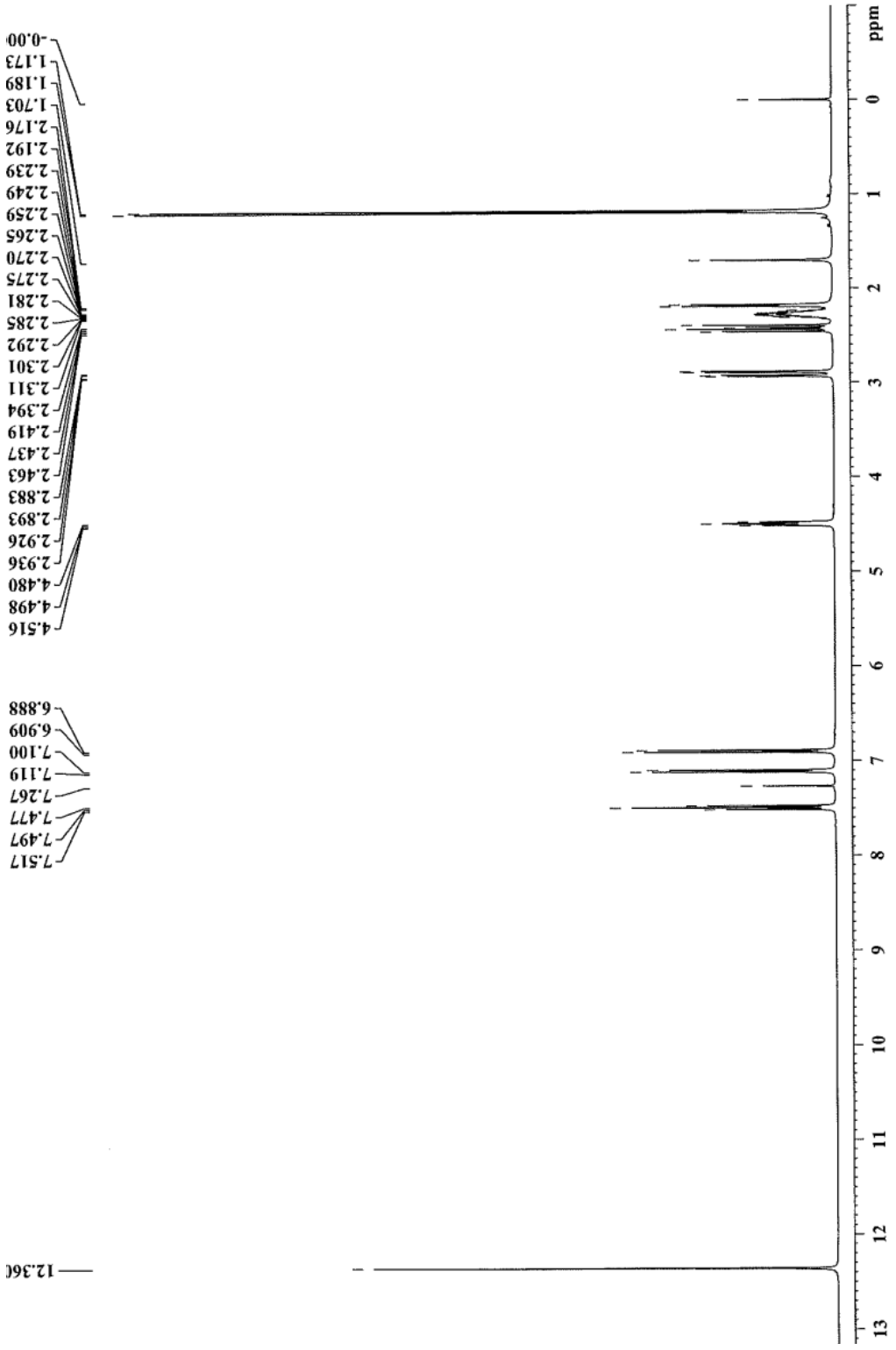

Figure 53. ^{1}H-NMR ($CDCl_3$; 500 MHz) of compound **6**

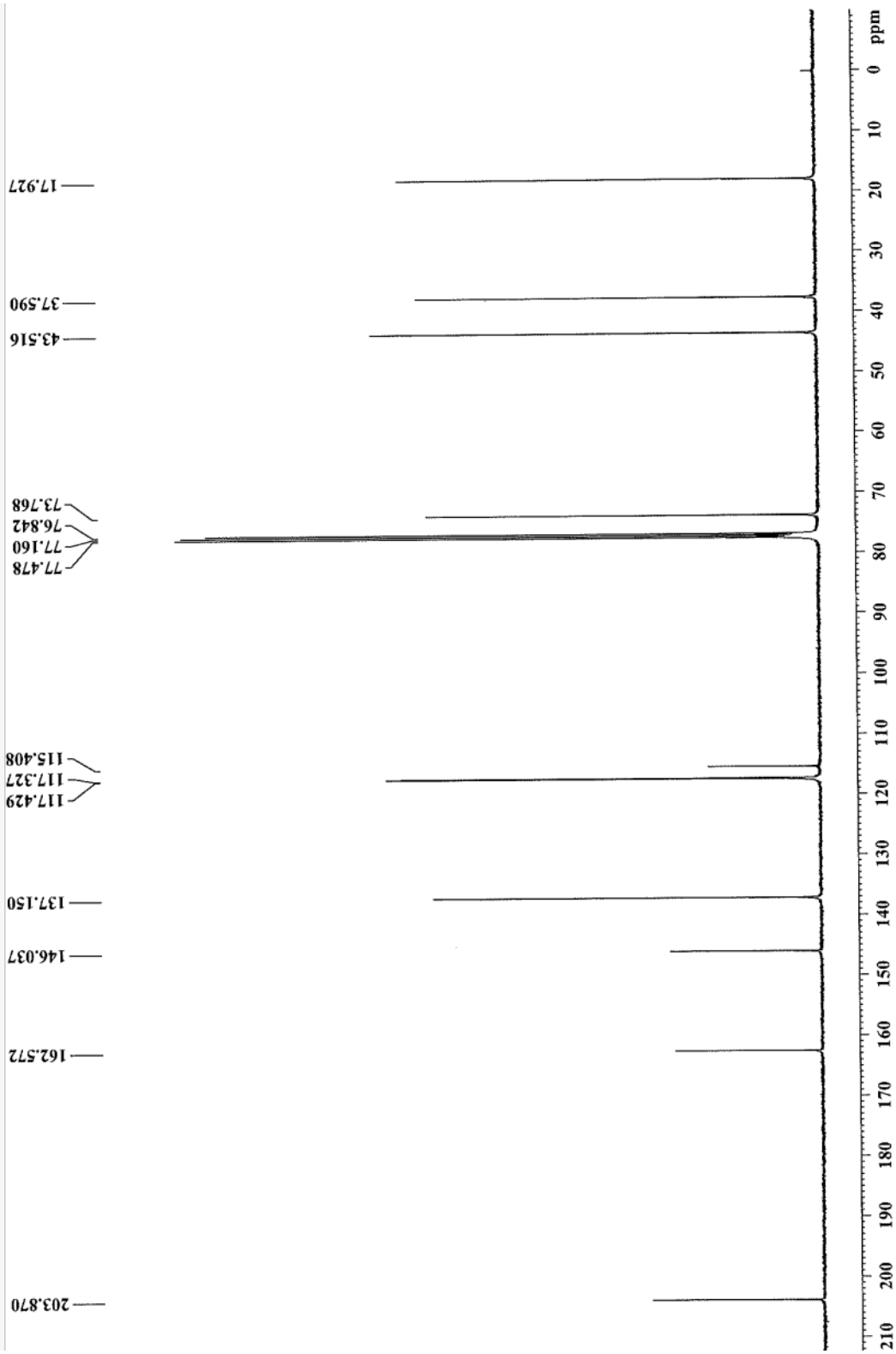

Figure 54. ^{13}C-NMR of compound **6**

