

การศึกษาทางพฤกษเคมีของลำต้นเถาเอ็นอ่อน



นางสาวมรติพย์ สมสุข

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จุฬาลงกรณ์มหาวิทยาลัย

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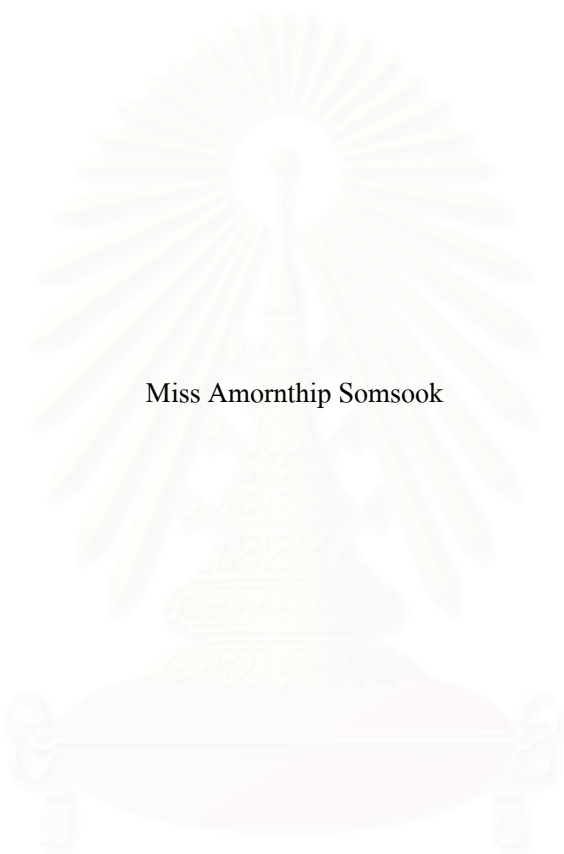
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PHYTOCHEMICAL STUDY OF *CRYPTOLEPIS BUCHANANI* STEM



Miss Amornthip Somsook

สถาบันวิทยบริการ  
A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Pharmacy Program in Pharmaceutical Botany

Department of Pharmaceutical Botany

Faculty of Pharmaceutical Sciences

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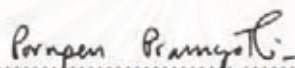
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Thesis Title                   PHYTOCHEMICAL STUDY OF *CRYPTOLEPIS BUCHANANI* STEM  
By                                 Miss Amornthip Somsook  
Field of Study                 Pharmaceutical Botany  
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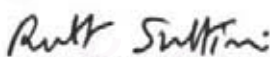
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(PHYTOCHEMICAL STUDY OF *CRYPTOLEPIS BUCHANANI* STEM)

อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.รพีพล ภาโววาท, 167 หน้า.

จากลำต้นของเถาเอ็นอ่อน วงศ์ Asclepiadaceae สามารถสกัดแยกสารใหม่ในกลุ่ม pregnane steroids ได้ 3 ชนิด คือ  $2\alpha,21$ -dihydroxypregn-4-ene-3,20-dione,  $2\alpha,21$ -dihydroxypregn-4,6-diene-3,20-dione และ  $2,21$ -dihydroxypregn-1,4,6-triene-3,20-dione รวมทั้งพบสารที่เคยมีรายงานมาแล้วอีก 4 ชนิด ได้แก่ สารกลุ่ม coumarin 1 ชนิด คือ scopoletin สารกลุ่ม anthraquinone 1 ชนิด คือ danthron และสารผสมในกลุ่ม stigmastane steroids 2 ชนิด คือ  $\beta$ -sitosterol กับ stigmasterol การพิสูจน์เอกลักษณ์ของสารเหล่านี้ ทำโดยการวิเคราะห์ข้อมูลทางสเปกโตรสโกปี จาก UV, IR, MS, 1-D NMR ( $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ ,  $^{13}\text{C-DEPT}$ ) และ 2-D NMR ( $^1\text{H-}^1\text{H COSY}$ ,  $^1\text{H-}^{13}\text{C HMQC}$ ,  $^1\text{H-}^{13}\text{C HMBC}$ ) ร่วมกับการเปรียบเทียบข้อมูลกับค่าที่ได้มีการรายงานไว้แล้ว

สถาบันวิทยบริการ  
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## 4976608133 : MAJOR PHARMACEUTICAL BOTANY

KEY WORD : *CRYPTOLEPIS BUCHANANI* / ASCLEPIADACEAE / PREGNANE / ANTHRAQUINONE / COUMARIN

AMORNTHIP SOMSOOK: PHYTOCHEMICAL STUDY OF *CRYPTOLEPIS BUCHANANI* STEM. THESIS PRINCIPAL ADVISOR: ASSOC. PROF. RAPEPOL BAVOVADA, Ph.D., 167 pp.

From the stem of *Cryptolepis buchanani* Roem. & Schult. (family Asclepiadaceae), three new naturally occurring pregnane steroids, 2 $\alpha$ ,21-dihydroxypregn-4-ene-3,20-dione, 2 $\alpha$ ,21-dihydroxypregn-4,6-diene-3,20-dione and 2,21-dihydroxypregn-1,4,6-triene-3,20-dione, were isolated together: a coumarin, scopoletin; an anthraquinone, danthron; and a mixture of steroids,  $\beta$ -sitosterol and stigmasterol. Identification and structure elucidation of these compounds were accomplished by analyses of their spectroscopic data: UV, IR, MS, 1-D NMR ( $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ ,  $^{13}\text{C-DEPT}$ ) and 2-D NMR ( $^1\text{H-}^1\text{H COSY}$ ,  $^1\text{H-}^{13}\text{C HMQC}$ ,  $^1\text{H-}^{13}\text{C HMBC}$ ) as well as comparison with reported values.

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## CONTENTS

	<b>Page</b>
ABSTRACT (THAI) .....	iv
ABSTRACT (ENGLISH) .....	v
ACKNOWLEDGEMENTS .....	vi
CONTENTS .....	vii
LIST OF TABLES.....	ix
LIST OF FIGURES .....	x
LIST OF SCHEMES .....	xiii
LIST OF ABBREVIATIONS .....	xiv
CHAPTER	
I INTRODUCTION.....	1
II HISTORICAL.....	6
Chemical constituents of Asclepiadaceous plants.....	6
Chemical constituents of <i>Cryptolepis</i> species.....	68
Ethnomedicinal uses of <i>Cryptolepis</i> species.....	74
Biological activities of <i>Cryptolepis</i> species.....	75
III EXPERIMENTAL.....	78
Source of Plant Material. ....	78
General Techniques.....	78
Extraction.....	81
Isolation.....	81
1. Fractionation of the hexane extract.....	81
1.1 Isolation of compound CB01.....	82
2. Fractionation of the chloroform extract.....	83
2.1 Isolation of compound CB02.....	83
2.2 Isolation of compound CB03.....	84
2.4 Isolation of compound CB04.....	84
2.5 Isolation of compound CB05.....	85
2.6 Isolation of compound CB06.....	85
Characterization of isolated compounds.....	86

CHAPTER	Page
IV RESULT AND DISSUSION.....	92
1. Structure elucidation of compound CB01.....	92
2. Structure elucidation of compound CB02.....	95
3. Structure elucidation of compound CB03.....	98
4. Structure elucidation of compound CB04.....	103
5. Structure elucidation of compound CB05.....	107
6. Structure elucidation of compound CB06.....	110
V CONCLUSION.....	113
REFERENCES.....	114
APPENDIX.....	131
VITA.....	167



สถาบันวิทยบริการ  
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## LIST OF TABLES

Table		page
1	Distribution of pregnane steroids in the family Asclepiadaceae .....	7
2	Chemical constituents of <i>Cryptolepis</i> species.....	68
3	Biological activities of <i>Cryptolepis</i> species.....	75
4	Combined fractions from the hexane extract.....	82
5	Combined fractions from the fraction H08.....	82
6	Combined fractions from the chloroform extract.....	83
7	Combined fractions from the fraction C02.....	83
8	Combined fractions from the fraction C05.....	84
9	Combined fractions from the fraction C054.....	85
10	Combined fractions from the fraction C053.....	85
11	Comparison of NMR spectral data of $\beta$ -sitosterol, stigmasterol and component CB01 (a mixture of CB01A and CB01B) (in CDCl <sub>3</sub> , 75 MHz).....	93
12	Comparison of NMR spectral data of danthron and compound CB02 (in CDCl <sub>3</sub> , 500 MHz).....	97
13	Comparison of NMR spectral data of $\beta$ -hydroxycortexone, deoxycorticosterone and compound CB03 (in acetone- <i>d</i> <sub>6</sub> , 500 MHz).....	101
14	NMR spectral data of compound CB04 (in CDCl <sub>3</sub> , 500 MHz).....	105
15	NMR spectral data of compound CB05 (in CDCl <sub>3</sub> , 500 MHz).....	109
16	Comparison of NMR spectral data of scopoletin and compound CB06 (in CDCl <sub>3</sub> , 500 MHz).....	112

## LIST OF FIGURES

Figure		Page
1	<i>Cryptolepis buchmanani</i> Roem & Schult.....	5
2	Pregnane steroids from Asclepiadaceous plants.....	36
3	Chemical structures of plants in <i>Cryptolepis</i> species.....	72
4	<sup>1</sup> H-NMR (300 MHz) Spectrum of compound CB01 (in CDCl <sub>3</sub> ).....	132
5	<sup>13</sup> C-NMR (75 MHz) Spectrum of compound CB01 (in CDCl <sub>3</sub> ).....	132
6	<sup>13</sup> C-NMR (75 MHz) Spectrum of compound CB01 (in CDCl <sub>3</sub> ) (expanded).....	133
7	DEPT 90 and 135 Spectrum of compound CB01.....	133
8	UV Spectrum of compound CB02 (in CDCl <sub>3</sub> ).....	134
9	IR Spectrum of compound CB02 (KBr disc).....	134
10	ES-TOF Mass spectrum of compound CB02.....	135
11	<sup>1</sup> H-NMR (500 MHz) Spectrum of compound CB02 (in CDCl <sub>3</sub> ).....	135
12	<sup>13</sup> C-NMR (125 MHz) Spectrum of compound CB02 (in CDCl <sub>3</sub> ).....	136
13	DEPT 90 and 135 Spectrum of compound CB02.....	136
14	<sup>1</sup> H- <sup>1</sup> H COSY Spectrum of compound CB02.....	137
15	HSQC Spectrum of compound CB02.....	137
16	HMBC Spectrum of compound CB02 .....	138
17	UV Spectrum of compound CB03 (in MeOH).....	138
18	IR Spectrum of compound CB03 (KBr disc).....	139
19	ES-TOF Mass spectrum of compound CB03.....	139
20	<sup>1</sup> H-NMR (500 MHz) Spectrum of compound CB03 (in acetone <i>d</i> <sub>6</sub> ).....	140
21	<sup>13</sup> C-NMR (500 MHz) Spectrum of compound CB03 (in CDCl <sub>3</sub> ).....	140
22	DEPT 90 and 135 Spectrum of compound CB03.....	141
23	<sup>1</sup> H- <sup>1</sup> H COSY Spectrum of compound CB03 .....	141
24	HSQC Spectrum of compound CB03 .....	142
25	HSQC Spectrum of compound CB03 (expanded).....	142
26	HSQC Spectrum of compound CB03 (expanded).....	143
27	HMBC Spectrum of compound CB03 .....	143
28	HMBC Spectrum of compound CB03 (expanded).....	144
29	HMBC Spectrum of compound CB03 (expanded).....	144

## LIST OF FIGURES (continued)

Figure		Page
30	HMBC Spectrum of compound CB03 (expanded).....	145
31	NOESY Spectrum of compound CB03.....	145
32	UV Spectrum of compound CB04 (in MeOH).....	146
33	IR Spectrum of compound CB04 (KBr disc).....	146
34	ES-TOF Mass spectrum of compound CB04.....	147
35	<sup>1</sup> H-NMR (500 MHz) Spectrum of compound CB04 (in CDCl <sub>3</sub> ).....	147
36	<sup>13</sup> C-NMR (500 MHz) Spectrum of compound CB04 (in CDCl <sub>3</sub> ).....	148
37	DEPT 90 and 135 Spectrum of compound CB04.....	148
38	<sup>1</sup> H- <sup>1</sup> H COSY Spectrum of compound CB04 .....	149
39	<sup>1</sup> H- <sup>1</sup> H COSY Spectrum of compound CB04(expanded).....	149
40	HSQC Spectrum of compound CB04.....	150
41	HSQC Spectrum of compound CB04 (expanded).....	150
42	HMBC Spectrum of compound CB04.....	151
43	HMBC Spectrum of compound CB04 (expanded).....	151
44	HMBC Spectrum of compound CB04 (expanded).....	152
45	HMBC Spectrum of compound CB04 (expanded).....	152
46	HMBC Spectrum of compound CB04 (expanded).....	153
47	NOESY Spectrum of compound CB04.....	153
48	UV Spectrum of compound CB05 (in MeOH).....	154
49	IR Spectrum of compound CB05 (KBr disc).....	154
50	ES-TOF Mass spectrum of compound CB05.....	155
51	<sup>1</sup> H-NMR (500 MHz) Spectrum of compound CB05 (in CDCl <sub>3</sub> ).....	155
52	<sup>13</sup> C-NMR (125 MHz) Spectrum of compound CB05 (in CDCl <sub>3</sub> ).....	156
53	DEPT 90 and 135 Spectrum of compound CB05.....	156
54	<sup>1</sup> H- <sup>1</sup> H COSY Spectrum of compound CB05.....	157
55	HMQC Spectrum of compound CB05.....	157
56	HMQC Spectrum of compound CB05 (expanded).....	158
57	HMBC Spectrum of compound CB05.....	158
58	HMBC Spectrum of compound CB05 (expanded).....	159

**LIST OF FIGURES (continued)**

<b>Figure</b>		<b>Page</b>
59	HMBC Spectrum of compound CB05 (expanded).....	159
60	NOESY Spectrum of compound CB05.....	160
61	UV Spectrum of compound CB06 (in MeOH).....	160
62	IR Spectrum of compound CB06 (KBr disc).....	161
63	ES-TOF Mass spectrum of compound CB06.....	161
64	<sup>1</sup> H-NMR (500 MHz) Spectrum of compound CB06 (in CDCl <sub>3</sub> ).....	162
65	<sup>13</sup> C-NMR (125 MHz) Spectrum of compound CB06 (in CDCl <sub>3</sub> ).....	162
66	DEPT 90 and 135 Spectrum of compound CB06.....	163
67	<sup>1</sup> H- <sup>1</sup> H COSY Spectrum of compound CB06.....	163
68	HSQC Spectrum of compound CB06 .....	164
69	HSQC Spectrum of compound CB06 (expanded).....	164
70	HMBC Spectrum of compound CB06 .....	165
71	HMBC Spectrum of compound CB06 (expanded).....	165
72	HMBC Spectrum of compound CB06 (expanded).....	166
73	NOESY Spectrum of compound CB06.....	166

## LIST OF SCHEMES

Scheme		Page
1	Extraction of <i>Cryptolepis buchanani</i> Roem. & Schult.....	84
2	Isolation of the hexane extract from <i>Cryptolepis buchanani</i> stem.....	90
3	Isolation of the chloroform extract from <i>Cryptolepis buchanani</i> stem .....	91



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## ABBREVIATIONS

$[\alpha]_D^{25}$	=	Specific rotation at 25 °C and sodium D line (589 nm)
$\delta$	=	Chemical shift
$\epsilon$	=	Molar absorptivity
Ac	=	acetyl
acetone- $d_6$	=	Deuterated acetone
aco	=	acovenose
Acr	=	acrolloyl
all	=	allose
Ami	=	<i>O</i> - aminobenzoyl
Ang	=	angeloyl
Anth	=	anthraniloyl
<i>br s</i>	=	broad singlet (for NMR spectra)
Bz	=	benzoyl
°C	=	degree Celsius
can	=	canarose
CDCl <sub>3</sub>	=	Deuterated chloroform
Cin	=	cinnamoyl
CHCl <sub>3</sub>	=	Chloroform
cm	=	centimeter
cm <sup>-1</sup>	=	reciprocal centimeter (unit of wave number)
<sup>13</sup> C-NMR	=	Carbon-13 Nuclear Magnetic Resonance
COSY	=	Correlated Spectroscopy
cym	=	cymarose
1-D	=	one dimensional
2-D	=	two dimensional
<i>d</i>	=	doublet (for NMR spectra)
<i>dd</i>	=	doublet of doublets (for NMR spectra)
<i>ddd</i>	=	doublet of doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
digno	=	diginose

digto	=	digitoxose
DMSO- $d_6$	=	deuterated dimethylsulfoxide
ESI TOFMS	=	Electrospray Ionization Time of Flight Mass Spectrometry
EtOAc	=	ethyl acetate
EtOH	=	ethanol
eV	=	electron volt
flu	=	flucose
g	=	gram
gal	=	galactose
glc	=	glucose
HBz	=	hydroxybenzoyl
$^1\text{H}$ NMR	=	Proton Nuclear Magnetic Resonance
$^1\text{H}$ - $^1\text{H}$ COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
HMBC	=	$^1\text{H}$ -detected Heteronuclear Multiple Bond Correlation
HMQC	=	$^1\text{H}$ -detected Heteronuclear Multiple Quantum Correlation
HSQC	=	Heteronuclear Single Quantum Correlation
Hz	=	Hertz
Ikem	=	ikemaoyl
IR	=	Infrared spectrum
Iso	=	isovalcroyl
$J$	=	coupling constant
KBr	=	potassium bromide
kg	=	kilogram
L	=	liter
$\lambda_{\text{max}}$	=	wavelength at maximal absorption
$m$	=	multiplet (for NMR spectra)
m	=	meter
mm	=	millimeter
$[\text{M}+\text{H}]^+$	=	Protonated molecular ion
MeBu	=	2-methylbutyryl
MeOH	=	methanol
mg	=	miligram

MHz	=	Megahertz
ml	=	milliliter
MS	=	Mass Spectrum
m/z	=	mass-to-charge ratio
nm	=	nanometer
Nic	=	nicotinoyl
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy
n.s.	=	not specified
ppm	=	part per million
Pro	=	propinoyl
ole	=	oleandrose
oli	=	olivose
<i>q</i>	=	quartet (for NMR spectra)
$\nu_{\max}$	=	wave number of maximal absorption
<i>s</i>	=	singlet (for NMR spectra)
sp.	=	species
<i>t</i>	=	triplet (for NMR spectra)
the	=	thetevose
Tig	=	tigloyl
TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet
var.	=	variety
xyl	=	xylose



## CHAPTER I

### INTRODUCTION

A large number of medicinal plants are distributed all over Thailand, many of which have been employed by native practitioners to treat diseases in the form of traditional remedies. It is considered that medicine of natural origins is usually safer than synthetic drugs. Therefore, it is quite interesting to investigate medicinal plants of Thailand, many of which still lack basic informations on its chemical constituents.

*Cryptolepis buchmanii* Roem. & Schult. has a long association with Thai folk medicine. The genus *Cryptolepis* belongs to the family Asclepiadaceae, a large and diverse family with about 315 genera and over 2,900 species mostly in warm regions with a few species in temperate habitats (Woodland, 2000). In Thailand, asclepiadaceous plants are arranged into about 45 genera and 150 species (ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้, 2544). The genus *Cryptolepis* comprises a number of glabrous, usually twining shrubs with opposite leaves. The flowers are small, in axillary lateral or terminal lax usually few-flowered pedunculate cymes. The calyx is 5-partite, while the corolla-tube is short, cylindric or campanulate with 5 lobes overlapping to the right in bud. The corona is 5 filiform or fleshy lobes or processes arising from about the middle of the corolla-tube. Stamens inserted towards the base of the corolla-tube; filaments free; anthers more or less triangular with the connective produced into a fleshy apiculus, united at their base to the dilated part of the style, more or less connivent into a cone; pollen-masses granular, cohering in pairs in each cell; pollen-carriers more or less spatulate. Style-apex shortly conical, not exerted beyond the anther. Follicles divaricate, terete, smooth. Seeds comose (Kirtikar and Basu, 1981)

The genus *Cryptolepis* includes about 73 species distributed throughout the tropical regions of Africa, Madagascar, Asia, Australia and Papua New Guinea. (Good, 1952; Paulo and Houghton, 2002; The International Plant Names Index, 2008). These species are:

- |                                                   |                                                 |
|---------------------------------------------------|-------------------------------------------------|
| <i>C. subgen. Phyllanthera</i> (Blume) P.I.Forst. | <i>C. africana</i> (Bullock) Venter & R.L.Verh. |
| <i>C. albicans</i> Jum. & H.Perrier               | <i>C. angolensis</i> Welw. ex Hiern             |
| <i>C. apiculata</i> K.Schum. ex Engl.             | <i>C. arbuscula</i> (Radcl.-Sm.) Venter         |
| <i>C. arenicola</i> Schltr.                       | <i>C. barteri</i> K.Schum.                      |

- C. baumii* N.E.Br.  
*C. brazzaei* Baill.  
*C. buxifolia* Chiov.  
*C. cryptolepioides* (Schltr.) Bullock  
*C. decidua* N.E.Br.  
*C. dubia* (Burm.f.) M.R.Almeida  
*C. edithae* Benth. & Hook.f.  
*C. elliotii* Schltr.  
*C. gillettii* Hutch. & E.A.Bruce  
*C. grandiflora* Wight  
*C. hensii* N.E.Br.  
*C. intricata* (Balf.f.) Venter  
*C. lancifolia* P.I.Forst.  
*C. laxa* Baill.  
*C. linearis* N.E.Br.  
*C. macrophylla* (Radcl.-Sm.) Venter  
*C. migiurtina* Chiov.  
*C. multinervosa* P.I.Forst.  
*C. newii* (Benth.) P.I.Forst.  
*C. nugaalensis* Venter & Thulin  
*C. oblongifolia* Schltr.  
*C. orbicularis* Chiov.  
*C. pauciflora* Wight  
*C. perakensis* (Gamble) P.I.Forst.
- C. bifida* (Blume) P.I.Forst.  
*C. buchanani* Roem. & Schult.  
*C. capensis* Schltr.  
*C. debeerstii* De Wild.  
*C. delagoensis* Schltr.  
*C. eburnea* (Pichon) Venter  
*C. elegans* Wall. & G.Don  
*C. filiformis* Wall.  
*C. gossweileri* S.Moore  
*C. grayi* P.I.Forst.  
*C. hypoglauca* K.Schum. ex Engl.  
*C. javanica* Blume  
*C. laurenti* De Wild.  
*C. laxiflora* Blume  
*C. longiflora* Regel  
*C. microphylla* Baill.  
*C. monteiroae* Oliver  
*C. myrtifolia* Hiern  
*C. nigritana* N.E.Br.  
*C. nymanii* (Schumann) P.I.Forst.  
*C. obtusa* N.E.Br.  
*C. papillata* P.I.Forst.  
*C. pendulina* (Venter & D.V.Field) P.I.Forst.  
*C. producta* N.E.Br.

<i>C. purpureus</i> (N.E.Br.) P.I.Forst.	<i>C. reticulata</i> (Roxb.) Steud.
<i>C. reticulata</i> Wall.	<i>C. ruspolii</i> Chiov.
<i>C. sanguinolenta</i> (Lindl.) Schltr.	<i>C. sinensis</i> Merr.
<i>C. sizenandi</i> Rolfe	<i>C. socotrana</i> (Balf.f.) Venter
<i>C. somaliensis</i> Venter & Thulin	<i>C. stefianinii</i> Chiov.
<i>C. suffruticosa</i> N.E.Br.	<i>C. transvaalensis</i> Schltr.
<i>C. triangularis</i> N.E.Br.	<i>C. volubilis</i> (Balf.f.) Schwartz
<i>C. welwitschii</i> Schltr.	<i>C. wightiana</i> Wall.
<i>C. yemenensis</i> Venter & R.L.Verh.	

In Thailand, at least two species of *Cryptolepis* have been recorded: *C. buchanani* and *C. elegans* (ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้, 2544)

*Cryptolepis buchanani* Roem. & Schult. (Figure 1) is an indigenous plant known in Thailand, as Thao en on (เถาเอ็นอ่อน). Mueai (central), Kuan (Shan-Mea Hong Son), No-o-mi (Karen-Mae Hong Son), Khrua thao en (Chiangmai), Tin pet khrua (Northern), Mon tin pet (Surat Thani), Ya-li-len (Pattani) or Khrua mak hoa toa (Northeastern) (สายสนม กิตติขจร, 2526; ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้, 2544). It is a large twining shrub; branches terete, glabrous. Leaves 7.5-12.5 by 3.8-6.3 cm., elliptic-oblong or sometimes oblong-lanceolate, rounded, retuse, or sometimes acute, apiculate, green above, whitish beneath, glabrous, base usually acute; main nerves numerous slender, nearly at right angles to the midrib, uniting in an intramarginal nerve; petioles 6-13 mm. long. Flowers greenish yellow, in short paniculate cymes; pedicels glabrous; bracts ovate, acute, with scarious margins. Calyx glabrous; segments 1.5 mm. long, ovate, subacute. Corolla tube 2 mm. long; lobes 6 mm. long, linear or linear-lanceolate, subacute; corona-scales clavate. Follicles 5-10 cm. long, straight, rigid, divaricate, gradually tapering to a blunt point from about the middle, where they are 1.3-2 cm. diam. Seeds 6-8 mm. long, ovate-oblong, black; coma rather more than 2.5 cm. long (Kirtikar and Basu, 1981).

The plant can be found throughout India, Kashmir, Laos, Myanmar, Nepal, Vietnam, Sri Lanka, Thailand and China. (Kirtikar and Basu, 1981; Wu and Raven, 1995).

In Thai folk medicine, the alcoholic extract of its stem is commonly used in the treatment of inflammatory conditions such as arthritis, muscle and joint pain (Panthong, Kanjanapothi and Taylor, 1986).

The main secondary metabolites found in the leaves and roots of *C. buchanani* are cardenolides and pseudo-alkaloids (nicotinoyl glycosides) (Dutta, Sharma and Sharma, 1980 Purushothaman *et al.*, 1988) Some of these compounds and crude extracts from the plant have been found to exhibit interesting biological activities e.g. antibacterial (Vasanth, Gopal and Roa, 1997), cardiogenic (Venkateswara, Sankara Rao and Vaidyanathan, 1987), muscle relaxant (Ikegami *et al.*, 1990) and anti-inflammatory activities (Laupattarakasem *et al.*, 2006)

Although *C. buchanani* has been frequently used in medicine, there are only a few reports on the phytochemical study of this plant. Preliminary examination revealed positive results for alkaloids, flavonoids, triterpenoids and steroids. Therefore, it is the purpose of this investigation to study the compounds in the stem of *C. buchanani*. The result of this investigation may serve as an additional information on the chemical nature of this plant family, which could be a valuable lead in the fields of chemotaxonomy and phytochemistry. The purposes of this research were as follows:

1. Isolation and purification of compounds from the stem of *Cryptolepis buchanani*.
2. Determination of chemical structure and physical properties of each isolated compound.

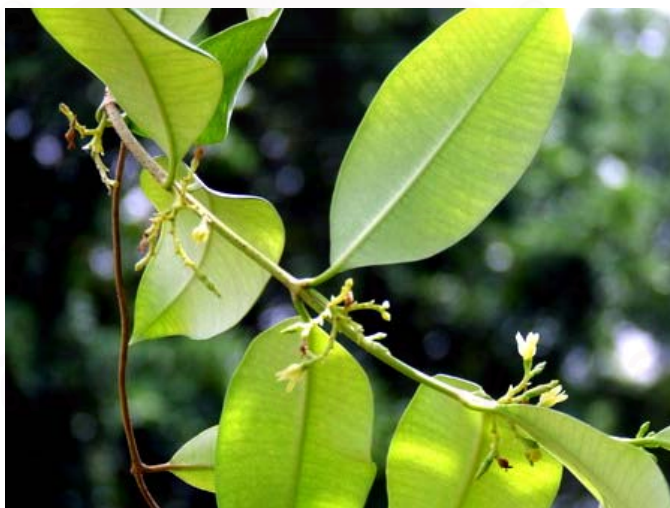


Figure 1. *Cryptolepis buchanani* Roem. & Schult.

## CHAPTER II

### HISTORICAL

#### Chemical constituents of asclepiadaceous plants

##### Pregnane Steroids

Several types of compounds have been recorded in previous phytochemical studies of plants belonging to the family Asclepiadaceae. The Asclepiadaceae was reported to be rich in pregnane and their glycosides (Khare *et al.*, 1986; Prakash *et al.*, 1991; El Sayed *et al.*, 1995; Halim and Khalil, 1996; Warashina and Noro, 1997; Al-Yahya, Abdel-Sattar and Guittet, 2000; Sigler *et al.*, 2000; Leo *et al.*, 2005; Abdel-Sattar *et al.*, 2007), drawing much attention in recent years due to their antitumor and anticancer (Ahsah *et al.*, 1973; Aquino and Pizza, 1995; Qin *et al.*, 1999; Pan *et al.*, 2003), platelet pro-aggregating (Piacente *et al.*, 1998), anti-fungal (Hu *et al.*, 1999), immunodulating and immunosuppressive (Li *et al.*, 2006b; Ye *et al.*, 2005), antiepilepsy (Mu *et al.*, 1986), antifertility (Yaun *et al.*, 1992), anti-inflammatory (Aquino and Pizza, 1995; Bai, Li and Koike, 2008; Innocenti *et al.*, 2005; Ramesh *et al.*, 1999), antinociceptive (Verma *et al.*, 2005) and digitlalis receptor binding activities (Thempleton *et al.*, 1993).

Pregnanes are C-21 steroidal compounds having the perhydro-1-2-cyclopentano phenanthrene ring system with  $\beta$ -orientated angular methyl group at C-10 and C-13, and a two carbon atom side chain at C-17. It is quite normal that pregnane derivatives of the Asclepiadaceae possess a  $14\beta$ -hydroxy moiety,  $\Delta^5$  unsaturation, and a  $\beta$ -hydroxy group at C-3 like in many other naturally occurring steroidal compounds. Pregnanes are the parent hydrocarbon of the pregnancy hormone, progesterone (pregn-4-ene-3,20-dione), and of the great majority of the corticosteroids and many other natural products, which together make the pregnanes the largest single group of steroids. Distribution of pregnane steroids in Asclepiadaceae as shown in Table 1

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Asclepias fruticosa</i>	Whole plant	Lineolon [1]		Warashina and Noro, 1994a
	Whole plant	[2]	Lineolon	Warashina and Noro, 1994a
	Whole plant	[3]	Lineolon	Warashina and Noro, 1994a
	Whole plant	[4]	Isolineolon	Warashina and Noro, 1994a
	Whole plant	[5]	Isolineolon	Warashina and Noro, 1994a
<i>A. incarnata</i>	Aerial part	[6]	Lineolon	Warashina and Noro, 2000
	Aerial part	[7]	Lineolon	Warashina and Noro, 2000
	Aerial part	[8]	Lineolon	Warashina and Noro, 2000
	Aerial part	[9]	Lineolon	Warashina and Noro, 2000
	Aerial part	[10]	Lineolon	Warashina and Noro, 2000
	Aerial part	[11]	Lineolon	Warashina and Noro, 2000
	Aerial part	[12]	Lineolon	Warashina and Noro, 2000
	Aerial part	[13]	Isolineolon	Warashina and Noro, 2000
	Aerial part	[14]	Isolineolon	Warashina and Noro, 2000
	Aerial part	[15]	Isolineolon	Warashina and Noro, 2000
	Aerial part	[16]	Isolineolon	Warashina and Noro, 2000

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>A. incarnata</i>	Aerial part	[17]	Isolineolon	Warashina and Noro, 2000
	Aerial part	[18]	Lineolon	Warashina and Noro, 2000
	Aerial part	[19]	Lineolon	Warashina and Noro, 2000
	Aerial part	[20]	Lineolon	Warashina and Noro, 2000
	Aerial part	[21]	Lineolon	Warashina and Noro, 2000
	Aerial part	[22]	Lineolon	Warashina and Noro, 2000
	Aerial part	[23]	Lineolon	Warashina and Noro, 2000
	Aerial part	[24]	Lineolon	Warashina and Noro, 2000
	Aerial part	[25]	Lineolon	Warashina and Noro, 2000
	Aerial part	[26]	15 $\beta$ -hydroxylineolon	Warashina and Noro, 2000
	Aerial part	[27]	15 $\beta$ -hydroxylineolon	Warashina and Noro, 2000
	Aerial part	[28]	15 $\beta$ -hydroxylineolon	Warashina and Noro, 2000
	Aerial part	[29]	15 $\beta$ -hydroxylineolon	Warashina and Noro, 2000
	Aerial part	[30]	15 $\beta$ -hydroxylineolon	Warashina and Noro, 2000
	Aerial part	[31]	15 $\beta$ -hydroxylineolon	Warashina and Noro, 2000
	Aerial part	[32]	15 $\beta$ -hydroxyisolineolon	Warashina and Noro, 2000



**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>A. incarnata</i>	Aerial part	[33]	Metaplexigenin	Warashina and Noro, 2000
	Aerial part	[34]	Metaplexigenin	Warashina and Noro, 2000
	Aerial part	[35]	Metaplexigenin	Warashina and Noro, 2000
	Aerial part	[36]	Metaplexigenin	Warashina and Noro, 2000
	Aerial part	[37]	Metaplexigenin	Warashina and Noro, 2000
	Aerial part	Deacylmetaplexigenin [38]	Sarcogenin	Warashina and Noro, 2000
	Aerial part	Rostratamine [39]	Sarcogenin	Warashina and Noro, 2000
<i>Boucerosia aucheriana</i>	Whole plant	Boucerocin [40]		Ahmad Usmanghani and Rizwani, 1988
	Whole plant	Dihydroboucerocin [41]	Dihydroboucerin	Ahmad, Usmanghani and Rizwani, 1988
	Aerial part	Bouceroside ANC [42]	Dihydroboucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside ANO [43]	Dihydroboucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside BNO [44]	Dihydroboucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside BNC [45]	Dihydroboucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside CNO [46]	Dihydroboucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside CNC [47]	Dihydroboucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside ADC [48]	Dihydroboucerin	Tanaka, Tsukamoto and Hayashi, 1990

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Boucerosia aucheriana</i>	Aerial part	Bouceroside ADO [49]	Boucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside BDO [50]	Boucerin	Tanaka, Tsukamoto and Hayashi, 1990
	Aerial part	Bouceroside BDC [51]	Boucerin	Tanaka, Tsukamoto and Hayashi, 1990
<i>Carallum dalzielii</i>	Whole plant	[52]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[53]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[54]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[55]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[56]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[57]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[58]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[59]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[60]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[61]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[62]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[63]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[64]	Tomentogenin	Leo <i>et al.</i> , 2005

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Carallum dalzielli</i>	Whole plant	[65]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[66]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[67]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[68]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[69]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[70]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[71]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[72]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[73]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[74]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[75]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[76]	Tomentogenin	Leo <i>et al.</i> , 2005
	Whole plant	[77]	Tomentogenin	Leo <i>et al.</i> , 2005
<i>C. retrospiciens</i>	Aerial part	Caretroside A [78]		Halim and Khalil, 1996
	Aerial part	12 $\beta$ -benzoyloxy-8 $\beta$ ,14 $\beta$ -dihydroxypregnane-20-one [79]		Halim and Khalil, 1996

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. russeliana</i>	Aerial part	Russelioside A [80]	Calocin	Al-Yahya, Abdel-Sattar and Guittet, 2000
	Aerial part	Russelioside B [81]	Calocin	Al-Yahya, Abdel-Sattar and Guittet, 2000
	Aerial part	Russelioside C [82]	Calocin	Al-Yahya, Abdel-Sattar and Guittet, 2000
	Aerial part	Russelioside D [83]	Calocin	Al-Yahya, Abdel-Sattar and Guittet, 2000
	Aerial part	Russelioside E [84]	Boucerin	Abdel-Sattar <i>et al.</i> , 2007
	Aerial part	Russelioside F [85]	Boucerin	Abdel-Sattar <i>et al.</i> , 2007
	Aerial part	Russelioside G [86]	Boucerin	Abdel-Sattar <i>et al.</i> , 2007
	Aerial part	Russelioside H [87]	Boucerin	Abdel-Sattar <i>et al.</i> , 2007
<i>C. tuberculata</i>	Whole plant	Caratuberside A [88]		Ahmad, Usmanghani and Rizwani, 1988
	Whole plant	Caratuberside B [89]		Ahmad, Usmanghani and Rizwani, 1988
	Whole plant	Caratuberside C [90]		Rizwani <i>et al.</i> , 1993
	Whole plant	Caratuberside D [91]		Rizwani <i>et al.</i> , 1993
<i>C. umbellata</i>	Whole plant	Carumbellosid I [92]	Calocin	Lin <i>et al.</i> , 1994
	Whole plant	Carumbelloside II [93]	Calocin	Lin <i>et al.</i> , 1994
	Whole plant	Carumbelloside III [94]	Calocin	Qiu <i>et al.</i> , 1997
	Whole plant	Carumbelloside IV [95]	Calocin	Qiu <i>et al.</i> , 1997

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. umbellata</i>	Whole plant	Carumbelloside V [96]	Calocin	Qiu <i>et al.</i> , 1997
<i>Cynanchum africanum</i>	n.s.	Cynafoside A [97]		Tsukamoto <i>et al.</i> , 1985
	n.s.	Cynafoside B [98]		Tsukamoto <i>et al.</i> , 1985
<i>C. ascyrifolium</i>	Root	[99]	Cynajapogenin A	Yoe <i>et al.</i> , 1998
	Root	[100]	Cynajapogenin A	Yoe <i>et al.</i> , 1998
	Root	[101]	Cynajapogenin A	Yoe <i>et al.</i> , 1998
<i>C. atratum</i>	Root	Atratoside A [102]		Zhang <i>et al.</i> , 1988
	Root	Atratoside B [103]		Zhang <i>et al.</i> , 1988
	Root	Atratoside C [104]		Zhang <i>et al.</i> , 1988
	Root	Atratoside D [105]		Zhang <i>et al.</i> , 1988
	Root	Cynanoside A [106]		Bai <i>et al.</i> , 2005
	Root	Cynanoside B [107]		Bai <i>et al.</i> , 2005
	Root	Cynanoside C [108]		Bai <i>et al.</i> , 2005
	Root	Cynanoside E [109]		Bai <i>et al.</i> , 2005
	Root	Cynanoside D [110]		Bai <i>et al.</i> , 2005
	Root	Cynanoside F [111]		Bai <i>et al.</i> , 2005

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. atratum</i>	Root	Cynanoside G [112]		Bai <i>et al.</i> , 2005
	Root	Cynanoside H [113]		Bai, Li and Koike, 2008
	Root	Cynanoside I [114]		Bai, Li and Koike, 2008
	Root	Cynanoside J [115]		Bai, Li and Koike, 2008
	Root	Cynanoside K [116]	Cynajapogenin A	Bai, Li and Koike, 2008
	Root	Cynanoside L [117]	Cynajapogenin A	Bai, Li and Koike, 2008
	Root	Cynanoside M [118]	Cynajapogenin A	Bai, Li and Koike, 2008
	Root	Cynanoside N [119]	Atratogenin A	Bai, Li and Koike, 2008
	Root	Cynanoside O [120]	1 $\beta$ -Hydroxyatratogenin A	Bai, Li and Koike, 2008
<i>C. boerharifolium</i>	n.s.	3 $\beta$ ,14 $\beta$ ,15 $\beta$ ,16 $\alpha$ - Tetrahydroypregnan-20-one [121]		Hayashi <i>et al.</i> , 1987
<i>C. caudatum</i>	Root	[122]	Cynanchogenin	Warashina and Noro, 1997
	Root	[123]	Cynanchogenin	Warashina and Noro, 1997
<i>C. caudatum</i>	Root	[124]	Cynanchogenin	Warashina and Noro, 1997
	Root	[125]	Cynanchogenin	Warashina and Noro, 1997
	Root	[126]	Cynanchogenin	Warashina and Noro, 1997

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. caudatum</i>	Root	[127]	Cynanchogenin	Warashina and Noro, 1997
	Root	[128]	Cynanchogenin	Warashina and Noro, 1997
	Root	[129]	Caudatin	Warashina and Noro, 1997
	Root	[130]	Caudatin	Warashina and Noro, 1997
	Root	[131]	Caudatin	Warashina and Noro, 1997
	Root	[ 132]	Caudatin	Warashina and Noro, 1997
	Root	[133]	Caudatin	Warashina and Noro, 1997
	Root	[134]	Caudatin	Warashina and Noro, 1997
	Root	[135]	Caudatin	Warashina and Noro, 1997
	Root	Gagamine [136]	Sarcostin	Warashina and Noro, 1997
	Aerial part	Sarcostin [137]		Warashina and Noro, 1995
	Aerial part	[138]	Sarcostin	Warashina and Noro, 1995
	Aerial part	[139]	Sarcostin	Warashina and Noro, 1995
	Aerial part	[140]	Sarcostin	Warashina and Noro, 1995
	Aerial part	[141]	Sarcostin	Warashina and Noro, 1995
Aerial part	[142]	Sarcostin	Warashina and Noro, 1995	

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. caudatum</i>	Aerial part	[143]	Sarcostin	Warashina and Noro, 1995
	Aerial part	[144]	Sarcostin	Warashina and Noro, 1995
	Aerial part	[145]	Sarcostin	Warashina and Noro, 1995
	Aerial part	[146]	Sarcostin	Warashina and Noro, 1995
	Root	Glycocynanchogenin [147]		Yamagishi <i>et al.</i> , 1972
	Root	Glycocaudatin [148]		Bando <i>et al.</i> , 1976
<i>C. chekiangense</i>	Root	Chekiangenoside A [149]	Cynajapogenin A	Li <i>et al.</i> , 2006a
	Root	Chekiangenoside B [150]	Glaucogenin A	Li <i>et al.</i> , 2006a
<i>C. glaucescens</i>	Root	Glaucogenin A [151]		Nakagawa, Hayashi and Wada, 1983
	Root	Glaucogenin B [152]		Nakagawa, Hayashi and Wada, 1983
	Root	Glaucoside A [153]	Glaucogenin A	Nakagawa, Hayashi and Wada, 1983
	Root	Glaucoside B [154]	Glaucogenin A	Nakagawa, Hayashi and Wada, 1983
	Root	Glaucoside C [155]	Glaucogenin A	Nakagawa, Hayashi and Wada, 1983
	Root	Glucoside D [156]	Glaucogenin A	Nakagawa, Hayashi and Wada, 1983
<i>C. forrestii</i>	Root	Cynaforroside B [157]		Liu <i>et al.</i> , 2006
	Root	Cynaforroside C [158]		Liu <i>et al.</i> , 2006



**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. forrestii</i>	Root	Cynaforroside D [159]		Liu <i>et al.</i> , 2006
	Root	Cynaforroside E [160]		Liu <i>et al.</i> , 2006
	Root	Cynaforroside F [161]		Liu <i>et al.</i> , 2006
	Root	Cynaforroside G [162]		Liu <i>et al.</i> , 2006
	Root	Cynaforroside H [163]		Liu <i>et al.</i> , 2006
	Root	Cynaforroside J [164]		Liu <i>et al.</i> , 2006
	Root	Cynatratoside A [165]		Liu <i>et al.</i> , 2006
	Root	Komaroside [166]		Liu <i>et al.</i> , 2006
	Root	Cynaforroside I [167]		Liu <i>et al.</i> , 2007a
	Root	Cynaforroside K [168]	Glucogenin C	Liu <i>et al.</i> , 2007a
	Root	Cynaforroside L [169]	Glucogenin C	Liu <i>et al.</i> , 2007a
	Root	Cynaforroside M [170]	Glucogenin C	Liu <i>et al.</i> , 2007a
	Root	Cynaforroside N [171]	Glucogenin C	Liu <i>et al.</i> , 2007a
	Root	Cynaforroside O [172]	Cynaforrogenin A	Liu <i>et al.</i> , 2007a
	Root	Cynaforroside P [173]	Cynaforrogenin A	Liu <i>et al.</i> , 2007a
	Root	Cynaforroside Q [174]	Glucogenin C	Liu <i>et al.</i> , 2007a

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. komarovii</i>	Root	Cynatratoside [175]		Wang <i>et al.</i> , 2004
	Root	Komaroside D [176]	Glucogenin A	Wang <i>et al.</i> , 2004
	Root	Komaroside E [177]	Glucogenin A	Wang <i>et al.</i> , 2004
	Root	Komaroside F [178]	Glucogenin A	Wang <i>et al.</i> , 2004
	Root	Komaroside G [179]	Glucogenin A	Wang <i>et al.</i> , 2004
	Root	Komaroside H [180]		Wang <i>et al.</i> , 2004
	Root	Hancoside A [181]		Wang <i>et al.</i> , 2004
	Root	Komaroside F [178]		Wang <i>et al.</i> , 2004
	Root	Komaroside G [179]		Wang <i>et al.</i> , 2004
	Root	Komaroside H [180]		Wang <i>et al.</i> , 2004
	Root	Hancoside A [181]		Wang <i>et al.</i> , 2004
<i>C. auriculatum</i>	Root	Auriculoside A [182]	Sarcogenin	Zhang <i>et al.</i> , 2000
	Root	Auriculoside B [183]	Sarcogenin	Zhang <i>et al.</i> , 2000
<i>C. otophyllum</i>	Root	Otophyllside H [184]	Sarcogenin	Ma <i>et al.</i> , 2007
	Root	Otophyllside I [185]	Sarcogenin	Ma <i>et al.</i> , 2007
	Root	Otophyllside J [186]	Sarcogenin	Ma <i>et al.</i> , 2007

