การสังเคราะห์สารอนุพันธ์ของแอนโดรกราโฟไลด์ที่มีความขมลดลง

นาย สันติ ตั้งประภา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
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ปีการศึกษา 2542

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# SYNTHESIS OF ANDROGRAPHOLIDE DERIVATIVE

# WITH REDUCED BITTERNESS

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Chemistry

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Chulalongkorn University

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| **Thesis Title** | SYNTHESIS OF ANDROGRAPHOLIDE DERIVATIVE WITH REDUCED BITTERNESS |
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สันติ ตั้งประภา : การสังเคราะห์สารอนุพันธ์ของแอนโดรกราโฟไลด์ที่มีความขมลดลง
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สังเคราะห์หรือดัดแปลงสูตรโครงสร้างของสารแอนโดรกราโฟไลด์ที่มีอยู่ในพืชสมุนไพร "ฟ้าทะลายโจร" ด้วยปฏิกิริยาการเกิดเอสเทอร์โดยใช้แอซิดคลอไรด์ ได้ผลิตภัณฑ์เป็นอนุพันธ์ของแอนโดรกราโฟไลด์ ไดอะซีเตต, 8-คลอโร-แอนโดรกราโฟไลด์ไตรอะซีเตต, แอนโดรกราโฟไลด์ไดบิวทิวเลต, แอนโดรกราโฟไลด์ไตรบิวทิวเลต, แอนโดรกราโฟไลด์ไตรเฮกซะโนเอต และ 14-ดีออกซี-11,12-ไดดีไฮโดรแอนโดรกราโฟไลด์ พาราไนโตรเบนโซเอต ทั้งนี้อนุพันธ์ที่ได้จากปฏิกิริยายังคงรักษาโครงสร้างของแอนโดรกราโฟไลด์เดิมไว้ แต่มีความขมน้อยลง ทำให้มีผลดีในการรักษาโรคท้องเสียในลูกสุกร และได้ศึกษาโครงสร้างของสารอนุพันธ์ที่สังเคราะห์ได้โดยวิธีทางสเปกโตรสโคปี นอกจากนั้นยังได้ศึกษาโดยใช้เทคนิคเอกซเรย์คริสตัลโลกราฟีของแอนโดรกราโฟไลด์และสารอนุพันธ์ เพื่อยืนยันโครงสร้างและศึกษาความสัมพันธ์ของพันธะไฮโดรเจนภายในโมเลกุลของสาร จนสามารถอธิบายกลไกเกี่ยวกับความขมของแอนโดรกราโฟไลด์ได้



ภาควิชา.....เภสัช.....  ลายมือชื่อนิสิต.....สันติ ตั้งประภา.....
สาขาวิชา.....เภสัชเคมี.....  ลายมือชื่ออาจารย์ที่ปรึกษา.....นาตยา งามโรจนวณิชย์.....
ปีการศึกษา.....2541.....  ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....อมร เพชรสม.....

SANTI TUNGPRAPA : SYNTHESIS OF ANDROGRAPHOLIDE DERIVATIVE WITH REDUCED BITTERNESS. THESIS ADVISOR : NATTAYA NGAMROJNA-VANICH, Ph.D. THESIS CO- ADVISSOR : ASSO.PROF. AMORN PETSOM , Ph.D. 89 pp. ISPN 974-334-018-1

The synthesis or modification of the structural formula of andrographolide, which can be found in the herb known as "Fa thalaai joan", was carried out through esterification by using acid chloride to give the product derivatives of andrographolide diacetate, 8-chloro-andrographolide triacetate, andrographolide dibutyrate, andrographolide tributyrate, andrographolide trihexanoate and 14-deoxy-11,12-didehydroandrographolide *p*-nitro monobenzoate. All this,the resulting derivative maintained the structural formula of original andrographolide. However, the bitter was decreased, which is considered to be beneficial in the treatment of diarrhea in baby pigs and the structure of the synthesized derivative was also studied through the use of spectroscopy. Furthermore, through the use of X-ray crystallography, the structures of andrographolide and its derivatives were confirmed. The relationships between the hydrogen bonds within the molecule of the substances can be used to explain the mechanism of bitterness taste of andrographolide.



ภาควิชา....เคมี.....  ลายมือชื่อนิสิต.....

สาขาวิชา....เคมีอินทรีย์....  ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา....2542.....  ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

# ACKNOWLEDGEMENTS

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| | | |
|---|---|---|
| kg | = | Kilogram |
| L | = | Liter |
| $M^+$ | = | Molecular ion |
| mg | = | Milligram |
| MHz | = | Megahertz |
| ml | = | Millilitre |
| mm | = | Millimetre |
| m.p. | = | Melting point |
| MeOH | = | Methanol |
| M | = | Molar |
| m/z | = | Mass to charge ratio |
| MS | = | Mass spectrometry |
| No. | = | Number |
| NMR | = | Nuclear Magnetic Resonance |
| ppm | = | Part per million |
| q | = | Quartet ( for NMR spectra ) |
| s | = | Singlet ( for NMR spectra ) |
| t | = | Triplet ( for NMR spectra ) |
| TLC | = | Thin layer Chromatography |

# CHAPTER I

## INTRODUCTION

A large number of plants in Thailand have been used as traditional medicine for a long time. Medicinal plants are plants that have the biological activities. Chemical constituents of plants were widely studied in order to use in therapeutic drugs or herbal medicine. In addition, one medicinal plant that is widely used in Thailand because of its effective for certain illness, readily available and inexpensive, is *Andrographis paniculata* Nees. as shown by literature surveys in Table 1.

*Andrographis paniculata* Nees. (Acanthaceae) is an annual herb common in Sri Lanka, India, China and South East Asia.[1] It is believed that every part of this plant can be used as antipyretic, antidysentery, antidiarrhoea and antiinflammatory agent etc.

*A. paniculata* Nees. is the plant in the family of "Acanthaceae". In Thailand, it is commonly known as "Fa thalaai joan" (Bangkok), "Nam lai pangpon" (Bangkok), "Yaa kannguu" (Songkhla), "Fa sang" (Chonburi), "Sam sib dee" (Roy-ed) and "Mekh thalai" (Yala) [1]

### General Characterization of the *A. paniculata* Nees. [2]

The botanical characteristics of *A. paniculata* Nees. are summarized as the following. The plants are annual herbs about 30-100 cm. high. The leaves are green, opposite and lanceolate. On the other hand, the calyx are small and have segments that are ovate and more than 2 mm. long. Flowers are small and solitary, while corolla is whitish or light pink in colour. Fruit is a linear-oblong capsule about 1.5 cm. long and 3-5 mm. wide. Furthermore, the stamens are inserted in the throat and far exserted. In addition,

within each fruit there are many seeds, each seed approximately 5-6 mm. long, rounded and subquadrate.

**Uses in traditional medicine.**

The active component of *A. paniculata* Nees. is andrographolide, a naturally occuring diterpenoid lactone. This specific extract has been shown to inhibit both platelet aggregation and smooth muscle proliferation, two biological markers of restenosis. Andrographolide inhibits blood clotting through the platelet activation pathway. Ultra-structural studies show significant in vitro inhibition of platelet aggregation through the arachidonic acid-induced pathway and platelet activation factor pathway. [3]

The alcoholic extract of *A. paniculata* Nees. exhibited significant antidiarrhoeal activity against *E. coli* enterotoxins in animal models. The activity was further located in *n*-butanol fraction, which led to the isolation of four diterpenes , andrographolide (1), neoandrographolide (2), deoxyandrographolide (3) and andrographiside (4). Among the four diterpenes , andrographolide and neoandrographolide showed similar activity to loperamide against *E. coli* LT and LT / ST enterotoxins. Andrographolide was found to be superior against ST enterotoxin, the most common cause of epidemics of neonatal diarrhoeal.[4]

Andrographolide exhibits protective effects in galactosamine and paracetamol induced intoxication in rats. Andrographolide was demonstrated to possess antiheptotoxic effect in carbon tetrachloride-induced hepatotoxicity in albino rats. The $LD_{50}$ of ethanolic extract of whole plant was determine to be > 1000 mg/kg, i.p. in mice.[5]

Dehydroandrographolide succinic acid monoester ( DASM ) derivative is the dehydroandrographolyl ester of succinic acid obtained from andrographolide and succinic anhydride. The DASM derivative has been found to be an inhibitor against the human immunodeficiency virus ( HIV ) in vitro.[6]

From above, the present communication deals with the antidiarrhoeal properties of the alcoholic extract, its fractions and pure compounds isolated from *A. paniculata* Nees. Antisecretory properties of four diterpenes isolated from this plant, especially andrographolide, have been compared with loperamide and tetracyclin. It found that have been better effective than both references compound. *A. paniculata* Nees. has been recommended to widely used as an antidiarrhoeal drug in baby pigs. However, this herb or isolated compound have a high bitterness taste until the baby pigs could not eat. So that, this research propose to solve the problem about the bitterness taste of andrographolide by synthetic modification of its functional group which involve the bitterness character of this compound but also maintain the therapeutic properties of the strating compound. Moreover, it was found that the yield of andrographolide, from extraction of *A. paniculata* Nees., have high amount to useful in therapeutic drugs or herbal medicine in the future.


**Figure 1** ***Andrographis paniculata*** **Nees.**

**The characteristic features of bitterness and sweetness of taste bud** [7]

It is proposed that the structure feature responsible for bitterness is similar to the sweet-tasting proton-donor / proton acceptor pair, except that the distance between the proton and the Lewis base site is 1.5 A°. Again it is suggested that the proton donor and acceptor sites of the proteins on the taste bud act as a receptor site and form hydrogen bonds with bitter-tasting molecule. This idea is demonstrated by comparing the molecular structure of compounds such as

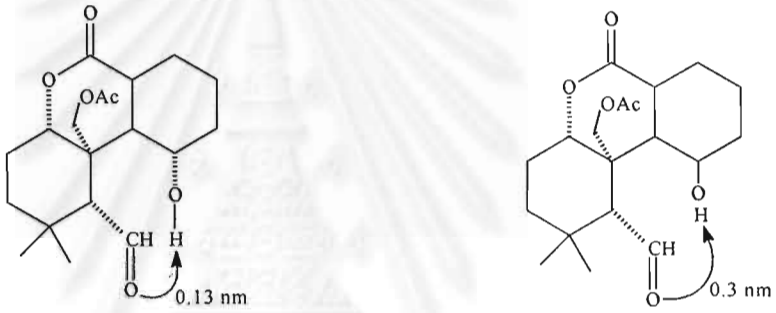
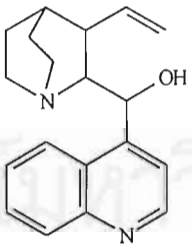



Figure 3

Alkaloids have pronounced bitter tastes. In quinine ( Figure 4 ) , for example, the OH and nitrogen of the quinuclidine ring may act as proton donor and acceptor, respectively.




Quinuclidine

Figure 4

It is generally accepted that there are four basic tastes; acidity, saltiness, sweetness and bitterness. The physiological interpretation of the first two sensations can be ascribed to the interaction of ions with the taste buds of the tongue.

**Sweetness**

It has been suggested that a common feature of this wide variety of compounds is the presence in the sweet tasting molecule of a weakly acidic proton such as is present in the alcoholic OH group , the imide or amide hydrogen , the proton adjacent to the nitro group or the single hydrogen in chloroform , together with a proton acceptor site ( Lewis base ) about 0.3 nm from the acidic proton . The oxygen of the alcoholic OH group , the oxygen of the carbonyl , sulphonyl or nitro-group , or even the electronegative chlorine atom, could function as a Lewis base.

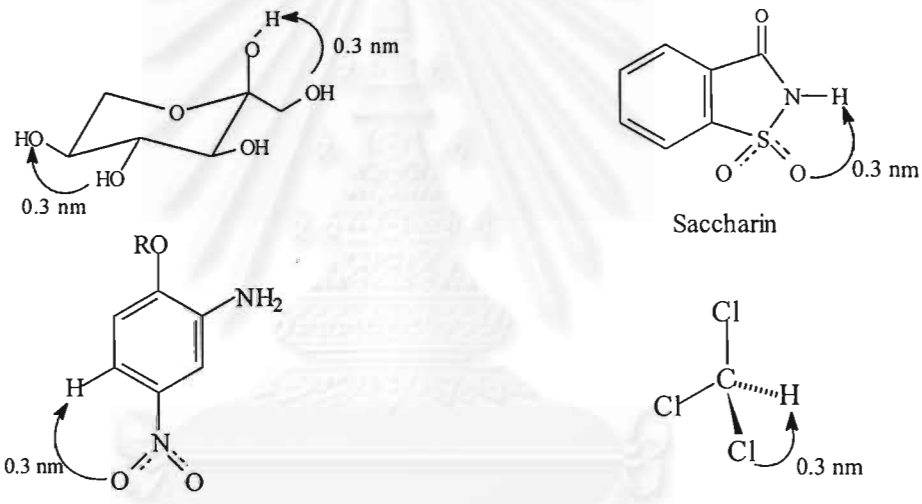



Figure 5

It is then assumed that, if these two groups, an acidic proton and a proton acceptor are at the correct distance from each other in the molecule, the taste bud responsible for detecting sweetness has a pair of complementary proton-donor / Lewis-base.

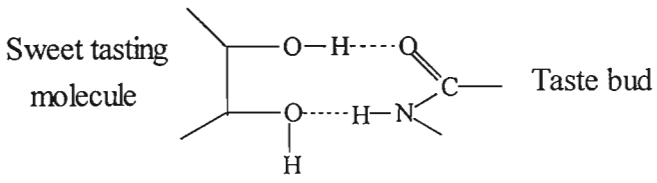



Figure 6

# CHAPTER II

# LITERATURE REVIEWS

**Previous Studies in chemical constituents of *A. paniculata* Nees.**

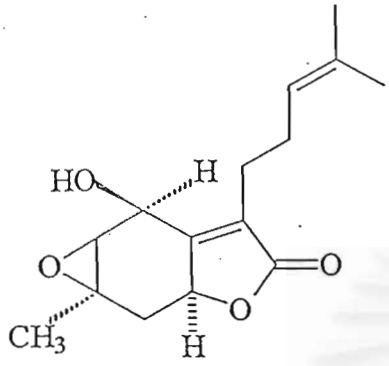
From the literature surveys , *A. paniculata* Nees. have been widely studied. Many lactones and flavonoids have been isolated and characterized in Table 1. and figure 2 below.

**Table 1. The chemical constituents of *A. paniculata* Nees.**

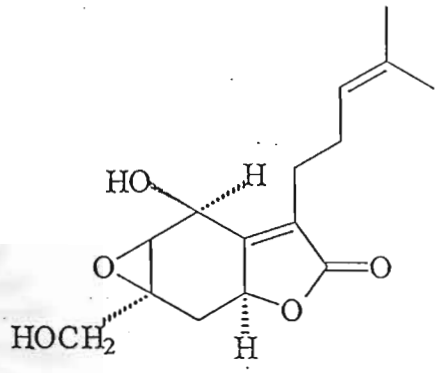
| Category | Chemical compound | Plant part | Reference |
|---|---|---|---|
| Sesquiterpene lactone | Paniculide A,B and C | Leaves, tissue culture | 8 |
| Diterpene lactone ( ent-labdane ) | Andrographolide | Leaves | 9 |
| | 14-Deoxyandrographolide | Leaves | 10 |
| | 14- deoxy-11,12- didehydroandrographolide | Whole plant | 10 |
| | Andrograpanin | Leaves | 10 |
| | 14-deoxy-11- oxoandrographolide | | 10 |
| | Deoxyandrographolide-19β-D-glucoside | Leaves | 11 |
| | Neoandrographolide | Leaves | 12 |
| | Andrographisside | Aerial parts | 13 |
| | 14- deoxy-11,12- didehydroandrographisside | Aerial parts | 13 |
| | 14- *epi*-andrographolide | | 13 |
| | Isoandrographolide | | 13 |
| | Andrograpanin | Leaves | 14 |

**Table 1. The chemical constituents in *A. paniculata Nees*. (continued)**

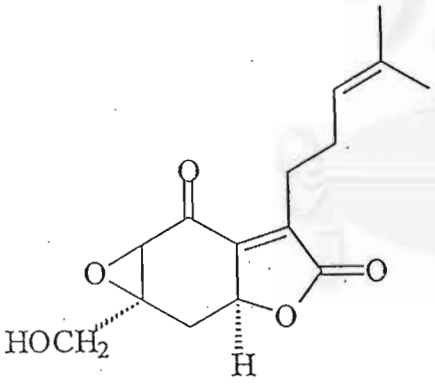
| Category | Chemical compound | Plant part | Reference |
|---|---|---|---|
| Flavonoids | Andrographin | Root | 15 |
| | Apigenin-4',7'- di- o- methyl ether | | 15 |
| | Mono- o- methyl- wightin | | 15 |
| | Panicolin | | 15 |
| | 5-Hydroxy-2',3',7,8- tetramethoxyflavone | Root | 16 |
| | (*dl*) -5-Hydroxy-7,8- dimethoxyflavone | | 16 |
| | 5-Hydroxy-7,8- dimethoxyflavone | | 16 |
| Flavanone | Andrographidine A | Root | 17 |
| Flavone | Andrographidine B,C,D,E,F | | 17 |



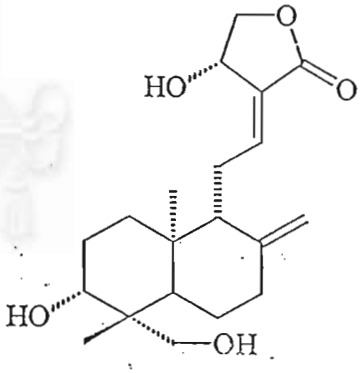

Paniculides A




Paniculides B

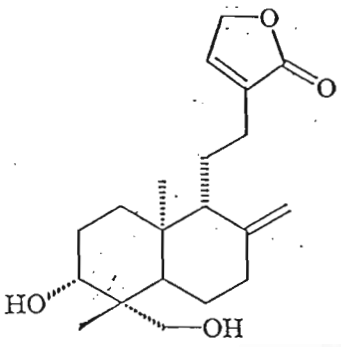



Paniculides C

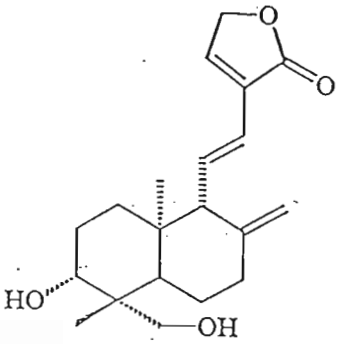



Andrographolide

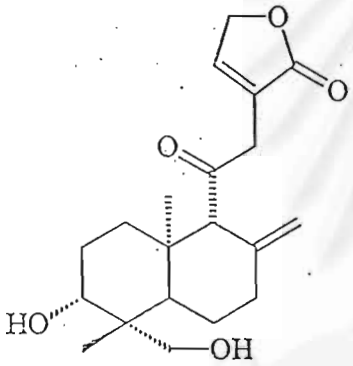
Figure 7 The chemical constituents of *A. paniculata* Nees.



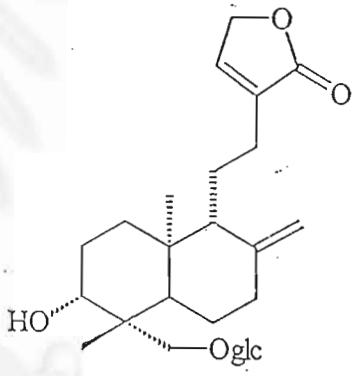

14-Deoxyandrographolide



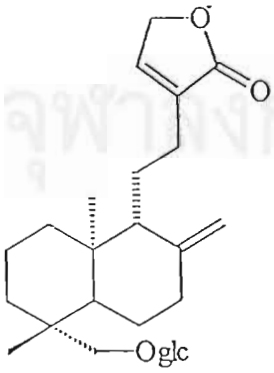

14-deoxy-11,12-didehydroandrographolide



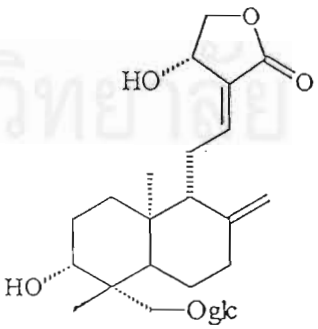

14-deoxy-11-oxoandrographolide




Deoxyandrographolide-19β-D-glucoside

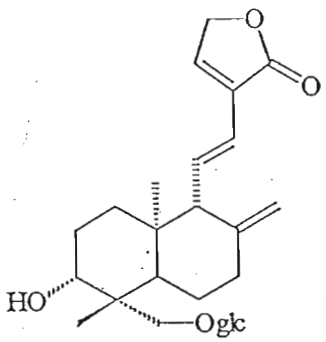



Neoandrographolide

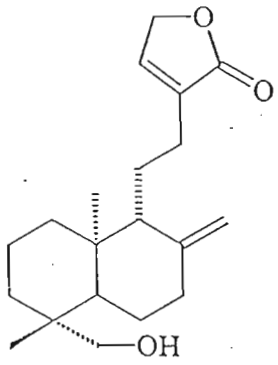



Andrographiside

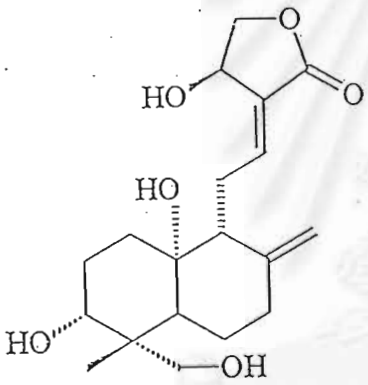
Figure 7 The chemical constituents of *A. paniculata* Nees. ( continue )



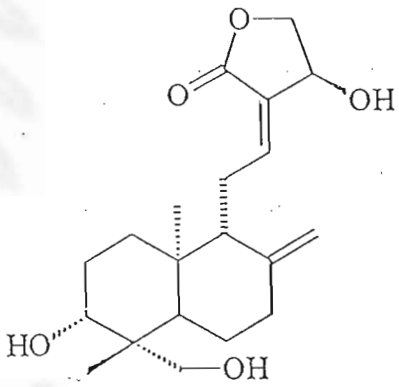

14-deoxy-11,12-didehydroandrographiside




Andrograpanin



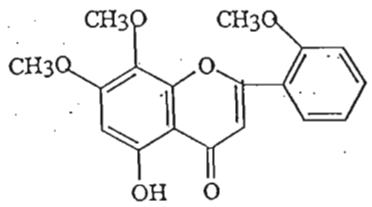

14-epi-andrographolide



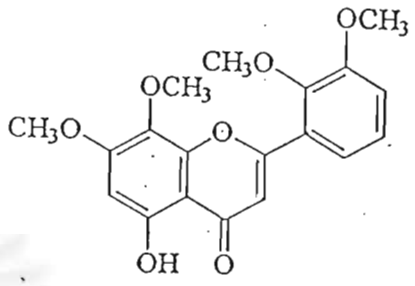

Isoandrographolide



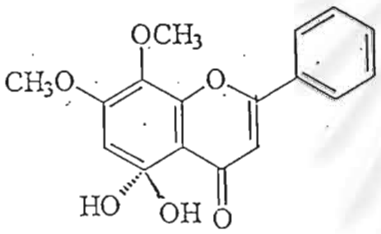
Figure 7 The chemical constituents of *A. paniculata* Nees. ( continue )



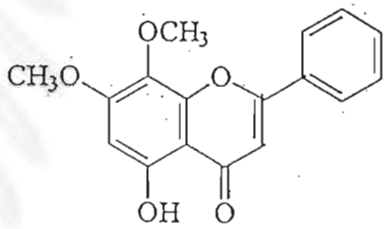
Andrographin



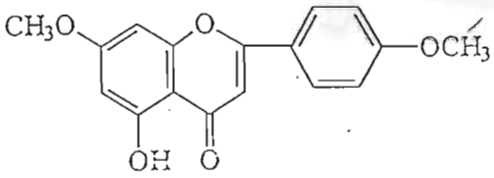
5-Hydroxy-2', 3', 7, 8-tetramethoxyflavone



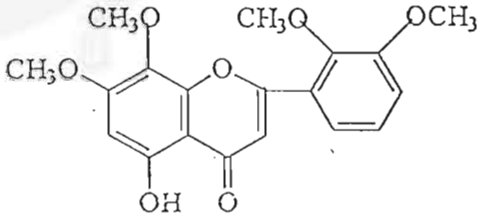
( dl ) – 5 – Hydroxy-7,8-dimethoxyflavone



5 – Hydroxy-7,8-dimethoxyflavone

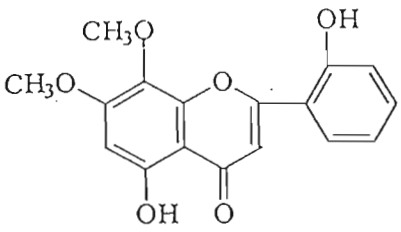


Apigenin - 4',7'- di-o-methyl ether

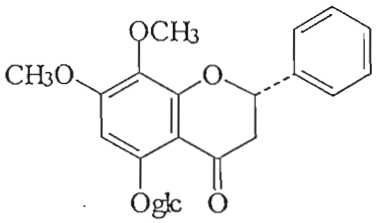


Mono - o – Methyl – Wightin

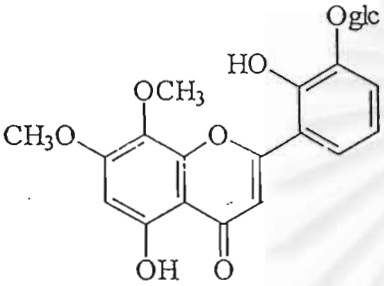
Figure 7 The chemical constituents of *A. paniculata* Nees. ( continue )



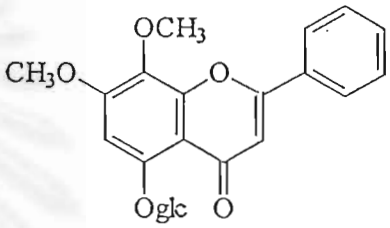
Panicolin



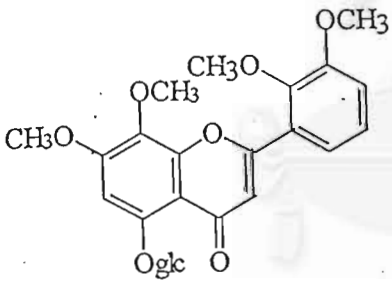
Andrographidine A



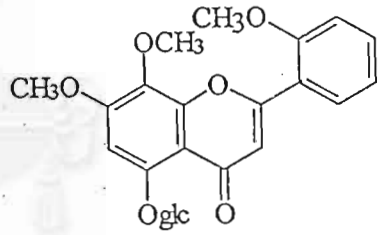
Andrographidine B



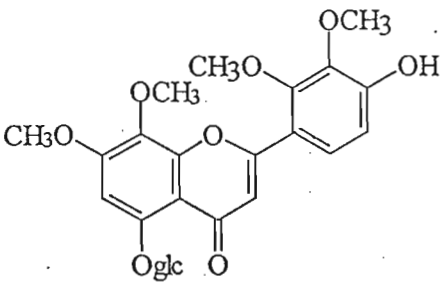
Andrographidine C



Andrographidine D



Andrographidine E



Andrographidine F

Figure 7 The chemical constituents of *A. paniculata* Nees. ( continue )

# CHAPTER III

# EXPERIMENTAL

## General experimental procedure

The IR absorption spectra were obtained on a Nicolet Impact 410 Fourier Transform Infrared Spectrophotometer using KBr or NaCl cell on neat samples. Low resolution mass spectra were obtained with a Fisons Instruments Mass Spectrometer model Trio 2000 in Electron Impact ( EI ) mode at 70 ev. $^{1}H$ and $^{13}C$ - NMR spectra were recorded on a Bruker Model AC - F200 spectrometer operated at 200.13 MHz for $^{1}H$ and 50.32 MHz for $^{13}C$- nuclei. Silica gel Art.7734.1000( 70 - 230mesh ASTM ) were purchased from Merck Company. Elemental Analyses were carried out at Chulalongkorn University Research Equipment Centre on a Perkin Elmer Elemental Analyzer model 2400 CHNS/O. X-ray diffraction data set was collected on SIEMEN SMART diffractrometer using Mo X-ray Tube and area detector at Department of Physics, Faculty of Science, Thammasart University.

**Plant material** . The plant material of *A. paniculata* Nees used in this study was collected from Nakornprathom province, Thailand in January 1999. The plant was identified by comparing with herbarium specimens ( BKF no.12195 ) in the Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

**Extraction Procedure.** The dried leaves of *A. paniculata* Nees (10 kg) were macerated with methanol for 3 days at room temperature and then filtered. The extract was evaporated under reduced pressure to yield 600 g of crude extract. The extract was dissolved in chloroform and partitioned with distilled water to give crude crystals. After

decolourisation with activated charcoal, crude crystals were recrystallized from methanol to give andrographolide ( 20 g, 0.2 % yield )

**Andrographolide ( Compound 1 )**

Colourless needles crystal, m.p. 230-231 $^{0}$ ( ref. m.p. 230-231 $^{0}$ )

$\nu_{max}$ (neat) / $cm^{-1}$ 3500-3150 , 2930, 1726, 1224, 1035

$^{1}$H-NMR ($CDCl_3$) δ 0.68 ( s ), 1.07 ( s ), 1.20 ( m ), 1.68 ( m), 1.82 ( td ), 2.31 ( m ), 2.49 ( m ), 3.22 ( m ), 3.30 ( s ), 3.84 ( dd ), 4.04 ( dd ), 4.12 ( dd ), 4.38 ( dd ), 4.63 ( brs ), 4.84 ( brd ), 5.05 ( brs ), 5.70 ( dd ) , 6.55 ( t )

$^{13}$C-NMR ( DMSO) δ 4.7 ($CH_3$) , 22.9 ($CH_3$) , 23.8 ($CH_2$) , 23.9 ($CH_2$) , 27.7 ($CH_2$), 36.4 ($CH_2$) , 37.4 ($CH_2$) , 38.7 ($CH_2$) , 42.1 (C) , 54.3 (CH) , 55.4 (CH) , 62.7 ($CH_2$) , 64.5 (CH) , 74.3 ($CH_2$) , 78.5 (CH) , 108.3 ($CH_2$) , 128.7 (C) , 146.5 (C) , 147.4 (CH) , 170.0 (C =O)

m/z ( EI ) 350(2) , 332(10) , 314(12) , 296(14)

**Preparation of 14-deoxy-11,12-didehydroandrographolide ( Compound 2 )**

Andrographolide (1.0 g, 0.28 mmole) was refluxed in dry pyridine ( 5 ml ) over night. The crude product was purified by column chromatography and used mixture of chloroform : methanol (80:20) as eluent to obtain 14-deoxy-11,12– didehydroandrogra pholide as white needles crystal. ( 0.54 g, 54 % yield ) m.p. 203 - 204 $^{0}$ ( ref. m.p. 204 - 205 $^{0}$ )

$\nu_{max}$ (neat) / $cm^{-1}$ 3450-3200, 2940, 1742, 1102, 1040

$^{1}$H-NMR ($CDCl_3$) δ 0.82 ( s ), 1.15 ( m ), 1.22 ( s ), 1.50 ( d ), 1.70 ( m ), 2.29 ( m ), 2.47 ( m ), 2.95 ( br ), 3.28 ( d ), 3.48 ( brt ), 4.18 ( d ), 4.50 ( d ), 4.50 - 4.74( brs ), 6.18 ( d ), 6.85 ( q ), 7.20 ( brt )

$^{13}$C-NMR ( $CDCl_3$) δ 15.9 ($CH_3$) , 22.7 ($CH_3$) , 23.0 ($CH_2$) , 28.1 ($CH_2$) , 36.6 ($CH_2$) ,

38.2 ($CH_2$) , 38.6 (C) , 42.9 (C) , 54.7 (CH) , 61.7 (CH) , 64.2 ($CH_2$) , 69.7 ($CH_2$) , 80.8 (CH) ,109.2 ($CH_2$) , 121.1 (CH) , 129.2 (C) , 136.0 (CH) , 143.0 (CH) , 148.1 (C) , 172.4 (C =O)

m/z ( EI ) 332(27) , 314(8) , 296(10)

**Esterification of andrographolide**

***General method*** Acylation of andrographolide with acid chloride

Andrographolide( 1.0 g, 0.28 mmole ) was dissolved in 50 ml of dichloromethane in100 ml round bottom flask. Acid chloride was slowly added dropwise with vigorously stirring overnight. After the reaction mixture was worked up, it was evaporated under reduced pressure to give crude product. It was dissolved in diethyl ether and partitioned with distilled water and sodium hydrogencarbonate for 3-4 times each, respectively. The solution was dried with anhydrous magnesium sulfate and evaporated under reduced pressure to remove ether, and then the residue was purified by column chromatography.