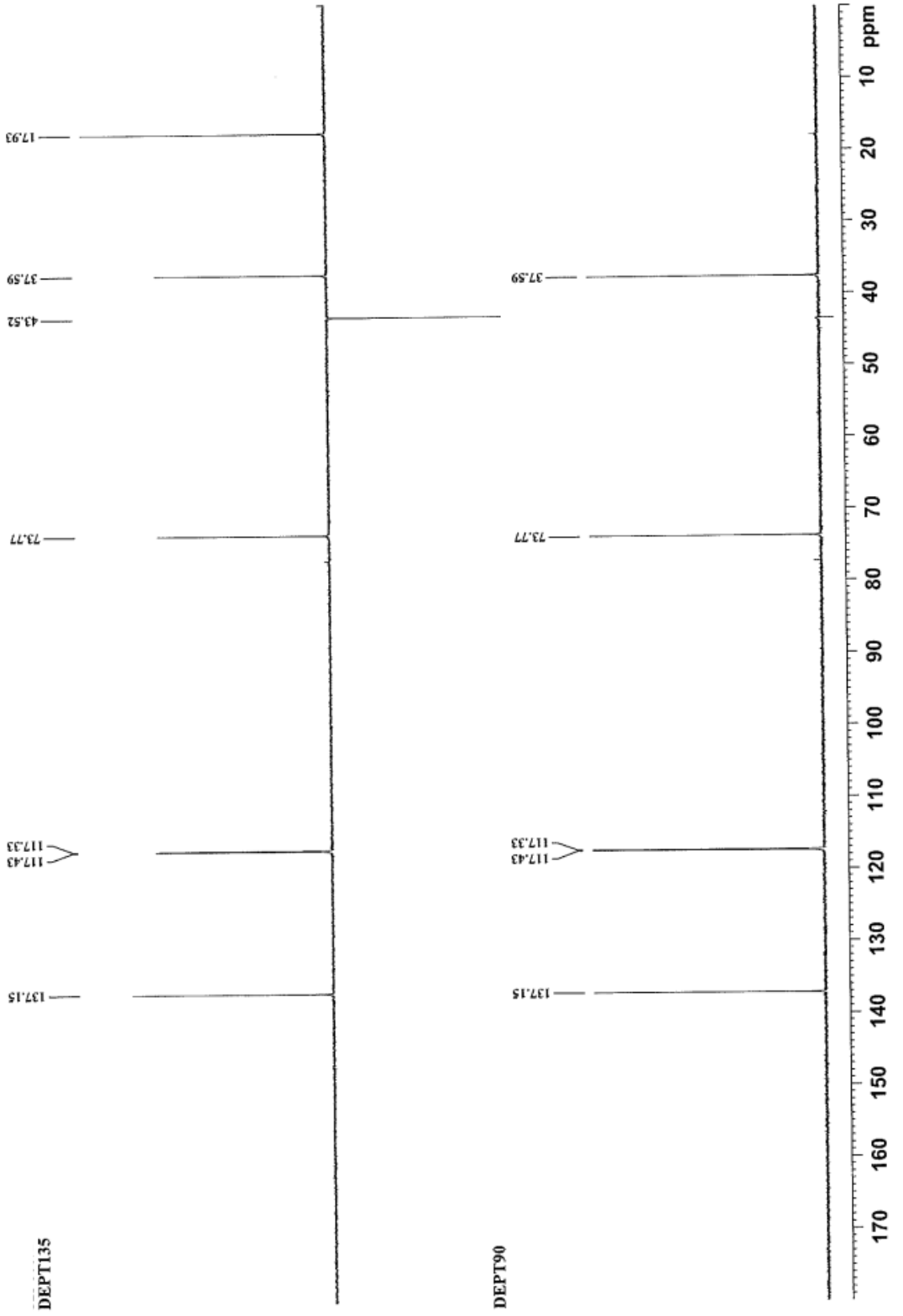

Figure 55. DEPT spectra of compound **6**

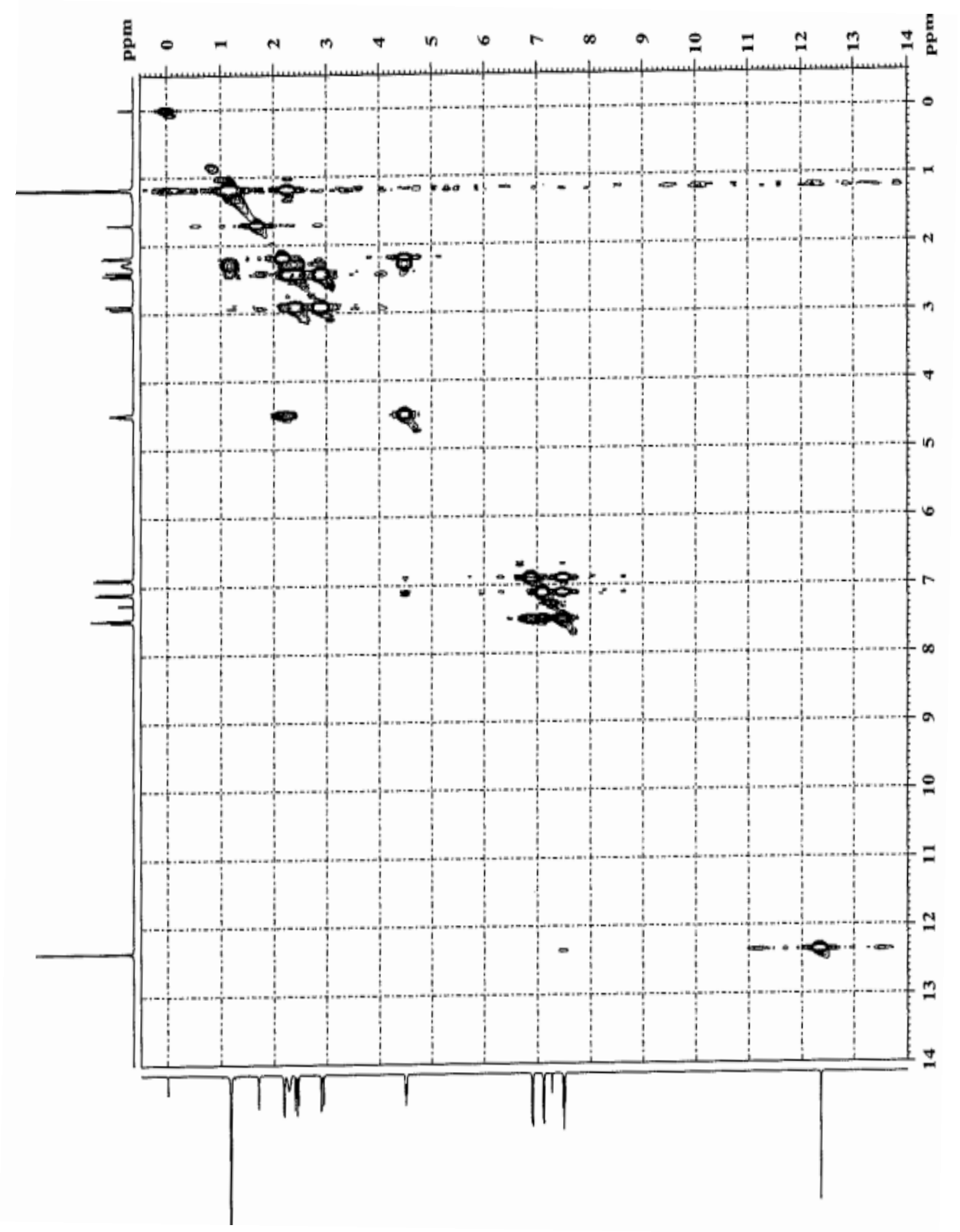

Figure 56. ^{1}H-^{1}H COSY spectra of compound **6**

