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จุฬาลงกรณ์มหาวิทยาลัย

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PHYTOCHEMICAL STUDY OF *DIOSPYROS RUBRA* LEC. STEM

Miss Shollada Posritong

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ชลลดา โพธิ์ศรีทอง : การศึกษาทางพฤกษเคมีของลำต้นพญารากดำ. (Phytochemical study of *Diospyros rubra* Lec. stem) อ. ที่ปรึกษา : อ.ดร.วิชชุดา ธนกิจเจริญพัฒน์, 136 หน้า. ISBN 974-17-2883-2

จากส่วนลำต้นของพญารากดำ (*Diospyros rubra* Lec.) สามารถแยกสารในกลุ่มไตรเทอร์ปีนอยด์ได้ 3 ชนิด คือ lupeol, betulin และ ursolic acid รวมทั้งได้สารผสมของ β-sitosterol และ stigmasterol การพิสูจน์เอกลักษณ์ของสารเหล่านี้ ทำโดยการวิเคราะห์ข้อมูล IR, MS, ^{1}H-NMR และ ^{13}C-NMR ร่วมกับการเปรียบเทียบกับค่าที่ได้มีรายงานไว้แล้ว

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ภาควิชา....เภสัชพฤกษศาสตร์.....ลายมือชื่อนิสิต.....
สาขาวิชา..เภสัชพฤกษศาสตร์.....ลายมือชื่ออาจารย์ที่ปรึกษา.....
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LEC. STEM. THESIS ADVISOR : WITCHUDA THANAKIJCHAROENPATH, PH.D.,

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From the stem of *Diospyros rubra* Lec., three triterpeniods including lupeol, betulin and ursolic acid, together with a mixture of β-sitosterol and stigmasterol, have been isolated. Identification of these compounds was accomplished by analysis of IR, MS, ^{1}H-NMR and ^{13}C-NMR data, as well as the comparison with reported values.

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Department.....Pharmaceutical Botany.....Student's signature.....

Field of study....Pharmaceutical Botany…..Advisor's signature.....….

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สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CONTENS

CONTENTS

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF FIGURES

page

LIST OF FIGURES

page

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

page

ABBREVIATION

br	=	broad (for NMR spectra)
°C	=	degree celsius
CC	=	column chromatography
$CDCl_3$	=	deuterated chloroform
$CHCl_3$	=	chloroform
cm	=	centimeter
^{13}C-NMR	=	Carbon-13 Nuclear Magnetic Resonance
δ	=	Chemical shift
d	=	doublet (for NMR spectra)
dd	=	doublet of doublet (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO-d_6	=	deuterated dimethylsulfoxide
EIMS	=	Electron Impact Mass Spectroscopy
EtOH	=	ethanol
eV	=	electron volt
g	=	gram
^{1}H-NMR	=	Proton Nuclear Magnetic Resonance
Hz	=	Hertz
IR	=	Infrared Spectroscopy
J	=	coupling constant
KBr	=	potassium bromide
L	=	liter
λ_{max}	=	wavelength at maximum absorption (nm)
m	=	multiplet (for NMR spectra)
m	=	meter
M^+	=	molecular ion
MeOH	=	methanol
mg	=	milligram

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

MHz = Megahertz

ml = milliliter

mm = millimeter

MS = Mass Spectrum

m/z = mass-to-charge ratio

ε = Molar absorptivity

nm = nanometer

NMR = Nuclear Magnetic Resonance

ppm = part per million

q = quartet (for NMR spectra)

rel. int. = relative intensity

s = singlet (for NMR spectra)

sp. = species

t = triplet (for NMR spectra)

TLC = Thin-Layer Chromatography

UV = Ultraviolet

var. = variety

ν_{max} = wavenumber at maximum absorption

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

Diospyros rubra Lec. (Figure 1) is a tree which belongs to the genus *Diospyros* of the family Ebenaceae. The plant is an evergreen tree that can grow up to 5 m and very commonly found on limestone hill in dry evergreen forest at altitudes of 10-500 m (Phengklai, 1981). Its leaves are (ovate-) oblong to lanceolate, 6-16 by 2-7 cm, with acute, obtuse or rounded base. The leaf apex is cuspidate with blunt tip, sometimes acute or obtuse. The leaf texture is subcoriaceous to coriaceous and the upper surface is glabrous while the lower surface is pubescent then glabrescent. The leaf has 8-12 pairs of secondary nerves, arched and anastomosing well away from the margin, more or less impressed on the upper surface but prominent on the lower surface. The scalariform veins are conspicuous. The petiole is 0.5-1 cm long, pubescent or glabrescent.

The male flowers are in cymose, inflorescence, 4-merous, sessile or subsessile. The calyx is broadly campanulate, 2-3 mm long, divided to two-third or to the base, sericeous outside but pubescent inside. The corolla is urceolate, 3-4 mm long, divided to one-third, glabrous on both sides except outside along the mid-line of the lobes down to the tube. The number of the stamens are 16-18, glabrous. The rudimentary ovary is hirsute. The female flowers are solitary, 4(-5)-merous, sessile or subsessile. The calyx and corolla are the same as those in male flowers but larger and the corolla is divided to half way. The ovary is globose, sericeous, 4-locular with single, sericeous style. The staminodes are absent.

The fruit of the plant is sessile, ellipsoid, orange or red when ripe, 1-2.5 by 1-2 cm, woody, with glabrous epicarp which is at least 2 mm thick, mostly singly seeded with faint line on seed coat. The base of the fruit is rounded, whereas the apex is acute or obtuse with short apiculus. The fruiting-calyx is divided to the base, pubescent outside, pubescent or woolly inside, accrescent. The lobes are reflexed, not plicate nor undulate, with inconspicuous nerves. The endosperm is ruminate (Phengklai, 1981).

Figure 1. *Diospyros rubra* Lec. (from Flora of Thailand, volume two part four, October 1981.)

The plant can be found throughout Thailand. Its Thai vernacular names are " Phaya rak dam" (General), "Khlai" (Nakhon Sawan), "Dam dong" (Southwestern), "Di mi" (Prachuap Khiri Khan), "Fai", "Muai dam khao", "Mak kuea ka" (Eastern), "Salang tua phu" (Southeastern) (ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้, 2544). It is also found in South China, Vietnam, and Cambodia (Phengklai, 1981).

Plants in the genus *Diospyros* are mostly found in the tropics. A few of them are found in the subtropics (Heywood, 1978).

In Thailand 62 species of *Diospyros* can be found, as follows (ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้, 2544)

1. *Diospyros andamanica* (Kurz) Bakh. var. *aequabilis* Bakh.
2. *D. apiculata* Hiern
3. *D. areolata* King & Gamble
4. *D. bambuseti* Fletcher
5. *D. bejaudii* Lec.
6. *D. borneensis* Hiern
7. *D. brandisiana* Kurz
8. *D. buxifolia* (Bl.) Hiern
9. *D. castanea* Fletcher
10. *D. cauliflora* Bl.
11. *D. coaetanea* (Craib) Fletcher
12. *D. collinsae* Craib
13. *D. confertiflora* (Hiern) Bakh.
14. *D. curranii* Merr.
15. *D. curraniopsis* Bakh.
16. *D. dasyphylla* Kurz
17. *D. decandra* Lour.
18. *D. dictyoneura* Hiern
19. *D. diepenhorstii* Miq.
20. *D. dumetorum* W.W. Smith.
21. *D. ehretioides* Wall. ex G. Don

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

22. *D. ferrea* (Willd.) Bakh.
    a. *D. ferrea* (Willd.) Bakh. var. *ferrea* (Willd.) Bakh.
    b. *D. ferrea* (Willd.) Bakh. var. *littorea* (R.Br.) Bakh.
23. *D. filipendula* Pierre ex Lec.
24. *D. frutescens* Bl.
25. *D. fulvopilosa* Fletcher
26. *D. glandulosa* Lace
27. *D. gracilis* Fletcher
28. *D. hasseltii* Zoll.
29. *D. insidiosa* Bakh.
30. *D. kaki* L.*
31. *D. kerrii* Craib
32. *D. kurzii* Hiern
33. *D. lanceifolia* Roxb.
34. *D. latisepala* Ridl.
35. *D. longipilosa* Phengklai
36. *D. malabarica* (Desr.) Kostel.
    a. *D. malabarica* (Desr.) Kostel. var. *malabarica* Kostel.
    b. *D. malabarica* (Desr.) Kostel. var. *siamensis* (Hochr.) Phengklai
37. *D. martabanica* C.B. Clarke
38. *D. mollis* Griff.
39. *D. montana* Roxb.
40. *D. oblonga* Wall. ex G. Don
41. *D. pendula* Hasselt ex Hassk.
42. *D. philippensis* A. DC.*
43. *D. pilosanthera* Blanco
44. *D. pilosula* (A.DC.) Hiern
45. *D. pubicalyx* Bakh.
46. *D. pyrrhocarpa* Miq.
47. *D. rhodocalyx* Kurz
48. *D. rubra* Lec.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

49. *D. scalariformis* Fletcher

50. *D. scortechinii* King & Gamble

51. *D. sumatrana* Miq.

52. *D. tahanensis* Bakh.

53. *D. thaiensis* Phengklai

54. *D. toposia* Ham.

    a. *D. toposia* Ham. var. *toposia* Ham.

    b. *D. toposia* Ham. var. *toposioides* (King & Gamble) Phengklai

55. *D. transitoria* Bakh.

56. *D. trianthos* Phengklai

57. *D. truncata* Zoll. ex Moritzi

58. *D. undulata* Wall. ex G. Don

    a. *D. undulata* Wall. ex G. Don var. *undulata*

    b. *D. undulata* Wall. ex G. Don var. *cratericalyx* (Craib) Bakh.

59. *D. variegata* Kurz

60. *D. venosa* Wall. ex A.DC.

61. *D. wallichii* King & Gamble

62. *D. winitii* Fletcher

* exotic plant

The importance of *Diospyros* species in traditional medicines have been known for a long time. In Thailand the fruit of *D. mollis* is used as anthelmintic (นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2542). The fruit of *D. rhodocalyx* is used as astringent to control bleeding and in the treatment of renal diseases (Sutthivaiyakit *et al*., 1995). The bark of *D. montana* is used as a remedy for vomiting, high fever and jaundice, while its gum is used to cure tuberculosis (Pardhasaradhi *et al*., 1990). The wood of *D. rubra* is used in the treatment of skin diseases, tuberculosis, renal disorders and urinary discharge (นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2542).

Out of 350 identified *Diospyros* species, more than 150 species have been investigated (Mallavadhani, Panda and Rao, 1998). Triterpenoids, steroids,

naphthoquinones, tannins and other groups of phytochemicals have been found. The triterpenoids and naphthoquinones are the groups of compounds which are found widespread and in almost all parts of the plants. Several compounds of these two groups have been shown to exert interesting bioactivities.

This study has been conducted in order to isolate and identify the chemical components from the stem of *D. rubra*, which is one of *Diospyros* species with no previous phytochemical study. The result obtained might add to the knowledge on chemical nature of the genus *Diospyros*, and provide useful information in the field of phytochemistry and chemotaxonomy.

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CHAPTER II

HISTORICAL

Chemical constituents of *Diospyros* species

A variety of chemical compounds have been isolated from plants in the genus *Diospyros* : naphthoquinones, triterpenoids, steroids, tannins, coumarins, etc. Distribution of these constituents in various parts of the plants is shown in Table 1. Two groups of compounds, naphthoquinones and triterpenoids, are found widespread and present in almost all parts of *Diospyros* plants. These compounds can be used as chemical markers of the genus for taxonomic study. The occurrence of triterpenoids and steroids in the genus *Diospyros*, is summarized as follows.

Table 1. Distribution of chemical constituents in various parts of *Diospyros* species.

Class of Compounds	Plant part
Carotenoids	Fruit
Tannins	Fruit, leaf
Sugars	Fruit, seed, root
Hydrocarbons	Fruit, seed, leaf
Lipids	Fruit, seed, bark
Aromatics	Fruit, root, bark
Flavonoids/coumarins	Fruit, leaf, root, sapwood
Terpenoids	Fruit, leaf, calyx, seed, root, bark, heartwood
Steroids	Leaf, root, bark, heartwood
Naphthoquinones	Fruit, leaf, root, bark, heartwood

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Triterpenoids

Triterpenoids can be found widespread in the genus *Diospyros*. These metabolites are detected in almost all parts of the plants. *Diospyros* triterpenoids isolated so far are all with pentacyclic core and belong to the lupane, ursane, oleanane, taraxerane or friedelane types. The first three types are more prevalent in the genus.

The most common group of triterpenoids found in *Diospyros* is the lupanes. These compounds accumulate in bark and heartwood. Major metabolites of this type are betulin, betulinic acid and lupeol.

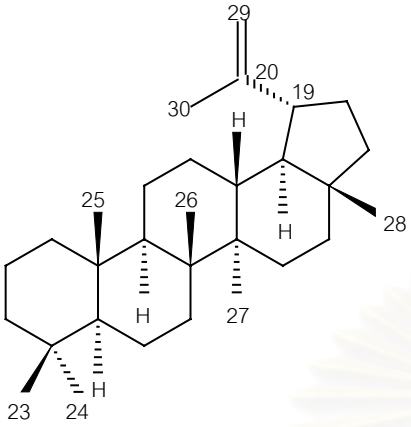
Another group of triterpenoids which are widely distributed in the genus *Diospyros* is the ursanes. Major metabolites of this type include α-amyrin, ursolic acid and baurenol. Ursolic acid accumulates in significant quantities in a number of *Diospyros* species and co-exists mostly with α-amyrin.

In the entire *Diospyros* genus, triterpene glycosides with oleanane skeleton were the only type being isolated. The aglycone of these glycosides is oleanolic acid which is the most abundant oleanane triterpenoid of the genus *Diospyros*.

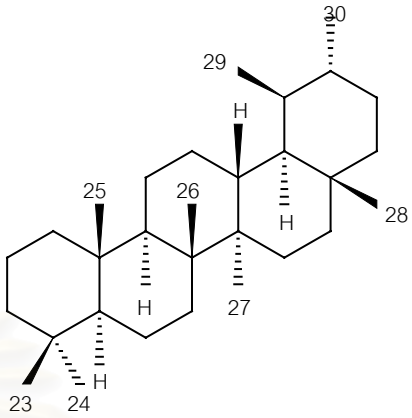
Only three metabolites of the taraxerane type have been found in the genus *Diospyros*. These metabolites are taraxerol, taraxerol acetate and taraxerone. It is interesting to note that further hydroxylation or unsaturation did not take place in this class of compounds and no significant biological activity has been reported for these metabolites.

Friedelanes do not seem to be widely represented in the genus *Diospyros*. There are only three reports on the isolation of friedelanes and related pentacyclic triterpenes (Mallavadhani, Panda and Rao, 1998).

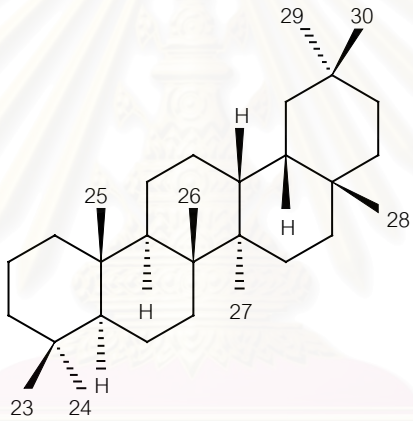
The occurrence of triterpenoids in the geus *Diospyros* is summarized in Table 2.



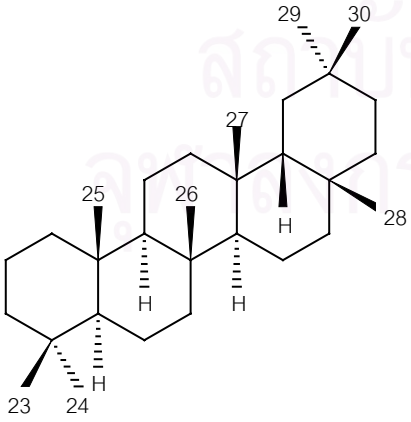
Lupane (A)



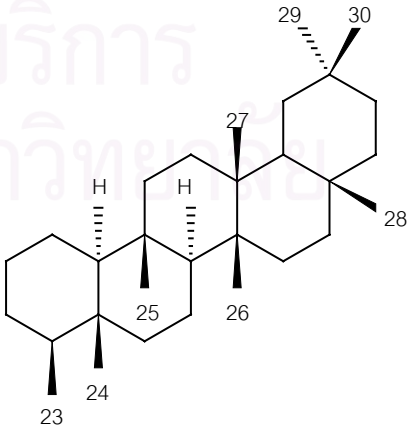
Ursane (B)



Oleanane (C)



Taraxerane (D)



Friedelane (E)

Table 2. Distribution of triterpenoids in the genus *Diospyros*.

Compounds	Sources	References
1. Friedelane type		
Friedelin **(1)**	*D. eriantha*	Chen, Yu and Huang,1992
	D. ferrea	Tiwarri, Masood and Minocha, 1979
	D. maritima	Higa, Orihara and Yogi,1998
	D. undulata var.*cratericalyx*	Aoonpakh, 2001
Friedelin-3-ol **(2)**	*D. eriantha*	Chen *et al*., 1992
	D. ferrea	Chandler and Hoope.,1979 Tiwari *et al*., 1979
2α-Hydroxyfriedelin **(3)**	*D. iturensis*	Zhong, Waterman and Jeffreys,1984
	D. sanza-minika	Zhong *et al*.,1984
2. Lupane type		
28-Acetyl-3-(*E*)-coumaroylbetulin **(4)**	*D. maritima*	Kuo, Chang and Kuo,1997b
Allobetulin **(5)**	*D. montana*	Lillie, Musgrave and Skoyles,1976a
Betulin **(6)**	*D. abyssinica*	Zhong *et al*.,1984
	D. argentea	Zakaria *et al*.,1984
	D. bipindensis	Waterman and Mbi,1979
	D. buxifolia	Bhakuni *et al*.,1971
	D. canaliculata	Zhong *et al*.,1984
	D. candolleana	Desai *et al*.,1970
	D. castanea	Musgrave and Skoyles,1974
	D. cauliflora	Musgrave and Skoyles,1974
	D. chevalieri	Zhong *et al*.,1984

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จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Betulin **(6)**	*D. chloroxylon*	Matsura *et al*.,1971
	D. cinnabarina	Waterman and Mbi, 1979 ; Zhong *et al*.,1984
	D. consolatae	Khan,Nkunya and Wevers, 1979, 1980; Paris and Prista, 1954
	D. cornii	Khan *et al*.,1980; Paris and Prista, 1954
	D. crassiflora	Zhong *et al*.,1984
	D. curranii	Musgrave and Skoyles,1974
	D. dendo	Zhong *et al*.,1984
	D. discolor	Zakaria *et al*.,1984
	D. ebenaster	Dominguez *et al*.,1979
	D. ebenum	Gupta and Mahadevan, 1967, 1968
	D. elliptifolia	Musgrave and Skoyles,1974
	D. embryopteris	Bhakuni *et al*.,1971
	D. eriantha	Chen *et al*.,1992
	D. evena	Musgrave and Skoyles,1974
	D. exsculpta	Bhakuni *et al*.,1971
	D. fragrans	Zhong *et al*.,1984
	D. gabunensis	Zhong *et al*.,1984
	D. gracilescens	Zhong *et al*.,1984
	D. guianensis	Braneton and Moretti, 1979
	D. hirsuta	Herath *et al*.,1978
	D. hoyleana	Zhong *et al*.,1984

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Betulin **(6)**	*D. indica*	Sundar Ramaiah *et al*.,1976
	D. ismailii	Zakaria *et al*.,1984
	D. iturensis	Zhong *et al*.,1984
	D. kaki	Andriamasy and Fouraste, 1978 ; Matsura *et al*.,1971
	D. kaki var. *sylvestris*	Tezuka *et al*.,1972
	D. kamerunensis	Zhong *et al*.,1984
	D. kirkii	Maria *et al*.,1980
	D. leucomelas	Recio *et al*.,1995a, 1995b
	D. longifolia	Zhong *et al*., 1984
	D. lotus	Yoshihira,Tezuka and Natori, 1971a; Zakaria *et al*.,1984
	D. maingayi	Musgrave and Skoyles,1974; Zakaria *et al*.,1984
	D. malanonilau	Singh and Prakash,1988
	D. mannii	Jeffreys, Zakaria and Waterman, 1983
	D. maritima	Tezuka *et al*.,1973
	D. melanoxylon	Gupta and Roa,1964; Rao, Rao and Sundar Ramaiah, 1964, 1966; Sankaram and Sidhu, 1964
	D. mespiliformis	Zhong *et al*.,1984
	D. microphylla	Bhakuni *et al*.,1971
	D. mollis	Musgrave and Skoyles, 1974 ; Yoshihira *et al*.,1971b

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Betulin **(6)**	*D. monobuttensis*	Zhong *et al*., 1984
	D. montana	Dutta,Dutta and Chakrarti, 1972 ; Misra, Nigam and Mitra 1972 ; Musgrave and Skoyles,1974
	D. moonii	Herath *et al*.,1978
	D. morrisiana	Yoshihira *et al*.,1971a
	D. obliquifolia	Waterman and Mbi,1979
	D. oblongifolia	Herath *et al*., 1978
	D. peregrina	Bhaumik *et al*.,1981 ; Dinda *et al*.,1995 ; Misra *et al*.,1971
	D. pseudo-malabarica	Musgrave and Skoyles, 1974
	D. quaesita	Herath *et al*.,1978
	D. rhodocalyx	Sutthivaiyakit *et al*., 1995
	D. rotundifolia	Gupta and Roa,1964
	D. sanza-minika	Musgrave and Skoyles,1974; Zhong *et al*.,1984
	D. siamang	Zakaria *et al*.,1984
	D. siamensis	Musgrave and Skoyles,1974
	D. siderophylla	Li *et al*.,1982
	D. singaporensis	Zakaria *et al*.,1984
	D. spinescens	Herath *et al*.,1978
	D. sumatrana	Zakaria *et al*.,1984
	D. sylvatica	Gupta and Roa,1964; Roa, Roa and Sundar Ramaiah, 1966

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Betulin **(6)**	*D. thwaitesii*	Herath *et al*.,1978
	D. tomentosa	Bhakuni *et al*.,1971
	D. undulata var.*cratericalyx*	Aoonpakh, 2001
	D. variegata	Musgrave and Skoyles,1974
	D. verrucosa	Khan, Kishimba and Lockslay, 1987a
	D. virginiana	Shukla and Kapadia,1989
	D. walkeri	Herath *et al*.,1978
	D. wallichii	Zakaria *et al*.,1984
	D. zenkeri	Zhong *et al*.,1984
Betulinic acid **(7)**	*D. abyssinica*	Zhong *et al*.,1984
	D. alboflavescena	Bouquet,1972
	D. argentea	Zakaria *et al*., 1984
	D. bipindensis	Waterman and Mbi,1979
	D. buxifolia	Bhakuni *et al*.,1971
	D. canaliculata	Zhong *et al*.,1984
	D. candolleana	Desai *et al*.,1970
	D. castanea	Musgrave and Skoyles, 1974
	D. cauliflora	Musgrave and Skoyles, 1974
	D. chevalieri	Zhong *et al*.,1984
	D. chloroxylon	Matsura *et al*.,1971
	D. cinnabarina	Waterman and Mbi,1979 ; Zhong *et al*.,1984
	D. consolatae	Khan *et al*., 1987a, 1987b, 1979, 1980
	D. crassiflora	Zhong *et al*.,1984
	D. curranii	Musgrave and Skoyles,1974

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Betulinic acid **(7)**	*D. dendo*	Zhong *et al*.,1984
	D. discolor	Lin, 1978 ; Zakaria et al., 1984
	D. ebenum	Brown and Thomson ,1965
	D. elliptifolia	Musgrave and Skoyles,1974
	D. embryopteris	Bhakuni *et al*.,1971
	D. eriantha	Chen *et al*., 1992
	D. evena	Musgrave and Skoyles, 1974
	D. exsculpta	Bhakuni *et al*.,1971
	D. ferrea	Bhakuni *et al*.,1971
	D. fragrans	Zhong *et al*.,1984
	D. gabunensis	Zhong *et al*.,1984
	D. gilleti	Bouquet , 1972
	D. gracilescens	Waterman and Mbi,1979 ; Zhong *et al*.,1984
	D. greeniwayi	Khan and Rwekika, 1992
	D. guaianensis	Braneton and Moretti, 1979
	D. hirsuta	Herath *et al*.,1978
	D. hoyleana	Bouquet,1973 ; Zhong *et al*.,1984
	D. ismailii	Zakaria *et al*.,1984
	D. iturensis	Zhong *et al*.,1984
	D. kaki	Andriamasy and Fouraste, 1978 ;Matsura *et al*.,1971
	D. kaki var. *sylvestris*	Tezuka *et al*.,1972
	D. kameerunensis	Zhong *et al*.,1984

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Betulinic acid **(7)**	*D. leucomelas*	Recio *et al*.,1995a, 1995b
	D. longiflora	Zhong *et al*.,1984
	D. lotus	Yoshihira *et al*., 1971a ; Zakaria *et al*.,1984
	D. mafiensis	Khan and Rwekika , 1999
	D. maingayi	Musgrave and Skoyles,1974; Zakaria *et al*.,1984
	D. mannii	Jeffreys *et al*.,1983
	D. maritima	Tezuka *et al*.,1973
	D. mespiliformis	Zhong *et al*.,1984
	D. monobuttensis	Zhong *et al*.,1984
	D. montana	Musgrave and Skoyles,1974; Likhitwitayawuid *et al*.,1999 ; Narayan *et al*.,1978
	D. moonii	Herath *et al*., 1978
	D. morrisiana	Yoshihira *et al*.,1971a
	D. obliquifolia	Waterman and Mbi , 1979
	D. palmeri	Dominguez *et al*.,1979
	D. peregrina	Dinda *et al*.,1995 ; Misra *et al*.,1971
	D. pseudo-malabarica	Musgrave and Skoyles,1974
	D. quaesita	Herath *et al*.,1978
	D. rhodocalyx	Sutthivaiyakit *et al*., 1995
	D. sanza-minika	Musgrave and Skoyles,1974; Zhong *et al*.,1984
	D. siamang	Zakaria *et al*.,1984

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Betulinic acid **(7)**	*D. siamensis*	Musgrave and Skoyles,1974
	D. siderophylla	Li *et al*.,1981
	D. singaporensis	Zakaria *et al*., 1984
	D. spinescens	Herath *et al*.,1978
	D. sumatrana	Zakaria *et al*.,1984
	D. sylvatica	Rao *et al*., 1966; Gupta and Rao, 1964
	D. thwaitesii	Herath *et al*.,1978
	D. tomentosa	Bhakuni *et al*.,1971
	D. verrucosa	Khan *et al*.,1980 ; Khan *et al*.,1987a
	D. virginiana	Shukla and Kapadia, 1989
	D. walkeri	Herath *et al*.,1978
	D. wallichii	Zakaria *et al*.,1984
	D. zenkeri	Zhong *et al*.,1984
Betulinaldehyde **(8)**	*D. canaliculata*	Zhong *et al*.,1984
	D. eriantha	Chen *et al*., 1992
3-(*E*)-Coumaroylbetulinaldehyde **(9)**	*D. maritima*	Chang and Kuo,1999
3-(*Z*)-Coumaroyllupeol **(10)**	*D. maritima*	Chang and Kuo,1998
3-(*E*)-Coumaroyl-28-palmitoyl betulin **(11)**	*D. maritima*	Chang and Kuo,1999
3-(*Z*)-Coumaroyl-28-palmitoylbetulin **(12)**	*D. maritima*	Chang and Kuo,1998

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
3-(*E*)- Coumaroylbetulin-28-yl ethylnonanedioate **(13)**	*D. maritima*	Kuo and Chang, 2000
3-(*E*)- Coumaroylbetulin-28-yl ethyl(*2R*)-2-hydroxysuccinate **(14)**	*D. maritima*	Kuo and Chang, 2000
3-(*E*)- Coumaroylbetulin-28-yl ethyl succinate **(15)**	*D. maritima*	Kuo and Chang, 2000
Epi-lupeol **(16)**	*D. ebenaster*	Dominguez *et al*.,1979
	D. palmeri	Dominguez *et al*.,1979
3-(*E*)-Feruloyl-28-palmitoylbetulin **(17)**	*D. maritma*	Chang and Kuo,1998
3-(*E*)-Feruloylbetulin **(18)**	*D. maritima*	Kuo *et al*.,1997b
Lupenone **(19)**	*D. mollis*	Yoshihira *et al*., 1971b
Lupeol **(20)**	*D. abyssinica*	Zhong *et al*.,1984
	D. acuta	Herath *et al*.,1978
	D. argentea	Zakaria *et al*.,1984
	D. bipindensis	Waterman and Mbi,1979
	D. buxifolia	Bhakuni *et al*.,1971
	D. canaliculata	Zhong *et al*.,1984
	D. candollenana	Desai *et al*.,1970
	D. castanea	Musgrave and Skoyles,1974
	D. cauliflora	Musgrave and Skoyles,1974
	D. chevalicri	Zhong *et al*.,1984
	D. cinnabarina	Waterman and Mbi,1979 ; Zhong *et al*.,1984
	D. consulatae	Khan *et al*., 1987b

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Lupeol (20)	*D. cordifolia*	Chandra and Shastry, 1989
	D. cornii	Khan *et al*.,1980
	D. crassiflora	Zhong *et al*.,1984
	D. curranii	Musgrave and Skoyles,1974
	D. dendo	Zhong *et al*.,1984
	D. diepenhorstii	Balza *et al*.,1989
	D. discolor	Zakaria *et al*.,1984
	D. ebenum	Gupta and Mahadevan,1967; Gupta and Mahadevan,1968
	D. ehretioides	Musgrave and Skoyles,1974
	D. elliptifolia	Musgrave and Skoyles,1974
	D. embryopteris	Bhakuni *et al*.,1971
	D. eriantha	Chen *et al*., 1992
	D. evena	Musgrave and Skoyles,1974
	D. exsculpta	Bhakuni *et al*.,1971
	D. fragrans	Zhong *et al*.,1984
	D. gabunensis	Zhong *et al*.,1984
	D. gracilescens	Waterman and Mbi,1979 ; Zhong *et al*.,1984
	D. greeniwayi	Khan and Rwekika ,1998
	D. guaianensis	Braneton and Moretti, 1979
	D. hirsuta	Herath *et al*.,1978
	D. hoyleana	Zhong *et al*.,1984
	D. ismailii	Zakaria *et al*.,1984
	D. iturensis	Zhong *et al*.,1984
	D. kaki var. *sylvestris*	Tezuka *et al*.,1972

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Lupeol (20)	*D. kamerunensis*	Zhong *et al*.,1984
	D. kirkii	Maria *et al*.,1979
	D. longiflora	Zhong *et al*., 1984
	D. lotus	Yoshihira *et al*., 1971a ; Zakaria *et al*.,1984
	D. mafiensis	Khan and Rwekika, 1992
	D. maingayi	Musgrave and Skoyles,1974; Zakaria *et al*.,1984
	D. mannii	Jeffreys *et al*., 1983
	D. maritima	Tezuka *et al*.,1973
	D. melanoxylon	Rao *et al*., 1964; Rao *et al*., 1966; Gupta and Rao, 1964
	D. mespiliformis	Zhong *et al*.,1984
	D. microphylla	Bhakuni *et al*.,1971
	D. mollis	Yoshihira *et al*., 1971b ; Musgrave and Skoyles,1974
	D. monobuttensis	Zhong *et al*.,1984
	D. montana	Musgrave and Skoyles,1974; Marayan, Row and Satyanarayana, 1978 ; Raj and Agrawal, 1979
	D. moonii	Herath *et al*.,1978
	D. morrisiana	Yoshihira *et al*.,1971a
	D. obliquifolia	Waterman and Mbi,1979
	D. oblongifolia	Herath *et al*.,1978
	D. oppositifolia	Herath *et al*.,1978

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Lupeol **(20)**	*D. peregrina*	Bhaumik *et al*.,1981 ; Dinda *et al*., 1995
	D. pseudo-malabarica	Musgrave *et al*.,1974
	D. quaesita	Herath *et al*.,1978
	D. quiloensis	Harper, Kemp and Tanock, 1970
	D. rheophytica	Herath *et al*.,1978
	D. rhodocalyx	Musgrave and Skoyles,1974; Sutthivaiyakit *et al*., 1995
	D. rotundifolia	Gupta and Rao, 1964
	D. sanza-minika	Musgrave and Skoyles,1974; Zhong *et al*.,1984
	D. siamang	Zakaria *et al*.,1984
	D. siamensis	Musgrave and Skoyles,1974
	D. siderophylla	Li *et al*.,1981
	D. singaporensis	Zakaria *et al*.,1984
	D. spinescens	Herath *et al*.,1978
	D. sumatrana	Zakaria *et al*.,1984
	D. thwaitesii	Herath *et al*.,1978
	D. tomentosa	Bhakuni *et al*.,1971
	D. toposia	Musgrave and Skoyles,1974
	D. variegata	Musgrave and Skoyles,1974
	D. walkeri	Herath *et al*.,1978
	D. zenkeri	Zhong *et al*.,1984
Oxyallobetulin **(21)**	*D. lotus*	Bhakuni *et al*.,1971 ; Yoshihira *et al*.,1971a