**Preparation of andrographolide diacetate ( Compound 3 )**

Acetyl chloride ( 4 ml, 5.63 mmole ) was added into a solution of andrographolide ( 1.0 g, 0.28 mmole ) in 50 ml $CH_2Cl_2$ and the reaction mixture was stirred for 12 hours at room temperature. The product was purified by column chromatography and used mixture of dichloromethane : methanol ( 90 : 10 ) as eluent to obtain andrographolide diacetate, after recrystallization from dichloromethane : hexane, the product was obtained white needles crystal ( 0.78 g, 78 % yields ). m.p. 164-165° ( Found : C, 65.98 ; H, 7.72 $C_{24}H_{34}O_7$ requires C, 66.34 ; H, 7.88 % )

$\nu_{max}$ (neat) / $cm^{-1}$ 3450-3530 , 2965 , 1732 , 1245 , 1050

$^1$H-NMR ($CDCl_3$) δ 0.74 ( s ), 1.01 ( s ), 1.38 ( m ), 1.81 ( m ), 2.02 ( s ), 2.10 ( s ),

4.25 ( m ), 4.55 ( m ), 4.88 ( s ), 5.89 ( d ), 6.98 ( td )

$^{13}$C-NMR ($CDCl_3$) δ 14.4 ($CH_3$) , 20.9 ($CH_3$) , 21.0 ($CH_3$) , 22.6 ($CH_3$) , 24.1 ($CH_2$) , 24.5 ($CH_2$) , 25.1 (C) , 36.8 ($CH_2$) , 37.7 ($CH_2$) , 38.8 (C) , 41.2 (C) , 55.0 (CH) , 55.6 (CH) , 64.5 ($CH_2$) , 67.7 (CH) , 71.5 ($CH_2$) , 79.6 (CH) ,108.7 ($CH_2$) ,124.0 (C) , 146.5 (C) , 150.0 (CH) , 169.0 (C=O) , 170.4 (C=O) , 170.8 (C=O)

m/z ( EI ) 416 (4) , 356 (8) , 314 (3) , 296 (23)

**Preparation of 8-Chloro-andrographolide triacetate ( Compound 4 )**

Acetyl chloride ( 7 ml, 9.85 mmole ) was added into a solution of andrographolide ( 1.0 g , 0.28 mmole ) together with 4- dimethylamino pyridine ( DMAP ) ( 0.1 g , 0.08 mmole ) as a catalyst. The reaction mixture was stirred at room temperature for 15 hours. After the reaction mixture was worked up, the product was purified by column chromatography and used mixture of dichloromethane : methanol ( 80 : 20 ) as eluent to obtain white plate crystal (0.87 g, 87 % yield) m.p.181-182 ° ( Found : C, 60.76 ; H, 7.213 $C_{26}H_{37}O_8Cl$ requires C, 60.87 ; H, 7.26 % )

$\nu_{max}$ (neat) / $cm^{-1}$ 2980 , 1736 , 1234 , 1029

$^1$H-NMR ($CDCl_3$) δ 0.88 ( s ), 0.98 ( s ), 1.19 ( d ), 1.51 ( s ), 1.87 ( dd ), 2.05 ( m ), 2.30 ( m ), 4.09 ( d ), 4.23 ( m ), 4.48 ( dd ), 5.92 ( d ), 7.08 ( td )

$^{13}$C-NMR ($CDCl_3$) δ 15.6($CH_3$) , 20.9($CH_3$) , 21.1 ($CH_3$)(x2) , 21.7 ($CH_2$) , 22.5 ($CH_3$), 23.2 ($CH_2$) , 26.3 ($CH_3$) , 27.7 ( $CH_2$ ) , 38.2 ($CH_2$) , 40.2 (CH) , 40.9 (C) , 46.5 (C) , 55.2 ($CH_2$) , 62.4 (CH) , 65.1 (CH) , 67.5 ($CH_2$) , 71.5 (CH) , 76.1 ($CH_2$) , 79.1 (CH) , 123.0 (C) , 151.6 (CH) , 169.0 (C=O) , 170.3 (C=O) , 170.5 (C=O) , 170.8 (C=O)

m/z ( EI ) 416(38) , 356(60) , 314(15) , 296(83)

**Preparation of Andrographolide dibutyrate ( Compound 5 )**

Butyryl chloride ( 4 ml , 3.86 mmole ) was added into a solution of andrographolide ( 1.0 g, 0.28 mmole ) in 50 ml $CH_2Cl_2$ and the reaction mixture was stirred for 12 hours at room temperature. The product was purified by column chromatography and used mixture of dichloromethane : methanol( 90 : 10 ) as eluent to result, andrographolide dibutyrate as yellow oil ( 0.75 g, 75 % yields ).

$\nu_{max}$ (neat) / $cm^{-1}$ 3380-3520, 2965, 1742, 1188, 1010

$^1$H-NMR ($CDCl_3$) δ 0.68 ( s ), 0.85 ( m ), 1.34 ( m ), 1.56 ( m ), 2.25 ( m ), 4.19 ( dd ), 4.50 ( m ), 4.84 ( s ), 5.84 ( d ), 6.94 ( td )

$^{13}$C-NMR ($CDCl_3$) δ 13.6 ($CH_3$) (x2) , 14.4 ($CH_3$) , 18.33 ($CH_2$) , 18.5($CH_2$) , 22.6 ($CH_3$) , 24.2 ($CH_2$) , 24.6 ($CH_2$) , 25.1 ($CH_2$) , 35.9 ($CH_2$) , 36.3 ($CH_2$) , 36.5 ($CH_2$) , 36.9 ($CH_2$) , 37.8 ($CH_2$) , 38.9 (C) , 41.2 (C) , 55.2 (CH) , 55.7 (CH) , 64.5 ($CH_2$) , 67.6 (CH) , 71.7 ($CH_2$) , 79.3 (CH) , 108.9 ($CH_2$) , 124.0 (C) , 146.5 (C) , 150.1 (CH) , 169.0 (C=O) , 173.1 (C=O) , 173.5 (C=O)

m/z ( EI ) 472(16), 384(28), 296(56)

**Preparation of andrographolide tributyrate ( Compound 6 )**

Butyryl chloride ( 6 ml, 5.79 mmole ) was added into a solution of andrographolide ( 1.0 g, 0.28 mmole ) in 50 ml $CH_2Cl_2$ and the reaction mixture was stirred for 15 hours at room temperature. The product was purified by column chromatography and used mixture of dichloromethane : methanol ( 90 :10 ) as eluent to give andrographolide tributyrate as yellow oil ( 0.84 g, 84 % yields ).

$\nu_{max}$ (neat) / $cm^{-1}$ 2960, 1731, 1183, 1004

$^1$H-NMR ( $CDCl_3$ ) δ 0.71 ( s ), 0.80 ( m ), 1.29 ( m ), 1.65 ( m ), 2.24 ( m ), 2.38 ( dd ), 4.56 ( m ), 4.83 ( s ), 5.85 ( d ), 6.88 ( td )

$^{13}$C-NMR ($CDCl_3$) δ 13.6 ($CH_3$)(x2), 13.7 ($CH_3$), 14.4 ($CH_3$), 18.4 ($CH_2$)(x2), 18.5 ($CH_2$),

22.6 ($CH_3$), 24.2 ($CH_2$), 24.6 ($CH_2$), 25.2 ($CH_2$), 35.9 ($CH_2$), 36.4 ($CH_2$), 36.5 ($CH_2$), 37.0 ($CH_2$), 37.8 ($CH_2$), 38.9 (C), 41.3 (C), 55.2 (CH), 55.7 (CH), 64.6 ($CH_2$), 67.6 (CH), 71.7 ($CH_2$), 79.4 (CH), 108.9 ($CH_2$), 124.0 (C), 146.5 (C),150.2 (CH), 169.1 (C=O), 173.2 (C=O), 173.6 (C=O), 179.0 (C=O)

m/z ( EI ) 472(20), 384(52), 296(100)

**Preparation of andrographolide trihexanoate ( Compound 7 )**

Hexanoyl chloride ( 5 ml, 5.38 mmole ) was added into a solution of andrographolide and the reaction mixture was stirred for 15 hours at room temperature. The product was purified by using column chromatography and used mixture of dichloromethane : methanol ( 80 : 20 ) as eluent to give andrographolide trihexanoate as yellow oil ( 0.87 g, 87 % yields ).

$\nu_{max}$ (neat) / $cm^{-1}$ 2955, 1741, 1173, 1009

$^1$H-NMR ( $CDCl_3$ ) δ 0.69 ( s ), 0.81 ( m ), 0.95 ( s ), 1.27 ( m ), 1.60 ( m ), 2.27 ( m ), 4.15 ( dd ), 4.46 ( m ), 4.78 ( s ), 5.84 ( d ), 6.90 ( td )

$^{13}$C-NMR ($CDCl_3$) δ 13.8 ($CH_3$)(x3), 14.4 ($CH_3$), 22.2 ($CH_2$)(x2), 22.6 ($CH_3$), 24.2 ($CH_2$), 24.4 ($CH_2$), 24.6 ($CH_2$), 24.7 ($CH_2$)(x2), 25.1 ($CH_2$), 31.2 ($CH_2$), 31.3 ($CH_2$)(x2), 33.9 ($CH_2$), 34.0 ($CH_2$), 34.4 ($CH_2$), 34.6 ($CH_2$), 36.9 ($CH_2$), 37.9 ($CH_2$), 38.9 (C), 41.3 (C), 55.2 (CH), 55.7 (CH), 64.6 ($CH_2$), 67.6 (CH), 71.7 ($CH_2$), 79.4 (CH),108.9 ($CH_2$),124.0 (C), 146.5 (C), 150.2 (CH), 169.2 (C=O), 173.4 (C=O) , 173.8 (C=O) , 179.2 (C=O)

m/z (EI) 528(8), 412(38), 314(28), 296(97)

**Preparation of 14-deoxy-11,12-didehydroandrographolide *p* - nitro monobenzoate ( Compound 8 )**

Andrographolide ( 1.0 g, 0.28 mmole ) was dissolved in 5 ml of redistilled pyridine in 100 ml round bottom flask. *p* - Nitro benzoyl chloride ( 3.0 g, 1.61 mmole )

was added in the reaction with stirring at room temperature until a solution was obtained. After that, the reaction mixture was refluxed on heating mantle for 12 hours and solvent ( pyridine ) was removed by reduced pressure distillation to obtained crude product. The crude product was then extracted with 50 ml of 5 % HCl for 3 - 4 times and purified by column chromatography and used mixture of chloroform : acetone ( 95 : 5 ) as eluent to result, 14- deoxy-11,12- didehydroandrographolide *p*-nitromonobenzoate as pale yellow solid ( 0.74 g, 74 % yields ). m.p. 160-161 °

$\nu_{max}$ (neat) / $cm^{-1}$ 3250-3530, 2935, 1757, 1526, 1275

$^{1}$H-NMR ($CDCl_3$) δ 0.88 ( s ), 1.27 ( s ), 1.82 ( m ), 2.40 ( dd ), 4.53 ( m ), 4.80 ( s ), 6.15 ( d ), 6.88 ( dd ), 7.22 ( brt ), 8.23 ( dd )

$^{13}$C-NMR ($CDCl_3$) δ 15.5 ($CH_3$) , 22.6 ($CH_3$) , 23.7 (CH) , 27.7 (CH) , 36.6 (CH) , 38.5 (CH) , 38.8 (C) , 42.9 (C) , 50.9 (C) , 54.6 (CH) , 61.6 (CH) , 66.2 ($CH_2$) , 69.7 ($CH_2$) , 78.9 (CH) , 109.4 ($CH_2$) , 121.3 (C) , 123.6 (CH)(x2) , 129.2 (C) , 130.7 (CH)(x2) , 135.7(CH), 143.1 (CH) , 147.8 (C) , 150.5 (C) , 164.9 (C=O) , 172.3 (C=O)

m/z ( EI ) 481(8) , 463(10) , 314(13) , 296(21)

## X-ray Diffraction Experiment

A colourless needle-shaped crystal of andrographolide ( compound 1 ) was obtained from methanol, while a colourless plate-shaped crystal of 8-Chloro-andrographolide triacetate ( compound 4 ) was obtained from dichloromethane. All data were collected at room temperature using graphite monochromated Mo Kα radiation ( lamda = 0.71069 $A^{o}$ ) on SIEMEN SMART diffractometer. The data were corrected for Lorentz and polarization effects. The crystal experimental data of compound 1 and compound 4 are given in Table 2 and 6 , respectively.

The structure was solved by direct methods using SHELXS-97 and refined by full matrix least-square on $F^2$ using SHELXL-97 with anisotropic thermal parameters for all the non-hydrogen atoms. All the hydrogen atoms were found in difference Fourier maps and were included in refinement. The fraction coordinates of non-hydrogen atoms and

selected bond distances and angles of compound 1 and compound 4 are listed in Table 3,4,7 and 8, respectively.

Table 2. Crystal data and structure refinement for Andrographolide (Compound 1)

| | |
|---|---|
| Empirical formula | $C_{20}H_{30}O_5$ |
| Formula weight | 350 |
| Temperature | 293(2) K |
| Wavelength | 0.71073 A |
| Crystal system, space group | Monoclinic , $P_2$ (1) |
| Unit cell dimensions | a = 6.5969(3) A alpha = 90 deg. |
| | b = 8.0652(4) A beta = 97.3980 (10) |
| | c = 18.1271(8) A gamma = 90 deg. |
| Volume | 956.43(8) $A^3$ |
| Z, Calculated density | 2 , 1.008 $mg/m^3$ |
| Absorption coefficient | 0.062 $mm^{-1}$ |
| F(000) | 310 |
| Theta range for data collection | 2.27 to 30.34 deg. |
| Index ranges | $-9 < h < 8$ , $-11 < k < 10$ , $-24 < l < 22$ |
| Reflection collected / unique | 6961 / 4708 [ R(int) = 0.0164 ] |
| Completeness to 2theta = 30.34 | 93.8 % |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data / restraints / parameters | 4708 / 1 / 342 |
| Goodness-of-fit on $F^2$ | 0.343 |
| Final R indices [I > 2 sigma( I )] | R1 = 0.0473, wR2 = 0.1696 |
| R indices ( all data ) | R1 = 0.0561, wR2 = 0.2261 |
| Absolute structure parameter | -0.6 (14) |
| Largest diff. peak and hole | 0.426 and – 0.169 e. $A^{-3}$ |

Table 3. Atomic coordinates ( x $10^4$ ) and equivalent isotropic displacement parameters ( $A^2$ x $10^3$ ) for Andrographolide. ( Compound 1 )

| | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| C (10) | 4074 (3) | 1362 (3) | 2387 (1) | 32 (1) |
| C (9) | 6051 (3) | 558 (3) | 2829 (1) | 33 (1) |
| O (3) | 11185 (3) | -138 (3) | 5850 (1) | 54 (1) |
| C (11) | 6380 (3) | 1013 (3) | 3663 (1) | 39 (1) |
| C (5) | 4122 (3) | 981 (3) | 1538 (1) | 36 (1) |
| C (16) | 11052 (3) | -216 (3) | 5099 (1) | 41 (1) |
| C (13) | 8940 (3) | 209 (3) | 4773 (1) | 36 (1) |
| O (5) | 12506 (3) | -611 (3) | 4786 (1) | 56 (1) |
| C (14) | 7636 (4) | 300 (3) | 5402 (1) | 42 (1) |
| C (12) | 8437 (3) | 462 (3) | 4041 (1) | 39 (1) |
| C (8) | 6070 (4) | -1288 (3) | 2679 (2) | 47 (1) |
| O (1) | 1209 (5) | 4814 (4) | 704 (2) | 81 (1) |
| C (4) | 2531 (3) | 1940 (4) | 967 (1) | 42 (1) |
| C (3) | 2688 (5) | 3818 (4) | 1167 (2) | 53 (1) |
| C (20) | 2189 (4) | 684 (5) | 2711 (1) | 51 (1) |
| C (2) | 2510 (6) | 4184 (4) | 1981 (2) | 58 (1) |
| C (6) | 4172 (5) | -899 (4) | 1393 (2) | 56 (1) |
| C (1) | 4172 (5) | 3263 (3) | 2494 (1) | 48 (1) |
| C (7) | 6033 (6) | -1699 (5) | 1857 (2) | 64 (1) |
| C (15) | 9278 (5) | 499 (6) | 6075 (2) | 61 (1) |
| C (18) | 3160 (5) | 1733 (7) | 179 (1) | 65 (1) |
| C (17) | 6027 (7) | -2446 (4) | 3201 (2) | 73 (1) |
| O (4) | 6529 (3) | -1197 (3) | 5473 (2) | 60 (1) |
| O (2) | -1126 (3) | 2105 (4) | 404 (1) | 64 (1) |
| C (19) | 323 (4) | 1300 (4) | 958 (1) | 50 (1) |

Table 4. Bond lengths ( A°) and angles ( deg ) for Andrographolide. ( Compound 1 )

| | | | | | |
|---|---|---|---|---|---|
| C(10) | - C(20) | 1.542 (3) | C(10) | - C(1) | 1.546 (3) |
| C(10) | - C(5) | 1.573 (3) | C(10) | - C(9) | 1.579 (3) |
| C(9) | - C(8) | 1.514 (3) | C(9) | - C(11) | 1.543 (3) |
| O(3) | - C(16) | 1.355 (3) | O(3) | - C(15) | 1.465 (4) |
| C(11) | - C(12) | 1.506 (3) | C(5) | - C(6) | 1.540 (4) |
| C(5) | - C(4) | 1.579 (3) | C(16) | - O(5) | 1.217 (4) |
| C(16) | - C(13) | 1.481 (3) | C(13) | - C(12) | 1.342 (3) |
| C(13) | - C(14) | 1.515 (3) | C(14) | - O(4) | 1.426 (3) |
| C(14) | - C(15) | 1.533 (4) | C(8) | - C(17) | 1.332 (5) |
| C(8) | - C(7) | 1.524 (4) | O(1) | - C(3) | 1.445 (3) |
| C(4) | - C(19) | 1.543 (3) | C(4) | - C(18) | 1.547 (3) |
| C(4) | - C(3) | 1.558 (4) | C(3) | - C(2) | 1.523 (4) |
| C(2) | - C(1) | 1.535 (3) | C(6) | - C(7) | 1.538 (5) |
| O(2) | - C(19) | 1.448 (3) | | | |

Table 4. Bond lengths ( A°) and angles ( deg ) for Andrographolide. ( Compound 1 )

| | | | |
|---|---|---|---|
| C(20) – C(10) – C(1) | 109.1(2) | C(20) – C(10) – C(5) | 114.91(18) |
| C(1) – C(10) – C(5) | 108.06(18) | C(20) – C(10) – C(9) | 108.38(17) |
| C(1) – C(10) – C(9) | 109.00(17) | C(5) – C(10) – C(9) | 107.22(16) |
| C(8) – C(9) – C(11) | 113.9(2) | C(8) – C(9) – C(10) | 109.79(18) |
| C(11) – C(9) – C(10) | 113.69(16) | C(16) – O(3) – C(15) | 110.46(19) |
| C(12) – C(11) – C(9) | 112.38(18) | C(6) – C(5) – C(10) | 111.29(19) |
| C(6) – C(5) – C(4) | 113.42(19) | C(10) – C(5) – C(4) | 116.75(18) |
| O(5) – C(16) – O(3) | 121.8(2) | O(5) – C(16) – C(13) | 129.2(2) |
| O(3) – C(16) – C(13) | 109.0(2) | C(12) – C(13) – C(16) | 122.0(2) |
| C(12) – C(13) – C(16) | 130.1(2) | C(16) – C(13) – C(14) | 107.91(19) |
| O(4) – C(14) – C(13) | 112.0(2) | O(4) – C(14) – C(15) | 109.5(2) |
| C(13) – C(14) – C(15) | 101.1(2) | C(13) – C(12) – C(11) | 126.1(2) |
| C(17) – C(8) – C(9) | 124.1(3) | C(17) – C(8) – C(3) | 122.9(3) |
| C(9) – C(8) – C(3) | 112.9(3) | C(19) – C(4) – C(18) | 108.6(2) |
| C(19) – C(4) – C(3) | 111.2(2) | C(18) – C(4) – C(3) | 107.6(3) |
| C(19) – C(4) – C(5) | 113.0(2) | C(18) – C(4) – C(5) | 108.5(2) |

Table 5. Hydrogen–bond geometry of Andrographolide

| D-H…A | D…A | D-H | H…A | < D-H…A |
|---|---|---|---|---|
| O(1)-H(1)…O(2) | 2.682 | 0.834 | 1.887 | 159.07 |
| O(2)-H(2)…O(1) | 2.726 | 0.834 | 1.920 | 162.30 |
| O(4)-H(4)…O(5) | 2.826 | 0.834 | 1.992 | 179.09 |

Table 6. Crystal data and structure refinement for 8-Chloro-andrographolide triacetate ( Compound 4 )

| | |
|---|---|
| Empirical formula | $C_{26}H_{37}0_8Cl$ |
| Formula weight | 513.01 |
| Temperature | 293(2) K |
| Wavelength | 0.71073 A |
| Crystal system, space group | Monoclinic , $P_2$ (1) |
| Unit cell dimensions | a = 8.3640(2) A alpha = 90 deg. |
| | b = 9.4503(3) A beta = 103.4150(10) |
| | c = 17.4323(6) A gamma = 90 deg. |
| Volume | 1340.29(7) $A^3$ |
| Z, Calculated density | 2 , 1.271 $mg/m^3$ |
| Absorption coefficient | 0.188 $mm^{-1}$ |
| F(000) | 548 |
| Theta range for data collection | 2.40 to 30.54 deg. |
| Index ranges | -11 < h < 11, -13 < k < 10, -24 < l < 24 |
| Reflection collected / unique | 9914 / 6058 [ R(int) = 0.0468 ] |
| Completeness to 2theta = 30.34 | 94.6 % |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data / restraints / parameters | 6058 / 1 / 445 |
| Goodness-of-fit on $F^2$ | 1.056 |
| Final R indices [I > 2 sigma( I )] | R1 = 0.0683, wR2 = 0.1110 |
| R indices ( all data ) | R1 = 0.1565, wR2 = 0.1421 |
| Absolute structure parameter | 0.06 (11) |
| Largest diff. peak and hole | 0.223 and – 0.221 e. $A^{-3}$ |

Table 7. Atomic coordinates ( x $10^4$ ) and equivalent isotropic displacement parameters ( $A^2$ x $10^3$ ) for 8-Chloro-andrographolide triacetate ( Compound 4 )

| | x | y | z | $B_{eq}$ |
|---|---|---|---|---|
| Cl(1) | 5443(2) | 3688(2) | 4365(1) | 89(1) |
| O(3) | 4274(4) | 4219(4) | 103(2) | 73(1) |
| O(5) | 6209(4) | 5077(4) | 1080(2) | 83(1) |
| O(2) | -3002(3) | 8391(4) | 3455(2) | 72(1) |
| O(6) | -5008(5) | 7542(5) | 2541(4) | 135(2) |
| O(1) | -1474(3) | 9283(3) | 2229(2) | 63(1) |
| O(7) | 309(5) | 10863(4) | 995(2) | 80(1) |
| O(4) | 3008(3) | 1476(3) | 922(2) | 54(1) |
| O(8) | 589(4) | 972(4) | 108(2) | 88(1) |
| C(1) | 1035(5) | 6007(5) | 2164(2) | 45(1) |
| C(2) | -344(6) | 7087(5) | 1924(3) | 49(1) |
| C(3) | -173(5) | 8255(4) | 2523(3) | 49(1) |
| C(4) | -247(5) | 7765(4) | 3354(3) | 47(1) |
| C(5) | 1114(5) | 6601(4) | 3584(2) | 38(1) |
| C(6) | 1327(7) | 6008(6) | 4416(3) | 58(1) |
| C(7) | 2982(7) | 5272(6) | 4667(3) | 63(2) |
| C(8) | 3205(5) | 4085(5) | 4116(2) | 52(1) |
| C(9) | 2814(5) | 4615(4) | 3255(2) | 40(1) |
| C(10) | 1099(4) | 5369(4) | 2988(2) | 37(1) |
| C(11) | 3153(6) | 3498(5) | 2658(3) | 46(1) |
| C(12) | 4124(5) | 4040(4) | 2114(3) | 46(1) |
| C(13) | 3901(4) | 3798(5) | 1353(2) | 47(1) |
| C(16) | 4950(6) | 4450(5) | 871(3) | 60(1) |
| C(15) | 2736(7) | 3462(7) | -1(3) | 68(2) |
| C(14) | 2596(5) | 2960(5) | 818(2) | 51(1) |
| C(20) | -352(6) | 4343(5) | 2914(3) | 51(1) |
| C(23) | -1071(6) | 10509(5) | 1950(3) | 51(1) |

| | | | | |
|---|---|---|---|---|
| C(24) | -2542(9) | 11369(6) | 1592(4) | 85(2) |
| C(18) | 214(6) | 9018(6) | 3924(3) | 65(1) |
| C(19) | -1941(6) | 7212(5) | 3386(3) | 57(1) |
| C(21) | -4469(5) | 8457(5) | 3006(3) | 64(1) |
| C(22) | -5306(7) | 9804(7) | 3082(5) | 84(2) |
| C(17) | 2336(9) | 2707(6) | 4270(3) | 69(2) |
| C(25) | 1856(6) | 556(6) | 526(3) | 64(1) |
| C(26) | 2360(8) | -901(7) | 677(4) | 80(2) |

Table 8. Bond lengths (A°) and angles (deg) for 8-Chloro-andrographolide triacetate ( compound 4 )

| | | | |
|---|---|---|---|
| Cl(1) – C(8) | 1.859(4) | C(4) – C(5) | 1.567(5) |
| O(3) – C(16) | 1.344(5) | C(5) – C(6) | 1.527(6) |
| O(3) – C(15) | 1.446(6) | C(5) – C(10) | 1.558(5) |
| O(5) – C(16) | 1.190(5) | C(6) – C(7) | 1.520(7) |
| O(2) – C(21) | 1.294(5) | C(7) – C(8) | 1.516(6) |
| O(2) – C(19) | 1.446(5) | C(8) – C(9) | 1.544(5) |
| O(6) – C(21) | 1.200(6) | C(8) – C(17) | 1.545(8) |
| O(1) – C(23) | 1.330(5) | C(9) – C(11) | 1.553(6) |
| O(1) – C(3) | 1.459(4) | C(9) – C(10) | 1.573(5) |
| O(7) – C(23) | 1.186(5) | C(10) – C(20) | 1.535(6) |
| O(4) – C(25) | 1.361(5) | C(11) – C(12) | 1.476(6) |
| O(4) – C(14) | 1.445(5) | C(12) – C(13) | 1.316(5) |
| O(8) – C(25) | 1.204(6) | C(13) – C(16) | 1.482(6) |
| C(1) – C(2) | 1.524(6) | C(13) – C(14) | 1.488(6) |
| C(1) – C(10) | 1.547(6) | C(15) – C(14) | 1.535(6) |
| C(2) – C(3) | 1.503(6) | C(23) – C(24) | 1.486(7) |
| C(3) – C(4) | 1.537(6) | C(21) – C(22) | 1.473(8) |
| C(4) – C(19) | 1.523(6) | C(25) – C(26) | 1.446(8) |
| C(4) – C(18) | 1.536(6) | | |

Table 8. Bond lengths (A°) and angles (deg) for 8-Chloro-andrographolide triacetate

| | | | |
|---|---|---|---|
| C(16) – O(3) – C(15) | 111.2(3) | C(21) – O(2) – C(19) | 120.1(4) |
| C(23) – O(1) – C(3) | 118.4(3) | C(25) – O(4) – C(14) | 115.9(4) |
| C(2) – C(1) – C(10) | 112.1(4) | C(3) – C(2) – C(1) | 110.9(3) |
| O(1) – C(3) – C(2) | 107.6(3) | O(1) – C(3) – C(4) | 110.0(4) |
| C(2) – C(3) – C(4) | 114.6(4) | C(19) – C(4) – C(18) | 109.7(4) |
| C(19) – C(4) – C(3) | 112.3(4) | C(18) – C(4) – C(3) | 108.6(4) |
| C(19) – C(4) – C(5) | 112.1(4) | C(18) – C(4) – C(5) | 108.5(3) |
| C(3) – C(4) – C(5) | 105.5(3) | C(6) – C(5) – C(10) | 109.9(3) |
| C(6) – C(5) – C(4) | 114.7(3) | C(10) – C(5) – C(4) | 117.5(3) |
| C(7) – C(6) – C(5) | 109.8(4) | C(8) – C(7) – C(6) | 113.2(4) |
| C(7) – C(8) – C(9) | 110.3(4) | C(7) – C(8) – C(17) | 112.4(4) |
| C(9) – C(8) – C(17) | 116.1(4) | C(7) – C(8) – Cl(1) | 105.5(3) |
| C(9) – C(8) – Cl(1) | 105.5(3) | C(17) – C(8) – Cl(1) | 106.2(3) |
| C(8) – C(9) – C(11) | 113.6(3) | C(8) – C(9) – C(10) | 114.1(3) |
| C(11) – C(9) – C(10) | 113.9(3) | C(20) – C(10) – C(1) | 108.2(4) |
| C(20) – C(10) – C(5) | 114.3(3) | C(1) – C(10) – C(5) | 108.7(3) |
| C(20) – C(10) – C(9) | 112.8(3) | C(1) – C(10) – C(9) | 106.5(3) |
| C(5) – C(10) – C(9) | 106.0(3) | C(12) – C(11) – C(9) | 114.1(4) |
| C(13) – C(12) – C(11) | 128.1(4) | C(12) – C(13) – C(16) | 122.3(4) |
| C(12) – C(13) – C(14) | 129.4(4) | C(16) – C(13) – C(14) | 108.1(4) |
| O(5) – C(16) – O(3) | 121.6(4) | O(5) – C(16) – C(13) | 129.2(4) |
| O(3) – C(16) – C(13) | 109.2(4) | O(3) – C(15) – C(14) | 107.1(4) |
| O(4) – C(14) – C(13) | 108.6(3) | O(4) – C(14) – C(15) | 110.5(4) |
| C(13) – C(14) – C(15) | 102.4(4) | O(7) – C(23) – O(1) | 123.2(4) |
| O(7) – C(23) – C(24) | 124.8(5) | O(1) – C(23) – C(24) | 112.0(5) |
| O(2) – C(19) – C(4) | 109.4(4) | O(6) – C(21) – O(2) | 121.9(5) |
| O(6) – C(21) – C(22) | 124.8(5) | O(2) – C(21) – C(23) | 113.1(5) |
| O(8) – C(25) – O(4) | 121.2(5) | O(8) – C(25) – C(26) | 126.9(5) |
| O(4) – C(25) – C(26) | 112.0(5) | | |

BITTERNESS TEST

Many of the medicinal plants have a bitter taste which are very useful to stimulate appetizer. WHO method is used to determine bitterness using a parnel of volunteer.