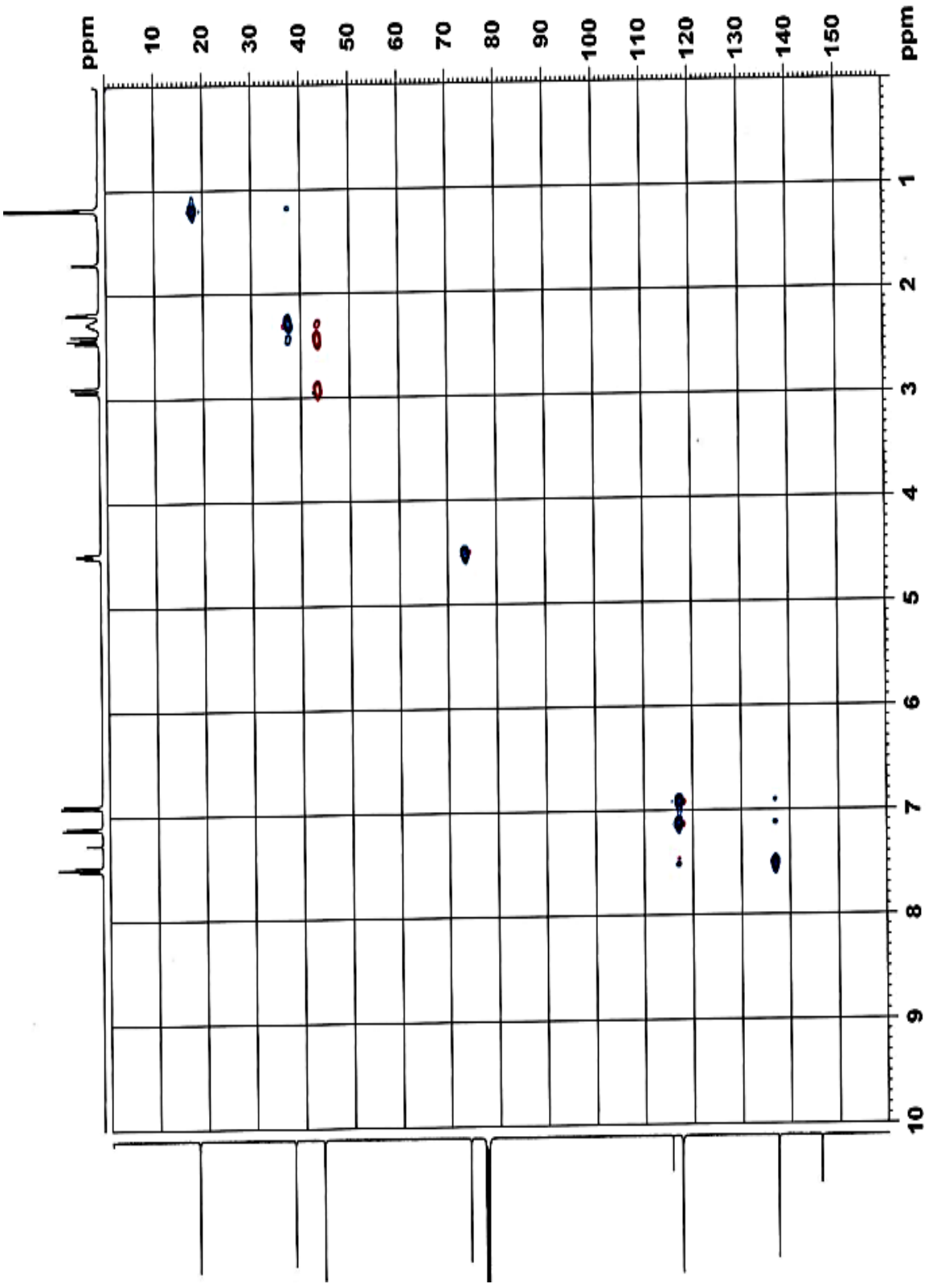

Figure 57. HSQC spectra of compound **6**

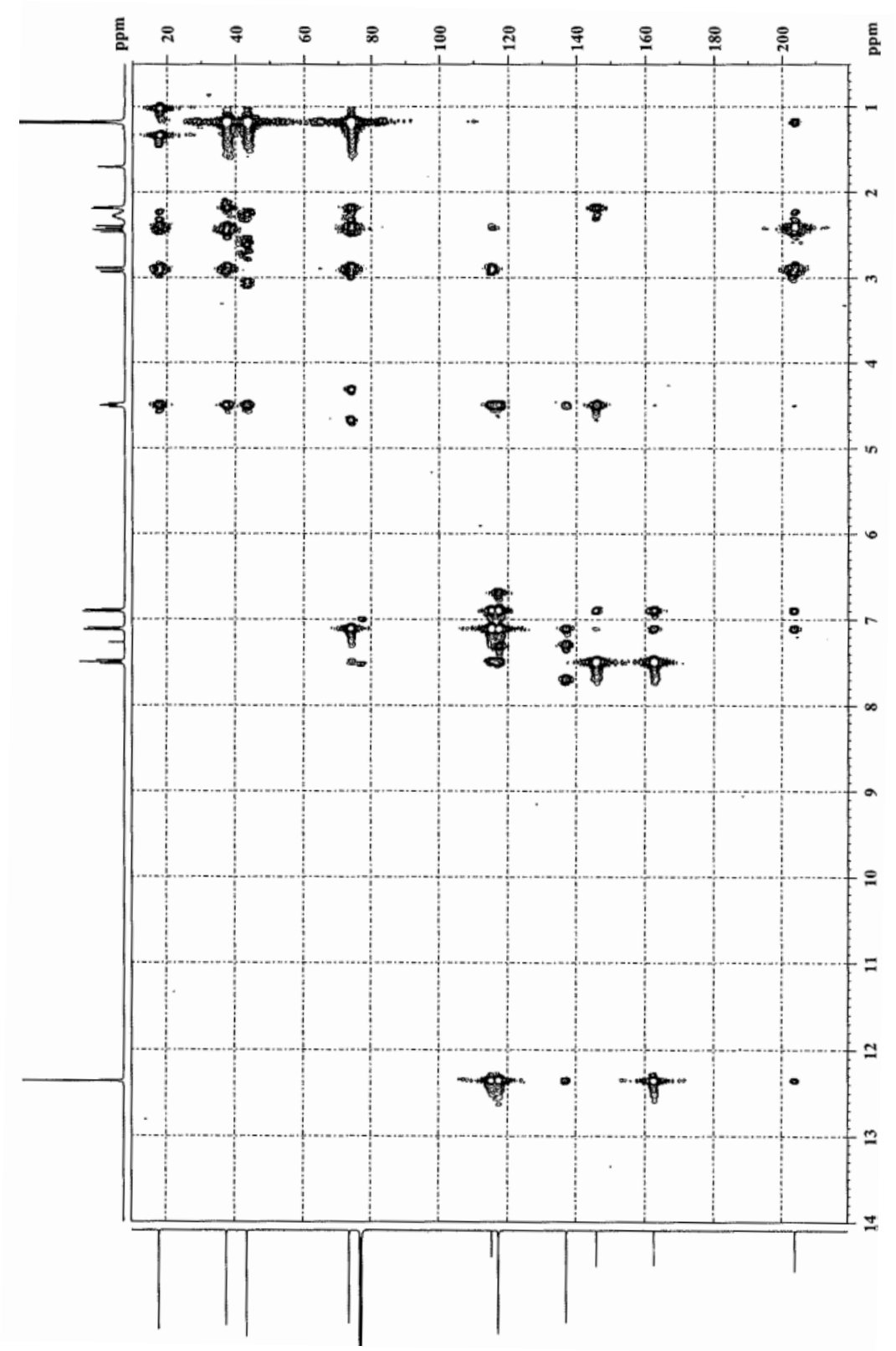

Figure 58. HMBC spectra of compound **6**

High resolution report

		Acquisition Date	27/5/2565 15:27:19
Analysis Name	\\10.222.72.169\Data\Taridaporn\JR006 neg.d		
Method	NaFormate_neg.m	Operator	Sutichai Ext: 3560
Sample Name	JR006 neg	Instrument	micrOTOF Bruker
		Calibrate by	Sodium Formate

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.3 Bar
Focus	Not active			Set Dry Heater	180 C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