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>C. otophyllum</i>	Root	Otophyllside K [187]	Sarcogenin	Ma <i>et al.</i> , 2007
	Root	Otophyllside L [188]	Sarcogenin	Ma <i>et al.</i> , 2007
	Root	Otophyllside M [189]	Sarcogenin	Ma <i>et al.</i> , 2007
	Rhizome	[190]	Caudatin	Zhao <i>et al.</i> , 2006
	Rhizome	[191]	Caudatin	Zhao <i>et al.</i> , 2006
<i>C. paniculatum</i>	n.s.	Neocynaponoside A [192]		Sugama and Hayashi, 1988
<i>C. taiwanianum</i>	Root	Taiwanoside A [193]	Metaplexigenin	Lin and Lin, 1995
	Root	Taiwanoside B [194]	Metaplexigenin	Lin and Lin, 1995
	Root	Taiwanoside C [195]	Metaplexigenin	Lin and Lin, 1995
<i>C. taiwanianum</i>	Root	Taiwanoside D [196]	Sarcogenin	Lin and Lin, 1995
	Root	Taiwanoside E [197]	Sarcogenin	Lin and Lin, 1995
	Root	Wilfooside C1N [198]	Caudatin	Tsukamoto, Hayashi and Mitsuhashi, 1985a.
	Root	Wilfooside C2N [199]	Caudatin	Tsukamoto, Hayashi and Mitsuhashi, 1985a.
	Root	Wilfooside M1N [200]	Sarcogenin	Tsukamoto, Hayashi and Mitsuhashi, 1985b.
	Root	Wilfooside K1N [201]	Sarcogenin	Tsukamoto, Hayashi and Mitsuhashi, 1985b.
<i>C. versicolor</i>	n.s.	Neocynaversicoside [202]		Sheng, Zhou and Zhou, 1990

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Dregea lanceolata</i>	Root	Dregealin [203]		Krishna <i>et al.</i> , 1990a
	Root	Ceolin [204]		Khishna <i>et al.</i> , 1990b
	Root	Drelin [205]		Khishna <i>et al.</i> , 1990b
	Root	Lancinin [206]		Khishna, Khare and Khare, 1991
<i>D. sinensis</i> var. <i>corrugata</i>	Rhizome	Dresgenin [207]		Qiu <i>et al.</i> , 1996
	Stem	[208]	Sarcotin	Liu <i>et al.</i> , 2007b
	Stem	[209]	Sarcotin	Liu <i>et al.</i> , 2007b
	Stem	[210]	Sarcotin	Liu <i>et al.</i> , 2007b
	Stem	[211]	Sarcotin	Liu <i>et al.</i> , 2007b
	Stem	[212]	Sarcotin	Liu <i>et al.</i> , 2007b
	Stem	[213]	Sarcotin	Liu <i>et al.</i> , 2007b
	Stem	[214]	Tayloron	Liu <i>et al.</i> , 2007b
	Stem	[215]	Tayloron	Liu <i>et al.</i> , 2007b
	Stem	[216]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
	Stem	[217]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
	Stem	[218]	Dihydrosarcotin	Liu <i>et al.</i> , 2008

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>D. sinensis</i>	Stem	[219]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
	Stem	[220]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
	Stem	[221]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
	Stem	[222]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
	Stem	[223]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
	Stem	[224]	Dihydrosarcotin	Liu <i>et al.</i> , 2008
<i>D. volubilis</i>	Flowers	Volubiloside A [225]		Sahu <i>et al.</i> , 2002
	Flowers	Volubiloside B [226]		Sahu <i>et al.</i> , 2002
	Flowers	Volubiloside C [227]		Sahu <i>et al.</i> , 2002
	Flowers	Volubiloside D [228]		Sahu <i>et al.</i> , 2002
	Flowers	Dregealol [230]		Panda <i>et al.</i> , 2003
	Flowers	Volubilogenone [231]		Panda <i>et al.</i> , 2003
	Flowers	Volubilol [232]		Panda <i>et al.</i> , 2003
	Flowers	<i>iso</i> -drevogenin P [233]		Panda <i>et al.</i> , 2003
	Flowers	17 $\alpha$ -marsdenin [234]		Panda <i>et al.</i> , 2003
	Flowers	Drevogenin [235]		Panda <i>et al.</i> , 2003

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Folotsia sarcostemmoides</i>	Aerial part	Folotsoside A [236]	Isolineolon	Rasoanaivo <i>et al.</i> , 1991
<i>Gongronema taylorii</i>	Root	Sarcostin [137]		Jaeggi, Weiss and Reichstein , 1967
	Seeds	Taylorone [237]	Drevogenin B	Jaeggi, Weiss and Reichstein , 1967
<i>Hemidesmus indicus</i>	Twigs	Desinine [238]	Calocin	Oberai, Khare and Khare, 1985
	Stem	Indicine [239]	Calocin	Prakash <i>et al.</i> , 1991
	Stem	Hemidine [240]	Calocin	Prakash <i>et al.</i> , 1991
	Stem	Hemidesine [241]	Calocin	Chandra, Deepak and Khare, 1993
	Stem	Emidine [242]	Sarcotin	Chandra, Deepak and Khare, 1993
	Stem	Medidesmine [243]	Calocin	Deepak, Srivastava and Khare, 1997
	Stem	Hemisine [244]	Calocin	Deepak, Srivastava and Khare, 1997
	Stem	Desmisine [245]	Calocin	Deepak, Srivastava and Khare, 1997
	Stem	Denicunine [246]	Calocin	Sigler <i>et al.</i> , 2000
	Stem	Heminine [247]		Sigler <i>et al.</i> , 2000
	Aerial part	Gordonoside A [248]		Acqua and innocenti, 2007
	Aerial part	Gordonoside B [249]		Acqua and innocenti, 2007

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Hoodia gordonii</i>	Aerial part	Gordonoside F [253]		Acqua and innocenti, 2007
	Aerial part	Gordonoside G [254]		Acqua and innocenti, 2007
	Aerial part	Gordonoside H [255]		Acqua and innocenti, 2007
	Aerial part	Gordonoside I [256]		Acqua and innocenti, 2007
	Aerial part	Gordonoside L [257]		Acqua and innocenti, 2007
	Aerial part	Hoodigoside A [258]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside B [259]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside C [260]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside D [262]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside E [263]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside F [264]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside G [265]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside H [266]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside I [267]		Pawar <i>et al.</i> , 2007a
	Aerial part	Hoodigoside J [268]		Pawar <i>et al.</i> , 2007a
Aerial part	Hoodigoside K [269]		Pawar <i>et al.</i> , 2007a	

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>H. gordonii</i>	Aerial part	Hoodigoside L [270]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside M [271]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside N [272]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside O [273]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside P [274]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside Q [275]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside R [276]	Calocin	Pawar, Shukla and Khan, 2007b
<i>H. gordonii</i>	Aerial part	Hoodigoside S [277]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside T [278]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside U [279]	Calocin	Pawar, Shukla and Khan, 2007b
	Aerial part	Hoodigoside V [280]	Calocin	Pawar, Shukla and Khan, 2007b
<i>Hoya carnosa</i>	Leaves	Drevogenin P [281]		Abe <i>et al.</i> , 1999
	Leaves	Drevogenin A [282]		Abe <i>et al.</i> , 1999
	Leaves	17 $\beta$ -marsdenin [283]		Abe <i>et al.</i> , 1999
	Leaves	Drebyssogenin [284]	Sarcotin	Abe <i>et al.</i> , 1999
	Leaves	Marsectohexol [285]	Sarcotin	Abe <i>et al.</i> , 1999



**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Hoya carnosa</i>	Leaves	[286]	Drevogenin P	Abe <i>et al.</i> , 1999
	Leaves	[287]	17 $\beta$ -marsdenin	Abe <i>et al.</i> , 1999
	Leaves	[288]	Drevogenin A	Abe <i>et al.</i> , 1999
	Leaves	[289]	17 $\beta$ -marsdenin	Abe <i>et al.</i> , 1999
	Leaves	11, 12- <i>O</i> -Diacetylmarsectohexol [290]		Abe <i>et al.</i> , 1999
	Leaves	Saguragenin [291]		Abe <i>et al.</i> , 1999
<i>Leptadenia hastata</i>	Bark	[292]	Sarcotin	Aquino <i>et al.</i> , 1995
	Bark	[293]	Sarcotin	Aquino <i>et al.</i> , 1995
	Bark	[294]	Sarcotin	Aquino <i>et al.</i> , 1995
<i>Leptadenia hastata</i>	Bark	[295]	Sarcotin	Aquino <i>et al.</i> , 1995
	Bark	[296]	Sarcotin	Aquino <i>et al.</i> , 1995
	Bark	Deacetylmataplexigenin [297]	Lineolon	Aquino <i>et al.</i> , 1995
	Bark	[298]	Sarcotin	Aquino <i>et al.</i> , 1995
	Bark	[299]	Sarcotin	Aquino <i>et al.</i> , 1995
	Bark	[300]	Sarcotin	Aquino <i>et al.</i> , 1995
<i>L. reticulata</i>	Root	Recticulin [301]	Calocin	Srivastava, Deepak and Khare, 1994

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>L. reticulata</i>	Root	Deniculatin [302]	Calocin	Srivastava, Deepak and Khare, 1994
	Root	Leptaculatin [303]	Calocin	Srivastava, Deepak and Khare, 1994
<i>Marsdenia condurango</i>	Bark	Condurangogluconide A [304]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide A <sub>0</sub> [305]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide A <sub>1</sub> [306]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide C [307]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide D <sub>0</sub> [308]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide E [309]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide E <sub>0</sub> [310]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide E <sub>2</sub> [311]		Berger, Junior and Kopanski, 1988
	Bark	Condurangogluconide E <sub>3</sub> [312]		Berger, Junior and Kopanski, 1988
<i>Marsdenia condurango</i>	Bark	Condurangogenin [313]		Berger, Junior and Kopanski, 1988
<i>M. erecta</i>	Leaves	Marsdenin [314]		Saner, Stöckel and Reichstein, 1972.
<i>M. flavescens</i>	Root bark	Flavescin [315]		Duff, Gellert and Rudzats, 1973
<i>M. koi</i>	Root	Marsdekoiside A [316]		Yuan <i>et al.</i> , 1992.
<i>M. rostrata</i>	Root	Rostratamine [317]		Gellert and Summons, 1973

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>M. rostrata</i>	Root	Metaplexigenin [318]		Schaub <i>et al.</i> , 1968
	Root	Dihydrorostratine [319]		Gellert <i>et al.</i> , 1973
<i>M. roylei</i>	Stem	Desacylkonduranggenin C [320]		Gupta <i>et al.</i> , 2003
	Stem	Deniagenin [321]		Gupta <i>et al.</i> , 2003
	Stem	Denin [322]		Gupta <i>et al.</i> , 2003
	Stem	Marsin [323]		Gupta <i>et al.</i> , 2003
<i>M. tenacissima</i>	Seed	Tenasogenin [324]		Singhal, Khare and Khare, 1980b
	Stem	Cissogenin [325]		Singhal, Khare and Khare, 1980a
	Stem	Tenacissosides A [326]		Miyakawa <i>et al.</i> , 1986
	Stem	Tenacissosides B [327]		Miyakawa <i>et al.</i> , 1986
	Stem	Tenacissosides C [328]		Miyakawa <i>et al.</i> , 1986
	Stem	Tenacissosides D [329]		Miyakawa <i>et al.</i> , 1986
<i>M. Tenacissima</i>	Stem	Tenacissosides E [330]		Qiu <i>et al.</i> , 1996
	Stem	Dresgenin [207]		Qiu <i>et al.</i> , 1996
	Stem	Marstenacigenin A [331]		Qiu <i>et al.</i> , 1996
	Stem	Marstenacigenin B [332]	Tenacigenin B	Lou <i>et al.</i> , 1993

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>M. Tenacissima</i>	Stem	[333]	Tenacigenin B	Lou <i>et al.</i> , 1993
	Stem	[334]	Tenacigenin B	Lou <i>et al.</i> , 1993
	Stem	[335]	Tenacigenin B	Lou <i>et al.</i> , 1993
	Stem	[336]	Tenacigenin B	Lou <i>et al.</i> , 1993
	Stem	[337]	Tenacigenin B	Lou <i>et al.</i> , 1993
	Stem	[338]	Tenacigenin B	Lou <i>et al.</i> , 1993
	Stem	Marsdenoside A [339]		Deng, Liao and Chen, 2005a
	Stem	Marsdenoside B [340]		Deng, Liao and Chen, 2005a
	Stem	Marsdenoside C [341]		Deng, Liao and Chen, 2005a
	Stem	Marsdenoside D [342]		Deng, Liao and Chen, 2005a
	Stem	Marsdenoside E [343]		Deng, Liao and Chen, 2005a
	Stem	Marsdenoside F [344]		Deng, Liao and Chen, 2005a
	Stem	Marsdenoside G [345]		Deng, Liao and Chen, 2005a
	Stem	Marsdenoside H [346]		Deng, Liao and Chen, 2005a
<i>M. tenacissima</i>	Stem	[347]	Tenacigenin B	Li <i>et al.</i> , 2007
	Stem	Tenacigenoside E [348]		Li <i>et al.</i> , 2007

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>M. tenacissima</i>	Stem	Tenacigenin A [349]		Deng, Liao and Chen, 2005b
<i>Metaplexis japonica</i>	Root	12- <i>O</i> -Acetylpergularin [350]		Warashina and Noro, 1998
	Root	12- <i>O</i> -Acetylpergularin [351]		Warashina and Noro, 1998
	Root	12- <i>O</i> -Acetylpergularin [352]		Warashina and Noro, 1998
	Root	12- <i>O</i> -Acetylpergularin [353]		Warashina and Noro, 1998
	Root	12- <i>O</i> -Acetylpergularin [354]		Warashina and Noro, 1998
	Root	12- <i>O</i> -Acetylpergularin [355]		Warashina and Noro, 1998
	Root	12- <i>O</i> -Acetylpergularin [356]		Warashina and Noro, 1998
	Root	12- <i>O</i> -Acetylpergularin [357]		Warashina and Noro, 1998
	Root	[358]	Sarcogenin	Warashina and Noro, 1998
	Root	[359]	Sarcogenin	Warashina and Noro, 1998
	Root	Deacylmetaplexigenin [360]		Warashina and Noro, 1998
	Leave	Digipurpurogenin II [361]		Mitsuhashi and Nomura, 1965
	Leave	Benzoylramamone [362]		Mitsuhashi and Nomura, 1965
<i>Orthenthera viminea</i>	Twig	Orgogenin [363]		Tiwari, Khare and Khare,1985
	Twig	Sarcostin [137]		Tiwari, Khare and Khare,1985

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Orthenthera viminea</i>	Twig	Sarcogenin [364]		Tiwari, Khare and Khare, 1985
	Twig	Therogenin [365]		Kuar, Khare and Khare, 1988
	Twig	Ornogenin [366]		Kuar, Khare and Khare, 1985
	Twig	Ornine [367]		Kuar, Khare and Khare, 1985
<i>Oxystelma esculentum</i>	Aerial part	Alpinoside A [368]	Sarcogenin	Hamed <i>et al.</i> , 2004
	Aerial part	Alpinoside B [369]	Sarcogenin	Hamed <i>et al.</i> , 2004
	Aerial part	Alpinoside C [370]	Sarcogenin	Hamed <i>et al.</i> , 2004
<i>Periploca calophylla</i>	Twig	Calocin [371]		Srivastava, Khare and Khare, 1982
	Twig	Plocigenin [372]		Deepak, Khare and Khare, 1985
	Twig	Plocin [373]		Deepak, Khare and Khare, 1985
<i>P. sepium</i>	Root bark	3 $\beta$ , 14 $\beta$ , 17 $\beta$ , 20-Tetratahydroxypregn-5-ene-21-O-methyl [374]		Xu, Takeya and Itokawa, 1996
	Root bark	3 $\beta$ , 17 $\beta$ , 20-Trihydroxypregn-5,14-ene-21-O-methyl [375]		Xu, Takeya and Itokawa, 1996
	Root bark	3 $\beta$ , 14 $\beta$ , 17 $\beta$ -Trihydroxypregn-5-ene-20-one-21-O-methyl [376]		Xu, Takeya and Itokawa, 1996

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Pergularia pallida</i>	Twig	Pallidine [377]		Khare <i>et al.</i> , 1984
	Twig	Pallidine [378]		Khare <i>et al.</i> , 1984
	Twig	Sarcogenin [364]		Khare <i>et al.</i> , 1986
<i>Sarcostemma australe</i>	n.d.	Sarcostin [137]		Shimizu and Mitsuhashi, 1968
<i>S. viminale</i>	n.d.	Metaplexigenin [379]		Schaub <i>et al.</i> , 1968
<i>S. brevistigma</i>	Twig	Sarcogenin [364]	Sarcogenin	Khare <i>et al.</i> , 1986
	Twig	Brevine [380]		Oberai, Khare and Khare, 1985c
	Twig	Brevinin [381]		Oberai, Khare and Khare, 1985a
	n.d.	Brogenin [382]		Khare <i>et al.</i> , 1987
<i>Solenostemma argel</i>	Aerial part	3 $\beta$ ,14 $\beta$ -Dihydroxypregn-5-ene-7,20-dione [383]		Kamel <i>et al.</i> , 2000
	Leaves	14 $\beta$ ,15 $\alpha$ -Dihydroxypregn-4-ene-3,20-dione [384]		Hassan <i>et al.</i> , 2001
	Leaves	3 $\beta$ ,14 $\beta$ ,15 $\alpha$ ,16 $\alpha$ -Tetrahydroxypregn-5-ene-20-one [385]		Hassan <i>et al.</i> , 2001
	Leaves	Solenoside A [386]		Innocenti <i>et al.</i> , 2005
<i>Stapelia gigantea</i>	Seed	Stapelogenin [387]		Eppenberger, Vetter and Reichstein, 1966
<i>S. grandiflora</i>	Root	Boucerin [388]		Bando <i>et al.</i> , 1974

**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>S. variegata</i>	Aerial part	Stavaroside A [389]	Sarcotin	El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside B [390]	Sarcotin	El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside C [391]		El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside D [392]		El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside E [393]	Sarcotin	El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside F [394]		El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside G [395]	Sarcotin	El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside H [396]		El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside I [397]		El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside J [398]		El Sayed <i>et al.</i> , 1995
	Aerial part	Stavaroside K [399]		El Sayed <i>et al.</i> , 1995
<i>Stephanotis mucronata</i>	Root	Stemucronatoside A [400]		Ye <i>et al.</i> , 2004
	Root	Stemucronatoside B [401]		Ye <i>et al.</i> , 2004
	Root	Stemucronatoside C [402]		Ye <i>et al.</i> , 2004
	Root	Stemucronatoside E [403]		Ye <i>et al.</i> , 2005
	Root	Stemucronatoside F [404]	Sarcotin	Ye <i>et al.</i> , 2005

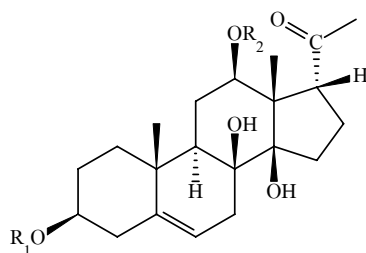


**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Stephanotis mucronata</i>	Root	Stemucronatoside G [405]	Sarcotin	Ye <i>et al.</i> , 2005
	Root	Stephanthraniline A [406]	Tomemtogenin	Ye <i>et al.</i> , 2006
	Root	Isogagamine [407]		Ye <i>et al.</i> , 2006
	Stem	Mucronatoside A [408]		Zhang <i>et al.</i> , 2003
	Stem	Mucronatoside B [409]		Zhang <i>et al.</i> , 2003
	Stem	Mucronatoside E [410]	Sarcogenin	Li <i>et al.</i> , 2006b
	Stem	Mucronatoside F [411]		Li <i>et al.</i> , 2006b
	Stem	Mucronatoside G [412]		Li <i>et al.</i> , 2006b
<i>Stephanotis mucronata</i>	Stem	Mucronatoside H [413]		Li <i>et al.</i> , 2006b
<i>Streptocaulon tomentosum</i>	Root	Pregn-5-ene-20-one-3 $\beta$ ,16 $\alpha$ -diol-d-O-[2,4-O-diacetyl- $\beta$ -digitalopyranosyl-(14)- $\beta$ -D-cymaropyranoside]-16-O-[ $\beta$ -D-glucopyranosides] [414]		Khine <i>et al.</i> , 2007
<i>Trachycalymma</i>	Root	3 $\beta$ -Hydroxypregn-14-ene-20-one [415]		Bach, Capitaine and Engel, 1968
<i>fimbriatum</i>	Root	3,8,14-Trihydroxypregnan-20-one [416]		Elber, Weiss and Reichstein, 1969
<i>Tylophora sylvatica</i>	Root	Tylogenin [417]		Gnabre <i>et al.</i> , 1991

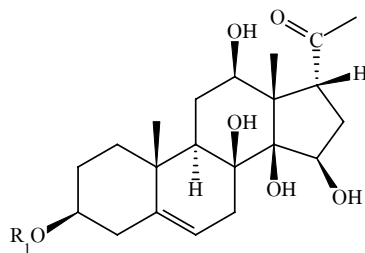
**Table1. Distribution of pregnane steroids in the family Asclepiadaceae (continued)**

Plant source	Plant part	Chemical compound	Genin	Reference
<i>Tylophora sylvatica</i>	Root	Tylophoroside [418]		Gnabre <i>et al.</i> , 1991
<i>Tylophora sylvatica</i>	n.d.	Acetylylophoroside [419]		Gnabre and Pinnas, 1991
<i>Vintoxicum hirundinariae</i>	Root	Cynatratoside E [420]		Lavault, Richomme and Bruneton, 1999
	Root	Cynatratoside C [421]	Glucogenin A	Lavault, Richomme and Bruneton, 1999
	Root	Hirundigoside B [422]	Glucogenin A	Lavault, Richomme and Bruneton, 1999
	Root	Hirundigoside C [423]	Glucogenin A	Lavault, Richomme and Bruneton, 1999
	Root	Hirundicoside D [424]		Lavault, Richomme and Bruneton, 1999
<i>V. officinale</i>	Root	Anhydrohirundigenin [425]		Kennard <i>et al.</i> , 1968; Stockel, Stocklin and Reichstein, 1969
	Root	Hirundoside A [426]		Kennard <i>et al.</i> , 1968; Stockel, Stocklin and Reichstein, 1969
	Root	Hirundigenin [427]		Kennard <i>et al.</i> , 1968; Stockel, Stocklin and Reichstein, 1969

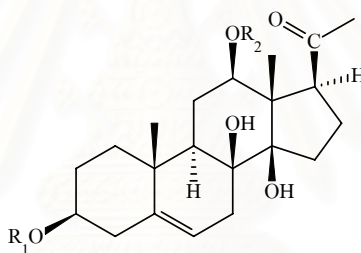


Compounds	R <sub>1</sub>	R <sub>2</sub>
[1]	H	H
[2]	$\beta$ -digto <sup>4</sup> $\beta$ -oli <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[3]	$\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[8]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[9]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	H
[10]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[11]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	H
[12]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[6]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Cin
[7]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Cin.
[18]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Ac
[19]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ac
[20]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Ac
[21]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ac
[22]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Ac
[23]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Nic
[24]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Nic
[25]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Nic
[297]	H	Nic

**Figure 2. Pregnane steroids from asclepiadaceous plants**

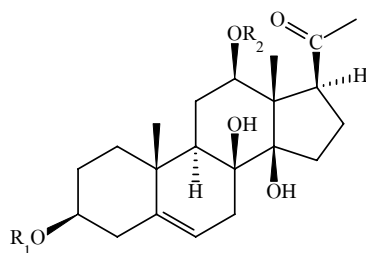


Compounds	R
[26]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole
[27]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc
[28]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -all
[29]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole
[30]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc
[31]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole
[164]	$\beta$ -glc <sup>2</sup> $\beta$ -glc

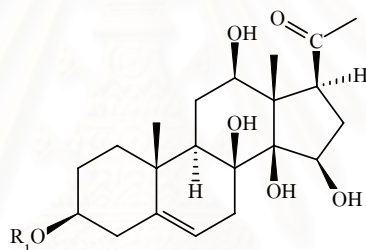


Compounds	R <sub>1</sub>	R <sub>2</sub>
[4]	$\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -dig <sup>4</sup> $\beta$ -ole	H
[5]	$\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole	H
[13]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[14]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	H
[15]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[16]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	H
[17]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[122]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ikem
[123]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ikem
[124]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Ikem

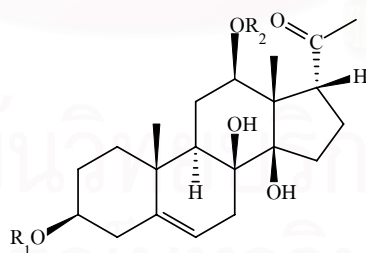
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compounds	R <sub>1</sub>	R <sub>2</sub>
[125]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Ikem
[126]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole	Ikem
[127]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ikem
[128]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cyme <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Ikem
[236]	H	Bz

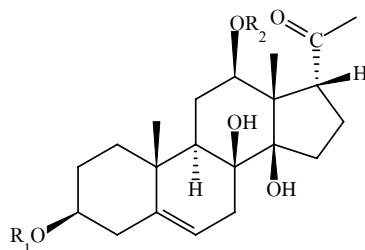


Compound	R <sub>1</sub>
[32]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole



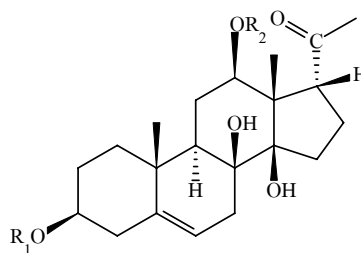
Compounds	R <sub>1</sub>	R <sub>2</sub>
[33]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Ac
[34]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Ac
[35]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ac

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



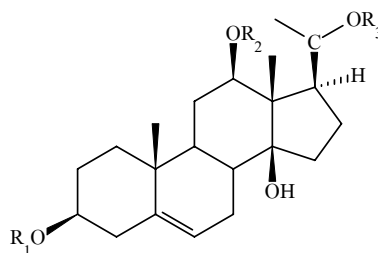
Compounds	R <sub>1</sub>	R <sub>2</sub>
[36]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Ac
[37]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ac
[379]	H	Ac
[38]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[39]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	Nic
[129]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ikem
[130]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ikem
[131]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -glc	Ikem
[132]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Ikem
[133]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole	Ikem
[134]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ikem
[135]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Ikem
[184]	$\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Hbz
[185]	$\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Hbz
[186]	$\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Hbz.
[187]	$\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Hbz.
[188]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Hbz.
[189]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Hbz.
[182]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	H
[183]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	H
[190]	$\beta$ -digno <sup>4</sup> $\beta$ -ole <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\alpha$ -cym	Ikem.
[191]	$\beta$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\beta$ -cym	Ikem.

**Figure 2. Pregnane steroids from asclepiadaceous plants (continued)**

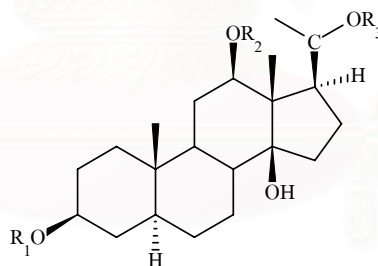


Compounds	R <sub>1</sub>	R <sub>2</sub>
[193]	$\beta$ -digto <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym	Ac
[194]	$\beta$ -digto <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	Ac
[195]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -cym	Ac
[196]	$\beta$ -digto <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	Cin
[197]	$\beta$ -digto <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	H
[198]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -cym	Ikem
[199]	$\beta$ -digto <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	Ikem
[200]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -cym	H
[201]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -cym	Cin
[358]	$\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	Ac
[359]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -digto	Ac
[360]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[364]	H	H
[368]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Cin
[369]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Cin
[370]	$\beta$ -cym <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Cin
[380]	$\alpha$ -digno <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\alpha$ -digno	H
[410]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	H

**Figure 2. Pregnane steroids from asclepiadaceous plants (continued)**



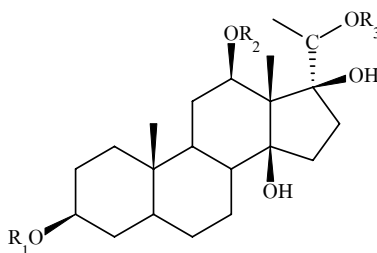
Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[40]	$\beta$ -glc <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-gal	Bz	H
[48]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	H
[49]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	H
[50]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	Ac
[51]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	Ac
[84]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -glu	Bz	Ac
[85]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Bz	Ac
[86]	$\beta$ -cym <sup>4</sup> $\beta$ -glc	Bz	Ac
[87]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -glu	Tig	Ac



Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[41]	H	H	H
[42]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	H
[43]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	H
[44]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	Ac
[45]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	Ac
[46]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	Bz
[47]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	Bz

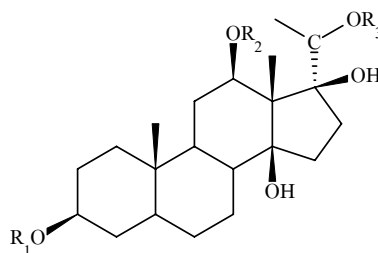
**Figure 2. Pregnane steroids from asclepiadaceous plants (continued)**



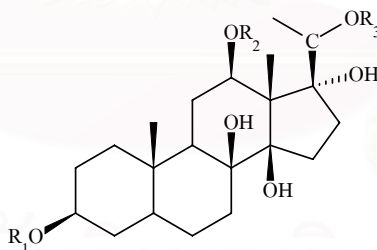


Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[52]	$\beta$ -ole <sup>4</sup> $\beta$ -the	H	H
[53]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Bz	Ac
[54]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Ami	Ac
[55]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Nic	Ac
[56]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Mebu	Ac
[57]	$\beta$ -cym. <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Bz	Ac
[58]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Ami	Ac
[59]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Nic	Ac
[60]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Mebu	Ac
[61]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc. <sup>4</sup> $\beta$ -glc	Bz	Ac
[62]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc. <sup>4</sup> $\beta$ -glc	Ami	Ac
[63]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc. <sup>4</sup> $\beta$ -glc	Nic	Ac
[64]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Mebu	Ac
[65]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Iso	Ac
[66]	$\beta$ -cym <sup>4</sup> $\beta$ -ole	Bz	Ac
[406]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all- $\beta$ - the <sup>4</sup> $\beta$ -glc	Anth	Ac
[67]	$\beta$ -cym <sup>4</sup> $\beta$ -ole	Mebu	Ac
[68]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Bz	Ac
[69]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Nic	Ac
[70]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Nic	Ac
[71]	$\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Bz	Ac
[72]	$\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Nic	Ac
[73]	$\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Bz	Ac

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

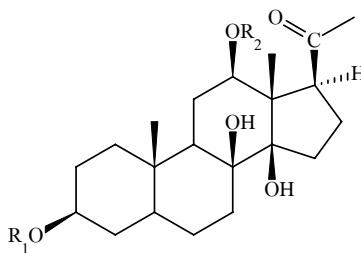


Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[74]	$\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Nic	Ac
[75]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Bz	Ac
[76]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Ami	Ac
[77]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Mebu	Ac
[207]	H	H	Bz
[331]	H	H	Cin
[332]	H	Bz	Bz

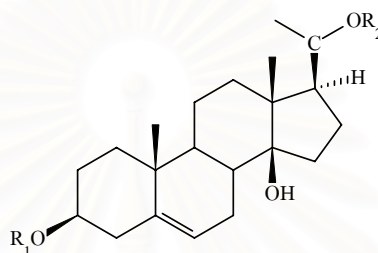


Compound	R	R <sub>1</sub>	R <sub>2</sub>
Caretroside A [78]	$\beta$ -glc <sup>6</sup> $\beta$ -glc <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-gal	Bz	Iso

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

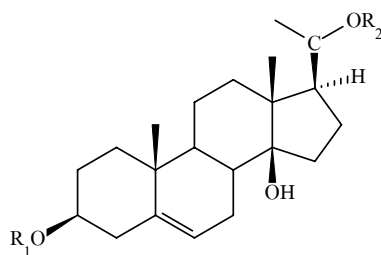


Compound	R <sub>1</sub>	R <sub>2</sub>
[79]	$\beta$ -ole <sup>4</sup> $\beta$ -cym	Bz

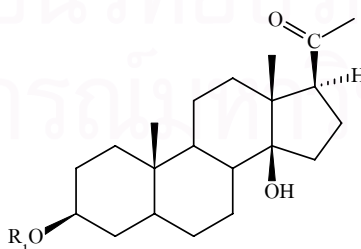


Compounds	R <sub>1</sub>	R <sub>2</sub>
[80]	$\beta$ -(6-deoxy-3-O-methyl)-gal.	$\beta$ -glc
[81]	$\beta$ -glc. <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-gal.	$\beta$ -glc
[82]	$\beta$ -glc. <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-gal.	H
[83]	$\beta$ -glc. <sup>6</sup> $\beta$ -glc. <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-gal	H
[92]	$\beta$ -glu. <sup>6</sup> $\beta$ -glu	H
[93]	$\beta$ -glu	H
[94]	$\beta$ -glu. <sup>4</sup> $\beta$ -digto	$\beta$ -glu.
[95]	$\beta$ -glu. <sup>4</sup> $\beta$ -digto	$\beta$ -(2-O-benzoyl)-glu
[96]	$\beta$ -(6-O-benzoyl)-glu. <sup>4</sup> $\beta$ -digto	$\beta$ -(2-O-benzoyl)-glu
[270]	$\beta$ -ole. <sup>4</sup> $\beta$ -(4-O-tigloyl)-the.	$\beta$ -glc. <sup>6</sup> $\beta$ -glc. <sup>6</sup> $\beta$ -glc
[271]	$\beta$ -ole. <sup>4</sup> $\beta$ -the	$\beta$ -glc. <sup>6</sup> $\beta$ -glc. <sup>6</sup> $\beta$ -glc
[272]	$\beta$ -ole. <sup>4</sup> $\beta$ -the	H
[273]	$\beta$ -ole. <sup>4</sup> $\beta$ -(4-O-tigloyl)-the	$\beta$ -glc. <sup>6</sup> $\beta$ -glc
[274]	$\beta$ -cym. <sup>6</sup> $\beta$ -(4-O-tigloyl)-ole	$\beta$ -glc
[275]	$\beta$ -cym. <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -(4-O-tigloyl)-the	$\beta$ -glc. <sup>6</sup> $\beta$ -glc. <sup>6</sup> $\beta$ -glc
[276]	$\beta$ -cym. <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -(4-O-tigloyl)-ole	$\beta$ -glc. <sup>6</sup> $\beta$ -glc. <sup>6</sup> $\beta$ -glc
[277]	$\beta$ -cym. <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -(4-O-tigloyl)-ole	$\beta$ -glc. <sup>6</sup> $\beta$ -glc. <sup>6</sup> $\beta$ -glc

**Figure 2. Pregnane steroids from asclepiadaceous plants (continued)**

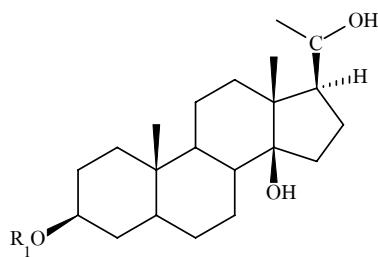


Compounds	R <sub>1</sub>	R <sub>2</sub>
[278]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(4- <i>O</i> -tigloyl)-ole	$\beta$ -glc <sup>6</sup> $\beta$ -glc
[279]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(4- <i>O</i> -tigloyl)-cym	$\beta$ -glc <sup>6</sup> $\beta$ -glc <sup>6</sup> $\beta$ -glc
[280]	$\beta$ -ole <sup>4</sup> $\beta$ -(4- <i>O</i> -tigloyl)-the	$\beta$ -glc
[371]	H	H
[239]	$\beta$ -digto	H
[240]	$\beta$ -boi	H
[241]	$\beta$ -ole <sup>4</sup> $\beta$ -digto	H
[242]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -digto	H
[246]	$\beta$ -ole <sup>4</sup> 3- <i>O</i> -methyl- $\beta$ -fuc	H
[247]	$\beta$ -digto <sup>4</sup> $\beta$ -cym	H
[245]	$\beta$ -digto <sup>4</sup> $\beta$ -xyl <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -xyl	H
[244]	$\beta$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> 3- <i>O</i> -methyl- $\beta$ -glc <sup>4</sup> $\beta$ -cym	H
[301]	$\beta$ -digto <sup>4</sup> $\beta$ -digto <sup>4</sup> 3- <i>O</i> -methyl- $\alpha$ -gal <sup>4</sup> $\beta$ -cym	H
[302]	$\beta$ -digto <sup>4</sup> 3- <i>O</i> -methyl- $\alpha$ -gal	H
[303]	$\beta$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	H

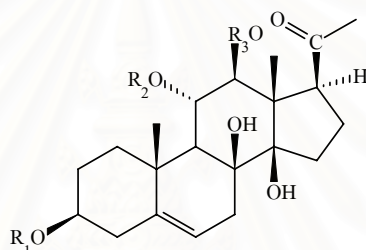


Compound	R <sub>1</sub>
[88]	$\beta$ -glc <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-gal

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

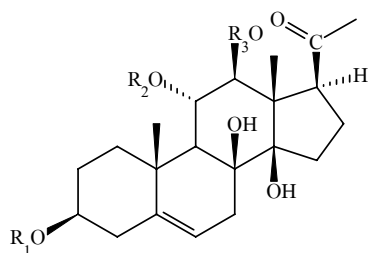


Compounds	R <sub>1</sub>
[89]	$\beta$ -glc <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-gal
[90]	$\beta$ -glc <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-gal
[91]	$\beta$ -glc <sup>6</sup> $\beta$ -glc <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-gal

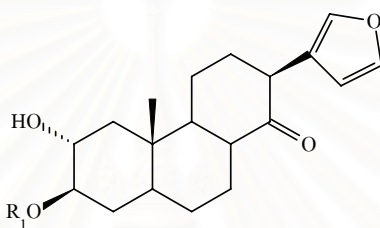


Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[97]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -glc <sup>4</sup> $\beta$ -glc	Bz	Ac
[98]	$\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -glc	Bz	Ac
[228]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -glc	H	H
[238]	$\beta$ -ole <sup>4</sup> $\beta$ -ole	H	Ac
[281]	H	H	H
[282]	H	Ac	Ac
[283]	H	Iso	Ac
[391]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Bz	Ac
[392]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Tig	Ac.
[394]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Ac	Ac
[396]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	H	H
[397]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -glc	Bz	Ang

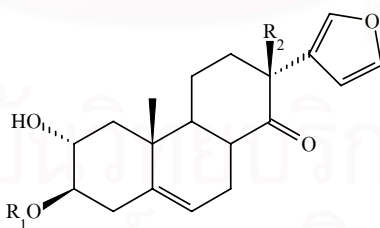
**Figure 2. Pregnane steroids from asclepiadaceae plants (continued)**



Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[398]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Bz	Ac
[399]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Tig	Ac

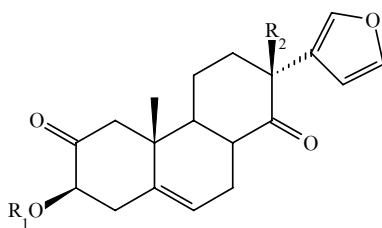


Compounds	R <sub>1</sub>
[99]	$\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole
[100]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc
[101]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc
[149]	$\beta$ -cym <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -glc

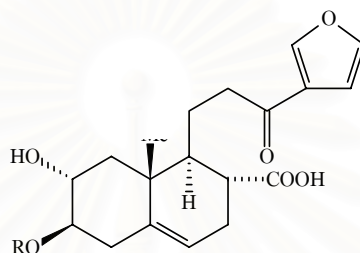


Compounds	R <sub>1</sub>	R <sub>2</sub>
[102]	$\beta$ -cym. <sup>4</sup> $\alpha$ -digno. <sup>4</sup> $\beta$ -cym	Me
[103]	$\beta$ -cym. <sup>4</sup> $\alpha$ -digno. <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -glc.	Me
[105]	$\beta$ -cym. <sup>4</sup> $\beta$ -digto. <sup>4</sup> $\alpha$ -ole.	H

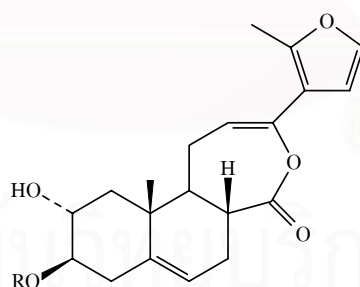
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compound	R <sub>1</sub>	R <sub>2</sub>
[104]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Me

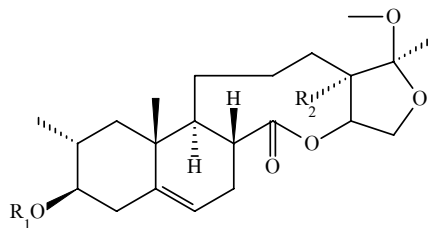


Compounds	R
[106]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym
[107]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc
[108]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -cy.