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Oxyallobetulin **(21)**	*D. montana*	Lillie, Musgrave and Skoyles,1976a
	D. morrisiana	Yoshihira *et al*.,1971a
Peregrinol **(22)**	*D. peregrina*	Jain and Yadav, 1994
3. Oleanane type		
β- Amyrin **(23)**	*D. lotus*	Yoshihira *et al*.,1971a
	D. morrisiana	Yan *et al*.,1989
Oleanolic acid **(24)**	*D. castanea*	Musgrave and Skoyles,1974
	D. cauliflora	Musgrave and Skoyles,1974
	D. curranii	Musgrave and Skoyles,1974
	D. evena	Musgrave and Skoyles,1974
	D. kaki	Matsura *et al*.,1977
	D. montana	Dutta *et al*.,1972 ; Misra.,1972; Musgrave and Skoyles,1974
	D. moonii	Herath *et al*.,1978
	D. oblongifolia	Herath *et al*.,1978
	D. tomentosa	Bhakuni *et al*.,1971
	D. zombensis	Gafner *et al*.,1987 ; Gafner and Rodriguez, 1988
Olean-12-ene-3-one **(25)**	*D. morrisiana*	Yan *et al*.,1989
Oleanolic acid glycosides	*D. peregrina*	Gupta and Tiwari, 1964
(26, 27, 28, 29)	*D. zombensis*	Gafner *et al*.,1987 ; Gafner and Rodriguez, 1988

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Oleanolic acid acetate **(30)**	*D. eriantha*	Chen *et al*.,1992
	D. lotus	Zakaria *et al*.,1984
Oleanolic acid palmitate **(31)**	*D. montana*	Misra *et al*.,1972
Oleanolic acid stearate **(32)**	*D. montana*	Misra *et al*.,1972
4. Taraxerane type		
Taraxerol **(33)**	*D. cordifolia*	Chandra and Shastry, 1989
	D. ferrea	Bhakuni *et al*.,1971
	D. hirsuta	Herath *et al*.,1978
	D. kaki	Zhong and Feng, 1987
	D. lotus	Bhakuni *et al*.,1971;
		Yoshihira *et al*.,1971a;
		Zakaria *et al*.,1984
	D. mollis	Yoshihira *et al*.,1971b
	D. morisiana	Yoshihira *et al*.,1971a
	D. nicaraguensis	Hasbun *et al*., 1988
Taraxerone **(34)**	*D. acuta*	Herath *et al*.,1978
	D. ferrea	Bhakuni *et al*.,1971
	D. lotus	Zakaria *et al*.,1984
	D. maritima	Kuo *et al*.,1997c
	D. moonii	Herath *et al*.,1978
	D. oblongifolia	Herath *et al*.,1978
	D. oppositifolia	Herath *et al*.,1978
	D. quaesita	Herath *et al*.,1978
	D. rheophytica	Herath *et al*.,1978
	D. rhodocalyx	Sutthivaiyakit *et al*., 1995

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Taraxerone **(34)**	*D. thwaitesii*	Herath *et al.*,1978
Taraxeryl acetate **(35)**	*D. maingayi*	Zakaria *et al.*,1984
	D. singaporensis	Zakaria *et al.*,1984
5. Ursane type		
3 - β - Acetoxy-urs-11-ene-28, 13-olide **(36)**	*D. eriantha*	Chen *et al.*,1992
α – Amyrenone **(37)**	*D. ebenum*	Gupta and Mahadevan, 1967; Sharma and Gupta, 1985
α - Amyrin **(38)**	*D. cornii*	Khan *et al.*,1980 ; Gafner *et al.*,1987
	D. ebenum	Brown and Thomson, 1965
	D. kaki	Andriamasy and Fouraste, 1978
	D. kirkii	Khan et al.,1980 ; Khan *et al.*, 1987
	D. mafiensis	Khan and Rwekika, 1999
	D. maingayi	Zakaria *et al.*,1984
	D. melanoxylon	Choudhury, 1973
	D. mespiliformis	Khan *et al.*,1980
	D. montana	Misra *et al.*,1972
	D. natalensis	Khan and Rwekika, 1992
	D. sylvatica	Rao and Rao, 1968

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

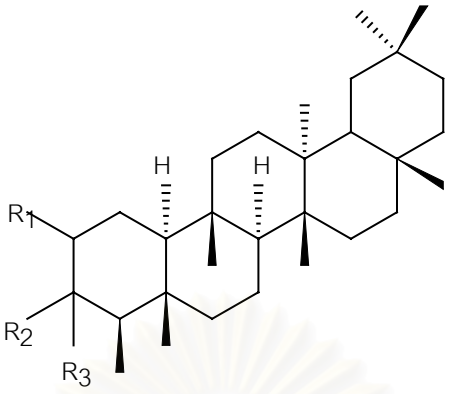
Compounds	Sources	References
Baurenol **(39)**	*D. ebenum*	Gupta and Mahadevan,1967 ; Sharma and Gupta, 1985
	D. kirkii	Khan *et al*.,1987b ; Khan *et al*.,1979
	D. melanoxylon	Rao *et al*., 1969
	D. mespiliformis	Khan *et al*.,1979
	D. sylvatica	Rao and Rao, 1968
Epi-uvaol **(40)**	*D. montana*	Dutta *et al*.,1972
19α-Hydroxyursolic acid **(41)**	*D. kaki*	Matsura and Iinuma, 1977
Marsformosanone **(42)**	*D. peregrina*	Bhaumik *et al*.,1981
Ursolic acid **(43)**	*D. castanea*	Musgrave and Skoyles, 1974
	D. cauliflora	Musgrave and Skoyles, 1974
	D. curranii	Musgrave and Skoyles,1974
	D. ebenum	Sharma and Gupta, 1985
	D. evena	Musgrave and Skoyles, 1974
	D. ferrea	Bhakuni *et al*.,1971
	D. hirsuta	Hearth *et al*.,1978
	D. kaki	Matsura *et al*.,1971 ; Matsura and Iinuma, 1977
	D. leucomelas	Recio *et al*., 1995a ; 1995b
	D. lotus	Yoshihira *et al*.,1971a ; Zakaria *et al*., 1984

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 2. Distribution of triterpenoids in the genus *Diospyros* (continued).

Compounds	Sources	References
Ursolic acid **(43)**	*D. montana*	Misra *et al*.,1972 ; Musgrave And Skoyles,1974; Zafar, Singh and Khan, 1991
	D. morrisiana	Yoshihira *et al*.,1971a
	D. quaesita	Herath *et al*.,1978
	D. tomentosa	Bhakuni *et al*.,1971
Ursolic acid acetate **(44)**	*D. eriantha*	Chen *et al*.,1992
	D. lotus	Yoshihira *et al*.,1971a
Ursolic acid palmitate **(45)**	*D. montana*	Misra *et al*.,1972
Ursolic acid stearate **(46)**	*D. montana*	Misra *et al*.,1972
Uvaol **(47)**	*D. lotus*	Zakaria *et al*.,1984
	D. maingayi	Zakaria *et al*.,1984
6. Miscellaneous		
Glut-5(6)-ene-3-β-ol **(48)**	*D. iturensis*	Zhong *et al*.,1984
	D. sanza-minika	Zhong *et al*.,1984

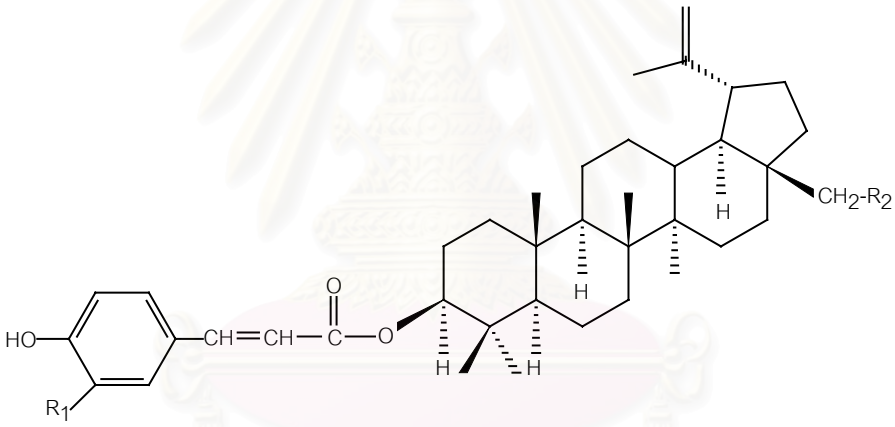
สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย



Friedelin **(1)** R_1 = H ; R_2 , R_3 = OH

Friedelin-3-ol **(2)** R_1 = H ; R_2 = β - H ; R_3 = α - OH

2α-hydroxyfriedelin (Cerin) **(3)** R_1 = α - OH ; R_2 , R_3 = =O



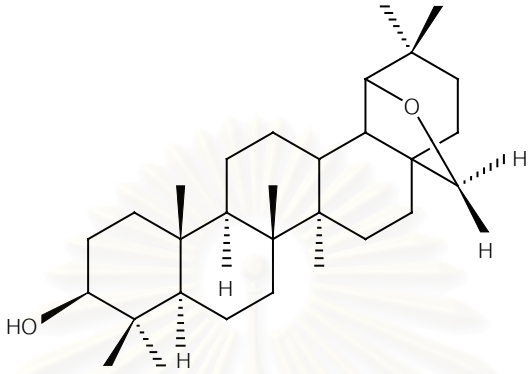
28-Acetyl-3-(*E*)-coumaroylbetulin **(4)**

R_1= H ; R_2= -O-C-CH_3 (O above C)

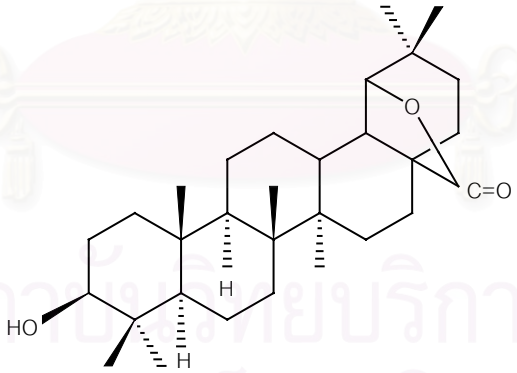
3-(*E*)-Feruloylbetulin **(18)**

R_1= OCH_3 ; R_2= -OH

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

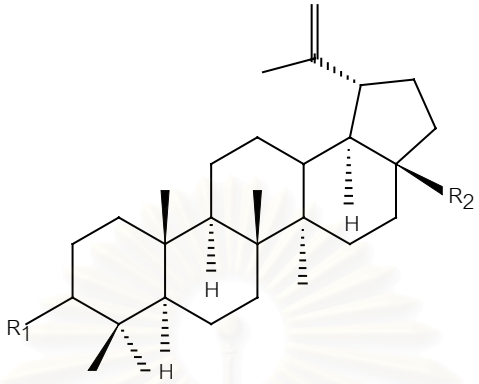


Allobetulin (5)



Oxyallobetulin (21)

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย



Betulin **(6)** R_1 = β- OH ; R_2 = CH_2OH

Betulinic acid **(7)** R_1 = β- OH ; R_2 = COOH

Betulinaldehyde **(8)** R_1 = β- OH ; R_2 = CHO

Epi-lupeol **(16)** R_1 = α- OH ; R_2 = CH_3

Lupenone **(19)** R_1 = =O ; R_2 = CH_3

Lupeol **(20)** R_1 = β- OH ; R_2 = CH_3

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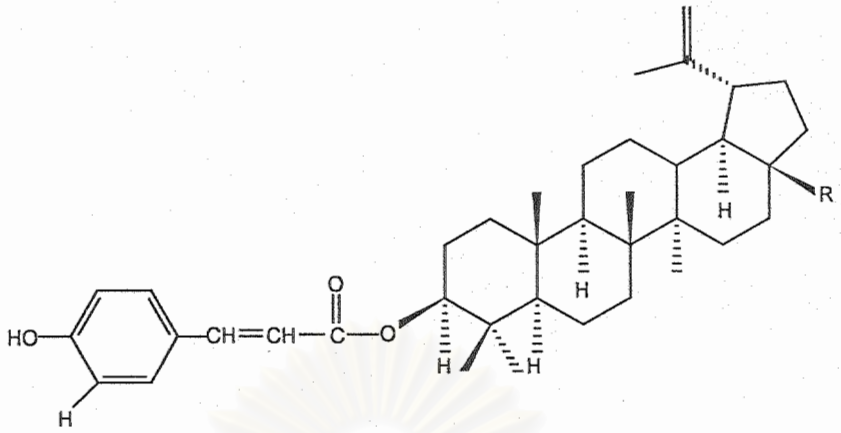

3-(*E*)-Coumaroylbetulinaldehyde **(9)** R = CHO

3-(*Z*)-Coumaroyllupeol **(10)** R = CH_3

3-(*E*)-Coumaroyl-28-palmitoylbetulin **(11)** R = $CH_2\text{-}O\text{-}C(=O)\text{-}CH_2\text{-}CH_2\text{-}(CH_2)_{10}CH_2\text{-}CH_2CH_3$

3-(*Z*)-Coumaroyl-28-palmitoylbetulin **(12)** R = $CH_2\text{-}O\text{-}C(=O)\text{-}CH_2\text{-}CH_2\text{-}(CH_2)_{12}CH_3$

3-(*E*)-Coumaroylbetulin-28-yl ethylnonanedioate **(13)** R = $CH_2\text{-}O\text{-}C(=O)\text{-}CH_2\text{-}(CH_2)_5CH_2\text{-}CO_2CH_2CH_3$

3-(*E*)-Coumaroylbetulin-28-yl ethyl(2*R*)-2-hydroxysuccinate **(14)** R = $CH_2\text{-}O\text{-}C(=O)\text{-}CH(OH)\text{-}CH_2\text{-}CO_2\text{-}CH_2CH_3$

3-(*E*)-Coumaroylbetulin-28-yl ethyl succinate **(15)** R = $CH_2\text{-}O\text{-}C(=O)\text{-}CH_2\text{-}CH_2\text{-}CO_2\text{-}CH_2CH_3$

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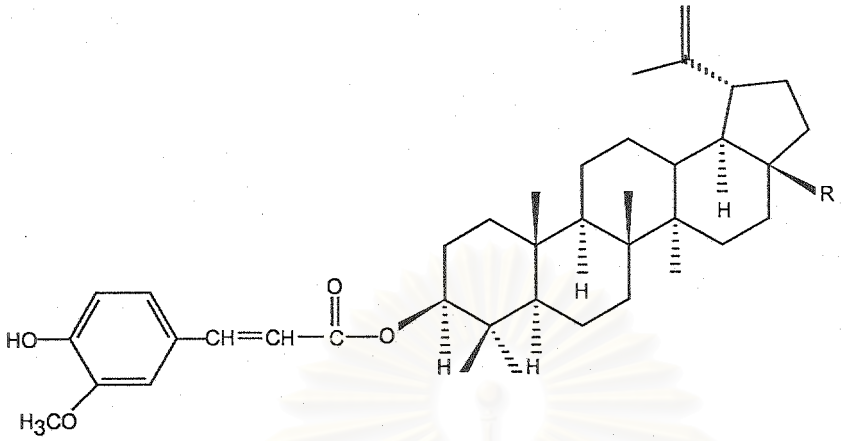

3-(*E*)-Feruloyl-28-palmitoylbetulin **(17)** R = CH_2-O-C(=O)-CH_2-$(CH_2)_{13}CH_3$

3-(*E*)-Feruloylbetulin **(18)** R = CH_2-OH

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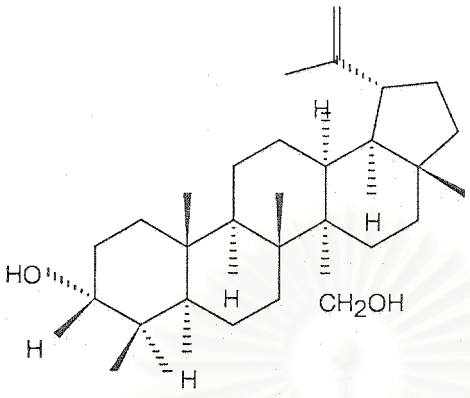
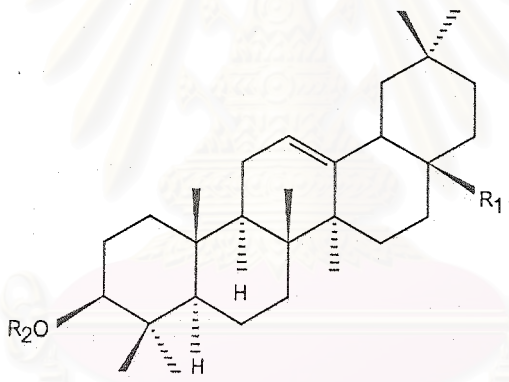


Peregrinol (22)



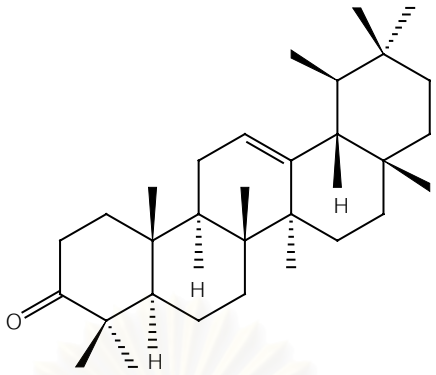
β - Amyrin (23) $R_1 = CH_3$; $R_2 = H$

Oleanolic acid (24) $R_1 = COOH$; $R_2 = H$

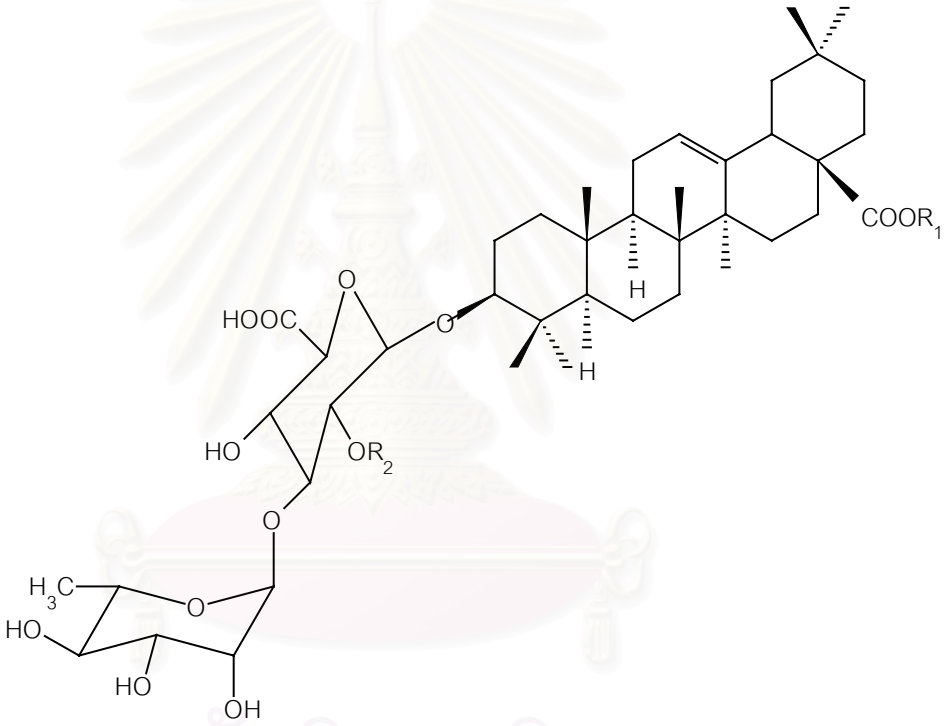
Oleanolic acid acetate (30) $R_1 = COOH$; $R_2 = COCH_3$

Oleanolic acid palmitate (31) $R_1 = COOH$; $R_2 = CO(CH_2)_{14}CH_3$

Oleanolic acid stearate (32) $R_1 = COOH$; $R_2 = CO(CH_2)_{16}CH_3$



Olean-12-ene-3-one **(25)**



Oleanolic acid glycosides **(26)** R_1 = R_2 = H

(27) R_1 = Glucosyl ; R_2 = H

(28) R_1 = Glucosyl ; R_2 = Xylosyl

(29) R_1 = H ; R_2 = Xylosyl

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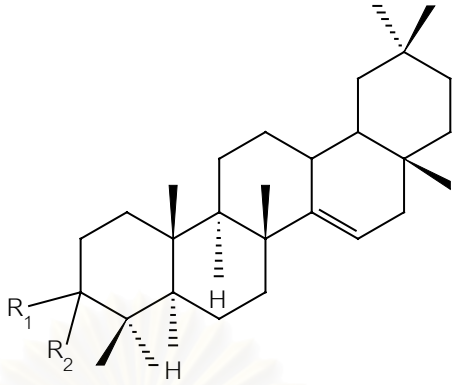
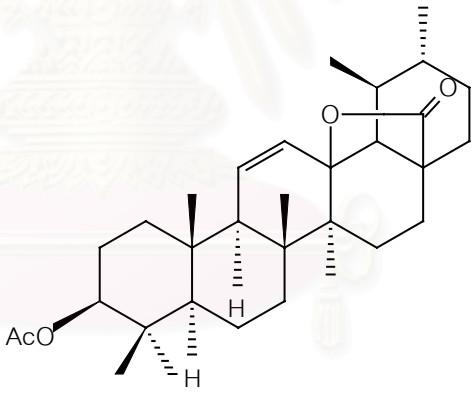


Taraxerol **(33)** R_1 = α- H ; R_2 = β- OH

Taraxerone **(34)** R_1, R_2 = =O

Taraxeryl acetate **(35)** R_1 = α- H ; R_2 = β- $OCOCH_3$



3β-Acetoxy-urs-11-ene-28,13-olide **(36)**

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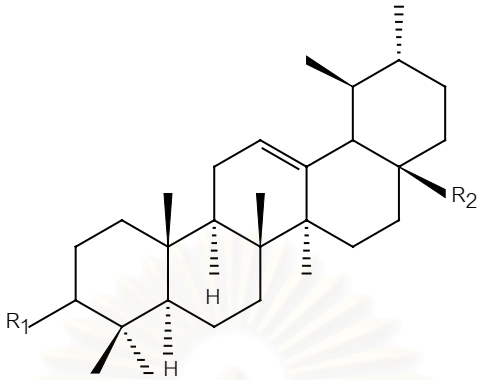

α – Amyrenone **(37)** R_1 = =O ; R_2 = -CH_3

α- Amyrin **(38)** R_1 = β- OH ; R_2 = CH_3

Epi-uvaol **(40)** R_1 = α- OH ; R_2 = CH_2OH

Ursolic acid **(43)** R_1 = β- OH ; R_2 = COOH

Ursolic acid acetate **(44)** R_1 = β- $OCOCH_3$; R_2 = COOH

Ursolic acid palmitate **(45)** R_1 = β- $OCO(CH_2)_{14}CH_3$; R_2 = COOH

Ursolic acid stearate **(46)** R_1 = β- $OCO(CH_2)_{16}CH_3$; R_2 = COOH

Uvaol **(47)** R_1 = β- OH ; R_2 = CH_2OH

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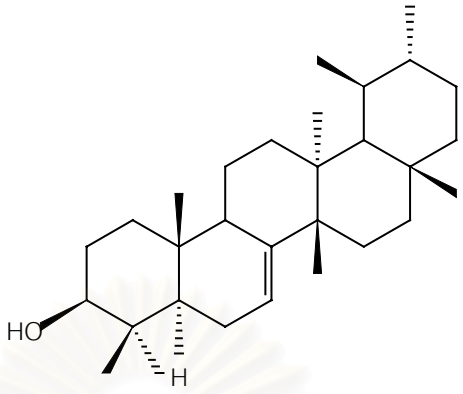
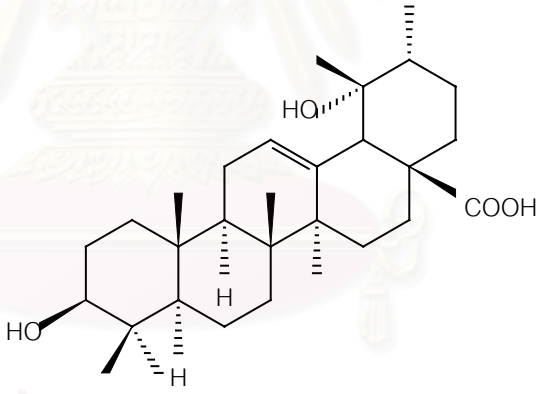


Baurenol (39)



19 α-Hydroxyursolic acid (41)

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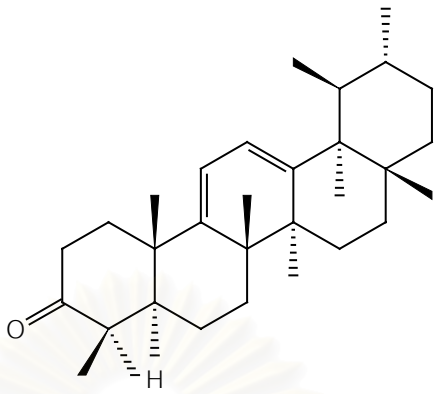
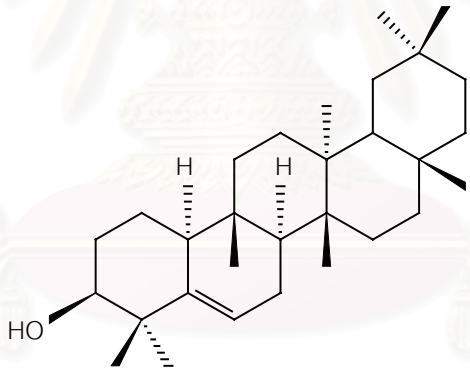


Marsformosanone (42)



Glut-5(6)-ene-3-β-ol (48)

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Steroids

Only six steroid compounds have been detected and isolated from *Diospyros* species. The most common steroid found is β-sitosterol, which occurs in both free and glycosidic forms. β-Sitosterol was found to accumulate in a number of species and so far it was detected in more than 30 *Diospyros* plants. Campesterol, Stigmasterol, Stigmasta-4-ene-3-one and Stigmasta-5,6-dihydro-22-en-3β-ol are the other steroids found in the *Diospyros* genus.

Table 3. Distribution of steroids in the genus *Diospyros*.

Compounds	Sources	References
β- Sitosterol **(49)**	*D. acuta*	Herath *et al*.,1978
	D. buxifolia	Bhakuni *et al*.,1971; Musgrave and Skoyles,1974
	D. chaetocarpa	Herath *et al*.,1978
	D. chloroxylon	Rao and Sunder, 1964 ; Sidhu *et al*.,1968
	D. cordifolia	Chandra and Shastry, 1989
	D. discolor	Rao *et al*., 1964 ; Sunder *et al*.,1976
	D. ebenaster	Dominguez *et al*.,1979
	D. ebenum	Sharma and Gupta, 1985
	D. eriantha	Chen *et al*., 1992
	D. ferrea	Bhakuni *et al*., 1971
	D. hirsuta	Herath *et al*., 1978

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Table 3. Distribution of steroids in the genus *Diospyros* (continued).

Compounds	Sources	References
β- Sitosterol **(49)**	*D. indica*	Sunder *et al*.,1976
	D. kaki	Rao *et al*., 1964 ; Matsura and Iinuma, 1977 ; Lin *et al*.,1989
	D. kirkii	Maria *et al*.,1980
	D. lotus	Bhakuni *et al*.,1971
	D. malanonilau	Bhakuni *et al*.,1971 ; Srivastava and Kharya, 1980
	D. melanoxylon	Gupta and Roa, 1964 ; Sankaram and Sidhu, 1964 ; Sidhu *et al*., 1968
	D. moonii	Dutta,Dutta and Chakrarti, 1972
	D. montana	Dutta, Dutta and Chakrarti, 1972 ; Goutum and Purohit, 1977 ; Misra, Nigam and Mitra, 1972 ; Raj and Agrawal, 1979
	D. morrisiana	Chen *et al*., 1989 ; 1992
	D. oblongifolia	Herath *et al*.,1978
	D. oppositifolia	Herath *et al*.,1978
	D. peregrina	Gupta and Roa, 1964 ; Gupta and Tiwari,1964 ; Misra *et al*., 1971 ; Misra, Nigam,and Mitra, 1972
	D. quaseita	Herath *et al*.,1978

Table 3. Distribution of steroids in the genus *Diospyros* (continued).

Compounds	Sources	References
β- Sitosterol **(49)**	*D. rheophytica*	Herath *et al*.,1978
	D. spinescens	Herath *et al*.,1978
	D. texana	Dominguez *et al*.,1979
	D. tomentosa	Bhakuni *et al*.,1971
	D. thwaitesii	Herath *et al*.,1978
	D. walkeri	Herath *et al*.,1978
β-Sitosterol glucoside **(50)**	*D. kaki*	Matsura and Iinuma, 1977
	D. montana	Dutta,Dutta and Chakrarti, 1972 ; Misra, Nigam and Mitra, 1972
	D. morrisiana	Chen *et al*.,1989
	D. peregrina	Misra *et al*., 1971 ; Misra, Nigam and Mitra, 1972
Stigmasterol **(51)**	*D. buxifolia*	Musgrave and Skoyles, 1974
	D. castanea	Musgrave and Skoyles, 1974
	D. cauliflora	Musgrave and Skoyles, 1974
	D. curranii	Musgrave and Skoyles,1974
	D. dipenhorstii	Musgrave and Skoyles, 1974
	D. ebenum	Sharma and Gupta.,1985
	D. evena	Musgrave and Skoyles, 1974
	D. kaki	Lin, Chou and Chen, 1988
	D. mollis	Musgrave and Skoyles, 1974

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Table 3. Distribution of steroids in the genus *Diospyros* (continued).

Compounds	Sources	References
Stigmasterol **(51)**	*D. montana*	Musgrave and Skoyles,1974; Dutta, Dutta and Chakrarti, 1972 ; Goutum and Purohit, 1977
	D. morrisiana	Chen *et al*.,1992
	D. sanza-minika	Musgrave and Skoyles,1974
Stigmasta-4-ene-3-one **(52)**	*D. morrisiana*	Musgrave and Skoyles,1974
Stigmasta-5,6-dihydro-22-en-3β-ol **(53)**	*D. morrisana*	Musgrave and Skoyles,1974
Campesterol **(54)**	*D. discolor*	Sidhu and Prasad, 1971
	D. kaki	Lin, Chou and Chen, 1988

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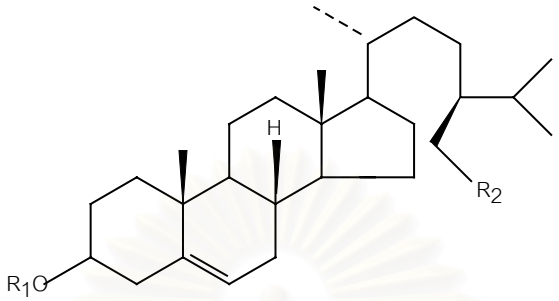
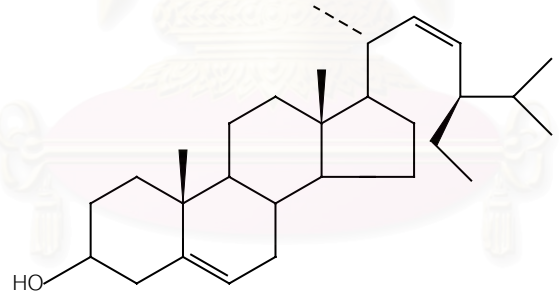


β- Sitosterol (49) $R_1 = H$; $R_2 = CH_3$

β-Sitosterol glucoside (50) R_1 = glucose ; $R_2 = CH_3$



Stigmasterol (51)

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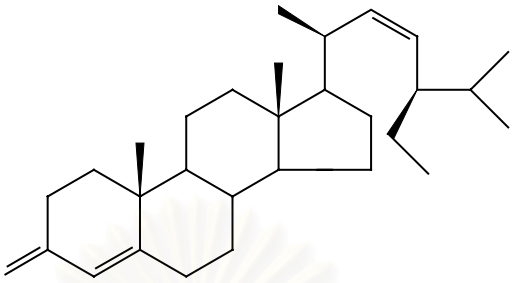
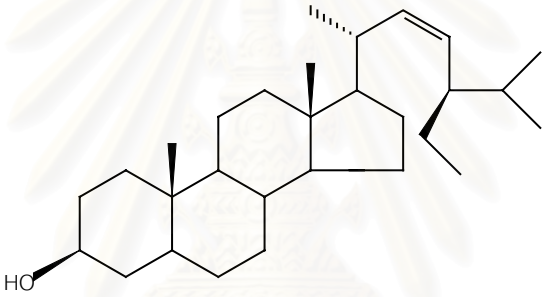
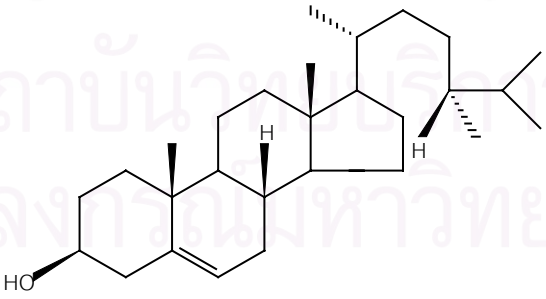


Stigmasta-4-en-3-one (52)



Stigmasta-5,6-dihydro-22-en-3β-ol (53)



Campesterol (54)

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Ethnomedicinal uses of *Diospyros* species

Plants in the genus *Diospyros* have been known for their medicinal uses since older times. Almost all parts of these plants have been used as medicines. Mallavadhani *et al.* (1998) have reviewed their uses in traditional medicines of various countries. In Thailand, medicinal uses of about 10 *Diospyros* species, have been recorded, as presented in Table 4.