Procedures for prepared stock solution

1. A solution was prepared by dissolving 0.1 g of Quinine sulfate in 100 ml of water.
2. Dilute 5 ml of the solution from (1) into 500 ml and keep as stock solution which contains 0.01 mg/ml of Quinine sulfate.
3. Dilute the stock solution of (2) to obtain several solutions of Quinine sulfate by transfer 250 ml of stock solution to 500 ml volumetric flask and make up to the mark with distilled water. After that, it was repeated dilution continue until 10 concentrations were obtained.

Procedures for prepared sample solution

Andrographolide

1. A solution was prepared by dissolving 0.1 g of andrographolide in a small amount of propylene glycol ( pharmaceutical grade ) and 100 ml of water, respectively.
2. Step (2) and (3) of the above were repeated to obtain 0.01 mg/ml solution of andrographolide.

Andrographolide diacetate and 8-Chloro-andrographolide triacetate

1. A solution was prepared by dissolving 1.0 g of each substance in a small amount of propylene glycol ( phamaceutical grade ) and 100 ml of water, respectively.
2. Step (2) and (3) of Quinine sulfate were repeated to obtain 0.1 mg/ml solution of andrographolide diacetate and 8-Chloro-andrographolide triacetate.

**Method of determination of bitterness.**

Ten volunteers served as testers. First, they had to rinse their mouths with water. Then they were given quinine sulfate and were asked to gargle the solution for 30 seconds. Once alter gargling, they then had to rinse theirs mouths with water again. Find the minimum value when the tester feel bitter and record data. For andrographolide, andro grapholide diacetate and 8-Chloro-andrographolide triacetate, the testing were carried out as same as Quinine sulfate.

Acid Hydrolysis of andrographolide diacetate ( compound 2 )

Andrographolide diacetate ( 500 mg, 0.12 mmole ) was dissolved in a mixture of EtOH ( 5 ml ) and HCl solution ( pH ≈ 5 ) in 100 ml round bottom flask. The reaction mixture was refluxed on silicone oil at 90 $^{0}$ C for 5 hours. After the reaction mixture was worked up, the product was purified by column chromatography and used mixture of chloroform : methanol ( 90 : 10 ) as eluent to give white oil ( 75 mg , 15 % yield )

# CHAPTER IV

# RESULTS AND DISCUSSION

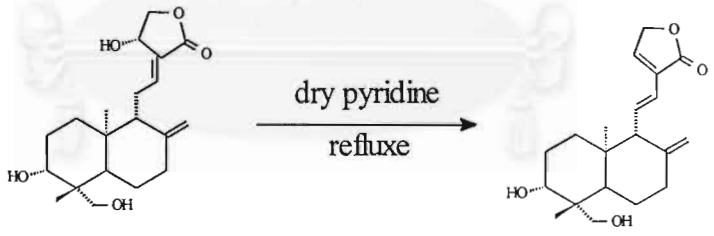
## Synthesis of andrographolide ester derivatives from *A. paniculata* Nees.

### General Discussion

The synthesis of andrographolide ester derivatives was accomplished in two to three steps starting from andrographolide. The andrographolide ester derivatives were characterized by IR, $^{1}$H-NMR , $^{13}$C-NMR , MS , Elemental Analysis and X-ray Analysis.

### Synthesis of 14-deoxy-11,12-didehydroandrographolide (2)

Figure 8 shows the preparation of 14-deoxy-11,12-didehydroandrographolide (2) from andrographolide and dry pyridine by refluxing overnight to obtain (2) in 65 % yield.




1 2

Figure 8

The spectroscopic data clearly confirmed the structure of (2). The IR spectrum of (2) is shown in figure 24 and the absorption peaks are assigned in Table 9 .

Table 9 : The IR absorption bands assignment of compound (2)

| Tentative assignments | Band type | Wavenumber ( $cm^{-1}$ ) |
|---|---|---|
| O-H streching vibration of alcohol | br | 3450-3200 |
| C-H streching vibration of $CH_2$, $CH_3$ | s | 2940 |
| C=O streching vibration of lactone ring | s | 1742 |
| C-O streching vibration | m | 1102 |
| C-H out of plane streching of exocyclic methylene | m | 909 |

The $^1$H-NMR spectrum of compound (2) ( Figure 25 ), clearly shown signal at 7.20 ( brt ) due to an olefinic proton conjugated with a lactone.

The $^{13}$C-NMR spectrum and DEPT experiments ( Figure 26, 27 ) revealed the presence of 20 nonequivalent carbons, of which 2 $CH_3$, 7 $CH_2$, 6 CH and 5 C hybridized carbons.

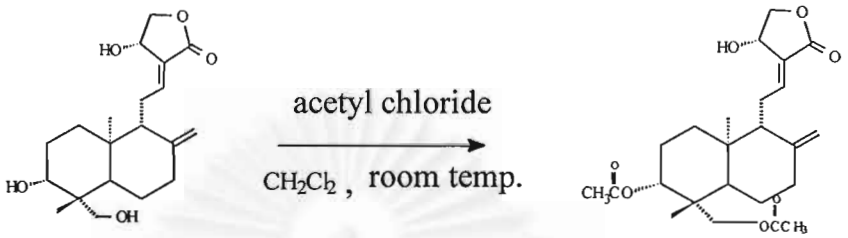
The $^{13}$C-NMR spectrum and DEPT experiments of compound (2) with that of compound (1) indicated that compound (2) differed from compound (1) only in having more one double bond and rearranged position of double bond due to dehydration mechanism between C-11 and C-14 positions.

MS ( figure 23 ) data gave the molecular ion peak at m/z 332 ( $C_{20}H_{28}O_4^+$, $M^+$ ) indicated the elimination of water of compound (1). The fragmentation ion peaks at m/z 314 and 296 were assigned as $C_{20}H_{26}O_3^+$ and $C_{20}H_{24}O_2^+$, due to the loss of water, respectively.

From this reaction, it indicated that andrographolide lost its 14-hydroxy group easily in pyridine solution.

**Synthesis of andrographolide diacetate (3)**

Figure 9 illustrated the preparation of andrographolide diacetate from the reaction of andrographolide and acetyl chloride. It was carried out in $CH_2Cl_2$ at room temperature for 12 hours to obtain (3) in 78 % yield.




1 3

Figure 9

The spectroscopic data clearly confirmed the structure of (3). The IR spectrum of (3) is shown in figure 29 and the absorption peaks are tabulated in Table 10 .

Table 10 : The IR absorption bands assignment of compound (3)

| Tentative assignments | Band type | Wavenumber ( $cm^{-1}$) |
|---|---|---|
| O-H streching vibration of alcohol | br | 3450 - 3530 |
| C-H streching vibration of $CH_2$, $CH_3$ | s | 2965 |
| C=O streching vibration of α,β-unsatura- | s | 1732 |
| ted-γ-lactone | m | 1245 |
| C-O streching vibration | m | 1050 |
| C-H out of plane streching of exocyclic | s | 896 |
| methylene | | |

The $^{13}$C-NMR spectrum and DEPT experiments ( Figure 31, 32 ) revealed the presence of 24 nonequivalent carbons, of which 4 $CH_3$, 7 $CH_2$, 5 CH and 8 C hybridized carbons.

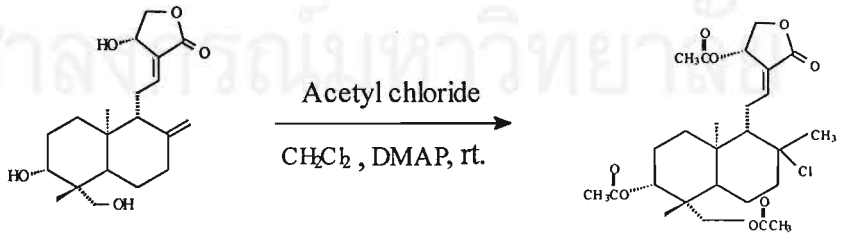
Comparison of $^{13}$C-NMR spectrum and DEPT experiments of compound (3) with that of compound (1) indicated that compound (3) differed from compound (1) only in having three carbonyl groups (C=O) and four methyl carbons ($CH_3$) which shown that it had two ester substituent groups but it was not dehydrated.

MS ( Figure 28 ) data gave the fragmentation ion peaks at m/z 416 ( $C_{24}H_{32}O_6^+$, $M^+$ ) indicated the elimination of water from compound (3). The fragmentation ion peaks at m/z 356, 314 ; due to the loss of acetic acid group ( $CH_3COOH$ ) and 296 were assigned as $C_{22}H_{28}O_4^+$, $C_{20}H_{26}O_3^+$ and $C_{20}H_{24}O_2^+$, respectively.

Elemental Analysis data confirmed the empirical formula of (3). It was very interesting to find that under acidic condition and room temperature the compound (3) was obtained without loosing the 14-hydroxy group.

**Synthesis of 8-Chloro-Andrographolide triacetate (4)**

Figure 10 shows the preparation of 8-Chloro-andrographolide triacetate from the reaction of andrographolide and acetyl chloride. It was catalyzed by 4-dimethylamino pyridine (DMAP) and the reaction was carried out in $CH_2Cl_2$ at room temperature for 15 hours to obtain (4) in 87 % yields.




1 4

Figure 10

The spectroscopic data clearly confirmed the structure of (4). The IR spectrum of (4) is shown in figure 34 and the absorption peaks are tabulated in Table 11.

Table 11 : The IR absorption bands assignment of compound (4)

| Tentative assignments | Band type | Wavenumber ( $cm^{-1}$) |
|---|---|---|
| C-H streching vibration of $CH_2$, $CH_3$ | s | 2945 |
| C=O streching vibration | s | 1736 |
| C-O streching vibration | m | 1234 |

The $^{13}$C-NMR spectrum and DEPT experiments ( Figure 36, 37 ) revealed the presence of 26 nonequivalent carbons, of which 6 $CH_3$, 7 $CH_2$, 6 CH and 7 C hybridized carbons.

Comparison of $^{13}$C-NMR spectrum and DEPT experiments of compound (4) with these of compound (1) indicated that compound (4) differed from compound (1) only in having four carbonyl groups (C=O) and six methyl carbons ($CH_3$) which shown that it having three ester substituent groups but it was not dehydration in the molecule. Moreover, the exocyclic methylene carbon was disappeared because chlorine atom was added at C-8 position.

MS ( Figure 33 ) data gave the fragmentation ion peaks at m/z 416 ($C_{24}H_{32}O_6^+$, $M^+$) indicated the elimination of acetic acid group ( $CH_3COOH$ ) and hydrochloric acid ( HCl ). The fragmentation ion peaks at m/z 356, 314, due to the loss of acetic group ($CH_3COOH$), and 296 were assigned as $C_{22}H_{28}O_4^+$, $C_{20}H_{26}O_3^+$ and $C_{20}H_{24}O_2^+$, respectively.

Elemental Analysis data confirmed the empirical formula of (4).

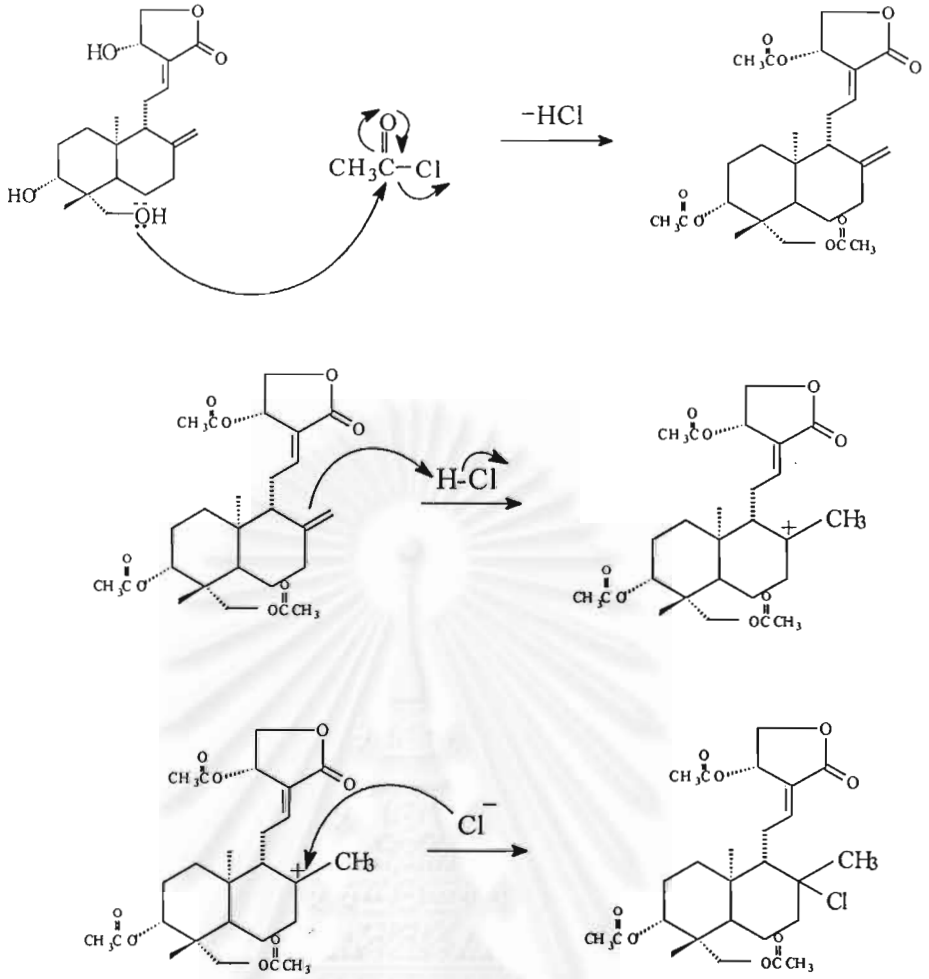
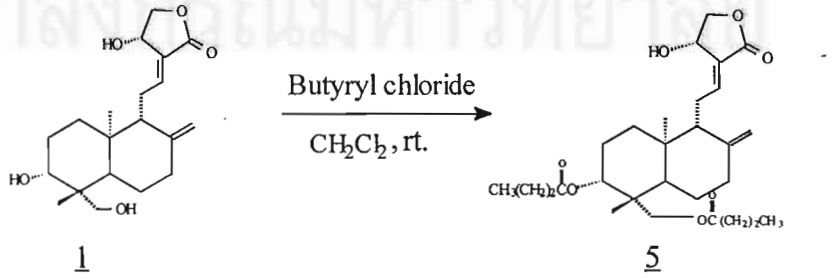


Figure 11 The esterification reaction mechanism of compound (4)

**Synthesis of andrographolide dibutyrate (5)**

Figure 12 shows the preparation of andrographolide dibutyrate (5) which the reaction was prepared between andrographolide and butyryl chloride. It was carried out in $CH_2Cl_2$ at room temperature for 12 hours to obtain (5) in 75 % yield.



1 5

Figure 12

The spectroscopic data clearly confirmed the structure of (5). The IR spectrum of (5) is shown in figure 39 and the absorption peaks are tabulated in Table 12.

Table 12 : The IR absorption bands assignment of compound (5)

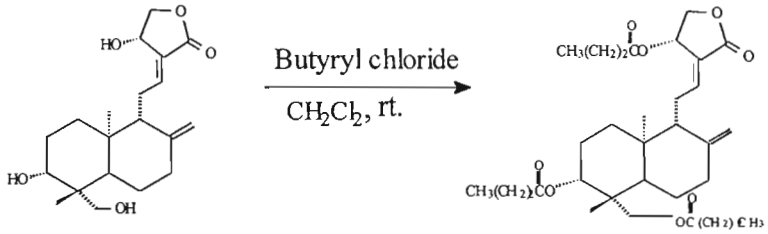
| Tentative assignments | Band type | Wavenumber ( $cm^{-1}$) |
|---|---|---|
| O-H streching vibration of alcohol | br | 3380 – 3520 |
| C-H streching vibration of $CH_2$,$CH_3$ | s | 2965 |
| C=O streching vibration | s | 1742 |
| C-O streching vibration | m | 1188 |
| C-H out of plane streching of exocyclic methylene | s | 896 |

The $^{13}$C-NMR spectrum and DEPT experiments ( Figure 41, 42 ) revealed the presence of 28 nonequivalent carbons, of which 4 $CH_3$, 12 $CH_2$, 5 CH and 7 C hybridized carbons. We found that having three carbonyl groups (C=O) and four methyl carbons ($CH_3$) which indicated that it had two ester substituent groups but it was not dehydrated.

MS ( Figure 38 ) data gave the fragmentation ion peaks at m/z 472 ( $C_{28}H_{40}O_6^+$, $M^+$ ) indicated the elimination of water ( $H_2O$ ). The fragmentation ion peaks at m/z 384 and 296 ; due to the loss of butyric acid group ( $CH_3(CH_2)_2COOH$ ), were assigned as $C_{24}H_{32}O_4^+$ and $C_{20}H_{24}O_2^+$, respectively.

**Synthesis of andrographolide tributyrate (6)**

Figure 13 illustrated the preparation of andrographolide tributyrate which the reaction was prepared between andrographolide and butyryl chloride. It was carried out in $CH_2Cl_2$ at room temperature for 12 hours to obtain (6) in 84 % yield.




1 6

Figure 13

The spectroscopic data clearly confirmed the structure of (6). The IR spectrum of (6) is shown in figure 44 and the absorption peaks are tabulated in Table 13.

Table 13 : The IR absorption bands assignment of compound (6)

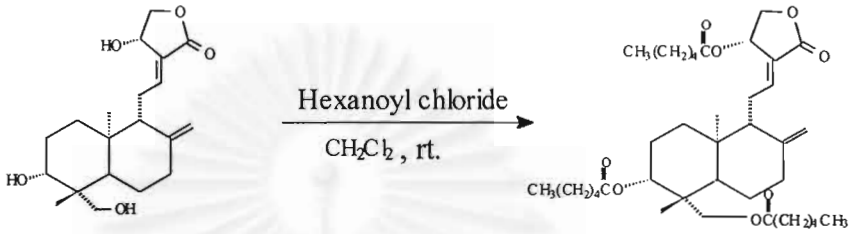
| Tentative assignments | Band type | Wavenumber ( $cm^{-1}$) |
|---|---|---|
| C-H streching vibration of $CH_2$,$CH_3$ | s | 2960 |
| C=O streching vibration | s | 1731 |
| C-O streching vibration | m | 1183 |
| C-H out of plane streching of exocyclic methylene | s | 897 |

The $^{13}$C-NMR spectrum and DEPT experiments ( Figure 46, 47 ) revealed the presence of 32 nonequivalent carbons, of which 5 $CH_3$, 14 $CH_2$, 5 CH and 8 C hybridized carbons. We found that having four carbonyl groups (C=O) and five methyl carbons groups ($CH_3$) which indicated that it had three ester substituent groups but it was not dehydrated.

MS ( Figure 43 ) data gave the fragmentation ion peaks at m/z 472 ( $C_{28}H_{40}O_6^+$, $M^+$ ) , 384 ( $C_{24}H_{32}O_4^+$ ) and 296 ( $C_{20}H_{24}O_2^+$ ), respectively, indicated the elimination of butyric acid ( $CH_3(CH_2)_2COOH$ ).

**Synthesis of andrographolide trihexanoate (7)**

Figure 14 illustrated the preparation of andrographolide trihexanoate which the reaction was prepared between andrographolide and hexanoyl chloride. It was carried out in $CH_2Cl_2$ at room temperature for 12 hours to give (7) in 87 % yield.




1 7

Figure 14

The spectroscopic data clearly confirmed the structure of (7). The IR spectrum of (7) is shown in figure 49 and the absorption peaks are tabulated in Table 14.

Table 14 : The IR absorption bands assignment of compound (7)

| Tentative assignments | Band type | Wavenumber ( $cm^{-1}$) |
|---|---|---|
| C-H streching vibration of $CH_2$,$CH_3$ | s | 2955 |
| C=O streching vibration | s | 1741 |
| C-O streching vibration | m | 1173 |
| C-H out of plane streching of exocyclic methylene | s | 895 |

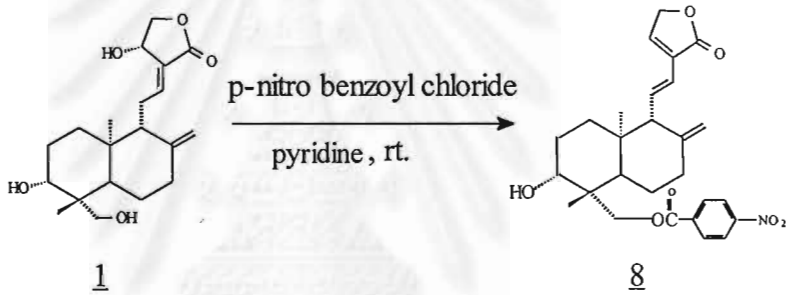
The $^{13}$C-NMR spectrum and DEPT experiments ( Figure 51, 52 ) revealed the presence of 38 nonequivalent carbons, of which 5 $CH_3$, 20 $CH_2$, 5 CH and 8 C hybridized

carbons. We found that having four carbonyl groups (C=O) and five methyl carbons group ($CH_3$) which indicated that it had three ester substituent groups but it was not dehydrated.

MS ( Figure 48 ) data gave the fragmentation ion peaks at m/z 528 ( $C_{32}H_{48}O_6^+$, $M^+$ ) , 412 ( $C_{26}H_{36}O_4^+$ ) and 296 ( $C_{20}H_{24}O_2^+$) , respectively, indicated the elimination of hexanoic acid ( $CH_3(CH_2)_4COOH$ ).

**Synthesis of 14-deoxy-11,12-didehydroandrographolide *p*-nitro monobenzoate (8)**

Figure 15 shows the preparation of 14-deoxy-11,12-didehydroandrographolide *p*-nitro monobenzoate (8) from andrographolide and *p*-nitro benzoyl chloride. Dry pyridine was dissolved the reaction mixture and refluxing overnight to obtain (8) in 74 % yield.




1 8

Figure 15

The spectroscopic data clearly confirmed the structure of (8). The IR spectrum of (8) is shown in figure 54 and the absorption peaks are tabulated in Table 15.

Table 15 : The IR absorption bands assignment of compound (8)

| Tentative assignments | Band type | Wavenumber ( $cm^{-1}$) |
|---|---|---|
| O-H streching vibration of alcohol | br | 3250-3530 |
| C-H streching vibration of $CH_2$,$CH_3$ | s | 2935 |
| C=O streching vibration | m | 1757,1526 |
| C-H out of plane streching of exocyclic methylene | s | 1010 |

The $^{1}$H-NMR spectrum of compound 8 ( Figure 55 ), clearly shown doublet of doublet signal at 8.12-8.33 which indicated *p*-substitution aromatic group of *p*-nitro benzoate and signal at 7.20 ( brt ) due to an olefinic proton conjugated with a lactone.

The $^{13}$C-NMR spectrum and DEPT experiments ( Figure 56, 57 ) revealed the presence of 27 nonequivalent carbons, of which 2 $CH_3$, 3 $CH_2$, 13 CH and 9 C hybridized carbons. We found that having two carbonyl groups (C=O) and two methyl groups ($CH_3$) which indicated that it had one ester substituent group. The signals at 123.6 and 130.7 ppm were assigned to p-substituent aromatic group. Moreover, $^{13}$C-NMR shown that compound 8 was occurred dehydration mechanism as similar to that of compound (2).

MS ( Figure 53 ) data gave the molecular ion peaks at m/z 481 ( $C_{27}H_{31}O_6N^+$, $M^+$ ). The fragmentation ion peaks at m/z 463 ( $C_{27}H_{29}O_5N^+$ ) indicated the elimination of water ( $H_2O$ ) and at m/z 296 ( $C_{20}H_{24}O_2^+$ ) due to the loss of p-nitro benzoic acid.

**X-ray Analysis**

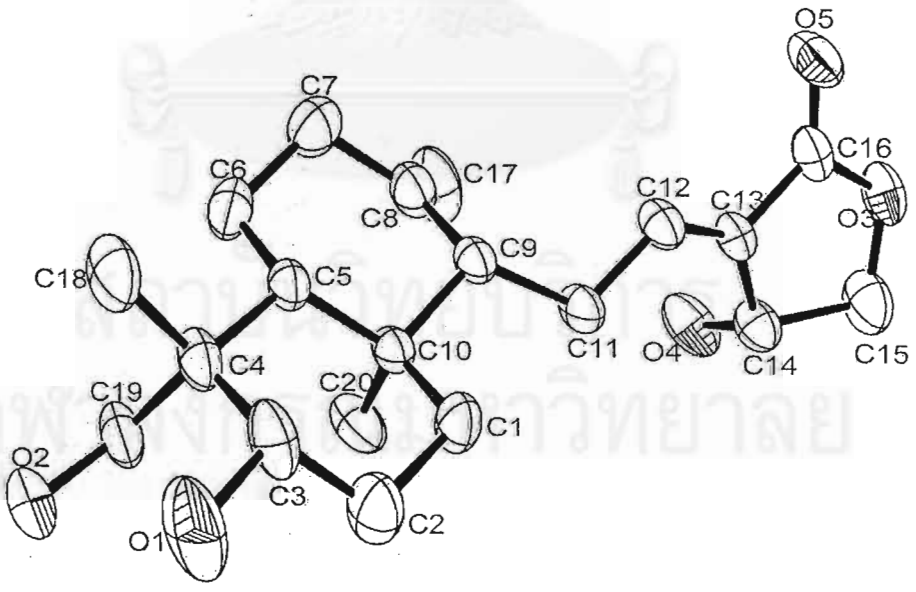



Figure 16 ORTEP diagram of andrographolide

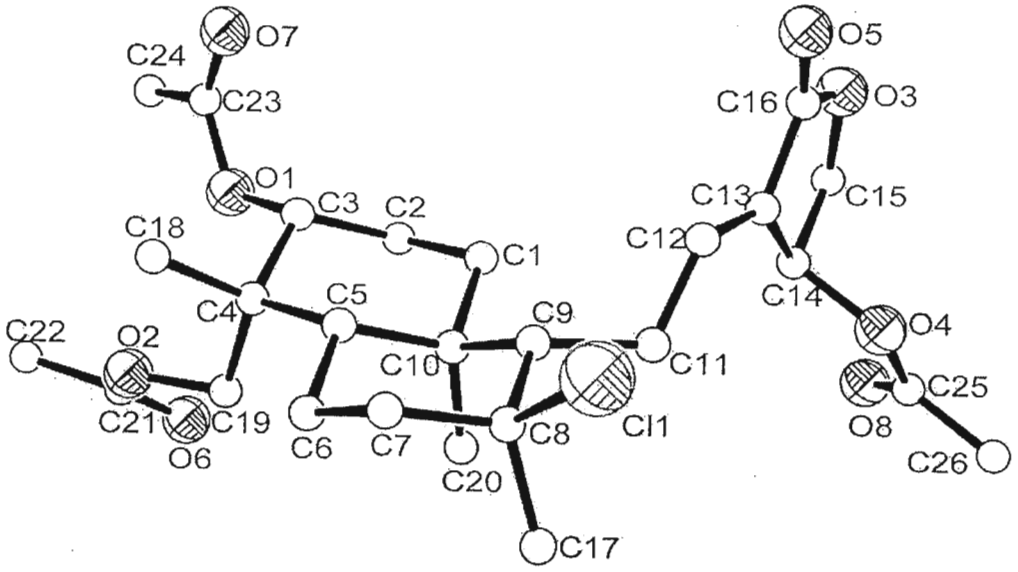



Figure 17 ORTEP diagram of 8-Chloro-andrographolide triacetate

Fig. 16 , 17 Gives a view of the molecule with the atom numbering. Bond distances and angles are given in Table 4 and 8 .

From data structure determinations of andrographolide, we showed that,

- The two central six-membered rings are in the chair conformations.
- The two extreme sides of andrographolide molecule are each involved with two oxygen functions in separate systems of infinite chains of hydrogen-bond molecules. One chain involves O(1) and O(2) with hydrogen bonds and the second chain involves with one intramolecular [ O(4)-H(4)...O(5) ] .( see Table 5. )
- The furan ring O(3) atom is not involved in hydrogen bonding.
- The conformation of the hydroxy group (OH) at C-3 and C-4 are same side.
- Fujita, T., et.al was reported X-ray Crystallographic analysis of andrographolide ( *Chem.Pharm.Bull.*,1984 ) and was determined the absolute configuration at C-14 as *R*.

From data structure determinations of 8-Chloro-andrographolide triacetate

- The conformation of title molecule is similar to that of the andrographolide ,which has bulky ester side chains at C-3,C-4 and C-14.

- The conformation of the two ester groups at C-3 and C-4 are trans with the torsion angle ( steric hindrance) cause both are opposite side and not occur H-bonds.
- The ester group at C-14 is nearly perpendicular to the plane of five-member ring, such these bulky results suggest that these ester groups play an important role to fix the orientation of the terminal five-membered ring and to have a lose molecular packing in the unit cell.
- The five membered ring is virtually planar and perpendicular with ester group at C-14.
- From the crystal structure of compound 4 was determined the absolute configulation at C-8 as *S* and this reaction was occurred as stereospecific, which was previously undecided.

Table 16. Show the results of bitterness test had a decrease in bitterness compared andrographolide derivatives with quinine sulfate and andrographolide.

| Compound | Quinine sulfate | Andrographolide |
|---|---|---|
| Andrographolide diacetate | 20 times | 200 times |
| 8-Chloro-andrographolide andrographolide | 40 times | 400 times |

From the structure of andrographolide, which has hydroxy groups in 3 positions where esterification can occur, it was found that the reaction takes place at the C-19 position first. Then the reaction occurs at the C-3 and C-14 positions, respectively. By directly testing the bitterness of andrographolide diacetate and 8-chloroandrographolide triacetate, it was found that both substances were not bitter at all. Therefore, it was concluded that the positions of the H-bonds of the hydroxy group that cause the bitterness in andrographolide, are the H-bonds at the O-1 and O-2 positions only, which can enter the position of the bitterness taste bud. As for the H-bond at the C-14 position, it has no effect.

Accordingly, since it is possible to change the hydroxy groups at the C-3 and C-4 positions to take place of other groups that are unable to result as H-bonds with the taste bud, such as the ester group, the physical characteristics of andrographolide also change. For example, the length of the bond and the angle between the C-3 and C-4 positions may change and will therefore cause the resulting derivative of andrographolide to not bitter taste.

As a result of having 10 individuals test the bitterness of andrographolide diacetate and 8-Chloro-andrographolide triacetate, by using the method by WHO, it was found that both substances had a decrease in bitterness when compared to the original andrographolide by 200 and 400 times, respectively ( see Table 16 ). The decrease in bitterness was due to the fact that the esterification at the C-3 and C-4 positions were in a trans conformation (opposite side) causing an increase in the distance between O-1 and O-2. Consequently, H-bonding that is to enter the bitterness taste bud will not occur and there will be no resulting bitterness taste.