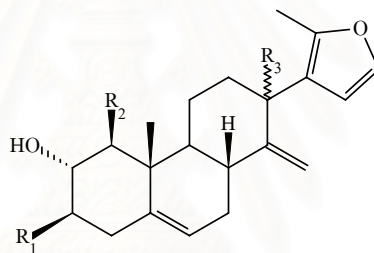


Compound	R
[110]	$\beta$ -cym <sup>4</sup> $\alpha$ -dign <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc

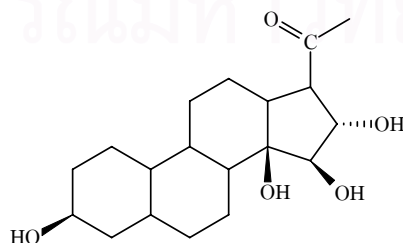
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compounds	R <sub>1</sub>	R <sub>2</sub>
Cynanoside E [109]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym	OH
Cynanoside F [111]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	OH
[112]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -cym	OH
Cynanoside H [113]	$\beta$ -cym <sup>4</sup> $\alpha$ -digto <sup>4</sup> $\beta$ -ole	OH
Cynanoside I [114]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	H
[115]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -cym	H



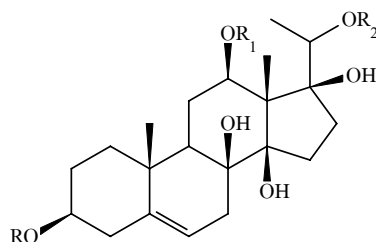
Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[116]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	H	$\beta$ -H
[117]	$\beta$ -cym <sup>4</sup> $\alpha$ -digto <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	H	$\beta$ -H
[118]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym	H	$\alpha$ -H
[119]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	H	$\beta$ -CH <sub>3</sub>
[120]	$\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	OH	$\beta$ -CH <sub>3</sub>



[121]

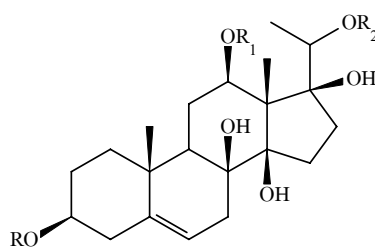
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



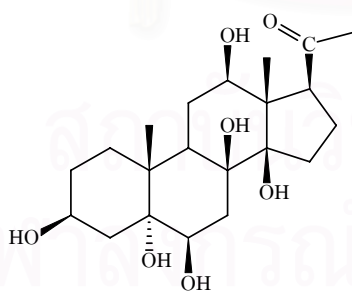


Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[136]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Cin	Nic
[137]	H	H	H
[138]	$\beta$ -cym	H	H
[139]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym	H	H
[140]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole	H	H
[141]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	H	H
[142]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym	H	H
[143]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole	H	H
[144]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole	H	H
[145]	$\beta$ -cym <sup>4</sup> $\beta$ -cym	H	H
[146]	$\beta$ -cym <sup>4</sup> $\beta$ -ole	H	H
[384]	$\beta$ -ole	Bz	Bz
[385]	$\beta$ -ole <sup>4</sup> $\beta$ -cym	Bz	Bz
[243]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -glc	H	H
[208]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Cin	Nic
[209]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Bz	H
[210]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Nic	Cin
[211]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Cin	H
[212]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Cin	H
[213]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Ac	Mebu
[292]	H	Ac	H
[293]	H	Bz	Bz

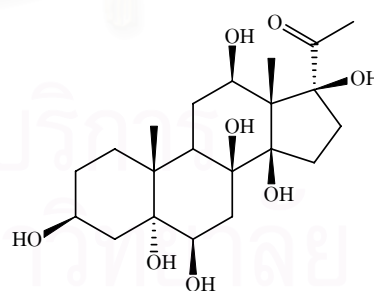
**Figure 2. Pregnane steroids from asclepiadaceous plants (continued)**



Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[294]	H	Bz	Cin
[295]	H	Cin	Ac
[296]	H	Nic	Ac
[298]	$\beta$ -cym.	Bz	Cin
[299]	$\beta$ -cym <sup>4</sup> $\beta$ -ole	Cin	H
[300]	$\beta$ -cym <sup>4</sup> $\beta$ -ole	Bz	Cin
[389]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Ang	Bz
[390]	$\beta$ -cym. <sup>4</sup> $\beta$ -cym. <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Ang	Tig
[393]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Bz.	H
[395]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Ac	Ac
[404]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the	O	Nic
[405]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-the	Tig	Ac

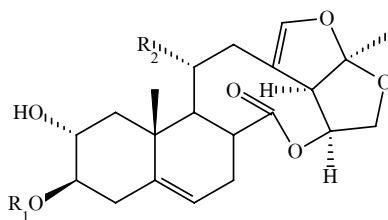


[147]

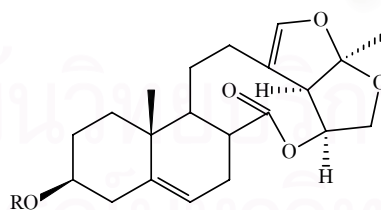


[148]

**Figure 2. Pregnane steroids from asclepiadaceous plants (continued)**

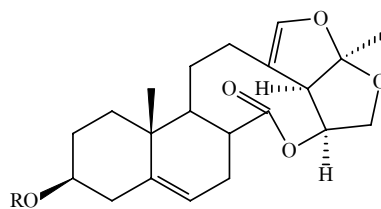


Compounds	R <sub>1</sub>	R <sub>2</sub>
[150]	$\beta$ -cym <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -glc	H
[151]	H	H
[152]	H	OH
[153]	$\beta$ -ole	H
[154]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -cym	H
[155]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -cym	H
[156]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -cym	H
[176]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\beta$ -glc	H
[177]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -glc	H
[178]	$\beta$ -ole <sup>4</sup> $\beta$ -(3-demethyl-2-deoxy)-the <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	H
[179]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\beta$ -glc	H
[421]	$\alpha$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H
[422]	$\beta$ -glc <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\alpha$ -digto <sup>4</sup> $\beta$ -ole	H
[423]	$\alpha$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole	H

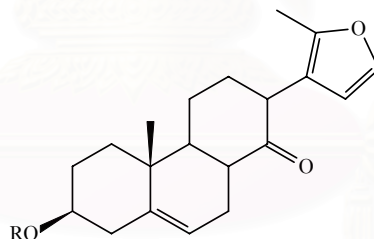


Compounds	R
[165]	$\beta$ -glc
[157]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[158]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[159]	$\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

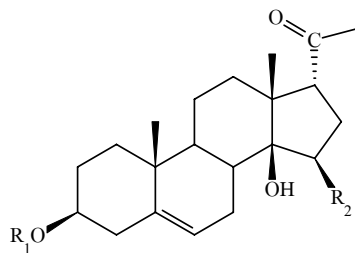


Compounds	R
[160]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>6</sup> $\beta$ -glc
[161]	$\beta$ -the <sup>4</sup> $\beta$ -(3-demethyl-2-deoxy)-the <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>6</sup> $\beta$ -glc
[166]	$\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>6</sup> $\beta$ -glc
[168]	$\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[169]	$\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[170]	$\beta$ -ole <sup>4</sup> $\beta$ -(3-demethyl-2-deoxy)-the <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[171]	$\beta$ -(3-demethyl-2-deoxy)-the <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[174]	$\beta$ -the <sup>4</sup> $\beta$ -(3-demethyl-2-deoxy)-the <sup>4</sup> $\beta$ -ole
[175]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\beta$ -glc
[420]	$\beta$ -glc <sup>4</sup> $\alpha$ -ole <sup>4</sup> $\alpha$ -digto <sup>4</sup> $\beta$ -ole

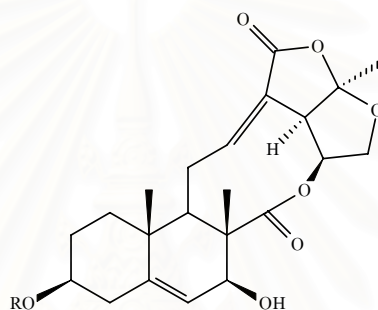


Compounds	R
[162]	$\beta$ -ole <sup>6</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[163]	$\beta$ -ole <sup>4</sup> $\beta$ -(3-demethyl-2-deoxy)-the <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>6</sup> $\beta$ -glc
[167]	$\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>6</sup> $\beta$ -glc
[172]	$\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc
[173]	$\beta$ -ole <sup>4</sup> $\beta$ -digt <sup>4</sup> $\alpha$ -cym <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc

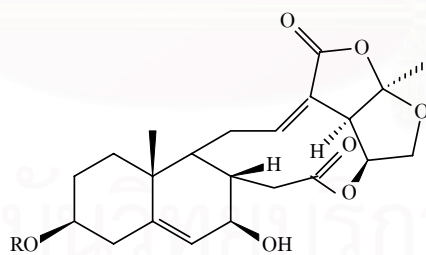
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compounds	R <sub>1</sub>	R <sub>2</sub>
[180]	$\beta$ -glu <sup>2</sup> $\beta$ -(6-sinapoyl)-glu	H
[181]	H	OH
[323]	$\alpha$ -fuc	H

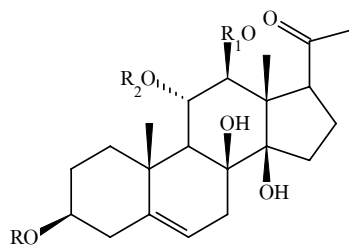


Compound	R
[192]	$\beta$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\alpha$ -cym

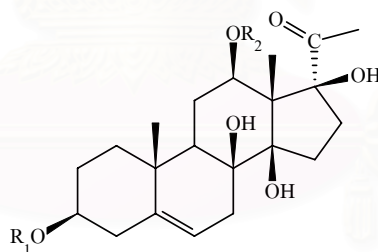


Compound	R
[202]	$\beta$ -the

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

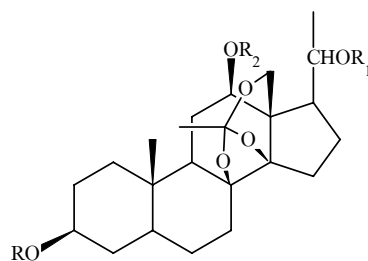


Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[314]	H	H	H
[203]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -cym	Ac	H
[204]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -boi	Ac	H
[205]	$\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\alpha$ -digno <sup>4</sup> $\alpha$ -dino	Ac	H
[206]	$\beta$ -ole <sup>4</sup> $\beta$ -cym	H	H
[284]	H	H	H
[285]	H	Ac	Ac
[286]	H	Ac	Iso
[287]	H	Ac	Bz

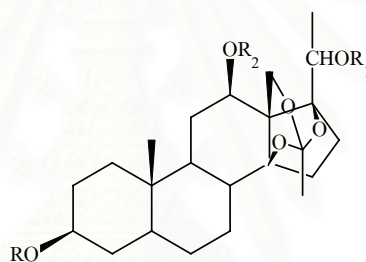


Compounds	R <sub>1</sub>	R <sub>2</sub>
[214]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Bz
[215]	$\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Cin
[237]	H	H

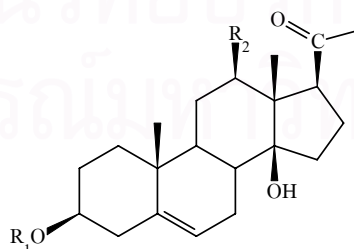
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[216]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Bz	Ac
[217]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Bz	Ac
[218]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Bz	Ac
[219]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Bz	Ac
[220]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Bz	Ac

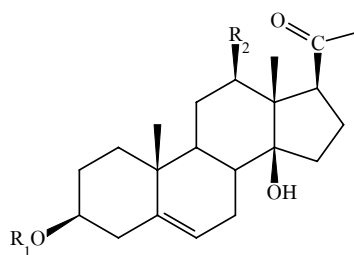


Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[221]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Bz	Ac
[222]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Bz	Ac
[223]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Bz	Ac
[224]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Bz	Ac



Compounds	R	R <sub>1</sub>
[248]	H	Tig
[249]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -the	Tig

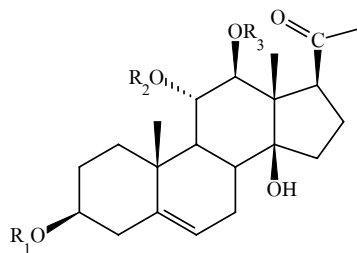
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



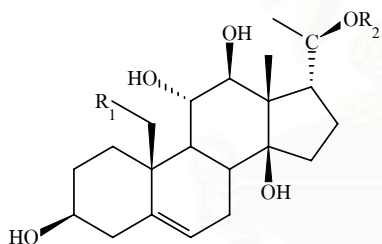
Compounds	R <sub>1</sub>	R <sub>2</sub>
[250]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the	Tig
[251]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -digto	Tig
[252]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole	Tig
[254]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole	Tig
[255]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	Tig
[256]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Tig
[257]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -digto	Tig
[257]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole	Tig
[258]	$\beta$ -cym <sup>4</sup> $\beta$ -the	Tig
[259]	$\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -the	Tig
[260]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the	Tig
[262]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -the	Tig
[263]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -glc	Tig
[264]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Tig
[265]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Tig
[266]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -glc	Tig
[267]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Tig
[268]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -glc	Tig
[269]	$\beta$ -cym <sup>4</sup> $\beta$ -glc <sup>6</sup> $\beta$ -glc	Tig
[361]	H	H
[362]	H	Bz

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

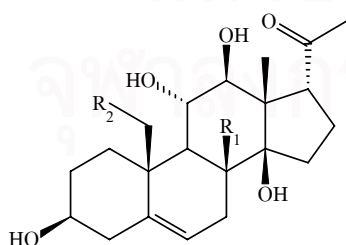




Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[225]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	H	H
[226]	$\beta$ -digto <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	H	H
[227]	H	H	H
[309]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Ac	Cin
[310]	$\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Ac	Cin
[311]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Ac	Cin
[312]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Cin	Ac
[313]	H	Cin	Ac

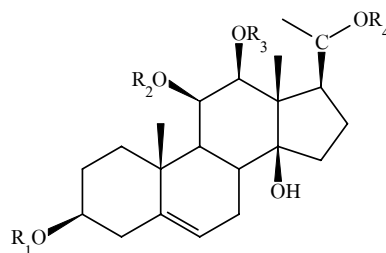


Compounds	R <sub>1</sub>	R <sub>2</sub>
[230]	H	Tig.
[232]	OH	H
[235]	H	H

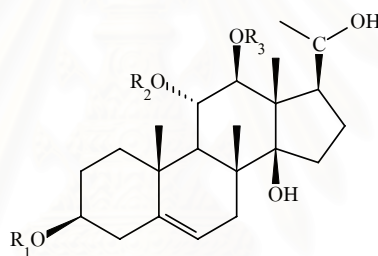


Compounds	R <sub>1</sub>	R <sub>2</sub>
[231]	H	OH
[233]	H	H
[234]	OH	H

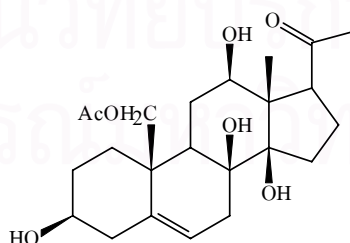
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
[288]	H	Ac	Ac	H
[320]	H	H	H	H
[322]	$\alpha$ -fuc <sup>4</sup> $\alpha$ -glc	H	H	H
[372]	H	H	Bz	Bz
[373]	$\beta$ -ole <sup>4</sup> $\beta$ -ole	H	Bz	Bz

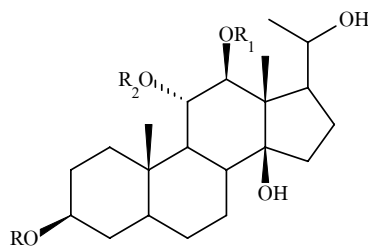


Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[289]	H	H	H
[290]	H	Ac	Ac

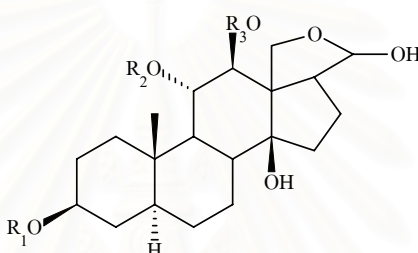


[291]

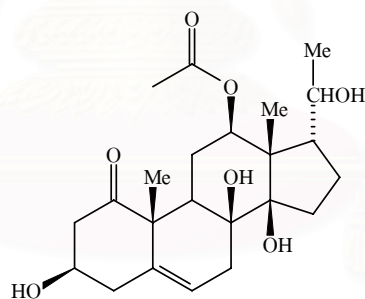
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



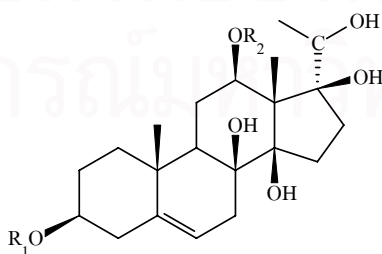
Compounds	R	R <sub>1</sub>	R <sub>2</sub>
[307]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Cin	Ac
[325]	H	H	H
[324]	H	H	Acr



Compound	R	R <sub>1</sub>	R <sub>2</sub>
[308]	$\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Cin	Ac

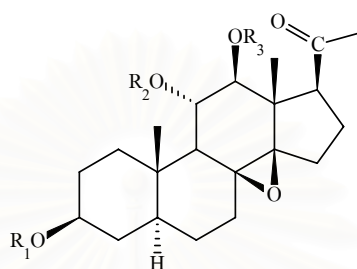
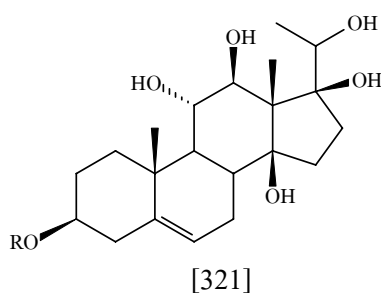


[315]

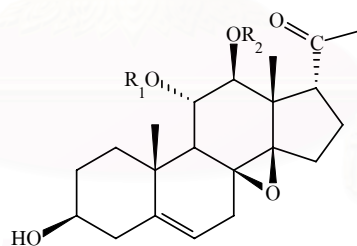


Compound	R <sub>1</sub>	R <sub>2</sub>
[316]	$\beta$ -cym <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all	Cin

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

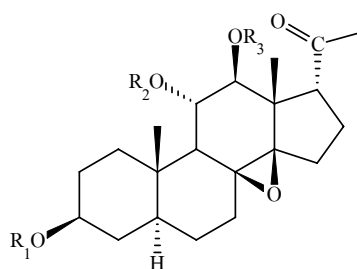


Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[326]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Ac	Tig
[327]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Tig	Tig
[328]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Bz	Tig
[329]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Mebu	Tig
[330]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -glc	Bz	Mebu

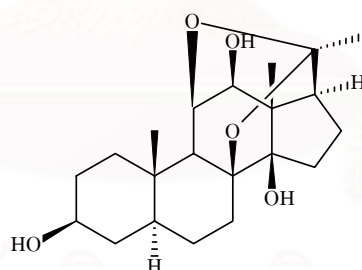


Compounds	R <sub>1</sub>	R <sub>2</sub>
[333]	Ac	Tig
[334]	Ac	Bz
[335]	Ac	Mebu
[336]	Tig	Mebu
[337]	Bz	Mebu
[338]	Tig	Tig

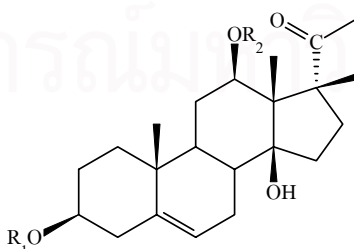
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[339]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Mebu	Tig
[340]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Tig	Tig
[341]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Mebu	Bz
[342]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	H	Mebu
[343]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Pro	Ac
[344]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Ac	Ac
[345]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Tig	H
[346]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all. <sup>4</sup> $\beta$ -glc	Mebu.	Ac
[347]	H	Tig	Tig
[348]	$\beta$ -ole <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all <sup>4</sup> $\beta$ -glc <sup>4</sup> $\beta$ -glc	Tig	Tig

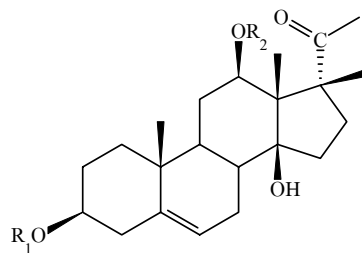


[349]

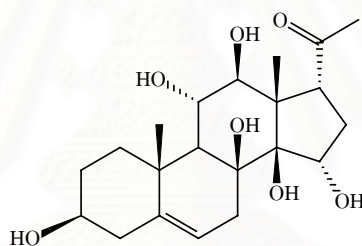


Compounds	R <sub>1</sub>	R <sub>2</sub>
[350]	$\beta$ -ole	Ac

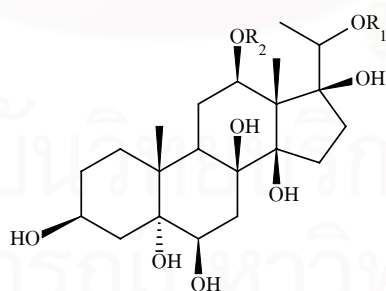
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compounds	R	R <sub>1</sub>
[351]	$\beta$ -ole <sup>4</sup> $\beta$ -ole	Ac
[352]	$\beta$ -ole <sup>4</sup> $\beta$ -can. <sup>4</sup> $\beta$ -ole	Ac
[353]	$\beta$ -ole <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym	Ac
[354]	$\beta$ -ole <sup>4</sup> $\beta$ -can. <sup>4</sup> $\beta$ -can. <sup>4</sup> $\beta$ -ole	Ac
[355]	$\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole <sup>4</sup> $\beta$ -ole	Ac
[356]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -dig	Ac
[357]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -can	Ac

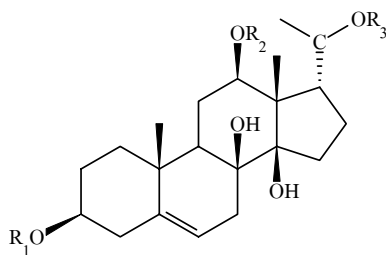


[ 363]

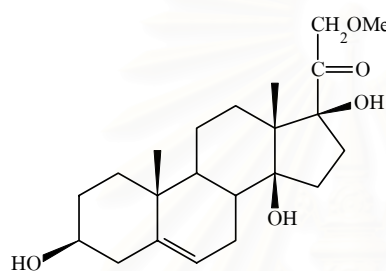


Compound	R <sub>1</sub>	R <sub>2</sub>
[365]	Cin	Cin

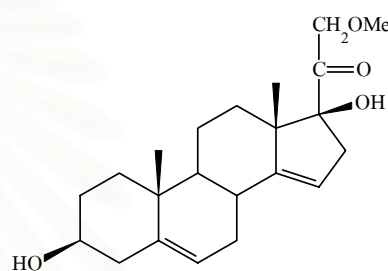
Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



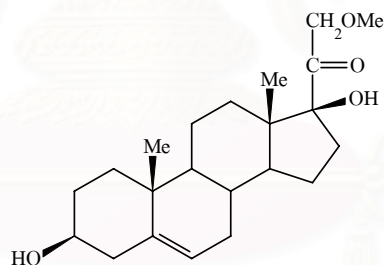
Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[366]	H	Cin	Cin
[367]	$\beta$ -cym <sup>4</sup> $\alpha$ -ole	Cin	Cin



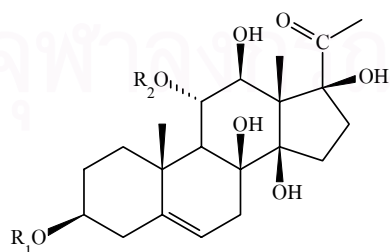
[374]



[375]

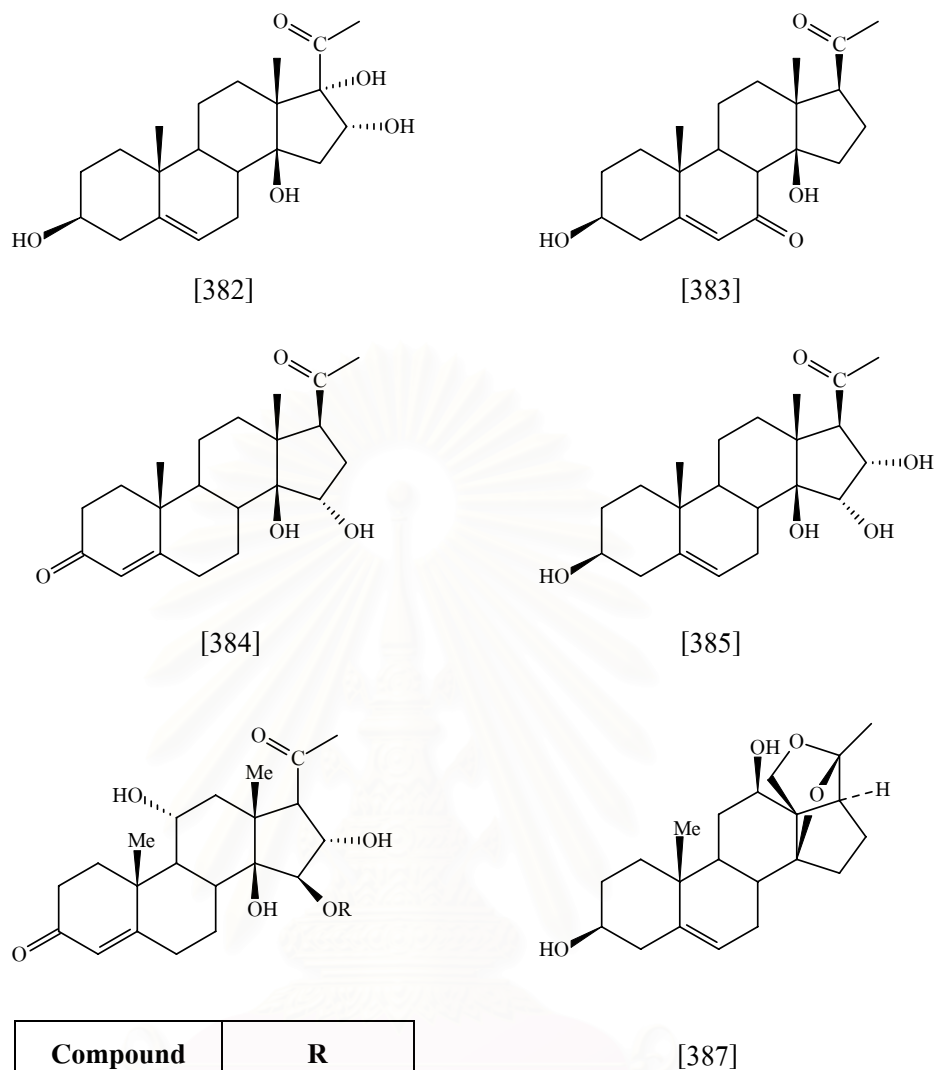


[376]

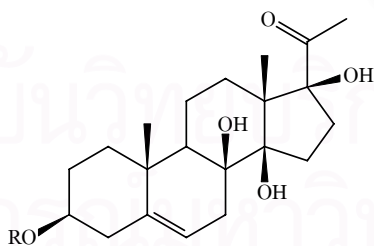


Compound	R	R <sub>1</sub>
[381]	$\alpha$ -digno <sup>4</sup> $\alpha$ -digno	Bz

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



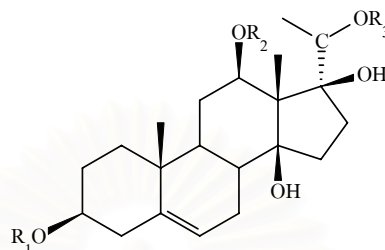
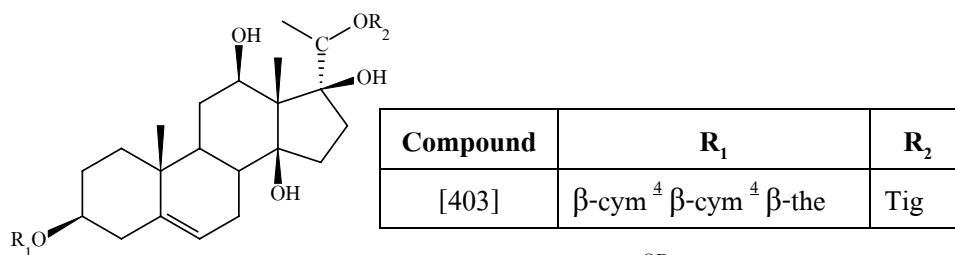
Compound	R
[386]	$\beta$ -xyl <sup>2</sup> $\beta$ -xyl



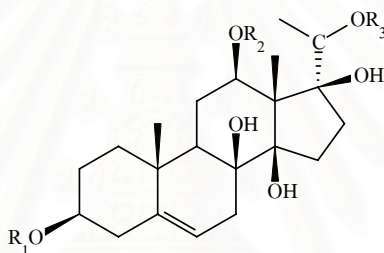
Compounds	R
[400]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all
[401]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the
[402]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3-O-methyl)-all <sup>4</sup> $\beta$ -cym

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)

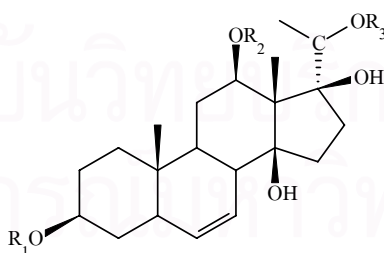




Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[407]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Nic	Cin

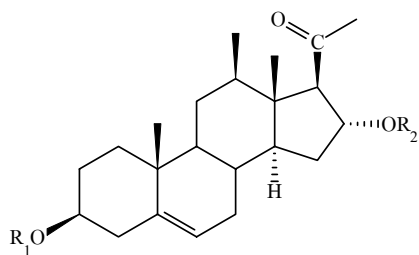


Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[408]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the	Tig	Ac
[409]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the	Tig	Ac
[411]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	H	H

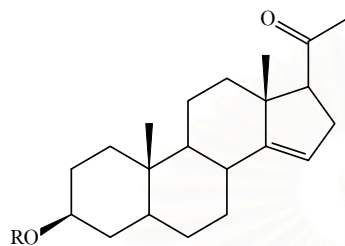


Compounds	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
[412]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -(6-deoxy-3- <i>O</i> -methyl)-all	Cin	Tig
[413]	$\beta$ -cym <sup>4</sup> $\beta$ -cym <sup>4</sup> $\beta$ -the	Cin	Ac

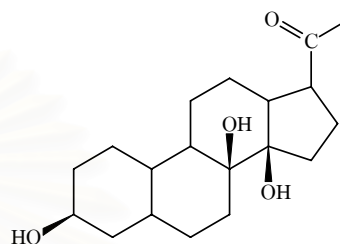
Figure 2. Pregnane steroids from asclepiadaceae plants (continued)



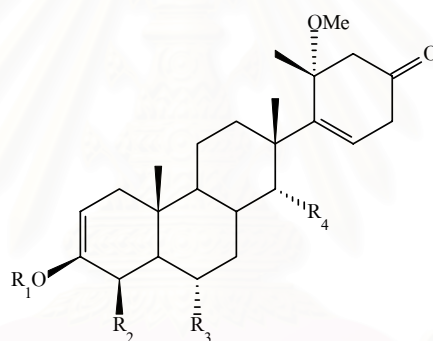
Compound	$R_1$	$R_2$
[414]	$\beta$ -cym <sup>4</sup> $\beta$ -digto	$\beta$ -glc



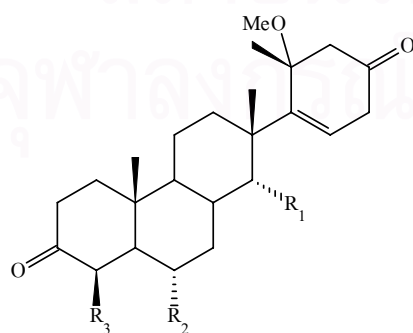
[415]



[416]

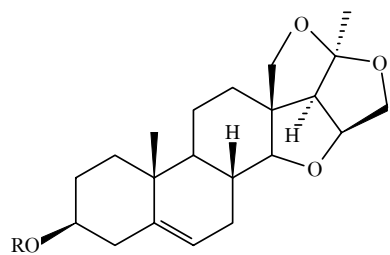


Compounds	R	$R_1$	$R_2$	$R_3$
[417]	$\beta$ -digto <sup>4</sup> $\beta$ -aco <sup>4</sup> $\beta$ -glc	H	H	H
[418]	$\beta$ -digto <sup>4</sup> $\beta$ -aco <sup>4</sup> $\beta$ -glc	OAc	OAc	OAc

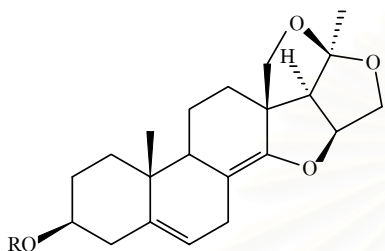


Compound	$R_1$	$R_2$	$R_3$
[419]	OAc	OAc	OAc

Figure 2. Pregnane steroids from asclepiadaceous plants (continued)



Compound	R
[424]	$\alpha$ -ole <sup>4</sup> $\beta$ -digto <sup>4</sup> $\beta$ -ole
[427]	H



Compounds	R
[425]	H
[426]	$\beta$ -(2,6-dideoxy-3-O-methyl)-glc

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Figure 2. Pregnane structures from Asclepiadaceous plants (continued)

### Chemical constituents of *Cryptolepis* species

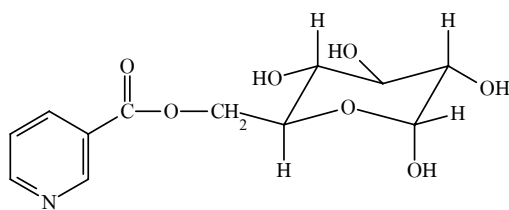
Only three *Cryptolepis* species have been phytochemically studied. The roots of *Cryptolepis sanguinolenta* were found to be rich in bioactive indole alkaloids (Paulo *et al.*, 2000a). This type of alkaloids is very common in the closely related Apocynaceae family but is seldom found in Asclepiadaceae. Alkaloids are rarely found in this family, the phenanthroindolizidines and phenanthroquinolizidines alkaloids were found in the genera *Tylophora* and *Cynanchum*. The steroidal alkaloids isolated from *C. obtusa* are of the pregnane type similar to those identified in Apocynaceae (Paulo *et al.*, 2000b). The main secondary metabolites previously found in the leaves and roots of *C. buchanani* are cardenolides and pseudo-alkaloids (nicotinoyl glycosides) (Dutta *et al.*, 1980; Purushothaman *et al.*, 1988)

**Table2. Chemical constituents of *Cryptolepis* species**

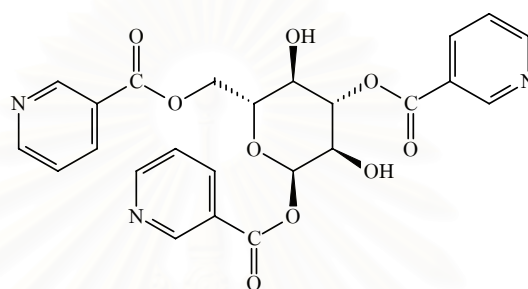
Plant source	Plant part	Chemical type / Chemical compound	Reference
<i>Cryptolepis buchanani</i>	Stem	<b>Alkaloids</b> Buchananine [428]	Dutta, Sharma and Sharma, 1978
	Stem	1, 3, 6- <i>O</i> -trinicotinoyl - $\alpha$ -D-glucopyranose [429]	Dutta, Sharma and Sharma, 1980
	Leaves	<b>Cardenolides</b> Cryposin [430]	Venkateswara, Rao and Vaidyanathan, 1987
	Leaves	Sarmentogenin [431]	Shah and Khare, 1981
	Leaves	Samentocymarin [432]	Purushothaman <i>et al.</i> , 1988
	Leaves	Cryptanoside A [433]	Purushothaman <i>et al.</i> , 1988
	Leaves	Cryptanoside B [434]	Purushothaman <i>et al.</i> , 1988
	Leaves	Cryptanoside C [435]	Purushothaman <i>et al.</i> , 1988
	Leaves	Cryptanoside D [436]	Purushothaman <i>et al.</i> , 1988
	Seed	<b>Fatty acid</b> 9-Oxo-cis-12- octadecenoic acid [437]	Daulatabad <i>et al.</i> , 1992

**Table2. Chemical constituents of *Cryptolepis* species (continued)**

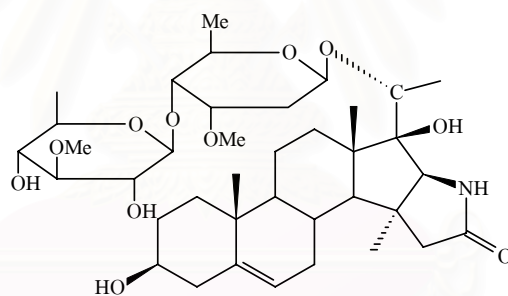
<b>Plant source</b>	<b>Plant part</b>	<b>Chemical type / Chemical compound</b>	<b>Reference</b>
<i>C. obtusa</i>	Root	<b>Alkaloids</b> Obtusine [438]	Paulo <i>et al.</i> , 2000b
	Root	Obtusolactam [439]	Paulo <i>et al.</i> , 2000b
	Root	<b>Steroids</b> $\beta$ -Sitosteryl-3-O- $\beta$ - glucopyranoside [440]	Paulo <i>et al.</i> , 2000b
	Root	<b>Flavonoids</b> Quercetin [441]	Paulo <i>et al.</i> , 1997
	Root	Isoquercetrin [442]	Paulo <i>et al.</i> , 1997
	Root	Rutin [443]	Paulo <i>et al.</i> , 1997
	<i>C. sanguinolenta</i>	Root	<b>Alkaloids</b> Cryptolepine [444]
Root		Quindoline [445]	Dwuma-Badu <i>et al.</i> , 1978
Root		Hydroxycryptolepine [446]	Paulo, Gomes and Houghton, 1995
Root		Cryptoheptine [447]	Paulo, Gomes and Houghton, 1995
Root		Cryptoquindoline [448]	Pousset <i>et al.</i> , 1995
Root		Isocryptolepine [449]	Cimanga <i>et al.</i> , 1996
Root		Biscryptolepine [450]	Cimanga <i>et al.</i> , 1996
Root		Neocryptolepine [451]	Tackie <i>et al.</i> , 1993
Root		Cryptospirolepine [452]	Tackie <i>et al.</i> , 1993



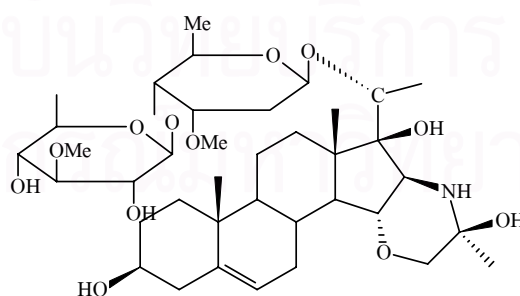
[428]



[429]



[438]



[439]

Figure 3. Chemical structures of plants in *Cryptolepis* species

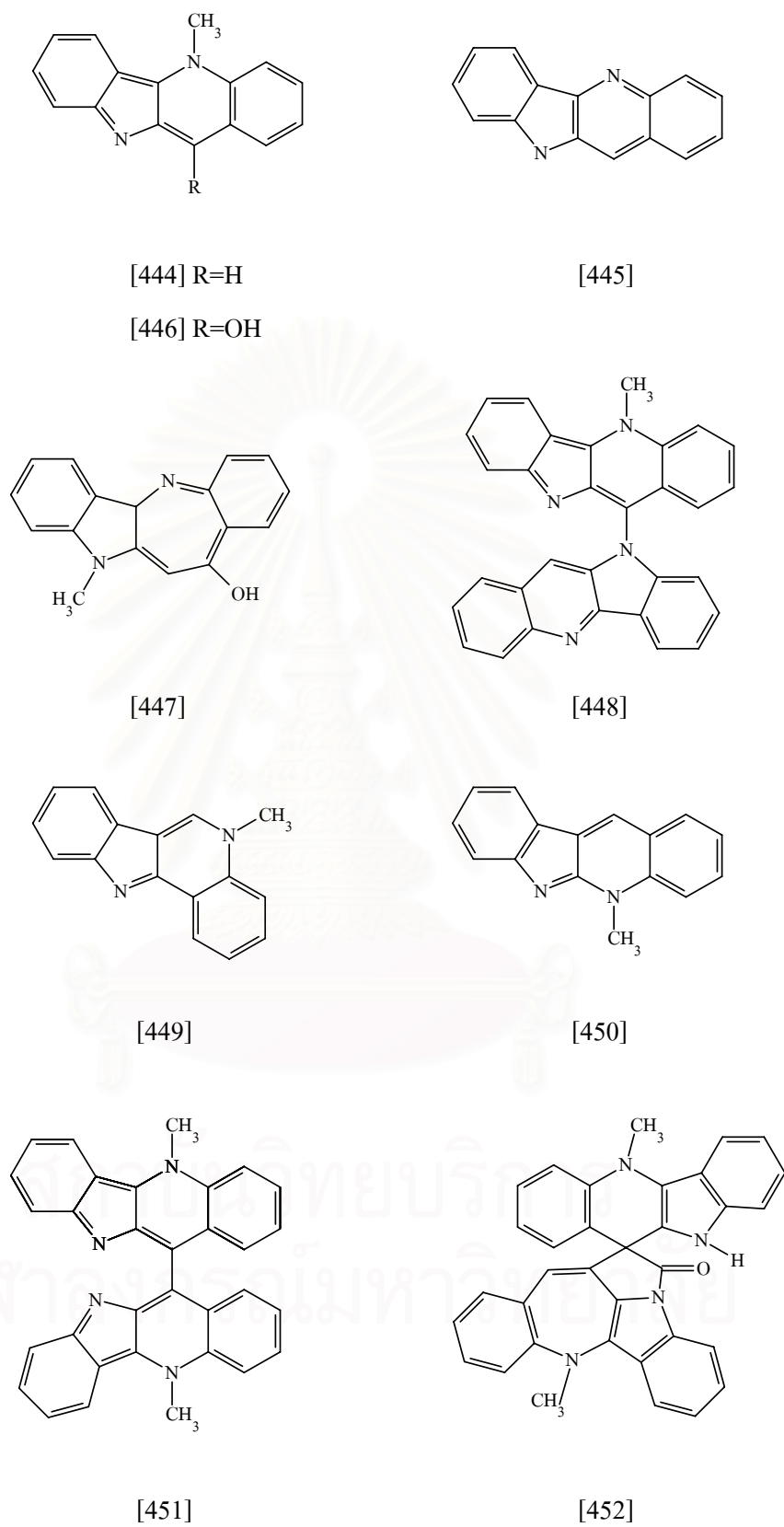
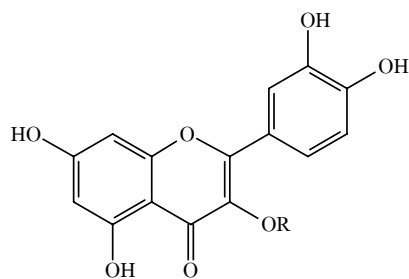


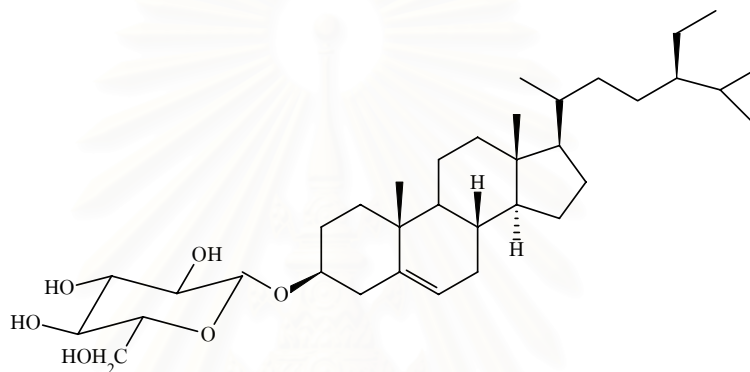
Figure 3. Chemical structures of plants in *Cryptolepis* species (continued)



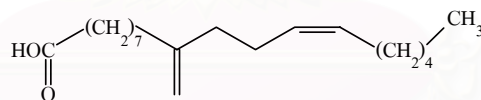
[441], R= H

[442], R= Glucose

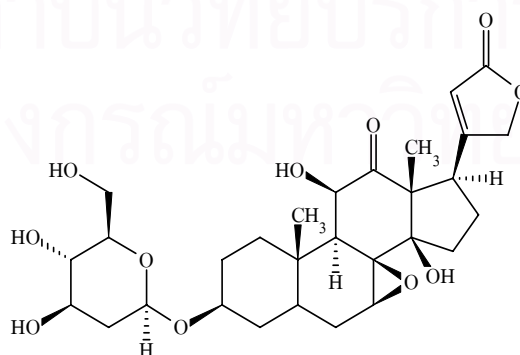
[443], R= Rutinose



[436]



[437]



Cryptosin [430]

Figure 3. Chemical structures of plants in *Cryptolepis* species (continued)



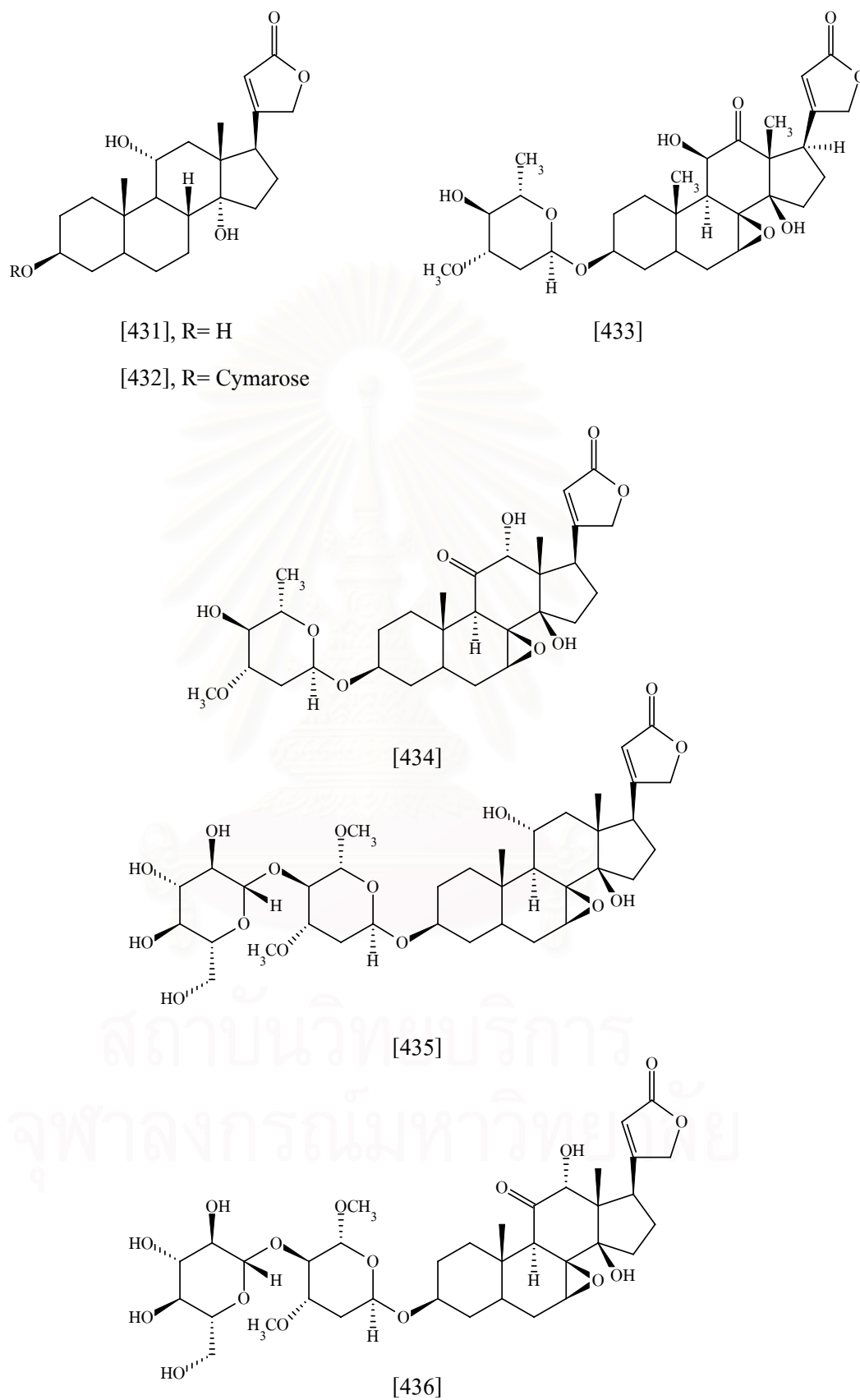


Figure 3. Chemical structures of plants in *Cryptolepis* species (continued)

### **Ethnomedicinal Uses of *Cryptolepis* species**

Plants of the genus *Cryptolepis* have been known for their uses in traditional medicine of several countries. Ethnomedicinal uses of these plants are as follows.

The stem of *Cryptolepis buchanani* is used in Thai folk medicine. The alcoholic extract of the stem of this plant is commonly used for the treatment of inflammatory conditions such as arthritis muscle joint and backpain, strain and sprain of tendon and muscle. The leaves and seeds are used as remedies for neuralgic pain and dyspepsia, respectively. (เส็งจ๋ยม พงษ์บุญรอด, 2519; สายสนม กิตติขจร, 2526; Panthong *et al.*, 1986). The plant is also used in herbal mixtures for tendon, muscle and blood tonic and to normalize menstruation, as a treatment of headache and paresis (วงศ์สถิตย์ นั้วกุล และคณะ, 2548)

In China, the root and fruits of *C. buchanani* are used for the treatment of fever and edema (Wu and Raven, 1995). In India, the plant is used in a preparation given to children to cure rickets. Decoctions of its stem are used by some rural people as a cure for paralysis. (Dutta, Sharma and Sharma, 1978). Its uses for the treatment of chronic rheumatism, dyspepsia, respiratory diseases dysuria, dysentery, leucorrhoea and uterine haemorrhage have also been reported (Khare, 2004).

The roots of *Cryptolepis sanguinolenta* is used in traditional Central and West African medicine to treat several infectious diseases. In Guinea Bissau the dried root, known as “Cuntesse”, is sold in the local market of Bandim and its decoction is used in the treatment of various fevers, hepatitis and jaundice. The leaves have been used in the treatment of malaria or powdered as a cicatrizant of wounds . In Ghana and Nigeria, the root has been used in the clinical therapy of malaria, rheumatism and urinary and upper respiratory tract infections. (Boye and Oku-Ampofo, 1990; Tackie *et al.*, 1993; Paulo, Duarte and Gomes, 1994; Paulo, Gomes and Houghton, 1995; Silva *et al.*, 1995; Olajide *et al.*, 2007).