Table 4. Uses of *Diospyros* species in Thai traditional medicine.

Species	Part used	Medicinal uses	References
D. decandra จันขาว	Wood	Antipyretic, anthelmntic, antiperspirant	นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2539
	Heart wood	Antipyretic, tonic	
	Fruit	Antidiarrhoeal	
D. rhodocalyx ตะโกนา	Root	Antipyretic, lactigenous	นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2541
	Stem	Antipyretic, antipruritic, tonic, diuretic, lactigenous	
	Stem bark	Diuretic, antitoothache	
	Bark	Digestive, diuretic, antitoothache	
	Wood	Tonic, antitoothache, aphrodisiac	
	Fruit	Antidiarrhoeal, astringent, antipruritic, anthelmintic	
D. peregrina ตะโก	Root	Anthelmintic, antidiarrhoeal	นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2541
	Bark	Anthelmintic, antidiarrhoeal, antidysentery and digestive	
	Stem bark	Astringent, tonic, anti-emetic	
	Fruit	Antidiarrhoeal, anthelmintic	

Table 4. Uses of *Diospyros* species in Thai traditional medicine (continued).

Species	Part used	Medicinal uses	References
D. peregrina	Fruit	Astringent	นันทวัน บุณยะประภัศร และ
ตะโก	Flower	Anthelmintic	อรนุช โชคชัยเจริญพร, 2541
D. ehretioides	Root	Antituberculosis, mucolytic	นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร, 2541
D. transitoria	Root	Anthelmintic	นันทวัน บุณยะประภัศร และ
	Wood	Anthelmintic	อรนุช โชคชัยเจริญพร, 2541
D. areolata	Root	Antidysentery, anthelmintic	นันทวัน บุณยะประภัศร และ
	Stem bark	Antidiarrhoeal, carminative	อรนุช โชคชัยเจริญพร, 2542
	Wood	Antidiarrhoeal	
	Gum	Antidysentery, antidiarrhoeal	
	Flower	Anthelmintic	
	Fruit	Astringent, anthelmintic, antidiarrhoeal	
D. malabarica	Root	Anthelmintic, antidiarrhoeal	นันทวัน บุณยะประภัศร และ
var. *siamensis*	Stem bark	Antidiarrhoeal, carminative	อรนุช โชคชัยเจริญพร, 2542
	Flower	Anthelmintic	
	Fruit	Astringent	
D. mollis	Root	Anthelmintic and antiemetic	นันทวัน บุณยะประภัศร และ
	Fruit, seed	Anthelmintic	อรนุช โชคชัยเจริญพร, 2542

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จุฬาลงกรณ์มหาวิทยาลัย

Table 4. Uses of *Diospyros* species in Thai traditional medicine (continued).

Species	Part used	Medicinal uses	References
D. rubra และ	Root, bark	Antitumor	นันทวัน บุณยะประภัศร
	Heart wood	Antituberculosis (duodenal & lung)	อรนุช โชคชัยเจริญพร, 2542
	Stem	For backache	
	Leaf	Anti-inflammatory	
	Wood, gum	Antituberculosis	
D. variegata	Root, bark	Antitumor	นันทวัน บุณยะประภัศร และ
	Heart wood	Antituberculosis	อรนุช โชคชัยเจริญพร, 2542
	Stem	Analgesic	
	Leaf, wood	For cure wounds	

Pharmacological Activity of *Diospyros* species

Many *Diospyros* species have been reported as exhibiting interesting bioactivity. The pharmacological activities of extracts and isolated compounds from these plants are summarized in Table 5 and Table 6, respectively.

Table 5. Pharmacological activities of *Diospyros* extracts.

Species	Part	Extract	Pharmacological activity	Refereces
D. chloroxylon	Aerial parts	50% EtOH	Antiviral	Dhar *et al*., 1973
D. cordifolia	NS	Alcohol	Anti-inflammatory,antipyretic, analgesic, CNS depressant, spasmolytic,produces bradycardia and hypotension	Singh *et al*., 1971 ; Kohli *et al*., 1972
D. embryopteris	Leaves	80% EtOH	Showed abolition of libido	Choudhary *et al*., 1990
D. exsculpta	Aerial parts	50% EtOH	Showed activity on cardiovascular system	Bhakuni *et al*., 1971
	Seeds	Unsaponified matter	Produced fall in blood pressure and increase in respiration,also showed anorexia, CNA depressant and antibacterial activities	

Table 5. Pharmacological activities of *Diospyros* extracts (continued).

Species	Part	Extract	Pharmacological activity	References
D. insignis	Aerial parts	50% EtOH	Antifertility	Dhawan *et al*., 1977
D. kaki	Leaves	Tannin	(i) Increases life span and decreases brain haemorrhage and infraction (ii)Showed scavenging action forwards active oxygen free radicals (iii)Inhibited lipid peroxidation	Uchida *et al*., 1990
	Leaves	MeOH	Showed hypotensive activity against urethane anaesthatised rats	Funayama and Hikino, 1979
	Fruit	---	(i) Showed strong detoxifying activity on various snake venoms	Fukami *et al*., 1979
	Fruit	---	(ii) Inactivated bacterial toxins	Fukami *et al*., 1979
D. leucomelas	Leaves	CH_2Cl_2& MeOH	Showed anti-inflammatory activity	Recio *et al*., 1995a Recio *et al*., 1995b

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Table 5. Pharmacological activities of *Diospyros* extracts (continued).

Species	Part	Extract	Pharmacological activity	References
D. melanoxylon	Seed	Unsaponified matter	Antibacterial	Aloskar *et al*., 1992
D. mespiliformis	Seed	----	Antibacterial	Lajubutu *et al*., 1995
D. montana	Leaves	Pet.ether, CCl_4,C_6H_6	Antibacterial	Goutam and Purohit, 1973
	Bark	90% EtOH	Inhibited the growth of Ehrlich ascites carcinoma in mice	Hazra *et al*., 1981
	---	---	Showed potent anti-inflammatory and antipyretic activities in rats and analgesic activity in mice	Singh *et al*., 1973

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จุฬาลงกรณ์มหาวิทยาลัย

Table 5. Pharmacological activities of *Diospyros* extracts (continued).

Species	Part	Extract	Pharmacological activity	References
D. montana	Bark	Alcohol	Showed CNS depressant activity, decrease locomoter activity and loss of righting reflux in mice and rats, spasmolytic activity on rabbit and guinea pig ileum and produced hypotension in anaesthetised dogs	Singh et al., 1971
D. morrisiana	Stem	Hexane	Showed significant cytotoxicity against of human KB and A-549 lung carcinoma, HCT-8 colon tumor and murine P-338 and L-1210 lymphocytic leukaemia	Yan *et al*., 1989
D. peregrina	Fruit	Ether	Antibacterial	Joshi and Magar, 1952
	NS	Alcohol	Anti-amoebic, anti-viral and hypoglycaemic activities	Dhar *et al*., 1968
	Aerial parts	50% EtOH	Showed activity on human epidermoid carcinoma of nasopharynx in tissue culture and diuretic activity	Dhawan *et al*., 1980

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 5. Pharmacological activities of *Diospyros* extracts (continued).

Species	Part	Extract	Pharmacological activity	References
D. peregrina	---	EtOAc	Significantly prevented rats from stress, gastric ulcers and hepatotoxicity	Singh et al., 1988
D. virginiana	Fruit	---	Produced tumors at the injection site in 66% or more of the treated rats	Kapadia *et al*., 1976
	Fruit	---	Showed cholesterol lowering activity	Ebihara *et al*., 1979 ; 1980
D. zombensis	Root bark	Petrol & $CHCl_3$	Showed cytotoxicity against human colon carcinoma cells	Gafner *et al*., 1987 Gafner *et al*., 1988 ; 1989

Remark ; NS : not specified.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 6. Pharmacological activities of compounds isolated from *Diospyros* species.

Compounds	Pharmacological activity	References
β-Amyrin	Moderately cytotoxic	Yan *et al*., 1989
Betulin	(i) Anti-inflammatory activity	Recio *et al*., 1995
	(ii) Active against the Walker-Carcinoma-256 tumor system	Misra and Pandey, 1989
Betulinic acid	(i) Showed potent anti-inflammatory activity against TPA-induced edema	Recio *et al*., 1995a Recio *et al*., 1995b
	(ii) Active against the Walker-Carcinoma-256 tumor system	Misra and Pandey, 1989
	(iii) Inhibited P-388 leukaemia growth	Chen *et al*., 1989
	(iv) Highly selective activity against human melanoma *in vitro* and *in vivo.*	Pisha *et al*., 1995

Table 6. Pharmacological activities of compounds isolated from *Diospyros* species (continued).

Compounds	Pharmacological activity	References
Lupeol	Active against the Walker-Carcinoma-256 tumor system	Misra and Pandey, 1989
Lupeol acetate	Inhibited stress induce ulcers in rats,also decreases incidence of gastric ulceration	Gupta *et al*., 1981
2α,3β-Dihydroxy-olean -12-ene-28-oic acid (maslinic acid)	(i) Showed anti-inflammatory effect and inhibitory effect on histamine induced ileum contraction	Shimizu *et al*., 1986
	(ii) Showed potent inhibitory activity against HIV-1 protease	Xu *et al*., 1996
Oleanolic acid	(i) inhibited 12-*O*-tetradecanoyl-phorbol-13-acetate induced Epstein-Barr virus activation	Ohigashi *et al*., 1986
	(ii)Showed potent antiarthritic and anti-inflammatory activities	Singh *et al*., 1994

Table 6. Pharmacological activities of compounds isolated from *Diospyros* species (continued).

Compounds	Pharmacological activity	References
Taraxerol	Inhibited stress induced ulcers in rats, decreases incidence of gastric ulceration	Gupta *et al*., 1981
Ursolic acid	(i) Anti-inflammatory activity	Recio *et al*., 1995
	(ii) inhibited 12-*O*-tetradecanoyl-phorbol-13-acetate induced Epstein-Barr virus activation	Ohigashi *et al*., 1986
	(iii)Suppressed tumor promoter induced inflammation of mouse ear	Hirota *et al*., 1990
	(iv) Inhibited stress induced ulcers in rats, also decreases incidence of gastric ulceration induced by pyrolic ligation	Gupta *et al*., 1981
	(v) Increases blood sugar concentration, glycogen and ATP contents in muscles, heart and uterus on intergastric administration into rats	Golovina and Vasilenko, 1976 Golovina and Vasilenko, 1978

จุฬาลงกรณ์มหาวิทยาลัย

Table 6. Pharmacological activities of compounds isolated from *Diospyros* species (continued).

Compounds	Pharmacological activity	References
Ursolic acid	(vi) Showed potent inhibitory activity against HIV-1 protease	Singh *et al*., 1994
Plumbagin	(i) stains the skin,produces blisters and effect mucous membrane	Roy *et al*., 1955
	(ii) inhibits the growth of all gram +ve and -ve test bacteria	Apandi *et al*., 1994
7-Methyljuglone	Showed cytotoxic activitiy against human colon carcinoma cells	Gafner *et al*., 1987 ; Gafner *et al*., 1988 ; Gafner *et al*., 1989 ; Marston *et al*., 1986
Diospyrin	(i) Inhibited the *in vivo* growth of Ehrlich Ascites Carcinoma (EAC). in Swiss Albino mice	Hazra *et al*., 1984

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 6. Pharmacological activities of single isolated metabolites of *Diospyros* species (continued).

Compounds	Pharmacological activity	References
Diospyrin	(ii) Showed *in vitro* activity against the protozoan *L. donovani*	Hazra *et al*., 1986
Isodiospyrin	Showed cytotoxic activity against HCT-8 colon tumer and P-388 lymphocytic leukaemia	Gafner *et al*., 1987 ; Gafner *et al*., 1988 ; Gafner *et al*., 1989 ; Yan *et al*., 1989
Diospyrol	Showed anthelmintic activity	Fukami *et al*., 1978 ; Fukami *et al*., 1979 ; Sen *et al*., 1974. Sen *et al*., 1975.
Astragalin	Inhibited the angiotensin converting enzyme activity	Kameda *et al*., 1987
Kaempferol-3-*O*-(2''-*O*-galloyl) -glucoside	Inhibited the angiotensin converting enzyme activity	Kameda *et al*., 1987

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 6. Pharmacological activities of single isolated metabolites of *Diospyros* species (continued).

Compounds	Pharmacological activity	References
Isoquereitrin	Inhibited the angiotensin converting enzyme activity	Kameda *et al*., 1987
Quercetin-3-*O-(2''-O-* galloyl) -glucoside	Inhibited the angiotensin converting enzyme activity	Kameda *et al*., 1987

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER III

EXPERIMENTAL

Source of Plant Material

The stem of *Diospyros rubra* Lec. were collected from Kao Soi-Dao National Park, Chanthaburi Province, Thailand, in April 2000. The plant was identified by comparison with the voucher specimen (BKF NO. 13260) at the Botanical Section, Royal Forest Department, Ministry of Agriculture and Co-operative, Thailand.

General Techniques

1. Chromatographic Techniques

   1.1 Thin-Layer Chromatography (TLC)

   Technique : one way ascending

   Stationary phase : Silica gel 60F 254, precoated plate layer thickness 0.2 mm.

   Solvent systems : Various solvent systems depending on materials.

   Distance : 10 cm.

   Temperature : 28-35°C (room temperature)

   Detection : 1) UV light (254 and 365)

   2) 10% sulfuric acid in ethanol and heating at 110°C

   1.2 Column Chromatography (CC)

   Column : Flat bottom glass column (various diameter)

   Stationary phase : Silica gel 60 (No. 9385, E. Merck) particle size 0.040-0.063 mm. (230-400 mesh ASTM)

Packing method : Dry and wet packing

Sample loading : Dry packing - The sample was dissolved in a small amount of suitable organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then loaded on the top of the column.

Wet packing - The sample was dissolved in a small amount of the eluent, then loaded on the top of the column.

Technique : Long and short column chromatography.

Solvent system : Various solvent systems depending on materials.

Detection : Fractions were examined by TLC observing under UV light at the wavelengths of 254 and 365 nm. The TLC plate was then sprayed with 10% sulfuric acid in ethanol and heated at 110°C. Fractions of similar chromatographic pattern were combined.

2. Spectroscopy

2.1 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and thin film) were obtained on a Perkin Elmer Infrared Spectrophotometer Model 283 (Pharmaceutical Research Equipment Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.2 Mass Spectra (MS)

The electron impact mass spectra (EIMS) were obtained on a Fisons VG Trio 2000 quadrupole mass spectrometer (Department of Chemistry, Faculty of Science, Mahidol University) operating at 70 eV.

2.3 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) Spectra

The ^{1}H and ^{13}C NMR spectra were obtained either on a Bruker Avance 400 Ultra Shield™ 400 MHz NMR spectrometer (Department of Chemistry, Faculty

of Science, Burapha University) or a Bruker Avance DPX-300 300 MHz NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

NMR solvents used in this study were deuterated chloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

3. Melting Points

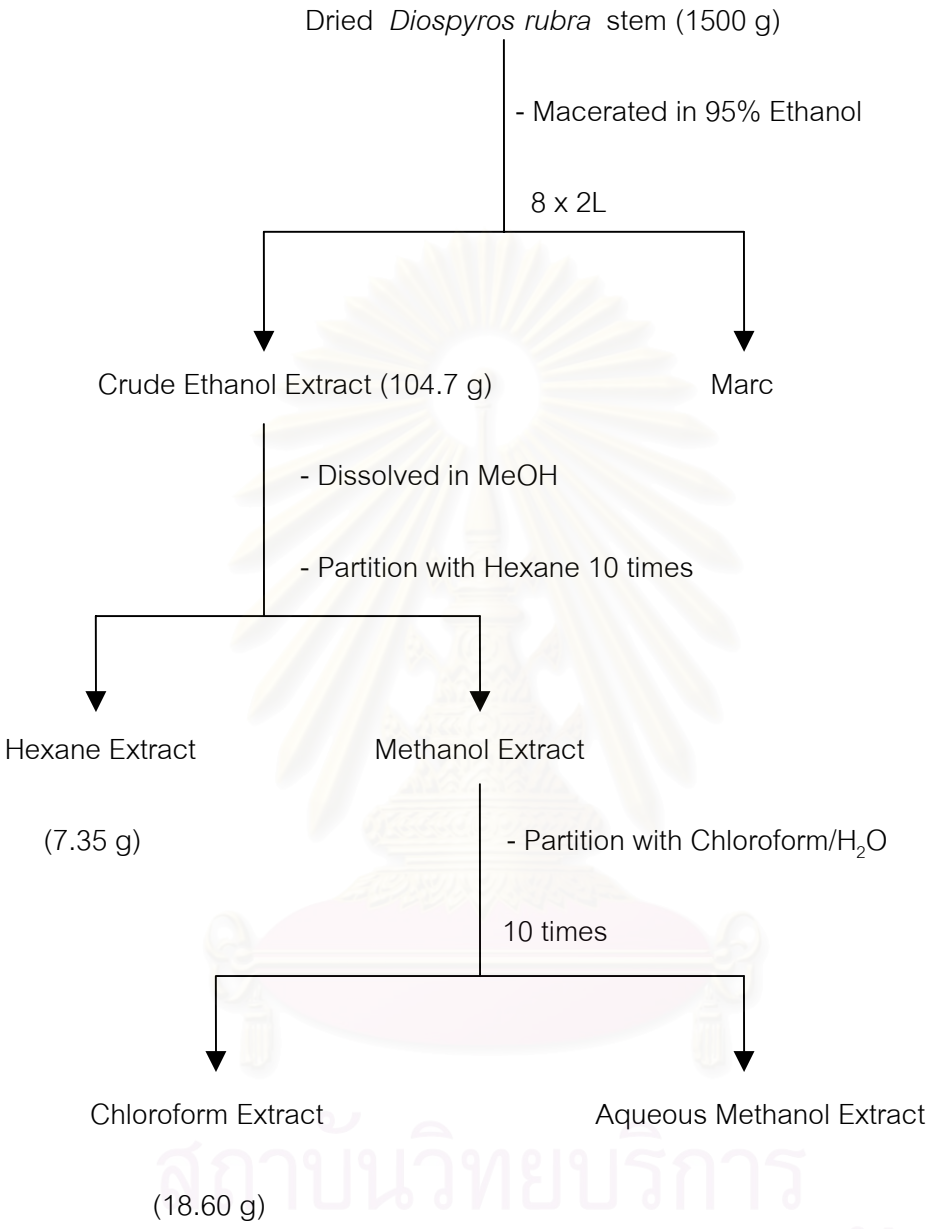
Melting points were obtained on a Gallenkamp Melting Point Apparatus Model MFB 595 (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

4. Solvents

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to used.

Extraction Procedure

The dried stem of *Diospyros rubra* Lec. (1500 g) were ground into small pieces, macerated eight times in 95% ethanol (2 liters 2 days each) and then filtered. The filtrate of each batch was combined and concentrated under reduced pressure to yield 104.7 g of dried crude extract (6.98% of dried weight). The ethanol extract was dissolved in methanol/H_2O, then partitioned (10 times) with 1 liter of hexane and chloroform, respectively. Each fraction was evaporated to dryness under reduced pressure to give 7.35 g of the hexane extract (0.49% of dried weight) and 18.60 g of the chloroform extract (1.24% of dried weight).



Scheme 1. Extraction of *Diospyros rubra* Lec. stem.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Isolation Procedure

1. Fractionation of the hexane extract

The hexane extract (7.35 g) was subjected to silica gel short column chromatography using hexane : chloroform (1 : 1) as the eluent. The extract was dissolved in a small volume of the eluent and blended with silica gel (60.53 g) until dried, then loaded to the top of a glass column (10.0 x 15 cm) already packed with a slurry of silica gel (500 g), and eluted with the eluent. One hundred and seventy-two 30-ml fractions were collected and combined according to their TLC patterns into seven major fractions, DRH 01 – DRH 07 (Table 7). The column was then washed down with methanol and the eluate was combined as fraction DRH 08.

Table 7. Combined fractions from the hexane extract.

Fraction	Number of eluates	Weight (g)
DRH 01	1 - 21	0.12
DRH 02	22 - 30	0.19
DRH 03	31 - 37	1.21
DRH 04	38 - 40	0.88
DRH 05	41 - 51	0.95
DRH 06	52 - 84	2.98
DRH 07	85 - 125	0.44
DRH 08	126 - 172	0.56

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

1.1 Isolation of compounds DR 1 and DR 2

Both fractions DRH 03 and DRH 04, which gave a major violet-blue spot on TLC plate, were combined and purified by recrystallization in methanol to give compound DR 1 as colorless needles (1.4025 g).

Fraction DRH 06 (2.98 g) was separated by column chromatography using silica gel (200 g, 5.0 x 26 cm) with hexane : chloroform (1 : 2) as the eluent. Each 30 ml fraction was collected and detected by TLC, using hexane : chloroform (2 : 3) as the developing solvent system. One hundred and two fractions were collected and combined according to their TLC patterns into six major fractions (DRH 09 – DRH 14) as shown in Table 8.

Table 8. Combined fractions from DRH 06.

Fraction	Number of eluates	Weight (g)
DRH 09	1 – 12	0.48
DRH 10	13 – 29	0.55
DRH 11	30 – 47	0.26
DRH 12	48 – 65	0.58
DRH 13	66 – 87	0.47
DRH 14	88 – 102	0.45

Fraction DRH 10 (0.55 g) was separated by column chromatography using silica gel (30 g, 2.0 x 20 cm) with hexane : chloroform (2 : 3) as the eluent. The fractional volume was about 30 ml. The eluates were collected and combined following TLC examination, with hexane : chloroform (2 : 3) as the developing solvent system, into ten fractions (DRH 15 - DRH 24) as shown in Table 9.

Table 9. Combined fractions from DRH 10.

Fraction	Number of eluates	Weight (g)
DRH 15	1 – 15	0.035
DRH 16	16 – 27	0.078
DRH 17	28 – 33	0.021
DRH 18	34 – 45	0.032
DRH 19	46 – 66	0.045
DRH 20	67 – 92	0.012
DRH 21	93 – 96	0.008
DRH 22	97	0.003
DRH 23	98 – 104	0.041
DRH 24	105 – 113	0.056

Fraction DRH 16 was recrystallized in methanol to give compound DR 1 (0.023 g), and fraction DRH 18 which gave a major violet-red spot upon TLC detection was recrystallized in methanol to give compound DR 2 as colorless needles (0.0074 g).

1.2 Isolation of compound DR 3

Fraction DRH 14 (0.45 g) was separated by silica gel column chromatography (50 g, 2.0x35 cm) with chloroform as the eluent. The fractional volume was about 15 ml. The eluates were collected and combined following TLC examination, with chloroform as the developing solvent system, into four fractions (DRH 25 – DRH 28) as shown in Table 10.

Table 10. Combined fractions from DRH 14.

Fraction	Number of eluates	Weight (g)
DRH 25	1 – 7	0.12
DRH 26	8 – 16	0.08
DRH 27	17 – 23	0.07
DRH 28	24 – 41	0.13

Fraction DRH 25 was separated by silica gel column chromatography (20 g, 2.0x20 cm) with chloroform as the eluent. The fractional volume was about 10 ml. The eluates were collected and combined following TLC examination, with chloroform as the developing solvent system, into five fractions (DRH 29 – DRH 33) as shown in Table 11.

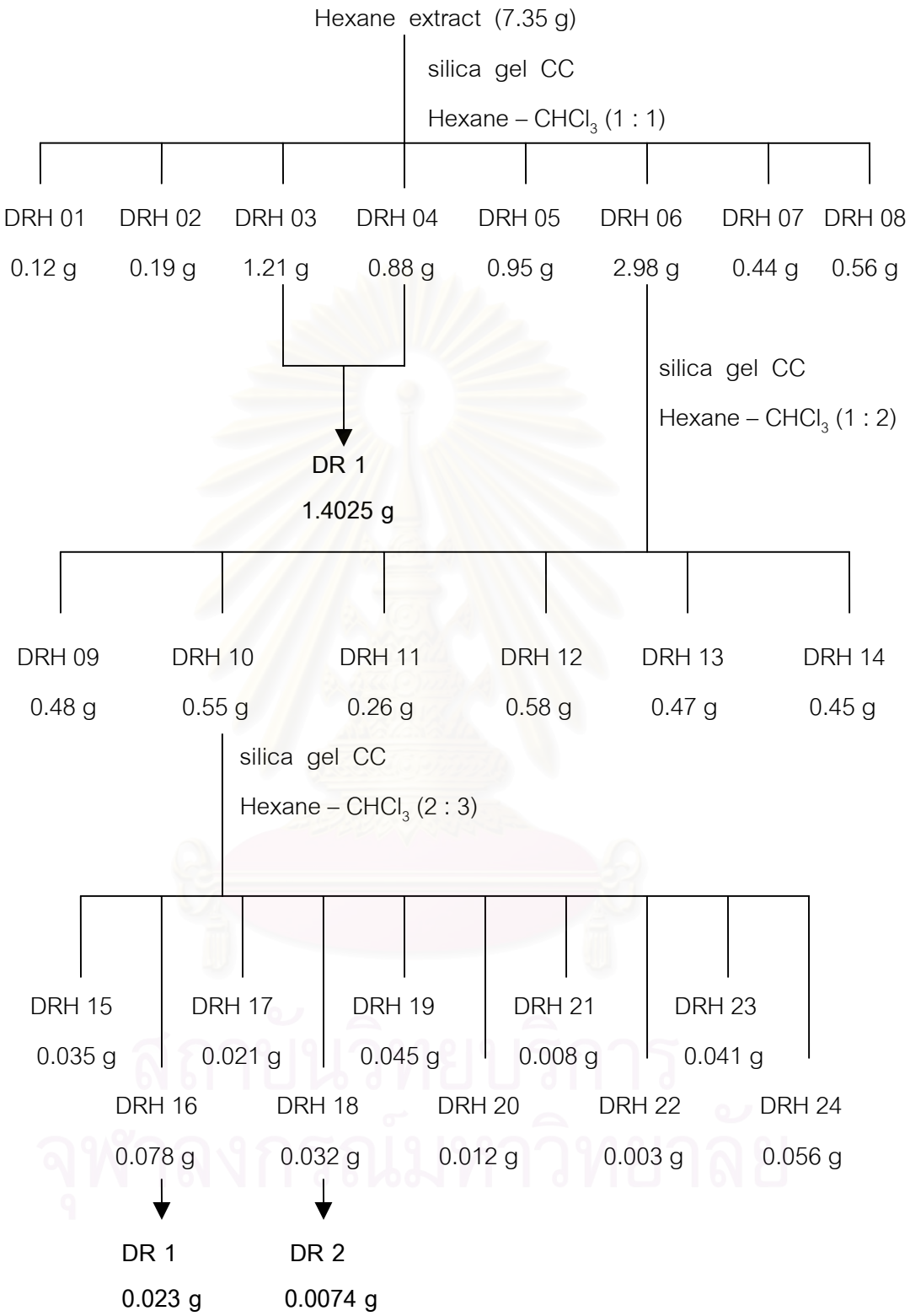
Table 11. Combined fractions from DRH 25.

Fraction	Number of eluates	Weight (g)
DRH 29	1 – 12	0.014
DRH 30	13 – 18	0.009
DRH 31	19 – 31	0.047
DRH 32	32 – 37	0.015
DRH 33	38 – 62	0.031

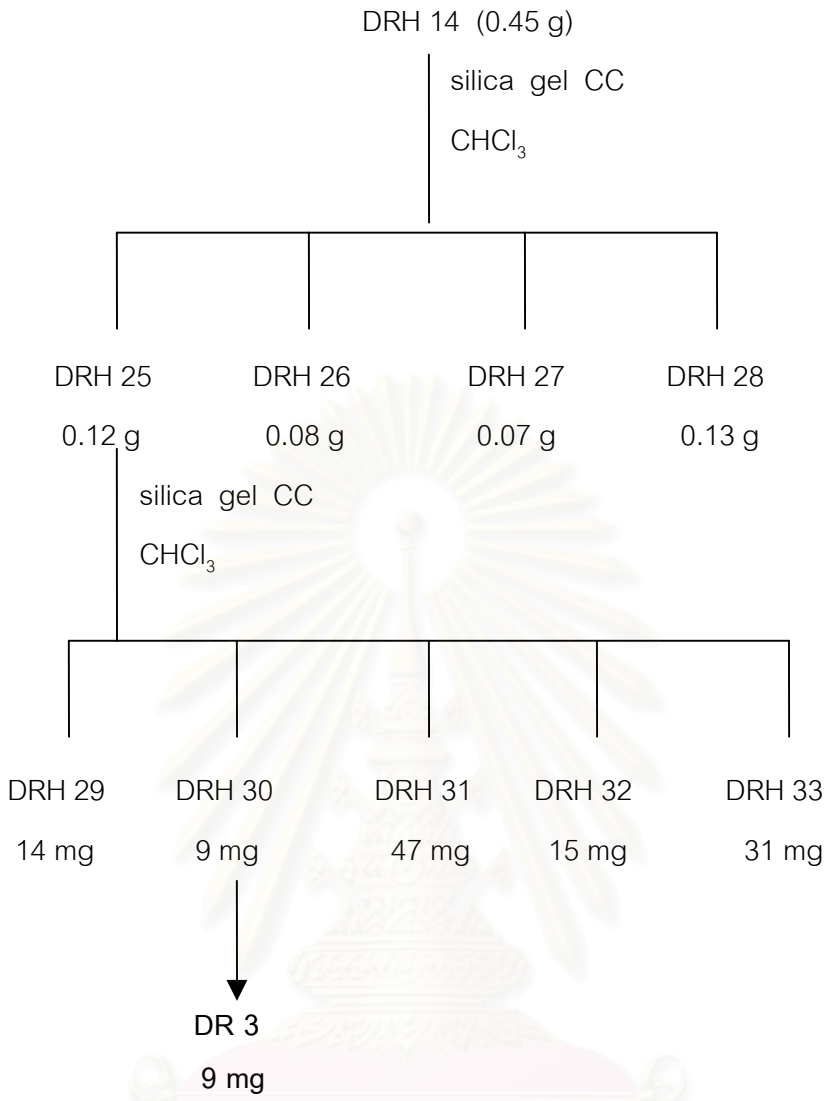
สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Fraction DRH 30 gave a major violet-blue spot upon TLC detection. It was therefore recrystallized in methanol to give compound DR 3 as white amorphous powder (9 mg).

The fractionation of the hexane extract is summarized in Scheme 2.



Scheme 2. Fractionation of hexane extract.



Scheme 2. Fractionation of hexane extract (continued).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

2. Fractionation of the chloroform extract

The chloroform extract (18.60 g) was subjected to silica gel column chromatography (500 g, 6.0x40 cm) using chloroform : methanol (98 : 2) as the eluent. The extract was dissolved in the eluent and blended with silica gel (65 g) until dried, then loaded on top of the column and eluted with the eluent. One hundred and eighty 30-ml fractions were collected and combined according to their TLC patterns into eight major fractions (DRC 01 – DRC 08) as shown in Table 12.

Table 12. Combined fractions from the chloroform extract.