Spectrum View

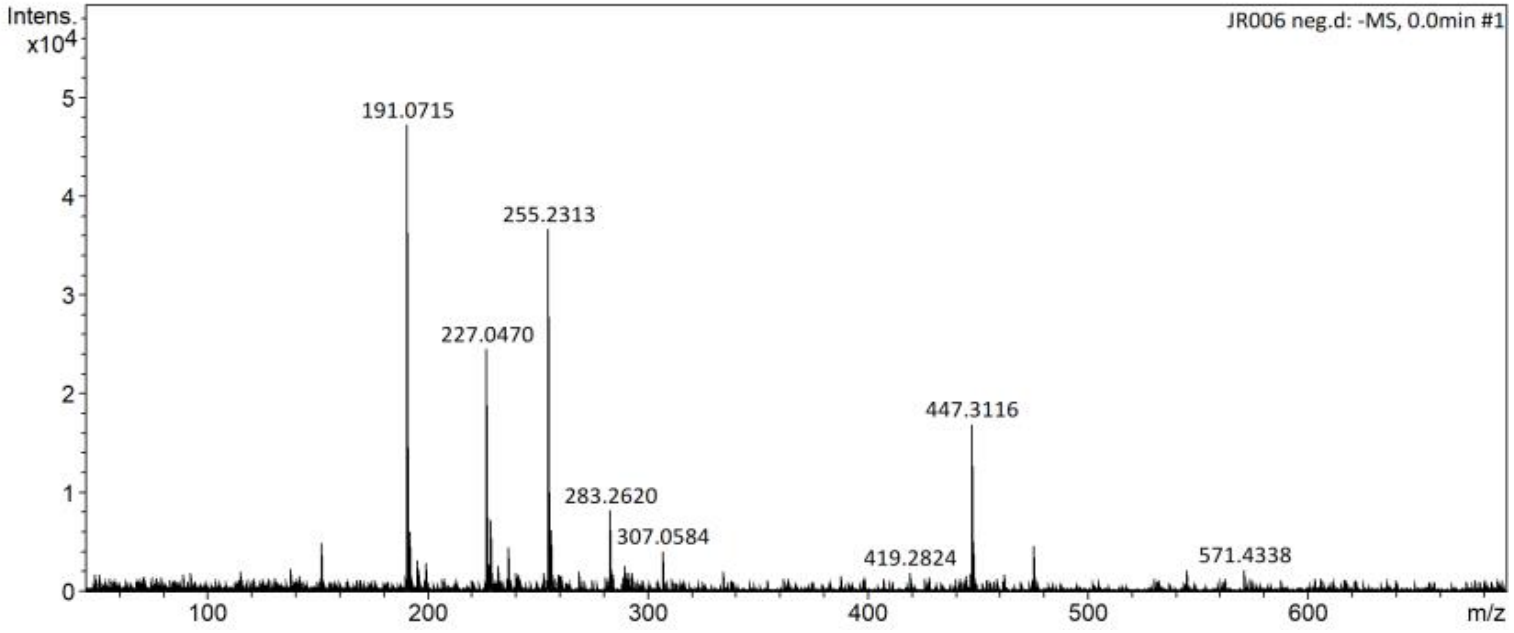

Figure 59. Mass spectroscopy of compound **6**

Antifungal assay

Antifungal activity of isolated compounds and crude extracts were measured by a zone of inhibition assay against clinical isolates of pathogenic fungi which are *A. flavus* and *T. marneffei*. The antifungal assay is shown in table 23.

Table 23. Antifungal activity of crude extracts and isolated compounds.

Crude extracts or compounds	Diameter of inhibition zones (mm)					
	A. flavus			*T. marneffei*		
	0.25 mg/disc	0.5 mg/disc	1.0 mg/disc	0.25 mg/disc	0.5 mg/disc	1.0 mg/disc
Crude CH_2Cl_2	-[a]	-	19.0	-	-	43.3
Crude EtOAc	-	-	IN[b]	-	-	IN
Crude 20%H2O:MeOH	-	-	IN	-	-	IN
Compound **1**	40.0	40.0	40.0	67.5	67.5	70.0
Compound **2**	IN	IN	IN	IN	IN	IN
Compound **3**	IN	IN	IN	IN	IN	IN
Compound **5**	IN	IN	7.0	12.0	15.0	20.0
Compound **6**	IN	IN	7.0	10.0	18.0	20.0

[a] -, not test. [b] IN, inactive.

The antifungal activities of crude extracts and compounds 1-3 and 5-6 were evaluated against *A. flavus* and *T. marneffei* (Table 23). Among all crude extracts of *P. indica* L. (CH_2Cl_2, EtOAc and 20%H_2O:MeOH extracts), It was found that only CH_2Cl_2 extract showed antifungal activity against *A. flavus* and *T. marneffei*, this suggests that CH_2Cl_2 extract is a source of antifungal agents. Compound **4** was not tested for its antifungal activity because of the low isolated yield (1.5 mg). Compounds **1**, **5** and **6** exhibited zones of inhibition of 40.0, 7.0, and 7.0 mm (at 1.0 mg/disc), respectively, for *A. flavus*. Compounds **5** and **6** exhibited zones of inhibition of 12.0 and 10.0 mm (at 0.25 mg/disc), respectively, for *T. marneffei*. Compound **1** showed a remarkable level of activity against *T. marneffei*, exhibiting zones of inhibition of 67.5, 67.5, and 70.0 mm at

0.25, 0.5 and 1.0 mg/disc, respectively. This is the first reported activity against *T. marneffei* for compound **1**. The linkage between the 8 and 8′ positions of plumbagin in compound **3** led to a significant loss of antifungal activity. Compounds **5** and **6** exhibited similar antifungal activity, indicating that the *cis*- and *trans*- configurations between H-3 and H-4 were not critical for their antifungal activity.