The aqueous extracts of *C. obtusa* roots are used in Mozambique as an anti-abortive , vermifuge and to treat abdominal pains (Paulo *et al.*, 2000b).

### Biological Activities of *Cryptolepis* Species

Ethnopharmacological and chemical studies on *Cryptolepis* plants are as yet not advanced enough to ascertain whether their various folk medicinal uses are supported by the pharmacological activity of the constituents. Two *Cryptolepis* species have been investigated pharmacologically and the results exhibited interesting bioactivities of their components (extracts/isolated compounds).

The biological activities of extracts and isolated compounds from *Cryptolepis* species are summarized in Table 3.

**Table 3. Biological Activities of *Cryptolepis* Species**

Plants	Extract/Isolated compound	Activity	Reference
<i>Cryptolepis buchanani</i>	Cryptosin [430]	Cardiotonic	Venkateswara, Sankara Rao and Vaidyanathan, 1987
	Cryptanoside A [433]	Antibacterial	Purushothaman <i>et al.</i> , 1988
	Cryptanoside C [435]	Antibacterial	Purushothaman <i>et al.</i> , 1988
	Methanol extract	Muscle relaxant	Ikegami <i>et al.</i> , 1990
	Ethanol extract	Immunomodulating	Kaul <i>et al.</i> , 2003
		Anti-inflammatory	Laupattarakasem, <i>et al.</i> , 2006
		Antibacterial	Vasanth, Gopal and Roa., 1997
	Chloroform extract	Antibacterial	Vasanth, Gopal and Roa., 1997
<i>C. sanguinolenta</i>	Cryptolepine [444]	Hypotensive	Paulo <i>et al.</i> , 1994
		Antimalarial	Noamesi <i>et al.</i> , 1991; Kirby <i>et al.</i> , 1995; Paulo <i>et al.</i> , 2000a; Lisgartent <i>et al.</i> , 2002

Table 3. Biological Activities of *Cryptolepis* Species (continued)

Plants	Extract/Isolated compound	Activity	Reference
<i>C. sanguinolenta</i>	Cryptolepine [444]	Antibacterial	Cimanga <i>et al.</i> , 1991; Paulo, Duarte and Gomes, 1994
		Antifungal	Mardenborough <i>et al.</i> , 1999
		Anti-inflammatory	Bamgbose and Noamesi, 1981; Olajide <i>et al.</i> ,2007
		Antidiarrhoeal	Paulo <i>et al.</i> , 1994
		Antipyretic	Paulo <i>et al.</i> , 1994
		Antihyperglycemic	Bierer <i>et al.</i> , 1998
		Anticonvulsant	Banerji <i>et al.</i> , 2005
		Genotoxicity (Hamster lung fibroblast cell line)	Ansah, Khan and Gooderham, 2005
		Cytotoxicity (melanoma cells) (leukemia cells)	Bonjean <i>et al.</i> , 1998; Dassonneville <i>et al.</i> , 2000
		Antimuscarinic	Rauwald <i>et al.</i> , 1992
	Antithrombotic	Oyekan, Btting and Noamesi, 1988	
	Cryptoheptine [447]	Antimalarial	Paulo <i>et al.</i> , 2000a
		Antibacterial	Paulo, Duarte and Gomes, 1994
	Cryptoquidoline [448]	Antibacterial	Cimanga <i>et al.</i> , 1998
Neocryptolepine [451]	Antibacterial	Cimanga <i>et al.</i> , 1998	
	Cytotoxicity (leukemia cells)	Dassonneville <i>et al.</i> , 2000	

**Table 3. Biological Activities of *Cryptolepis* Species (continued)**

<b>Plants</b>	<b>Extract/Isolated compound</b>	<b>Activity</b>	<b>Reference</b>
<i>C. sanguinolenta</i>	Neocryptolepine [451]	Antimalarial	Van <i>et al.</i> 2005
	Hydroxycryptolepine [446]	Antioxidant	Cimanga <i>et al.</i> 2000
	Biscryptolepine [450]	Antibacterial	Cimanga <i>et al.</i> , 1998
	Ethanol extract	Antimicrobial	Silva <i>et al.</i> , 1995



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## CHAPTER III

### EXPERIMENTAL

#### Source of Plant Material

The stem of *Cryptolepis buchanani* used in this study was collected from Phuvour wildlife sanctuary, Amphoe Bung Khla, Nongkhai province, Thailand on September 28, 2006. A voucher specimen no. 150913 was deposited in the herbarium of Royal Forest Department, Bangkok, Thailand.

#### General Techniques

##### 1. Chromatographic Technique

###### 1.1 Analytical Thin-Layer Chromatography (TLC)

Technique	: One dimension, ascending
Stationary phase	: Silica gel 60 F <sub>254</sub> (E. Merck) precoated plate
Layer thickness	: 0.2 mm
Solvent system	: Various solvent systems depending on materials
Distance	: 5 cm
Temperature	: Laboratory temperature 30-35 °C
Detection	: 1) UV light at the wavelengths of 254 and 365 nm : 2) 10% sulfuric acid in ethanol, heating at 110 °C

###### 1.2 Column Chromatography (CC)

Column	: Flat bottom glass column (various diameters)
Absorbent	: 1) Silica gel 60 (No. 7734, E. Merck) particle size 0.063-0.200 mm (70-230 mesh ASTM) : 2) Silica gel 60 (No. 9385, E. Merck) particle size 0.040-0.063 mm (230-400 mesh ASTM)
Packing method	: Wet loading
Sample loading	: 1) Dry packing

The sample was dissolved in a small volume of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then loaded on the top of the column.

: 2) Wet loading

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

Solvent system : Various solvent systems depending on materials

### 1.3 Gel Filtration Chromatography

Stationary phase : Sephadex<sup>TM</sup> LH-20

Packing method : Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample loading : The sample was dissolved in a small volume of the eluent and applied on top of the column.

Solvent system : Methanol 100 %  
Methanol: Chloroform (1: 1)

### 1.4 Preparative Thin Layer Chromatography (PTLC)

Stationary phase : Kieselgel 60 F<sub>254</sub>

Layer thickness : 1 mm.

Distance : 15 cm

Temperature : Laboratory temperature 30-35 °C

Detection : UV light at the wavelengths of 254 and 365 nm

Solvent : Hexane: Chloroform (3: 1)

## 2. Recrystallization Technique

The compounds were recrystallized from their less soluble single solvents or mixtures. Each compound was dissolved completely in selected solvent until saturated and let standing at room temperature until amorphous powder or crystals were formed.

## 3. Spectroscopy

### 3.1 Ultraviolet (UV) Absorption Spectra

UV spectra (in chloroform and methanol) were obtained on a Shimadzu UV-160A spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University)

### 3.2 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and thin film) were obtained on a Perkin Elmer Infrared Spectrophotometer Model 1760X (Scientific and Technological Research Equipment Center, Chulalongkorn University)

### 3.3 Mass Spectra (MS)

Electrospray ionization Time of Flight (ESI-TOF) mass spectra were recorded on a Micromass LCT mass spectrometer (National Center for Genetic Engineering and Biotechnology, Thailand).

### 3.4 Proton and Carbon 13 Nuclear Magnetic Resonance ( $^1\text{H}$ and $^{13}\text{C}$ - NMR) Spectra

The  $^1\text{H}$ -NMR (300 MHz) and  $^{13}\text{C}$ -NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

The  $^1\text{H}$ -NMR (500 MHz) and  $^{13}\text{C}$ -NMR (125 MHz) spectra were obtained with a JEOL JMN-A 500 spectrometer, Varian <sup>unity</sup>INOVA NMR spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University) and Bruker-AV 500 MHz spectrometer (National Center for Genetic Engineering and Biotechnology, Thailand).

NMR solvents used in this study were deuterated chloroform ( $\text{CDCl}_3$ ) and deuterated acetone ( $\text{acetone-}d_6$ ). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

## 4. Physical Properties

### 4.1 Melting Points

Melting points were obtained on a Fisher/Johns melting point apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

### 4.2 Optical Rotation

Optical rotation were measured on a Perkin-Elmer Polarimeter model 341 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

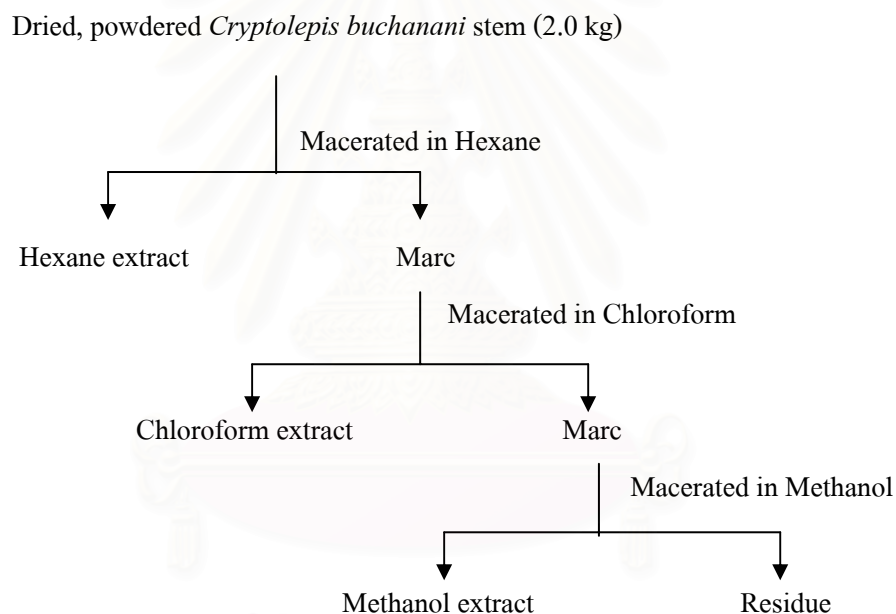
## 5. Solvents

Throughout this work, all organic solvents used in the extraction and isolation procedures were of commercial grade and were redistilled prior to use.



## Extraction

The dried, powdered stem of *Cryptolepis buchanani* (2.0 kg) was repeatedly macerated by with hexane (5 x 8L, 3 days each) at room temperature. Each combined hexane extract was filtered, evaporated under reduced pressure and to give the hexane extract 35.27 g: 1.76 % based on dried weight of the stem. The remaining marc was air-dried and consequently extracted with chloroform (5 x 8L, 3 days each) and methanol (5 x 8L, 3 days each) in the same manner to give, on evaporation, chloroform extract 23.59 g: 1.18 % based on dried weight of the stem and methanol extract 32.00 g: 1.60 % based on dried weight of the stem, respectively. These extracts were subjected to column chromatography for the further separation and purification.



**Scheme 1. Extraction of *Cryptolepis buchanani* stem.**

## Isolation

### 1. The Hexane extract of *C. buchanani* stem.

The hexane extract (10.0 g) was subjected to a silica gel column (300 g, 10 × 20 cm) eluted with hexane-chloroform (3 : 1) to give 130 fractions of 30 ml each. The fractions were combined according to their TLC pattern into 10 major fractions (H01-H10), as shown in Table 4. Finally, the column was washed down with methanol to give fraction H11.

**Table 4. Combined fractions from the hexane extract**

<b>Fraction</b>	<b>Number of eluates</b>	<b>Weight (g)</b>
H01	1-13	0.62
H02	14-27	0.98
H03	28-37	0.64
H04	38-45	0.30
H05	46-53	0.27
H06	54-69	0.59
H07	70-83	0.55
H08	84-104	0.92
H09	105-114	0.47
H10	115-130	0.75
H11	methanol eluted	2.38

### 1.1 Isolation of compound CB01

Fraction H08 (0.92 g) was subjected to silica gel column chromatography (30 g, 2x30 cm) eluted with hexane-acetone (4:1) to give 87 fraction of 20 ml each. The fractions were combined according to their TLC pattern into 6 major fractions (H081-H086), as shown in Table 5. Finally, the column was washed down with methanol to give fraction H087.

**Table 5. Combined fractions from the fraction H08**

<b>Fraction</b>	<b>Number of eluates</b>	<b>Weight (mg)</b>
H081	1-7	32.5
H082	8-13	22.3
H083	14-25	57.9
H084	26-31	102.2
H085	32-43	115.7
H086	44-45	134.5
H087	methanol eluted	239.4

Fraction H085, which displayed one major pink-violet spot on TLC upon detection with 10% H<sub>2</sub>SO<sub>4</sub>, was recrystallized in methanol to give 104.2 mg (0.018 % yield) of component CB01 as colorless needles. The fractionation of hexane extract is summarized in Scheme 2.

## 2. The Chloroform extract of *C. buchanani* stem.

The chloroform extract (20 g) was subjected to silica gel column chromatography (600 g, 10x23.5 cm) eluted with chloroform-methanol mixture of increasing polarity (from 97:3 to 1:9) to give 230 fractions of 30 ml each. The fractions were combined according to their TLC pattern into 6 major fractions (C01-C06), as shown in Table 6. Finally, the column was washed down with methanol to give fraction C07.

**Table 6. Combined fractions from the chloroform extract**

Fraction	Number of eluates	Weight (g)
C01	1-32	1.03
C02	33-60	1.19
C03	61-109	3.15
C04	110-138	1.41
C05	139-189	3.77
C06	190-230	3.93
C07	methanol eluted	4.01

### 2.1 Isolation of Compound C02

Fraction C02 (1.19 g) was subjected to silica gel column chromatography (40 g, 5x40 cm) eluted with hexane-chloroform (3:2) to give 45 fractions of 20 ml each. The fractions were combined from their TLC pattern into 6 major fractions, as shown in Table 8. The column was then washed down with methanol to give fraction fraction C027.

**Table 7. Combined fractions from the fraction C02**

Fraction	Number of eluates	Weight (mg)
C021	1-9	66.1
C022	10-14	76.2
C023	15-21	85.4

**Table 7. Combined fractions from the fraction C02**

<b>Fraction</b>	<b>Number of eluates</b>	<b>Weight (mg)</b>
C024	22-29	161.7
C025	30-36	104.1
C026	37-45	213.7
C027	methanol eluted	398.7

Fraction C023 (85.4 mg) displayed one major yellow-orange spot on TLC plate detection under UV light. It was further purified on a Sephadex LH 20 column (1x80 cm), using methanol as the eluent to give 11.9 mg (0.00070 % yield) of compound CB02 as yellow-orange crystals.

## 2.2 Isolation of compounds CB03 and CB04

Fraction C05 (3.77 g) was further chromatographed on a silica gel column (120 g, 5x40 cm) eluted with chloroform-ethyl acetate (9:1) to give 130 fractions of 30 ml each. The fractions were then combined into 5 major fractions (C051-C055), as shown in Table 7.

**Table 8. Combined fractions from the fraction C05**

<b>Fraction</b>	<b>Number of eluates</b>	<b>Weight (g)</b>
C051	1-48	0.77
C052	49-64	0.75
C053	65-79	0.53
C054	80-101	0.64
C055	102-130	0.91

Fraction C054 (0.64 g) showed interesting spots on TLC. It was subjected to Sephadex LH-20 gel filtration (2x120 cm) using methanol as the eluent. Twenty five fractions (5 ml each) were collected and monitored by silica gel TLC with chloroform-ethyl acetate (9:1) as the mobile phase. Those fraction of similar TLC pattern were combined into 3 major ones, as shown in Table 9.

**Table 9. Combined fractions from the fraction C054**

Fraction	Number of elutes	Weight (mg)
C0541	1-9	185.27
C0542	10-18	103.11
C0543	19-25	311.20

Fraction C0542 (103.11 mg) displayed two major spots ( $R_f = 0.40$  and  $0.38$ ) on TLC plates when detected under UV light. The two major bands were separate by preparative TLC, using hexane-acetone (3:1) as the solvent to give 13.0 mg (0.00076 % yield) and 16.0 mg (0.00094 % yield) of compound CB03 and CB04, respectively.

### 2.3 Isolation of compounds CB05 and CB06

Fraction CB053 (0.53 g), was subjected to silica gel column chromatography (20 g, 2x30 cm) eluted with hexane-acetone (3:1) to give 60 fractions of 20 ml each. The fractions were combined according to their similar TLC patterns into 4 major fractions (CB0531-CB0534), as shown in Table 10.

**Table 10. Combined fractions from the fraction C053**

Fraction	Number of elutes	Weight (mg)
C0531	1-13	97.3
C0532	14-28	66.4
C0533	19-47	67.8
C0534	48-60	185.2

Fraction CB0533 (0.53 g) displaying two major spots on TLC plate when detected under UV light. It was further separated by Sephadex LH 20 column (2x60 cm), using methanol as the eluent to give 6.5 mg (0.00038 % yield) of compound CB05 as pale yellow needles and 12.7 mg (0.00074 % yield) of compound CB06 as yellow needles.

The fractionation of hexane extract is summarized in Scheme 3.

## Characterization of isolated compounds

### 1. Component CB01

Appearance	: colorless needles
Solubility	: Soluble in chloroform, hexane
$^1\text{H-NMR}$ ( $\delta$ ppm, 300 MHz, $\text{CDCl}_3$ ) :	
	0.68 (3H, <i>s</i> ), 0.78 (3H, <i>s</i> ), 0.80 (3H, <i>s</i> ), 0.85 (3H, <i>m</i> ), 0.92 (3H, <i>m</i> ), 1.00 (3H, <i>m</i> ), 3.50 (1H, <i>m</i> ), 5.12 (1H, <i>dd</i> ), 5.10 (1H, <i>dd</i> ), 5.33 (1H, <i>d</i> , $J = 4.5$ Hz) (Figure 4)
$^{13}\text{C-NMR}$ ( $\delta$ ppm, 75 MHz, $\text{CDCl}_3$ )	
	11.9, 12.0, 12.2, 18.8, 19.0, 19.4, 19.8, 21.1, 23.1, 24.3, 26.1, 28.2, 28.9, 31.7, 31.9, 34.0, 36.2, 36.5, 37.2, 39.7, 39.8, 40.5, 42.2, 42.3, 45.9, 50.2, 51.2, 56.0, 56.1, 56.8, 56.9, 71.8, 121.7, 129.3, 138.2 and 140.7 (Figure 5-6)

### 2. Compound CB02

Appearance	: yellow-orange needles
Solubility	: Soluble in acetone, chloroform, ethyl acetate
Melting point	: 188-190 °C
UV $\lambda_{\text{max}}$ nm (log $\epsilon$ ), in $\text{CHCl}_3$	: 227 (4.52), 253 (4.41), 285 (4.15), 430 (4.14) (Figure 8)
IR $\nu_{\text{max}}$ (KBr disc) $\text{cm}^{-1}$	: 3434, 1626, 1470, 1376, 1282, 1209, 1159, 839, 745 (Figure 9)
HR ESI TOFMS( $m/z$ )	: $[\text{M} + \text{H}]^+$ 241.0505 (Figure 10)
$^1\text{H-NMR}$ ( $\delta$ ppm, 500 MHz, $\text{CDCl}_3$ )	
	7.29 (1H, <i>dd</i> , $J = 8.5, 1.0$ Hz), 7.67 (1H, <i>t</i> , $J = 8.0$ Hz), 7.82 (1H, <i>dd</i> , $J = 7.5, 1.0$ Hz), 12.05 (OH, <i>s</i> ) (Figure 11)
$^{13}\text{C-NMR}$ ( $\delta$ ppm, 125 MHz, $\text{CDCl}_3$ )	
	115.8, 120.0, 124.6, 133.6, 137.3, 162.5, 182.7, 193.1 (Figure 12)

### 3. Compound CB03

Appearance	: colorless needles
Solubility	: Soluble in acetone, chloroform, ethyl acetate
Melting Point	: 171-172 °C
$[\alpha]_{\text{D}}^{25}$	: + 85 ° (C= 0.02 in $\text{CHCl}_3$ )
UV $\lambda_{\text{max}}$ nm (log $\epsilon$ ), in MeOH	: 241 (4.28) (Figure 17)

IR  $\nu_{\max}$  (KBr disc)  $\text{cm}^{-1}$  : 3475, 2937, 2914, 1694, 1661, 1609, 1450,  
1387, 1222, 1237, 1070, 1087, 905 (Figure 18)

ESI TOFMS ( $m/z$ ) :  $[\text{M} + \text{Na}]^+$  369 (Figure 19)

$^1\text{H-NMR}$  ( $\delta$  ppm, 500 MHz, acetone- $d_6$ )

0.67 (1H, *s*), 1.05 (1H, *m*), 1.08 (1H, *m*), 1.24 (1H, *m*), 1.30 (1H, *m*), 1.33 (1H, *s*),  
1.45 (1H, *m*), 1.51 (1H, *dd*,  $J = 13.0, 4.0$  Hz), 1.48 (1H, *m*), 1.65 (1H, *m*), 1.66 (1H,  
*dd*,  $J = 7, 3.5$  Hz), 1.73 (1H, *m*), 1.75 (1H, *m*), 1.88 (1H, *m*), 1.95 (1H, *m*), 2.18 (1H,  
*m*), 2.27 (1H, *dd*,  $J = 13.0, 5.0$  Hz), 2.32 (1H, *ddd*,  $J = 14.5, 9.5, 2.5$  Hz), 2.46 (1H,  
*tdd*,  $J = 14.5, 5.5, 1.5$  Hz), 2.63 (1H, *t*,  $J = 9.0$  Hz), 3.68, (1H, *t*,  $J = 5.0$  Hz), 3.92 (1H,  
*d*,  $J = 3.0$  Hz), 4.15 (1H, *d*,  $J = 5.0$  Hz), 4.21 (1H, *ddd*,  $J = 13.0, 5.0, 3.0$  Hz), 5.70  
(1H, *d*,  $J = 1.5$  Hz) (Figure 20)

$^{13}\text{C-NMR}$  ( $\delta$  ppm, 125 MHz, acetone- $d_6$ )

13.7, 18.2, 21.5, 23.4, 24.9, 32.7, 32.9, 35.7, 38.9, 41.1, 44.9, 45.3, 55.1, 56.6, 59.1,  
69.9, 70.0, 121.2, 172.4, 200.0, 210.9 (Figure 21)

#### 4. Compound CB04

Appearance : colorless needles

Solubility : Soluble in acetone, chloroform, ethyl acetate

Melting point : 181-182 °C

$[\alpha]_{\text{D}}^{25}$  : + 225 ° (C = 0.02 in  $\text{CHCl}_3$ )

UV  $\lambda_{\max}$  nm (log  $\epsilon$ ), in MeOH : 283 (3.49) (Figure 32)

IR  $\nu_{\max}$  (KBr disc)  $\text{cm}^{-1}$  : 3468, 2941, 2926, 2972, 2860, 2876, 1696,  
1650, 1616, 1449, 1388, 1242, 1087, 1067,  
903 (Figure 33)

ESI TOFMS ( $m/z$ ) :  $[\text{M} + \text{Na}]^+$  367 (Figure 34)

$^1\text{H-NMR}$  ( $\delta$  ppm, 500 MHz, acetone- $d_6$ )

0.72 (1H, *s*), 1.23 (1H, *s*), 1.30 (1H, *m*), 1.45 (1H, *m*), 1.50 (1H, *m*), 1.53 (1H, *m*),  
1.56 (1H, *t*,  $J = 12.5$  Hz), 1.58 (1H, *m*), 1.65 (1H, *m*), 1.96 (1H, *m*), 2.02 (1H, *dd*,  $J$   
 $= 8.5, 2.5$  Hz), 2.23 (1H, *t*,  $J = 9$  Hz), 2.28 (1H, *m*), 2.30 (1H, *m*), 2.7 (1H, *t*,  $J = 9$   
Hz), 3.69 (1H, *t*,  $J = 5$  Hz), 3.97 (1H, *dd*,  $J = 5$  Hz), 4.17 (1H, *d*,  $J = 5.5$  Hz), 4.36  
(1H, *ddd*,  $J = 13.5, 5.5, 2.6$  Hz), 5.69 (1H, *s*), 6.18 (1H, *dd*,  $J = 9.5, 2$  Hz), 6.23 (1H,  
*dd*,  $J = 9.5, 2.5$  Hz) (Figure 35)

$^{13}\text{C-NMR}$  ( $\delta$  ppm, 125 MHz, acetone- $d_6$ )

13.6, 17.3, 21.3, 23.0, 24.5, 37.9, 38.7, 38.8, 43.7, 45.8, 51.7, 54.3, 58.9, 69.9, 70.3, 121.8, 128.2, 141.7, 165.0, 199.9, 210.8 (Figure 36)

## 5. Compound CB05

Appearance : pale-yellow needles

Solubility : Soluble in acetone, chloroform, ethyl acetate

Melting point : 129-130 °C

$[\alpha]_D^{25}$  : + 70° (C= 0.02 in CHCl<sub>3</sub>)

UV  $\lambda_{\max}$  nm (log  $\epsilon$ ), in MeOH : 224 (4.32), 284 (4.26) (Figure 48)

IR  $\nu_{\max}$  (KBr disc) cm<sup>-1</sup> : 3444, 3259, 2965, 2940, 2911, 2865, 1715, 1615, 1631, 1422, 1218, 1189, 1067, 882 (Figure 49)

HR ESI TOFMS( *m/z*) : [M+ Na]<sup>+</sup> 365 (Figure 50)

<sup>1</sup>H-NMR ( $\delta$  ppm, 500 MHz, CHCl<sub>3</sub>)  
 0.77 (3H, *s*), 1.25 (3H, *s*), 1.40 (1H, *m*), 1.40 (1H, *m*), 1.43 (1H, *m*), 1.55 (1H, *m*), 1.65 (1H, *m*), 1.85 (1H, *m*), 1.90 (1H, *m*), 1.95 (1H, *m*), 2.02 (1H, *m*), 2.28 (1H, *m*), 2.30 (1H, *m*), 2.52 (1H, *t*, *J* = 9.3 Hz), 3.26 (1H, *br s*), 4.20 (1H, *d*, *J* = 5.8 Hz), 4.21 (1H, *d*, *J* = 5.8 Hz), 6.09 (1H, *dd*, *J* = 9.7, 1.6 Hz), 6.27, (1H, *s*), 6.13 (1H, *s*), 6.30 (1H, *dd*, *J* = 9.9, 2.9 Hz), 6.44 (1H, *br s*) (Figure 51)

<sup>13</sup>C-NMR ( $\delta$  ppm, 125 MHz, CDCl<sub>3</sub>)  
 13.5, 21.7, 22.1, 23.1, 23.9, 38.1, 38.2, 41.5, 45.0, 49.4, 53.9, 58.7, 69.5, 120.8, 121.2, 127.7, 138.7, 146.7, 165.7, 181.4, 209.9 (Figure 52)

## 6. Compound CB06

Appearance : yellow needles

Solubility : Soluble in acetone, chloroform, ethyl acetate, methanol

Melting point : 200 °C

UV  $\lambda_{\max}$  nm (log  $\epsilon$ ), in CHCl<sub>3</sub> : 345 (4.45), 298 (4.25), 252 (3.78) (Figure 61)

IR  $\nu_{\max}$  (KBr disc) cm<sup>-1</sup> : 3337, 1703, 1608, 1566, 1509, 1290, 1139, 1018, 922, 862, 591 (Figure 62)

ESI TOFMS ( *m/z*) : [M+ H]<sup>+</sup> 193 (Figure 63)

<sup>1</sup>H-NMR ( $\delta$  ppm, 300 MHz, CDCl<sub>3</sub>)



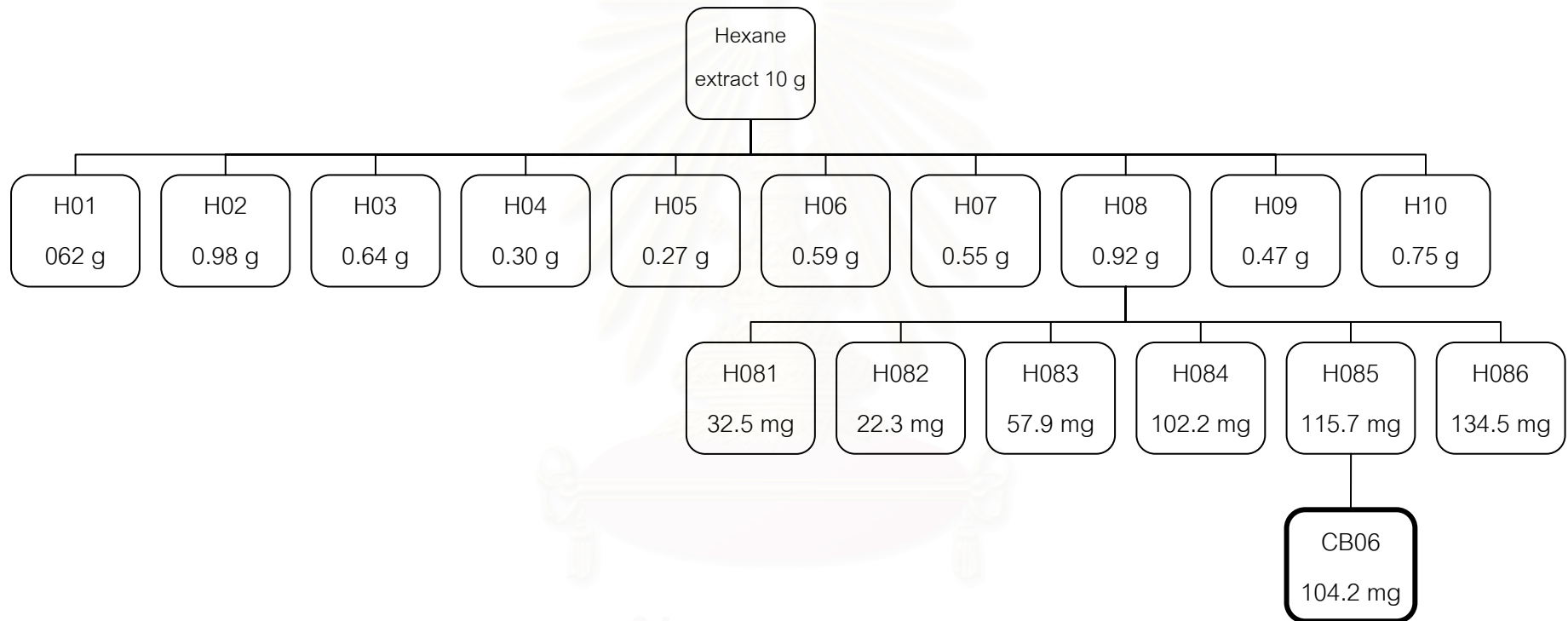
3.93 (3H, *s*), 6.16 (1H, *br s*), 6.24 (1H, *d*,  $J = 9.5$  Hz), 6.82 (1H, *s*), 6.89 (1H, *s*),  
7.57 (1H, *d*,  $J = 9.5$  Hz) (Figure 64)

$^{13}\text{C}$ -NMR ( $\delta$  ppm, 75 MHz,  $\text{CDCl}_3$ )

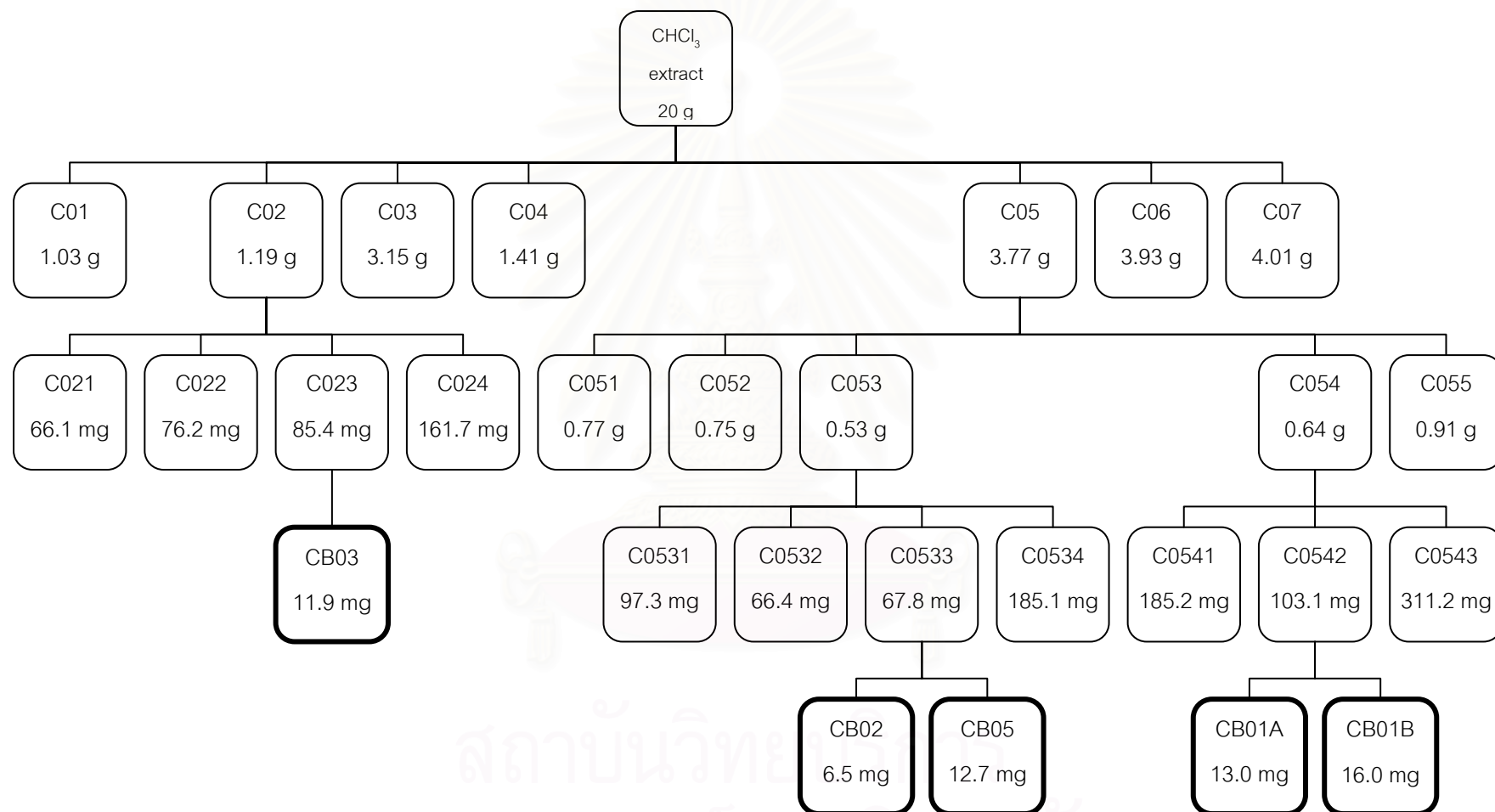
56.4, 103.2, 107.5, 111.5, 113.4, 143.3, 144.0, 149.7, 150.2, 161.4 (Figure 65)



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**Scheme 2. Isolation of the hexane extract from *Cryptolepis buchanani* stem**



**Scheme 3. Isolation of the chloroform extract from *Cryptolepis buchanani* stem**

## CHAPTER IV

### RESULTS AND DISCUSSION

Investigation of the hexane and chloroform extracts of *Cryptolepis buchanani* stem led to the isolation of compounds CB01, CB02, CB03, CB04, CB05, and CB06. The identification and structure elucidation of the isolated compounds were done by analysis of the spectroscopic data (UV, IR, MS and NMR), as well as comparison with the data of related compounds. The details are as follows.

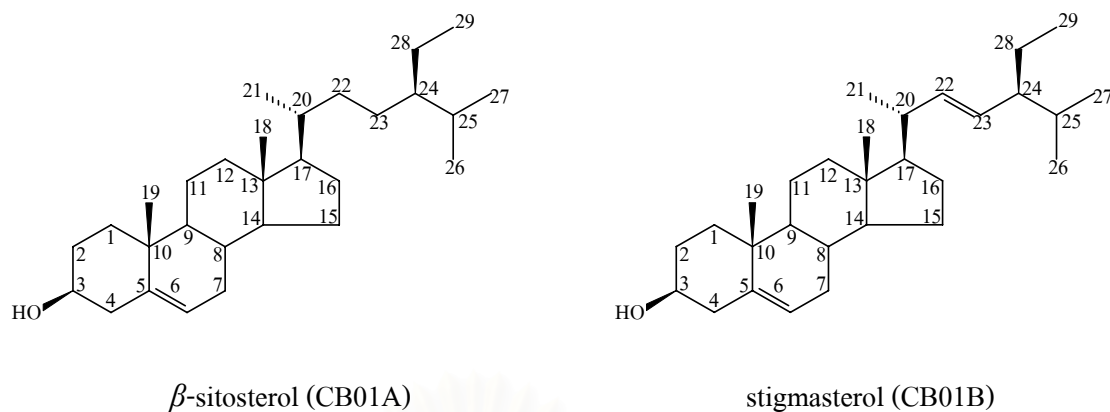
#### 1. Structure identification of compound CB01

Compound CB01 was obtained as colorless needles (104.2 mg, 0.018 % yield). It gave purple color upon spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heated. Liebermann-Burchard test of this compound gave positive green color, suggesting the presence of a steroidal skeleton.

The <sup>1</sup>H-NMR spectrum (Figure 4) gave evidences which suggested that CB06 is a mixture of  $\beta$ -sitosterol and stigmasterol. The signal at  $\delta$  5.33 ppm (1H, *d*, *J*= 4.5 Hz) was assignable to H-6 of both  $\beta$ -sitosterol and stigmasterol, while two signal at  $\delta$  5.09 and 5.00 ppm were assignable to olefinic proton at H-22 and H-23 of stigmasterol, respectively. A multiplet at  $\delta$  3.50 ppm was assigned as methine proton of hydroxyl-substituted position 3 of both  $\beta$ -sitosterol and stigmasterol. The ratio of CB01A ( $\beta$ -sitosterol) and CB01B (stigmasterol) in the mixture was deduced from the integration value between H-6 and H-22 or H-23 to be 4:1:1

The <sup>13</sup>C NMR spectrum (Figure 5-6) and DEPT experiment (Figure 7) showed signals of 29 carbons. The signals of  $\beta$ -sitosterol were more prominent than those of stigmasterol. However, four olefinic carbon signals could be observed at  $\delta$  140.7, 138.2, 129.3 and 121.7 ppm. The two signals at  $\delta$  138.2 and 129.3 ppm were assignable to C-22 and C-23 of stigmasterol, whereas the other two signals at  $\delta$  140.7 and 121.7 ppm were assignable to C-5 and C-6 of both  $\beta$ -sitosterol and stigmasterol, respectively.

Therefore, it was concluded that CB01 is a mixture (4:1) of  $\beta$ -sitosterol and stigmasterol. These two steroids are common phytosterols widely distributed in the plant kingdom. Comparison of <sup>13</sup>C NMR spectral data of CB01A and CB01B with reported data of  $\beta$ -sitosterol and stigmasterol (De-Eknamkul and Potduang, 2003), is shown in Table 16.



**Table 11. Comparison of  $^{13}\text{C}$  NMR spectral data of  $\beta$ -sitosterol, stigmasterol and compound CB01 (a mixture of CB01A and CB01B) (in  $\text{CDCl}_3$ , 75 MHz)**

Position	$\delta$ C (ppm)			
	CB01A	CB01B	$\beta$ -sitosterol *	stigmasterol *
1	37.2	37.2	37.2	37.2
2	31.7	31.7	31.6	31.6
3	71.8	71.8	71.8	71.8
4	42.2	42.3	42.2	42.3
5	140.7	140.7	140.7	140.7
6	121.7	121.7	121.7	121.7
7	31.9	31.9	31.9	31.9
8	31.9	31.9	31.9	31.9
9	50.2	50.2	50.1	50.1
10	36.5	36.5	36.5	36.5
11	21.1	21.1	21.1	21.1
12	39.8	39.7	39.7	39.7
13	42.3	42.3	42.3	42.3
14	56.8	56.9	56.7	56.8
15	24.3	24.3	24.3	24.3
16	28.2	28.9	28.2	28.9
17	56.1	56.0	56.0	55.9
18	11.9	12.0	11.8	12.0

**Table 11. Comparison of  $^{13}\text{C}$  NMR spectral data of  $\beta$ -sitosterol, stigmasterol and compound CB01 (a mixture of CB06A and CB06B) (in  $\text{CDCl}_3$ , 75 MHz)**

Position	$\delta\text{ C (ppm)}$			
	CB01A	CB01B	$\beta$ -sitosterol *	stigmasterol *
19	19.4	19.4	19.4	19.4
20	36.2	40.5	36.1	40.5
21	18.8	21.1	18.8	21.1
22	34.0	138.2	33.9	138.3
23	26.1	129.3	26.0	129.2
24	45.9	51.2	45.8	51.2
25	29.2	31.9	29.1	31.9
26	19.8	21.1	19.8	21.2
27	19.0	19.0	19.0	19.0
28	23.1	26.1	23.0	25.4
29	12.0	12.2	12.0	12.2

\* De Eknankul and Potduang, 2003 (in  $\text{CDCl}_3$ )

## 2. Structure identification of compound CB02

Compound CB02 was obtained as yellow-orange needles (11.9 mg, 0.00070 % yield). This compound displayed yellow-orange color under UV light, suggesting the present of a highly conjugated chromophore structure. Its molecular formula was determined as  $C_{14}H_8O_4$  from HR ESI TOF mass spectrum (Figure 10), with its  $[M+H]^+$  peak at  $m/z$  241. The presence of only eight carbon signals in its  $^{13}C$  NMR spectrum (Figure 12) could be due to the result of some equivalent and superimposition of signals. The IR absorption bands (Figure 9) suggested the presence of hydroxyl group ( $3434\text{ cm}^{-1}$ ), carbonyl group ( $1626\text{ cm}^{-1}$ ) and aromatic ring ( $1602$ ,  $1577$  and  $1470\text{ cm}^{-1}$ ).

The  $^1H$ -NMR spectrum (Figure 11) showed a signal of chelated phenolic hydroxyl groups at  $\delta$  12.05 ppm (1-OH and 8-OH, 1H, s). The spectrum indicated the present of three typical aromatic proton signals frequently recognized in quinones at  $\delta$  7.29 ppm (1H, *dd*,  $J=8.5$ , 1.0 Hz), 7.67 (1H, *t*,  $J=8.0$  Hz) and 7.82 ppm (1H, *dd*,  $J=7.5$ , 1.0 Hz), which could be assigned to H-2 and H-7, H-3 and H-6 and H-4 and H-5 of a symmetrical anthraquinone structure, respectively.

The DEPT (Figure 13) experiments were performed to differentiate these signals into six methine carbons at  $\delta$  124.6 (C-2, C-7), 137.3 (C-3, C-6) and 120.0 ppm (C-4, C-5), and six quaternary carbons at  $\delta$  162.5 (C-1, C-8), 133.6 (C-4a, C-5a) and 115.8 (C-1a, C-8a), and two quinone carbonyl signal at  $\delta$  193.1 (C-9) and 182.7 ppm (C-10).

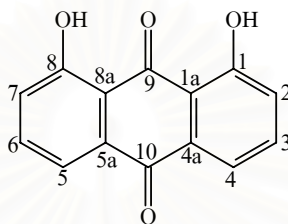
HMBC experiment exhibited long-range correlations from the hydroxy proton signal at  $\delta$  12.05 ppm to C-1, C-8 ( $\delta$  162.5 ppm), suggesting the presence of hydroxy group at C-1 and C-8. The three signals of methine proton at  $\delta$  7.29, 7.67 and 6.89 ppm were assigned to H-2 and H-7, H-3 and H-6 and H-4 and H-5, respectively, and confirmed by the HMBC correlations as shown in Table 14.

Thus, this compound was identified as 1,8-dihydroxy-9,10-anthracenedione or danthron from these spectroscopic data and comparison with literature (Khalafy and Bruce, 2002). This is the first report of its occurrence in the genus *Cryptolepis*.

Danthron had been widely used as a laxative, but was later found to be carcinogenic in human (Ausra *et al.*, 2002; Hui *et al.*, 2005; Mueller *et al.*, 1996; Van-Gorkom *et al.*, 2002). It is the precursor for the topical antipsoriatic drug anthralin or 1,8-dihydroxy 9-anthrone (Ashton *et al.*, 1983; Kemeny, Ruzicka and Braun-Falco, 1990). Danthron has been shown to possess various

biological activities, such as antifungal (Chi-Hoon and Hoi-Seon, 2005), antioxidant and radical scavenging activities (Malterud *et al.*, 1993).

Anthraquinones are distributed fairly widely in moulds especially in *Aspergillus* and *Penicillium spp.* They are uncommon in higher fungi but are found more frequently in lichens. Therefore, danthron found in this study might originated from lichens on the surface of *C. buchanani* stem.