Fraction	Number of eluates	Weight (g)
DRC 01	1 – 26	1.7450
DRC 02	27 – 59	2.4864
DRC 03	60 – 74	0.9560
DRC 04	75 – 91	1.5400
DRC 05	92 – 105	1.0073
DRC 06	106 – 138	2.0581
DRC 07	139 – 150	0.9850
DRC 08	151 – 180	6.2146

Fraction DRC 07 was separated by silica gel column chromatography (50 g, 2.0x20 cm) with chloroform : methanol (95 : 5) as the eluent. The fractional volume was about 30 ml. The eluates were collected and combined following TLC examination, with chloroform : methanol (95 : 5) as the developing solvent system, into four fractions (DRC 09 – DRC 12) as shown in Table 13.

Table 13. Combined fractions from DRC 07.

Fraction	Number of eluates	Weight (g)
DRC 09	1 – 6	0.0485
DRC 10	7 – 19	0.2378
DRC 11	20 – 26	0.0540
DRC 12	27 – 45	0.3710

Fraction DRC 11 gave a major violet-red spot upon TLC detection with 10% sulfuric acid. It was therefore recrystallized in methanol to give compound DR 4 as colorless needles (0.054 g).

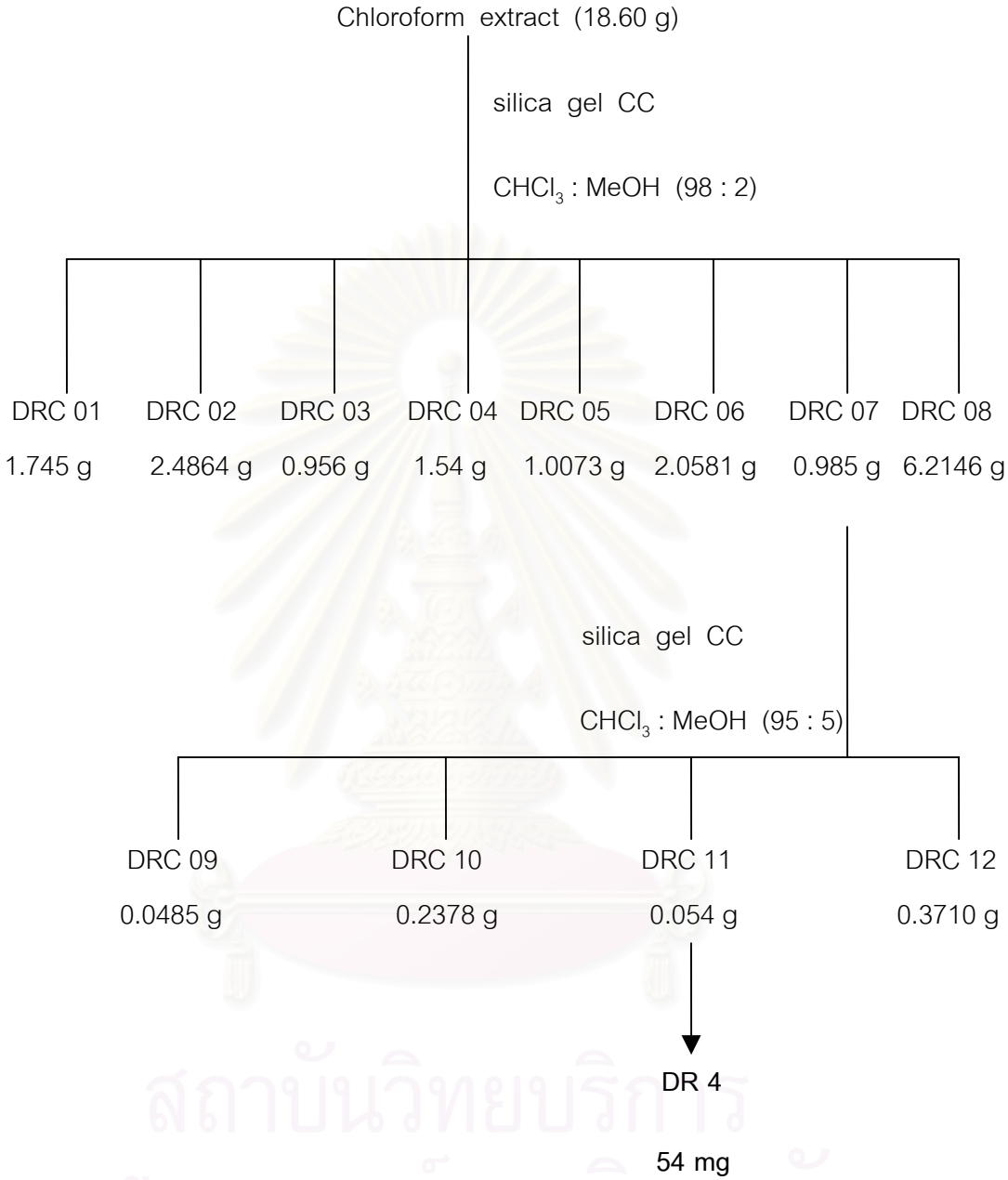
The fractionation of the chloroform extract is summarized in Scheme 3.

All isolated compounds were test with Liebermann-Burchard reagent and the results are shown in Table 14. The total amount of each compounds is also shown in the same table.

Table 14. Result of Liebermann-Burchard test and the total amount of isolated compounds.

Compound	Color with Liebermann-Burchard test	Total amount	
		Weight (g)	% yield
DR 1	Violet	1.4260	0.0950
DR 2	Blue	0.0074	0.0005
DR 3	Red – violet	0.0090	0.0006
DR 4	Red – violet	0.0540	0.0040

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย



Scheme 3. Fractionation of chloroform extract.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Characterization of isolated compounds

1. **Compound DR 1 (1.426 g, 0.095% yield)**

Appearance : Colorless needles (methanol)

Solubility : Soluble in chloroform

Melting point : 215 – 216 °C

EIMS *m/z* (% relative intensity) : 426(16), 411(20), 409(32), 393(11), 383(5), 257(23), 218(81), 205(46), 191(47), 189(100), 176(15), 148(31), 124(49), 108(37), 95(19), 80(27), 67(14) and 56(8) (Figure 2, page 79)

IR ν_{max} (thin film) cm^{-1} : 3436, 2934, 2863, 1644, 1454, 1378, 1040, 881, 758, 476 (Figure 3, page 80)

^{1}H – NMR (δ ppm, 400 MHz, $CDCl_3$) (Figures 6a-6c, pages 84-86)

4.70 (1H, *s*), 4.60 (1H, *s*), 3.20 (1H, *dd J* = 5.2, 5.2 Hz), 2.40 (1H, *dt*), 1.91 (1H, *m*), 1.71 (3H, *s*), 1.05 (3H, *s*), 0.99 (3H, *s*), 0.97 (3H, *s*), 0.85 (3H, *s*), 0.81 (3H, *s*), 0.79 (3H, *s*)

^{13}C – NMR (δ ppm, 100 MHz, $CDCl_3$) (Figures 4a-4b, pages 81-82)

150.9 (*s*), 109.3 (*t*), 79.0 (*d*), 55.3 (*d*), 50.5 (*d*), 48.3 (*d*), 48.0 (*d*), 43.0 (*s*), 42.8 (*s*), 40.9 (*s*), 40.0 (*t*), 38.9 (*s*), 38.7 (*t*), 38.1 (*d*), 37.2 (*s*), 35.6 (*t*), 34.3 (*t*), 29.9 (*t*), 28.0 (*q*), 27.4 (*t*), 27.5 (*t*), 25.2 (*t*), 20.9 (*t*), 19.3 (*q*), 18.3 (*t*), 18.0 (*q*), 16.1 (*q*), 16.0 (*q*), 15.3 (*q*), 14.6 (*q*)

2. **Compound DR 2 (0.0074 g, 0.0005% yield)**

Appearance : Colorless needles (methanol)

Solubility : Soluble in hexane, chloroform

Melting point : 140 –141 °C

EIMS *m/z* : 414(73), 396(64), 381(42)379(17), 329(75), 273(34),

(% relative intensity) 255(58), 231(46), 213(100), 199(56), 173(45), 159(75), 108(57), 95(57), 91(39), 81(34) and 56(10) (Figure 7,page 90)

IR ν_{max} (thin film) cm^{-1} : 3436, 2933, 2868, 2371, 2289,1639, 1465, 1372, 1101, 1050, 876, 476 (Figure 8, page 91)

^{1}H – NMR (δ ppm, 400 MHz, $CDCl_3$) (Figures 9a-9b, pages 92-93)

5.36 (1H, *d J* = 5.2 Hz), 5.14 (1H, *dd J* = 15.2, 8.8 Hz), 5.01 (1H, *dd J* = 15.2, 8.8 Hz), 3.54 (1H, *m*), 1.02 (3H, *s*), 0.93 (3H, *d*), 0.88 (3H, *t*), 0.85 (3H, *d*), 0.82 (3H, *d*), 0.70 (3H, *s*)

^{13}C – NMR (δ ppm, 100 MHz, $CDCl_3$) (Figures 10a-10b, pages 94-95)

140.7 (*s*), 121.7 (*d*), 71.8 (*d*), 56.7 (*d*), 56.0 (*d*), 50.1 (*d*), 45.8 (*d*), 42.3 (*t*), 42.3 (*s*), 39.7 (*t*), 37.2 (*t*), 36.5 (*s*), 36.1 (*d*), 33.9 (*t*), 31.9 (*d*), 31.9 (*t*), 31.6 (*t*), 29.1 (*d*), 28.2 (*t*), 26.0 (*t*), 24.3 (*t*), 23.0 (*t*), 21.0 (*t*), 19.8 (*q*), 19.4 (*q*), 19.0 (*q*), 18.8 (*q*), 11.9 (*q*), 11.8 (*q*)

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

3. **Compound DR 3 (9 mg, 0.0006% yield)**

Appearance : White amorphous powder (methanol)

Solubility : Soluble in chloroform

Melting point : 260 - 261 °C

EIMS *m/z* : 443(5), 442(12), 427(10), 411(32), 393(16),207(47),

(% relative intensity) 203(100), 189(98), 95(48), 79(27), 67(24) and 55(11)

(Figure 13, page 102)

IR ν_{max} (thin film) cm^{-1}: 3388, 2939, 2868, 1645, 1458, 1369, 1028, 987, 883, 472 (Figure 14, page 103)

^{1}H – NMR (δ ppm, 400 MHz, $CDCl_3$) (Figures 17a-17b, pages 107-108)

4.65 (1H, *s*), 4.55 (1H, *s*), 3.75 (1H, *d J* = 10.8 Hz), 3.29 (1H, *d J* = 10.8 Hz), 3.14 (1H, *dd J* = 4.8, 4.8 Hz), 1.65 (3H, *s*), 1.00 (3H, *s*), 0.95 (3H, *s*), 0.94 (3H, *s*), 0.80 (3H, *s*) and 0.73 (3H, *s*)

^{13}C – NMR (δ ppm, 100 MHz, $CDCl_3$) (Figures 15a-15b, pages 104-105)

150.9 (*s*), 110.1 (*t*), 78.7 (*d*), 61.0 (*t*), 56.4 (*d*), 51.4 (*d*), 48.8 (*d*), 48.6 (s), 48.2 (*d*), 42.8 (*s*), 40.8 (*s*), 39.2 (*s*), 38.4 (*t*), 37.6 (*d*), 37.0 (*s*), 34.7 (*t*), 34.4 (*t*), 30.2 (*t*), 29.6 (*t*), 27.8 (*q*), 27.5 (*t*), 26.6 (*t*), 25.7 (*t*), 21.2 (*t*), 20.0 (*q*), 18.7 (*t*), 17.1 (*q*), 16.4 (*q*), 15.8 (*q*) and 14.6 (*q*)

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

4. Compound DR 4 (54 mg, 0.004% yield)

Appearance : Colorless needles (methanol)

Solubility : Soluble in chloroform, methanol

Melting point : 259 °C

EIMS *m/z* : 438(2), 300(3), 248(100), 219(24), 203(68), 189(20),

(% relative intensity) 147(11), 133(61), 119(15), 95(6), 67(5) and 55(4)

(Figure 18, page 114)

IR ν_{max} (thin film) cm^{-1} : 3460, 2925, 1689, 1637, 1261, 1089, 1029, 802

(Figure 19, page 115)

^{1}H – NMR (δ ppm, 400 MHz, DMSO-d_6) (Figures 22a-22b, pages 120-121)

5.11 (1H, *t*), 2.98 (1H, *m*), 1.02 (3H, *s*), 0.88 (3H, *d* *J* = 7.2 Hz), 0.88 (3H, *s*), 0.85 (3H, *s*), 0.79 (3H, *d* *J* = 6.4 Hz), 0.73 (3H, *s*), 0.66 (3H, *s*)

^{13}C – NMR (δ ppm, 75 MHz, DMSO-d_6) (Figures 20a-20b, pages 116-117)

178.4 (*s*), 138.3 (*s*), 124.7 (*d*), 77.0 (*d*), 54.9 (*d*), 52.5 (*d*), 47.1 (*s*), 46.9 (*d*), 41.8 (*s*), 38.8 (*s*), 38.6 (*d*), 38.5 (*d*), 38.5 (*s*), 38.3 (*t*), 36.6 (*s*), 36.4 (*t*), 32.8 (*t*), 30.3 (*t*), 28.4 (*q*), 27.6 (*t*), 27.1 (*t*), 23.9 (*t*), 23.4 (*q*), 23.0 (*t*), 21.2 (*q*), 18.1 (*t*), 17.1 (*q*), 17.0 (*q*), 16.2 (*q*), 15.3 (*q*)

สถาบันวิทยบริการ
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CHAPTER IV

RESULTS AND DISCUSSION

Chromatographic separation of the hexane and chloroform extracts of the stem of *Diospyros rubra* Lec. led to the isolation of four chemical constituents. The identification of these compounds was based on analysis of their spectroscopic data (IR, NMR and mass spectra) and also confirmed by comparison with those values previously reported in the literature. The details can be discussed as follows.

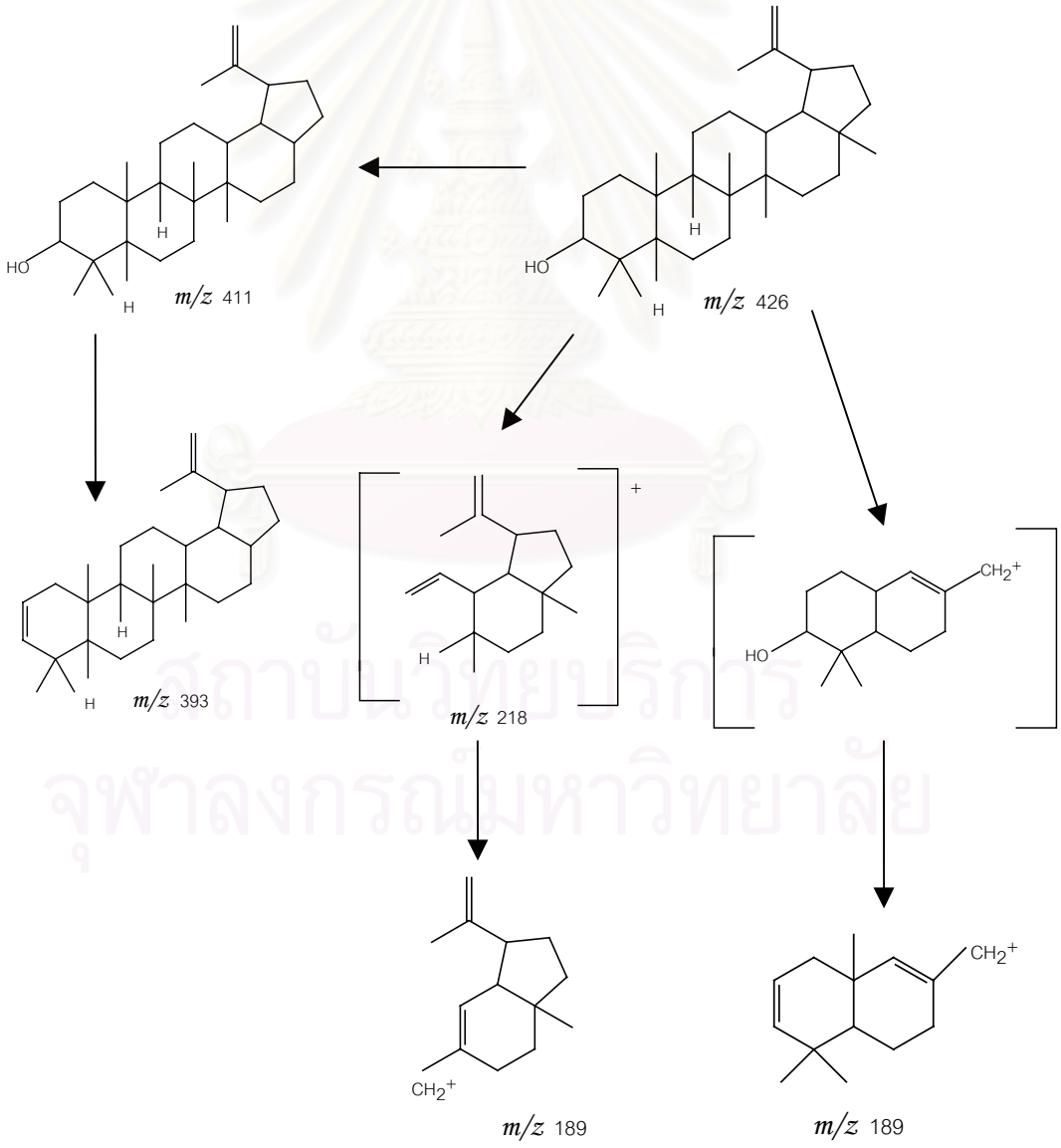
1. Identification of compound DR 1

Compound DR 1 was obtained as colorless needles (1.426 g, 0.095 % yield) from fractions DRH 03, DRH 04 and DRH 16 of the hexane extract. The compound gave violet color to Lieberman-Burchard reagent, suggesting that it is a triterpenoid. The EIMS spectrum of this compound (Figure 2) showed a molecular ion peak at *m/z* 426 which corresponded to the molecular formula of $C_{30}H_{50}O$. Successive losses of methyl and water produced fragment peaks at *m/z* 411 and 393, respectively. Intense fragment peaks at *m/z* 189 and 218 were suggestive of a pentacyclic triterpenoid with the lupane skeleton (Budzikiewicz, Wilson and Djerassi, 1963 ; Ogunkoya, 1981). These prominent peaks were the results of cleavage at different positions across the C-ring of the lupane skeleton as shown in Scheme 4. The presence of the alcohol functionality in the molecule was indicated by an IR absorption band at 3436 cm^{-1} (Figure 3).

The ^{13}C – NMR spectrum of DR 1 (Figure 4a – 4b) showed 30 carbon signals, supportive of a triterpenoid structure. The DEPT-90 and DEPT-135 experiments (Figure 5) were employed to classify these signals into those of six quaternary carbons at δ 37.2, 38.9, 40.9, 42.8, 43.0 and 150.9 ppm, six methine carbons at 38.1, 48.0, 48.3, 50.5, 55.3 and 79.0 ppm, eleven methylene carbons at

δ 18.3, 20.9, 25.2, 27.4, 27.5, 29.9, 34.3, 35.6, 38.7, 40.0 and 109.3 ppm, and seven methyl carbons at δ 14.6, 15.3, 16.0, 16.1, 18.0, 19.3 and 28.0 ppm. The two most downfield carbon signals at 150.9 and 109.3 ppm represents the disubstituted double bond at C–20 and C-29 in the lupane skeleton.

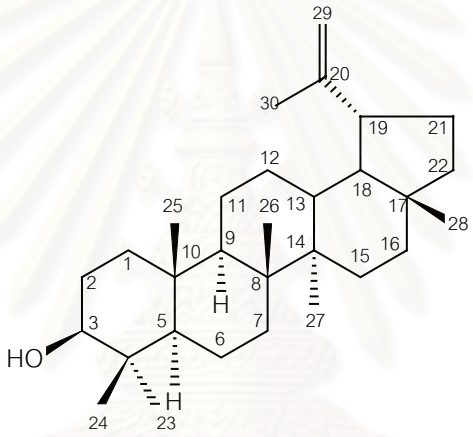
The ^{1}H – NMR spectrum (Figure 6a – 6c) showed seven singlets of tertiary methyls at δ 0.79 (H_3-24), 0.81 (H_3-28), 0.85 (H_3-25), 0.97 (H_3-27), 0.99 (H_3-23), 1.05 (H_3-26) and 1.71 ppm (H_3-30). The presence of exomethylene protons (H_2-29) could be observed as two downfield singlets (br) at δ 4.60 and 4.70 ppm. A double doublet at δ 3.20 ppm was attributable to the carbinylic proton (H-3).



Scheme 4. Mass fragmentation of compound DR 1

All spectroscopic data of DR 1 are in accordance with the structure of lupeol, a known triterpenoid of the lupane type. Comparison of the ^{13}C-NMR data of this compound with those previously reported for lupeol (Mahato and Kundu, 1994), is shown in Table 15.

Therefore, it was concluded that compound DR 1 is lupeol, the structure of which is shown below.



Lupeol ($C_{30}H_{50}O$)

Lupeol was previously isolated from several *Diospyros* species. It could be found in almost all parts of the plants, especially in the bark and heartwood. The compound have been reported as active against the Walker-Carcinoma-256 tumor system (Misra and Pandey, 1989). Lupeol was also revealed to possess antifungal and germination inhibitory activities (Higa *et al.*, 1998).

Table 15. Comparison of ^{13}C-NMR data of lupeol (in $CDCl_3$) and compound DR 1 (in $CDCl_3$).

Carbon	Chemical shift (δ) ppm	
	Lupeol	DR 1
1	38.7	38.7
2	27.4	27.5
3	78.9	79.0
4	38.8	38.9
5	55.3	55.3
6	18.3	18.3
7	34.2	34.3
8	40.8	40.9
9	50.4	50.5
10	37.1	37.2
11	20.9	20.9
12	25.1	25.2
13	38.0	38.1
14	42.8	42.8
15	27.4	27.4
16	35.5	35.6
17	43.0	43.0
18	48.2	48.3
19	47.9	48.0
20	150.9	150.9
21	29.8	29.9
22	40.0	40.0
23	28.0	28.0
24	15.4	15.3
25	16.1	16.1
26	15.9	16.0
27	14.5	14.6
28	18.0	18.0
29	109.3	109.3
30	19.3	19.3

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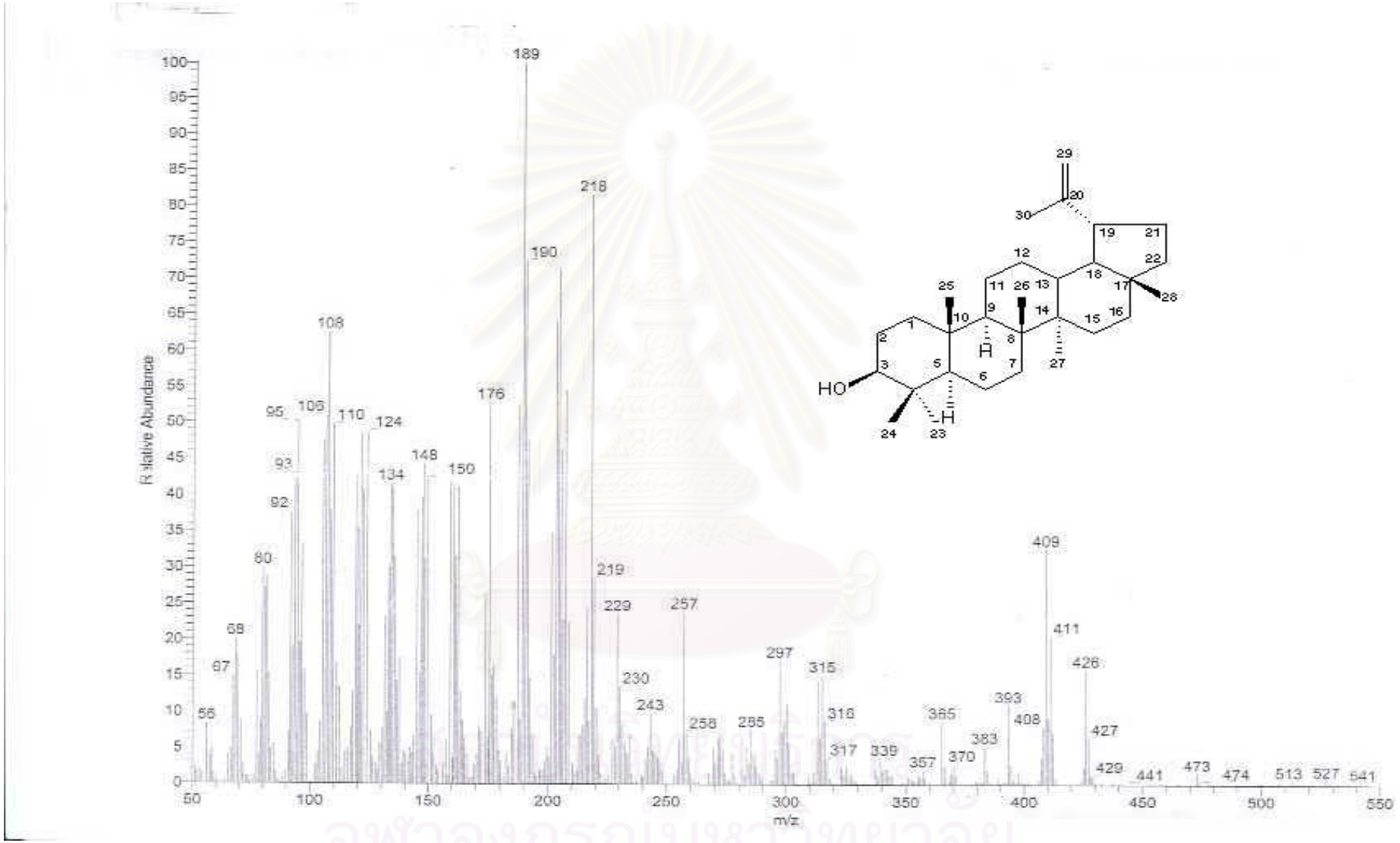

Figure 2. EIMS of compound DR 1

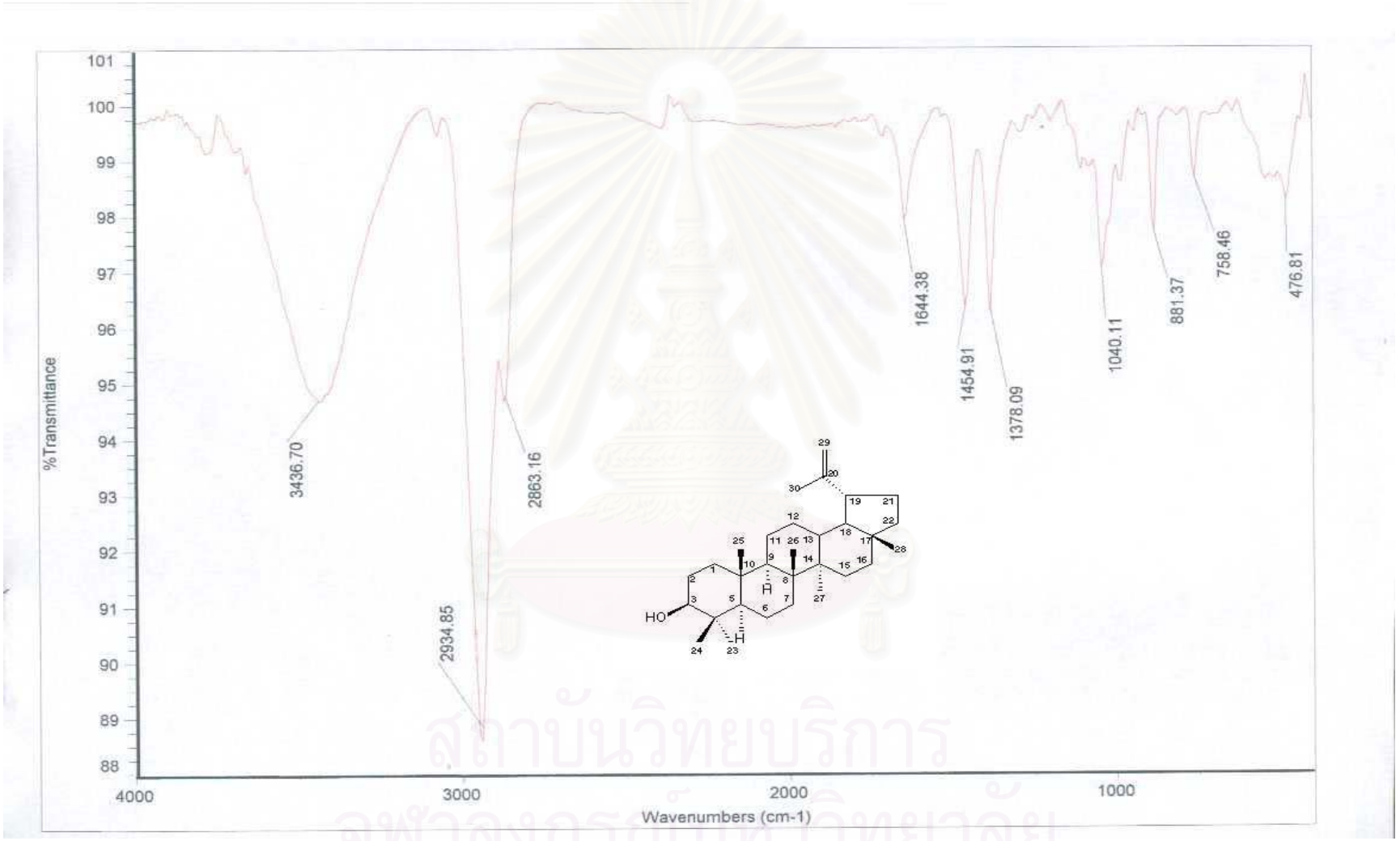

Figure 3. IR spectrum of compound DR 1

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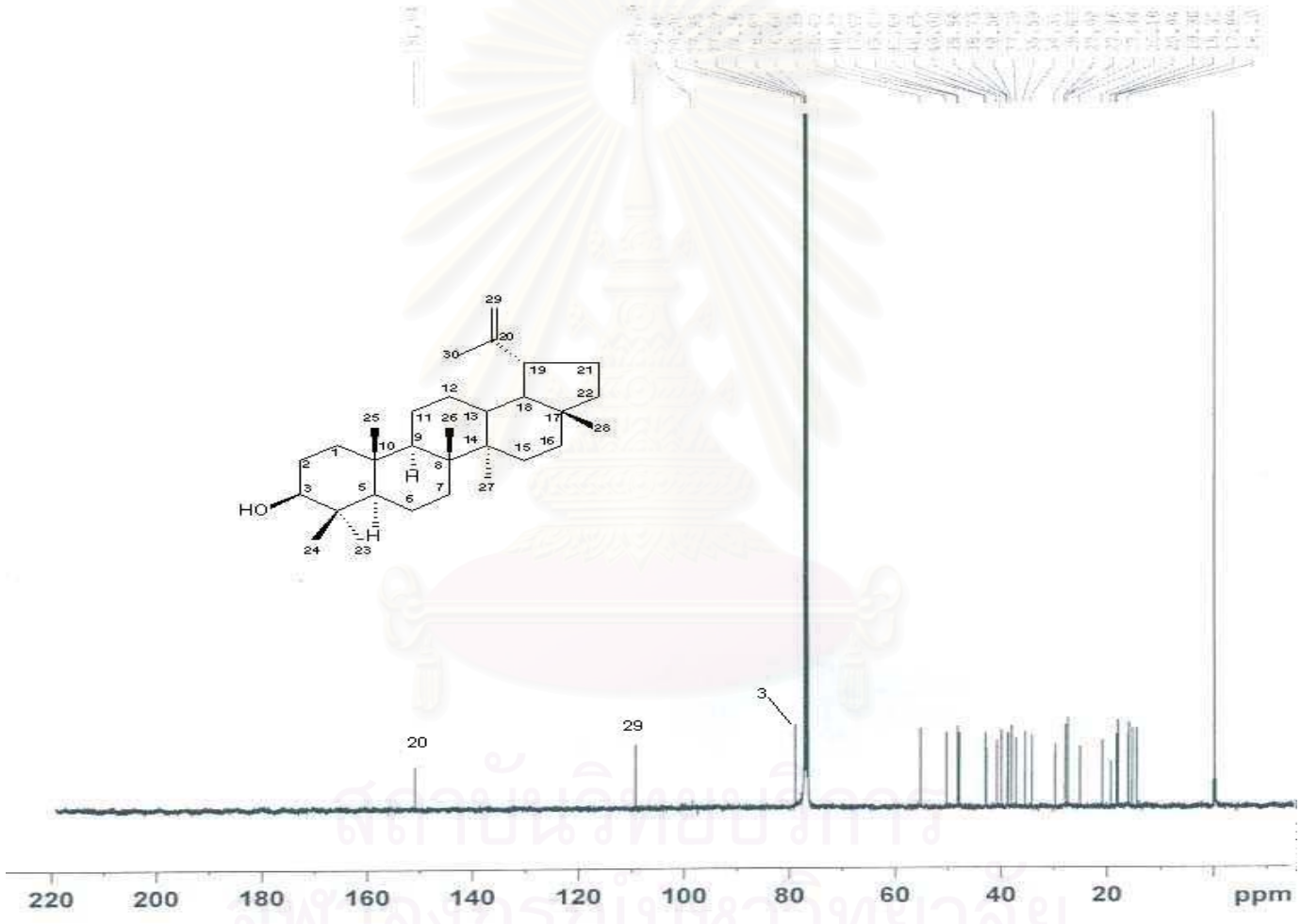