From $^{13}$C-NMR spectrum of acid hydrolysis of andrographolide diacetate ( Figure 18 ), by using HCl aqueous solution ( pH $\approx$ 5 ) and refluxed at 90$^{0}$C, was showed that andrographolide monoacetate was occurred after 1 hr. of reaction. Moreover, it was found andrographolide in this reaction after 5 hr. else. This fact indicated that andrographolide ester derivative could be hydrolyte by acid hydrolysis reaction to give andrographolide recovery.

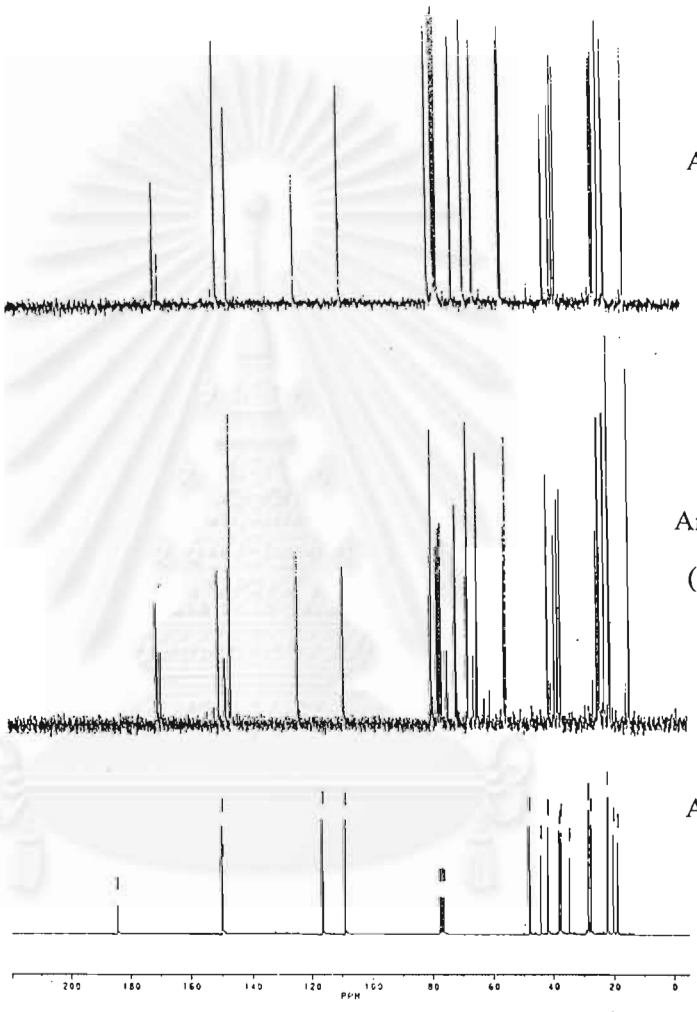



Figure 18 The $^{13}$C-NMR spectrum of acid hydrolysis of andrographolide diacetate ( compound 3 )

# CHAPTER IV

# CONCLUSION

Andrographolide were extracted from dried leaves of *A. paniculata* Nees.( 10 kg ) with MeOH to give colourless needles crystal ( 20 g, 0.2 % yield ). Andrographolide ester derivative, compound (3)-(8), were prepared by esterification reaction from andrographolide and acid chloride e.g. acetyl chloride, butyryl chloride, hexanoyl chloride and *p*-nitro benzoyl chloride to give andrographolide diacetate ( 0.78 g, 78 % yields ), 8-Chloro-andrographolide triacetate ( 0.87 g, 87 % yields ), andrographolide dibutyrate ( 0.75 g, 75 % yields ), andrographolide tributyrate ( 0.84 g, 84 % yields ), andrographolide trihexanoate ( 0.87 g, 87 % yields ) and 14-deoxy-11,12-didehydroandrographolide *p*-nitro monobenzoate ( 0.74 g , 74 % yields ), respectively. Almost, it was found that the derivative maintained the structure formula of andrographolide, not dehydration at C-14 position, except compound (8) was occurred.

Compound (3) and (4) were test bitterness by using the method by WHO, it was found that both substances had a reduced bitterness when compared to the quinine sulfate by 20 and 40 times and original andrographolide by 200 and 400 times, respectively. Futhermore, it was found that andrographolide diacetate could be hydrolyted by acid hydrolysis reaction at condition of HCl aqueous solution ( pH≈ 5 ) to give the original andrographolide ( 75 mg, 15 % yields ). This result data clearly confirmed that andrographolide ester derivatives is considered to be beneficial in treatment of diarrhea in baby pigs in the future.