Danthron (Compound CB02)

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**Table 12. Comparison of NMR spectral data of danthron and compound CB02 (in CDCl<sub>3</sub>, 500 MHz)**

Position	Compound CB02			Danthron	
	<sup>1</sup> H NMR	<sup>13</sup> C NMR	HMBC	<sup>1</sup> H NMR*	<sup>13</sup> C NMR**
1	12.05 ( <i>br s</i> , 1-OH)	162.5	C-1, C-2, C-1a	12.08 ( <i>br s</i> , 1-OH)	162.5
2	7.29 (1H, <i>dd</i> , <i>J</i> =8.5, <i>J</i> =1.0 Hz)	124.6	C-1, C-3 C-4, C-1a	7.31 (1H, <i>dd</i> , <i>J</i> =8.4, <i>J</i> =1.3 Hz)	124.6
3	7.67 (1H, <i>t</i> , <i>J</i> =8.0 Hz)	137.3	C-1, C-4, C-4a, C- 1a	7.31 (1H, <i>t</i> , <i>J</i> =8.0 Hz)	137.2
4	7.82 (1H, <i>dd</i> , <i>J</i> =7.5, <i>J</i> =1.0 Hz)	120.0	C-2, C-3, C-10, C- 1a	7.70 (1H, <i>dd</i> , <i>J</i> =7.7, <i>J</i> =1.3 Hz)	120.0
5	7.82 (1H, <i>dd</i> , <i>J</i> =7.5, <i>J</i> =1.0 Hz)	120.0	C-6, C-7, C-10, C- 8a	7.84 (1H, <i>dd</i> , <i>J</i> =7.7, <i>J</i> =1.3 Hz)	120.0
6	7.67 (1H, <i>t</i> , <i>J</i> =8.0 Hz)	137.3	C-5, C-8, C-5a, C- 8a	7.31 (1H, <i>t</i> , <i>J</i> =8.0 Hz)	137.2
7	7.29 (1H, <i>dd</i> , <i>J</i> =8.5, <i>J</i> =1.0 Hz)	124.6	C-5, C-6, C-8, C-8a	7.31 (1H, <i>dd</i> , <i>J</i> =8.4, <i>J</i> =1.3 Hz)	124.6
8	12.05 ( <i>br s</i> 8-OH + 8-OH)	162.5	C-8, C-7, C-8a	12.08 ( <i>br s</i> , 8-OH)	162.5
9		193.1			
10		182.7			
4a, 5a		133.6			133.6
1a, 8a		115.8			115.8

\* Khalafy and Bruce, 2002 (in CDCl<sub>3</sub>, 220 MHz)

\*\* Ruangrunsi *et al.*, 1993 (in CDCl<sub>3</sub>, 125 MHz)

### 3. Structure elucidation of compound CB03

Compound CB03 was obtained as colorless needles (13.0 mg, 0.00076 % yield). It gave purple color upon spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heated. Liebermann-Burchard test of this compound gave green color, suggesting the presence of a steroidal skeleton. Its molecular formula was determined as C<sub>21</sub>H<sub>30</sub>O<sub>4</sub> from ESI TOF mass spectrum (Figure 19), with its [M+Na]<sup>+</sup> peak at *m/z* 369. The IR spectrum (Figure 18) suggested the presence of hydroxyl group at 3475 cm<sup>-1</sup>, carbonyl and conjugated carbonyl groups at 1694 and 1661 cm<sup>-1</sup>, which the absorption band at 1609 cm<sup>-1</sup> was due to stretching vibration of the olefinic bond.

The <sup>1</sup>H-NMR spectrum (Figure 20) showed two methyl singlet signals at δ 0.67 (H-18) and 1.33 ppm (H-19). Six methine protons resonated at δ 1.66 (H-8), 1.05 (H-9), 1.24 (H-14), 2.63 (H-17). The doublet at δ 5.70 ppm could be assigned to the olefinic proton H-4. An oxymethine proton resonated at δ 4.21 ppm (H-2), while oxymethylene protons appeared at δ 4.15 ppm (H-21). The signal between δ 1.20-2.40 ppm were the signals of methylene protons at δ 1.51 (H-1α and 2.27 (H-1β), 2.32 (H-6α and 2.46 (H-6β), 1.08 (H-7α) and 1.88 (H-7β), 1.48 (H-11α) and 1.65 (H-11β), 1.45 (H-12α and 1.95 (H-12β), 1.30 (H-15α) and 1.75 (H-15β), 1.73 (H-16α) and 2.18 (H-16β) ppm. Two hydroxyl proton signals appeared as split into doublets (*J*= 3 Hz) and triplets (*J*= 5 Hz) due to the neighboring methine proton at δ 3.92 ppm (2-OH) and methylene protons at δ 3.68 ppm (21-OH), respectively.

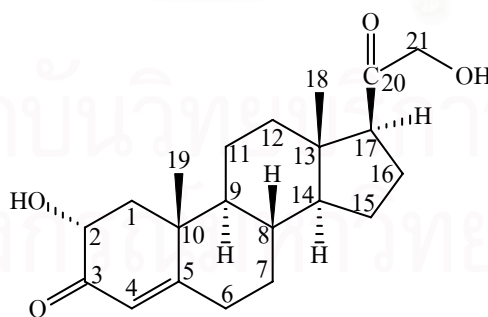
The <sup>13</sup>C NMR spectrum (Figure 21) showed the signals of 21 carbon atoms, supporting the assignment of this compound as a pregnane steroid. The DEPT 90 and 135 (Figure 22) experiments were performed to differentiate these signals into those of two methyl carbons at δ 13.7 (C-18) and 18.2 ppm (C-19), eight methylene carbons at δ 45.3 (C-1), 32.9 (C-6), 32.7 (C-7), 21.5 (C-11), 38.9 (C-12) 24.9 (C-15), 23.4 (C-16) and 69.9 ppm (C-21). Six methine carbons resonated at δ 35.7 (C-8), 55.1 (C-9), 56.6 (C-14) and 59.1 (C-17) In addition, two distinct downfield methine signals assignable to oxygenated and olefinic carbons at δ 70.0 (C-2) and 121.2 (C-4) ppm, respectively. α, β-unsaturation causes an olefinic signal to shift downfield to δ 172.4 (C-5), which two carbonyl signals at δ 200.0 (C-3) and 210.9 (C-20) ppm were assignable to keto carbonyl moieties.

The <sup>1</sup>H-<sup>1</sup>H COSY (Figure 23) and HMBC experiment (Figures 27-30, Table 13) assisted in the elucidation of the chemical structure of compound CB03. Two hydroxyl groups which resonated at δ 3.92 and 3.68 ppm should be placed at C-2 and C-21 as confirmed by the HMBC correlations with C-2 and C-21, respectively. The proton signal of H-2 at δ 4.21 ppm and 2-OH

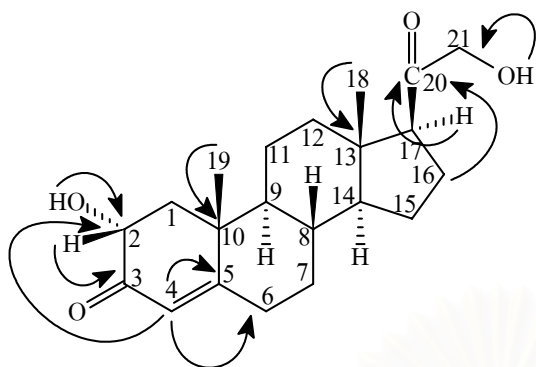
exhibited correlations with C-3 ( $\delta$  200.0 ppm), whereas the signals at  $\delta$  2.63 (H-17),  $\delta$  2.18 (H-16) and 21-OH ppm exhibited correlations with C-20, confirming the positions of keto carbonyl at C-3 and C-20, respectively. The signal of the olefinic H-4 ( $\delta$  5.70 ppm) showed two-bond correlations with C-5 and three-bond correlation with C-2, C-6 and C-10, confirming the position of double bond between C-4 and C-5.

The stereochemistry of the structure was determined basic on its NOESY (Figure 31). Correlations between H-2 and H-19, H-8 and H-19, H-8 and H-18 and H-18 and H-19 showed that they were in the same orientation. According to the literature, pregnanes have previously been isolated from several plants of the family Asclepiadaceae (Table 1. and Figure 2.) supporting that the 18, 19 methyl protons were both  $\beta$ -oriented and, therefore, H-2 and H-8 were  $\beta$ -oriented too. Hence, 2-OH, H-9, H-14 and H-17 which showed no NOESY with the former group must be  $\alpha$ -oriented and revealed that the ketone at C-17 should be  $\beta$ -oriented.

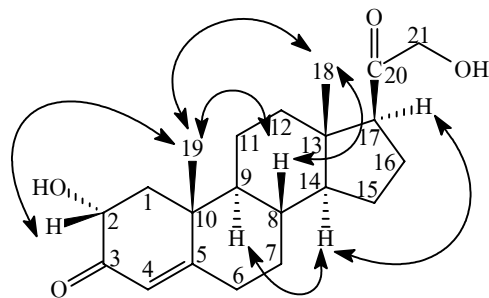
Elucidation of CB03 structure was also done by comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  -NMR data of this compound with the previously reported of the  $^1\text{H}$ -NMR data  $2\beta$ -hydroxycortexone (Laskin *et al.*, 1965) and  $^{13}\text{C}$  -NMR data of deoxycorticosterone (Hunter and Carragher, 2003). Compound CB03 was identified as a  $2\alpha$ , 21-dihydroxypregn-4-ene-3, 20-dione. Although this Compound has been previously obtained from semi-synthesis (Christain *et al.*, 1989) and synthesis (Hill, 1991), this is the first time it has been found in a naturally occurring compound. Comparison of their  $^1\text{H}$  and  $^{13}\text{C}$ -NMR data is presented in Table 13.



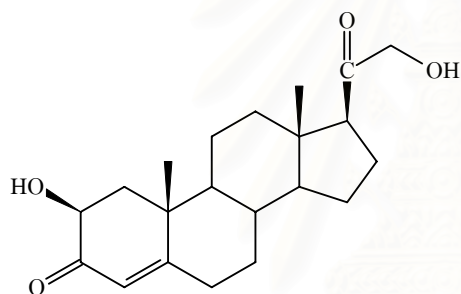
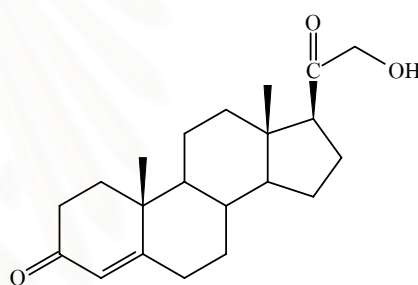
CB03



Major HMBC correlation of Compound CB03



Major NOESY correlation of Compound CB03

 $2\beta$ -hydroxycortexone

Deoxycorticosterone

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**Table 13. Comparison of NMR spectral data of 2 $\beta$ -hydroxycortexone, deoxycorticosterone and compound CB03 ( in acetone- $d_6$ , 500 HMz)**

Position	Compound CB03			2 $\beta$ -hydroxy cortexone	Deoxycor ticesterone
	<sup>1</sup> H NMR	<sup>13</sup> C NMR	HMBC	<sup>1</sup> H NMR*	<sup>13</sup> C NMR**
1	$\alpha$ 1.51 (1H, <i>dd</i> , $J=13.0, 4.0$ Hz) $\beta$ 2.27 (1H, <i>dd</i> , $J=13.0, 5.0$ Hz)	45.3	C-2, C-3, C-5, C- 9, C-10, C-19		36.4
2	4.21 (1H, <i>ddd</i> , $J=13.0, 5.0, 3.0$ Hz)	70.0	C-1, C-3	4.15	34.6
3		200.0			200.1
4	5.7(1H, <i>d</i> , $J=1.5$ Hz)	121.2	C-2, C-5, C-6, C- 10	5.89	124.7
5		172.4			171.4
6	$\alpha$ 2.32 (1H, <i>ddd</i> , $J=14.5, 9.5, 2.5$ Hz) $\beta$ 2.46(1H, <i>tdd</i> , $J=14.5, 5.5, 1.5$ Hz)	32.9	C-4, C-5, C-7, C-8		33.4
7	$\alpha$ 1.08 (1H, <i>m</i> ) $\beta$ 1.88 (1H, <i>m</i> )	32.7	C-5, C-6, C-8, C- 9, C-14		32.6
8	1.66 (1H, <i>dd</i> , $J=$ 7, 3.5 Hz)	35.7	C-6, C-7, C-9, C- 11, C-13, C-14		36.2
9	1.05 (1H, <i>m</i> )	55.1	C-1, C-10, C-11, C-14, C-19		54.3
10		41.1			39.2
11	$\alpha$ 1.48 (1H, <i>m</i> ) $\beta$ 1.65 (1H, <i>m</i> )	21.5	C-8, C-9, C-10, C- 12, C-13		21.6

**Table 13. Comparison of NMR spectral data of 2 $\beta$ -hydroxycortexone, deoxycorticosterone and compound CB03 ( in acetone- $d_6$ , 500 HMz)**

Position	Compound CB03			2 $\beta$ -hydroxy cortexone	Deoxycor tosterone
	<sup>1</sup> H NMR	<sup>13</sup> C NMR	HMBC	<sup>1</sup> H NMR*	<sup>13</sup> C NMR**
12	$\alpha$ 1.95 (1H, <i>m</i> ) $\beta$ 1.45 (1H, <i>m</i> )	38.9	C-9, C-11, C-13, C-14, C-17, C-18		39.1
13		44.9			45.4
14	1.24 (1H, <i>m</i> )	56.6	C-7, C-8, C-12, C- 13, C-18		56.8
15	$\alpha$ 1.30 (1H, <i>m</i> ) $\beta$ 1.75 (1H, <i>m</i> )	24.9	C-8, C-13, C-14, C-16, C-17		25.2
16	$\alpha$ 1.73(1H, <i>m</i> ) $\beta$ 2.18 (1H, <i>m</i> )	23.4	C-13, C-14, C-15, C-17, C-20		23.6
17	2.63 (1H, <i>t</i> , <i>J</i> = 9 Hz)	59.1	C-12, C-13, C-16, C-18, C-20		59.7
18	0.67 (3H, <i>s</i> )	13.7	C-12, C-13, C-14, C-17	0.70	14.2
19	1.33 (3H, <i>s</i> )	18.2	C-1, C-9, C-10	1.19	18.1
20		210.9			210.8
21	4.15 (1H, <i>d</i> , <i>J</i> =5.0 Hz)	69.9	C-20		70.1
2-OH	3.92 (1H, <i>d</i> , <i>J</i> =3.0 Hz)		C-1, C-2, C-3	3.47 (1H, <i>d</i> , <i>J</i> =2 Hz)	
21-OH	3.68 (1H, <i>t</i> , <i>J</i> =5 Hz)		C-21, C-20	3.21 (1H, <i>t</i> , <i>J</i> =4.5 Hz)	

\* Laskin *et al.*, 1965

\*\*Hunter and Carragher, 2003 (in CDCl<sub>3</sub>, 360 MHz)

#### 4. Structure elucidation of compound CB04

Compound CB04 was obtained as colorless needles (16.0 mg, 0.00094 % yield). It gave purple color upon spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heated. Liebermann-Burchard test of this compound gave green color, suggesting the presence of a steroidal skeleton. Its molecular formula was determined as C<sub>21</sub>H<sub>28</sub>O<sub>4</sub> from ESI TOF mass spectrum (Figure 34), with its [M+Na]<sup>+</sup> peak at *m/z* 367. The IR spectrum (Figure 33) suggested the presence of hydroxyl group at 3468 cm<sup>-1</sup>, carbonyl and conjugated carbonyl group at 1696 and 1650 cm<sup>-1</sup>, the absorption band at 1578 cm<sup>-1</sup> was due to stretching vibration of the olefinic bond.

Comparison of <sup>1</sup>H and <sup>13</sup>C-NMR data of compounds CB04 and CB03 indicated that they are similar, except compound CB04 contains an additional double bond.

The <sup>1</sup>H-NMR spectrum (Figure 35) showed two singlet signals of methyl group at δ 0.72 (H-18) and 1.23 ppm (H-19). The signals at δ 5.69, 6.23 and 6.18 ppm which could be assigned as olefinic proton H-4, H-6 and H-7, respectively. An oxymethine proton was observed at δ 4.36 ppm, while oxymethylene proton appeared at δ 4.17 ppm. Two hydroxyl proton signals appeared as split into doublets (*J*= 5 Hz) and triplets (*J*= 5 Hz) due to the neighboring methine proton at δ 3.97 ppm (2-OH) and methylene protons at δ 3.69 ppm (21-OH), respectively.

The <sup>13</sup>C NMR spectrum (Figure 36) showed the signals of 21 carbon atoms, supporting the assignment of this compound as pregnane steroid. The DEPT (Figure 37) and HSQC (Figures 40-41) experiments were performed to differentiate these signals into two methyl carbon at δ 17.3 (C-19) and 13.6 (C-18) ppm, Two carbonyl carbon signals appeared at δ 199.9 (C-3) and δ 210.8 (C-20) ppm and four olefinic carbons resonances were located at δ 121.8 (C-4), 165.0 (C-5), 128.2 (C-6) and 141.7 (C-7) ppm. Two signal for oxygenated carbon were observed at δ 70.3 (C-2) and 69.9 (C-21) ppm

The <sup>1</sup>H-<sup>1</sup>H COSY (Figures 38-39) and HMBC experiment (Figures 43-46, Table 14) assisted in the elucidation of the chemical structure of compound CB04. Two hydroxyl signals at δ 3.97 and 3.69 ppm should be to place at C-2 and C-21 as confirmed by the HMBC correlations with C-2 and C-21, respectively. The proton signal at δ 4.21 (H-2) ppm exhibited correlation with C-3 (δ 199.9 ppm), whereas the signals at δ 2.7 (H-17) and δ 2.23 (H-16) ppm exhibited correlation with C-20, confirming position of keto carbonyl at C-3 and C-20, respectively. The signal of the olefinic H-6 (δ 6.23 ppm) showed two-bond correlation with C-5 and three-bond correlation with C-4 and C-8, and H-7 (δ 6.18 ppm) showed two-bond correlation with C-8 and

three-bond correlation with C-5, C-9 and C-14, confirming the position double bond between C-6 and C-7.

Compound CB04 showed similar NOESY correlations to those of compound CB03, (Figure 47), suggesting that H-2, H-8, H-18 and H-19 were in  $\beta$ -oriented, whereas 2-OH, H-9, H-14 and H-17 were in  $\alpha$ -oriented.

Therefore, the elucidation of the structure of CB04 was mainly accomplished by comparison of the  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  chemical shift data with CB03 (Table 13). Based on above spectral evidence, compound CB04 was identified as a new naturally occurring pregnane steroid,  $2\alpha, 21$ -dihydroxypregn-4,6-diene-3, 20-dione.  $^1\text{H}$  and  $^{13}\text{C-NMR}$  data of compound CB04 is presented in Table 14.

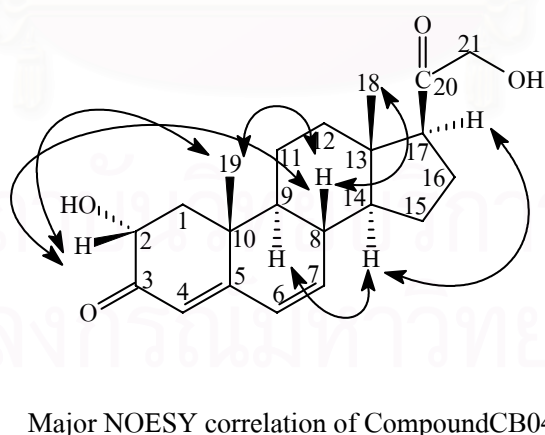
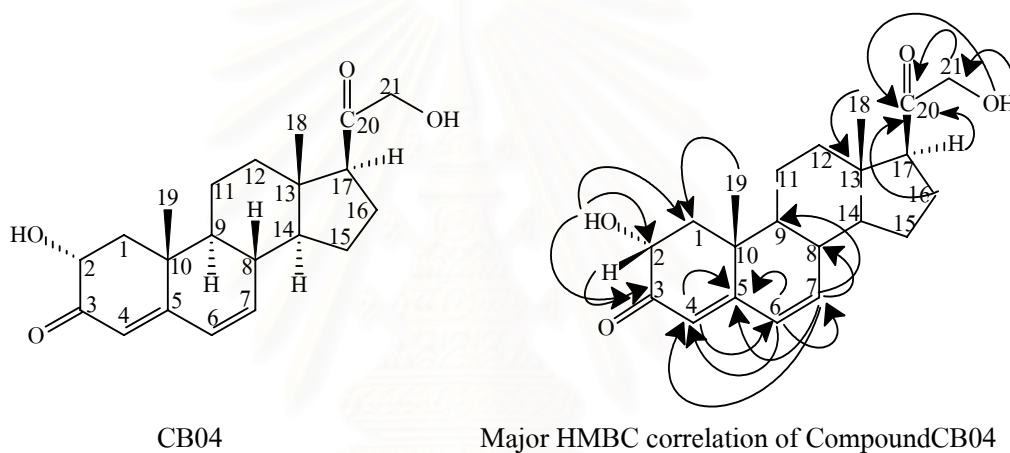




Table 14. NMR spectral data of compound CB04 ( in acetone- $d_6$ , 500 HMz)

Position	CB04		
	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR	HMBC
1	$\alpha$ 1.58 (1H, <i>dd</i> , $J = 13.0, 4.0$ Hz) $\beta$ 2.28 (1H, <i>dd</i> , $J = 13.0, 5.0$ Hz)	43.7	C-2, C-3, C-5, C-9, C-10, C-19
2	4.36 (1H, <i>ddd</i> , $J = 13.0, 5.0, 2.5$ Hz)	70.3	C-1, C-3
3		199.9	
4	5.69 (1H, <i>s</i> )	121.8	C-2, C-5, C-6, C-10
5		165.0	
6	6.23 (1H, <i>dd</i> , $J = 9.5, 2.5$ Hz)	128.2	C-4, C-5, C-8
7	6.18 (1H, <i>dd</i> , $J = 9.5, 2.0$ Hz)	141.7	C-5, C-8, C-9, C-14
8	2.30 (1H, <i>m</i> )	37.9	C-6, C-7, C-10, C-14
9	1.3 (1H, <i>m</i> )	51.7	C-1, C-5, C-10
10		38.7	
11	$\alpha$ 1.65 (1H, <i>m</i> ) $\beta$ 1.56 (1H, <i>t</i> , $J = 12.5$ )	21.3	C-8, C-9, C-10, C-12
12	$\alpha$ 1.53 (1H, <i>m</i> ) $\beta$ 2.02 (1H, <i>dd</i> , $J = 8.5, 2.5$ Hz)	38.8	C-9, C-11, C-13, C-14, C-18
13		45.8	
14	1.45 (1H, <i>m</i> )	54.3	C-7, C-8, C-9, C-12, C-15, C-16, C-18
15	$\alpha$ 1.96 (1H, <i>m</i> ) $\beta$ 1.5 (1H, <i>m</i> )	24.5	C-8, C-14, C-16
16	$\alpha$ 1.78 (1H, <i>m</i> ) $\beta$ 2.23 (1H, <i>m</i> )	23.0	C-13, C-14, C-15, C-17, C-20
17	2.7 (1H, <i>t</i> , $J = 9$ Hz)	58.9	C-12, C-13, C-16, C-18, C-20
18	0.72 (3H, <i>s</i> )	13.6	C-12, C-13, C-14, C-17
19	1.23 (3H, <i>s</i> )	17.3	C-1, C-5, C-9, C-10
20		210.8	

Table 14. NMR spectral data of compound CB04 ( in acetone- $d_6$ , 500 HMz)

Position	CB04		
	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR	HMBC
21	4.17 (1H, <i>d</i> , $J = 5.5$ Hz)	69.9	C-20
2-OH	3.97 (1H, <i>dd</i> , $J = 5.0$ Hz)		C-1, C-2, C-3
21-OH	3.69 (1H, <i>t</i> , $J = 5.0$ Hz)		C-20



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## 5. Structure elucidation of compound CB05

Compound CB05 was obtained as pale-yellow needles (6.5 mg, 0.00038 % yield). It gave purple color upon the spraying with 10 % H<sub>2</sub>SO<sub>4</sub> in ethanol and heated. Liebermann-Burchard test of this compound gave green color, suggesting the presence of a steroidal skeleton. Its molecular formula was determined as C<sub>21</sub>H<sub>26</sub>O<sub>4</sub> from HR ESI TOF mass spectrum (Figure 50), with its [M+Na]<sup>+</sup> peak at *m/z* 365. The IR spectrum (Figure 49) suggested the presence of hydroxyl group at 3444 cm<sup>-1</sup>, carbonyl and conjugated carbonyl group at 1715, 1631, 1615 cm<sup>-1</sup>.

Comparison of <sup>1</sup>H and <sup>13</sup>C-NMR data of compound CB05 which those of compound CB04 indicated that compound CB05 had a distinct similarity to compound CB04, excepted only the structure of compound CB05 contain an additional double bond.

The <sup>1</sup>H-NMR spectrum (Figure 51) showed two singlet signals of methyl group at δ 0.77 (H-18) and 1.25 ppm (H-19). The signals at δ 6.27, 6.13, 6.30 and 6.09 ppm could be assigned to the olefinic proton H-1, H-4, H-6 and H-7. An oxymethylene resonated at δ 4.20 ppm. Hydroxy group on ring A showed signal at δ 6.44 ppm, which downfield than compound CB03 and CB04 because its was attributable to the olefinic carbon at C-2.

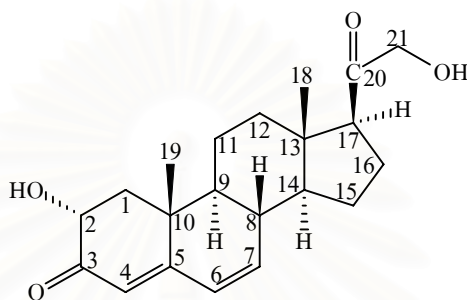
The <sup>13</sup>C NMR spectrum (Figure 52) showed the signals of 21 carbon atoms, supporting to assignment of this compound as pregnane steroid. The DEPT 90 and 135 (Figure 53) and HMQC (Figures 55-56) experiments were performed to differentiate these signals into two methyl carbon at δ 21.7 (C-19) and 13.5 (C-18) ppm, Two carbonyl carbon signals appeared at δ 181.4 (C-3) and δ 209.9 (C-20) ppm and six olefinic carbons resonances were located at δ 120.8 (C-1), 146.7 (C-2), 121.2 (C-4), 165.7 (C-5), 127.7 (C-6) and 138.7 (C-7) ppm. A signal for one carbon-bearing oxygen was observed at δ 69.5 (C-21) ppm.

The <sup>1</sup>H-<sup>1</sup>H COSY (Figure 54) and HMBC experiment (Figure 55-59, Table 15) assisted in the elucidation of the chemical structure of compound CB05. Ketone carbonyls could be assigned to C-3 and C-20 of which according to HMBC correlations of OH-(δ 6.44 ppm) and methine H-17 (δ 2.52 ppm) and oxymethylene H-21 (δ 4.20 ppm) to these carbon resonance at 181.4 (C-3) and 209.9 (C-20) ppm, respectively. The signal of olefinic H-1 (δ 6.27 ppm) showed two-bond correlations with C-2 and C-10 and three bond correlation with C-3, C-5, C-9 and C-19, confirming the position of double bond between C-1 and C-2. Cross peak between the signals of both 2-OH and C-2, established the position of hydroxyl substitutions at C-2.

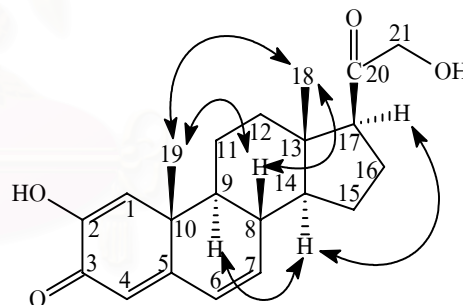
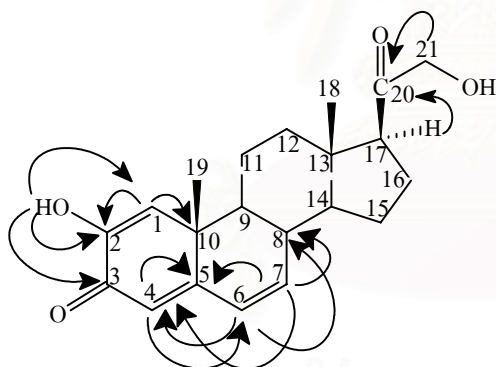
Compound CB05 showed similar NOESY correlations to those of compound CB03 and CB04 (Figure 60), suggested that H-8, H-18 and H-19 were in β-orientated, H-9, H-14 and

H-17 were in  $\alpha$ -oriented.

The elucidation of the structure of CB05 was mainly accomplished by comparison of the  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR chemical shift data with CB04 (Table 14). Base on above spectral evidence, compound CB05 was identified as a new naturally occurring pregnane steroid, 2,21-dihydroxypregn-1,4,6-triene-3,20-dione.  $^1\text{H}$  and  $^{13}\text{C}$ -NMR data of compound CB05 is presented in Table 15.



CB05



Major HMBC correlation of Compound CB05    Major NOESY correlation of Compound CB05

Table 15. NMR spectral data of compound CB05 ( in CDCl<sub>3</sub>, 500 MHz)

Position	Compound CB05		
	<sup>1</sup> H NMR	<sup>13</sup> C NMR	HMBC
1	6.27 (1H, <i>s</i> )	120.8	C-2, C-3, C-5, C-9, C-10, C-19
2		146.7	
3		181.4	
4	6.13 (1H, <i>s</i> )	121.2	C-2, C-5, C-6, C-10
5		165.7	
6	6.30 (1H, <i>dd</i> , <i>J</i> = 10.0, 2.9 Hz)	127.7	C-4, C-8, C-10
7	6.09 (1H, <i>dd</i> , <i>J</i> = 10.0, 1.6 Hz)	138.7	C-5, C-6, C-8, C-9, C-14
8	2.28 (1H, <i>m</i> )	38.1	C-6, C-7, C-14
9	1.43 (1H, <i>m</i> )	49.4	C-8, C-11, C-12, C-19
10		41.5	
11	$\alpha$ 1.65 (1H, <i>m</i> ) $\beta$ 1.90 (1H, <i>m</i> )	22.1	C-12
12	$\alpha$ 1.40 (1H, <i>m</i> ) $\beta$ 2.02 (1H, <i>dt</i> , <i>J</i> = 12.5, 3 Hz)	38.2	C-9, C-11, C-14
13		45.0	
14	1.40 (1H, <i>m</i> )	53.9	C-15
15	$\alpha$ 1.55 (1H, <i>m</i> ) $\beta$ 1.95 (1H, <i>m</i> )	23.9	C-13, C-14, C-16
16	$\alpha$ 1.85 (1H, <i>m</i> ) $\beta$ 2.30 (1H, <i>m</i> )	23.1	C-14, C-15
17	2.52 (1H, <i>t</i> , <i>J</i> =9.3)	58.7	C-12, C-13, C-16, C-18, C-20
18	0.77 (3H, <i>s</i> )	13.5	C-12, C-13, C-14, C-17
19	1.25 (3H, <i>s</i> )	21.7	C-1, C-5, C-9, C-10
20		209.9	
21	4.20 (1H, <i>d</i> , <i>J</i> = 5.8 Hz)	69.5	C-20
2-OH	6.44 (1H, <i>s</i> )		C-1, C-2, C-3
21-OH	3.26 (1H, <i>br s</i> )		

## 6. Structure identification of compound CB06

Compound CB06 was obtained as yellow needles (12.7 mg, 0.00074 % yield). This compound fluoresced under UV light, suggesting that it might be a coumarin. Its molecular formula was determined as  $C_{10}H_8O_4$  according to its ESI TOF mass spectrum (Figure 63), with its  $[M+H]^+$  peak at  $m/z$  193. The IR absorption peaks (Figure 62) suggested the presence of hydroxyl group ( $3337\text{ cm}^{-1}$ ), carbonyl group ( $1703\text{ cm}^{-1}$ ) and aromatic ring ( $1608, 1566$  and  $1509\text{ cm}^{-1}$ )

The  $^1\text{H-NMR}$  spectrum (Figure 64) showed two coupled doublets signals at  $\delta$  6.24 and 7.57 ppm (1H each,  $d, J=9.5$  Hz) which could be assigned to H-3 and H-4 of a coumarin skeleton, respectively. The 6, 7-disubstituted aromatic ring was suggested by two singlets at  $\delta$  6.82 and 6.89 ppm (1H each,  $s$ ) assignable to H-5 and H-8, respectively. The presence of a singlet at  $\delta$  3.93 ppm integrated for three protons represents one methoxy substituent.

The  $^{13}\text{C NMR}$  spectrum (Figure 65) showed the signals of 10 carbon atoms. The DEPT (Figure 66) and HSQC (Figure 68-69) experiments differentiated these signals into those of one methoxy carbon at  $\delta$  56.4 ppm, four methine carbons at  $\delta$  113.4 (C-3), 143.3 (C-4), 107.5 (C-5) and 103.2 ppm (C-8), four quaternary carbons at  $\delta$  111.5 (C-4a), 144.0 (C-6), 149.7 (C-7) and 150.2 ppm (C-8a), and the most downfield quaternary signal at  $\delta$  161.4 ppm was assignable to carbonyl moiety at C-2.

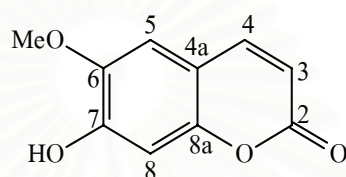
HMBC experiment (Figures 70-72) exhibited long-range correlation between the methoxy proton at  $\delta$  3.93 ppm and C-6 ( $\delta$  144.0 ppm), suggesting the presence of methoxy group at C-6. The NOESY experiment (Figure 73) showed cross peaks between H-5 and both H-4 and 6-OCH<sub>3</sub>, confirming the position of the methoxy group.

From all of the above spectroscopic data and comparison with previously published data (Tsukamoto *et al.*, 1985; Sibanda *et al.*, 1989), compound CB06 was identified as a 7-hydroxyl 6-methoxycoumarin named scopoletin.

Scopoletin has previously been isolated from several plants of the family Rutaceae e.g. from *Haphophyllum vulcanifolium* bark (Chang *et al.*, 1997), *H. obtusifolium* (Gashimov and Kuznetsova, 1975), *Pelea anisata* fruits (Elpern and Mitchell, 1984), *Murraya paniculata* foliage (Steck, 1972), *Clausena anisata* (Ojewole *et al.*, 2002), and also from other plants families such as from *Olea africana* bark (Oleaceae) (Tsukamoto *et al.*, 1984), *Coptis trifolia* whole plants (Ranunculaceae) (Mizuno *et al.*, 1992), *Sonchus gomerensis* (Compositae) (Mansour, Saleh and Boulos, 1983), *Pelargonium sidoides* roots (Geraniaceae) (Kayser and Kolodziej, 1995), *Impatiens balsamina* roots (Balsaminaceae) (Panichayupakaranant *et al.*, 1995), *Diospyros*

*hirsuta* bark (Ebenaceae) (Herath *et al.*, 1978), *Bupleurum fruticosum* roots (Apiaceae) (Pistelli *et al.*, 1996) and *Magonia glabrata* fruit bark (Sapindaceae) (Lemos *et al.*, 2006)

Scopoletin has been shown to possess various biological activities, for examples, antifungal (Valle *et al.*, 1997), hepatoprotective (Mohamed *et al.*, 2005), antioxidant (Shaw *et al.*, 2003), antiproliferative (Liu *et al.*, 2001), antitumor (Cassady *et al.*, 1979), immunomodulatory (Manuele *et al.*, 2006) and anti-inflammatory action against the release of PGE<sub>2</sub>, TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and suppression of cox-2 expression (Kim *et al.*, 2004).



Scopoletin (Compound CB06)

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**Table 15. Comparison of NMR spectral data of scopoletin and compound CB06 (in CDCl<sub>3</sub>, 500 MHz)**

Position	Compound CB06			Scopoletin	
	<sup>1</sup> H NMR	<sup>13</sup> C NMR	HMBC	<sup>1</sup> H NMR*	<sup>13</sup> C NMR**
2		161.4			160.2
3	6.24(1H, <i>d</i> , <i>J</i> = 9.5 Hz)	113.4	C-2, C-4a	6.17(1H, <i>d</i> , <i>J</i> =10 Hz)	112.5
4	7.57(1H, <i>d</i> , <i>J</i> = 9.5 Hz)	143.3	C-2, C-5, C-8a	7.83(1H, <i>d</i> , <i>J</i> =10 Hz)	142.3
4a		111.5			110.5
5	6.82(1H, <i>s</i> )	107.5	C-4, C-7, C-6, C-8a	7.14(1H, <i>s</i> )	107.0
6		144.0			143.2
7		149.7			149.2
8	6.89(1H, <i>s</i> )	103.2	C-4a, C-6, C-7, C-8a	6.74(1H, <i>s</i> )	102.5
8a		150.2			149.8
6-OCH <sub>3</sub>	3.93(3H, <i>s</i> )	56.4	C-6	3.80(3H, <i>s</i> )	55.2
7-OH	6.16(1H, <i>br s</i> )				-

\* Tsukamoto *et al.*, 1985 (in DMSO-*d*<sub>6</sub>, 90 MHz)

\*\* Sibanda *et al.*, 1989 (in DMSO-*d*<sub>6</sub>, 125 MHz)



## CHAPTER V

### CONCLUSION

Three pregnane steroids, an anthraquinone, a coumarin, and a mixture of two phytosterols were isolated from the stem of *Cryptolepis buchanani* Roem & Schult (Asclepiadaceae). Their chemical structures were determined using spectroscopic techniques. Three of them were identified as new naturally occurring pregnanes named 2 $\alpha$ ,21-dihydroxypregn-4-ene-3,20-dione, 2 $\alpha$ ,21-dihydroxypregn-4,6-diene-3,20-dione and 2,21-dihydroxypregn-1,4,6-triene-3,20-dione. The remainder was the known coumarin, scopoletin, the anthraquinone, danthron and a mixture (4:1) of  $\beta$ -sitosterol and stigmasterol.

The structures of pregnane steroids isolated from *C. buchanani* are closely related to biologically active steroid hormones, especially glucocorticoids which possessed significant in anti-inflammatory activities. It is possible that these compounds should possess anti-inflammatory activities as well. It may be harmonized with Thai folk medicinal usages, such as arthritis, muscle and joint pain. Furthermore the results obtained could be valuable information for future phytochemical studies of this plants as well as chemotaxonomy of the Asclepiadaceae, Tribe and Genus levels.

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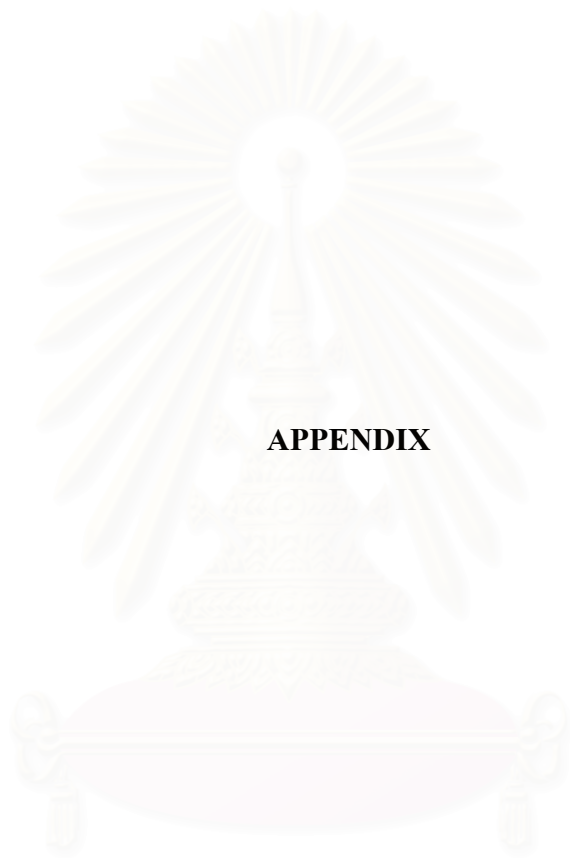
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**APPENDIX**

สถาบันวิทยบริการ  
จุฬาลงกรณ์มหาวิทยาลัย

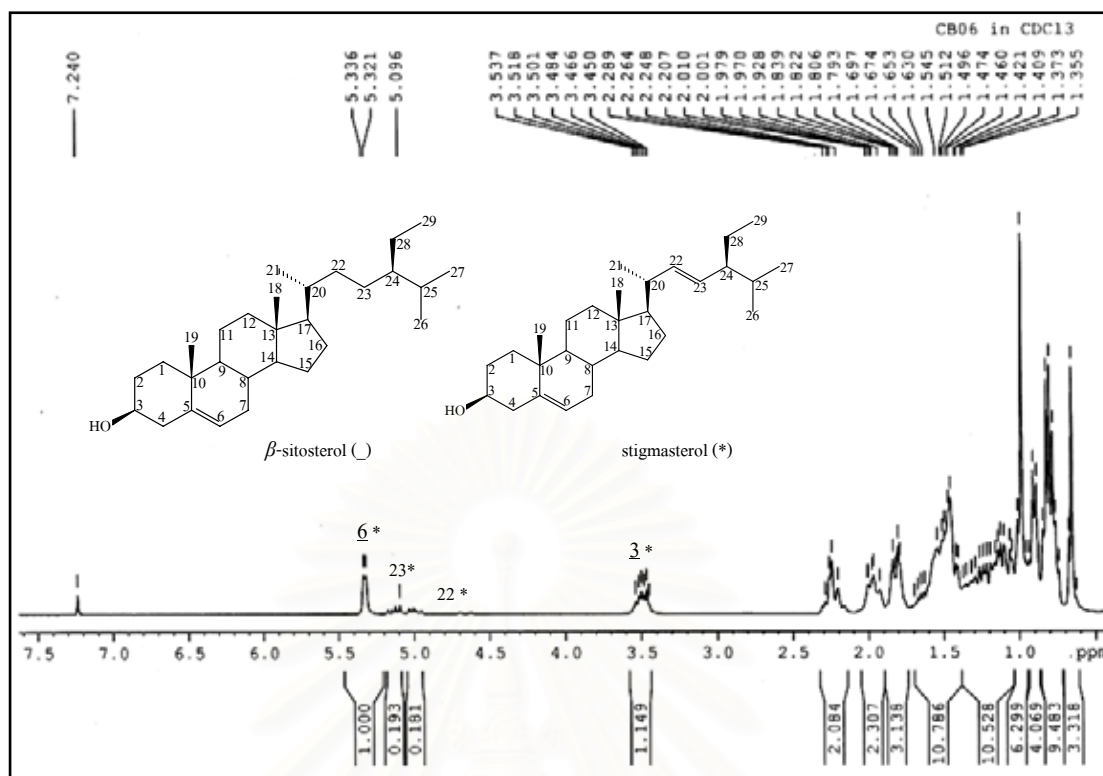


Figure 4.  $^1\text{H}$  NMR (300 MHz) Spectrum of component CB01 (in  $\text{CDCl}_3$ )

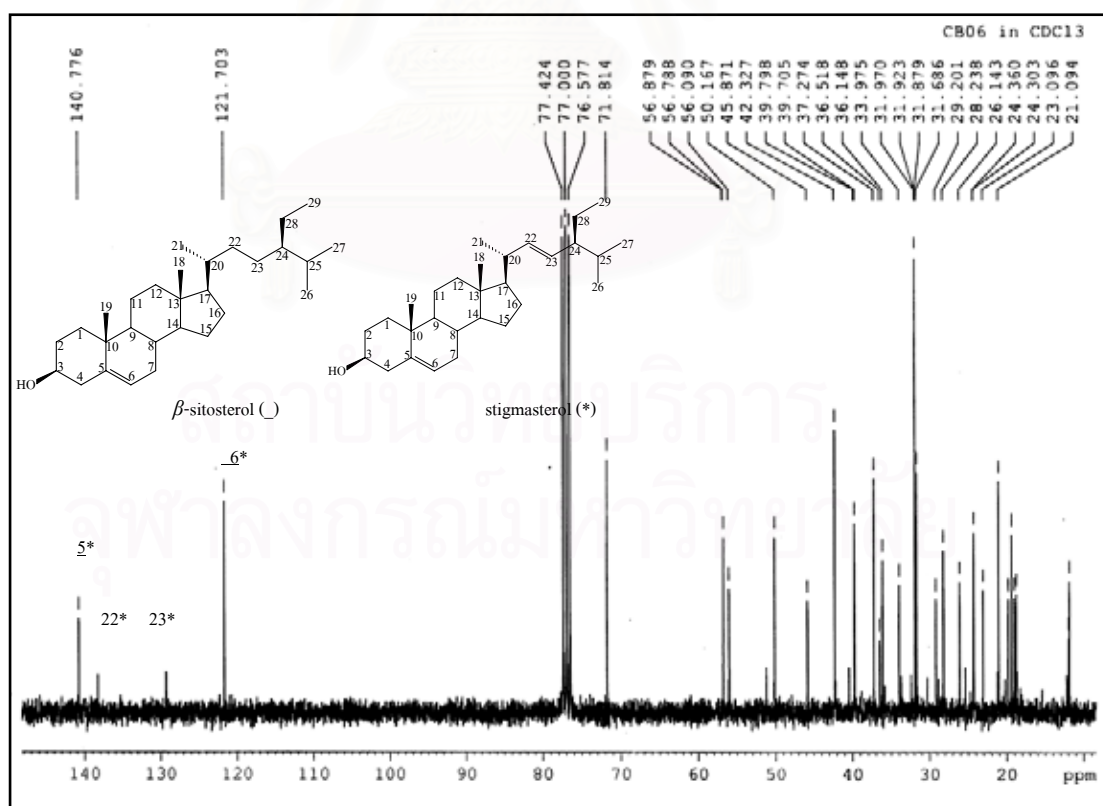


Figure 5.  $^{13}\text{C}$  NMR (75 MHz) Spectrum of component CB01 (in  $\text{CDCl}_3$ )