Figure 4a. The 100 MHz ^{13}C-NMR spectrum of compound DR 1

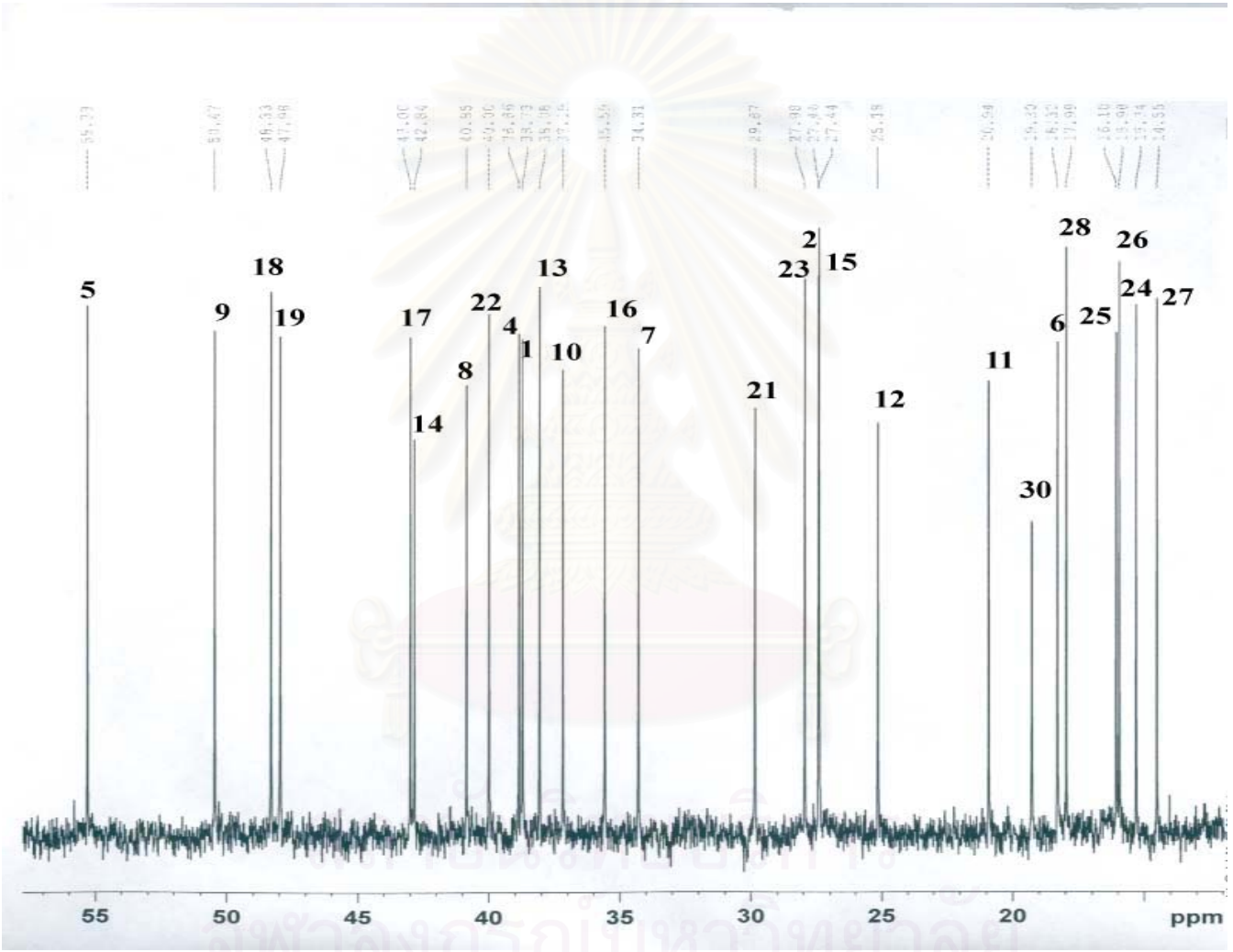

Figure 4b. The 100 MHz ^{13}C-NMR spectrum of compound DR 1 (expanded)

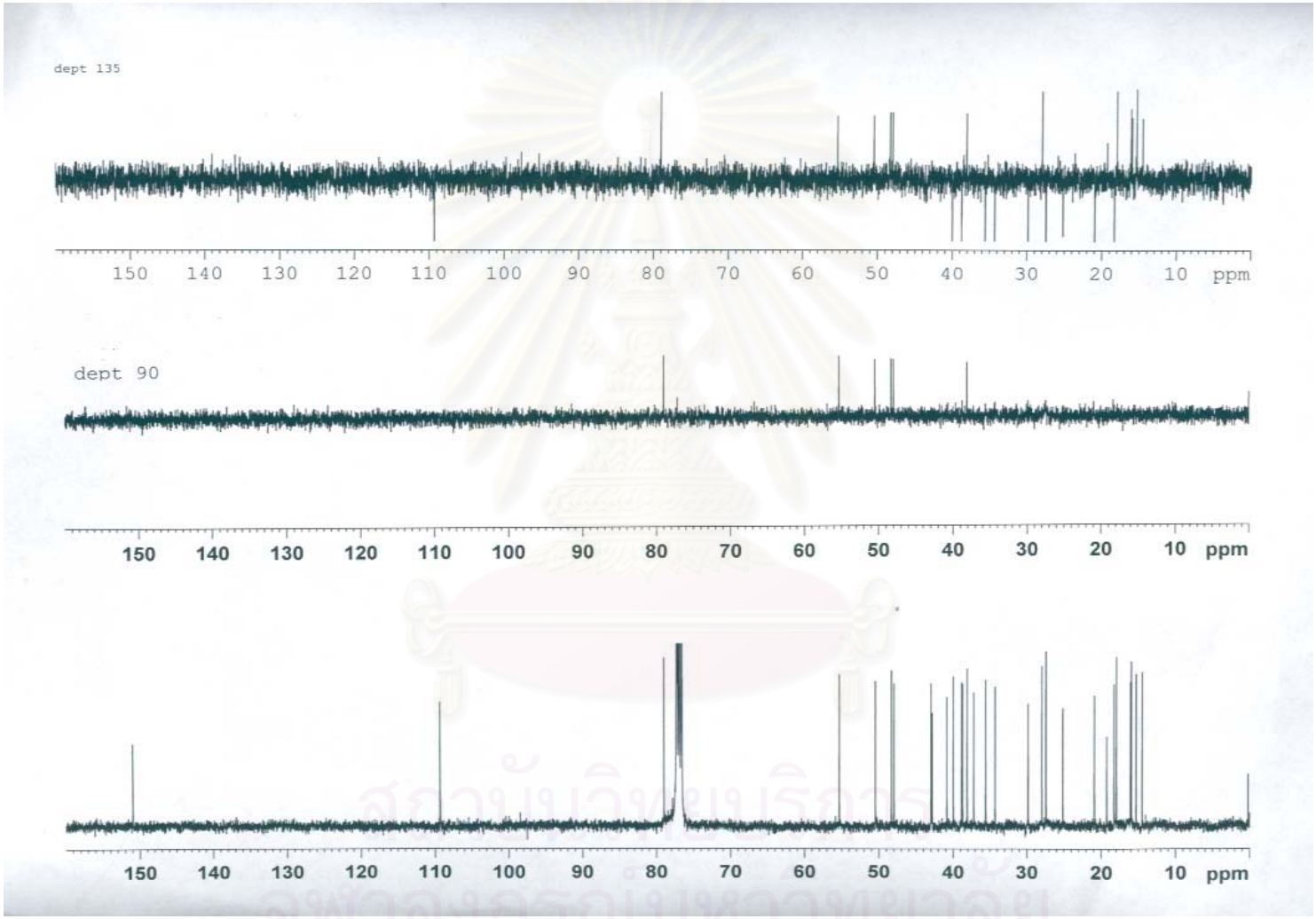

Figure 5. The 100 MHz ^{13}C-DEPT NMR spectra of compound DR 1

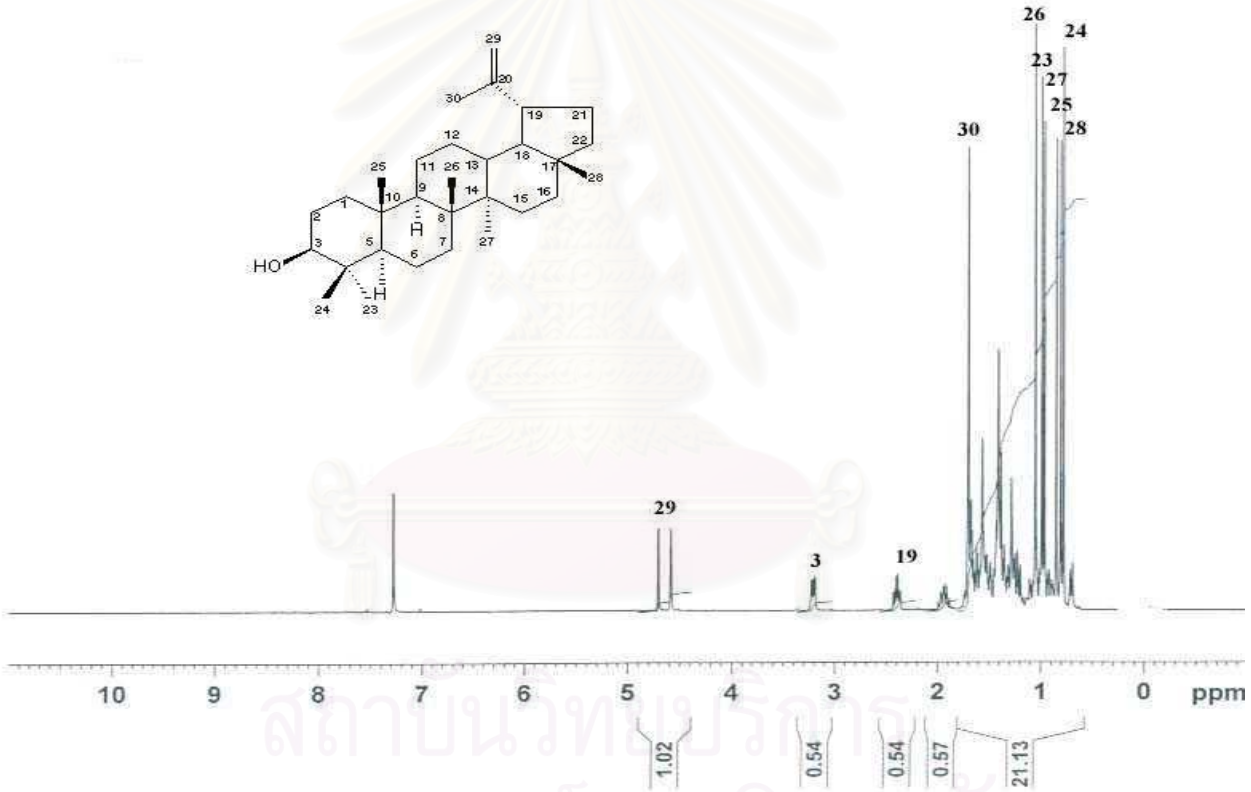

Figure 6a. The 400 MHz ^{1}H-NMR spectrum of compound DR 1

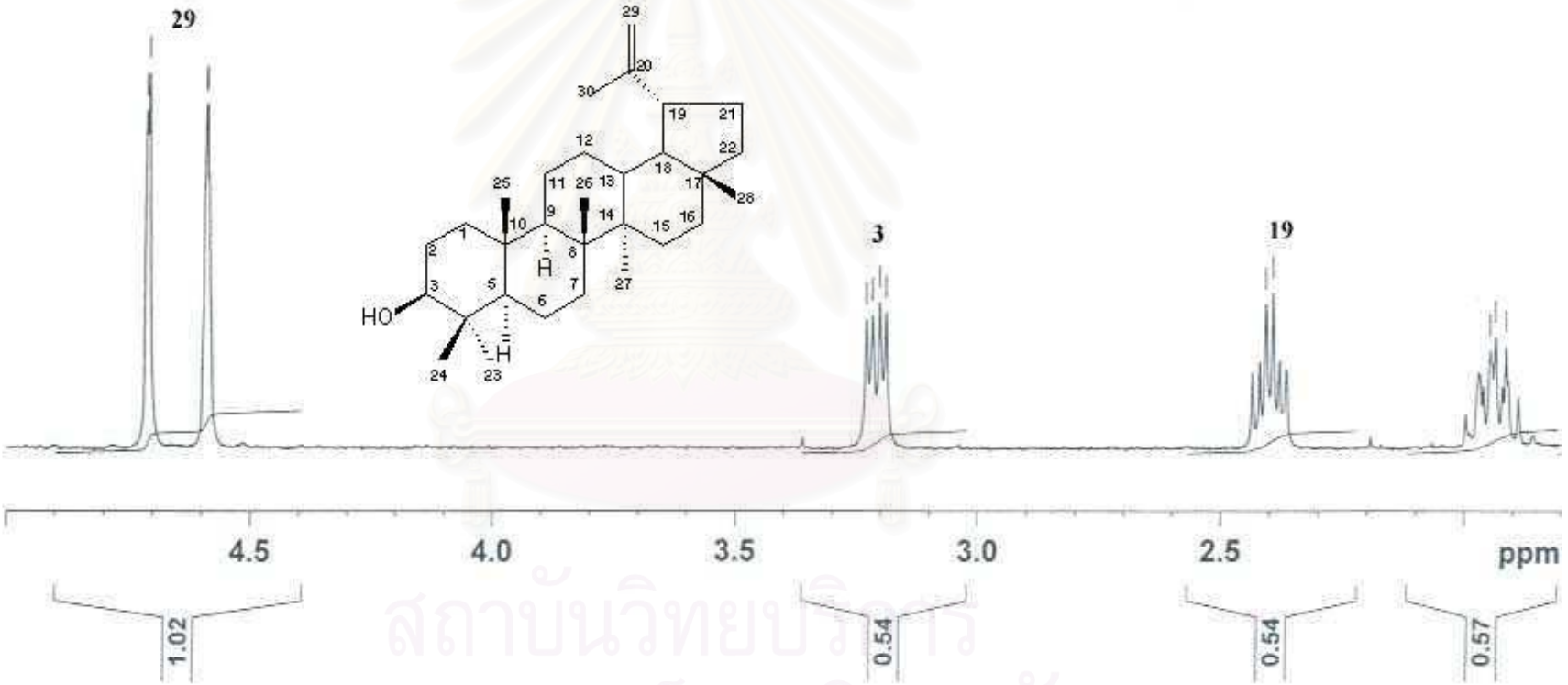

Figure 6b. The 400 MHz ^{1}H-NMR spectrum of compound DR 1 (expanded)

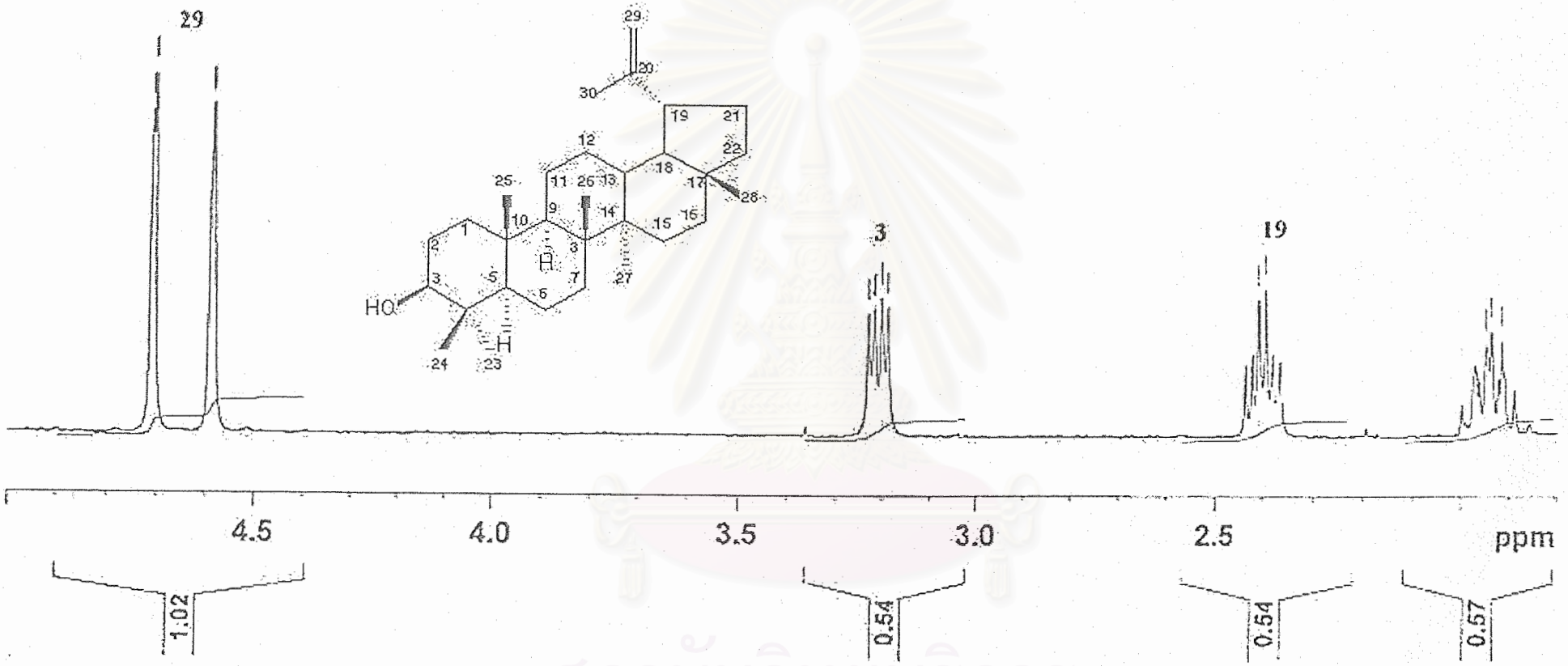

Figure 6c. The 400 MHz ^{1}H-NMR spectrum of compound DR 1 (expanded)

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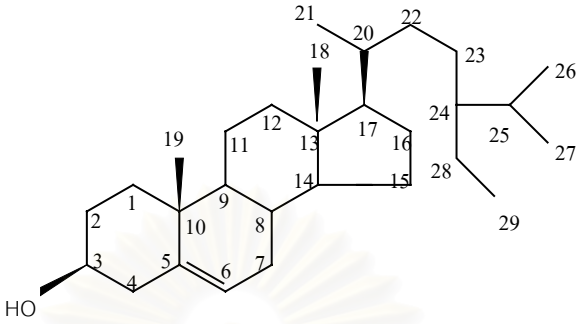
2. Identification of compound DR 2

Compound DR 2 was obtained as colorless needles (74 mg, 0.0005 % yield) from fraction DRH 16 of the hexane extract. The compound gave blue color to Lieberman-Burchard reagent, suggesting the presence of the steroid nucleus. The EIMS spectrum of this compound (Figure 7) showed a molecular ion peak at *m/z* 414, which corresponded to molecular formula of $C_{29}H_{50}O$. The IR spectrum displayed the OH absorption at 3436 cm^{-1}(Figure 8).

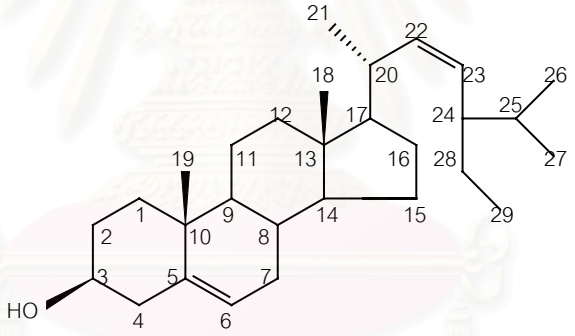
The ^{1}H-NMR spectrum (Figure 9a-9b) gave evidences which suggested that DR 2 is a mixture of β-sitosterol and stigmasterol. Two double doublets at δ 5.01 (*J* = 15.2, 8.8 Hz) and 5.14 (*J* = 15.2, 8.8 Hz) ppm were attributable to H-22 and H-23 of stigmasterol, respectively, while a doublet (*J* = 5.2 Hz) at δ 5.36 ppm was assignable to H-6 of both β-sitosterol and stigmasterol. A multiplet at δ 3.54 ppm was attributable to the methine proton of hydroxy-substitued position 3. The ratio of β-sitosterol and stigmasterol in the mixture, as deduced from the integration of peak areas, is 3 : 2.

In the ^{13}C-NMR spectrum (Figures 10a-10b), 29 carbon signals of β-sitosterol were evident, while the signals of stigmasterol were hardly observed. However, the signals for C-22 and C-23 of stigmasterol could be observed at δ 137.8 and 129.1 ppm, respectively, in the DEPT-90 NMR spectrum (Figure 11).

Therefore, it was concluded that DR 2 is a mixture of β-sitosterol and stigmasterol, both of which are common phytosterols widely distributed in the plant kingdom. Comparison of ^{13}C-NMR data of DR 2 with the reported data of β-sitosterol (Rubinstein *et al*., 1976) and stigmasterol (Rubinstein *et al*., 1976) is shown in Table 16. The structures of β-sitosterol and stigmasterol are shown below.



β- Sitosterol



Stigmasterol

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Table 16. Comparison of ^{13}C-NMR data of β- sitosterol (in $CDCl_3$), stigmasterol (in $CDCl_3$) and compound DR 2 (in $CDCl_3$).

Carbon	Chemical shift (δ) ppm		
	β- sitosterol	Stigmasterol	DR 2
1	37.2	37.2	37.3
2	31.7	31.7	31.7
3	71.7	71.8	71.8
4	42.3	42.4	42.3
5	140.8	140.8	140.8
6	121.7	121.7	121.7
7	31.9	32.0	31.9
8	31.9	32.0	31.9
9	50.1	50.2	50.2
10	36.5	36.6	36.5
11	21.1	21.1	21.1
12	39.8	39.7	39.8
13	42.3	42.4	42.3
14	56.8	56.9	56.8
15	24.3	24.4	24.3
16	28.2	29.0	28.3
17	56.0	56.1	56.1
18	11.9	12.1	12.0
19	19.4	19.4	19.4
20	36.1	40.5	36.2
21	18.8	21.1	18.8
22	33.9	138.0	34.0[a] (137.8)[b]
23	26.1	129.3	26.1[a] (129.1)[b]
24	45.8	51.3	45.9
25	29.1	32.0	29.2
26	19.8	21.3	19.8
27	19.0	19.0	19.0
28	23.1	25.4	23.1
29	11.9	12.3	11.9