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# APPENDIX

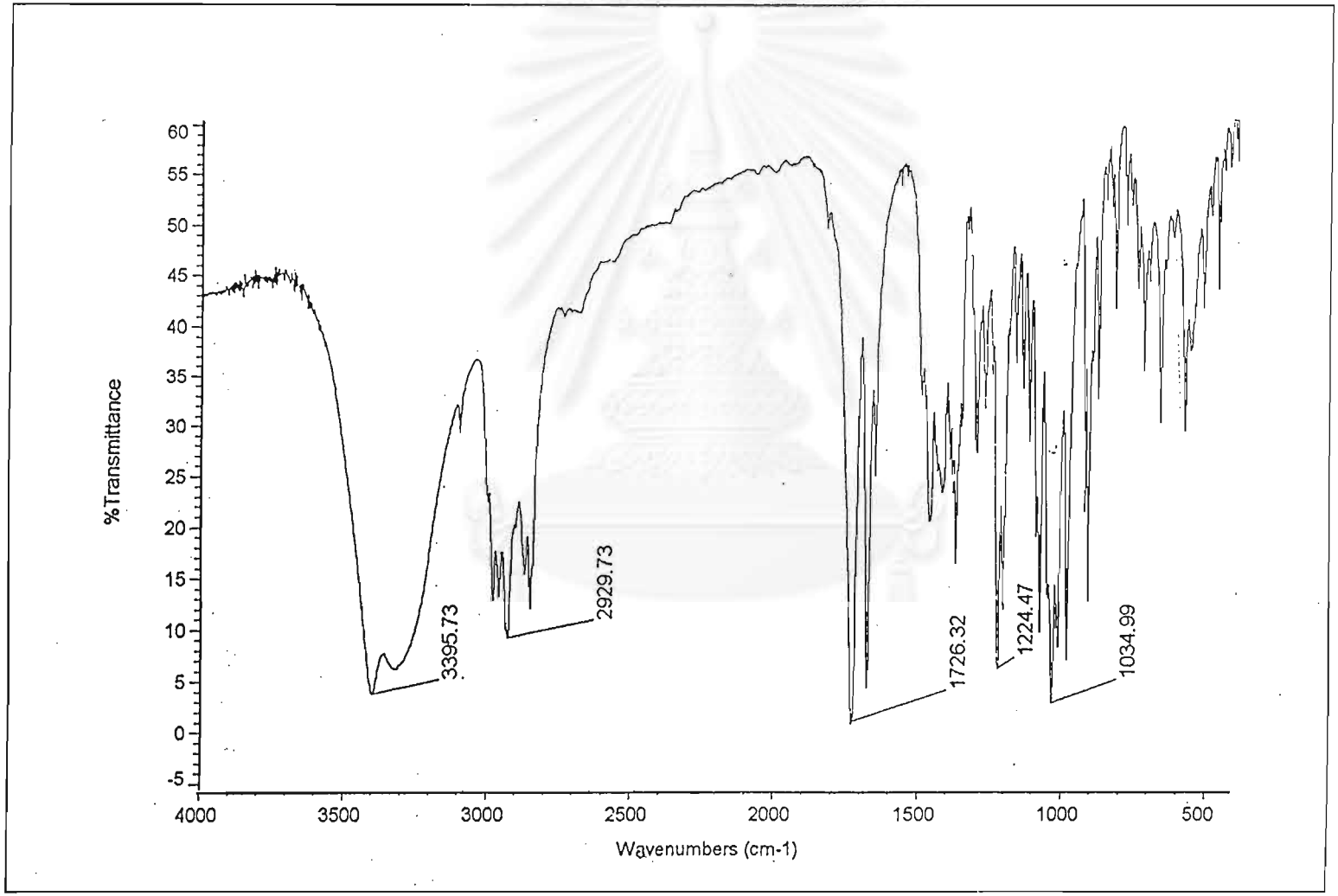


**Figure** 19 The IR spectrum of compound 1

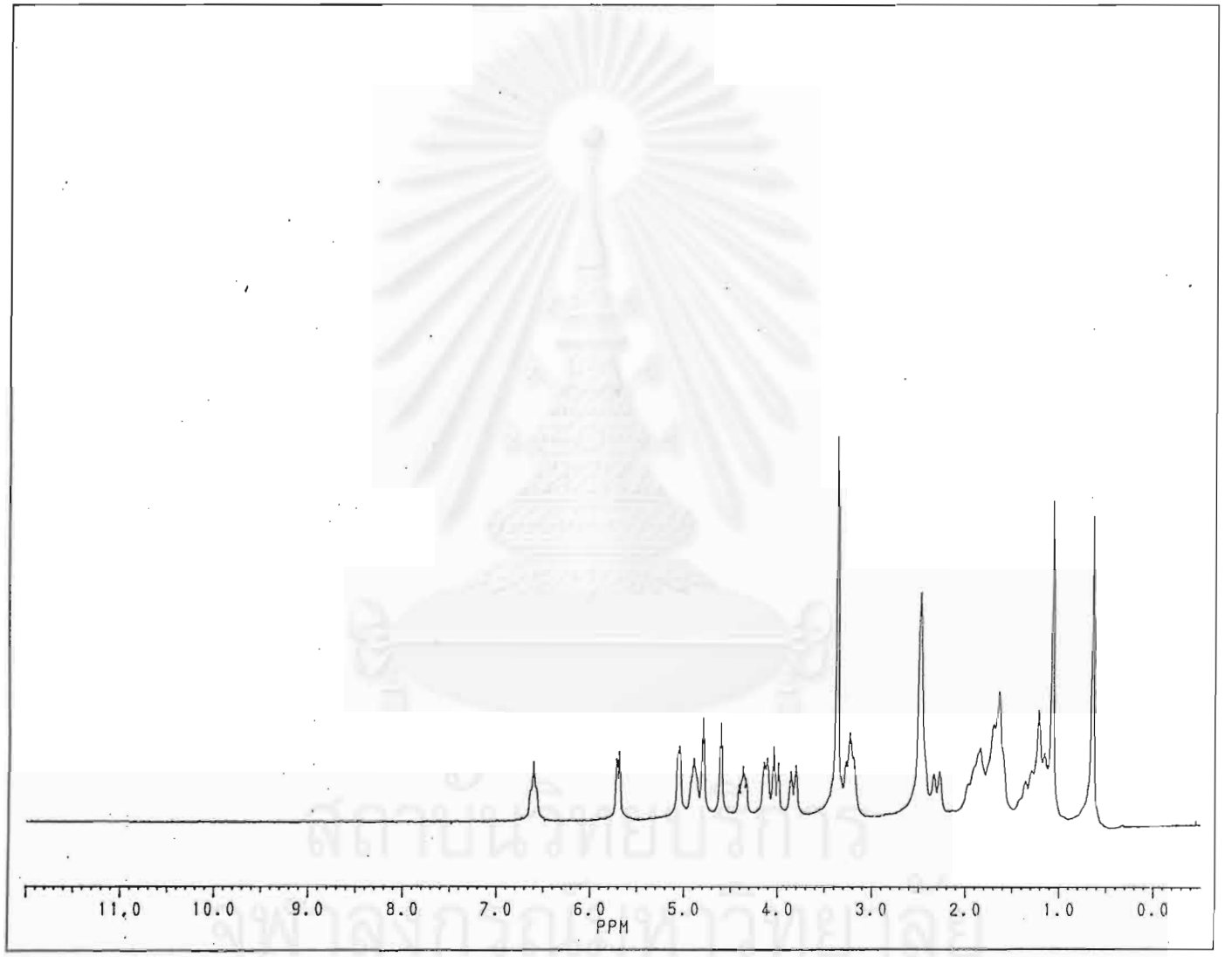


**Figure** 20 The $^{1}$H-NMR spectrum of compound 1

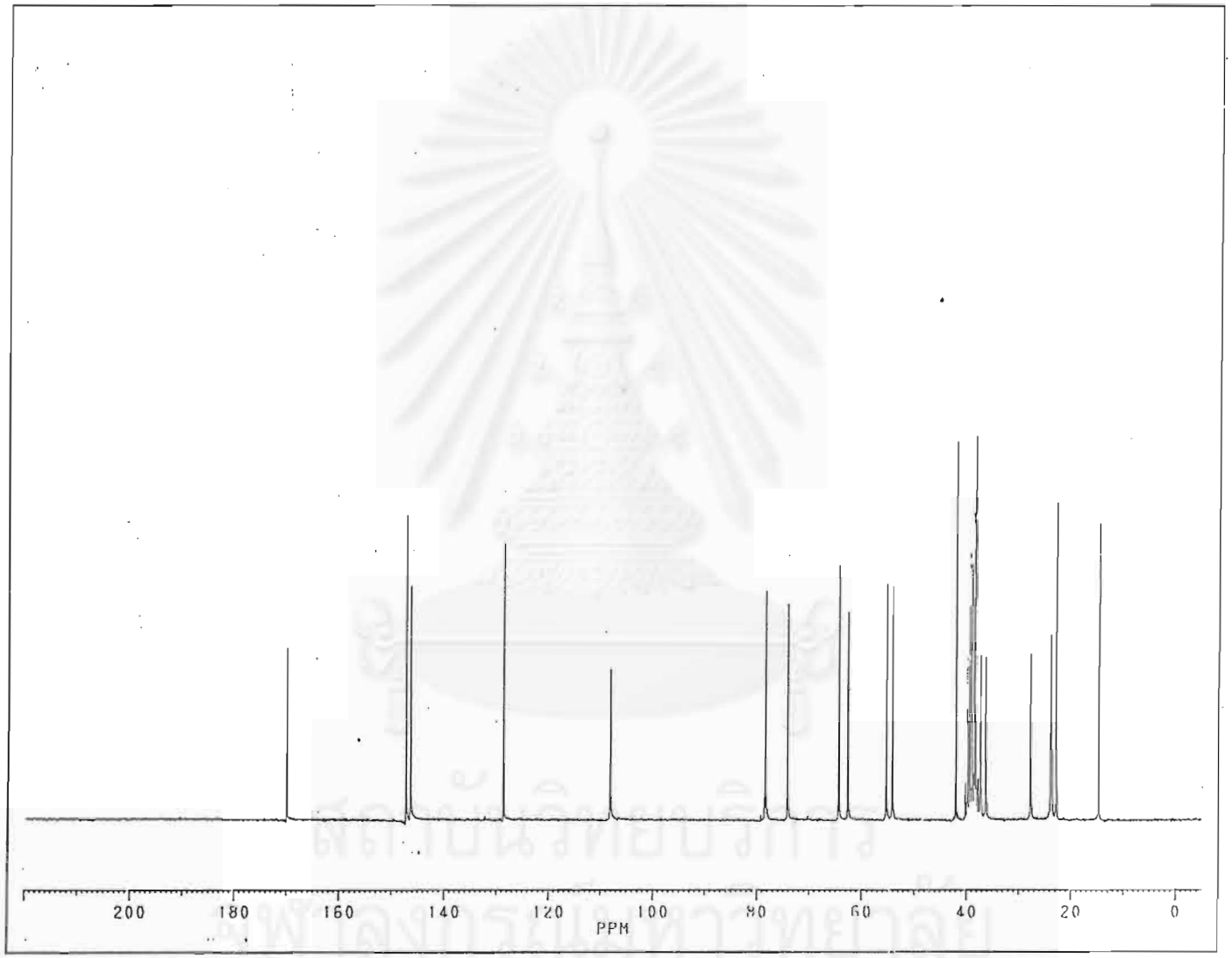


**Figure** 21 The $^{13}$C-NMR spectrum of compound 1

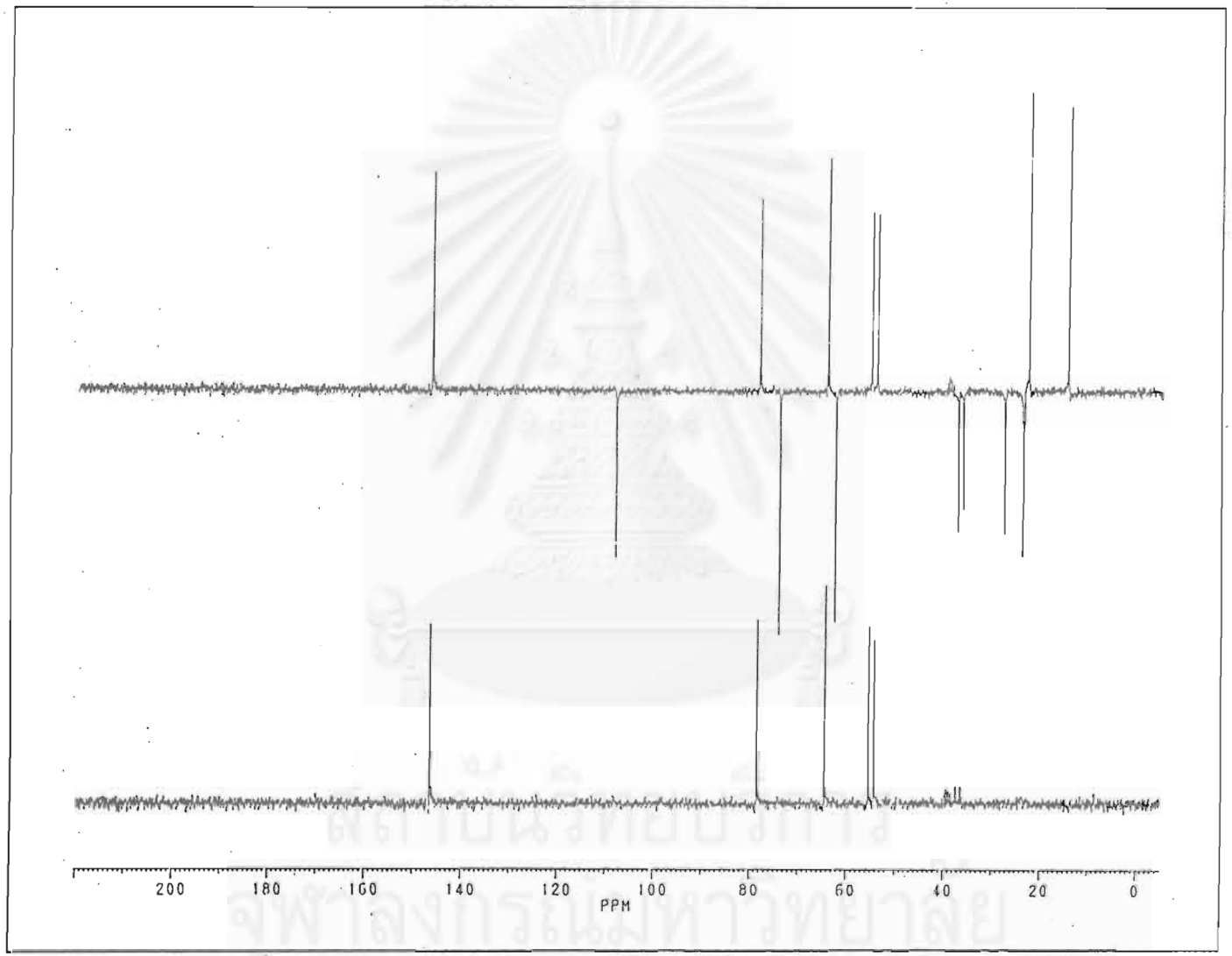


**Figure 22** DEPT-135, 90 $^{13}$C-NMR of compound *1*

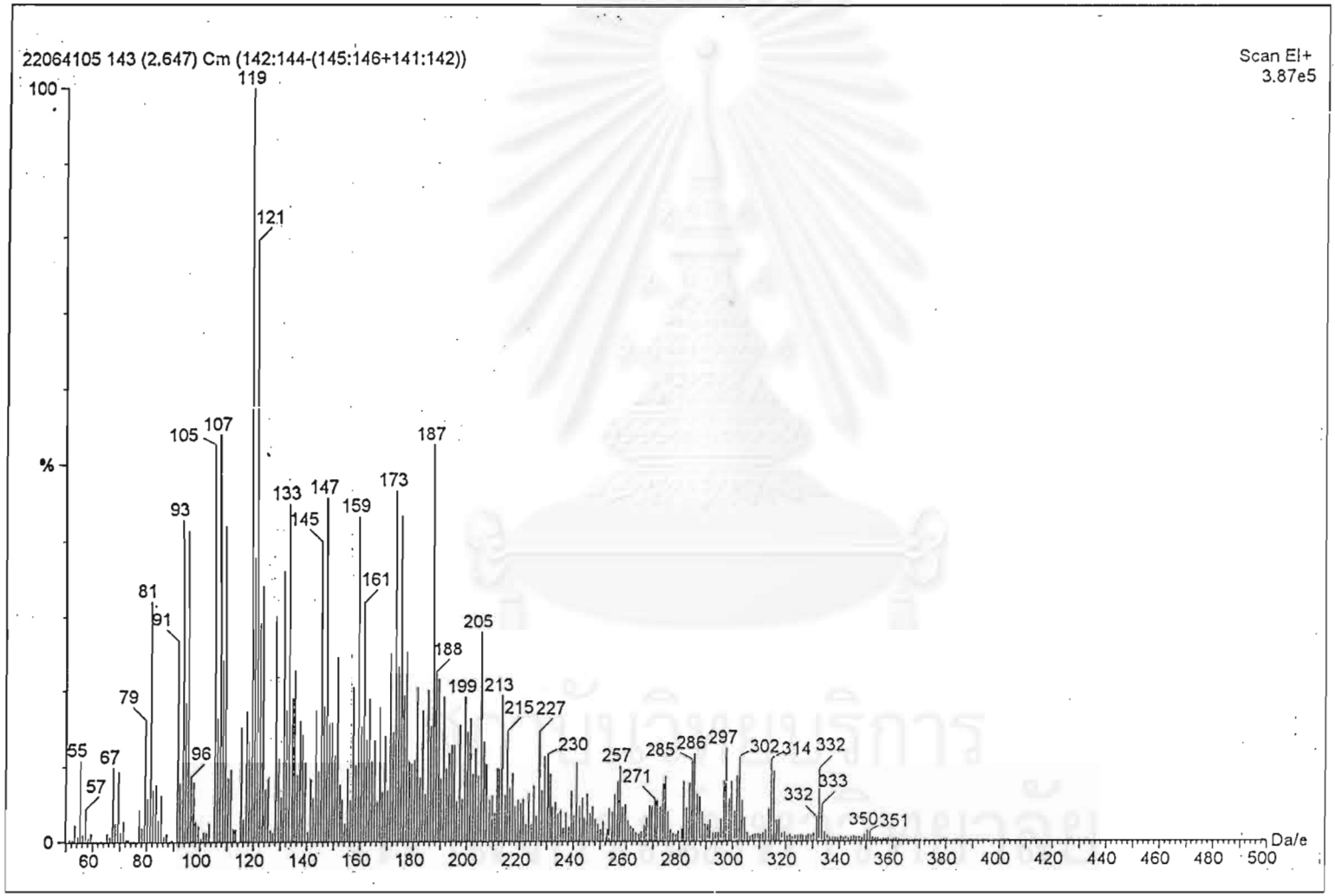


**Figure** 23 The EI MS spectrum of compound 1

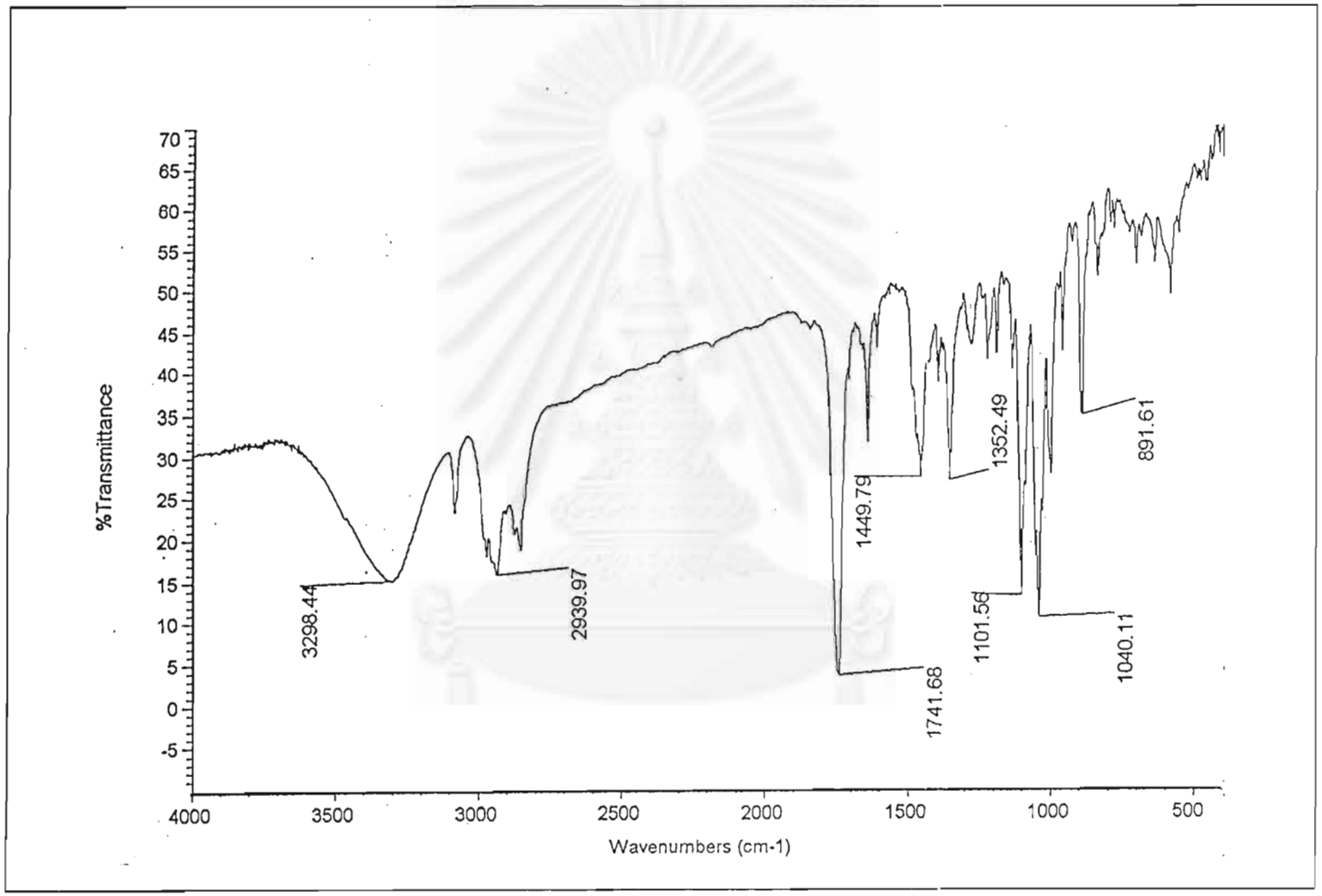


**Figure** 24 The IR spectrum of compound 2

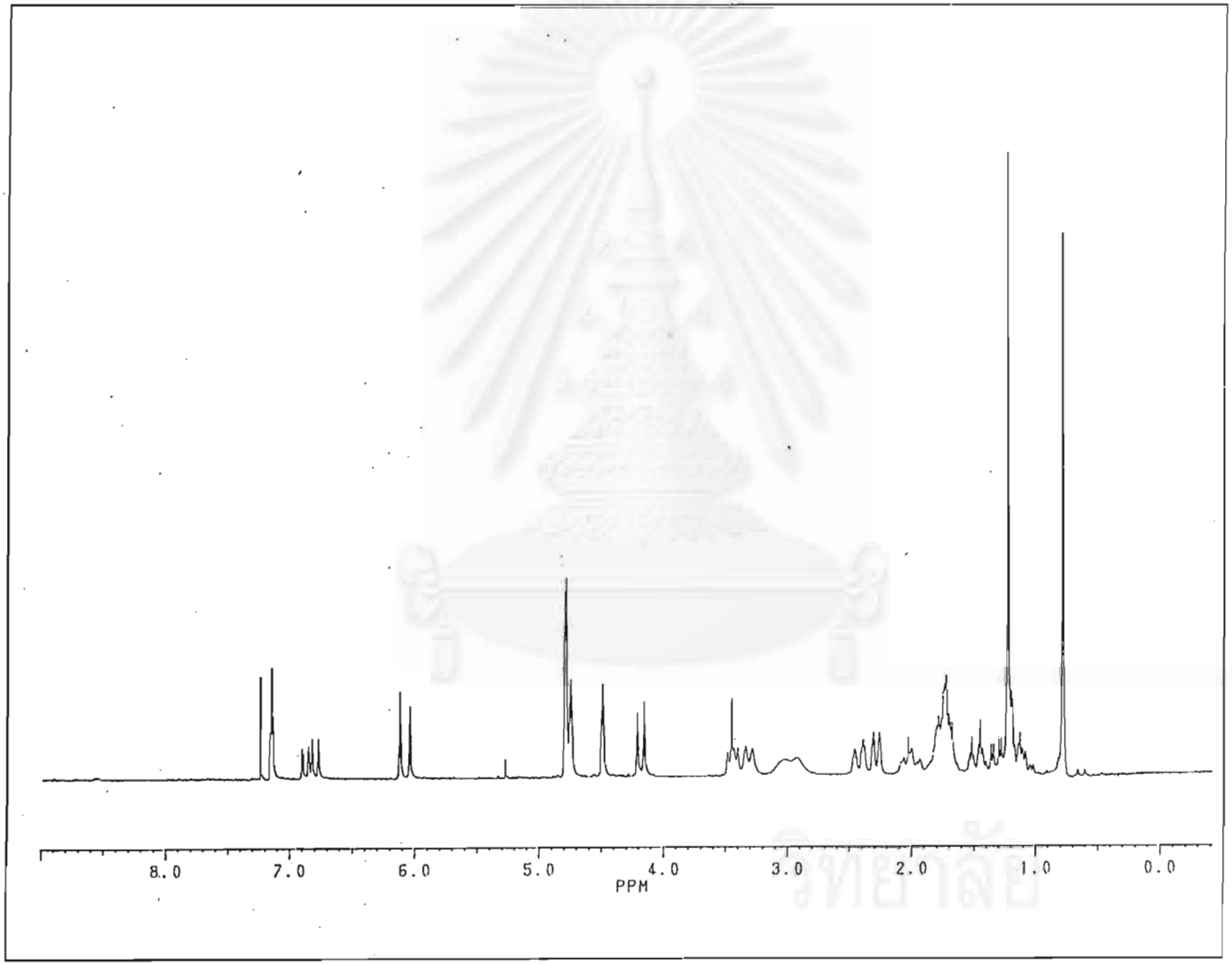


**Figure** 25 The $^{1}$H-NMR spectrum of compound 2

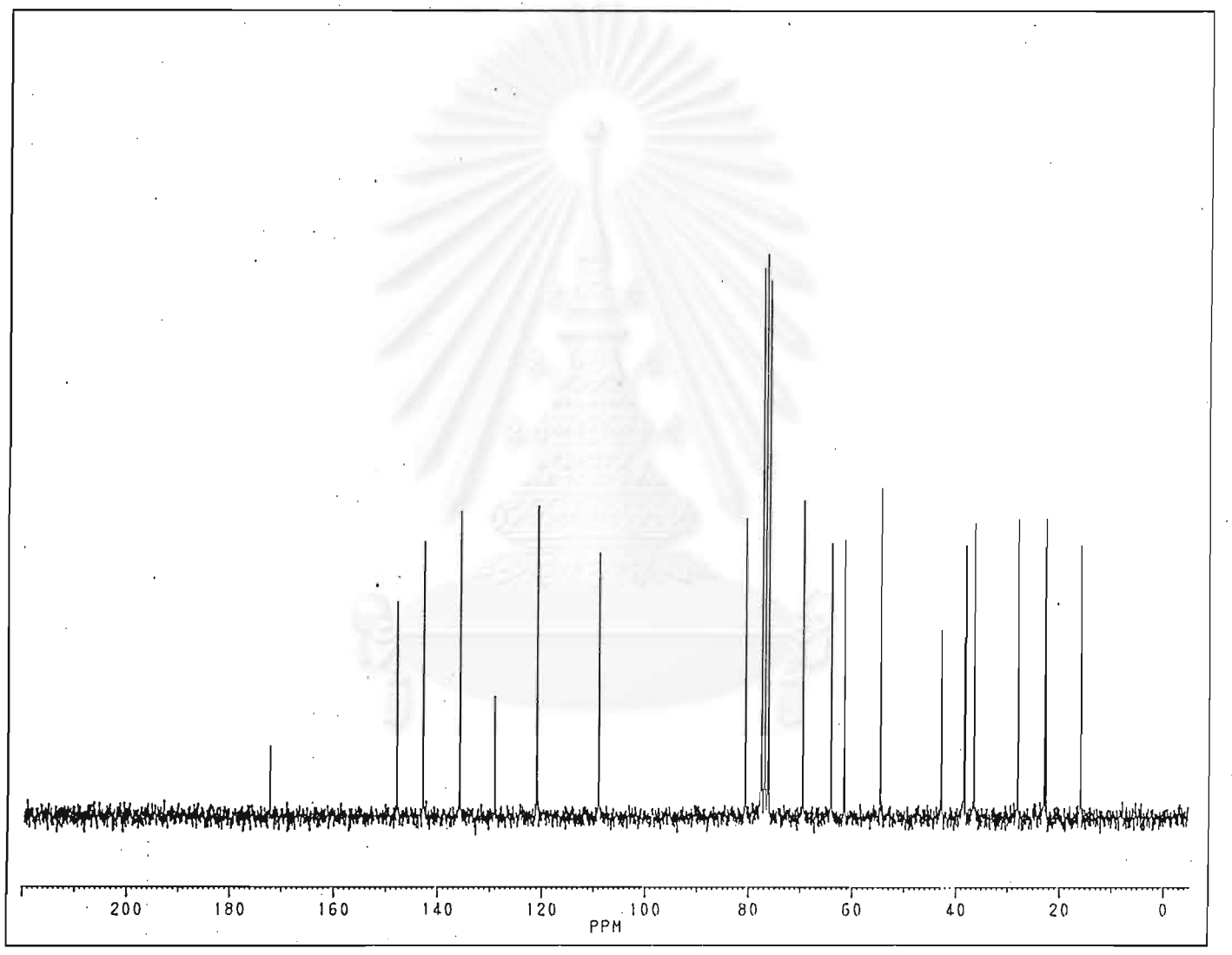


**Figure** 26 The $^{13}$C-NMR spectrum of compound 2

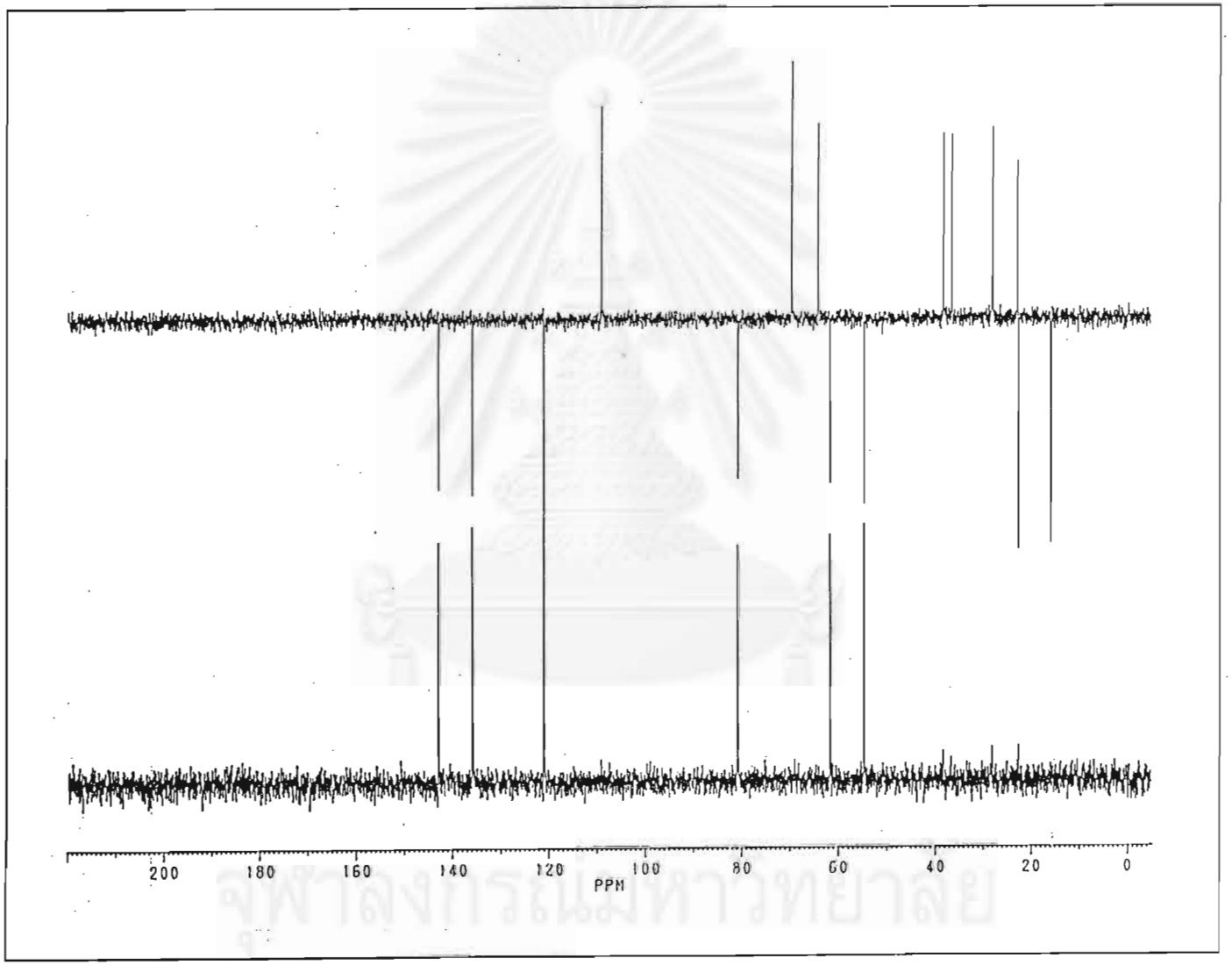


**Figure** 27 DEPT-135, 90 $^{13}$C-NMR of compound 2

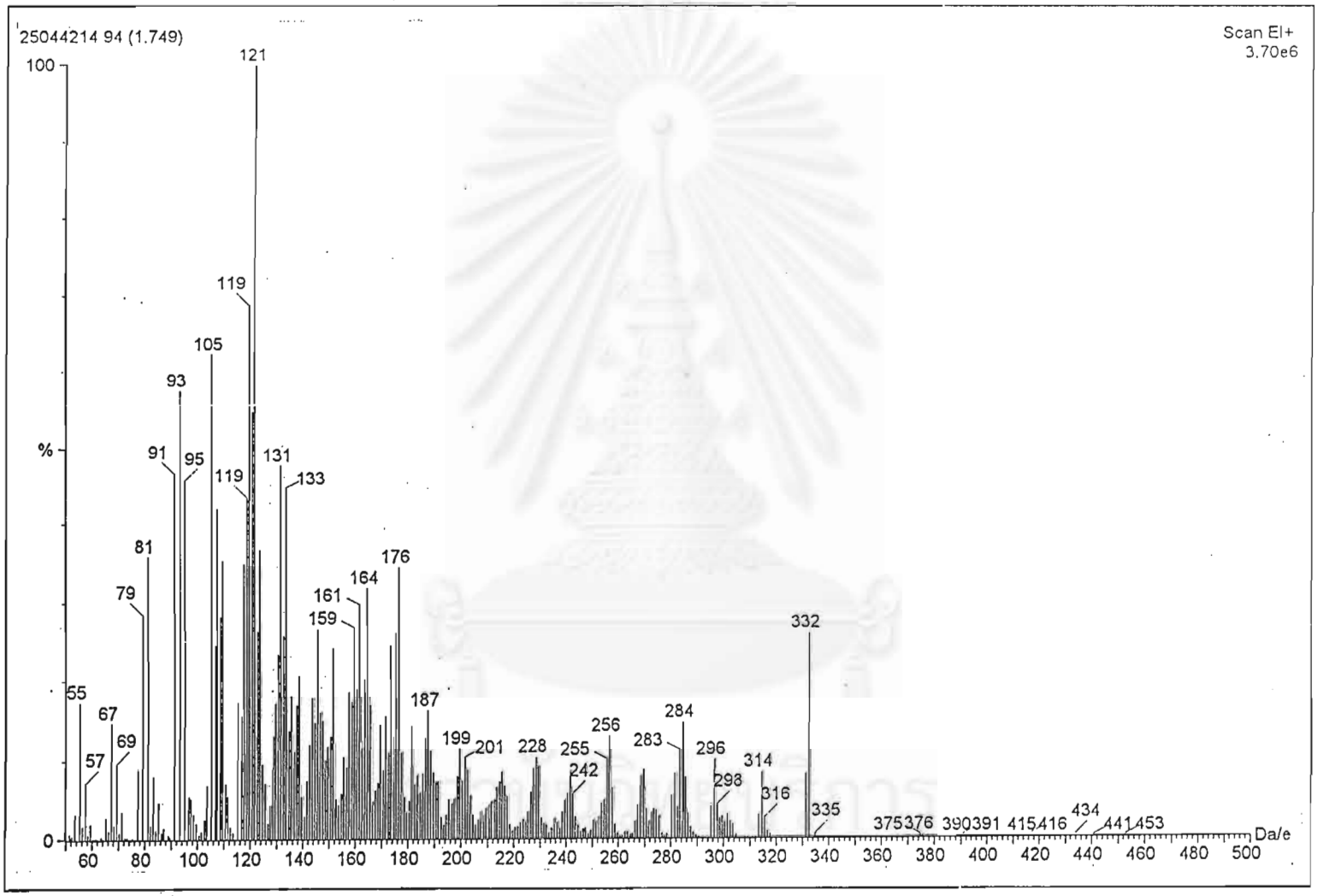


**Figure** 28 The EI MS spectrum of compound 2

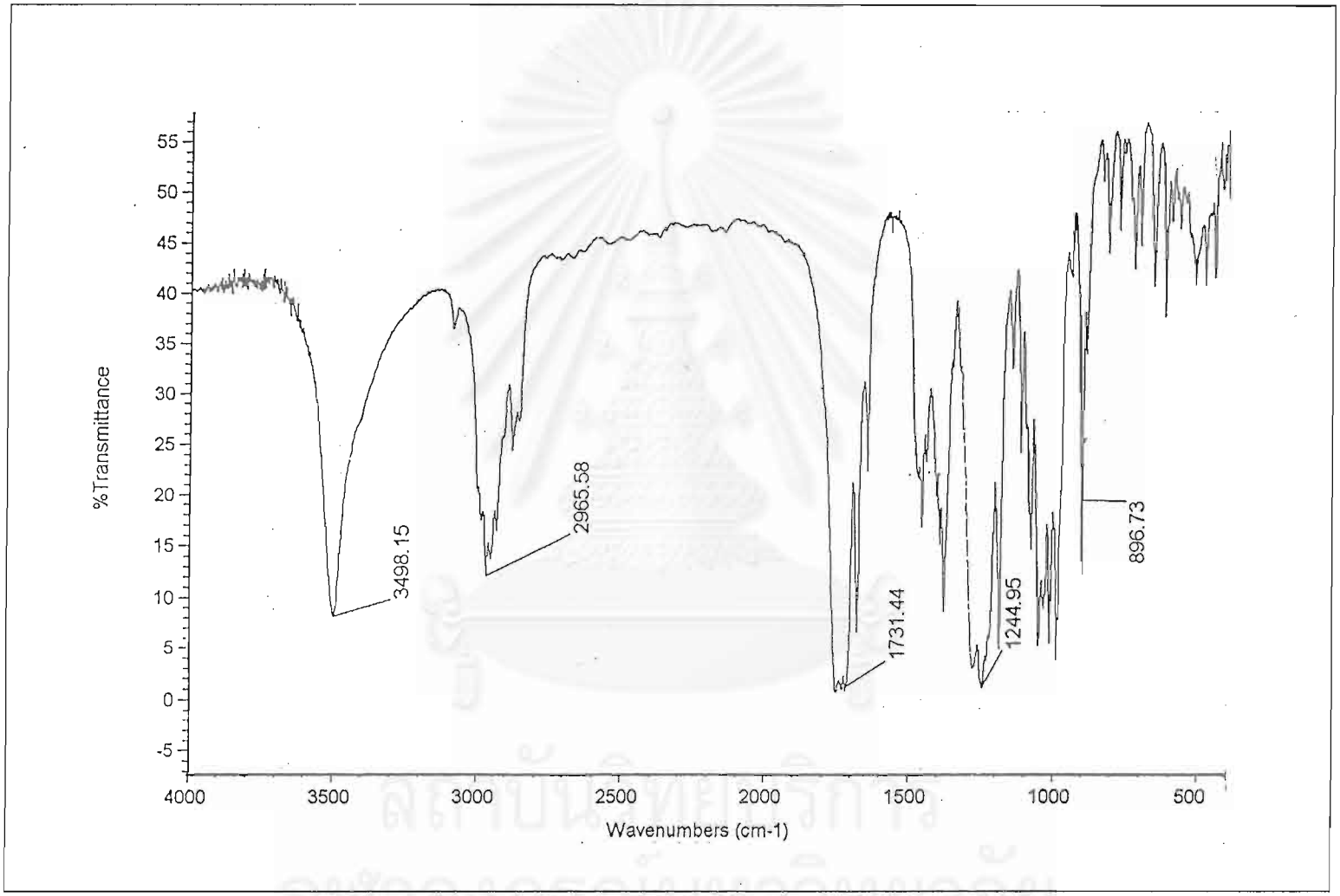