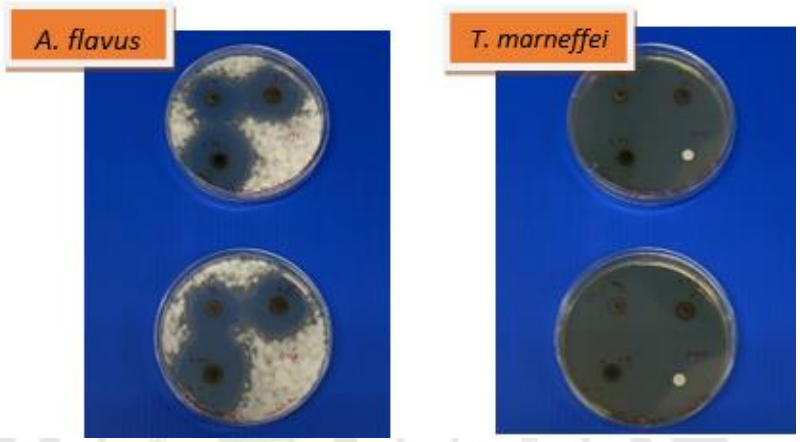

Figure 60. Antifungal against *A. flavus* and *T. marneffei* of CH_2Cl_2 extract

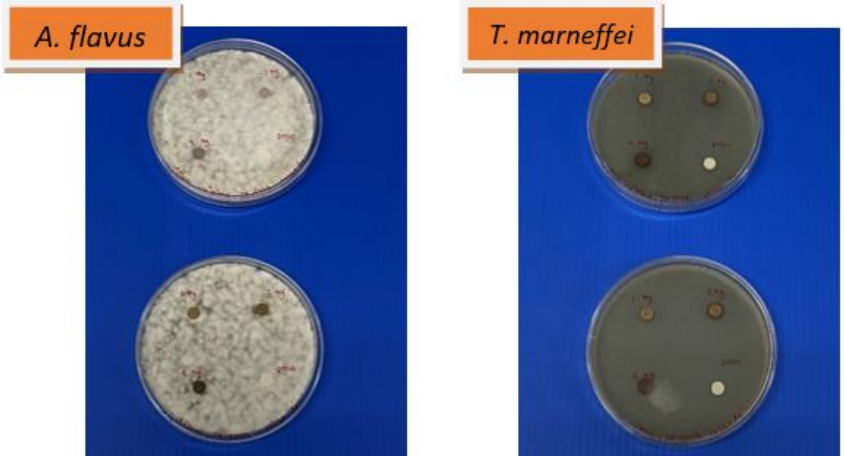

Figure 61. Antifungal against *A. flavus* and *T. marneffei* of EtOAc extract

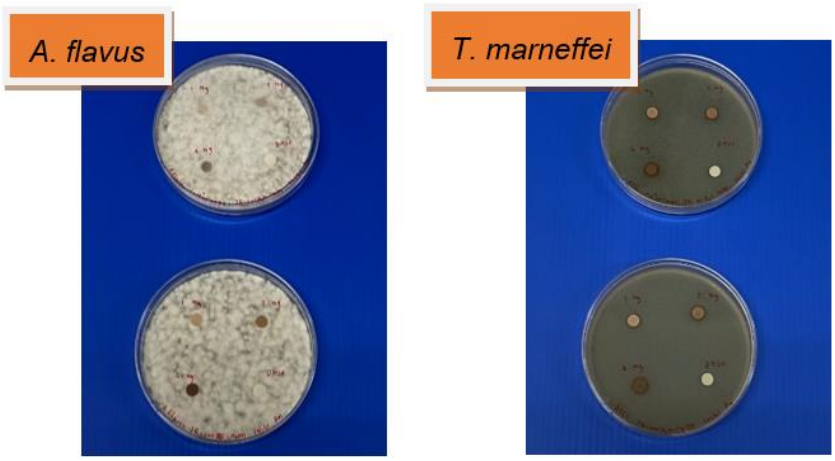

Figure 62. Antifungal against *A. flavus* and *T. marneffei* of 20%H_2O:MeOH extract