a - the signal of β- sitosterol

b – the signal of stigmasterol

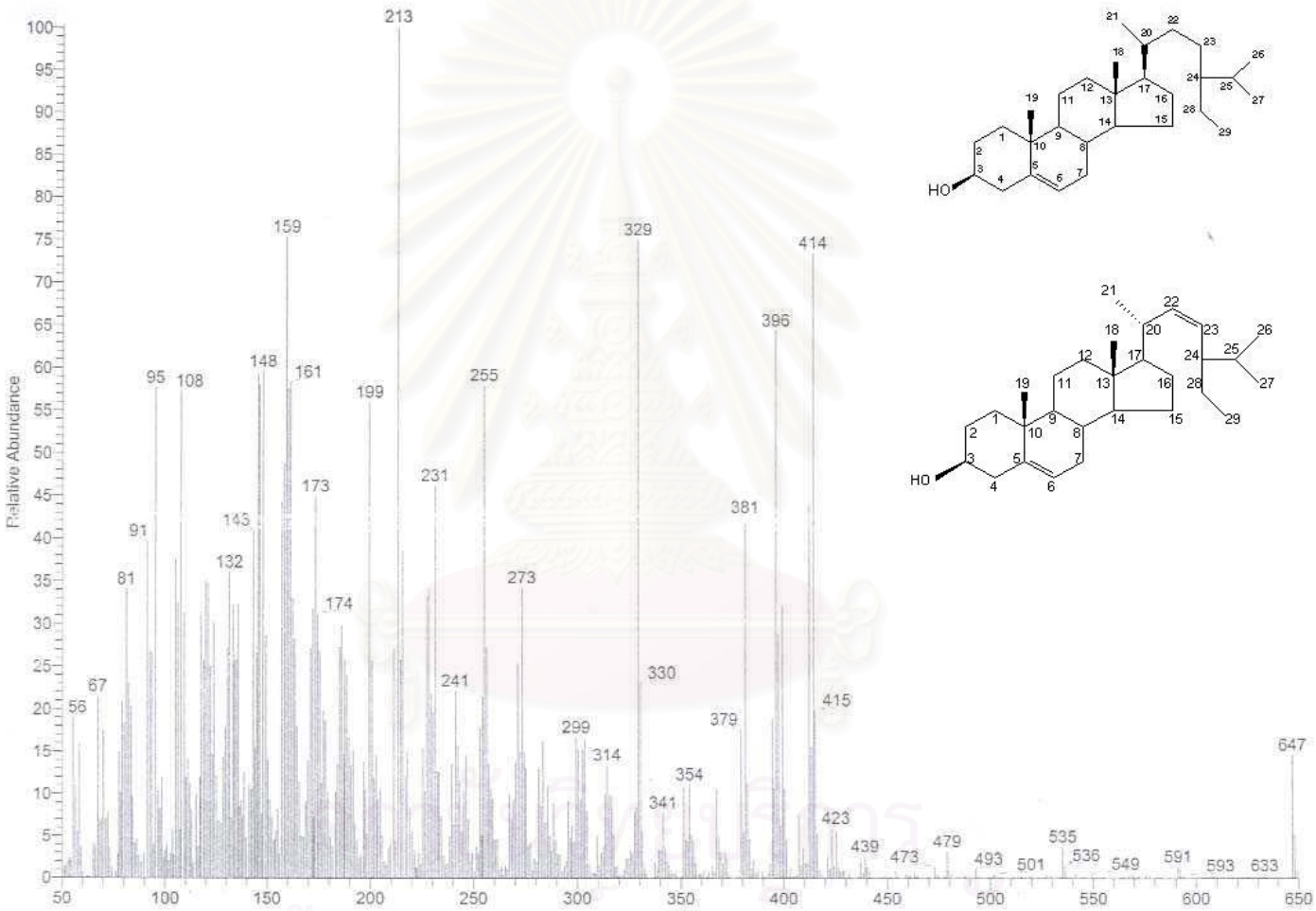

Figure 7. EIMS of compound DR 2

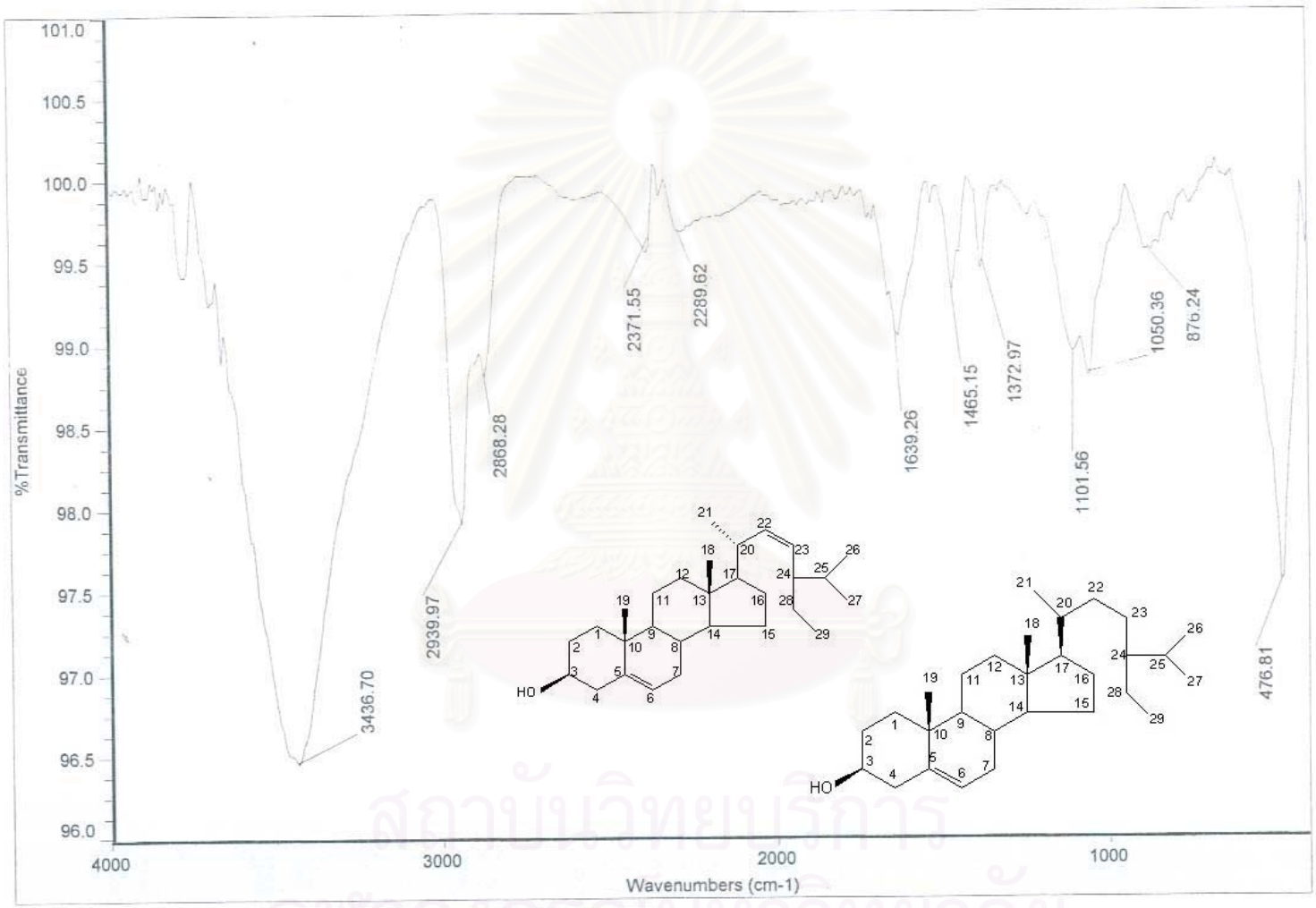

Figure 8. IR spectrum of compound DR 2

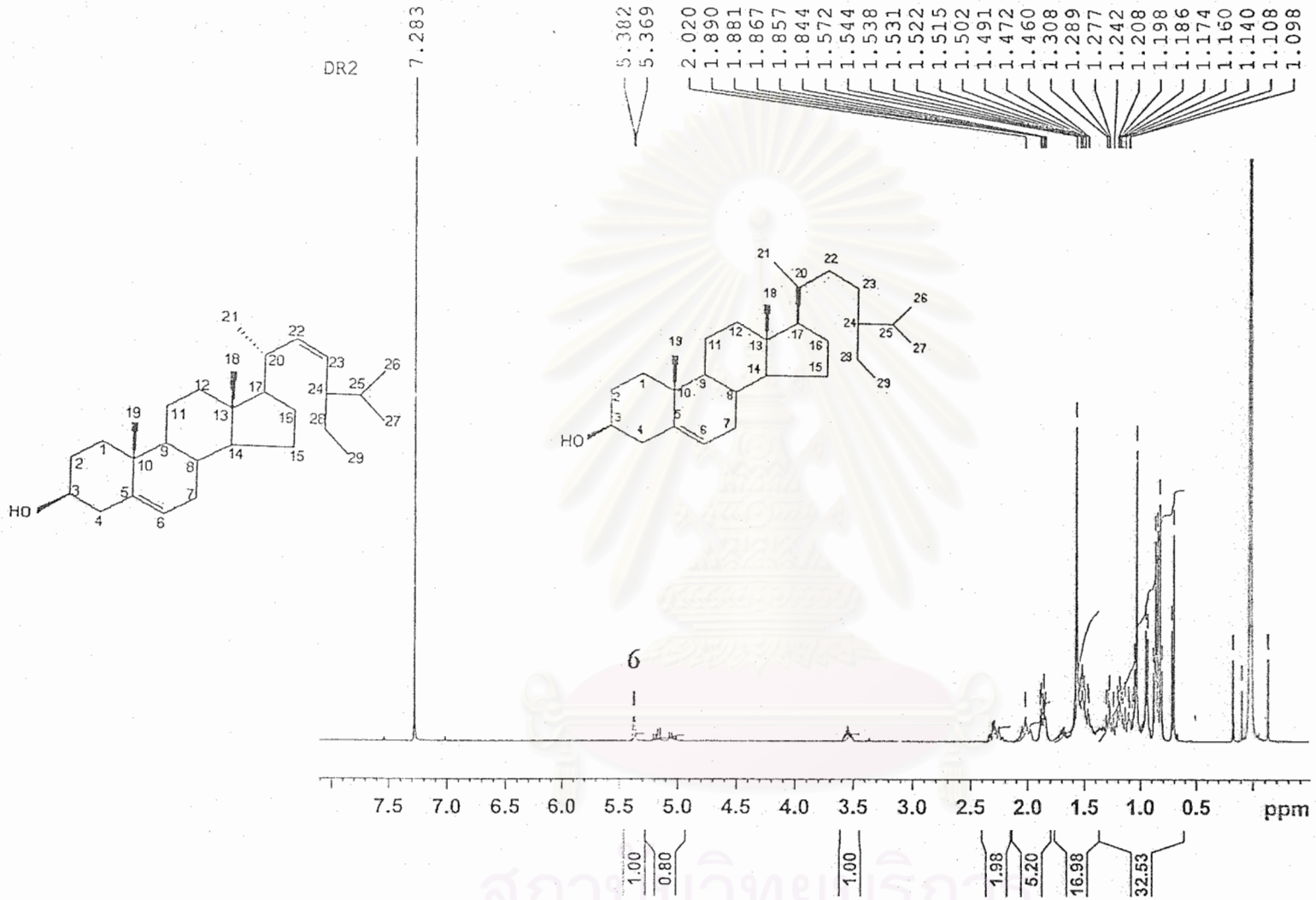

Figure 9a. The 400 MHz ^{1}H-NMR spectrum of compound DR 2

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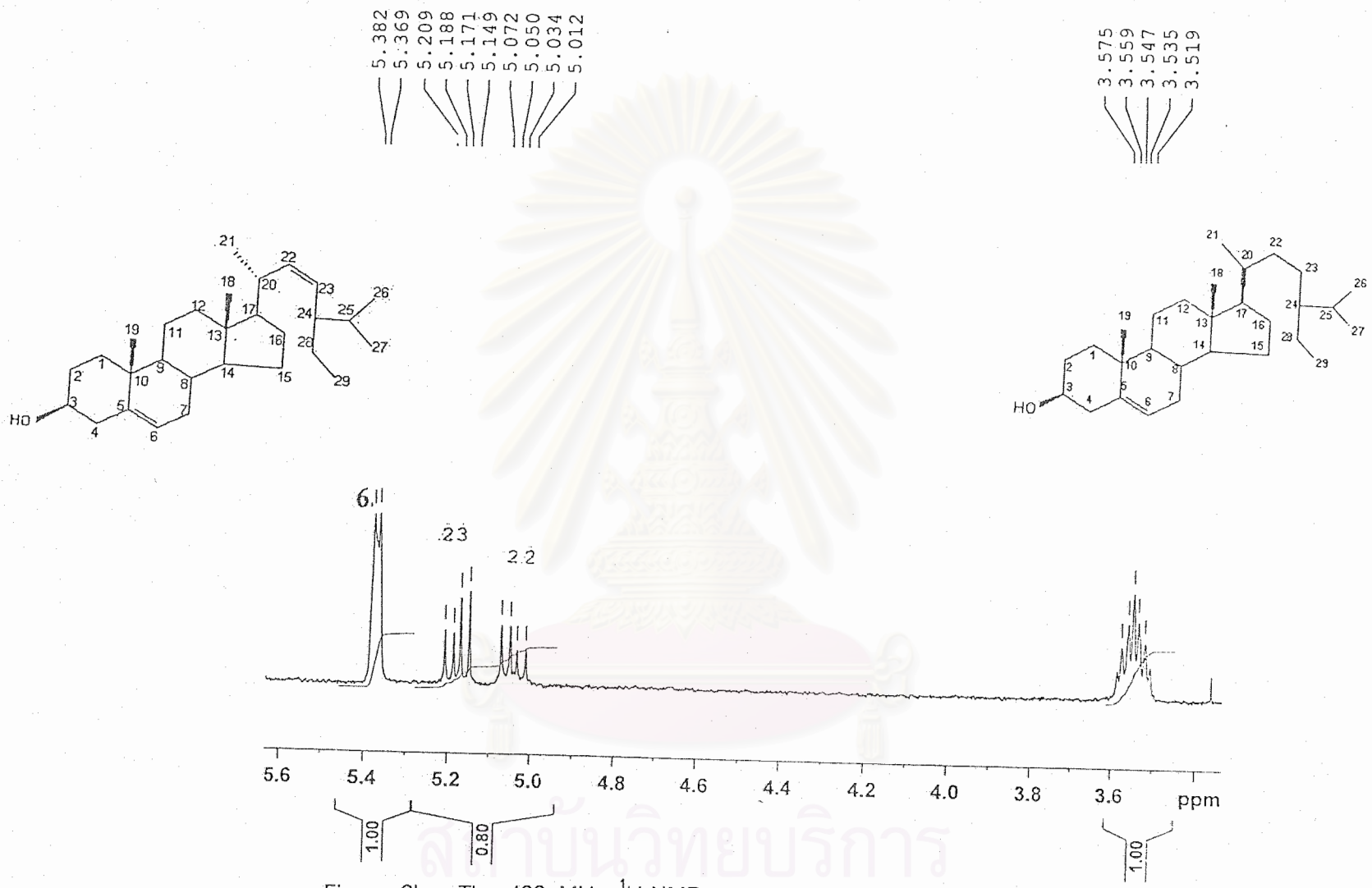

Figure 9b. The 400 MHz ^{1}H-NMR spectrum of compound DR 2 (expanded)

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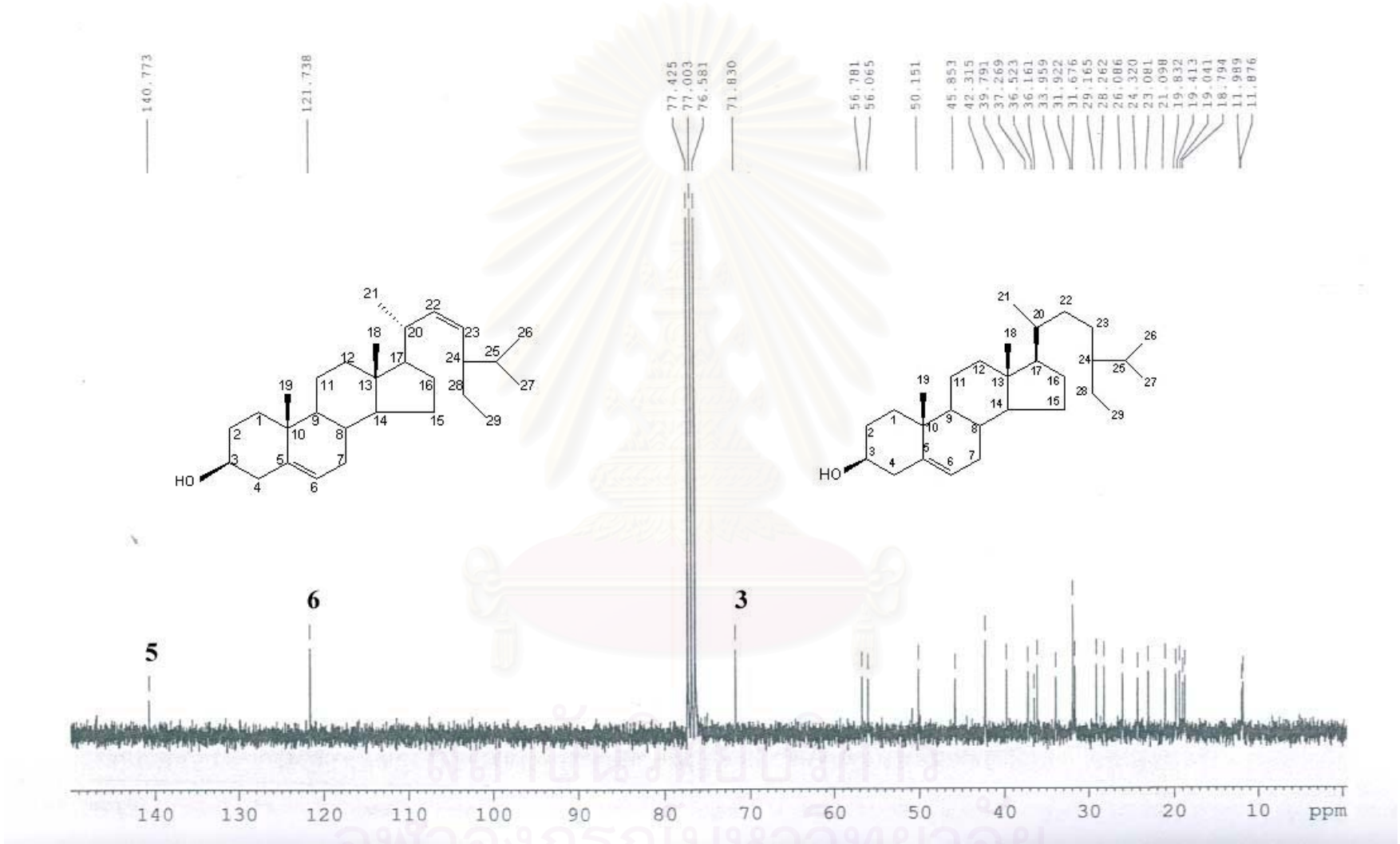

Figure 10a. The 100 MHz ^{13}C-NMR spectrum of compound DR 2

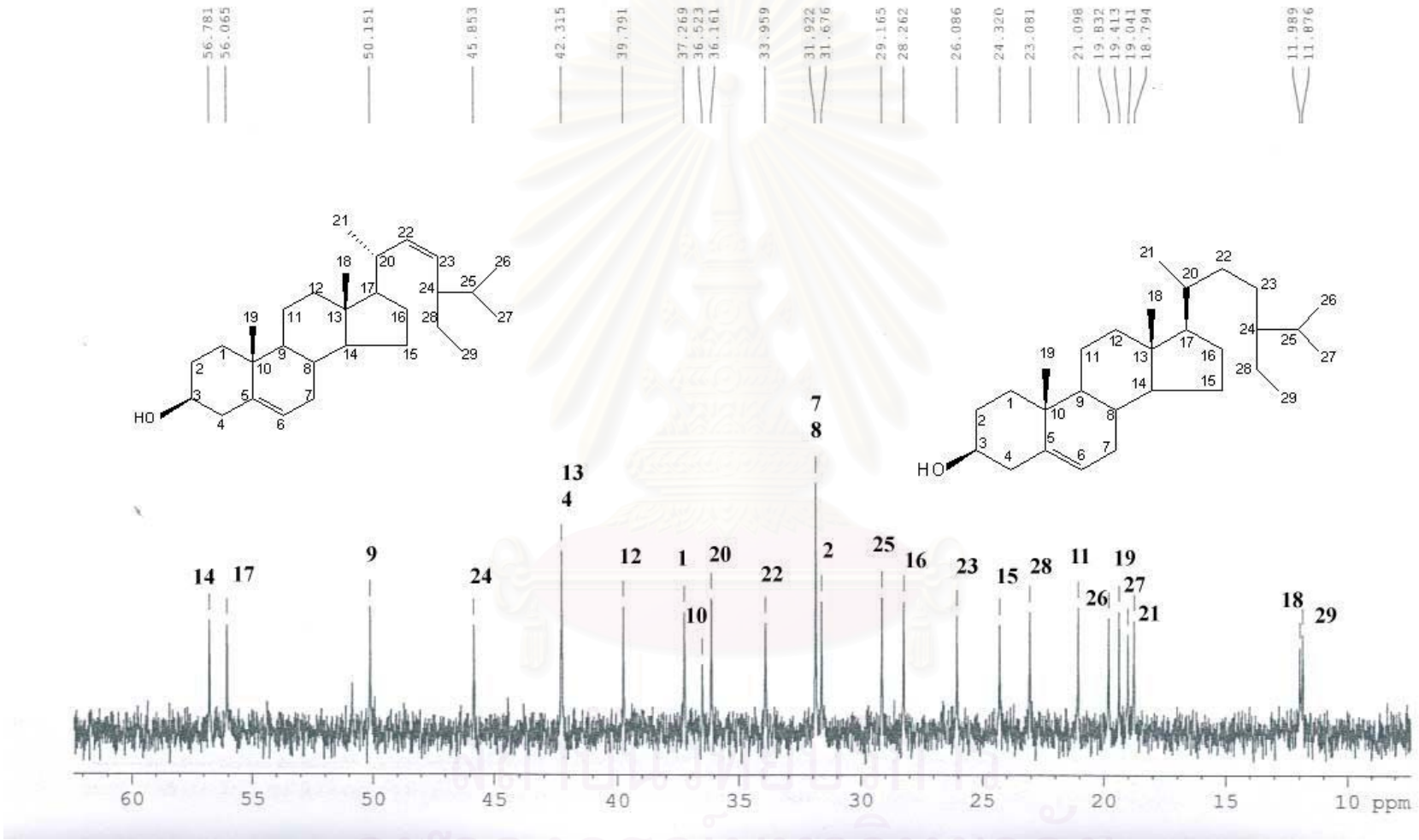

Figure 10b. The 100 MHz ^{13}C-NMR spectrum of compound DR 2 (expanded)

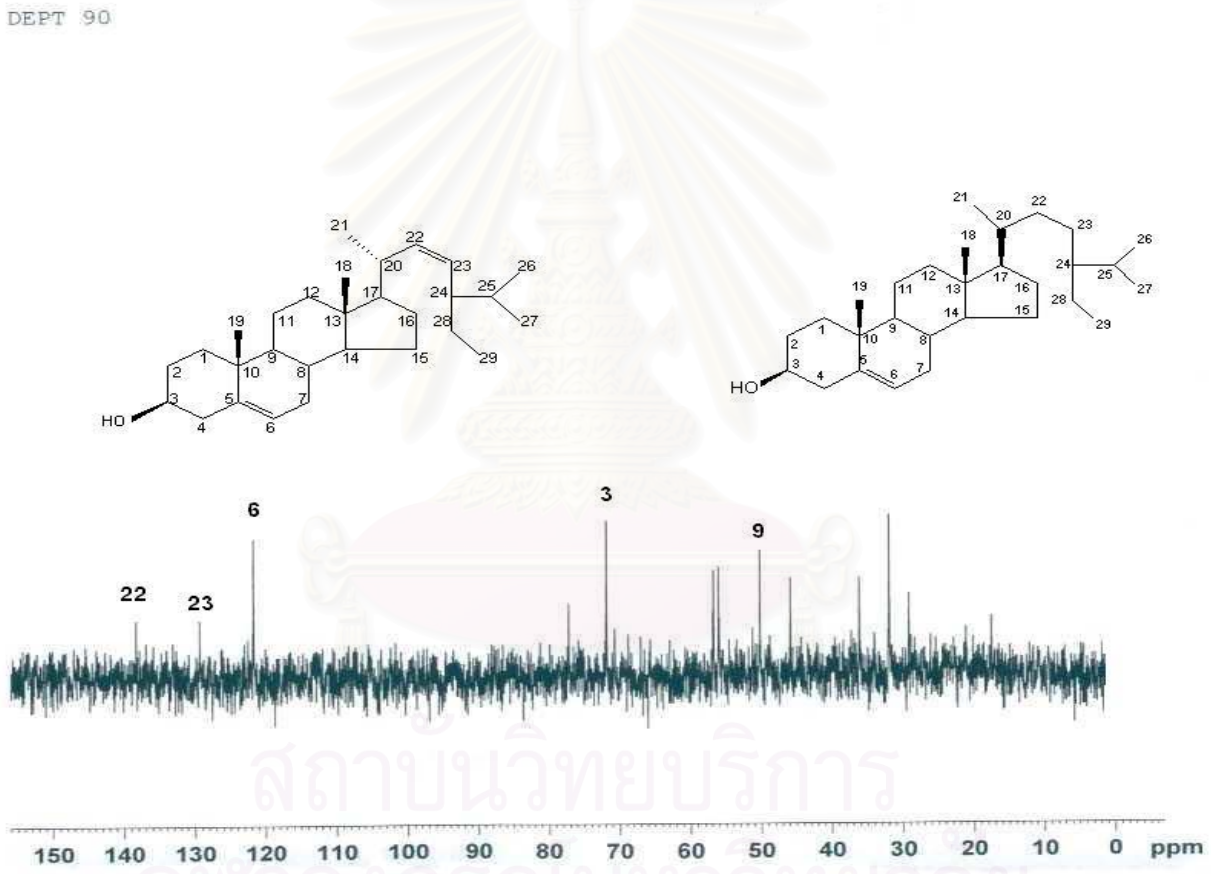

Figure 11. The 100 MHz ^{13}C-DEPT 90 NMR spectra of compound DR 2

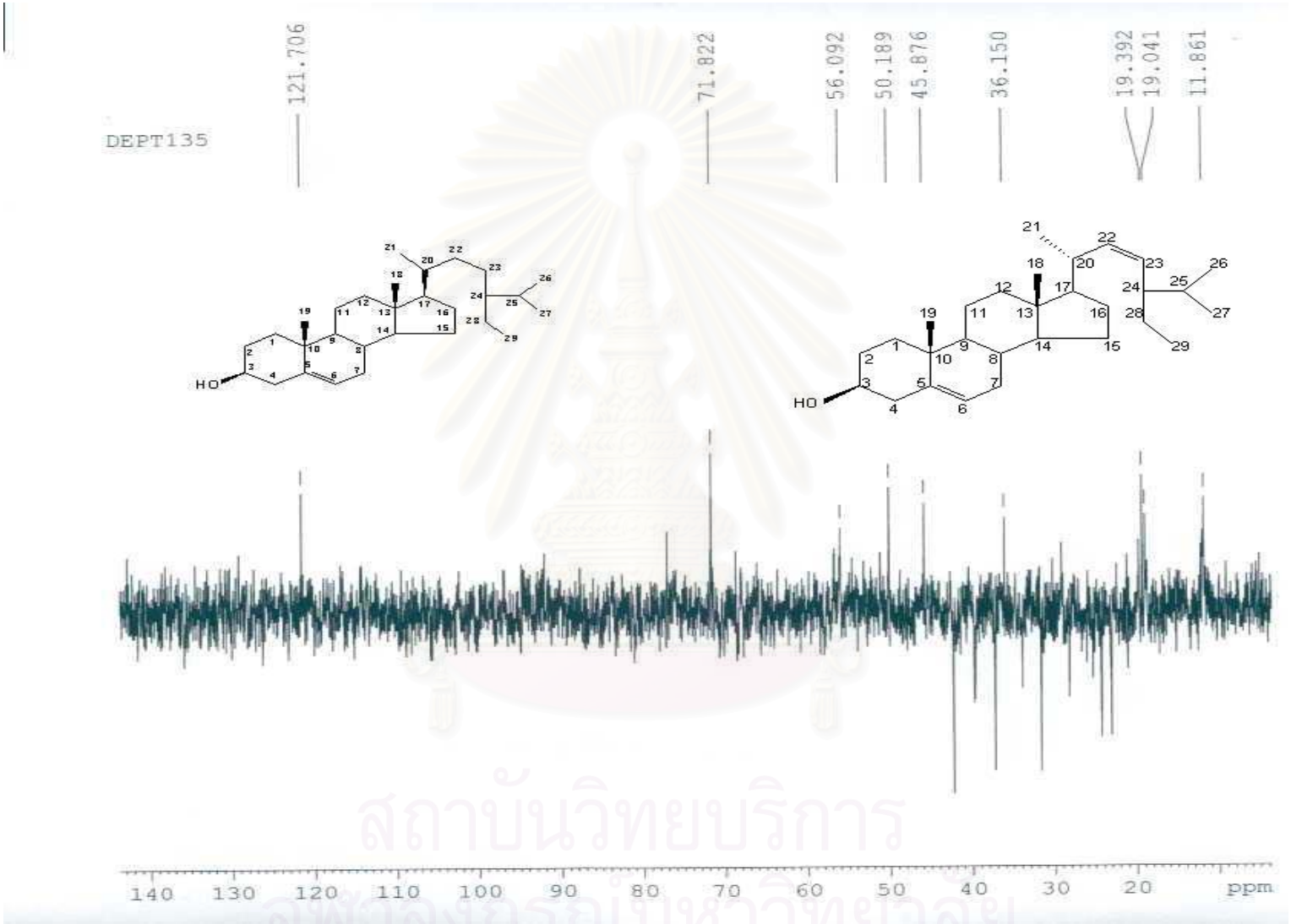

Figure 12. The 100 MHz ^{13}C-DEPT 135 NMR spectra of compound DR 2

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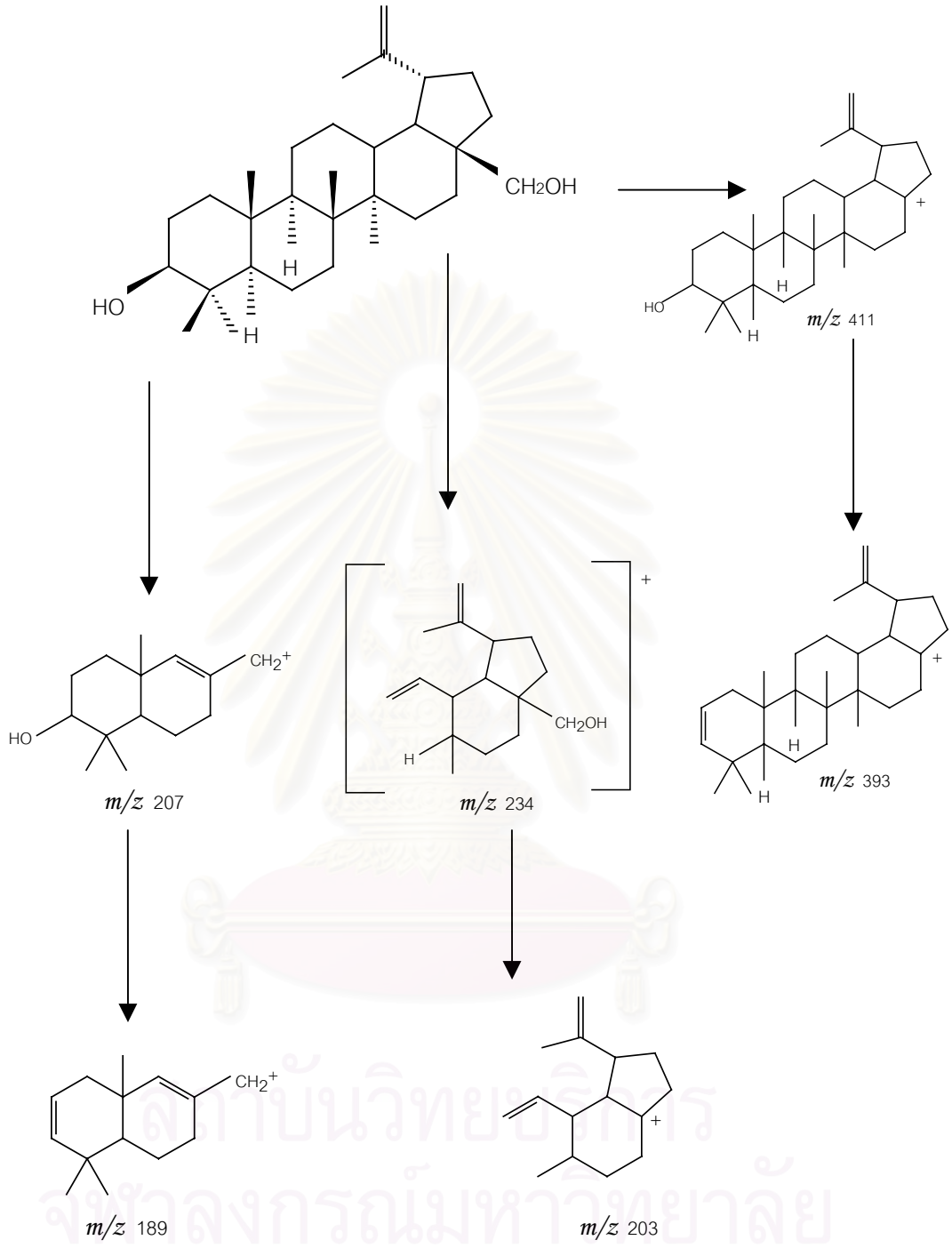
3. Identification of compound DR 3

Compound DR 3 was obtained as white amorphous powder from fraction DRH 30 of the hexane extract (9 mg, 0.0006% yield). The compound gave red-violet color to Liebermann-Burchard reagent, suggesting its triterpenoid nature. The EIMS spectrum of this compound (Figure 13) showed a molecular ion peak at *m/z* 442, corresponding to the molecular formula $C_{30}H_{50}O_2$. Mass fragment peaks at *m/z* 411 (M^+-CH_2OH) and 393 (M^+-CH_2OH-H_2O) suggested the presence of a primary alcoholic group and a hydroxyl group, respectively. An intense fragment peak at *m/z* 189 was suggestive of a lupane-type triterpenoid (Ogunkoya, 1981). This peak, as well as other peaks at *m/z* 203, 207 and 234, was the results of cleavage across the C-ring of the lupane skeleton (Scheme 5). The presence of a hydroxyl group in the molecule was also confirmed by an absorption band at 3388 cm^{-1} in the IR spectrum (Figure 14).

The ^{13}C-NMR spectrum (Figure 15a – 15b) showed the signals of 30 carbon atoms, supportive of a triterpenoid structure. The DEPT-90 and DEPT-135 experiments (Figure 16) helped in classifying the signals into those of six quaternary carbons at δ 37.0, 39.2, 40.8, 42.8, 48.6 and 150.9 ppm, six methine carbons at δ 37.6, 48.2, 48.8, 51.4, 56.4 and 78.7 ppm, twelve methylene carbons at δ 18.7, 21.2, 25.7, 26.6, 27.5, 29.6, 30.2, 34.4, 34.7, 38.3, 61.0 and 110.1 ppm, and six methyl carbons at δ 14.6, 15.8, 16.4, 17.1, 20.0 and 27.8 ppm.

The ^{1}H-NMR spectrum of DR 3 (Figure 17a-17b) displayed six methyl singlets at δ 0.73 (H_3-24), 0.80 (H_3-25), 0.94 (H_3-27), 0.95 (H_3-23), 1.00 (H_3-26) and 1.65 ppm (H_3-30). The presence of exomethylene protons could be observed as two downfield singlets (br) at δ 4.55 and 4.65 ppm (H_2-29), while a pair of doublets at δ 3.29 (*J*= 10.8) and 3.75 (*J*= 10.8) ppm could be attributed to hydroxy methylene protons (H_2-28). Another one proton double doublet at δ 3.14 (*J*= 4.8) ppm could be assigned to the carbinylic proton (H-3).



Scheme 5. Mass fragmentation of compound DR 3

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All spectroscopic data of DR 3 are in accordance with betulin, a known triterpenoid of the lupane type. Comparison of the ^{13}C-NMR data of this compound with those previously reported for betulin (Tinto *et al*., 1992) is shown in Table 17.

Table 17. Comparison of ^{13}C-NMR data of betulin (in $CDCl_3$) and compound DR 3 (in $CDCl_3$).

Carbon	Chemical shift (δ) ppm		Carbon	Chemical shift (δ) ppm	
	Betulin	DR 3		Betulin	DR 3
1	38.8	38.4	16	29.2	29.6
2	27.2	27.5	17	47.8	48.6
3	78.9	78.7	18	48.8	48.8
4	38.9	39.2	19	47.8	48.2
5	55.3	56.4	20	150.6	150.9
6	18.3	18.7	21	29.8	30.2
7	34.3	34.7	22	34.0	34.4
8	40.9	40.8	23	28.0	27.8
9	50.4	51.4	24	15.4	15.8
10	37.2	37.0	25	16.1	17.1
11	20.9	21.2	26	16.0	16.4
12	25.3	25.7	27	14.8	14.6
13	37.3	37.6	28	60.2	61.0
14	42.7	42.8	29	109.6	110.1
15	27.0	26.6	30	19.1	20.0

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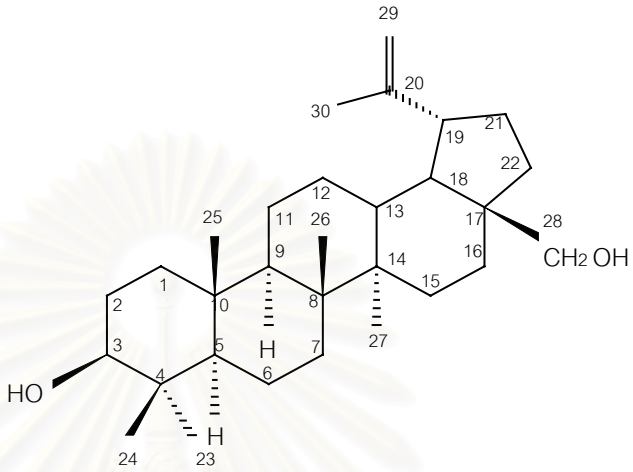
Therefore, it was concluded that DR 3 is betulin, the structure of which is shown below.



Betulin ($C_{30}H_{50}O_2$)

Similar to lupeol, betulin has been isolated from several plants of the genus *Diospyros*. The compound has been used as antiseptic (Batta and Rangaswami, 1973). It was demonstrated as having inhibitory effect against Epstein – Barr virus activation (Konoshima *et al.,* 1987), *in vitro* antitumor activity against human epidermoid carcinoma of nasopharynx (Miles *et al.,* 1974) and Walker-256 tumor system (Sheth *et al.,* 1973).