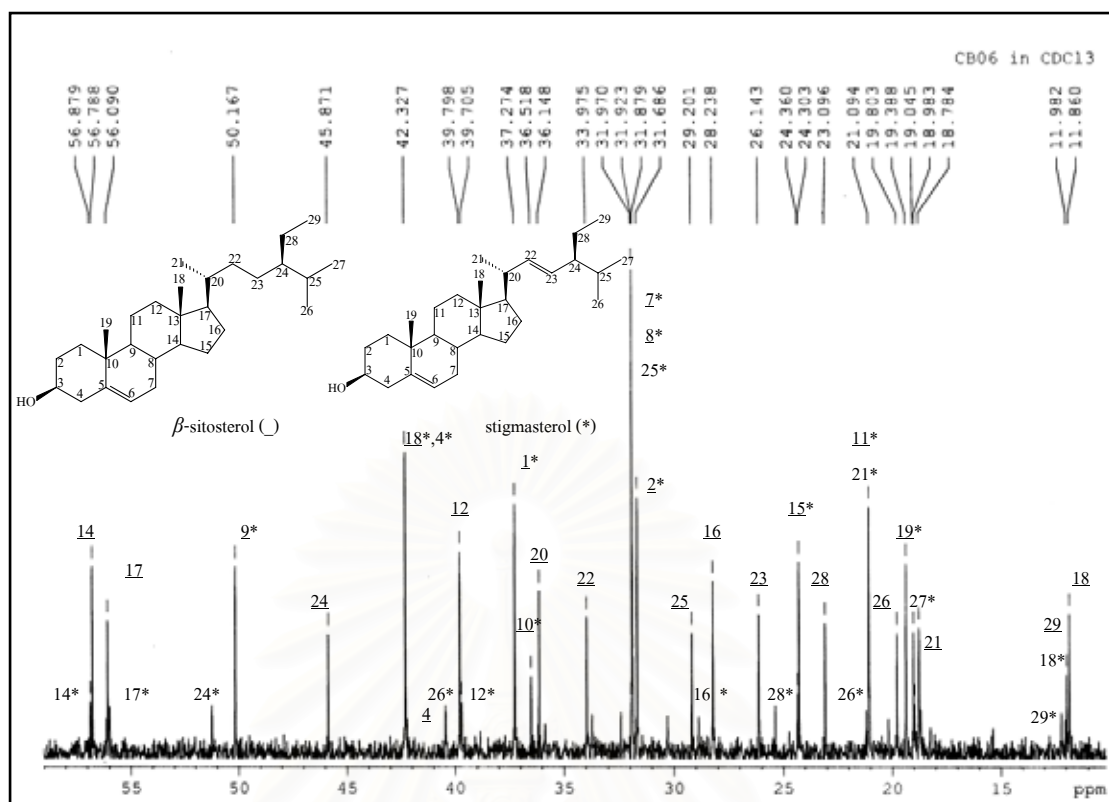


Figure 6. <sup>13</sup>C NMR (75 MHz) Spectrum of component CB01 (in CDCl<sub>3</sub>)

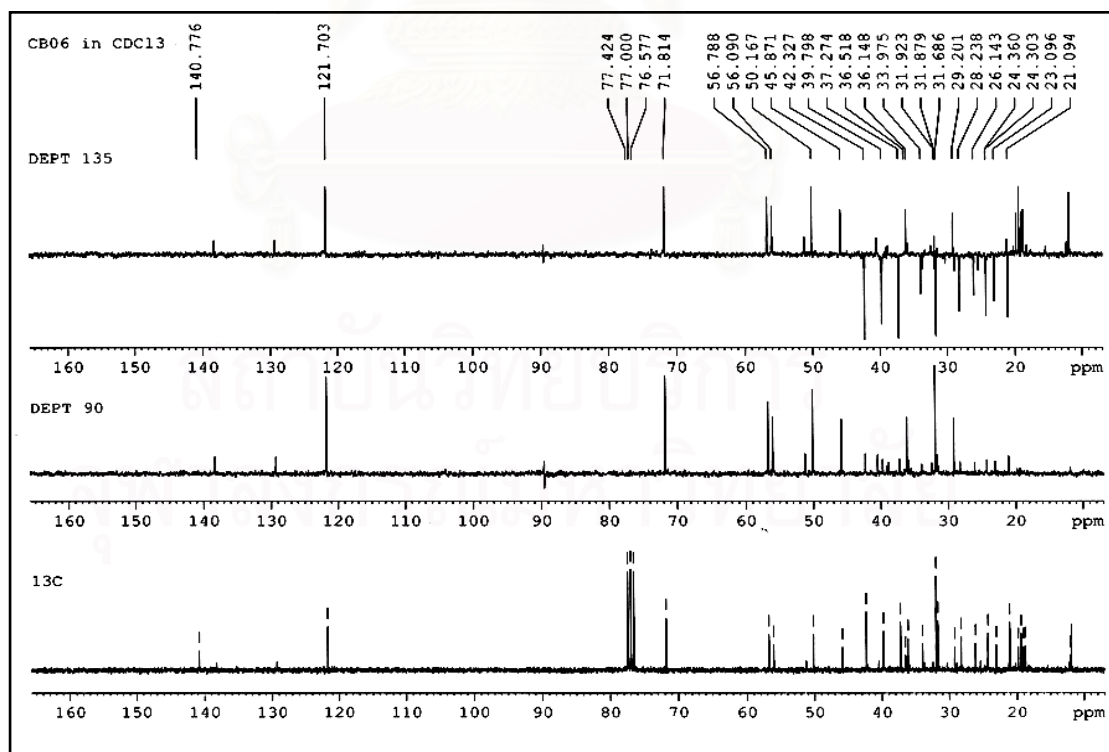


Figure 7. DEPT 90 and 135 Spectrum of component CB01

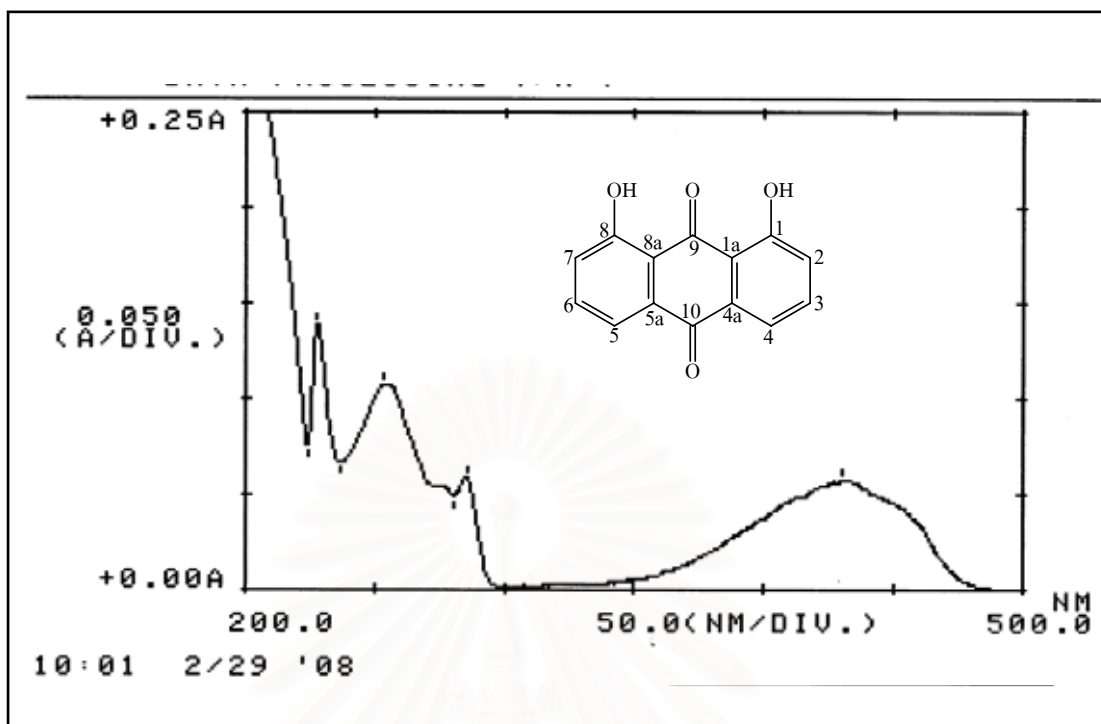


Figure 8. UV Spectrum of compound CB02 (in CDCl<sub>3</sub>)

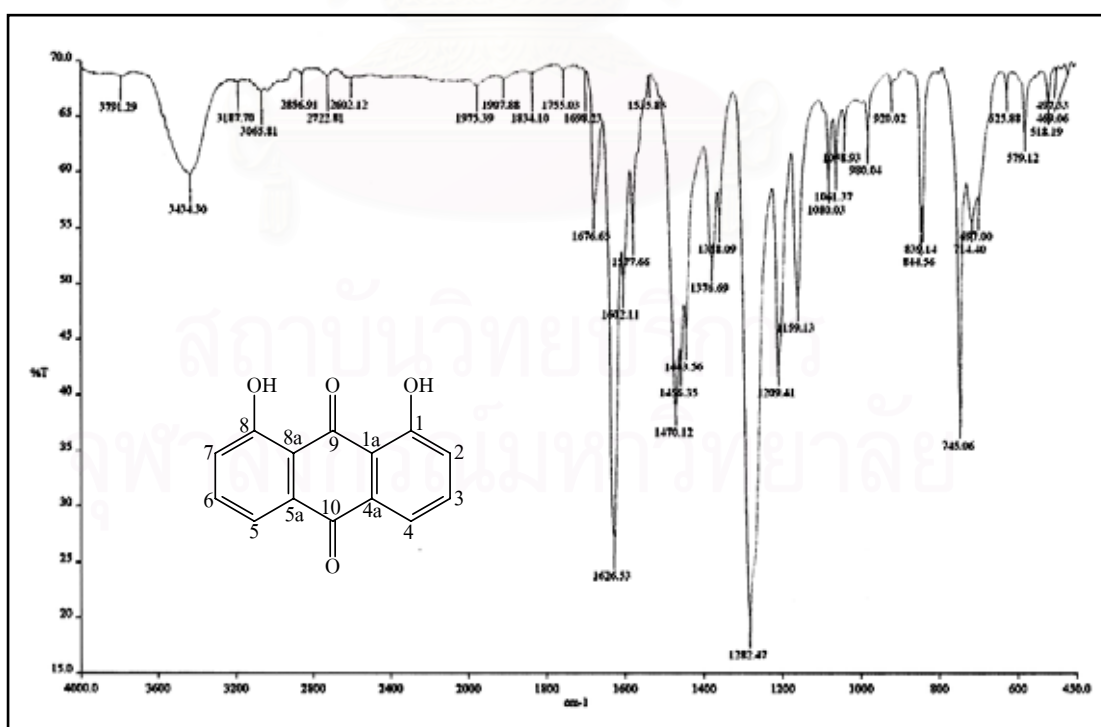


Figure 9. IR Spectrum of compound CB02 (KBr disc)

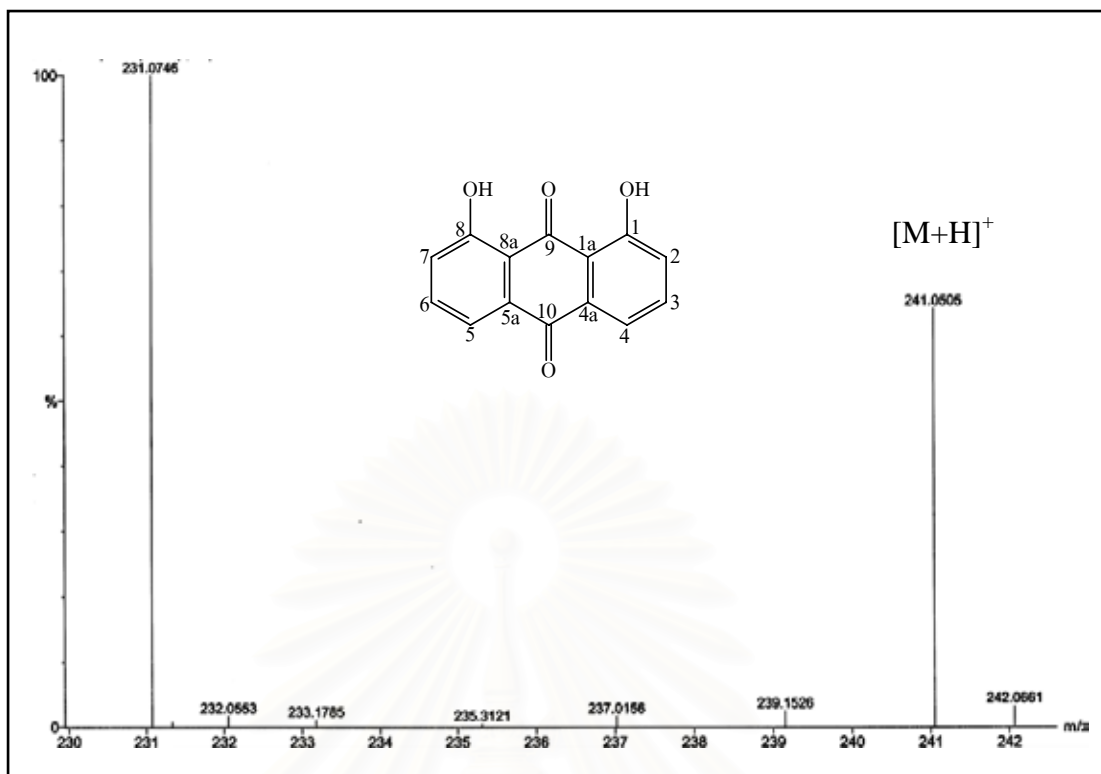


Figure 10. ESI TOF Mass spectrum of compound CB02

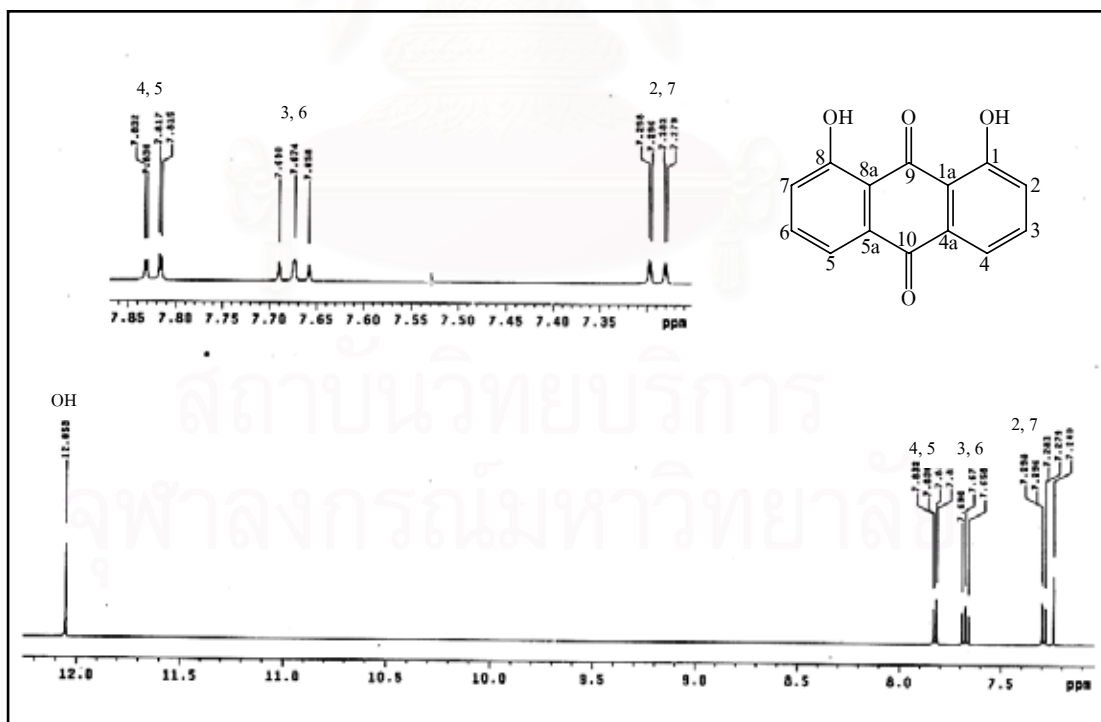


Figure 11.  $^1\text{H-NMR}$  (500 MHz) Spectrum of compound CB02 (in  $\text{CDCl}_3$ )

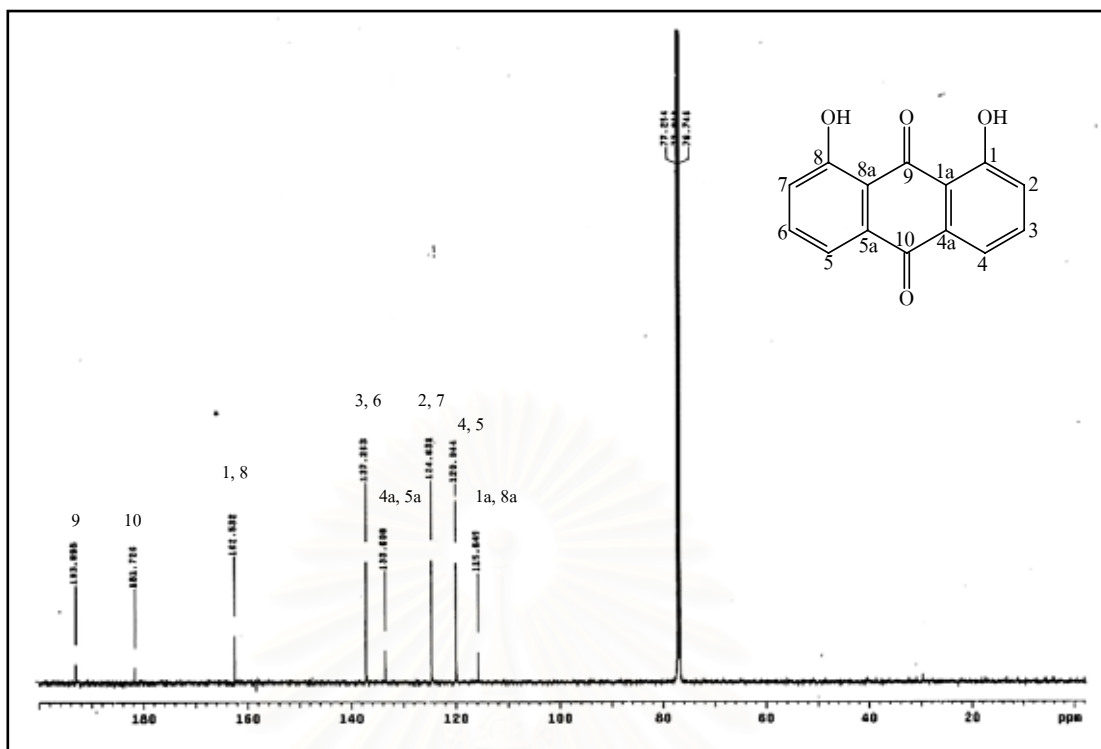


Figure 12.  $^{13}\text{C}$ -NMR (500 MHz) Spectrum of compound CB02 (in  $\text{CDCl}_3$ )

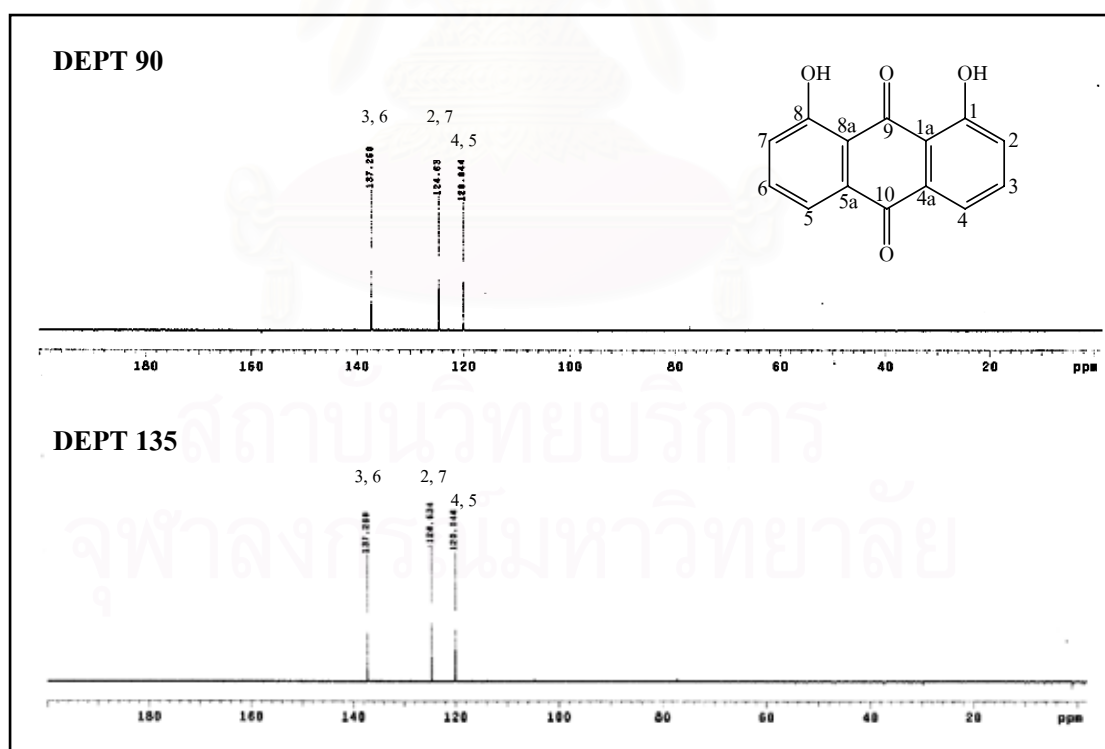


Figure 13. DEPT 90 and 135 Spectrum of compound CB02



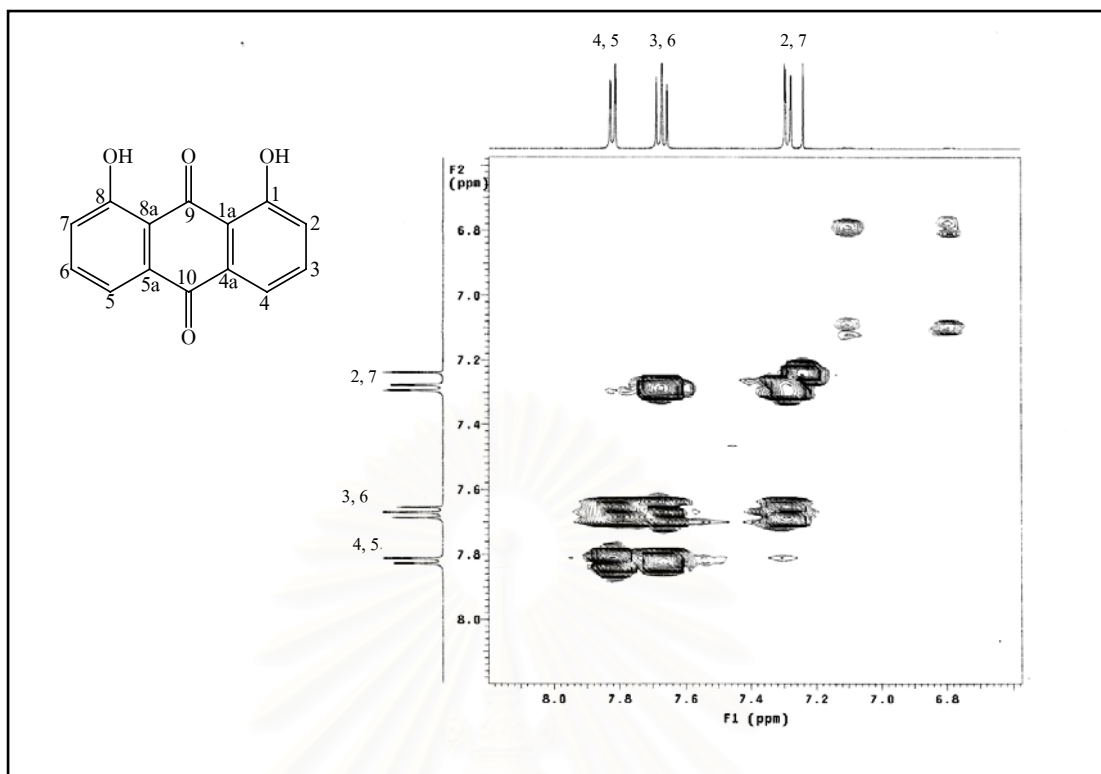


Figure 14.  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of compound CB02

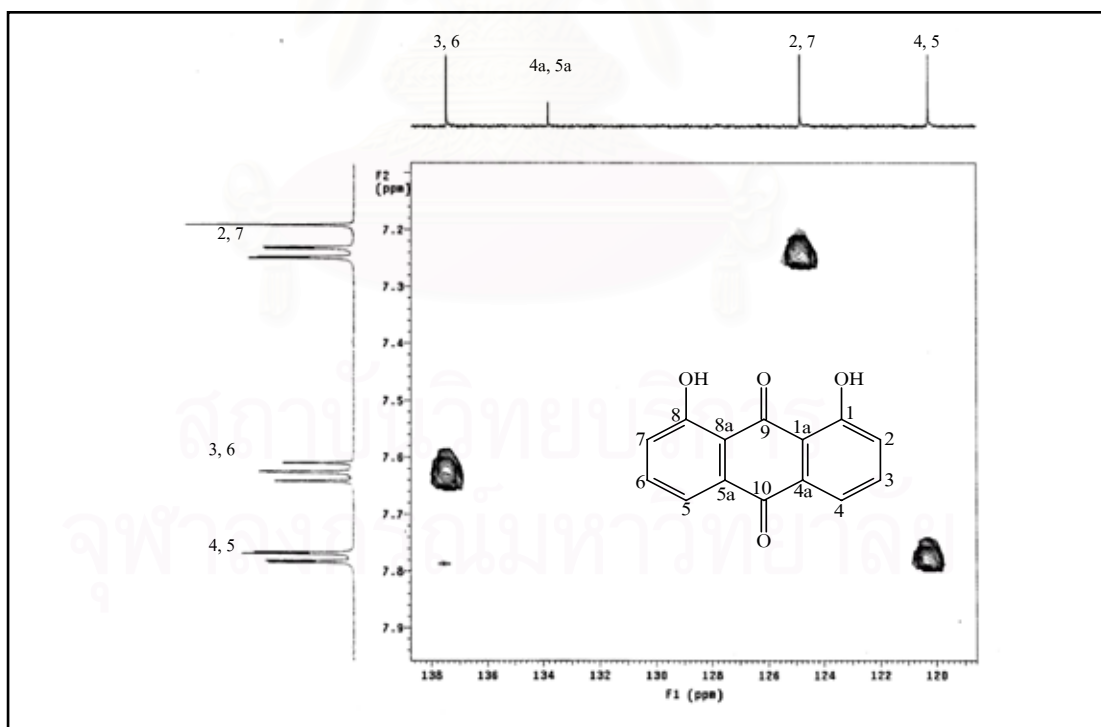


Figure 15. HSQC Spectrum of compound CB02

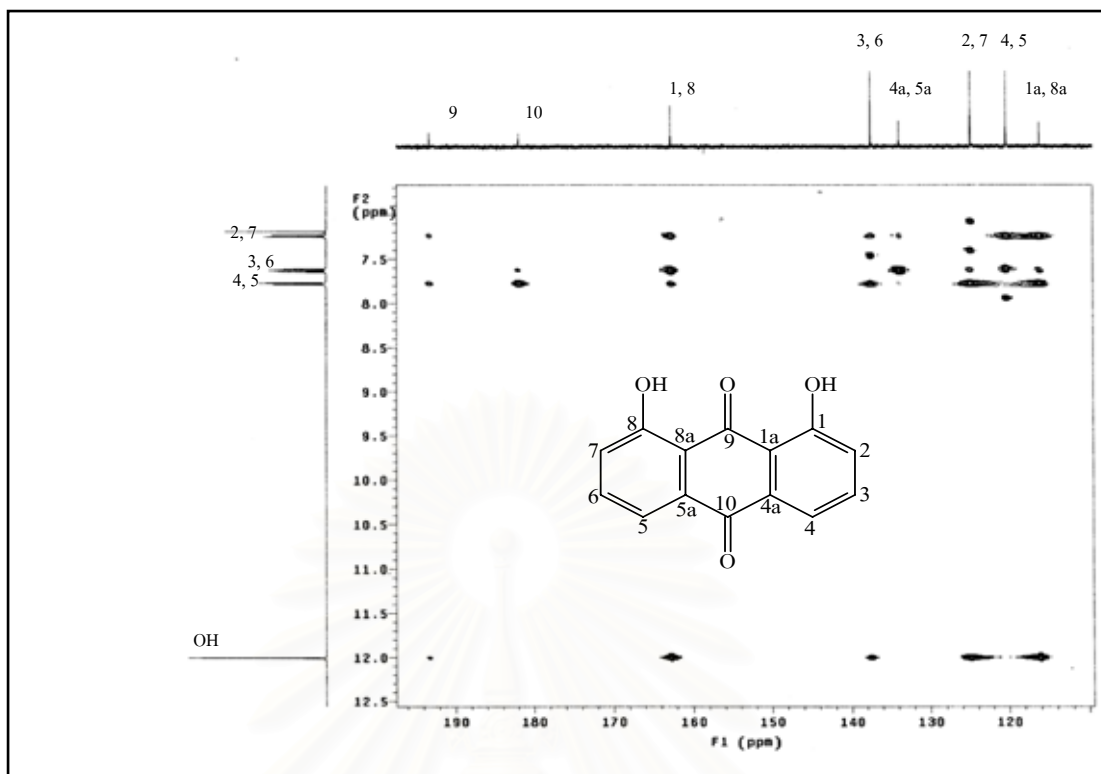


Figure 16. HMBC Spectrum of compound CB02

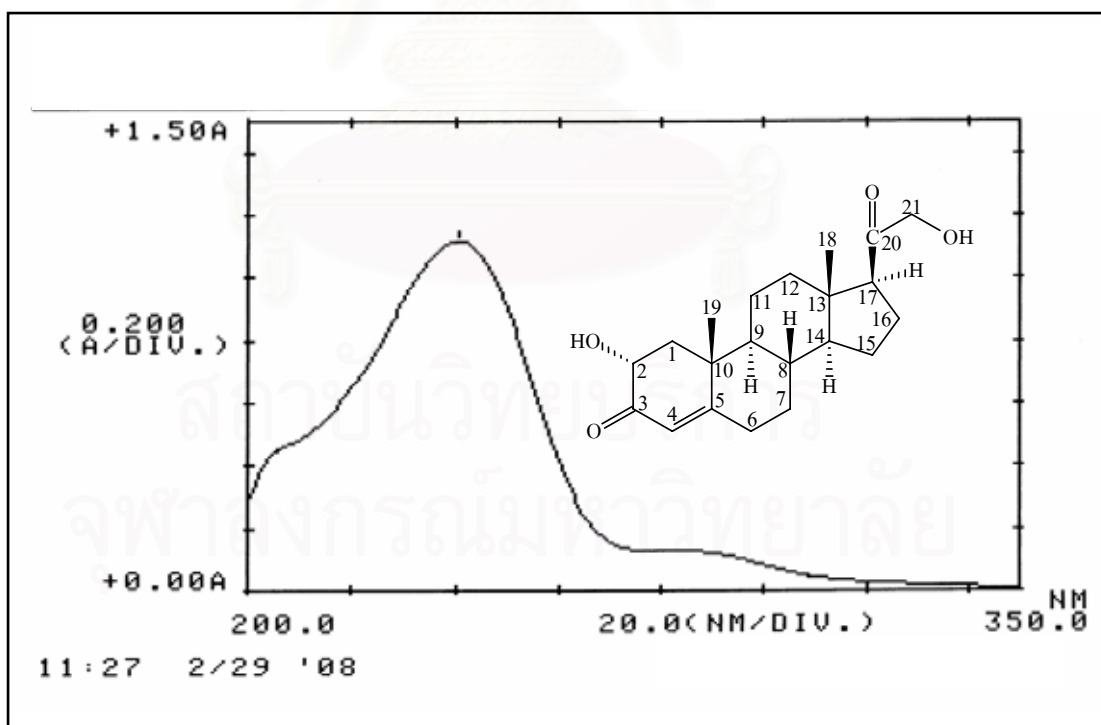


Figure 17. UV Spectrum of compound CB03 (in MeOH)

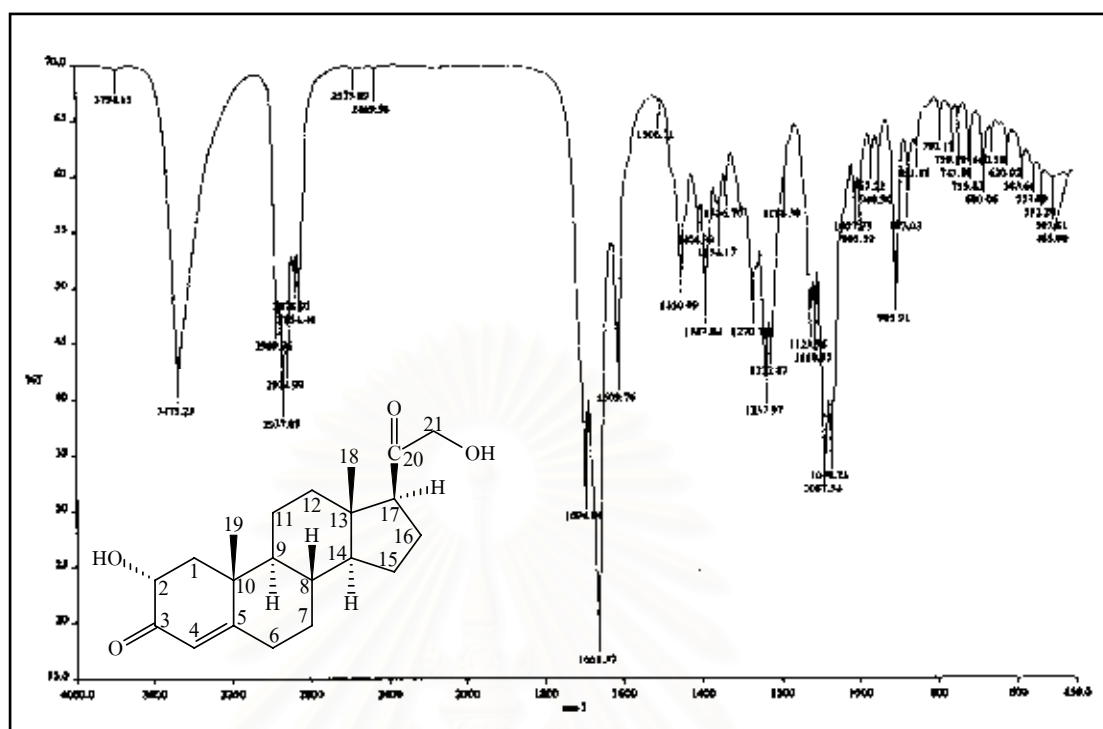


Figure 18. IR Spectrum of compound CB03 (KBr disc)

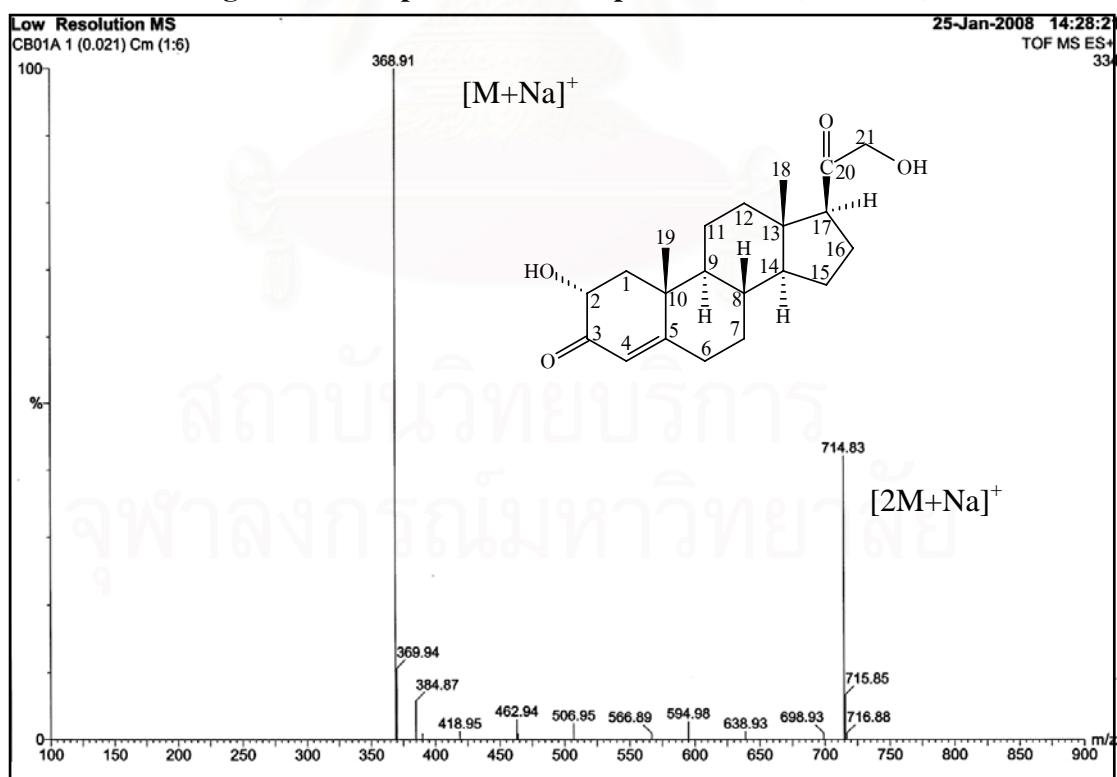


Figure 19. ESI TOF Mass spectrum of compound CB03

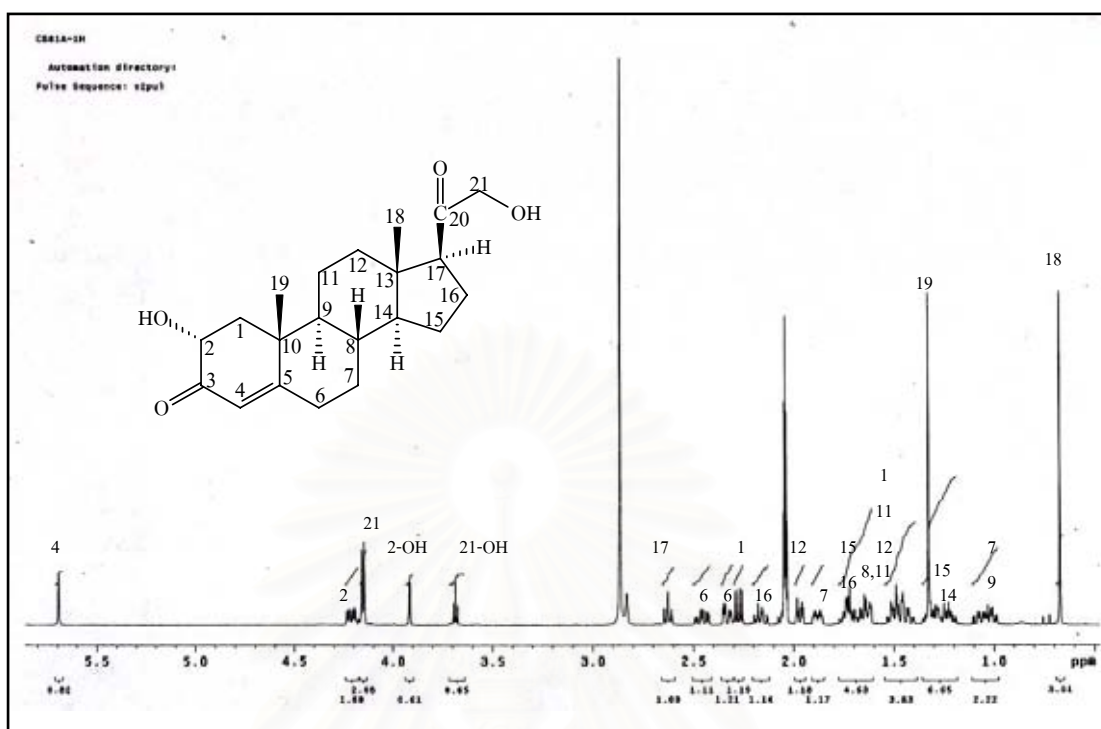


Figure 20.  $^1\text{H-NMR}$  (500 MHz) Spectrum of compound CB03 (in acetone- $d_6$ )

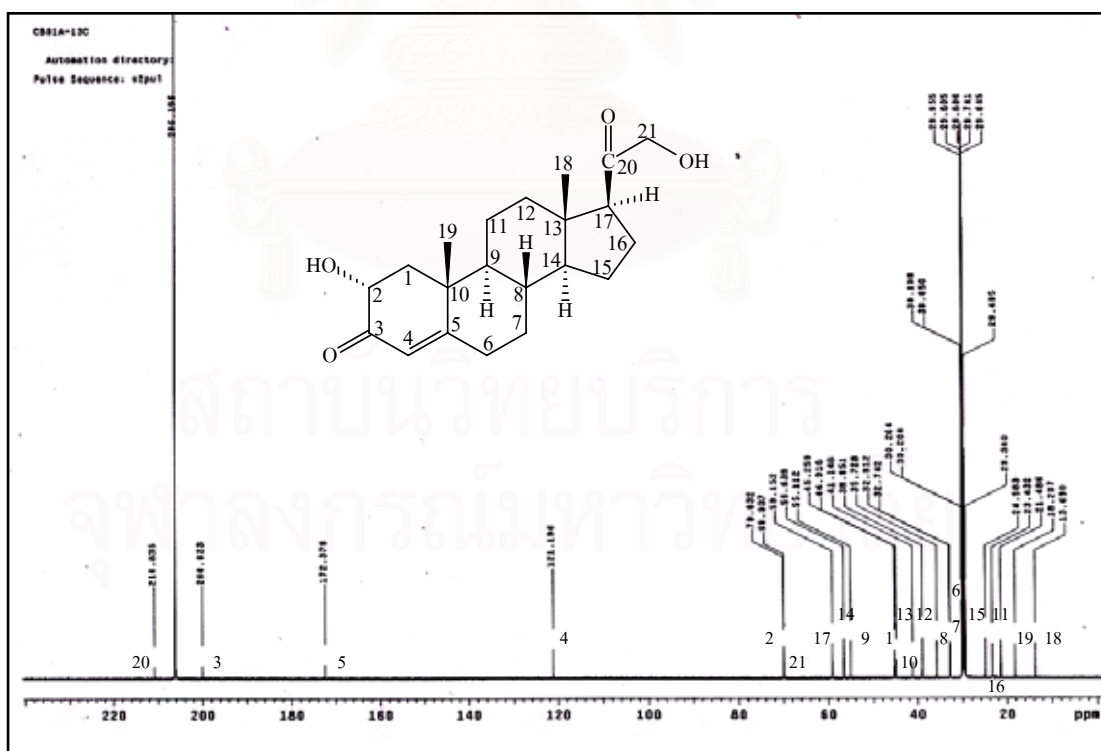


Figure 21.  $^{13}\text{C-NMR}$  (500 MHz) Spectrum of compound CB03 (in acetone- $d_6$ )

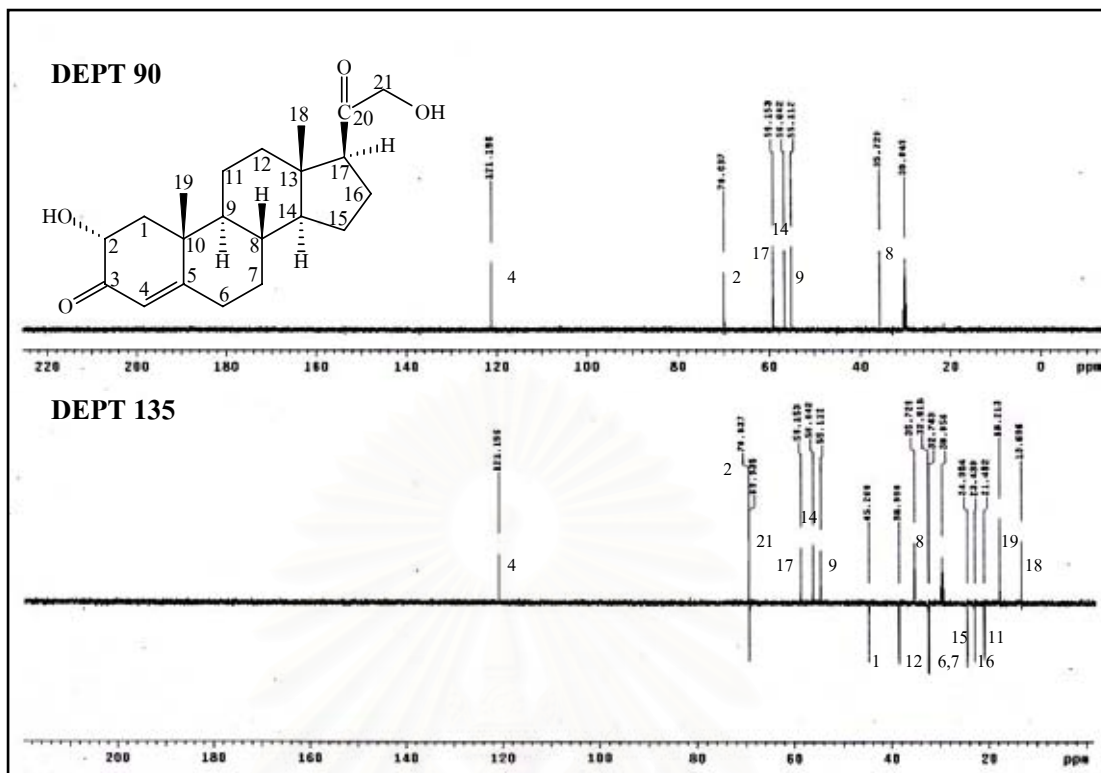


Figure 22. DEPT 90 and 135 Spectrum of compound CB03

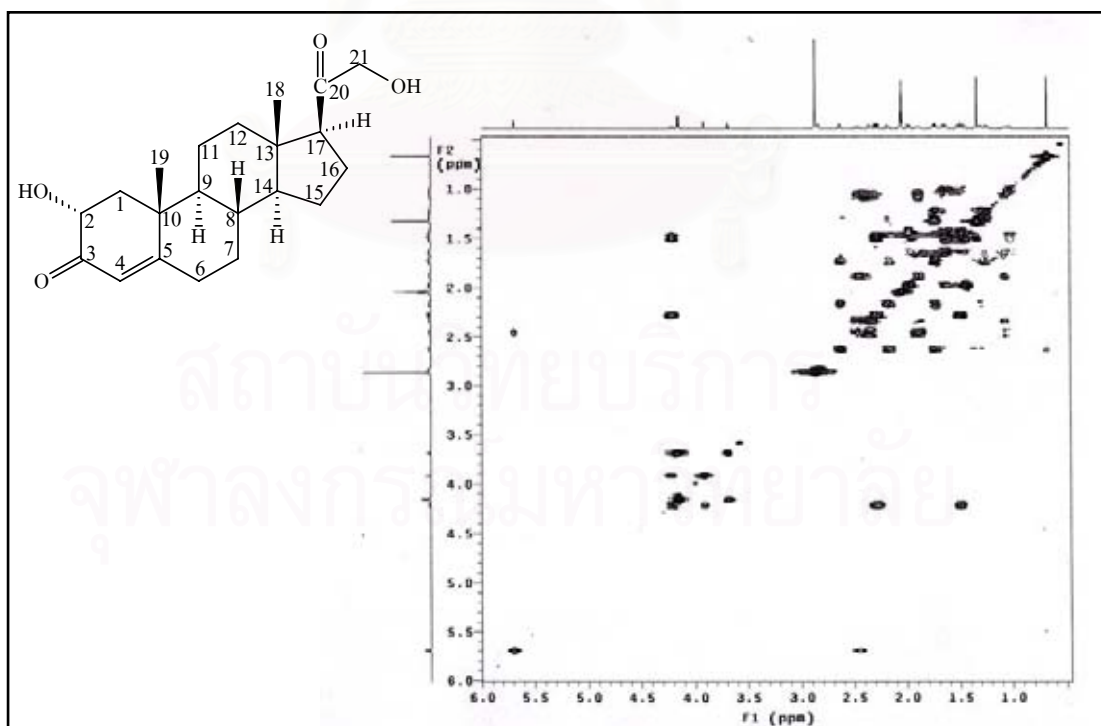


Figure 23.  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of compound CB03