**Figure 29** The IR spectrum of compound 3

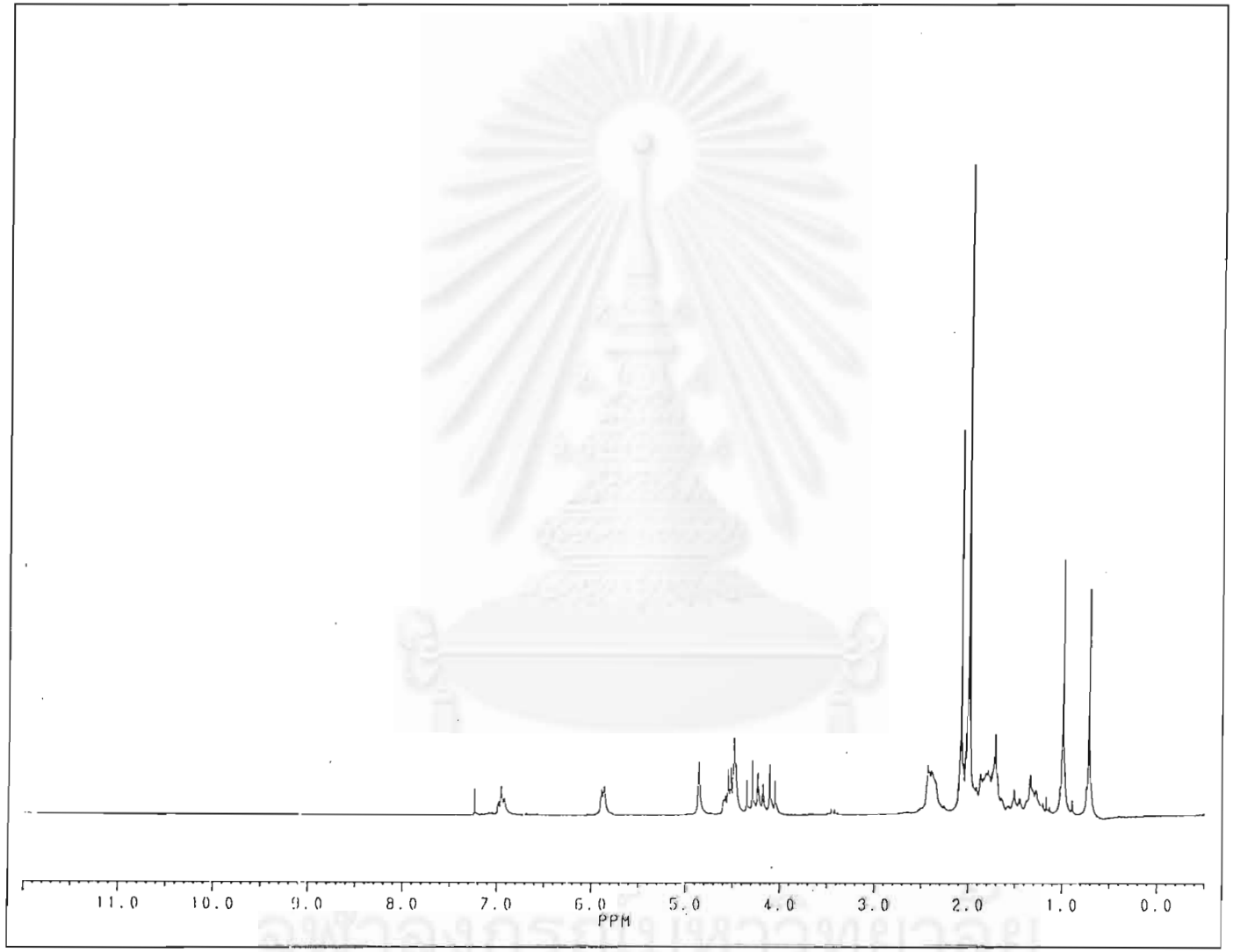


**Figure** 30 The $^{1}$H-NMR spectrum of compound 3

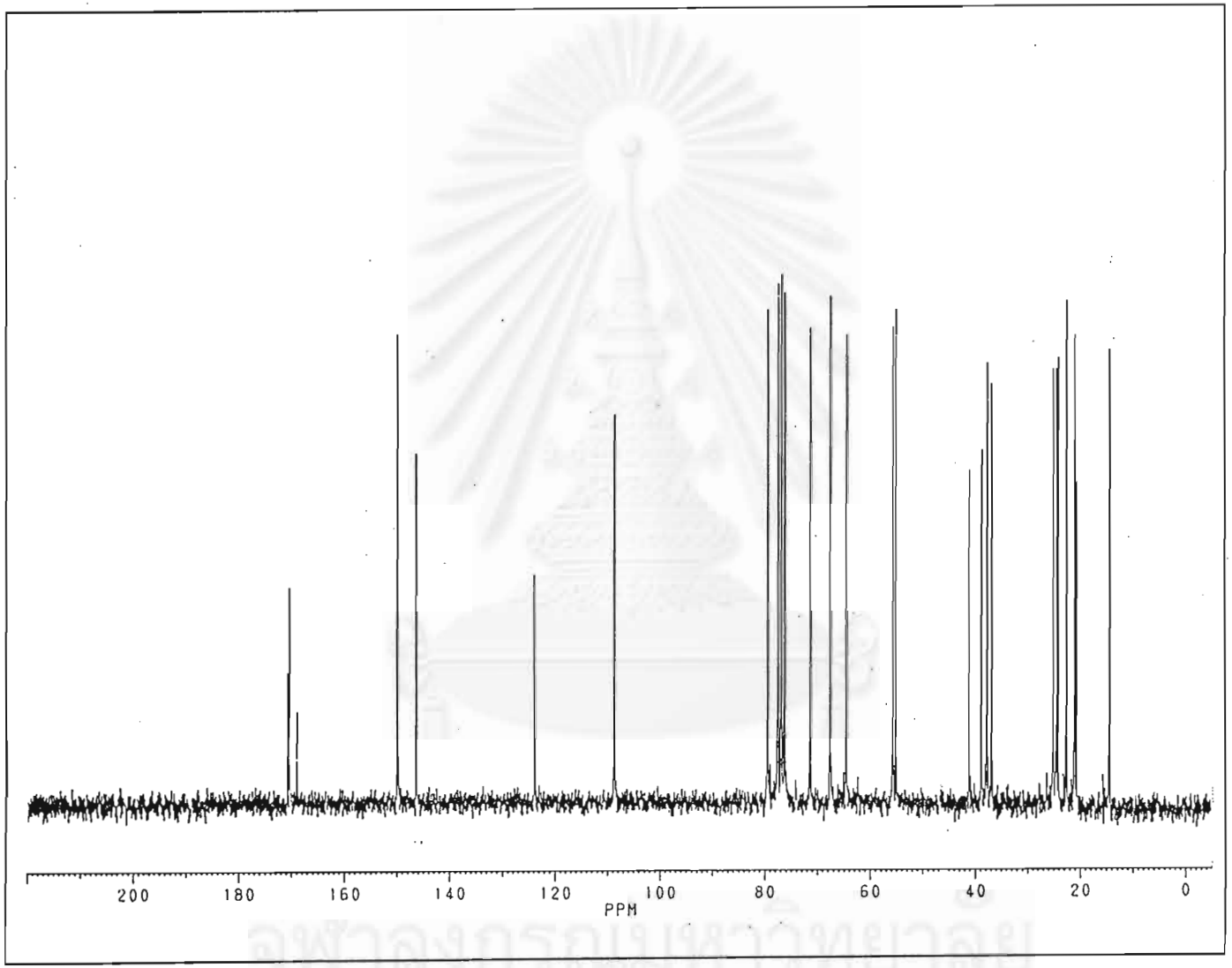


**Figure** 31 The $^{13}$C-NMR spectrum of compound 3

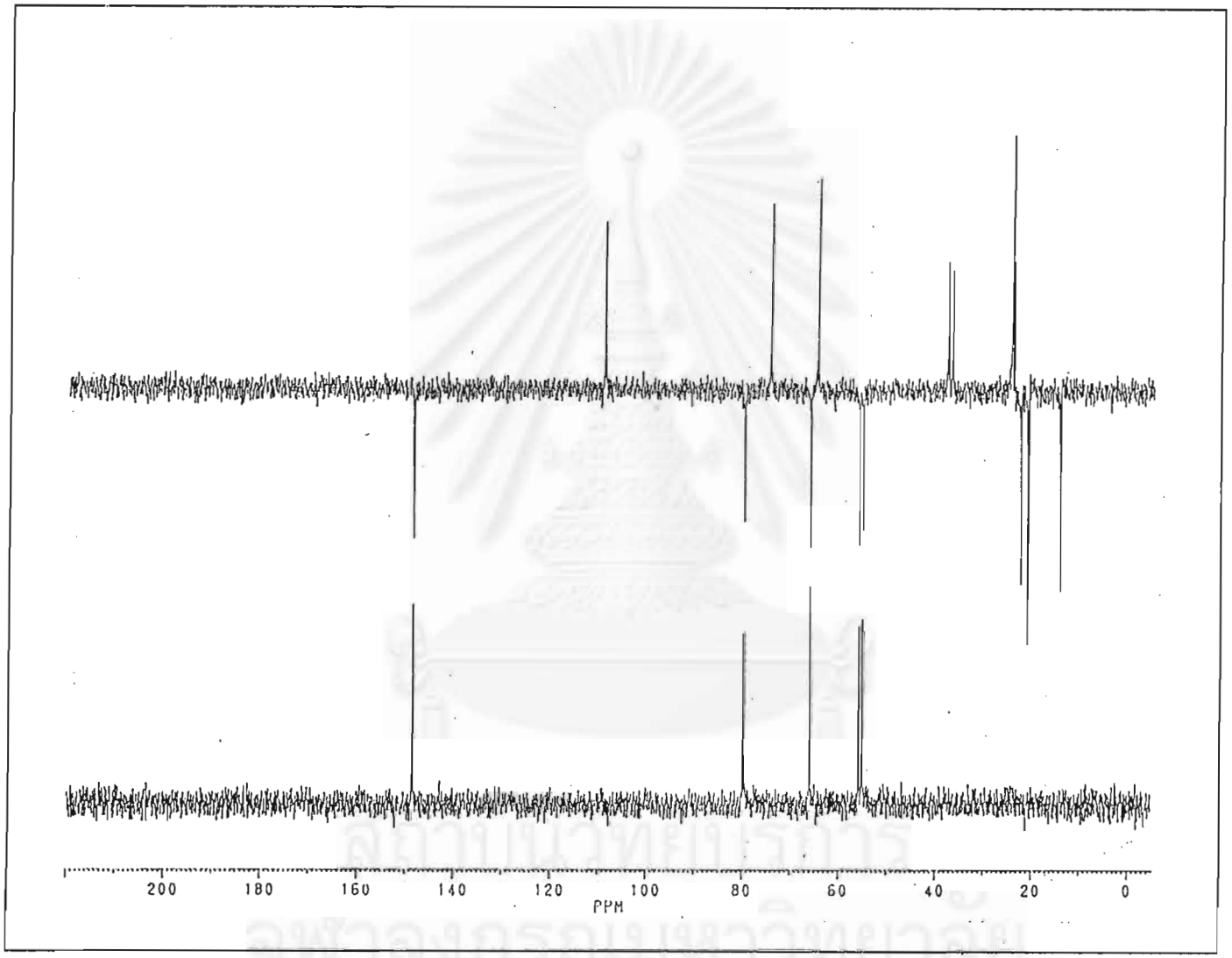


**Figure** 32 DEPT-135, 90 $^{13}$C-NMR of compound 3

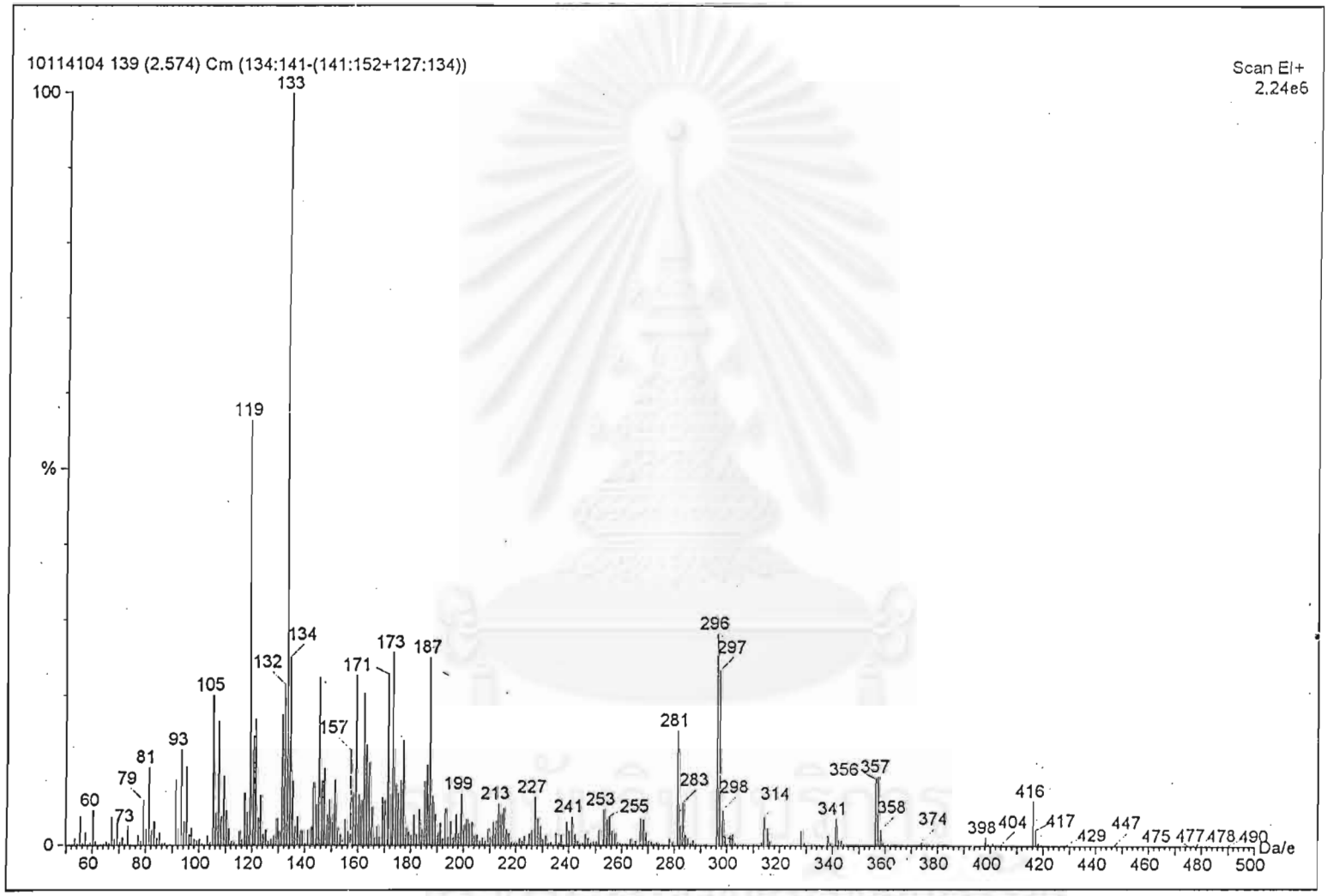


**Figure** 33 The EI MS spectrum of compound 3

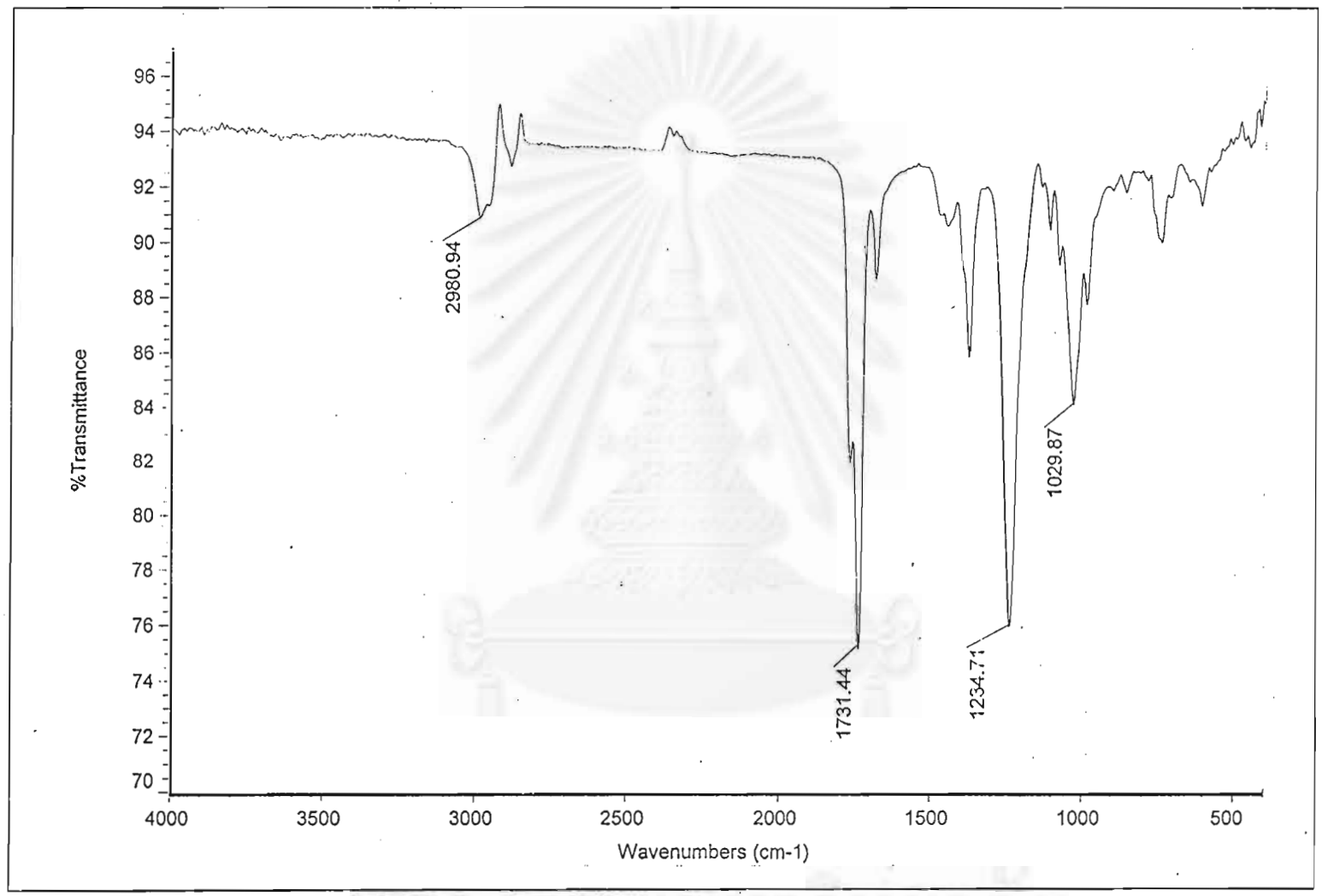


**Figure** 34 The IR spectrum of compound 4

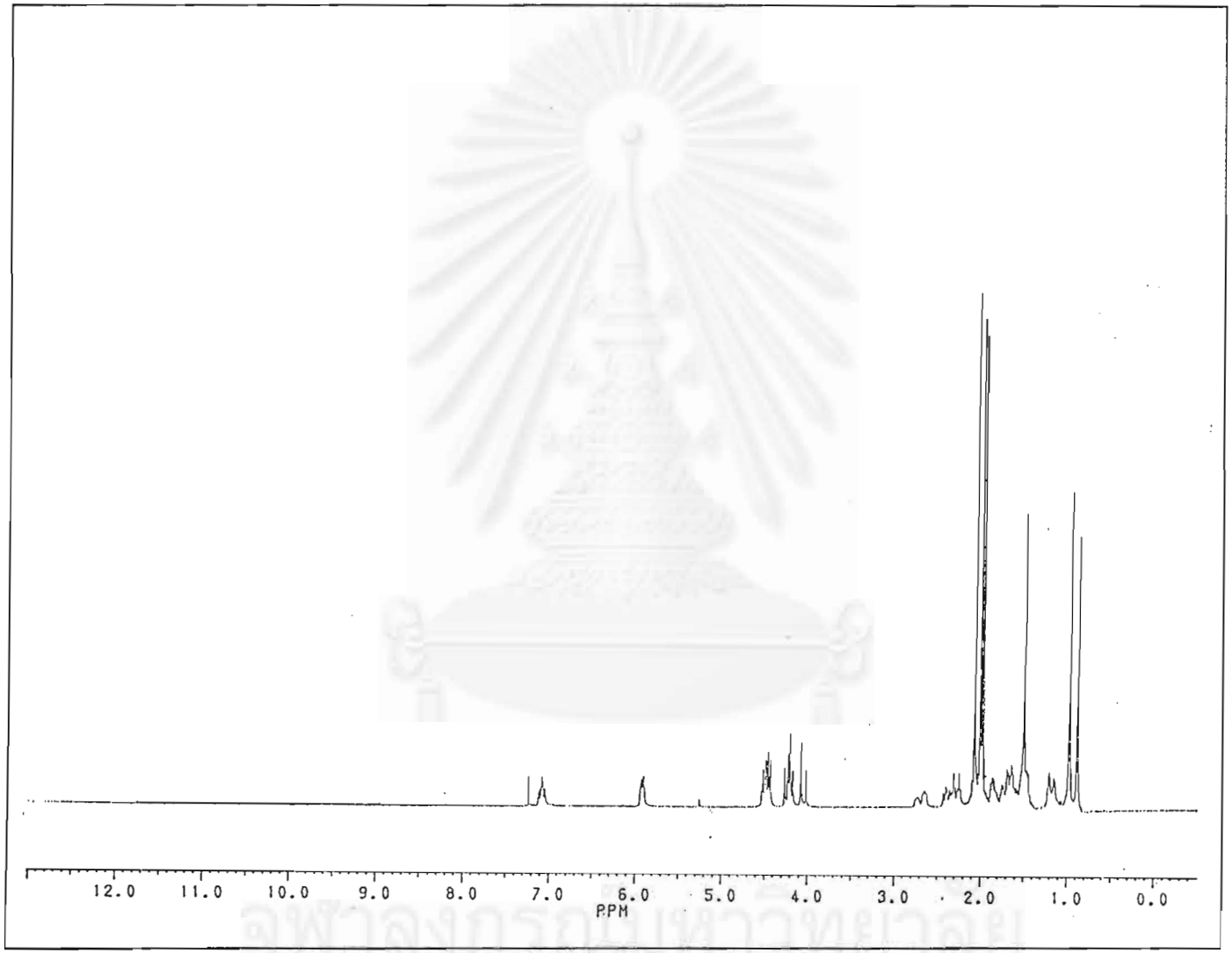


**Figure** 35 The $^{1}$H-NMR spectrum of compound 4

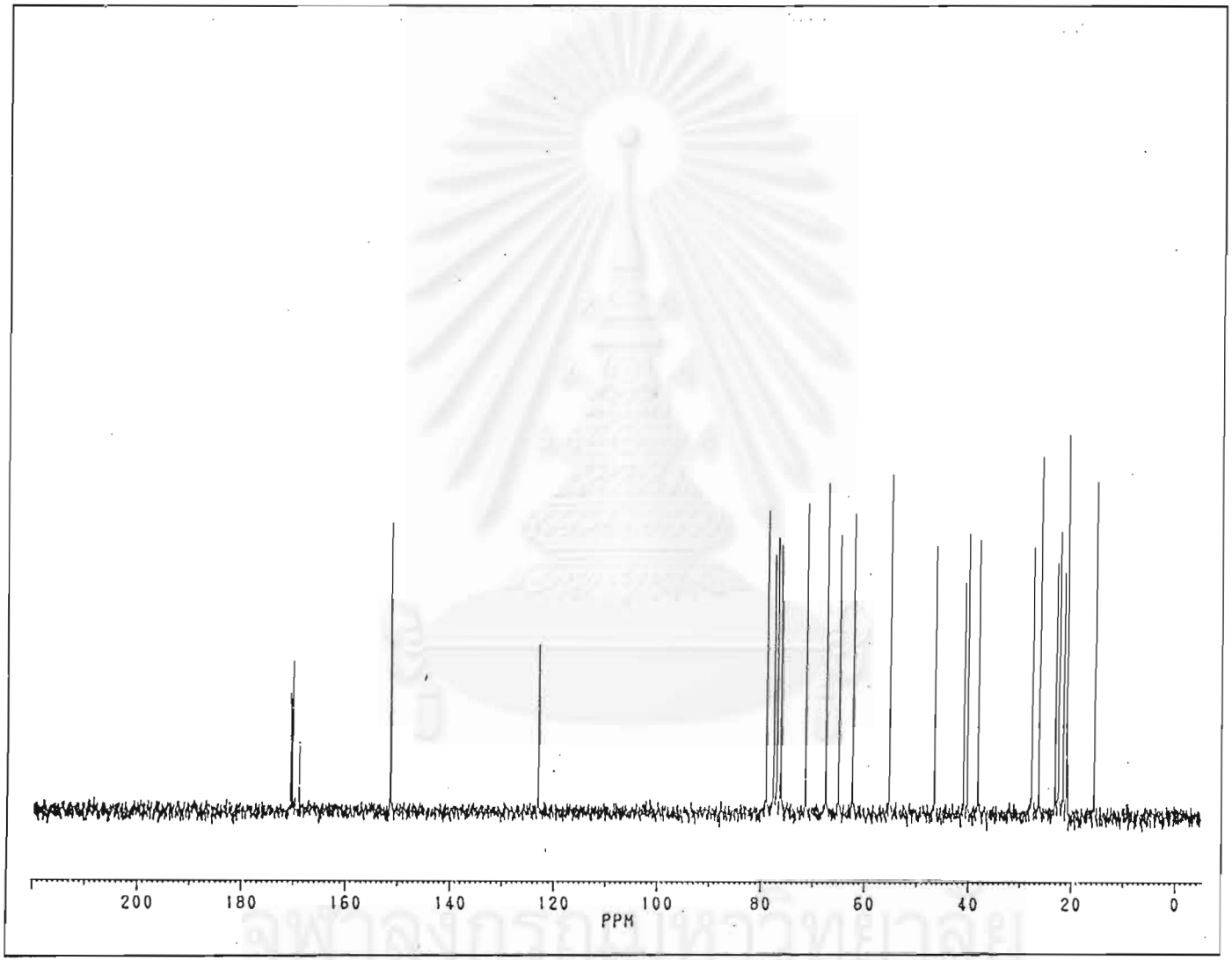


**Figure** 36 The $^{13}$C-NMR spectrum of compound 4

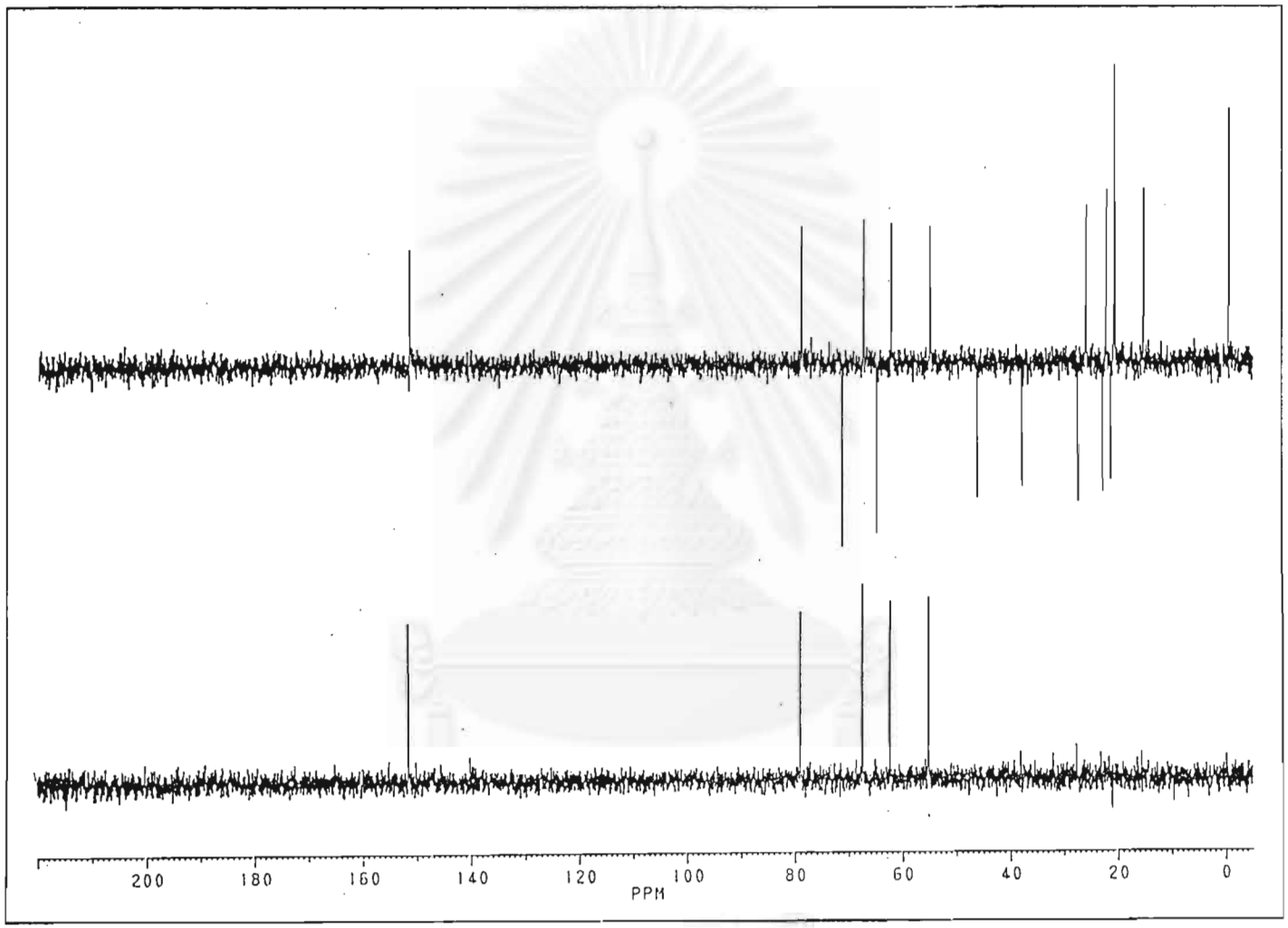


**Figure** 37 DEPT-135, 90 $^{13}$C-NMR of compound 4

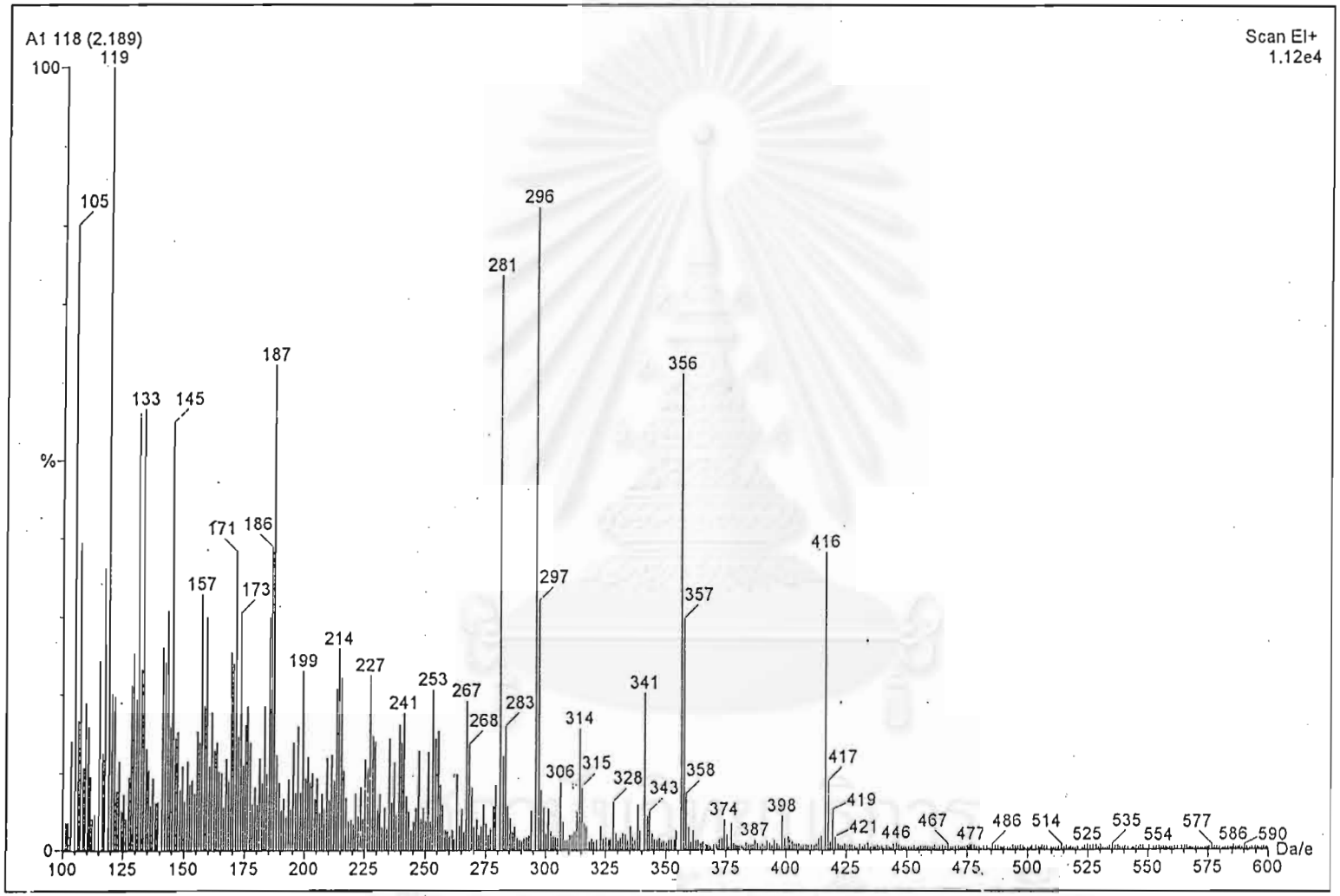


**Figure** 38 The EI MS spectrum of compound 4

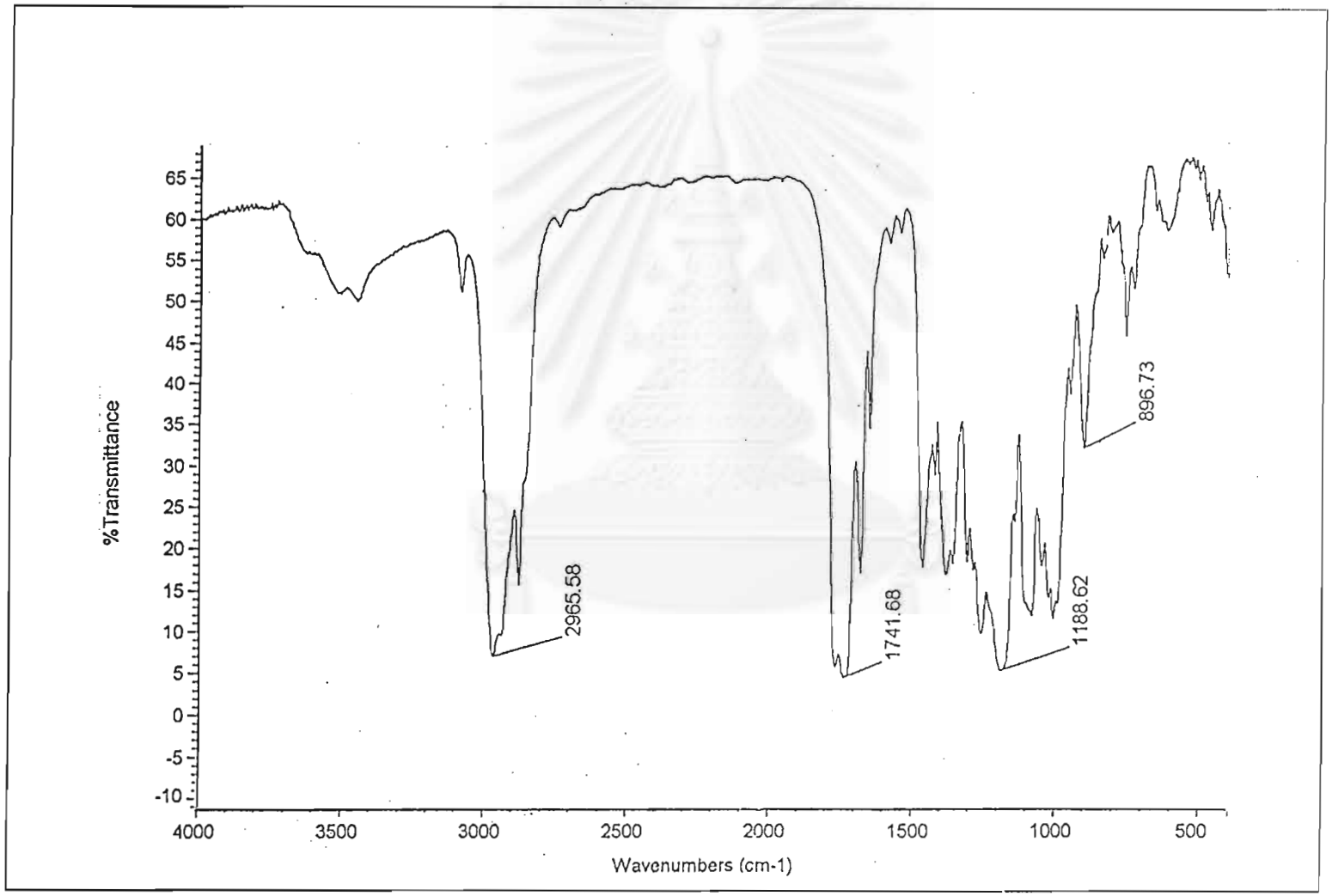


**Figure** 39 The IR spectrum of compound 5

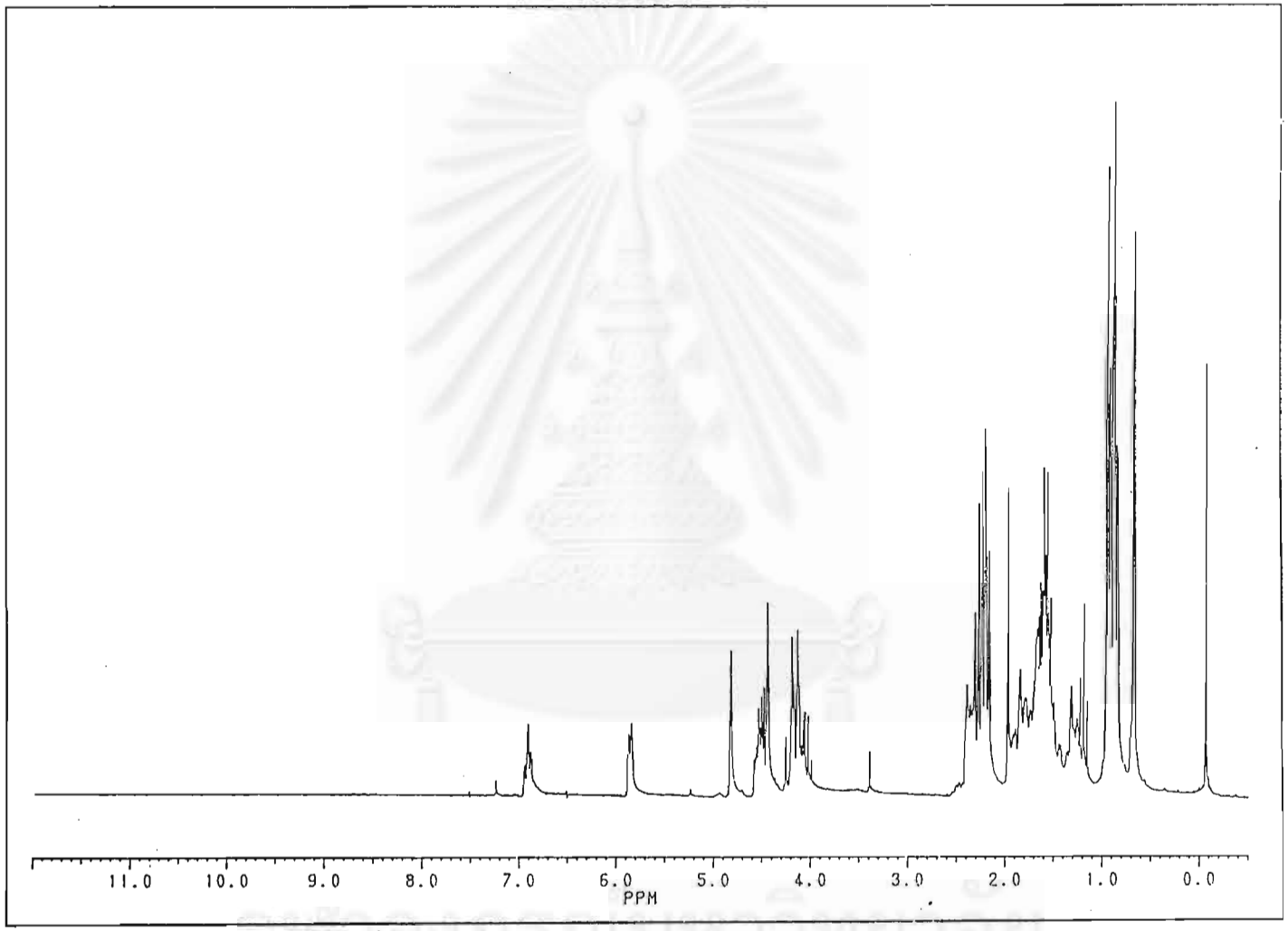


**Figure** 40 The $^{1}$H-NMR spectrum of compound 5

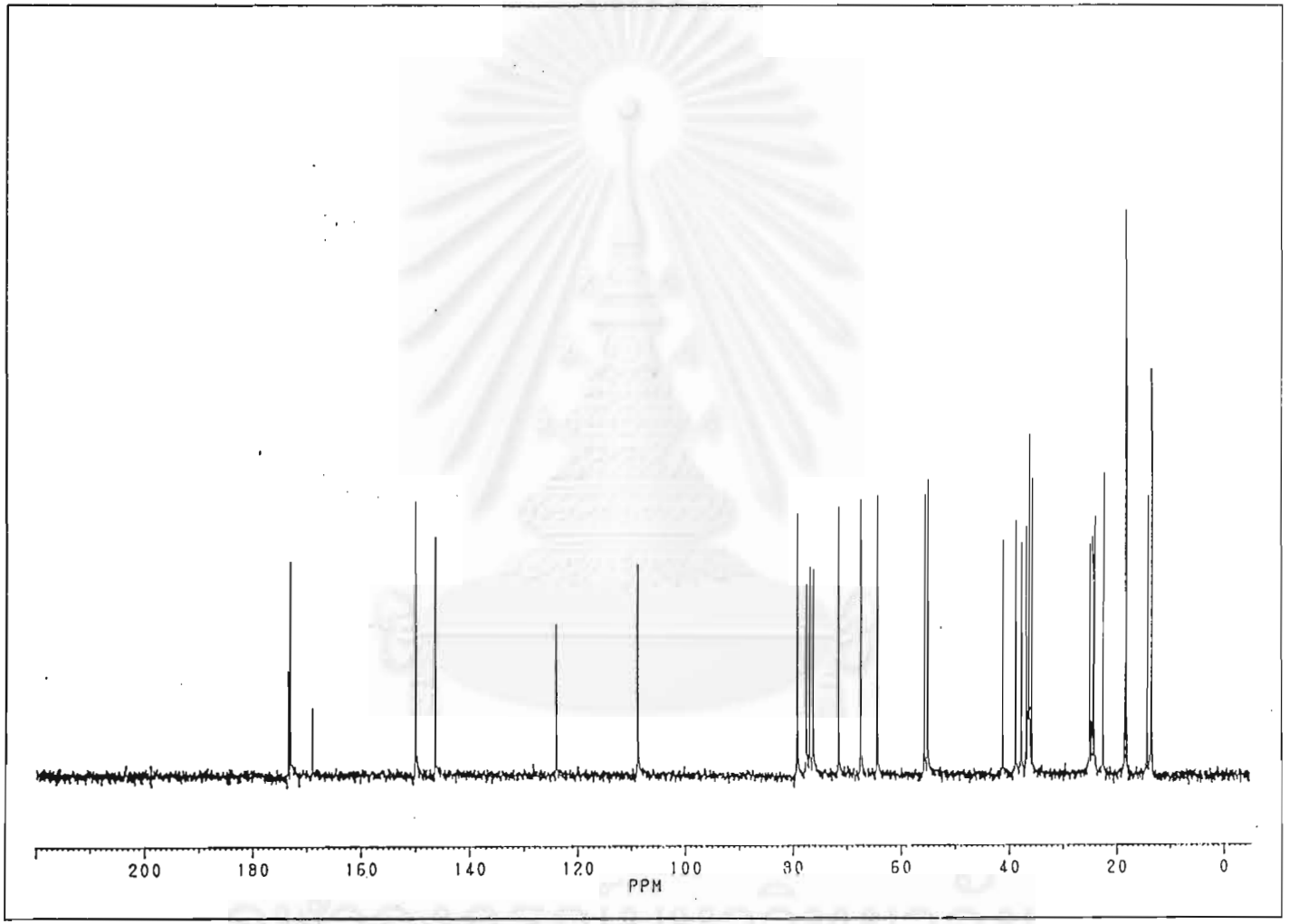