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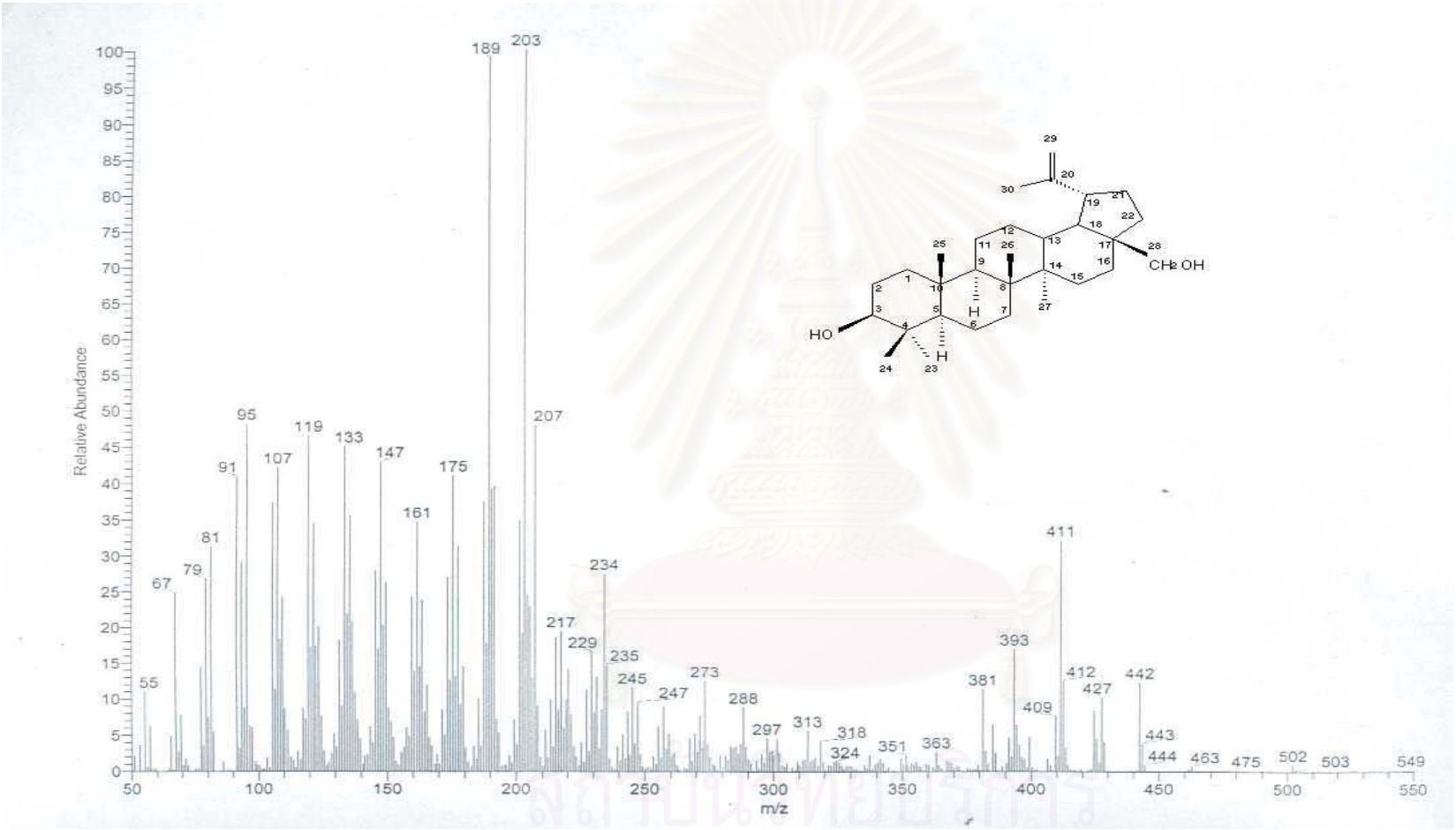


Figure 13. EIMS of compound DR 3

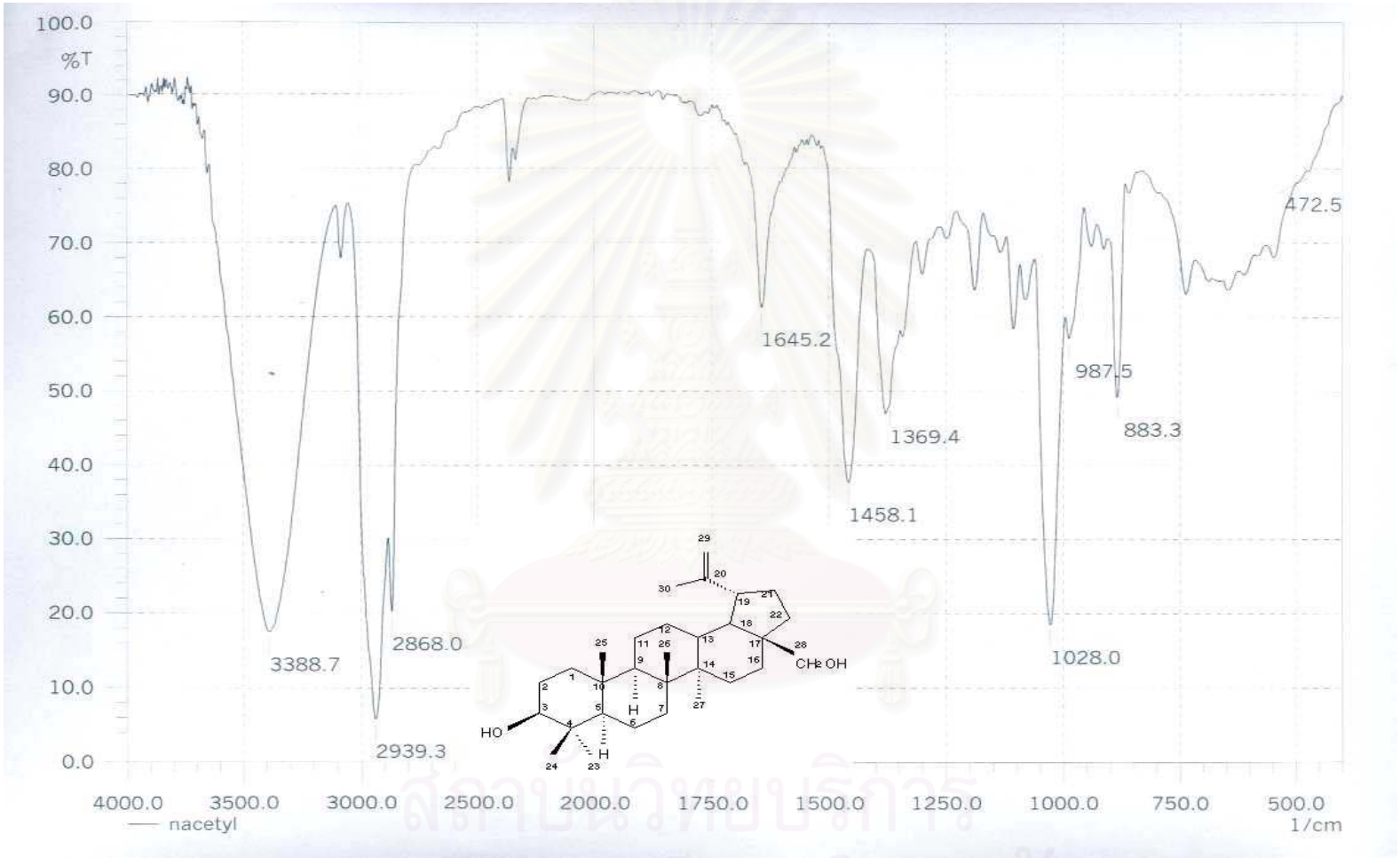


Figure 14. IR spectrum of compound DR 3

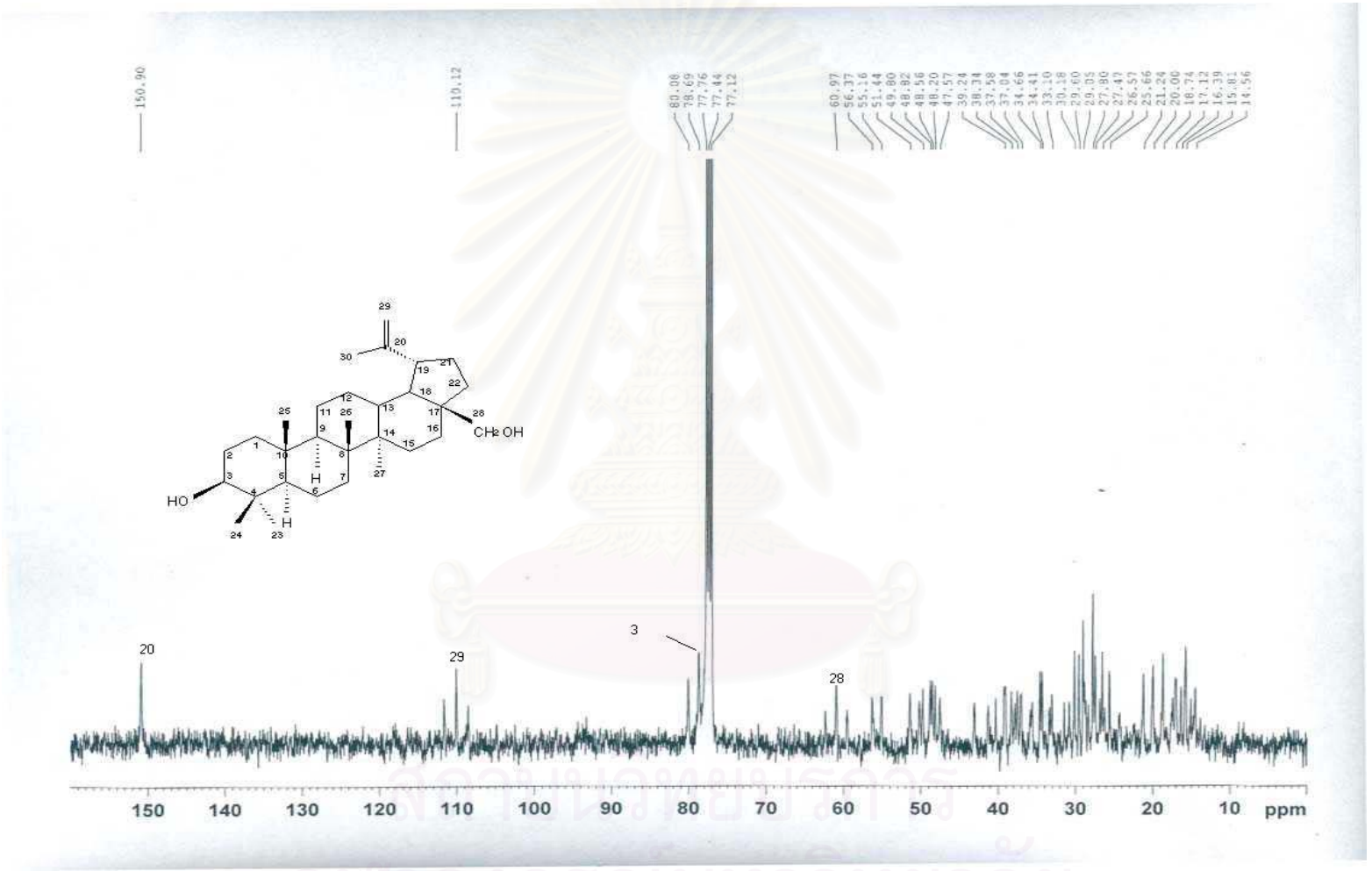


Figure 15a. The 100 MHz ^{13}C-NMR spectrum of compound DR 3 (in $CDCl_3$)

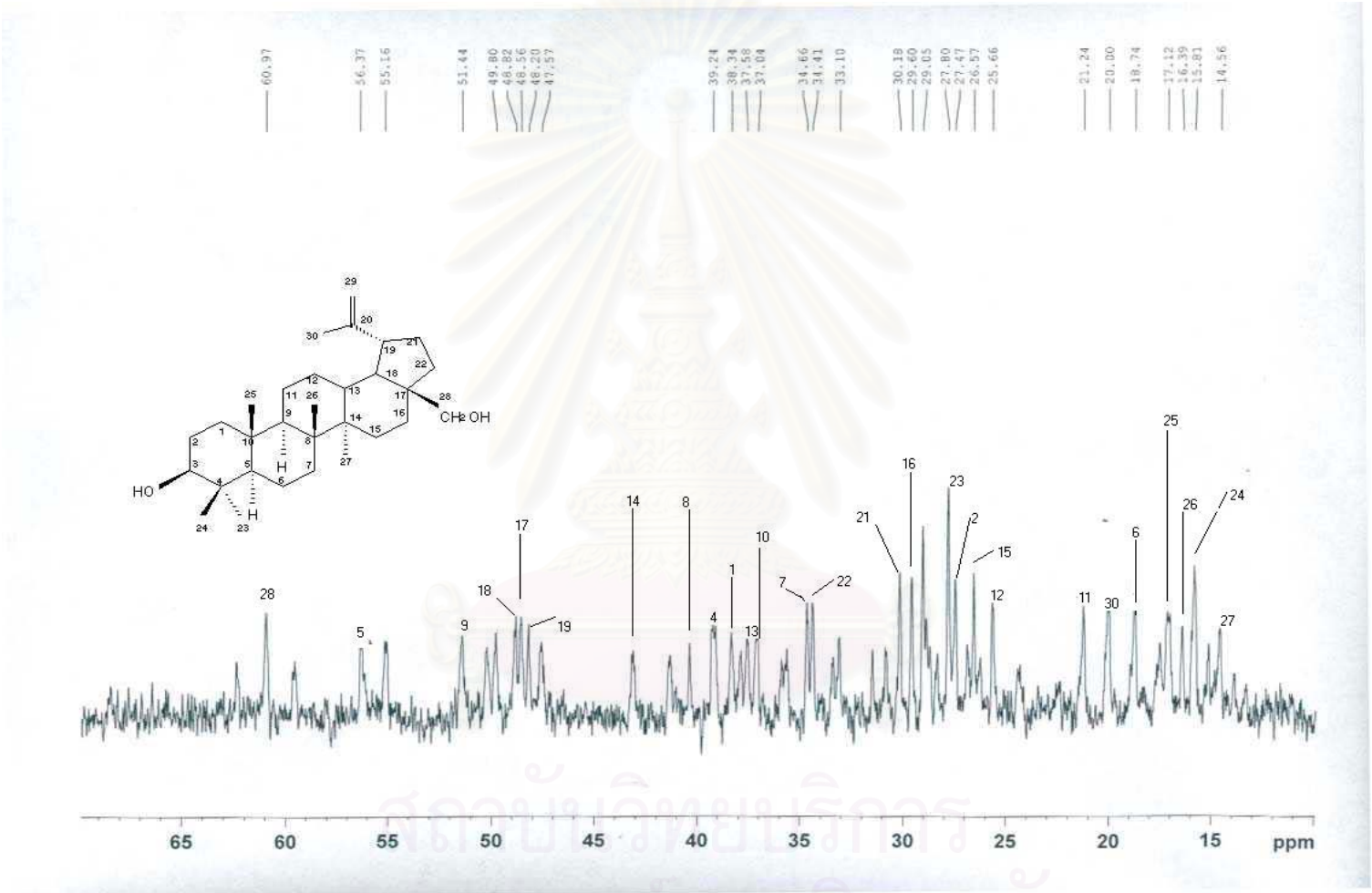


Figure 15b. The 100 MHz ^{13}C-NMR spectrum of compound DR 3 (in $CDCl_3$) (expanded)

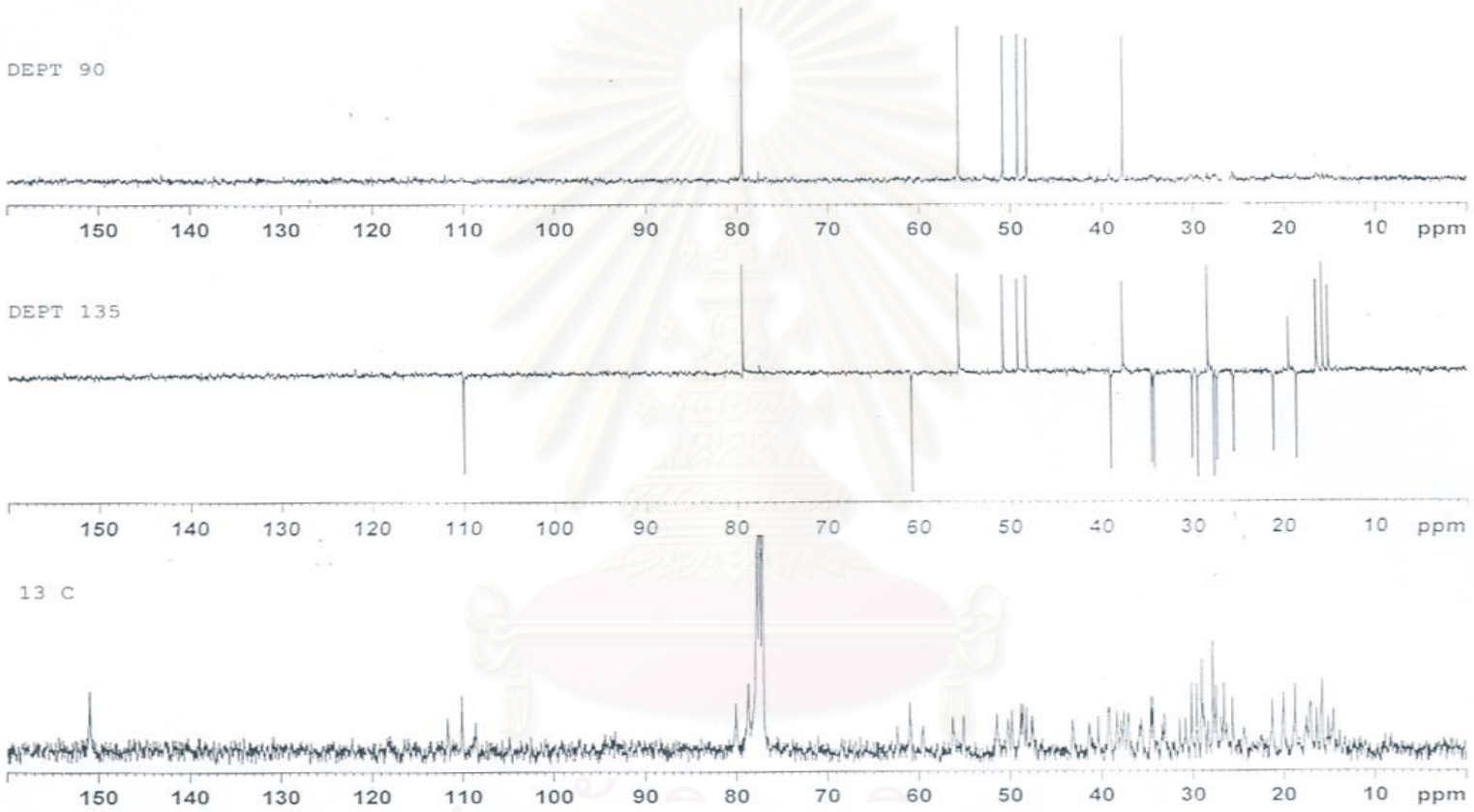


Figure 16. The 100 MHz ^{13}C-DEPT NMR spectra of compound DR 3 (in $CDCl_3$)

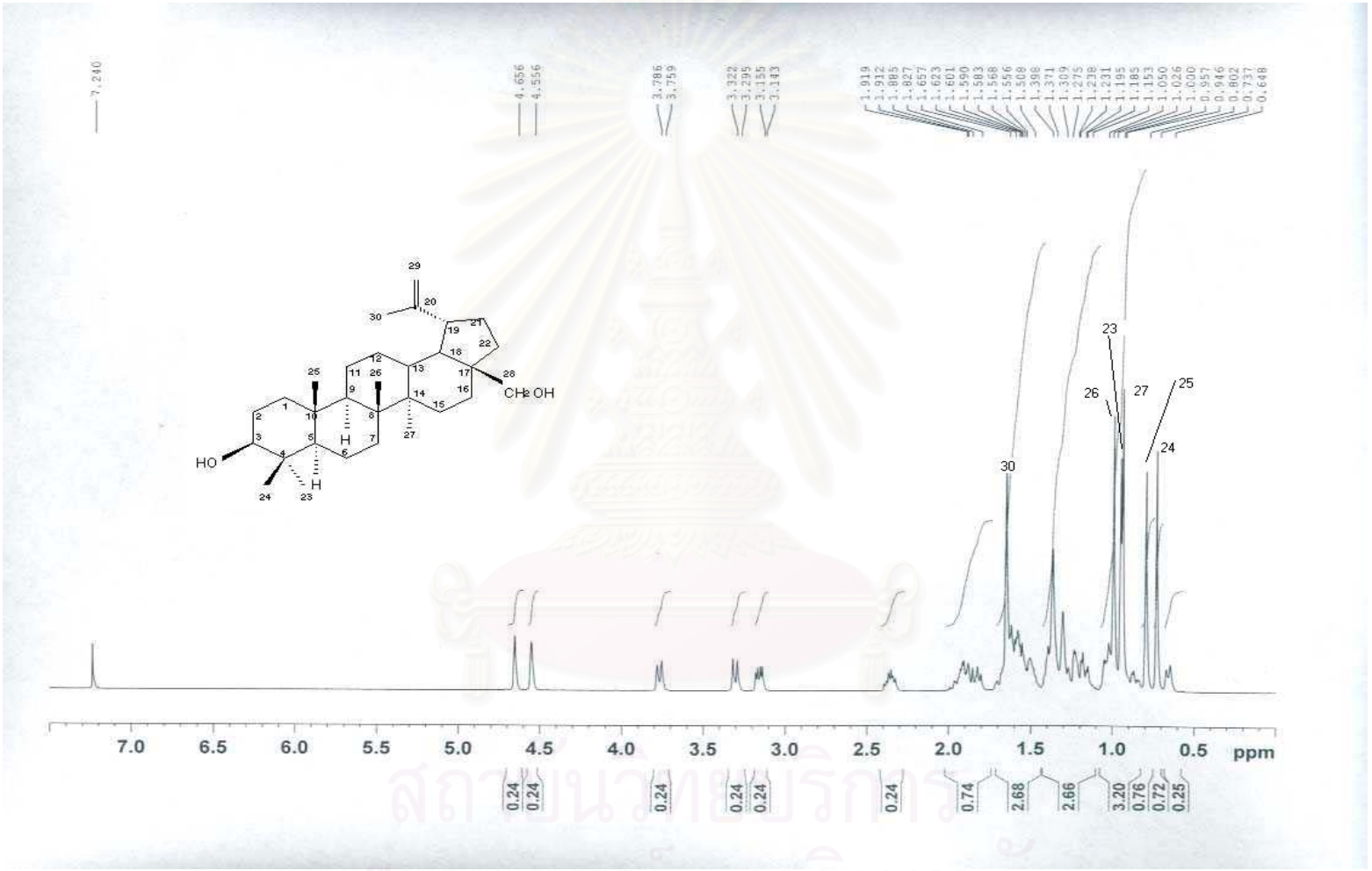


Figure 17a. The 400 MHz ^{1}H NMR spectrum of compound DR 3 (in $CDCl_3$)

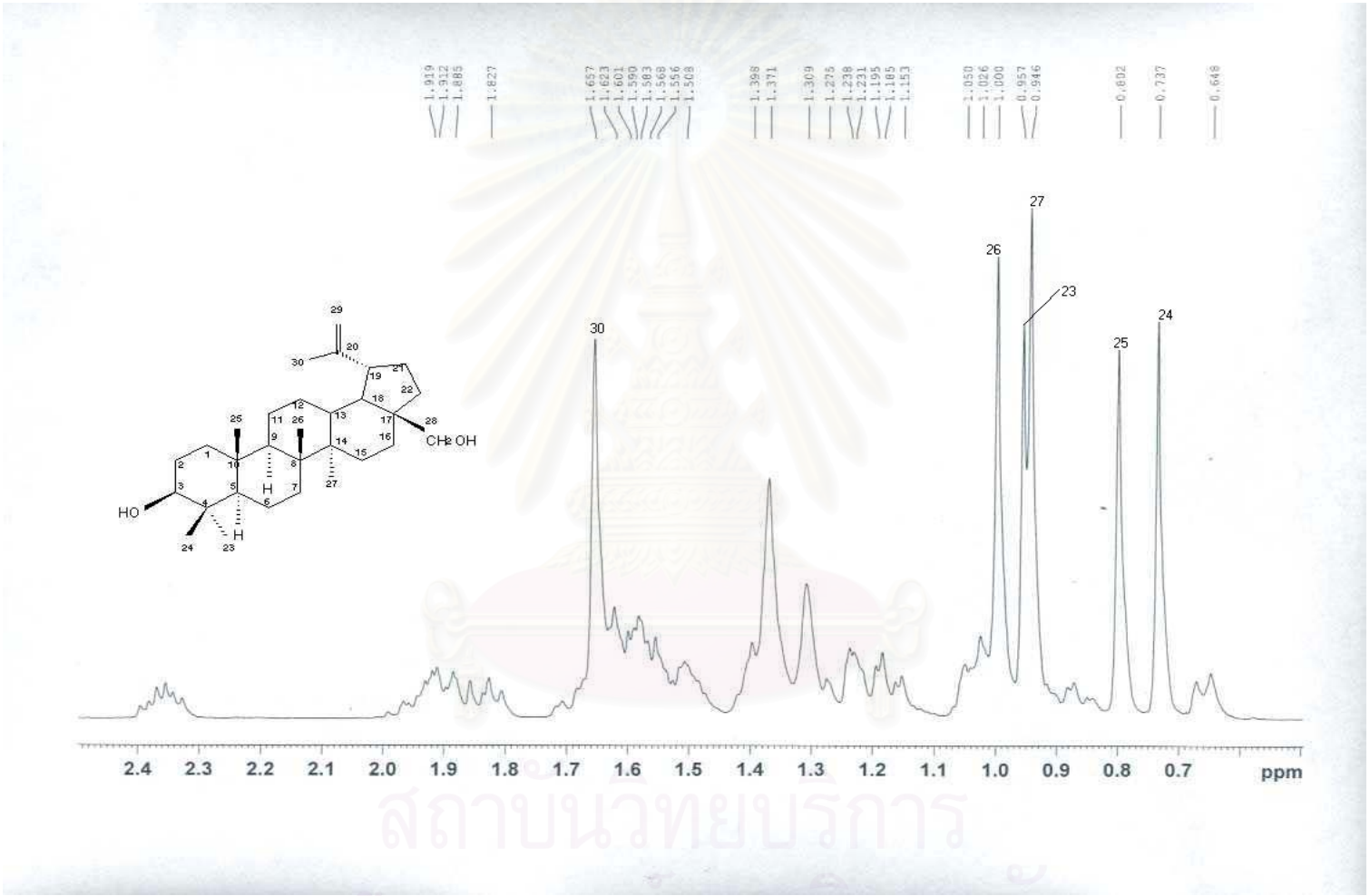


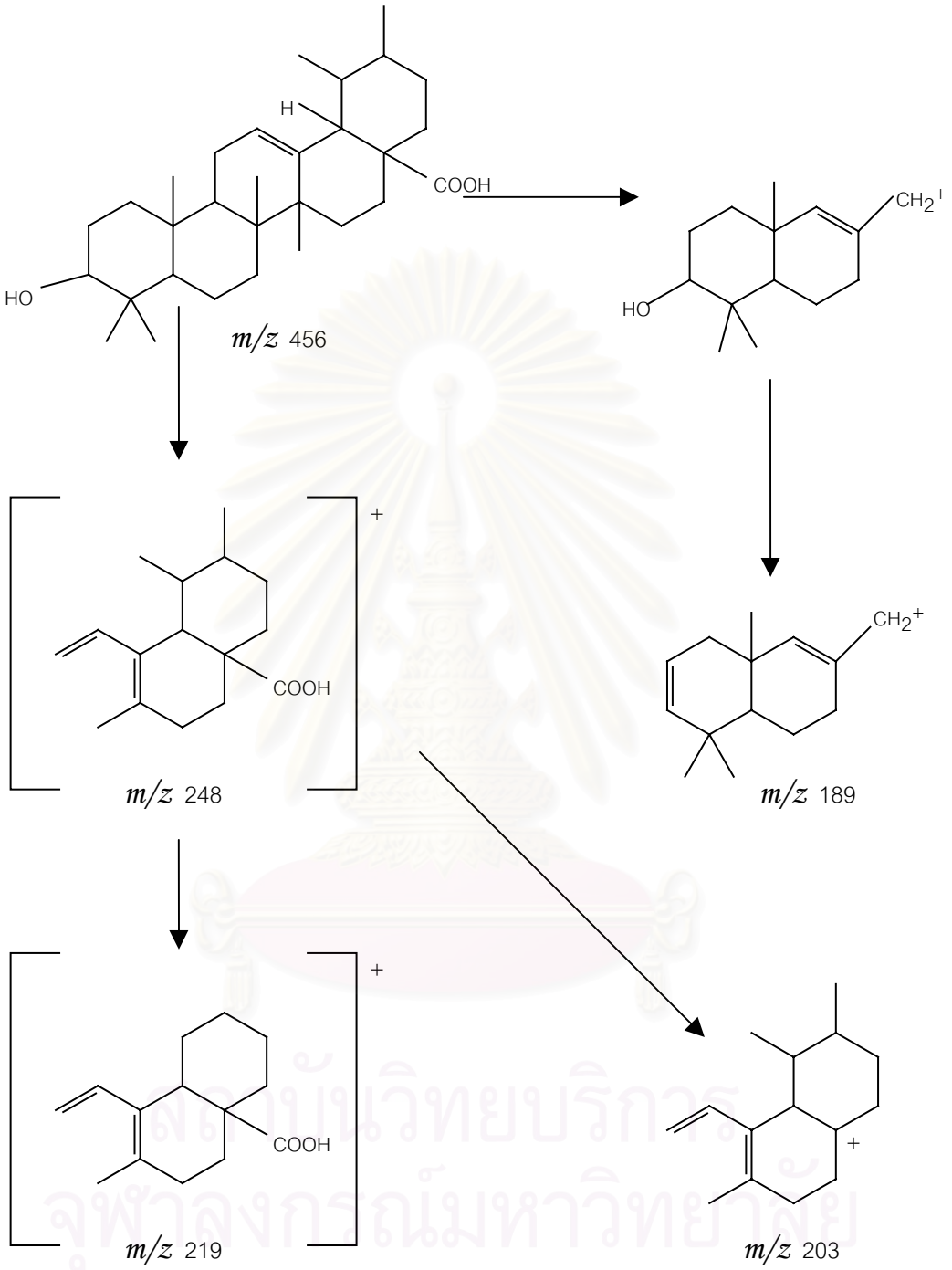
Figure 17b. The 400 MHz ^{1}H NMR spectrum of compound DR 3 (in $CDCl_3$) (expanded)

4. Identification of compound DR 4

Compound DR 4 was obtained as colorless needles (54 mg, 0.0004% yield) from fraction DRC 11 of the chloroform extract. The compound gave red-violet color to Liebermann-Burchard reagent, suggesting its triterpenoid nature. The base peak at *m/z* 248 in the EIMS (Figure 18), resulting from the cleavage through a retro-Dieals-Alder reaction, is characteristic of a C-12 unsaturated triterpenoid with the oleanane or ursane skeleton containing carboxylic group in ring D or E (Ogunkoya, 1981). The further losses of the methyl group and the carboxylic group led to fragment peaks at *m/z* 219 and 203, respectively. A peak at *m/z* 189 was produced by the loss of water from the other product of the retro-Dieals-Alder fragmentation (Scheme 6). The presence of the carboxylic functionality in the molecule was confirmed by an IR absorption band at 1689 cm^{-1} (Figure 19). A broad band at 3460 cm^{-1} indicated the presence of the hydroxyl group.

The ^{13}C-NMR spectrum of DR 4 (Figure 20a-20b) showed 30 carbon signals, supportive of a triterpenoid structure. The DEPT-90 and DEPT-135 (Figure 21a-21b) experiments were employed to classify these signals into those of seven quaternary carbons at δ 178.4 (C-28), 138.3 (C-13), 47.1 (C-17), 41.8 (C-14), 38.8 (C-8), 38.5 (C-4) and 36.6 ppm (C-10), seven methine carbons at δ 124.7 (C-12), 77.0 (C-3), 54.9 (C-5), 52.5 (C-18), 46.9 (C-9), 38.6 (C-19) and 38.5 ppm (C-20), nine methylene carbons at δ 38.3 (C-1), 36.4 (C-22), 32.8 (C-7), 30.3 (C-21), 27.6 (C-15), 27.1 (C-2), 23.9 (C-16), 23.0 (C-11) and 18.1 ppm (C-6), and seven methyl carbons at δ 28.4 (C-23), 23.4 (C-27), 21.2 (C-30), 17.1 (C-29), 17.0 (C-26), 16.2 (C-25) and 15.3 ppm (C-24). The most downfield carbon signal at δ 178.4 ppm represents the carbonyl carbon of the carboxylic group and two downfield carbon signals at δ 138.3 and 124.7 ppm represents the double bond between C-12 and C-13 of the compound.

The ^{1}H-NMR spectrum of DR 4 (Figure 22a-22b) showed the signals of methyl protons at δ 0.66 (H_3-25, *s*), 0.73 (H_3-26, *s*), 0.79 (H_3-29, *d* *J*=6.4 Hz),



Scheme 6. Mass fragmentation of compound DR 4

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0.85 (H_3-24, *s*), 0.88 (H_3-23, *s*), 0.88 (H_3-30, *d* *J*=7.2 Hz) and 1.02 ppm (H_3-27, *s*). The signal at δ 5.11 ppm could be assigned to the olefinic proton (H-12) and the signal at δ 2.98 ppm could be assigned to the methine proton of hydroxy – substituted position 3 (H-3).

Comparison of ^{13}C-NMR data of DR 4 with the reported data of ursolic acid (Lin *et al*., 1987) and methyl ursolate (Zhang and Yu, 1990) suggested that DR 4 is ursolic acid. Almost all carbon assignments of DR 4 are in agreement with the reported data of ursolic acid except for the assignments of signals for C-11 and C-29 at δ 23.0 and 17.1 ppm (DR 4) instead of δ 17.1 and 23.2 ppm (ursolic acid), respectively. However, the assignments of C-11 and C-29 of DR 4 were found to be in agreement with the reported data of methyl ursolate (δ 23.3 and 16.9 ppm, respectively), and the DEPT experiment of DR 4 also indicated that the carbon signal at δ 17.1 ppm is due to a methyl carbon (C-29), while the signal at δ 23.0 ppm is due to a methylene carbon (C-11).

All spectroscopic data of DR 4 are in accordance with ursolic acid, a known triterpenoid of the ursane type. Comparison of the ^{13}C-NMR data of this compound with the literature value of ursolic acid (Lin *et al*., 1987) is shown in Table 18.

สถาบันวิทยบริการ
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Table 18. Comparison of ^{13}C-NMR data of ursolic acid (in pyridine-d_5) and DR 4 (in DMSO-d_6).