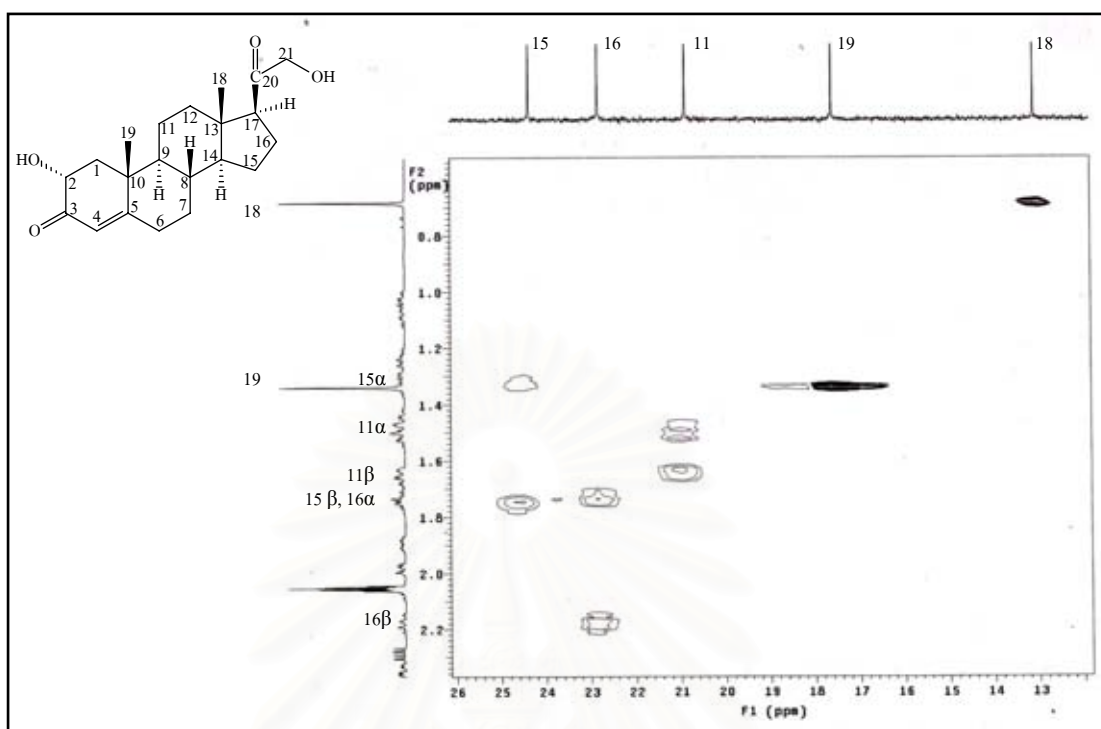


Figure 24. HSQC Spectrum of compound CB03

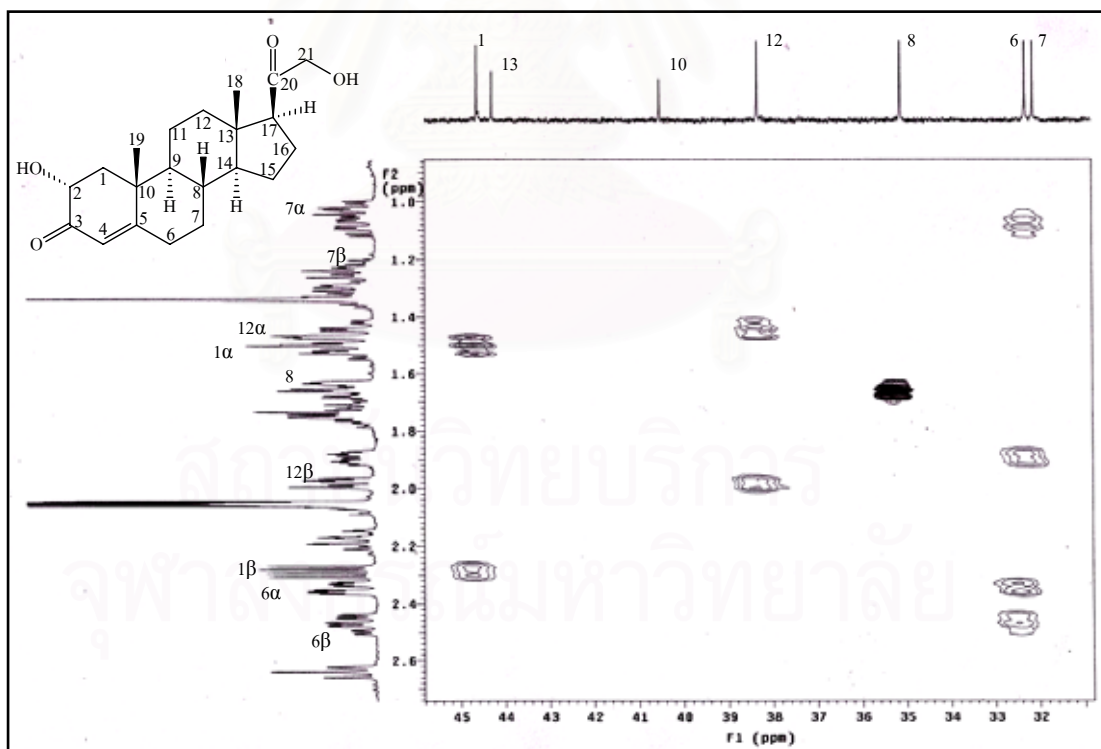


Figure 25. HSQC Spectrum of compound CB03 (expanded)

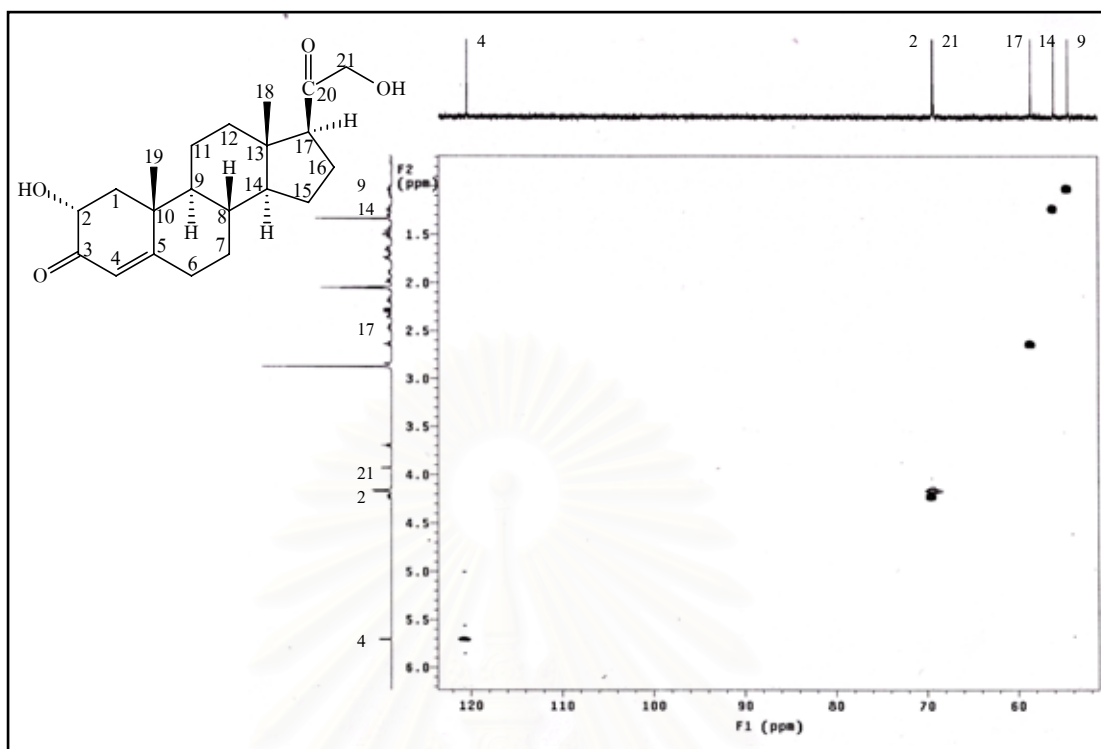


Figure 26. HSQC Spectrum of compound CB03 (expanded)

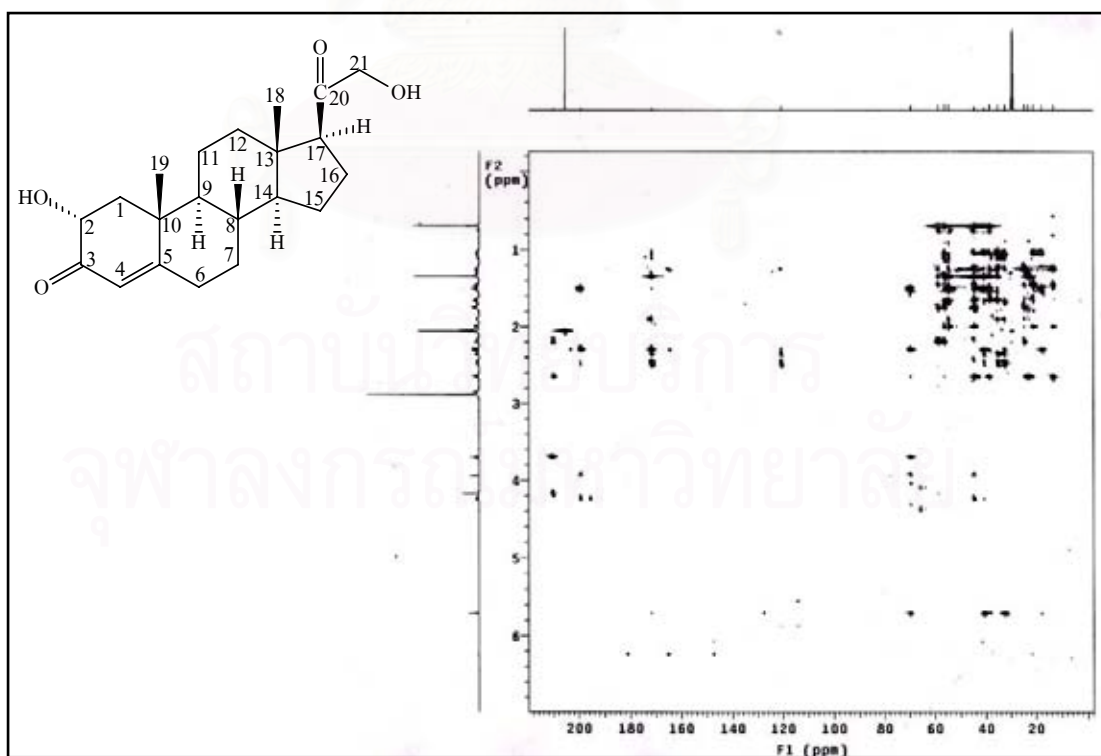


Figure 27. HMBC Spectrum of compound CB03

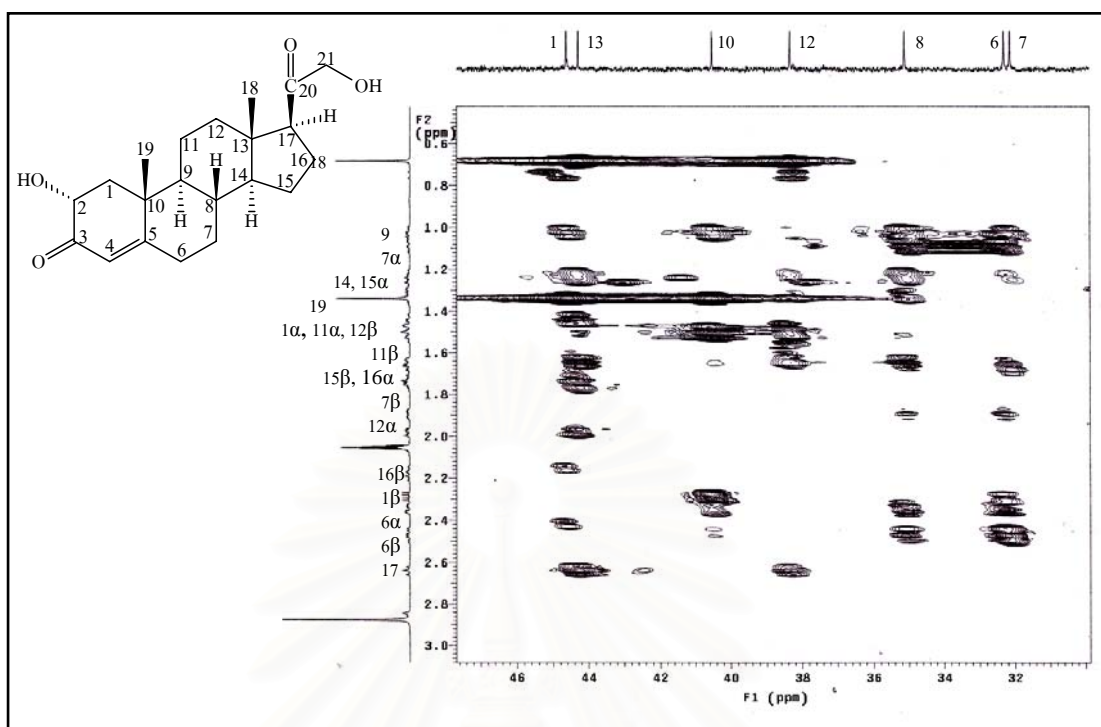


Figure 28. HMBC Spectrum of compound CB03 (expanded)

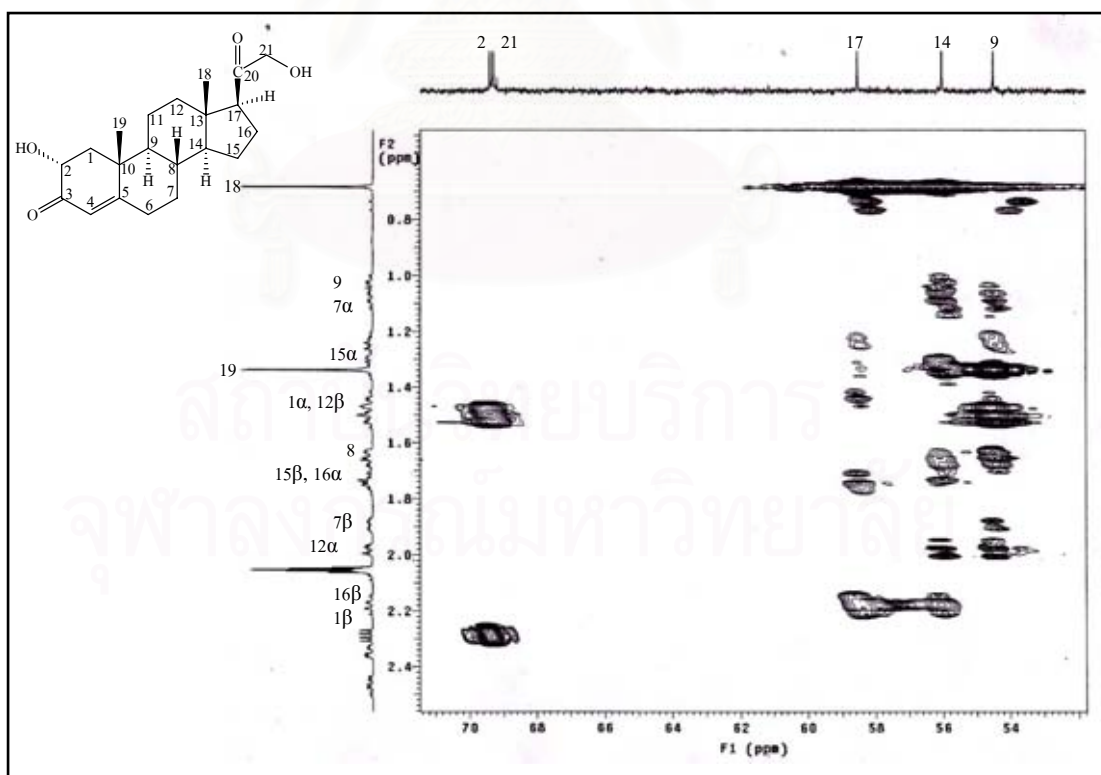


Figure 29. HMBC Spectrum of compound CB03 (expanded)



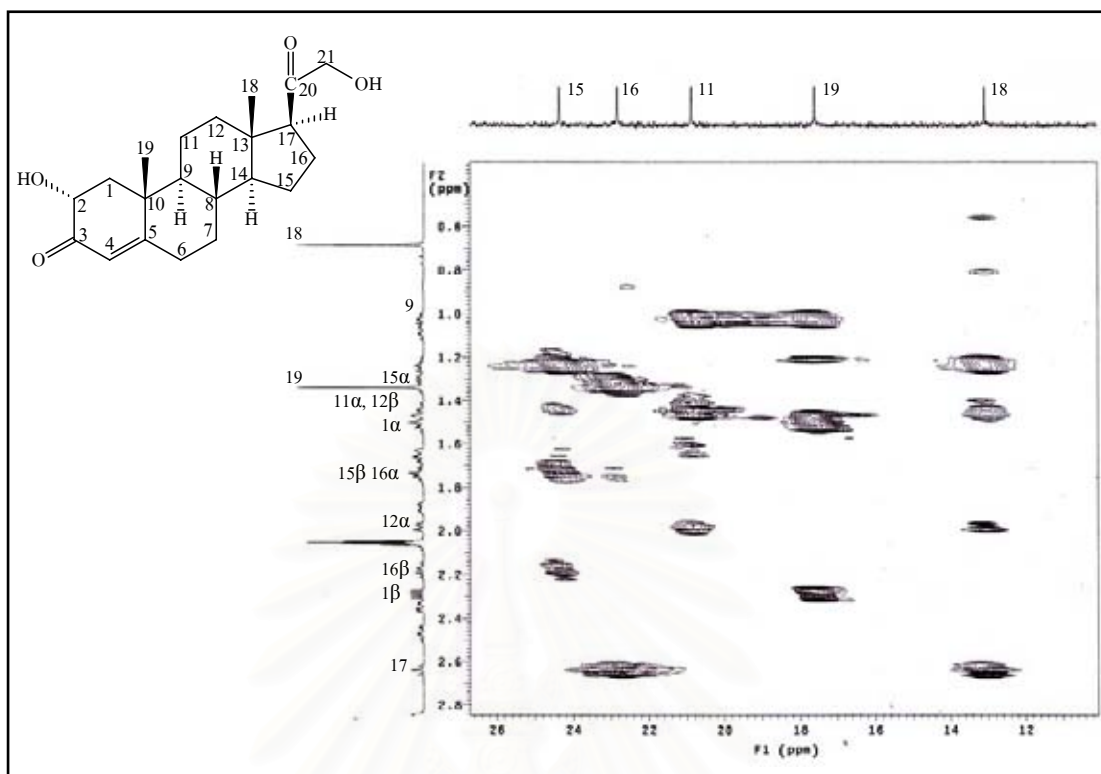


Figure 30. HMBC Spectrum of compound CB03 (expanded)

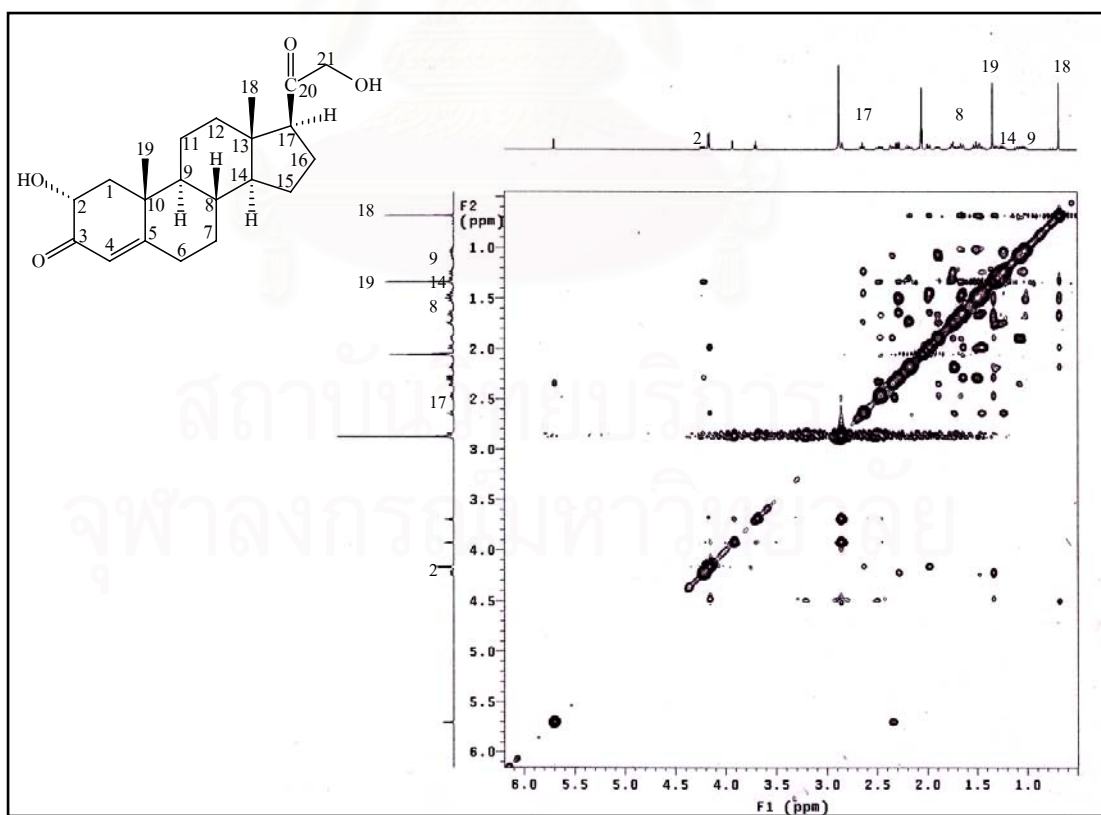


Figure 31. NOESY Spectrum of compound CB03

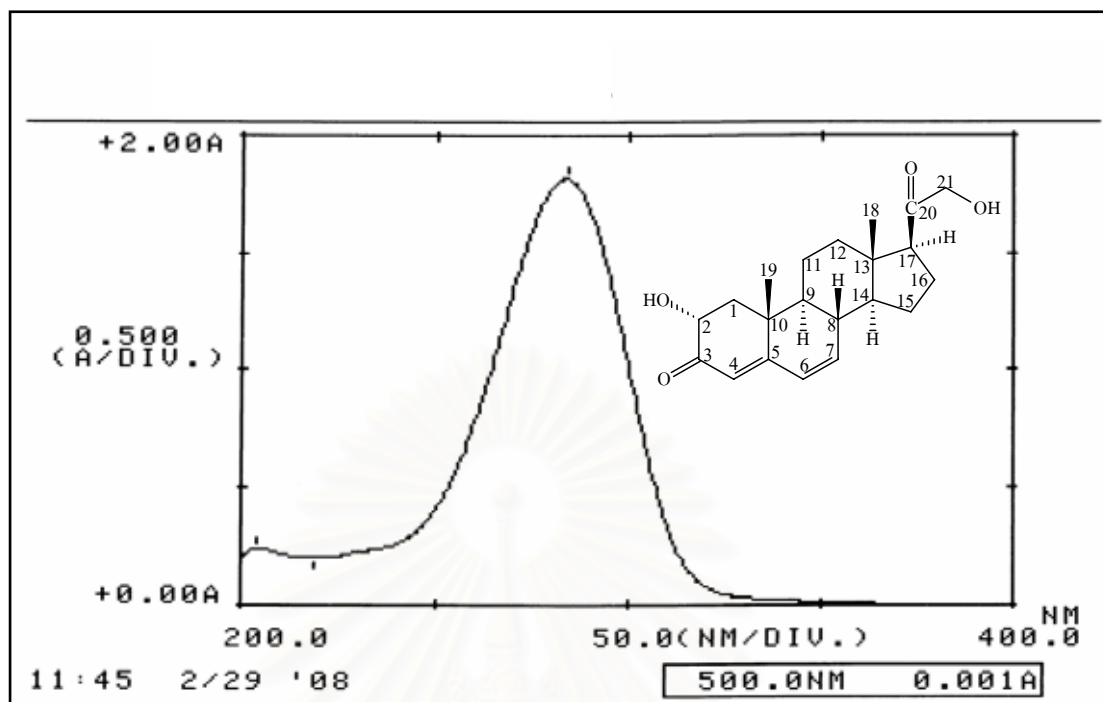


Figure 32. UV Spectrum of compound CB04 (in MeOH)

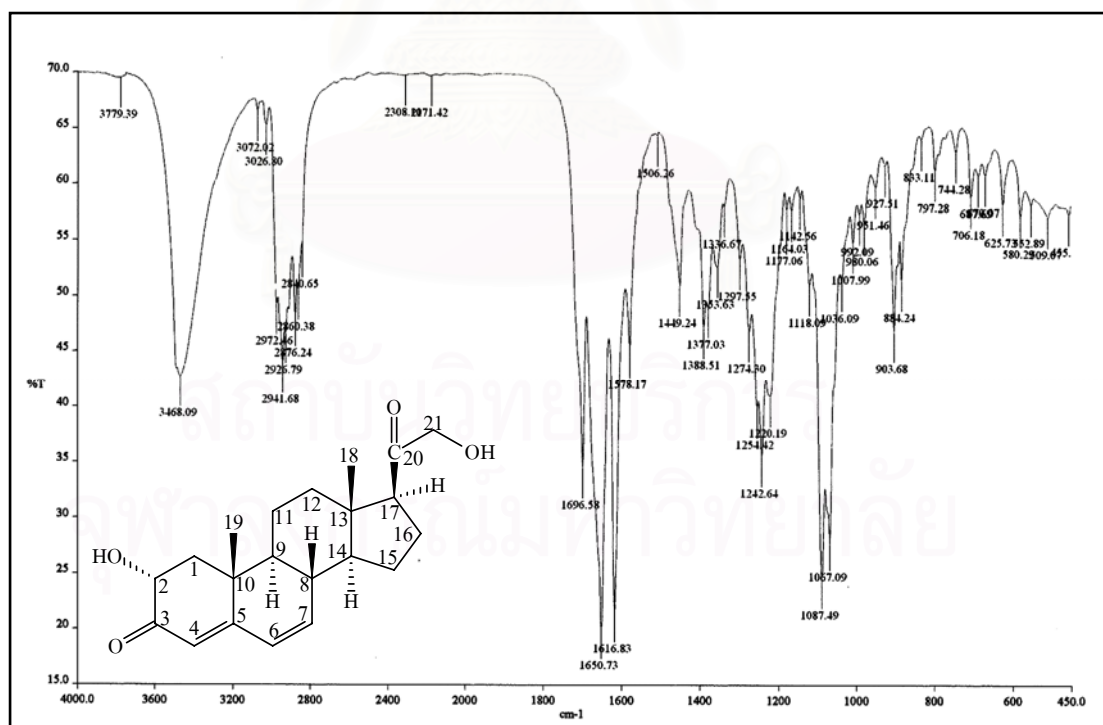


Figure 33. IR Spectrum of compound CB04 (KBr disc)

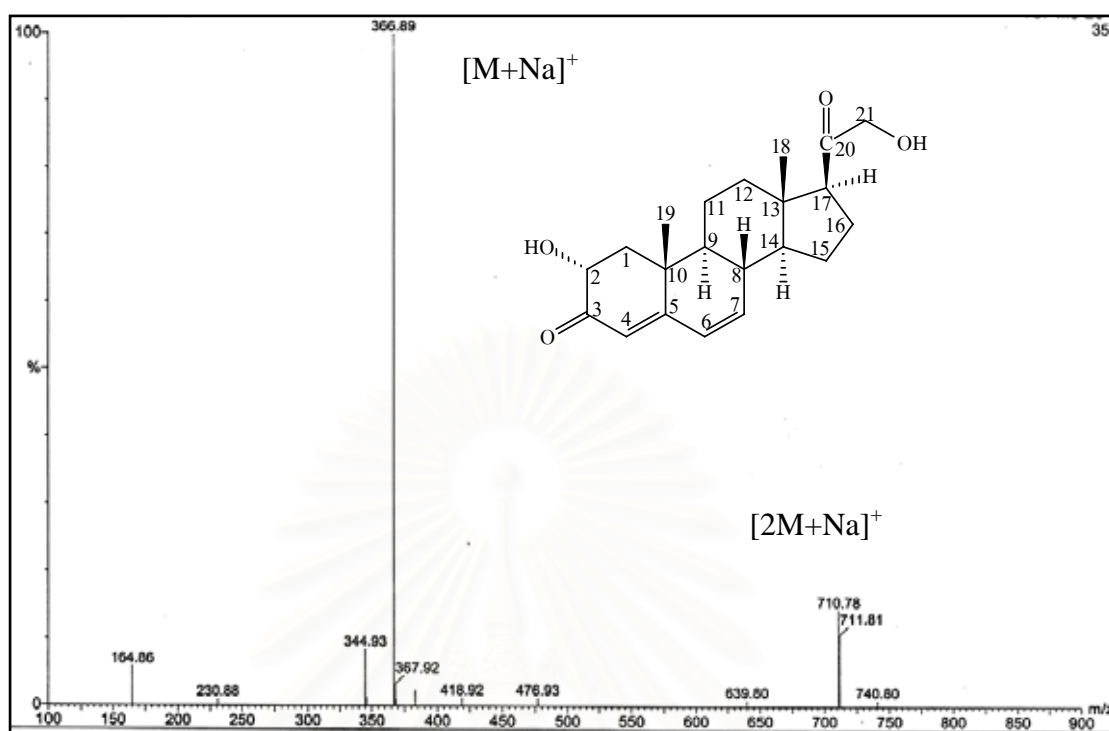


Figure 34. ESI TOF Mass spectrum of compound CB04

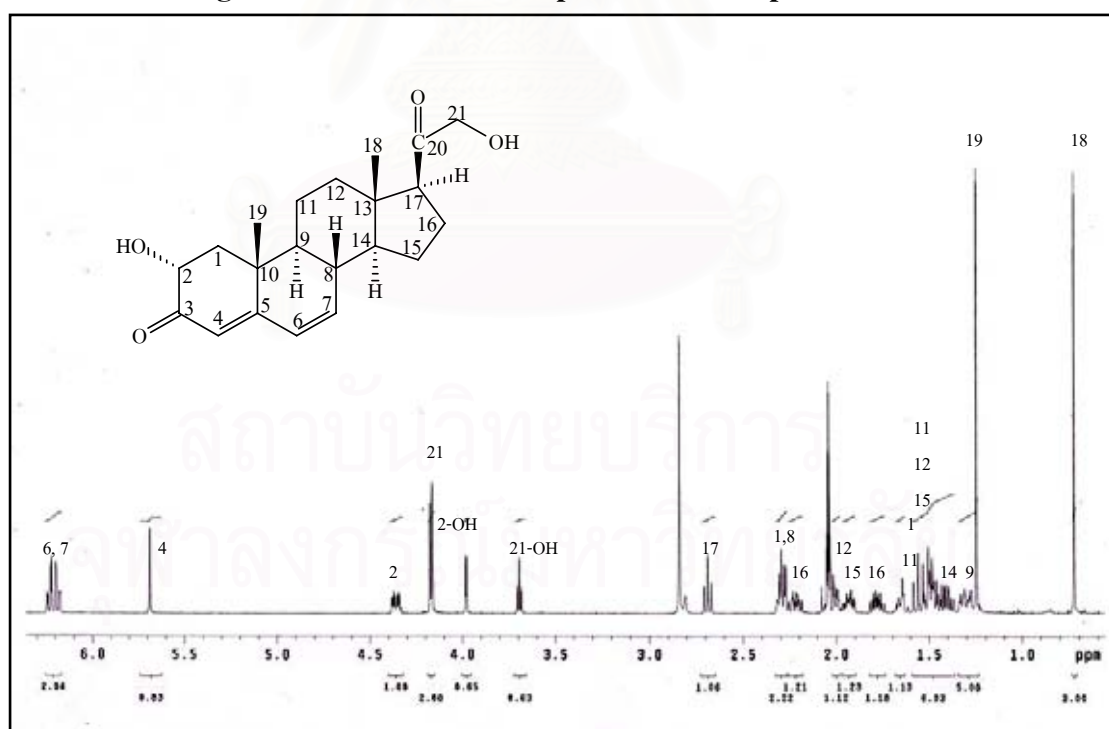


Figure 35.  $^1\text{H-NMR}$  (500 MHz) Spectrum of compound CB04 (in acetone- $d_6$ )

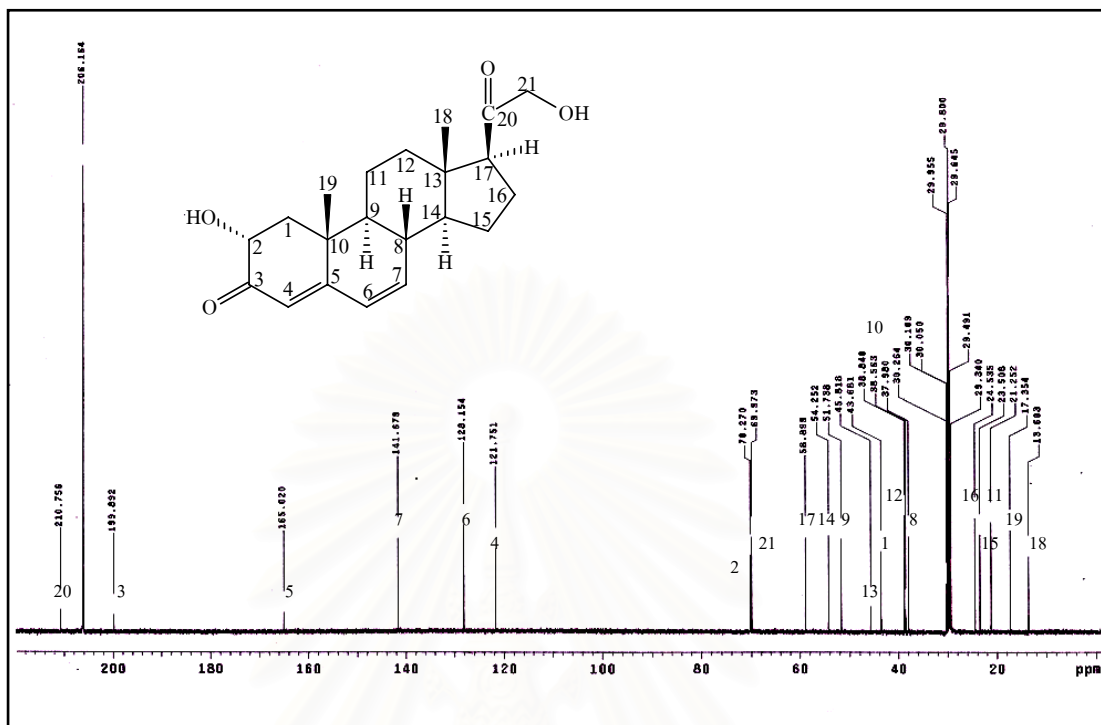


Figure 36.  $^{13}\text{C-NMR}$  (500 MHz) Spectrum of compound CB04 (in acetone- $d_6$ )

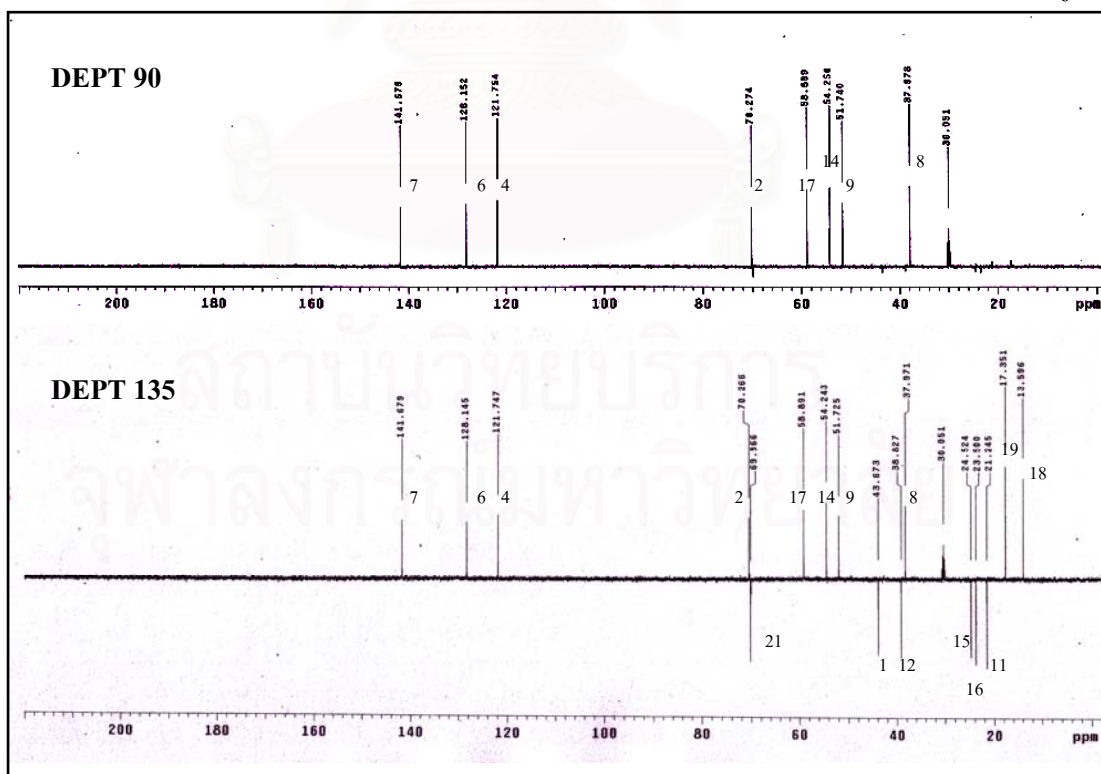


Figure 37. DEPT 90 and 135 Spectrum of compound CB04

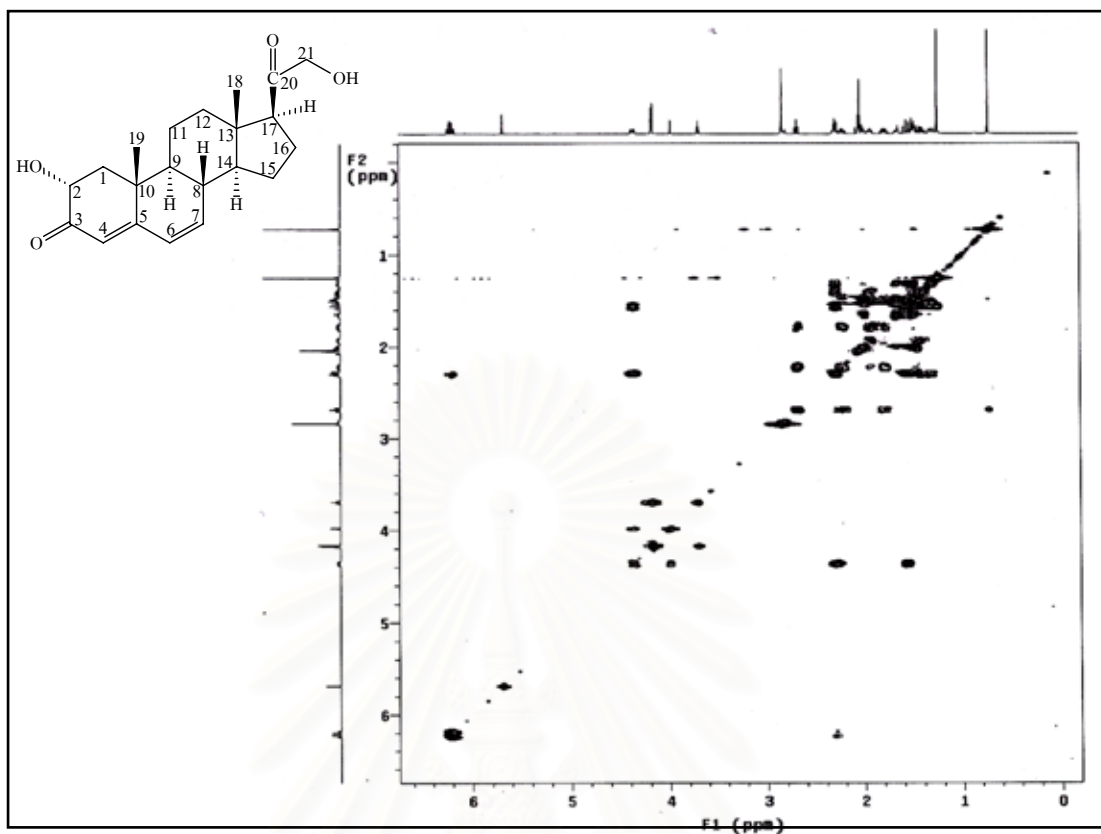


Figure 38.  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of compound CB04

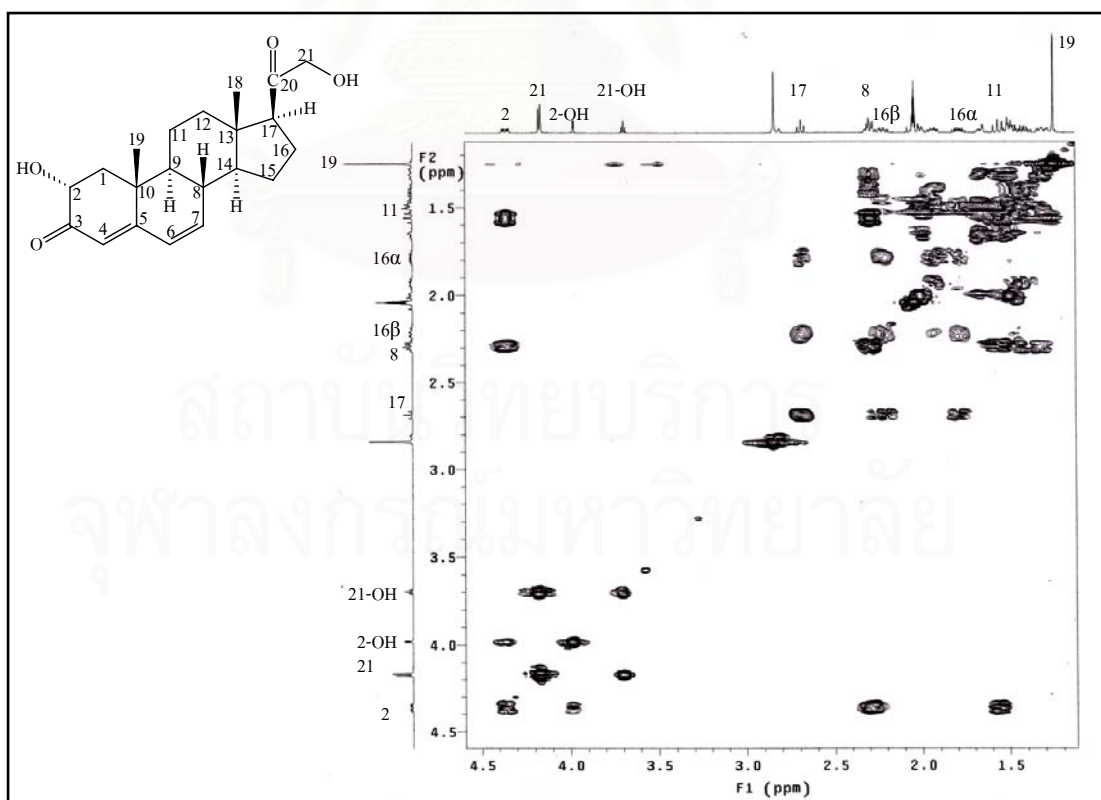


Figure 39.  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of compound CB04 (expanded)