**Figure** 41 The $^{13}$C-NMR spectrum of compound 5

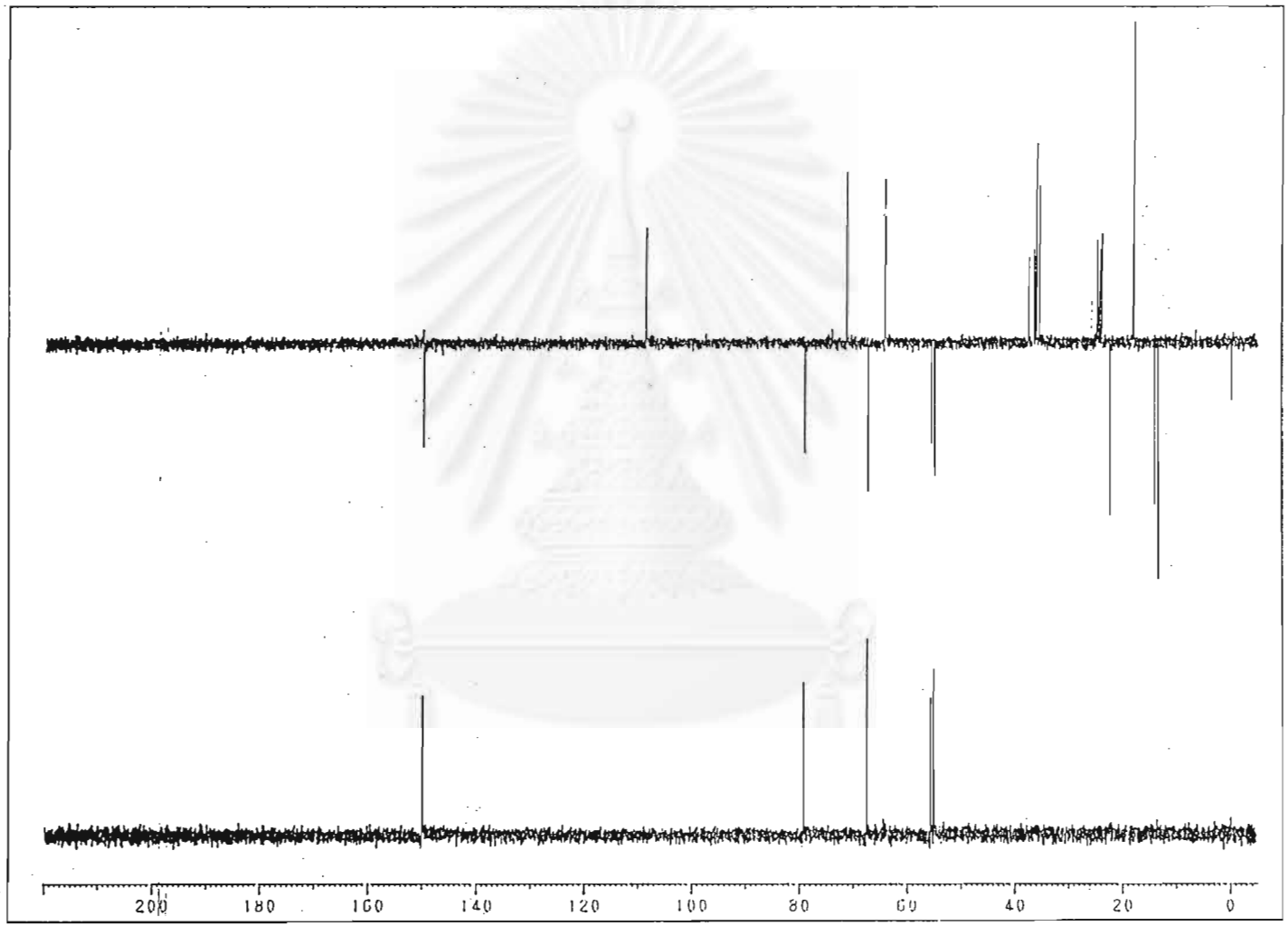


**Figure** 42 DEPT-135, 90 $^{13}$C-NMR of compound 5

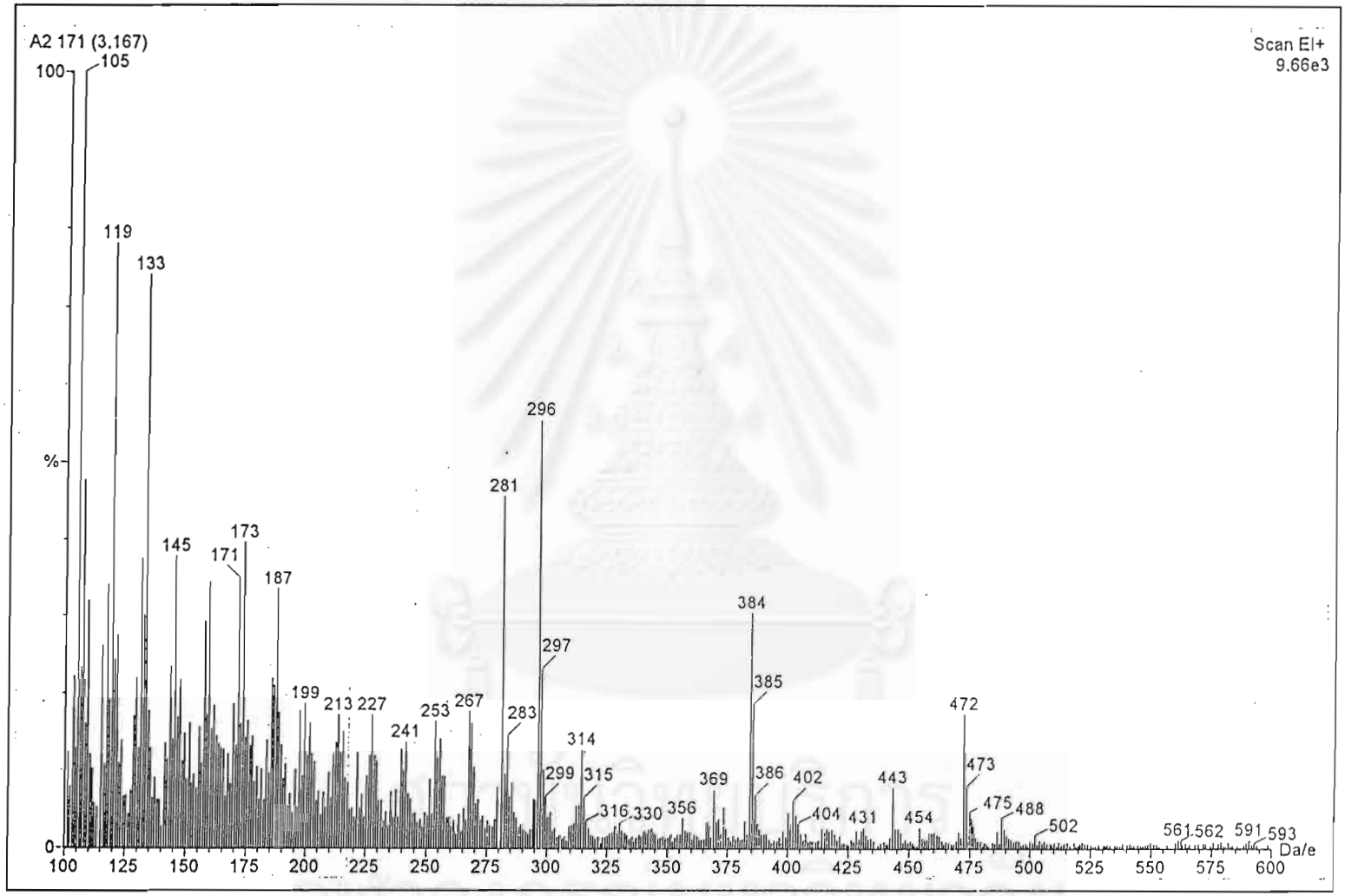


**Figure** 43 The EI MS spectrum of compound 5

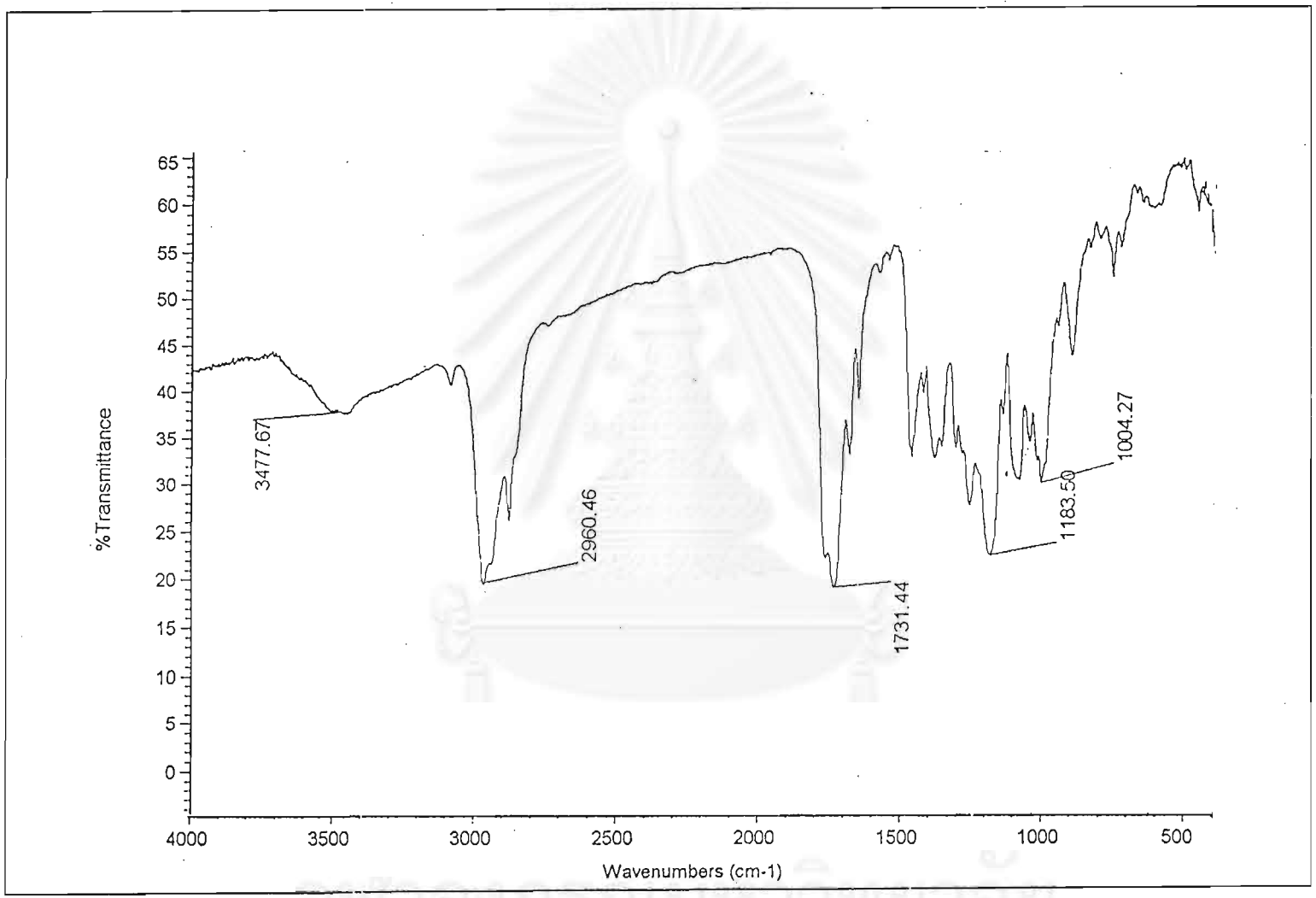


**Figure** 44 The IR spectrum of compound 6

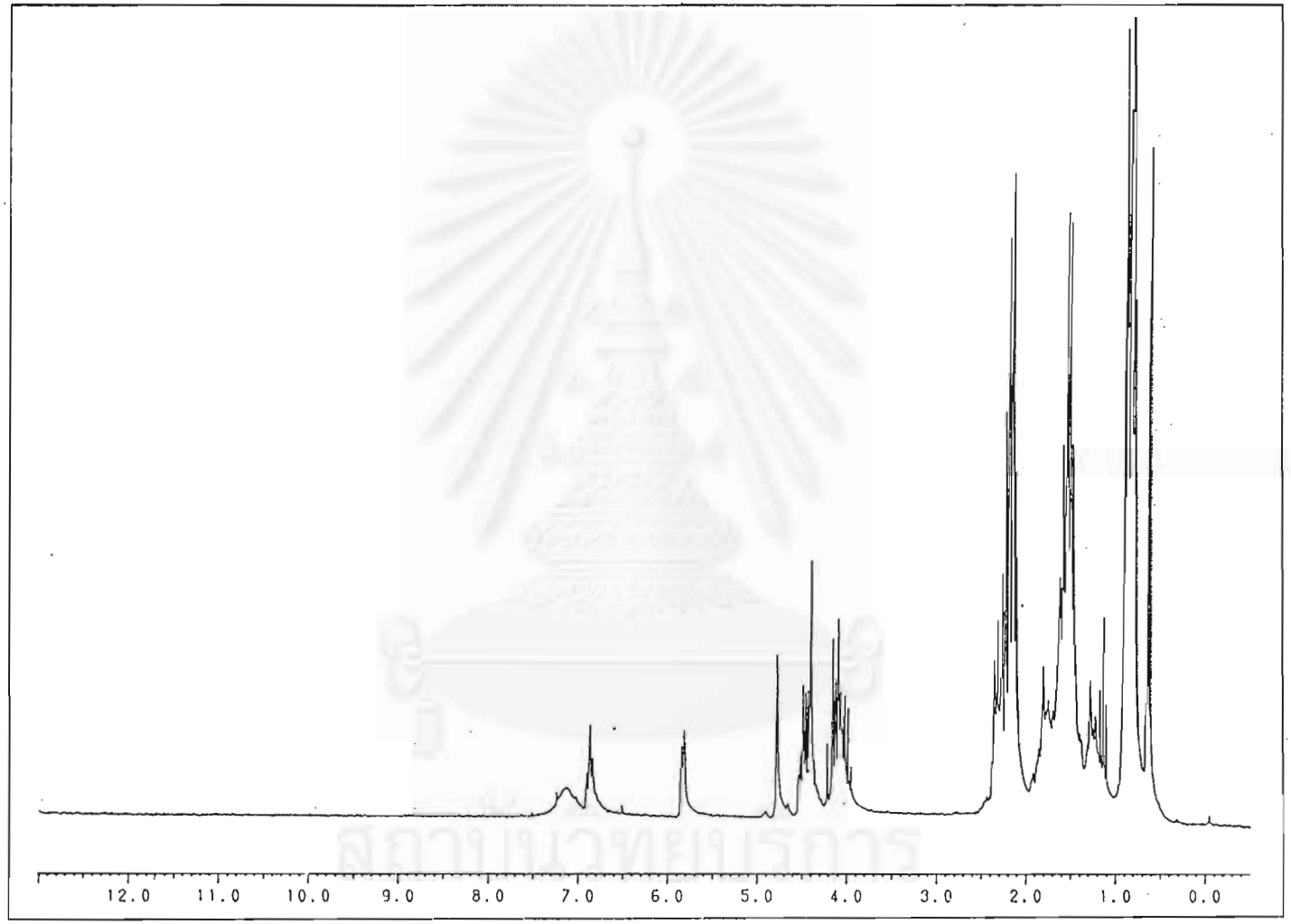


**Figure** 45 The $^{1}$H-NMR spectrum of compound 6

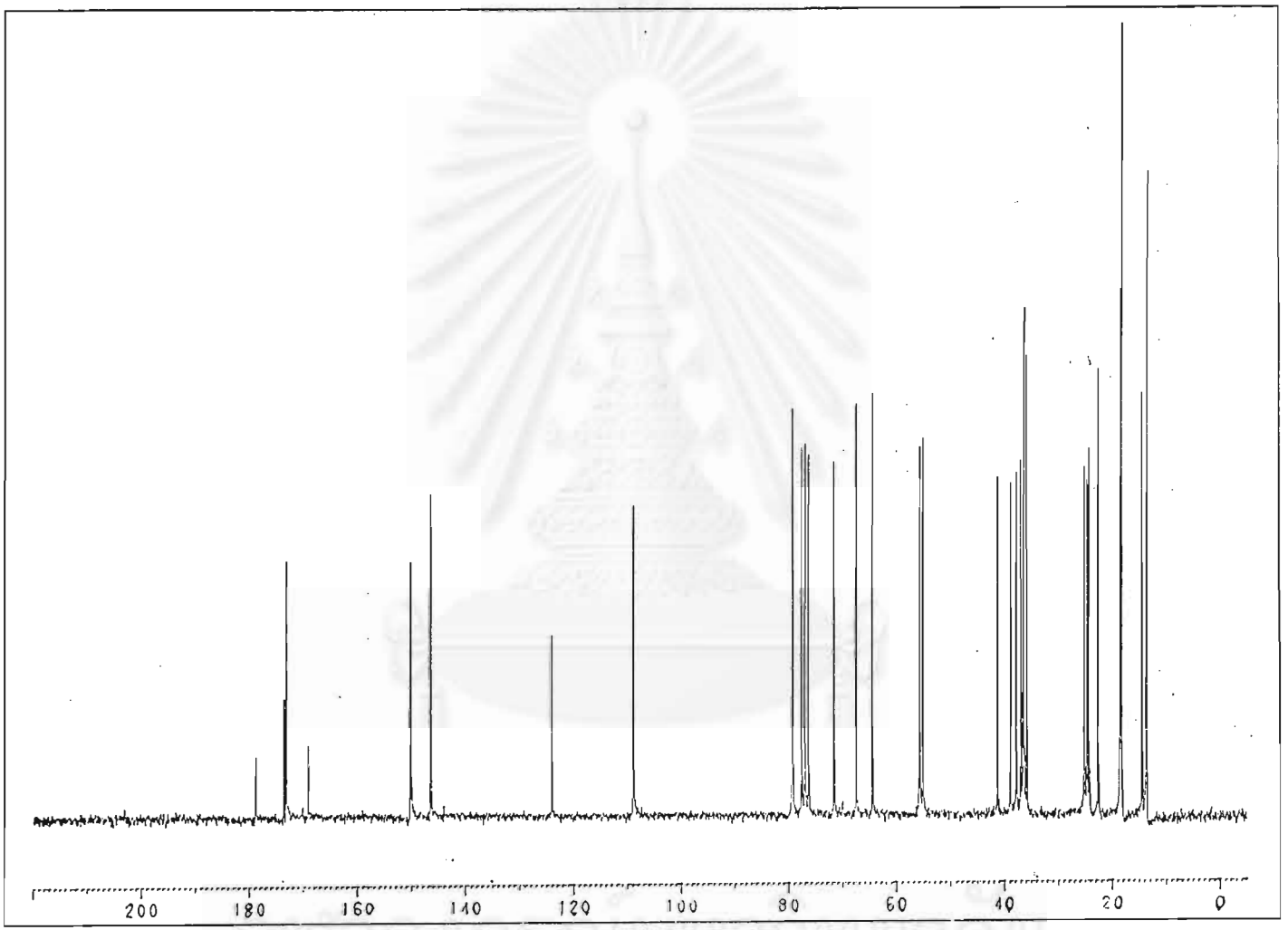


**Figure** 46 The $^{13}$C-NMR spectrum of compound 6

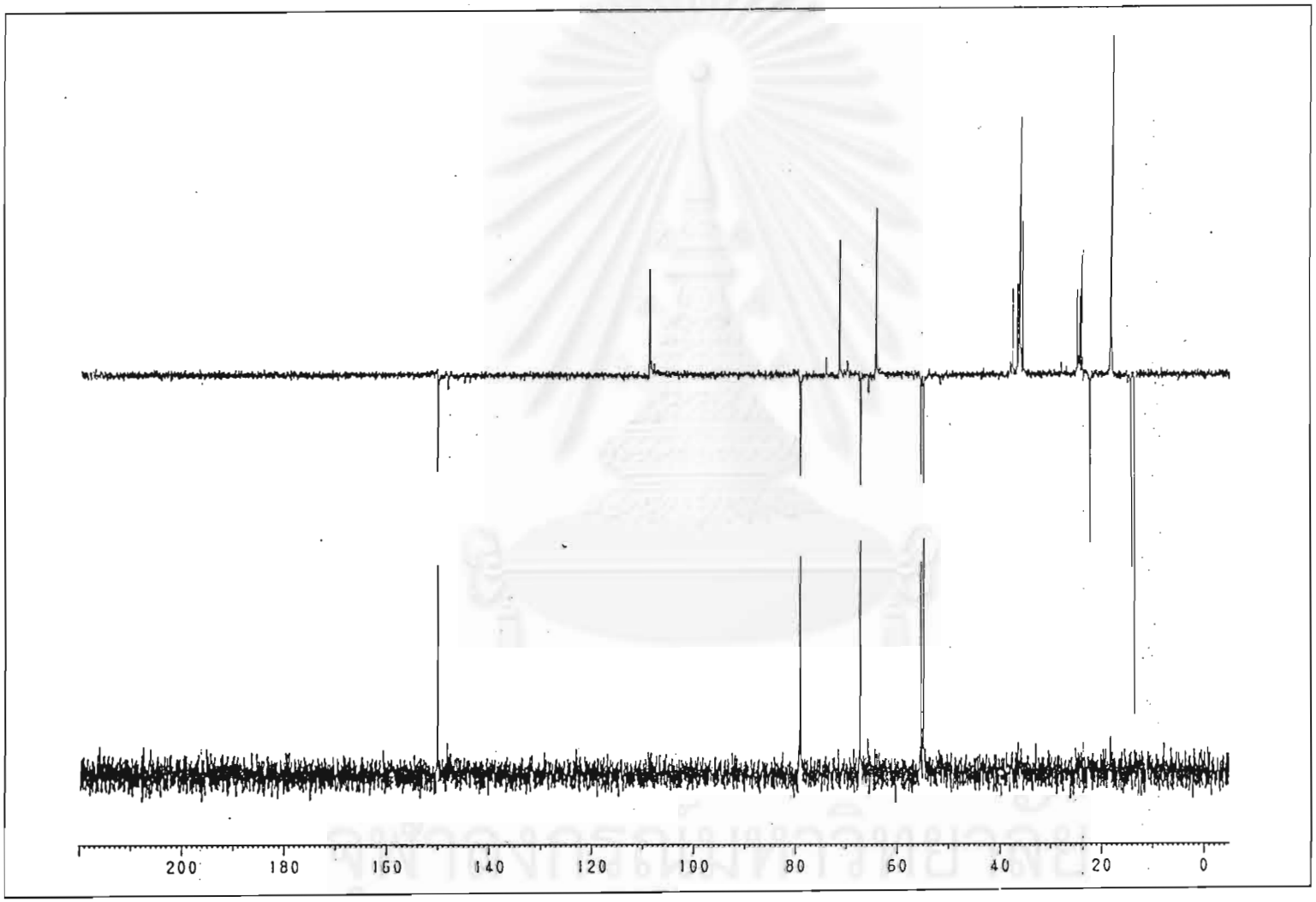


**Figure** 47 DEPT-135, 90 $^{13}$C-NMR of compound 6

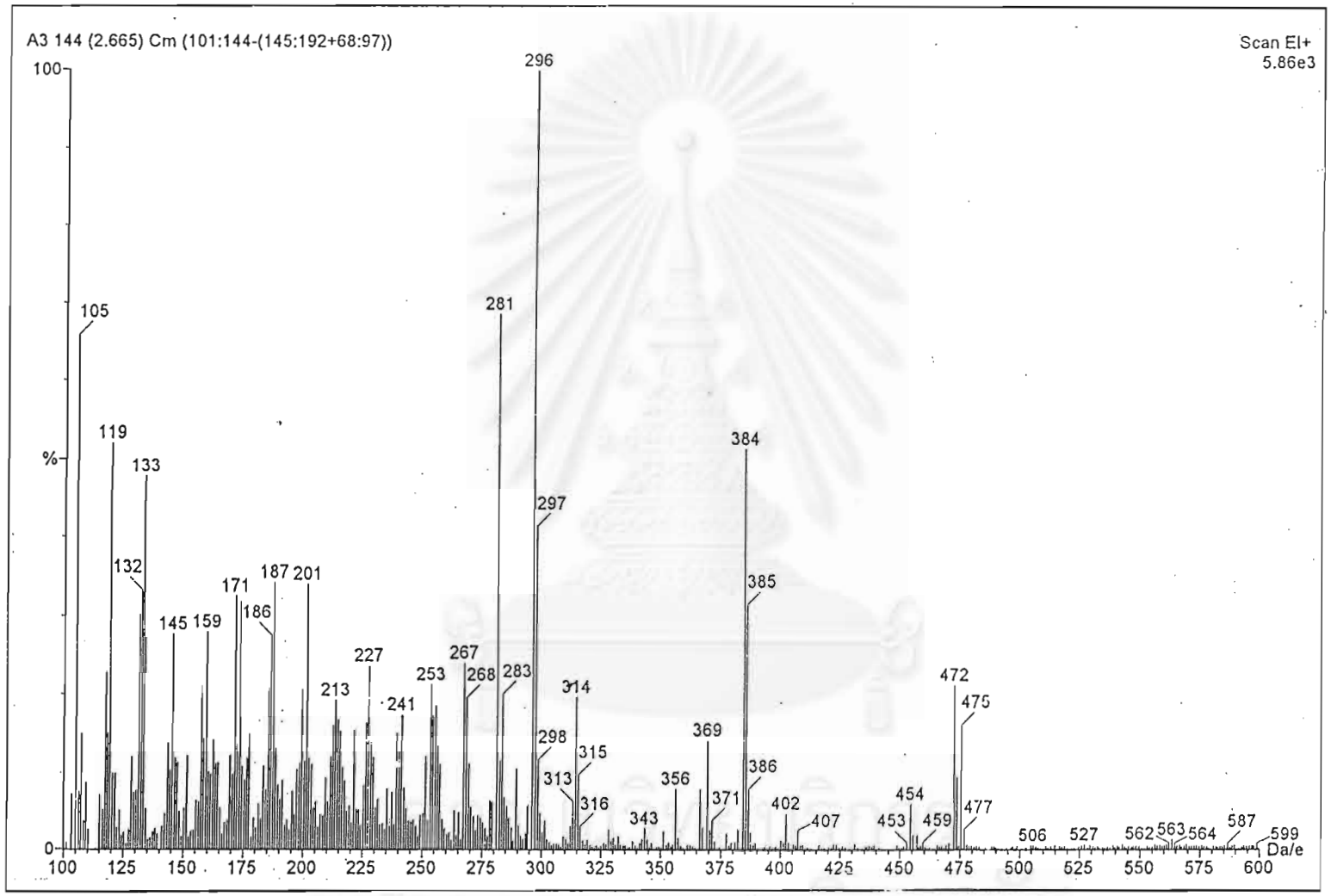


**Figure** 48 The EI MS spectrum of compound 6

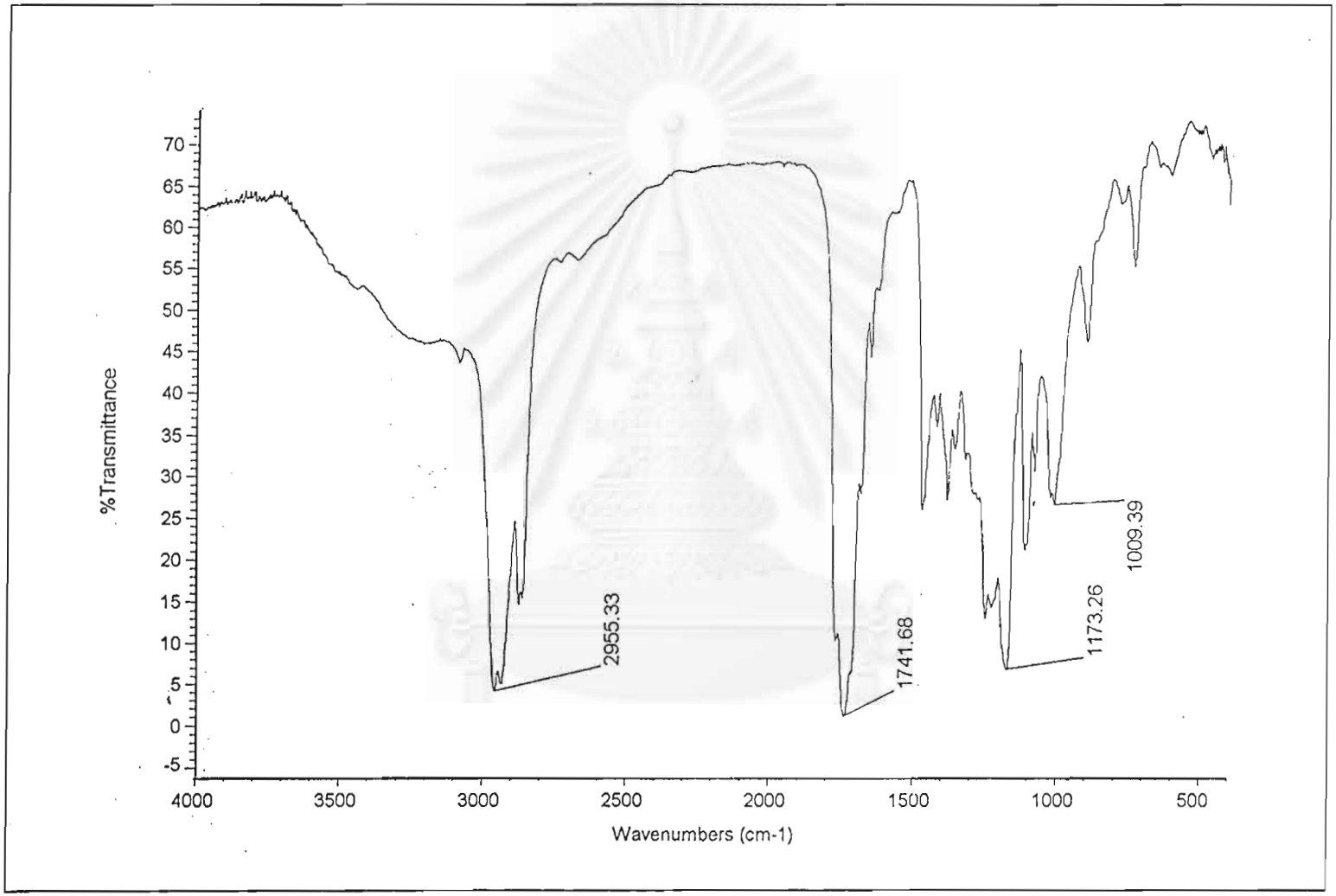


**Figure** 49 The IR spectrum of compound 7

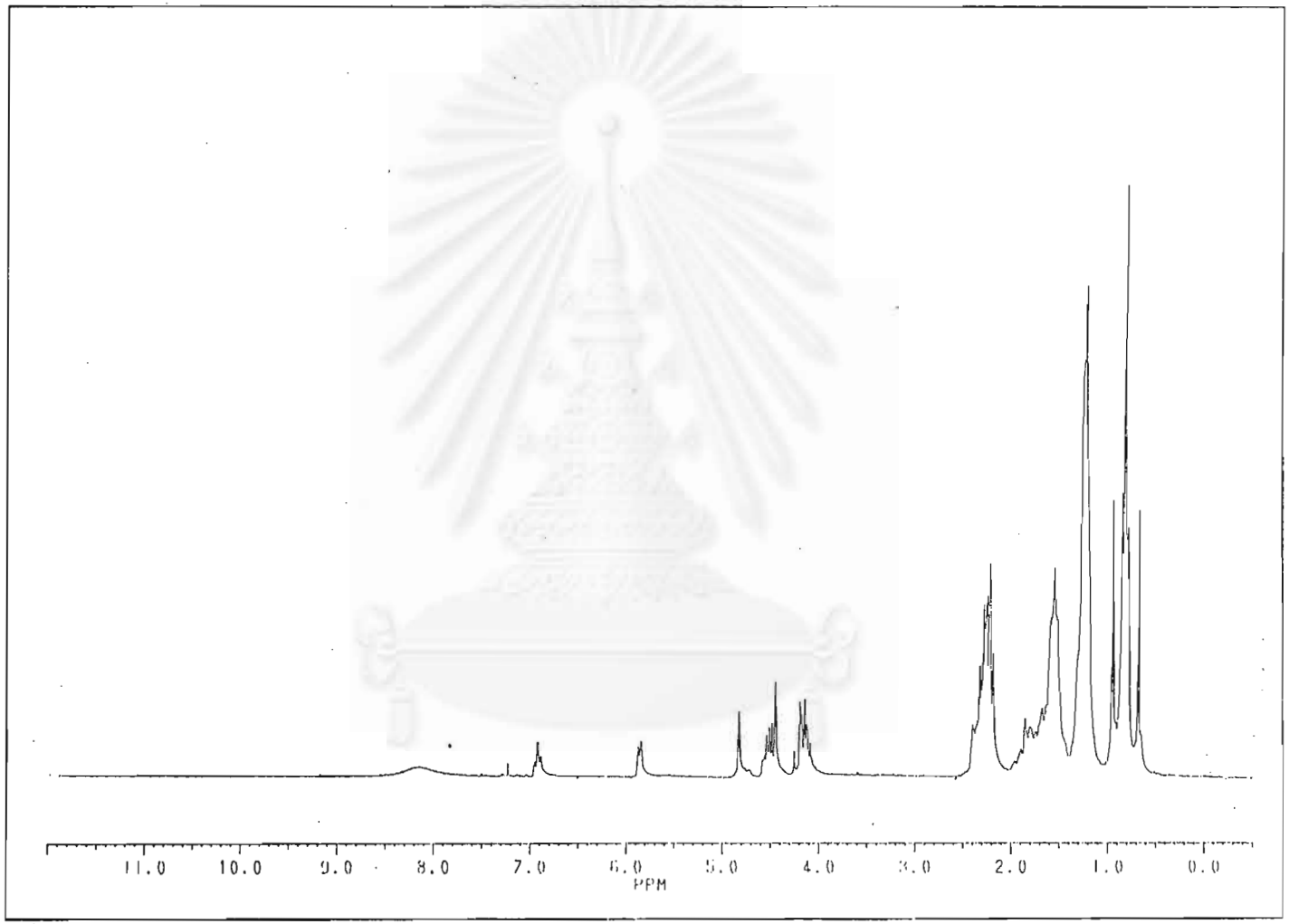


**Figure** 50 The $^{1}$H-NMR spectrum of compound 7

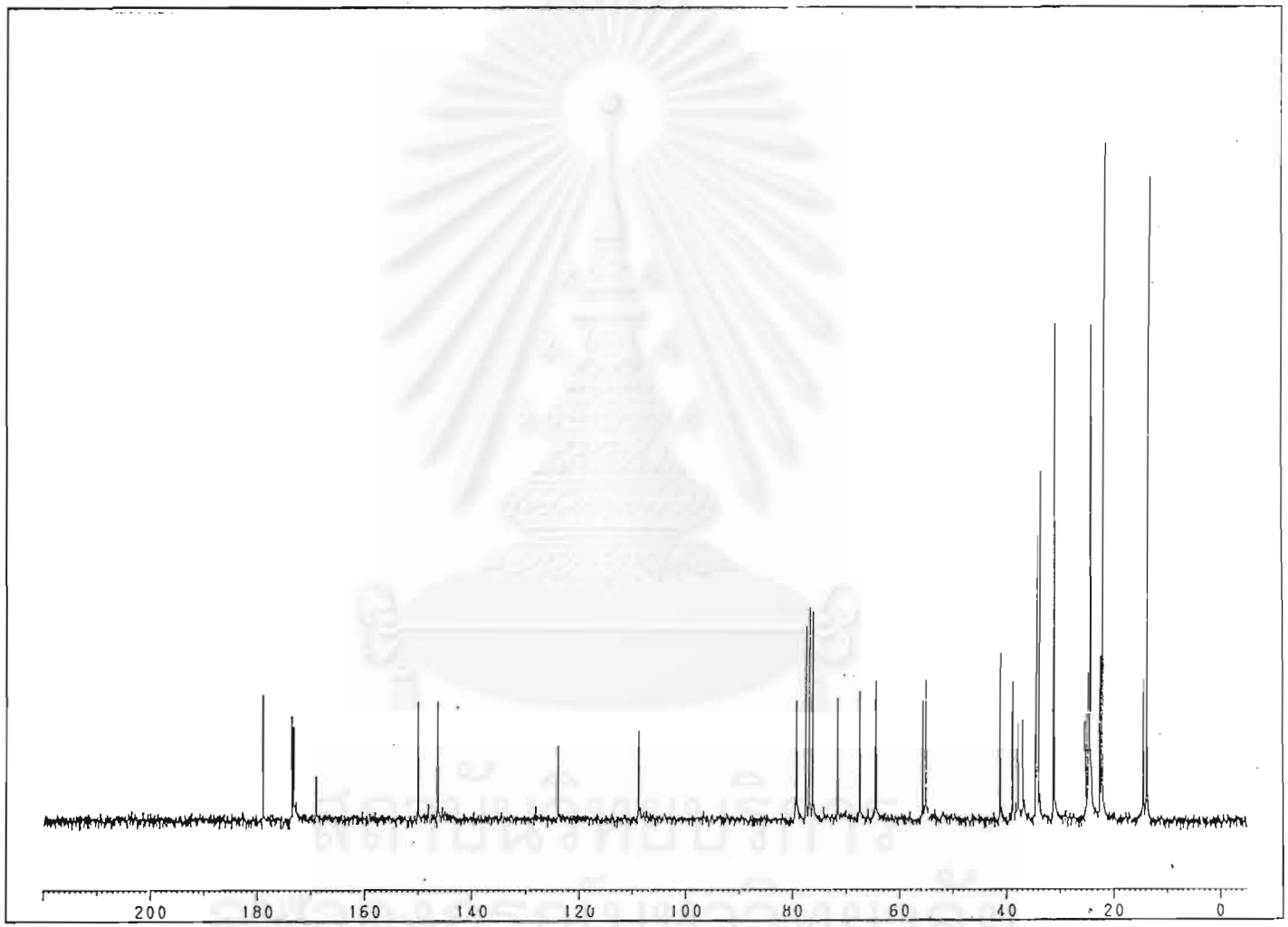


**Figure** 51 The $^{13}$C-NMR spectrum of compound 7

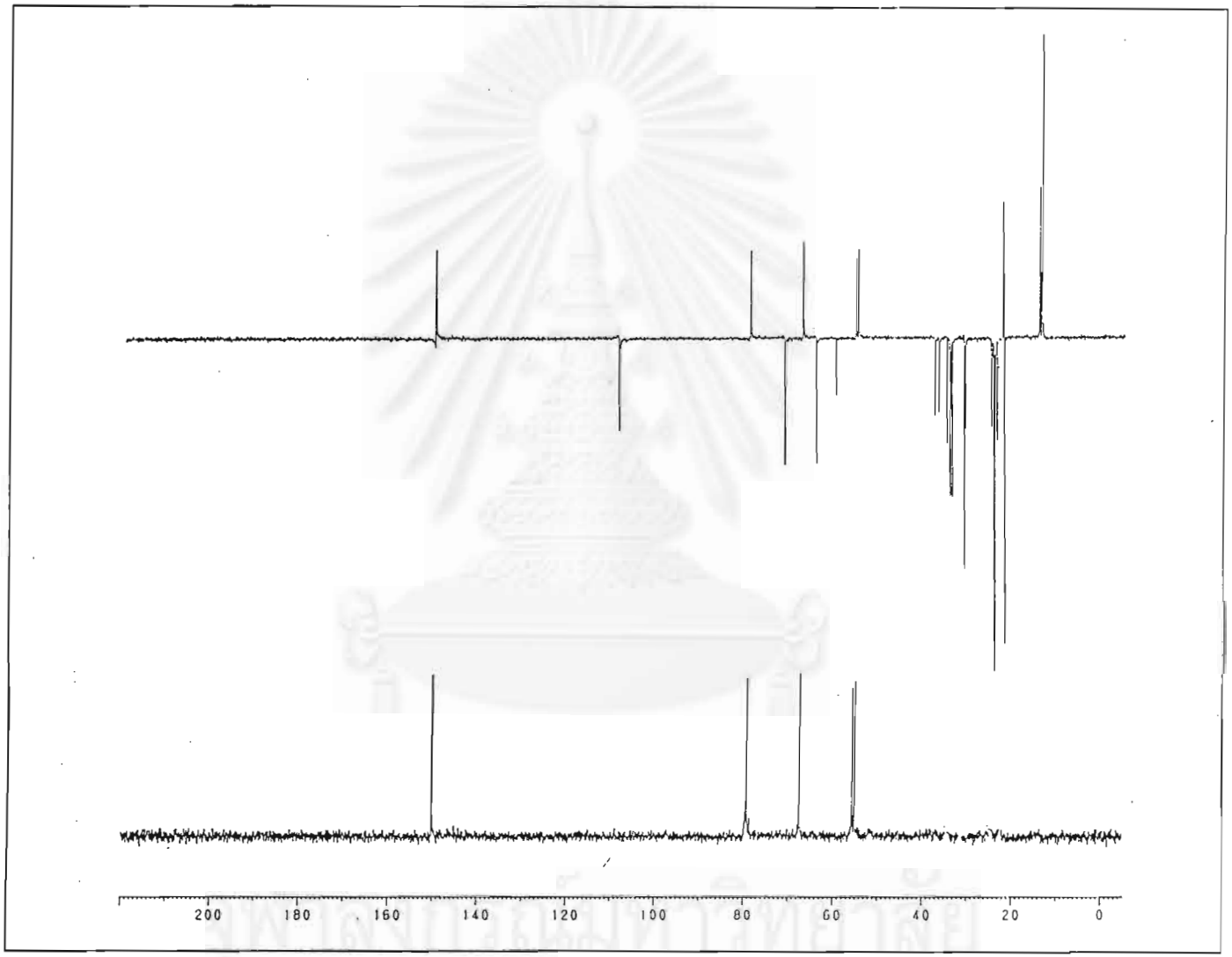