Carbon	Chemical shift (δ) ppm		Carbon	Chemical shift (δ) ppm	
	Ursolic acid	DR 4		Ursolic acid	DR 4
1	38.7	38.3	16	24.2	23.9
2	27.2	27.1	17	47.5	47.1
3	78.2	77.0	18	52.7	52.5
4	38.8	38.5	19	39.1	38.6
5	55.2	54.9	20	38.8	38.5
6	18.3	18.1	21	30.7	30.3
7	33.0	32.8	22	36.7	36.4
8	39.5	38.8	23	28.0	28.4
9	47.5	46.9	24	15.7	15.3
10	36.9	36.6	25	15.4	16.2
11	17.1	23.0	26	17.0	17.0
12	125.2	124.7	27	23.5	23.4
13	138.3	138.3	28	179.9	178.4
14	42.0	41.8	29	23.2	17.1
15	28.2	27.6	30	21.2	21.2

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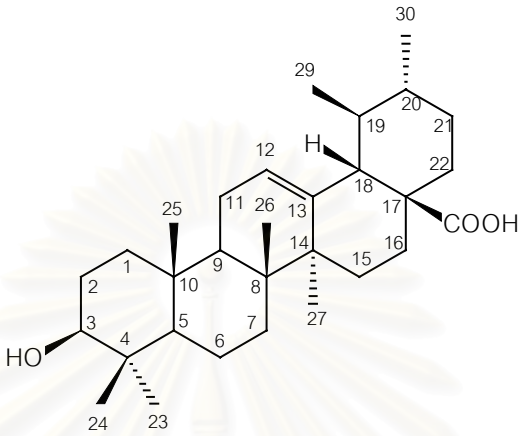
Therefore, it was concluded that DR 4 is ursolic acid, the structure of which is shown below.



Ursolic acid ($C_{30}H_{48}O_3$)

Similar to lupeol and betulin, ursolic acid has been isolated from several plants of the genus *Diospyros*. The compound has been reported possessing anti-inflammatory activity (Recio *et al*., 1995a ; Recio *et al*., 1995b). It was also demonstrated as exhibiting inhibitory effect against Epstein-Barr virus activation (Ohigashi *et al*., 1986), and inhibitory effect against HIV-1 protease (Singh, Singh and Bani, 1994).

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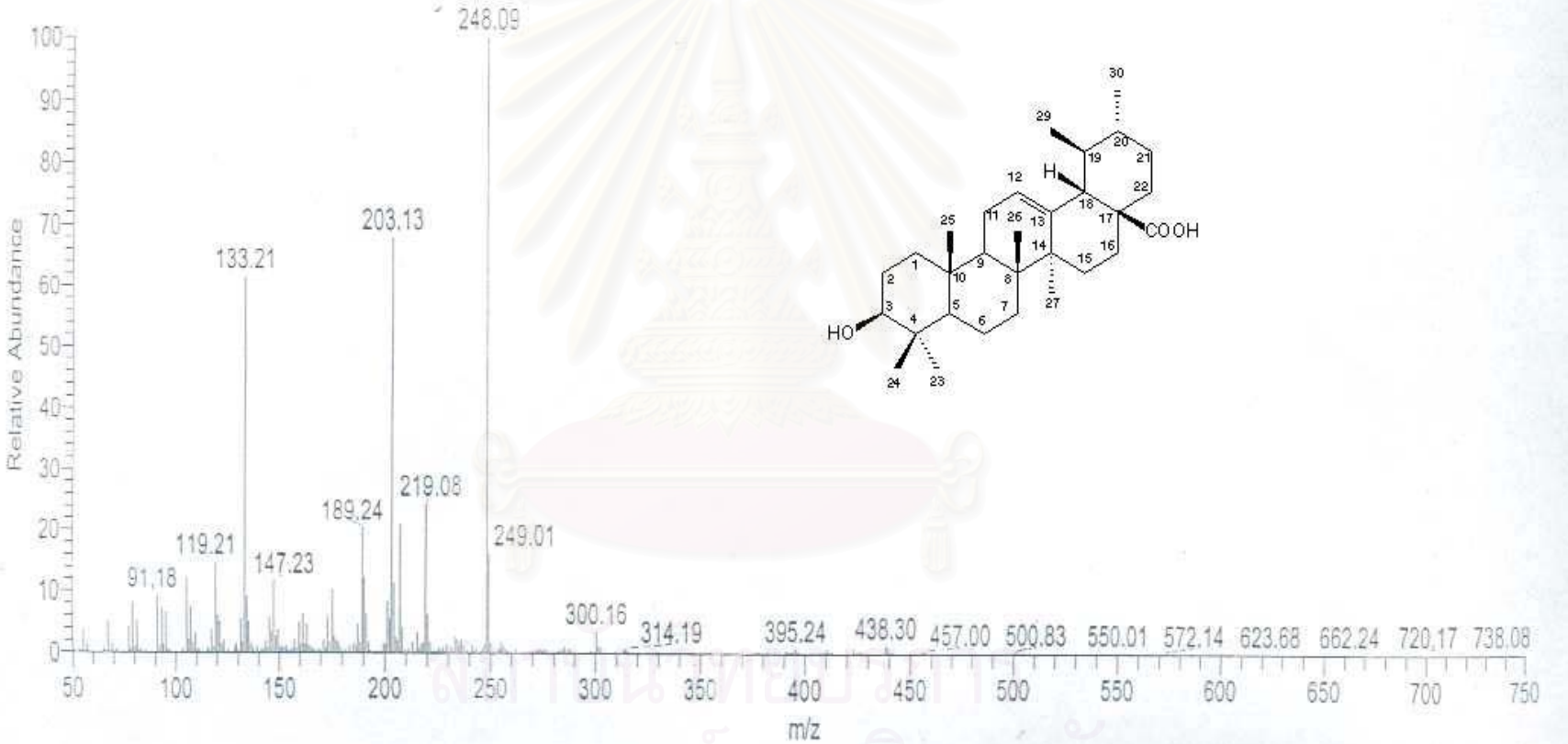

Figure 18. EIMS of compound DR 4

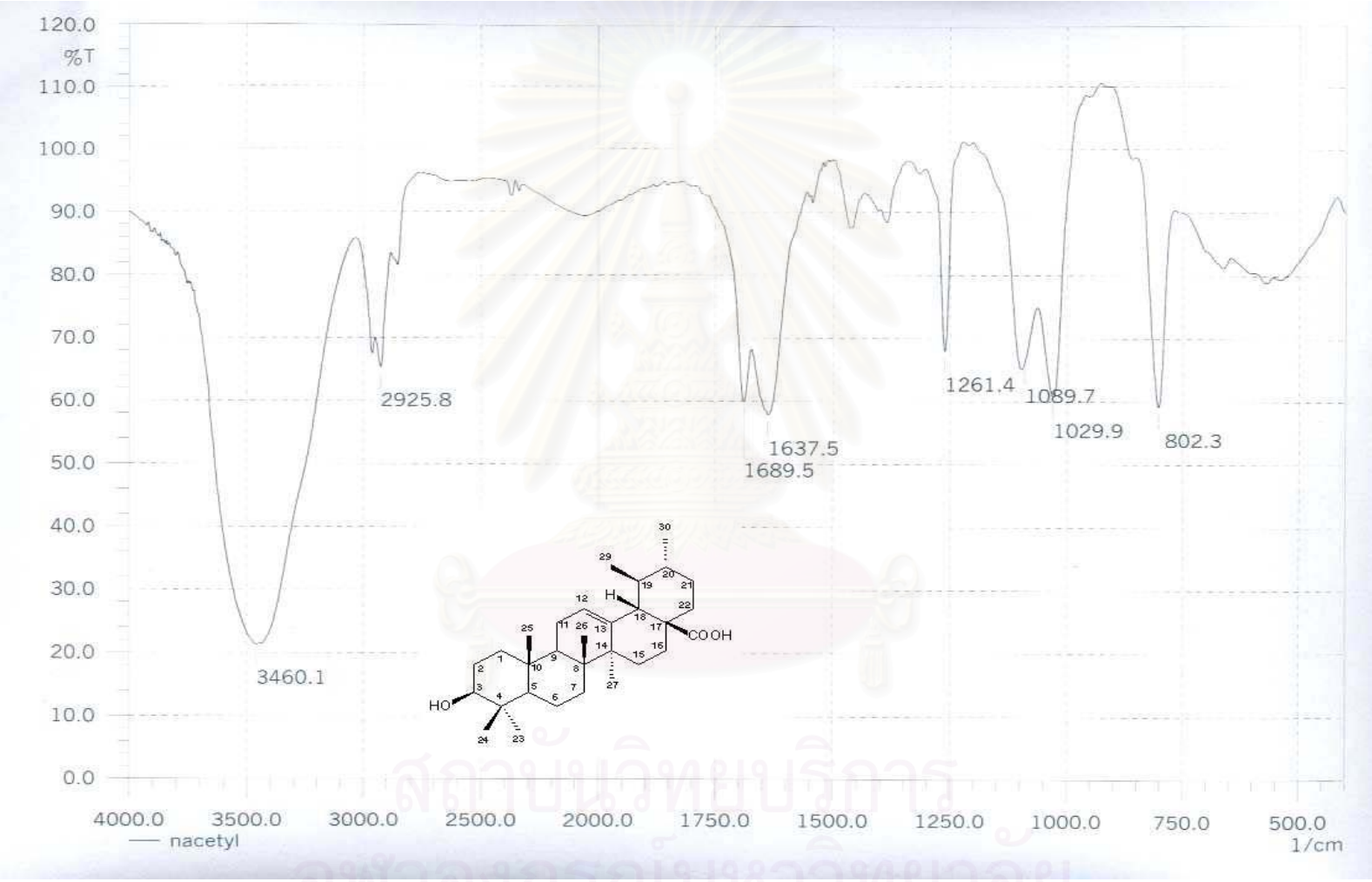

Figure 19. IR spectrum of compound DR 4

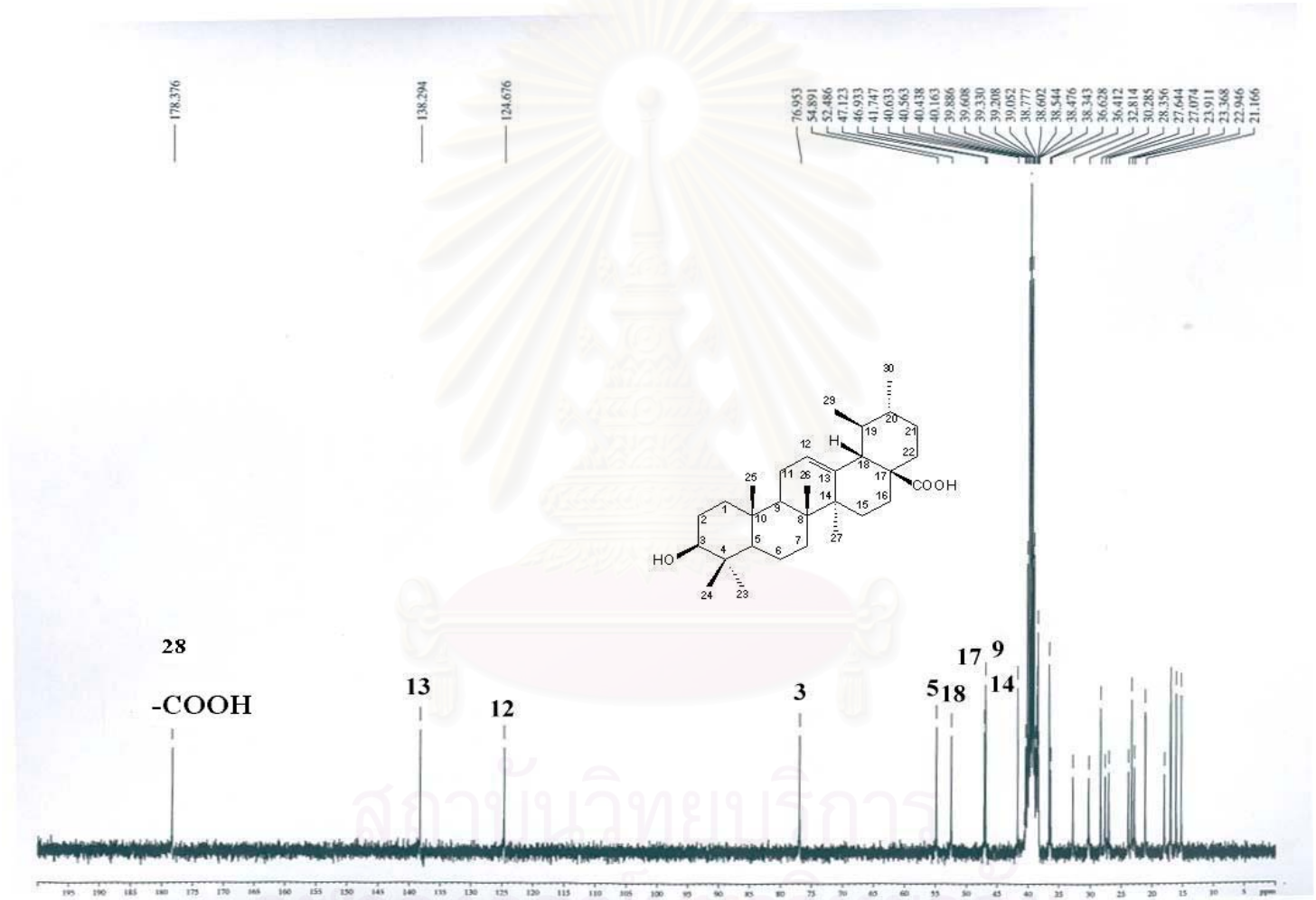

Figure 20a. The 100 MHz ^{13}C-NMR spectrum of compound DR 4 (in DMSO-d_6)

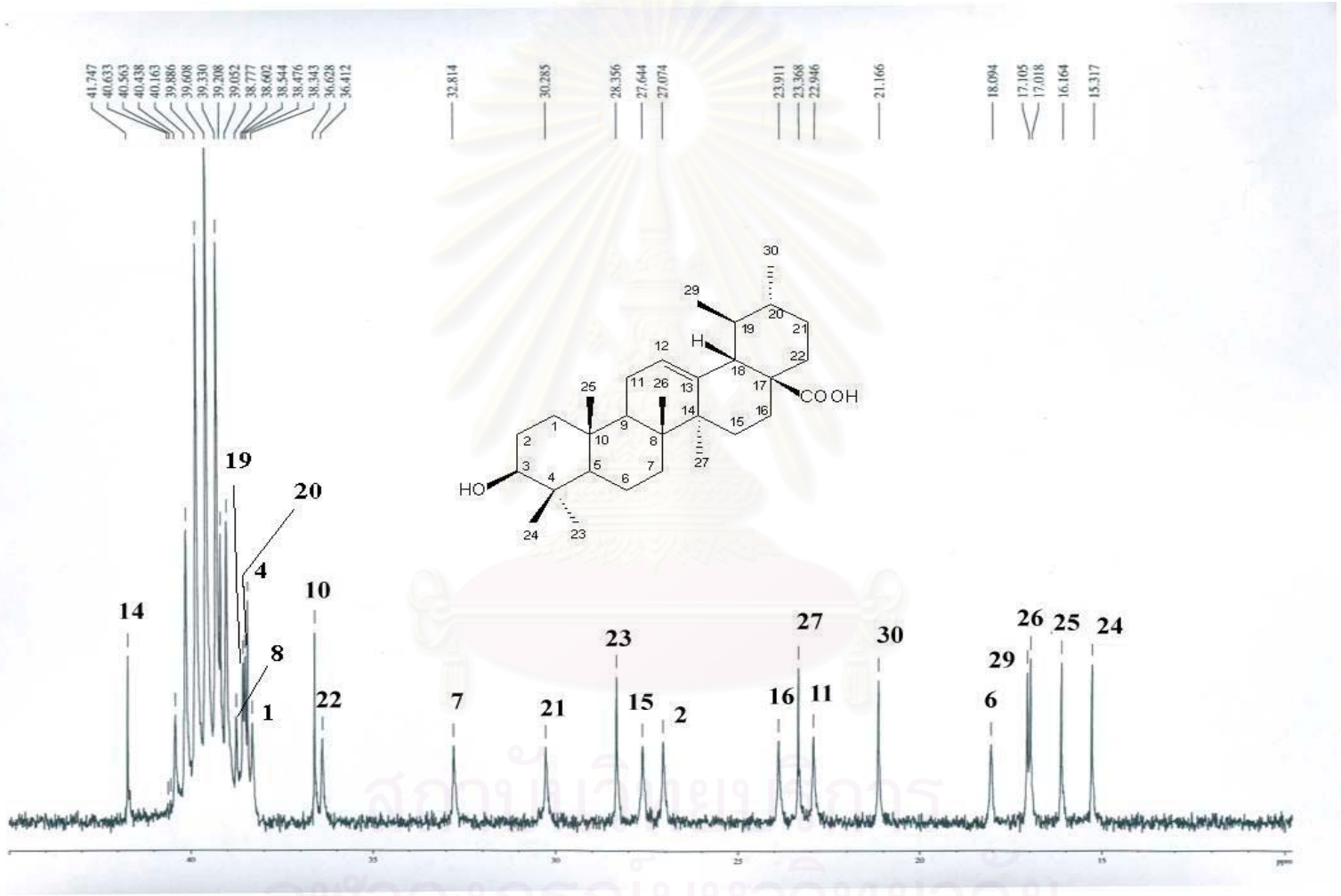

Figure 20b. The 100 MHz ^{13}C-NMR spectrum of compound DR 4 (in DMSO-d_6) (expanded)

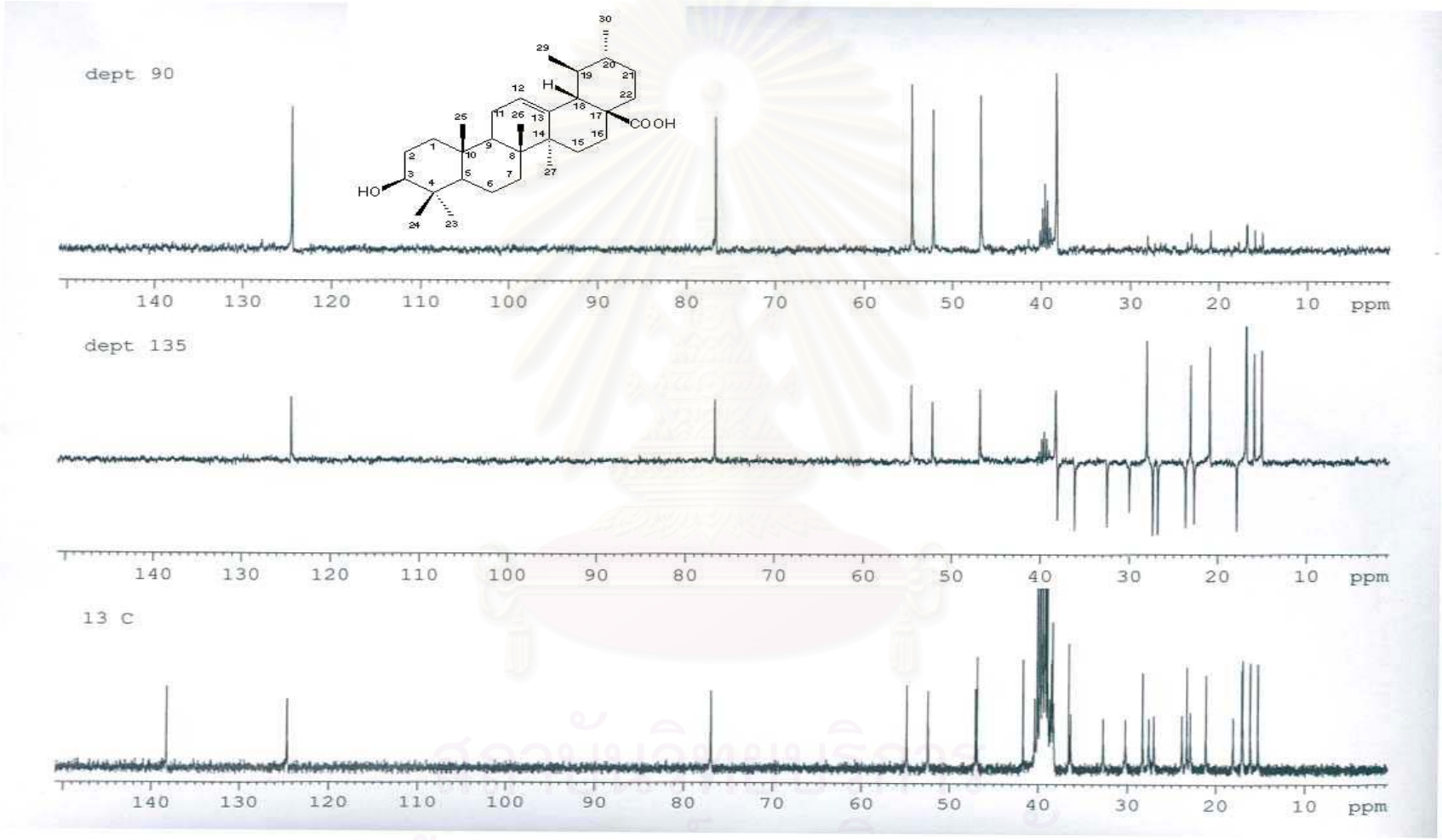

Figure 21a. The 100 MHz ^{13}C-DEPT NMR spectrum of compound DR 4 (in DMSO-d_6)

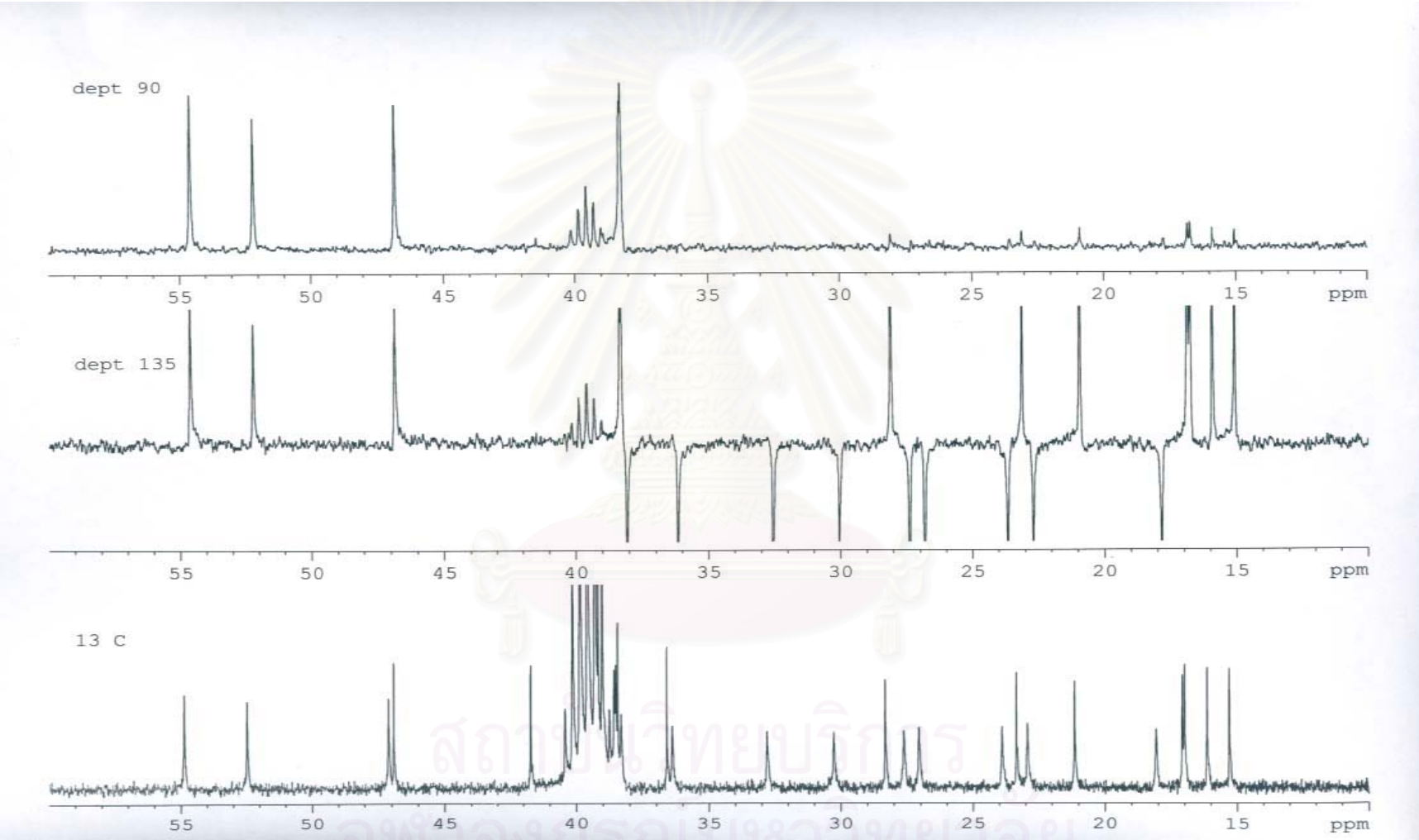

Figure 21b. The 100 MHz ^{13}C-DEPT NMR spectrum of compound DR 4 (in DMSO-d_6) (expanded)

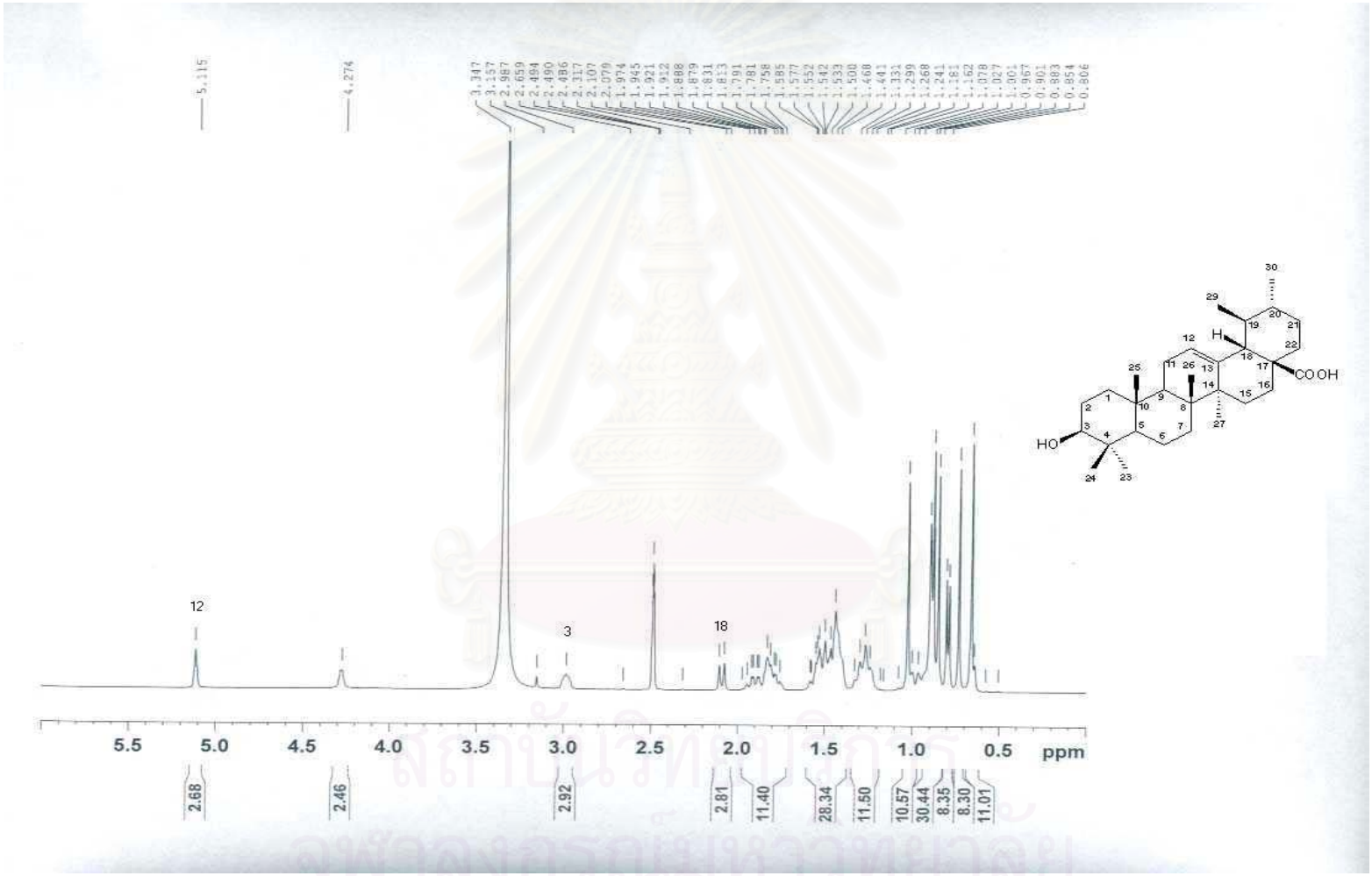

Figure 22a. The 400 MHz ^{13}H-NMR spectrum of compound DR 4 (in DMSO-d_6)

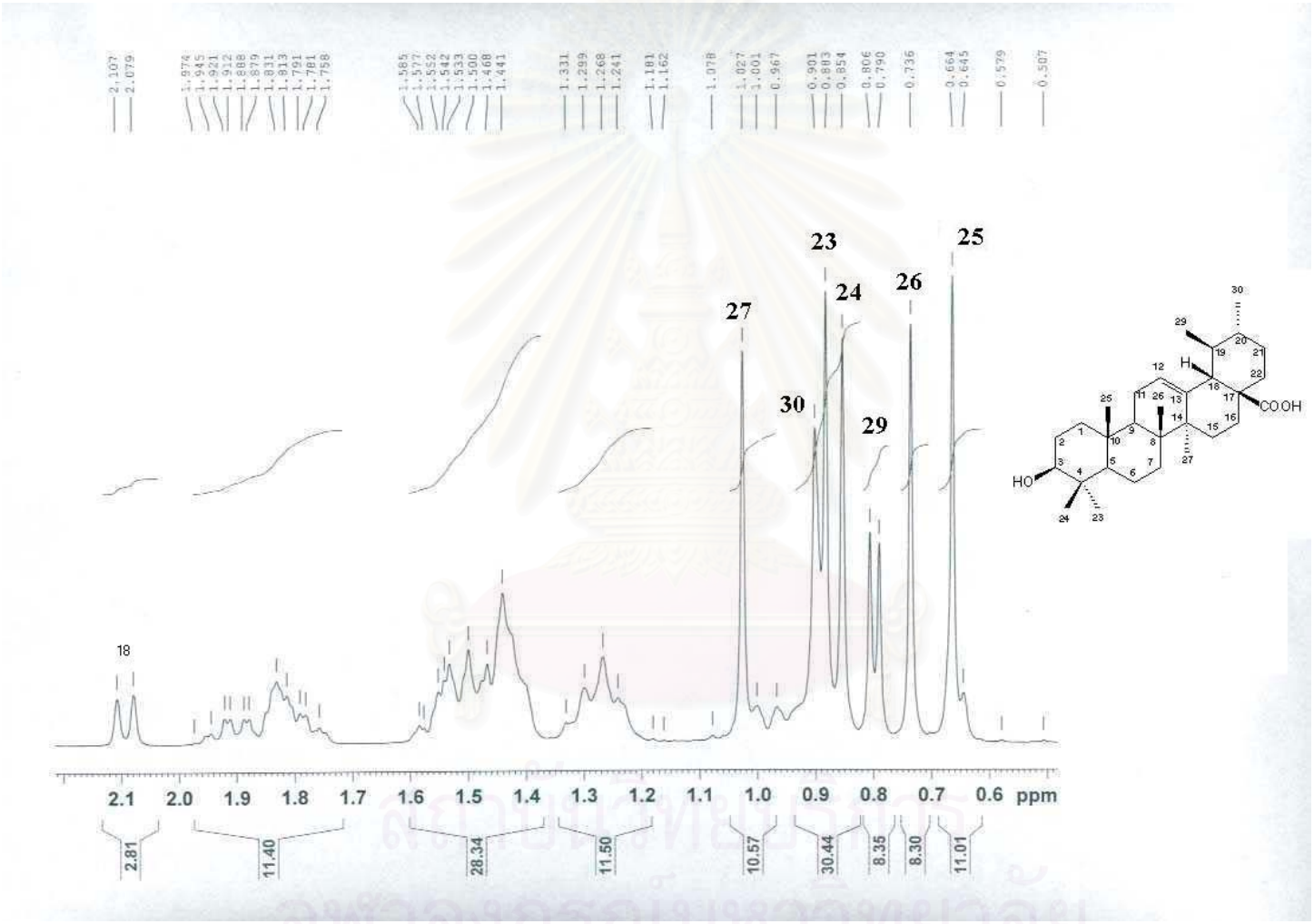

Figure 22b. The 400 MHz ^{13}H-NMR spectrum of compound DR 4 (in DMSO-d_6) (expanded)

CHAPTER V

CONCLUSION

In the present investigation of *Diospyros rubra* Lec. , three triterpenoids and a mixture of steroids were isolated from the stem of the plants by chromatographic techniques.

Two lupane – type triterpenoids, lupeol and betulin, together with a mixture of β-sitosterol and stigmasterol, were isolated from the hexane extract, while an ursane – type triterpenoids, ursolic acid, was isolated from the chloroform extract. The identification of isolated compounds was accomplished by analysis of their spectroscopic data.

This is the first report of the chemical constituents of this *Diospyros* species and the data obtained would be valuable in the chemotaxonomic and phytochemical studies of this plant genus.

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