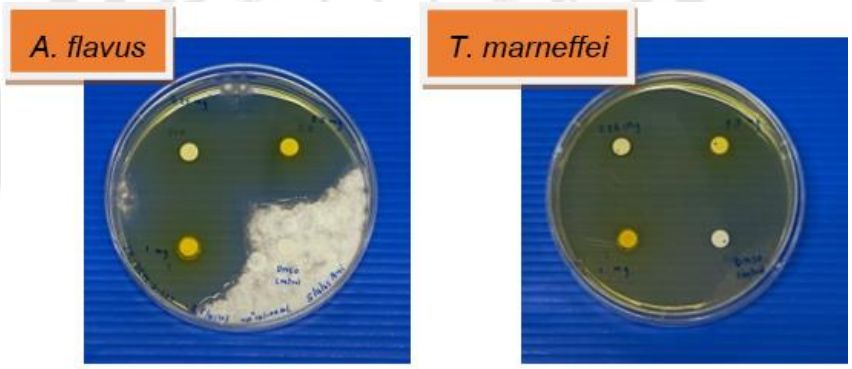

Figure 63. Antifungal against *A. flavus* and *T. marneffei* of compound **1**

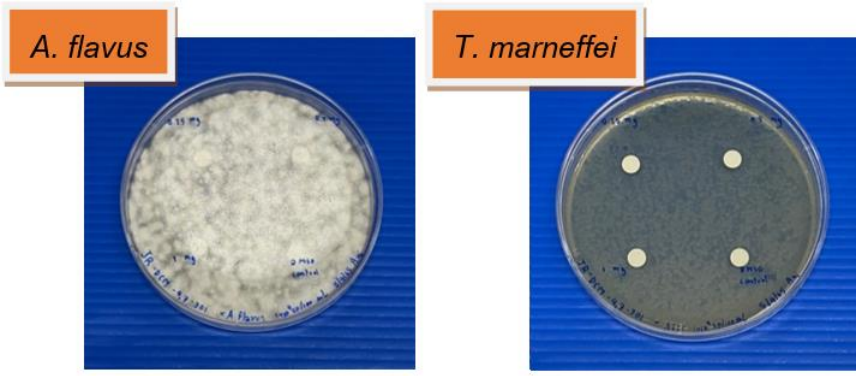

Figure 64. Antifungal against *A. flavus* and *T. marneffei* of compound **2**

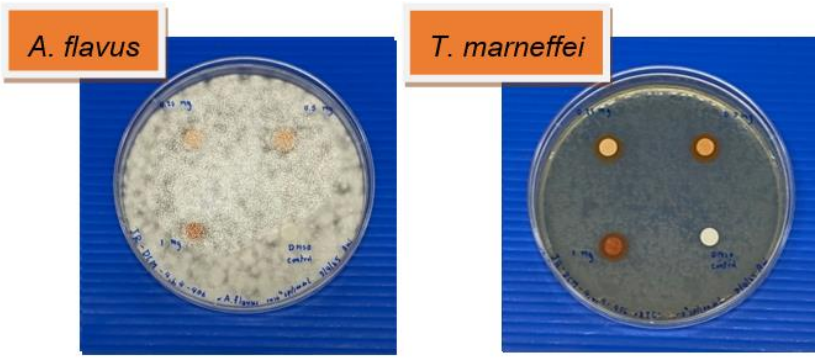

Figure 65. Antifungal against *A. flavus* and *T. marneffei* of compound **3**

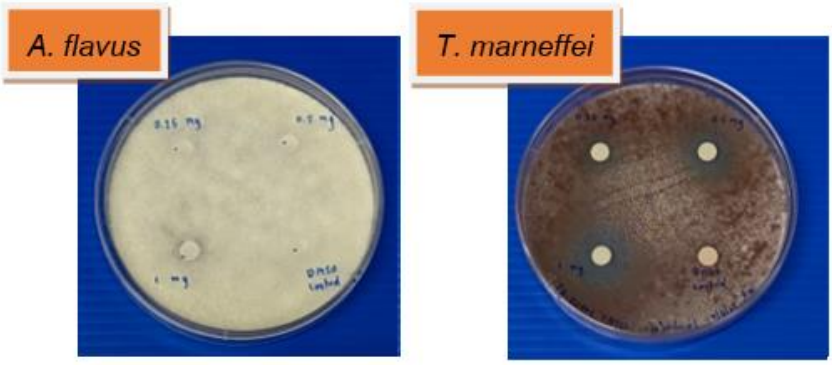

Figure 66. Antifungal against *A. flavus* and *T. marneffei* of compound **5**

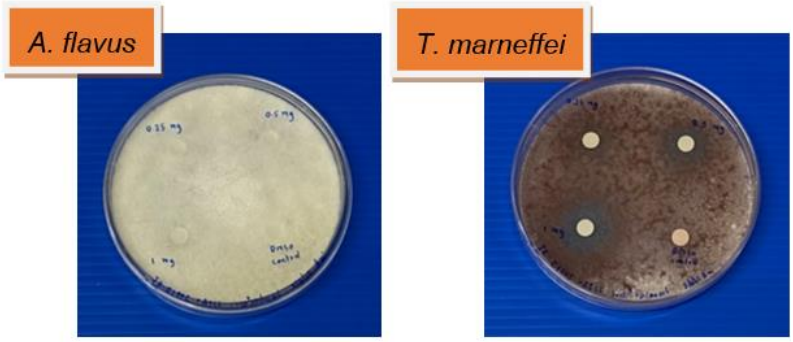

Figure 67. Antifungal against *A. flavus* and *T. marneffei* of compound **6**

CHAPTER 5
CONCLUSION

The present study has described the isolation of six constituents, plumbagin (**1**), β-sitosterol (**2**), maritinone (**3**), 7,7'-biplumbagin (**4**), *cis*-isoshinanolone (**5**) and *trans*-isoshinanolone (**6**), extracted from the branches of *P. indica* L. by chromatography techniques and those constituents were elucidated structure by spectroscopy techniques.

From the antifungal assay, among all crude extracts of *P. indica* L. (CH_2Cl_2, EtOAc and 20%H_2O:MeOH extracts), It was found that only dichloromethane (CH_2Cl_2) extract showed antifungal activity against *A. flavus* and *T. marneffei*, this suggests that CH_2Cl_2 extract is a source of antifungal agents. Therefore, CH_2Cl_2 is the best solvent for extracting antifungal agents. This information was useful for isolating antifungal agents from *P. indica* L.

The antifungal activity of some of the compounds was also evaluated, plumbagin (**1**), *cis*-isoshinanolone (**5**) and *trans*-isoshinanolone (**6**) displaying inhibition against *A. flavus* and *T. marneffei*, while β-sitosterol (**2**), maritinone (**3**) exhibiting no antifungal activity. From the comparison between plumbagin (**1**) and maritinone (**3**), the linkage between the 8 and 8' positions of plumbagin in maritinone (**3**) led to a significant loss of antifungal activity. These results suggest that plumbagin can be considered the active chemical constituent in *P. indica* L. responsible for the antifungal activity against *A. flavus* and *T. marneffei*.

This is the first report on the isolation of maritinone (**3**), 7,7'-biplumbagin (**4**) and *trans*-isoshinanolone (**6**) from *P. indica* L. and this is the first study to report on the antifungal activity of plumbagin (**1**), *cis*-isoshinanolone (**5**) and *trans*-isoshinanolone (**6**) against *A. flavus* and *T. marneffei*.

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VITA

NAME	Sirawit Manyaem
DATE OF BIRTH	24 December 1994
PLACE OF BIRTH	Nakhon Si Thammarat
INSTITUTIONS ATTENDED	In 2013 High School Certificate, Benjamarachutit School, Nakhon Si Thammarat In 2017 Department of chemistry, Faculty of Science, Srinakharinwirot university
HOME ADDRESS	260/1 Srithammarat Road, Nai Mueang Subdistrict, Mueang District, Nakhon Si Thammarat, 80000