**Figure** 52 DEPT-135, 90 $^{13}$C-NMR of compound 7

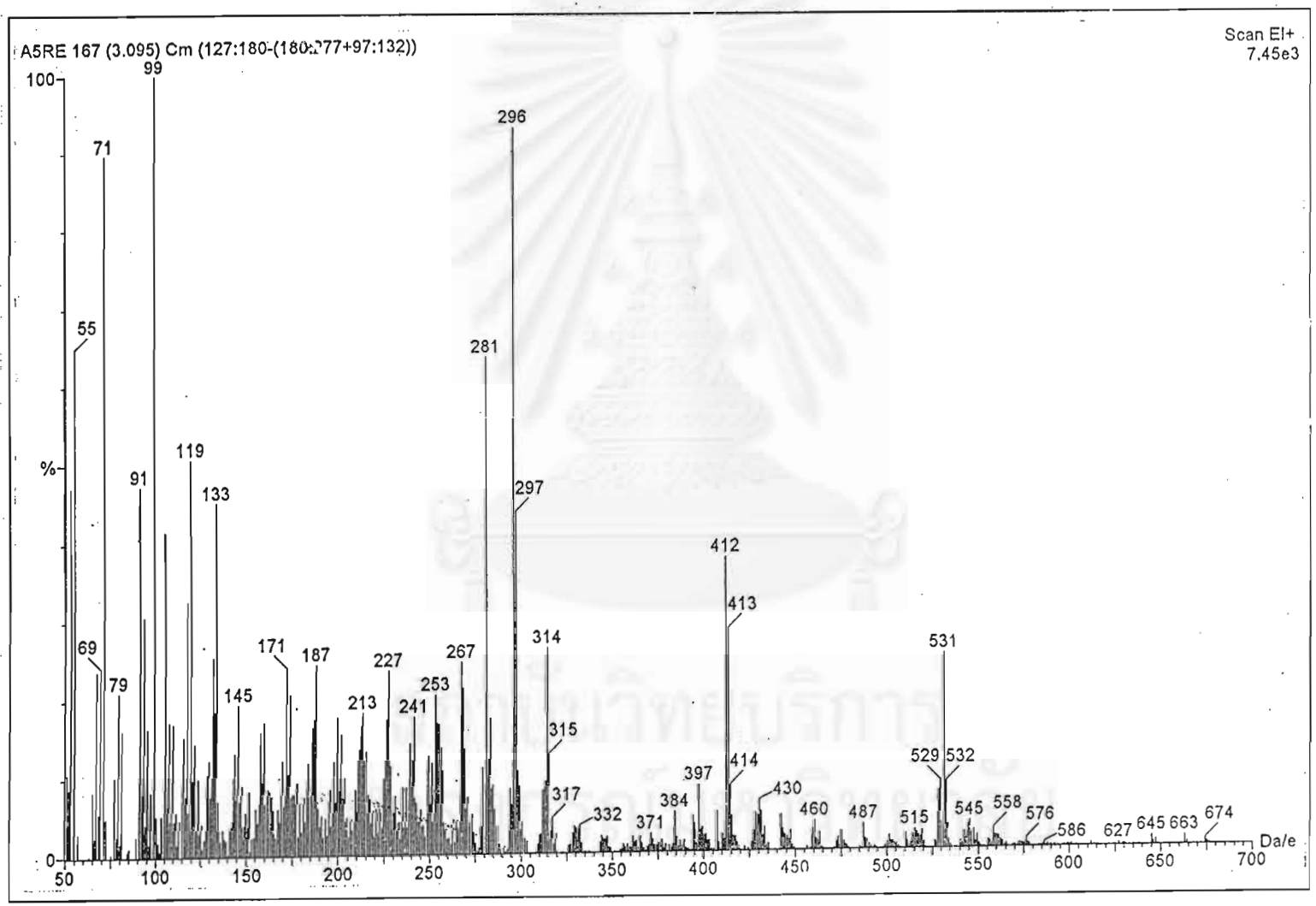


**Figure** 53 The EI MS spectrum of compound 7

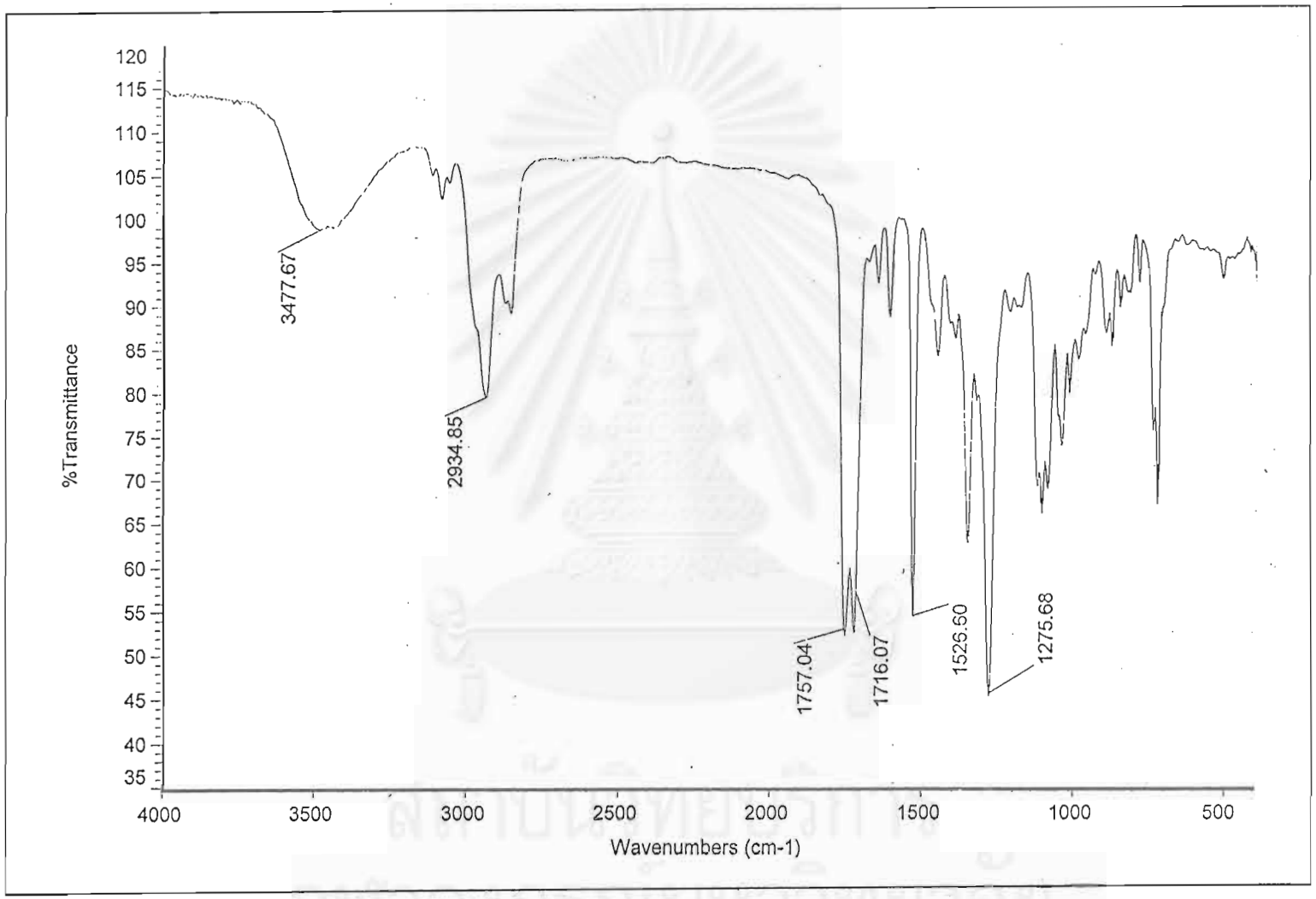


**Figure** 54 The IR spectrum of compound 8

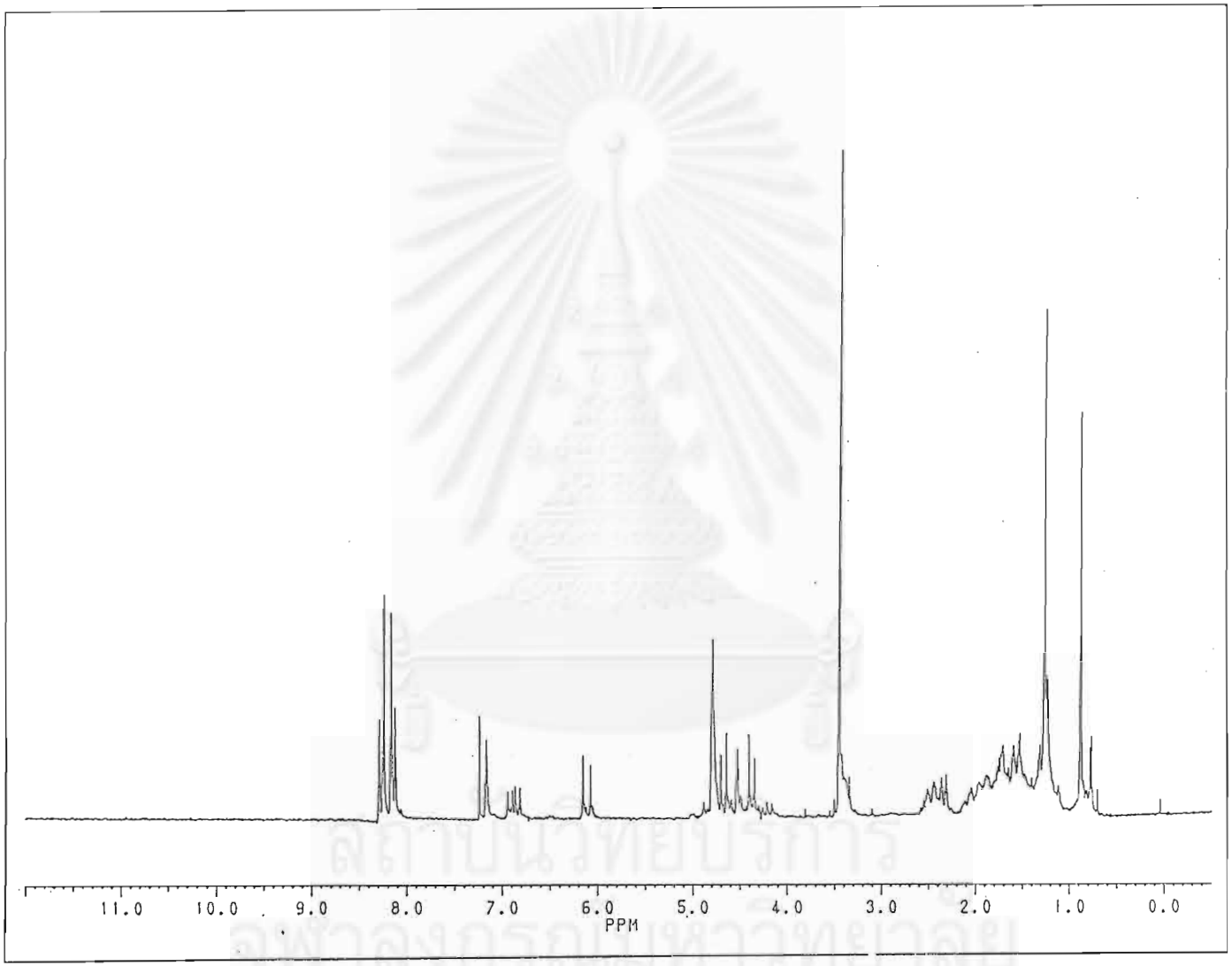


**Figure** 55 The $^{1}$H-NMR spectrum of compound 8

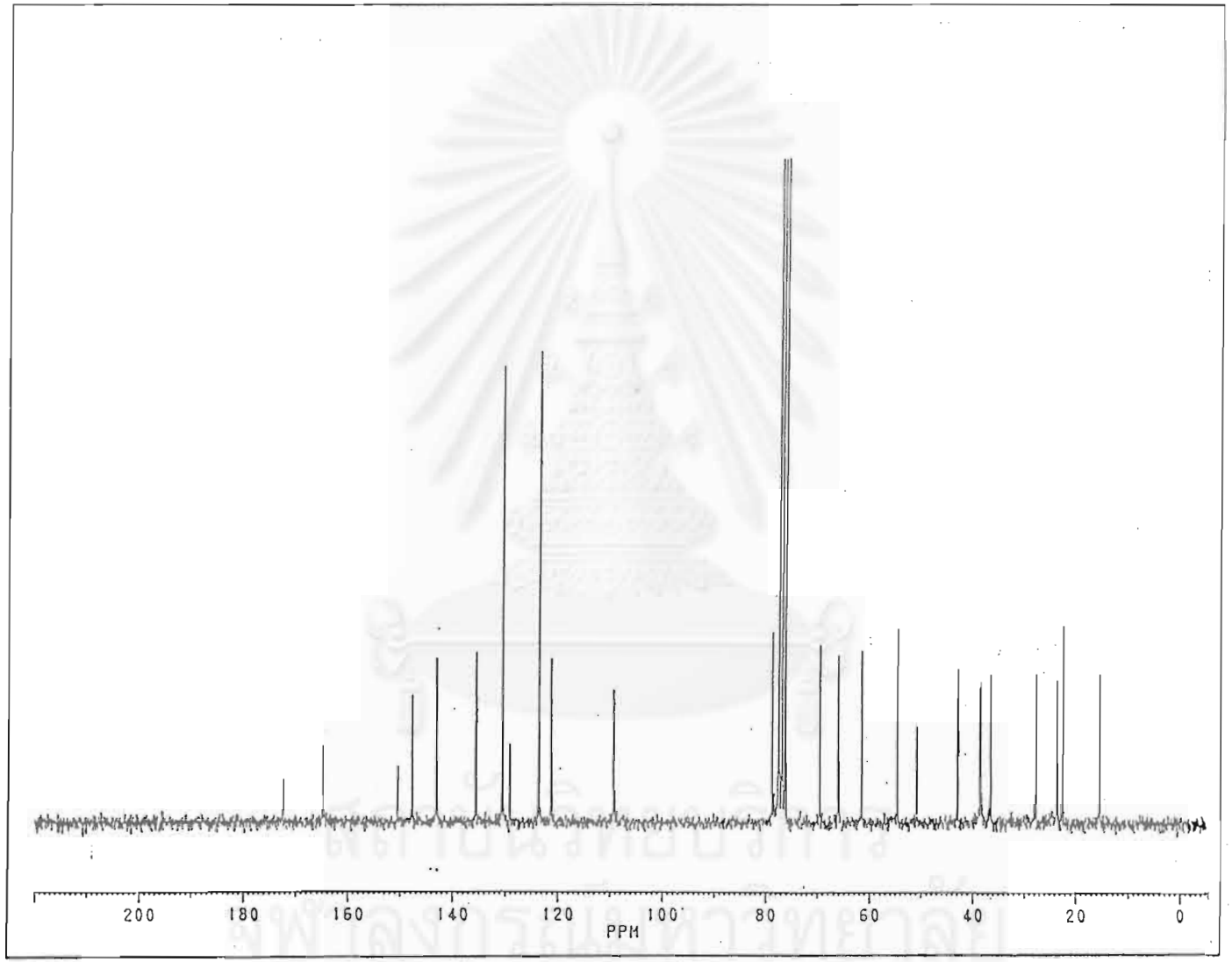


**Figure** 56 The $^{13}$C-NMR spectrum of compound 8

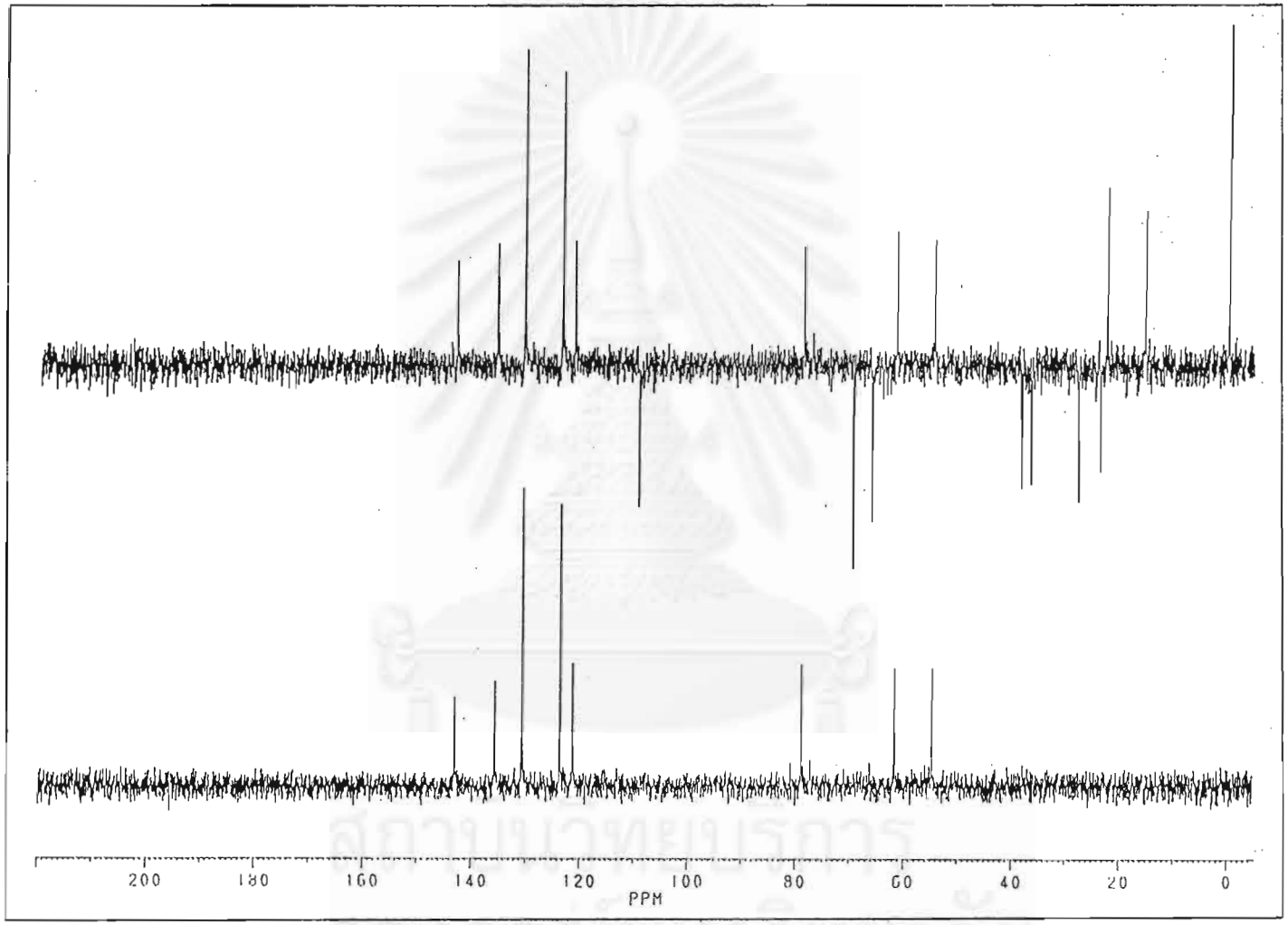


**Figure** 57 DEPT-135, 90 $^{13}$C-NMR of compound 8

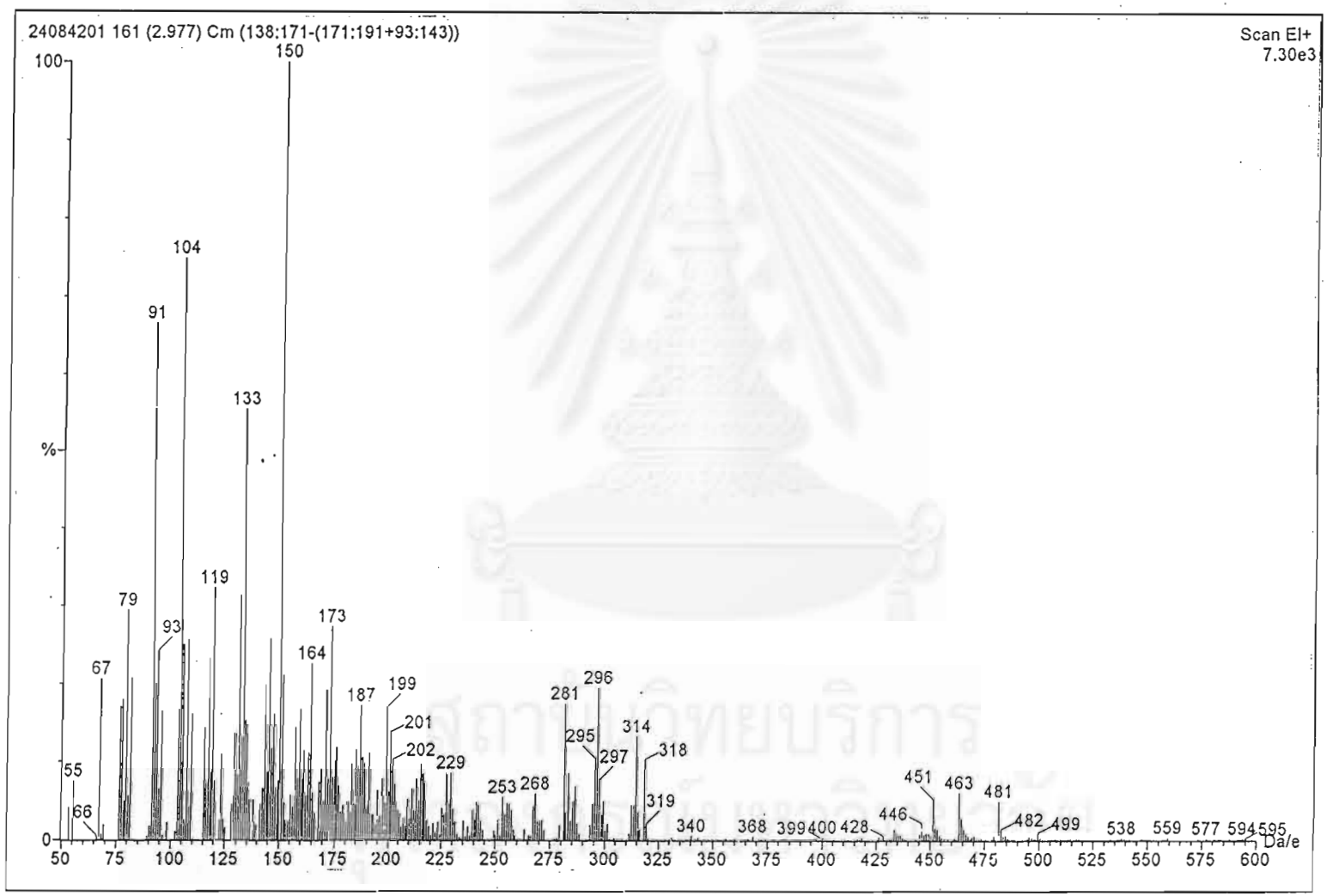


**Figure** 58 The EI MS spectrum of compound 8

# VITA

Mr. Santi Tungprapa was born on November 10,1974 in Phatthalung province, Thailand. He graduated with Bachelor Degree of Science of Chemistry at Chulalongkorn University in 1996. In the same year, he was admitted into a Master Degree program in organic chemistry at Chulalongkorn University. During his study toward the Master's degree, he received financial support from Department of Chemistry, Faculty of Science, Chulalongkorn University in 1997-1999.