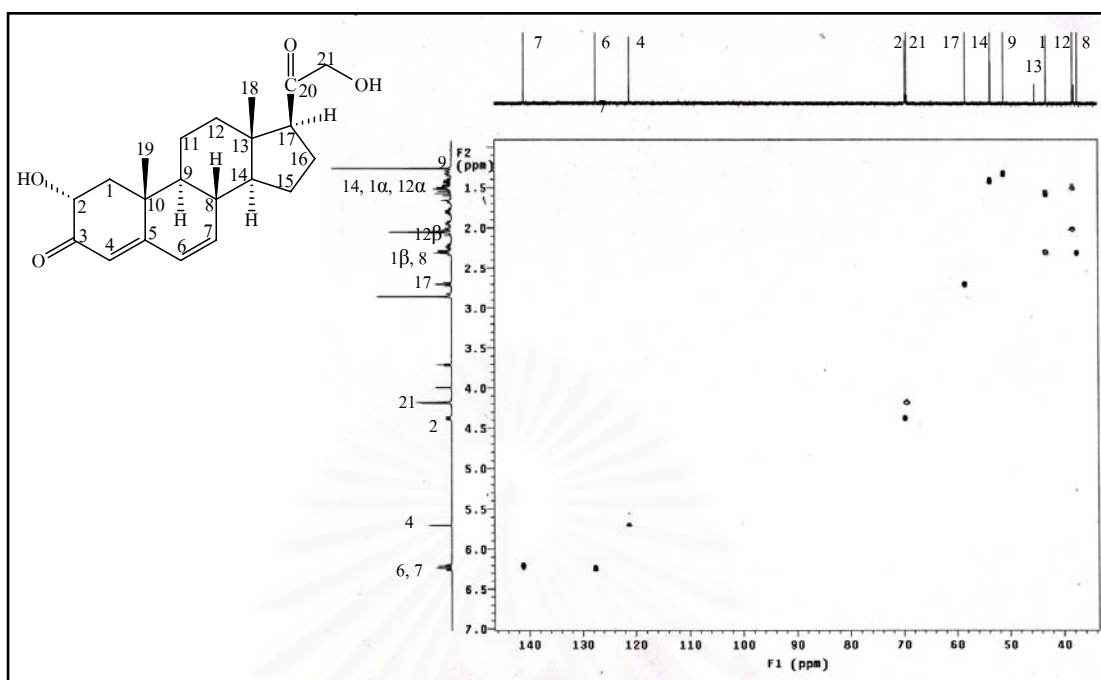


Figure 40. HSQC Spectrum of compound CB04

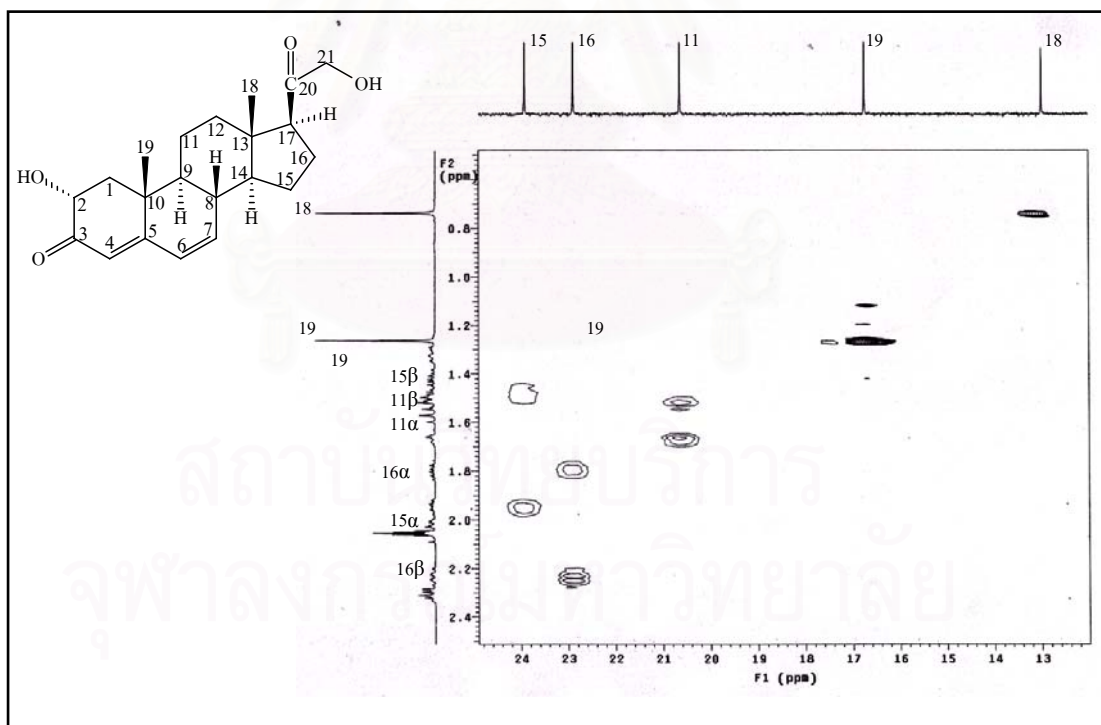


Figure 41. HSQC Spectrum of compound CB04 (expanded)

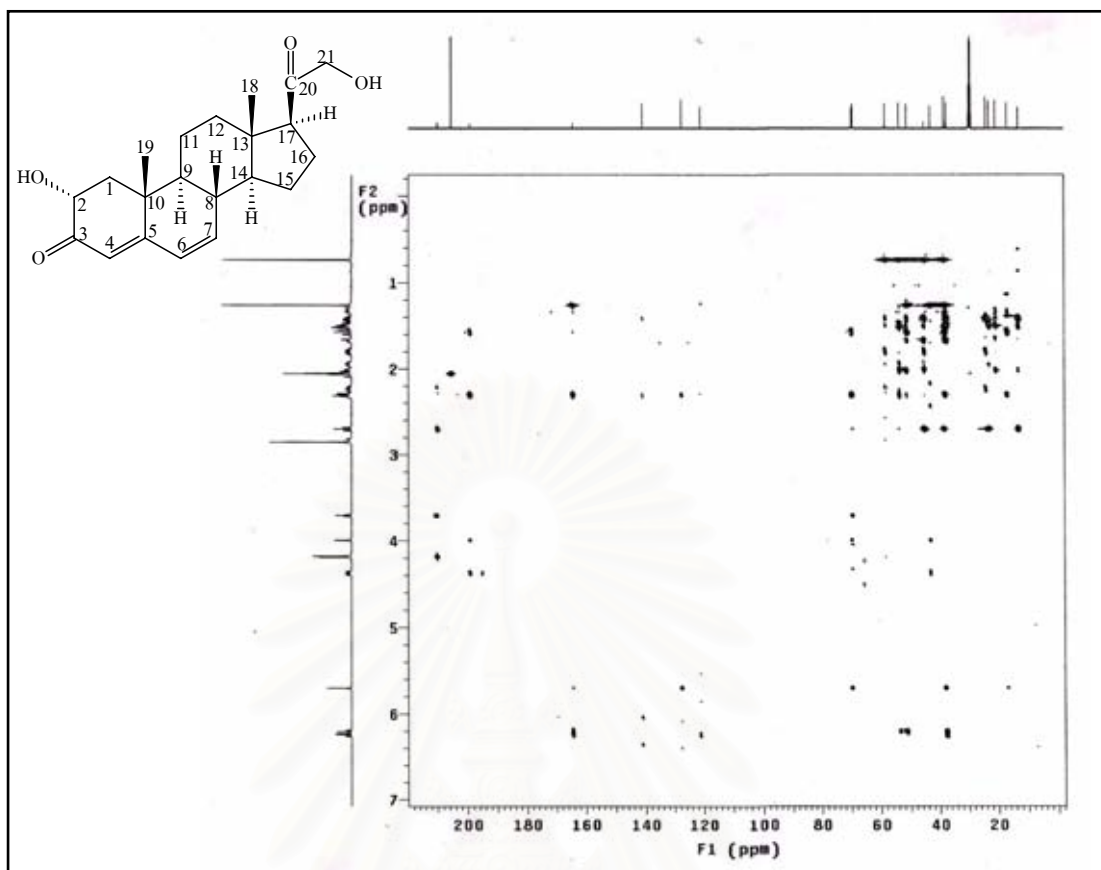


Figure 42. HMBC Spectrum of compound CB04

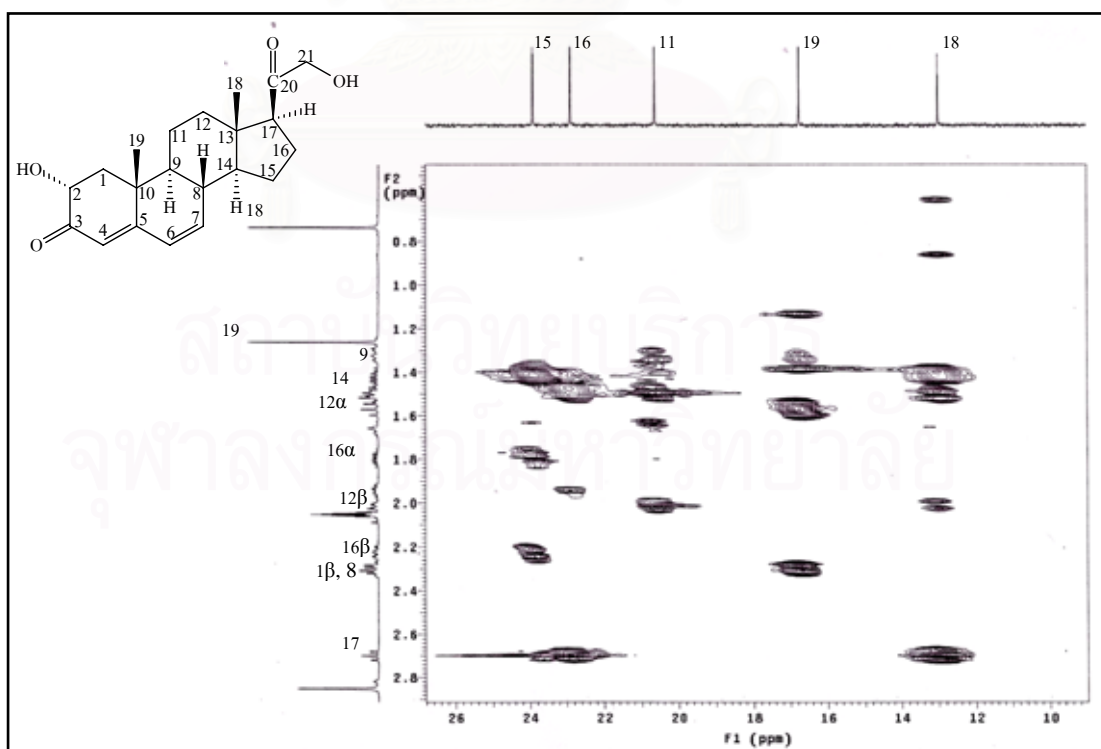


Figure 43. HMBC Spectrum of compound CB04 (expanded)

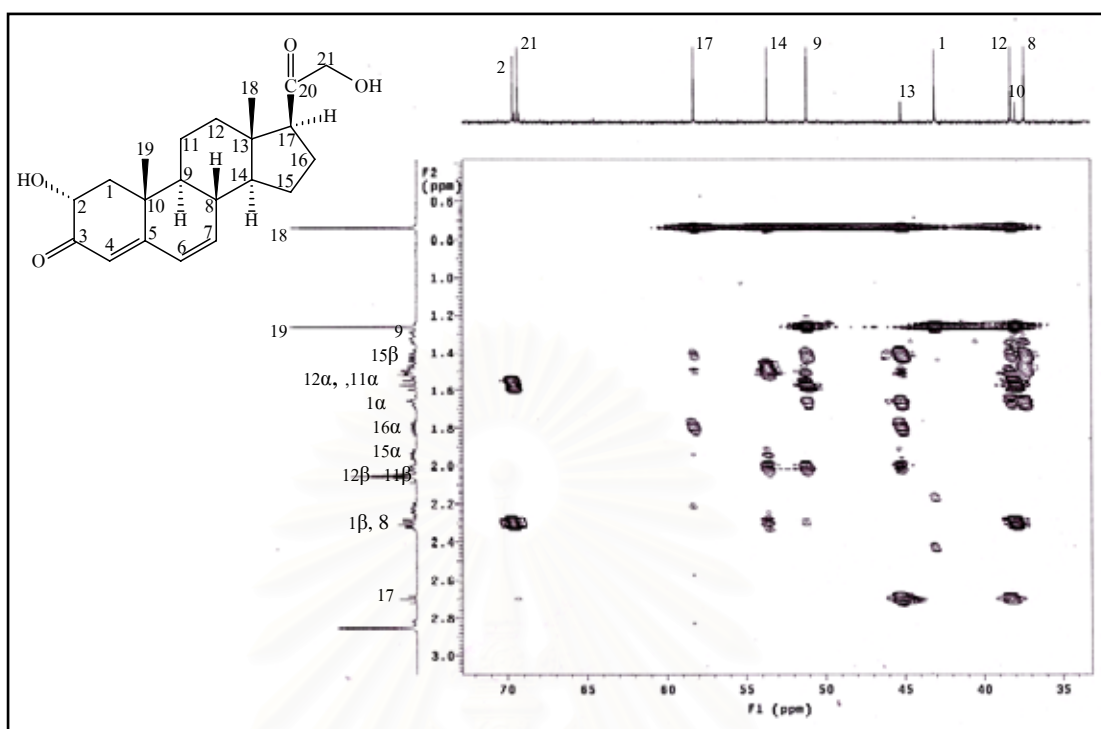


Figure 44. HMBC Spectrum of compound CB04 (expanded)

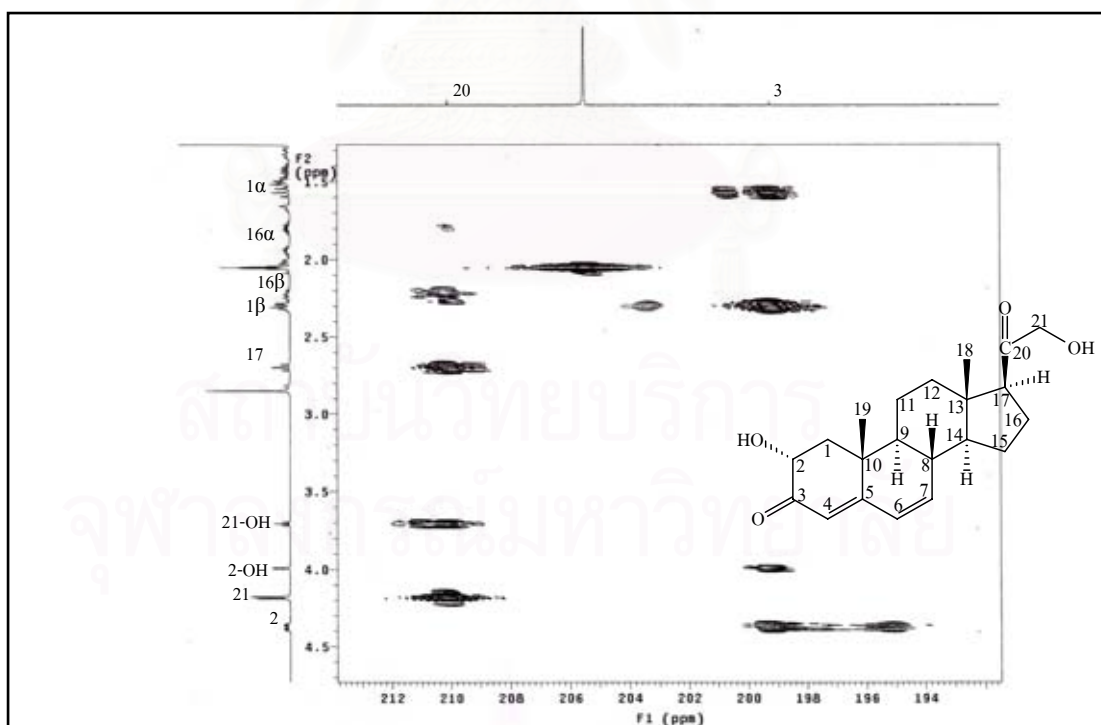


Figure 45. HMBC Spectrum of compound CB04 (expanded)



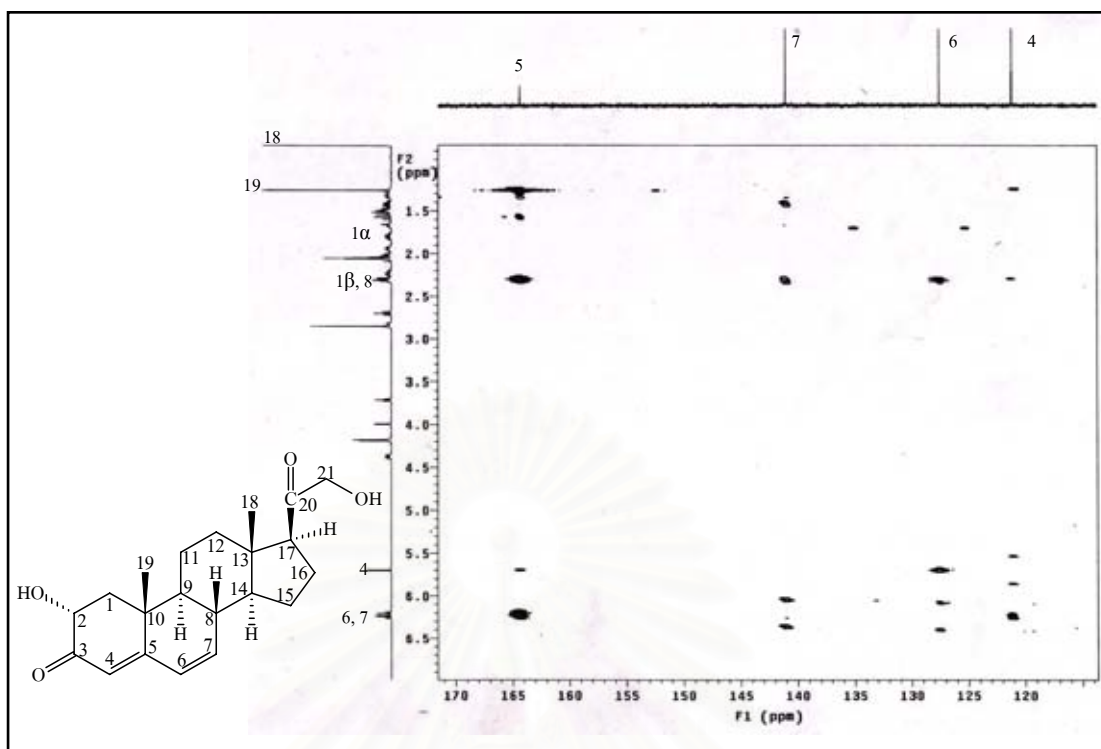


Figure 46. HMBC Spectrum of compound CB04 (expanded)

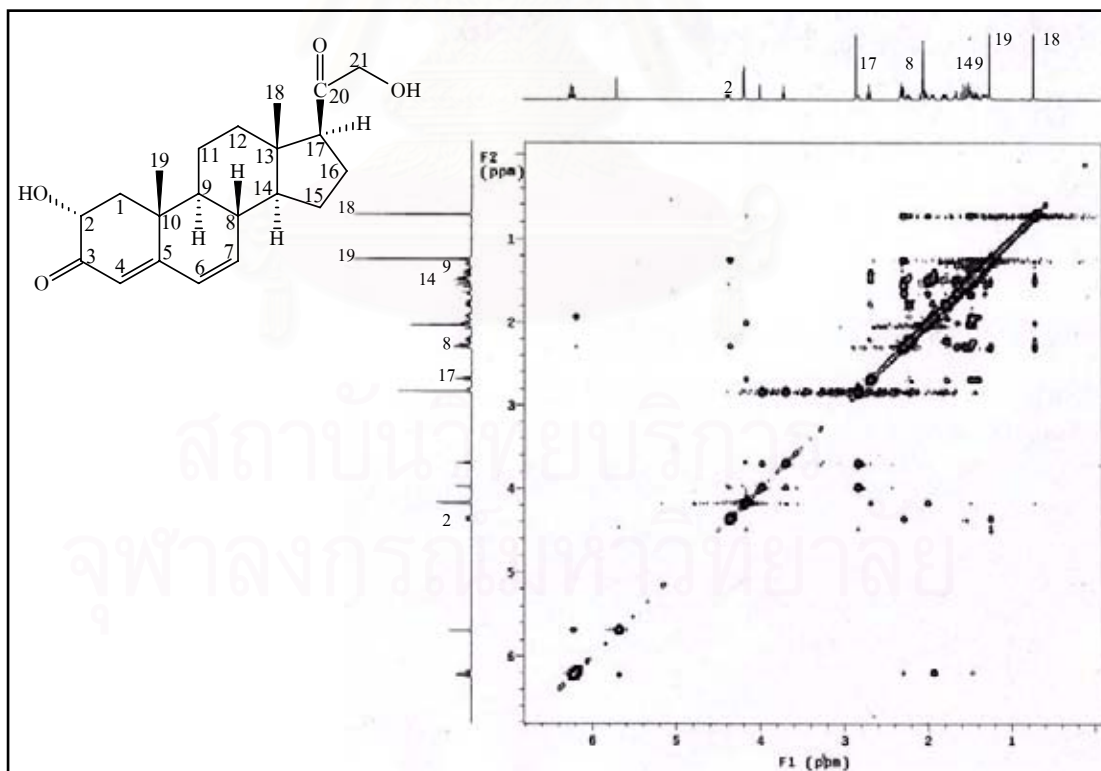


Figure 47. NOESY Spectrum of compound CB04

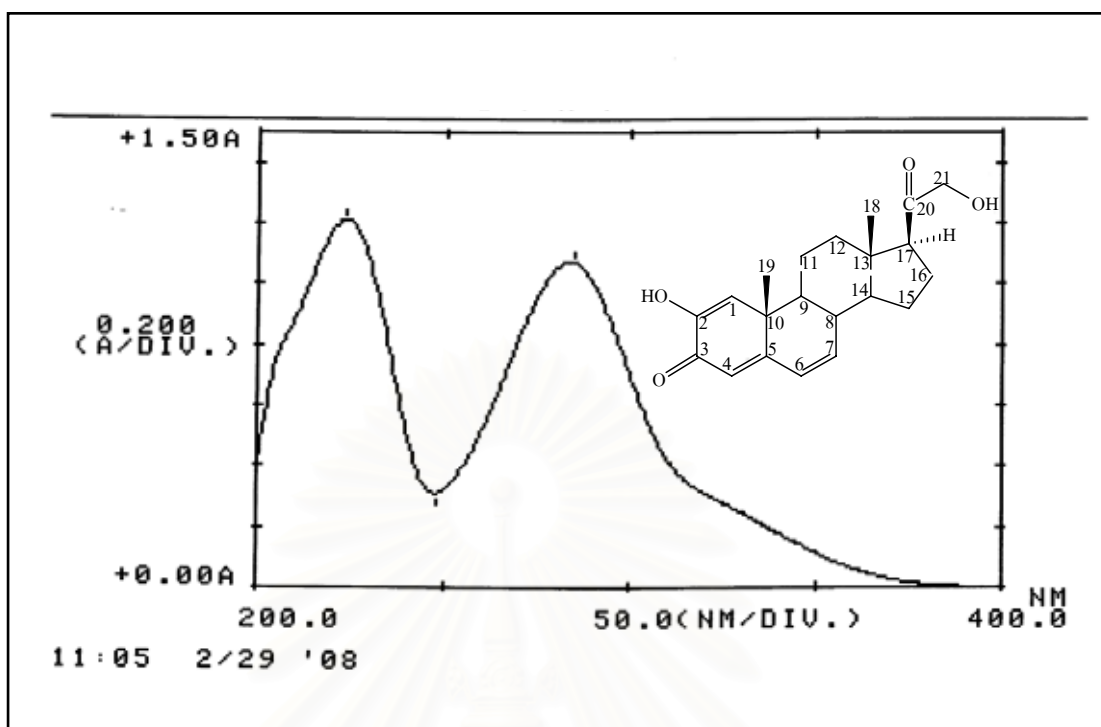


Figure 48. UV Spectrum of compound CB05 (in MeOH)

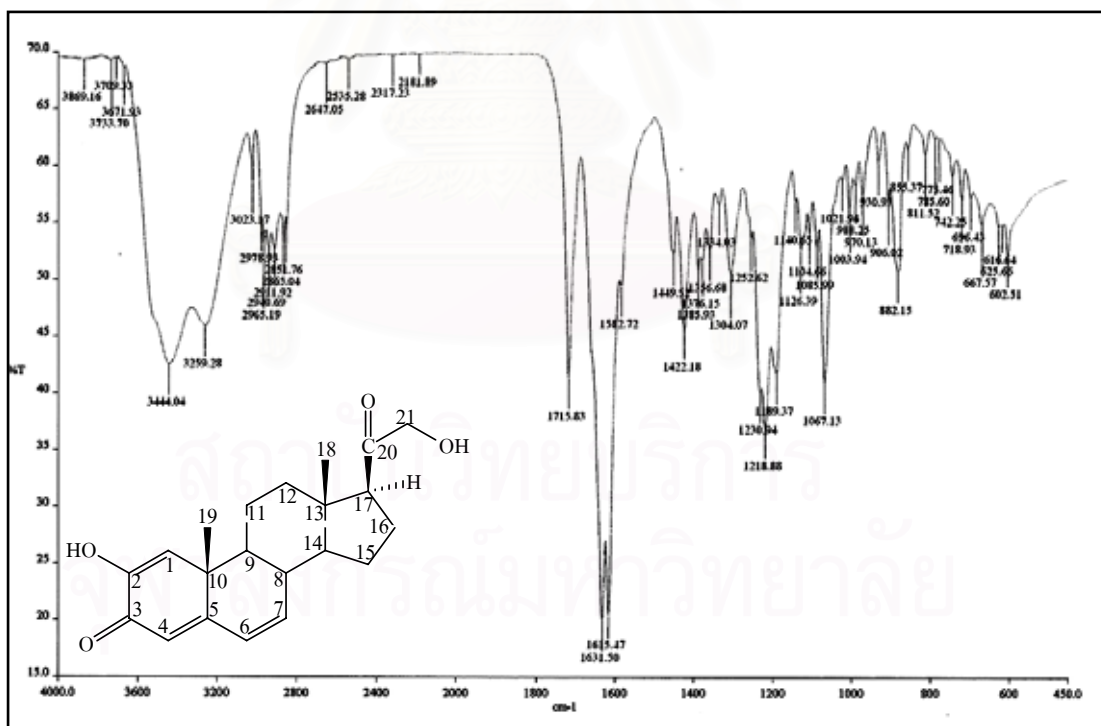


Figure 49. IR Spectrum of compound CB05 (KBr disc)

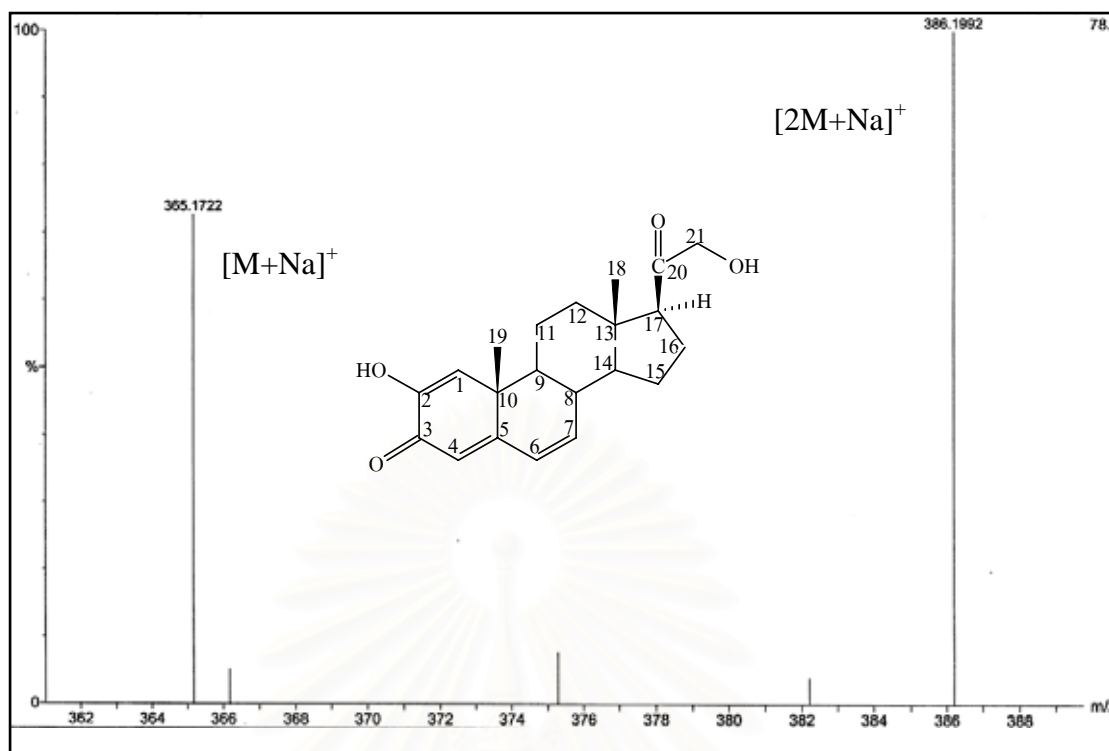


Figure 50. ESI TOF Mass spectrum of compound CB05

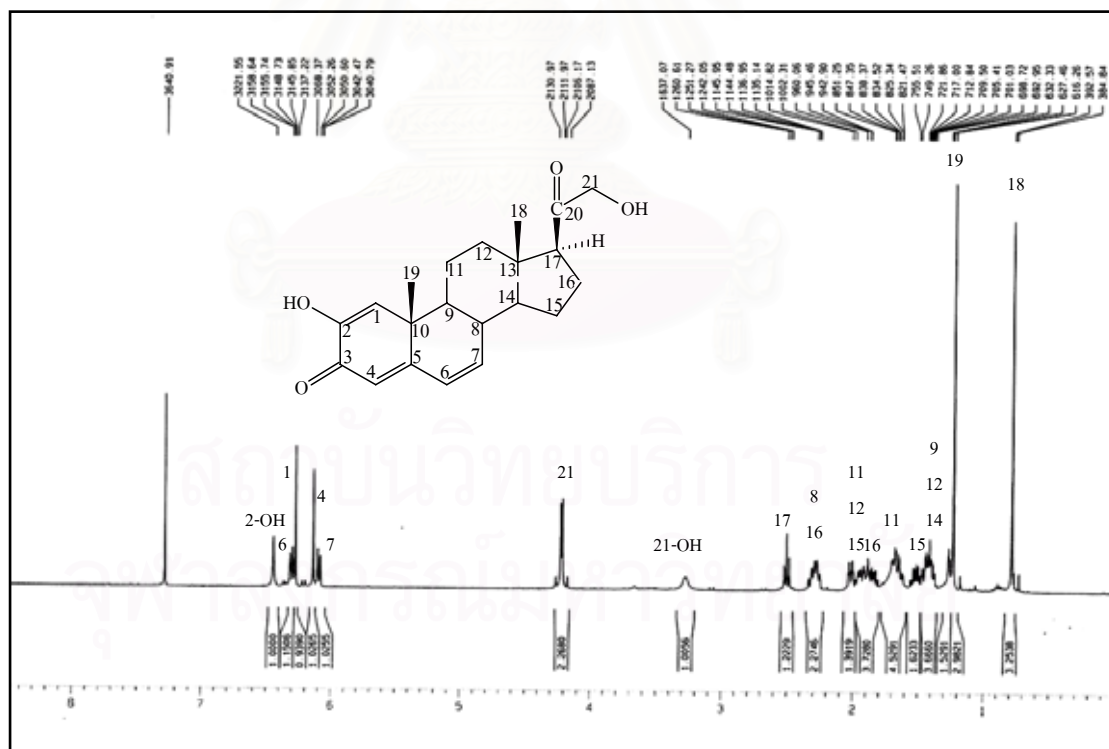


Figure 51.  $^1\text{H}$  NMR (500 MHz) Spectrum of compound CB05 (in  $\text{CDCl}_3$ )

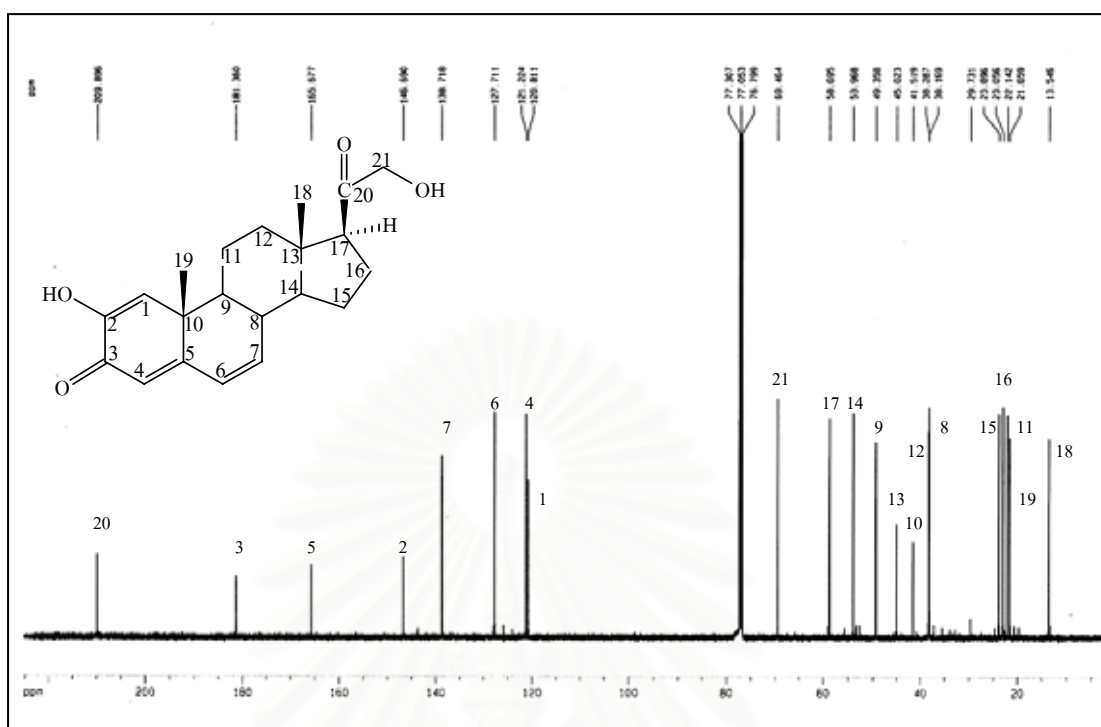


Figure 52.  $^{13}\text{C}$  NMR (125 MHz) Spectrum of compound CB05 (in  $\text{CDCl}_3$ )

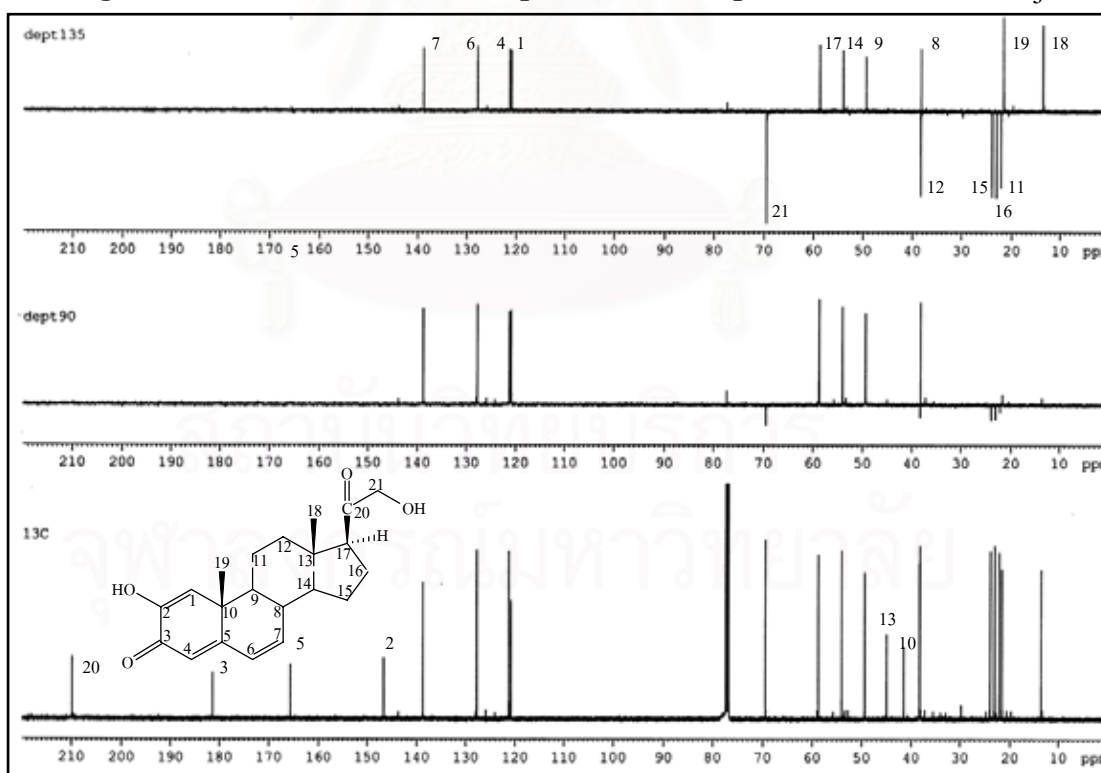


Figure 53. DEPT 90 and 135 Spectrum of compound CB05

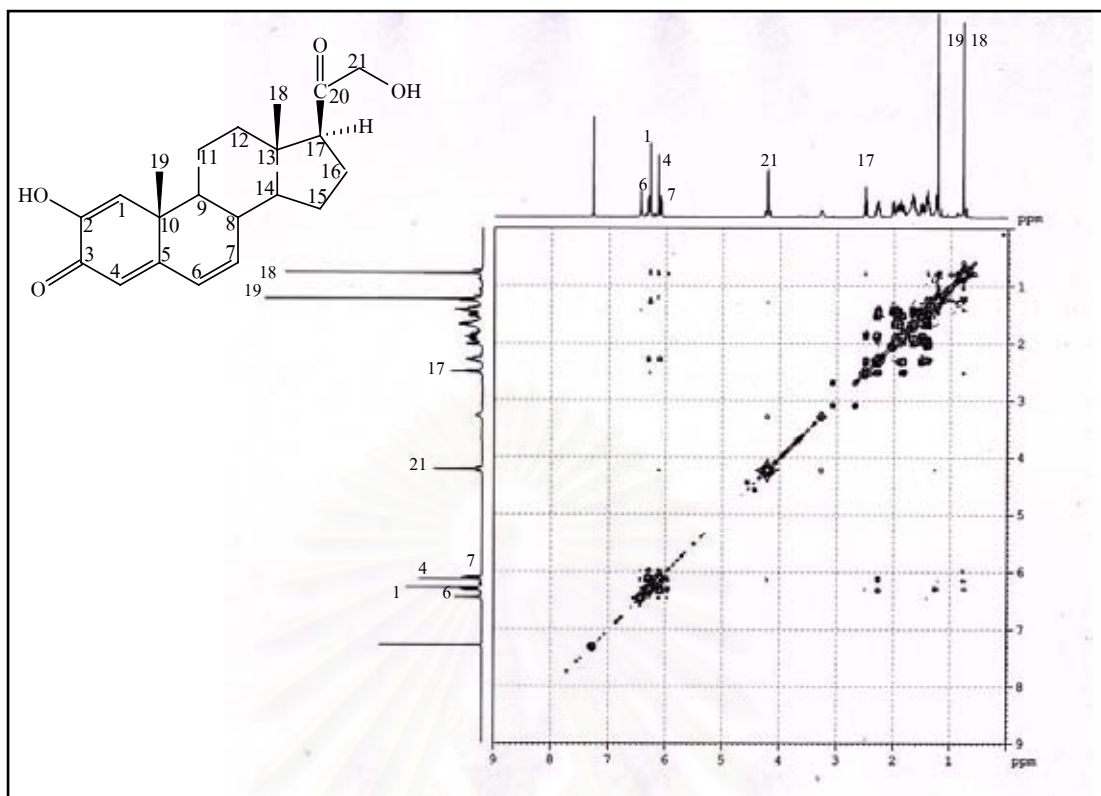


Figure 54.  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of compound CB05

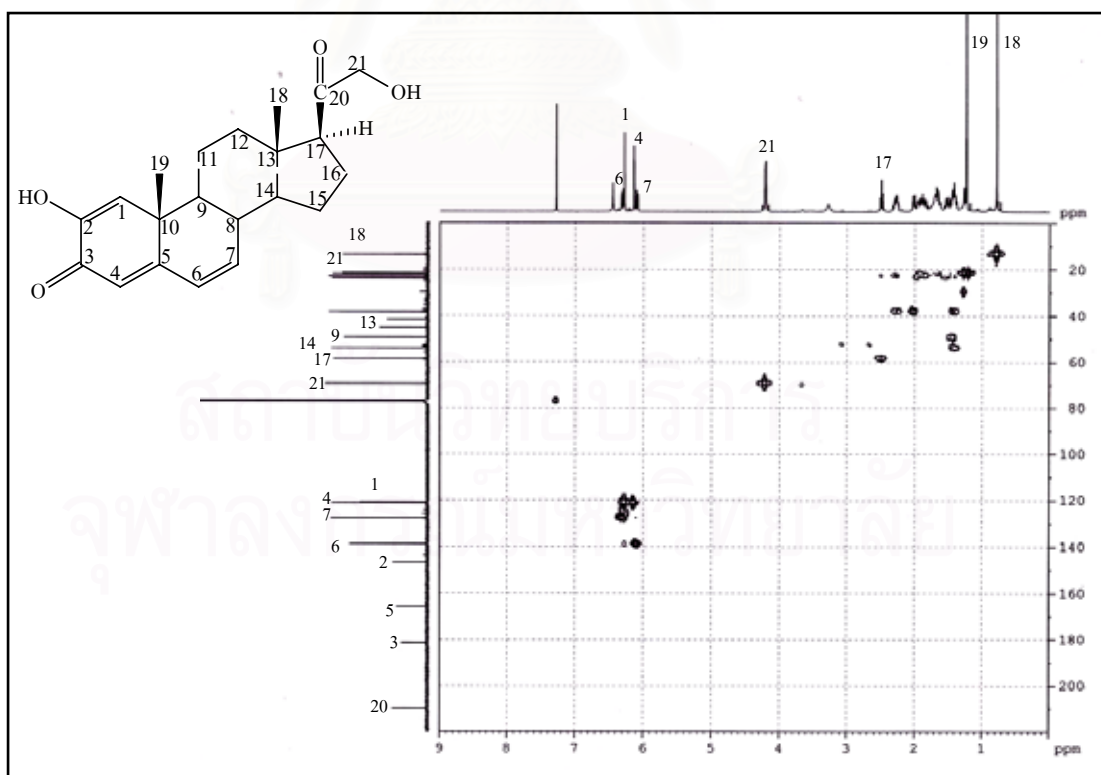


Figure 55. HMQC Spectrum of compound CB05

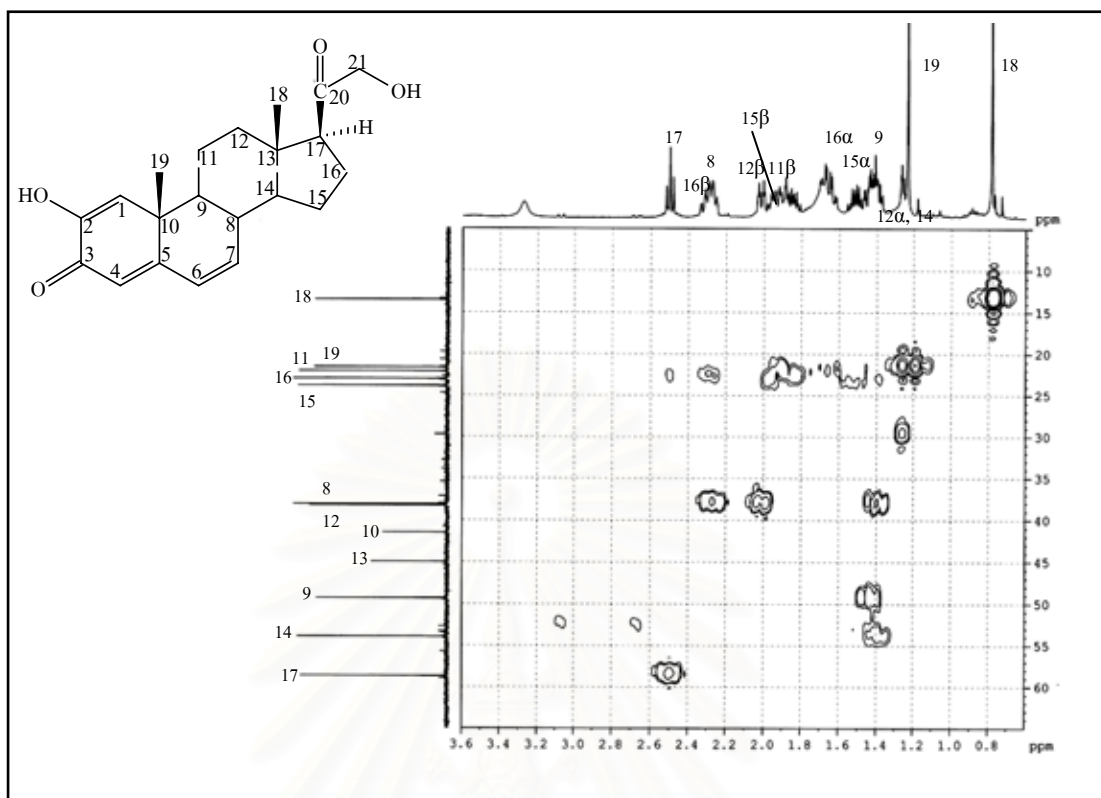


Figure 56. HMQC Spectrum of compound CB05 (expanded)

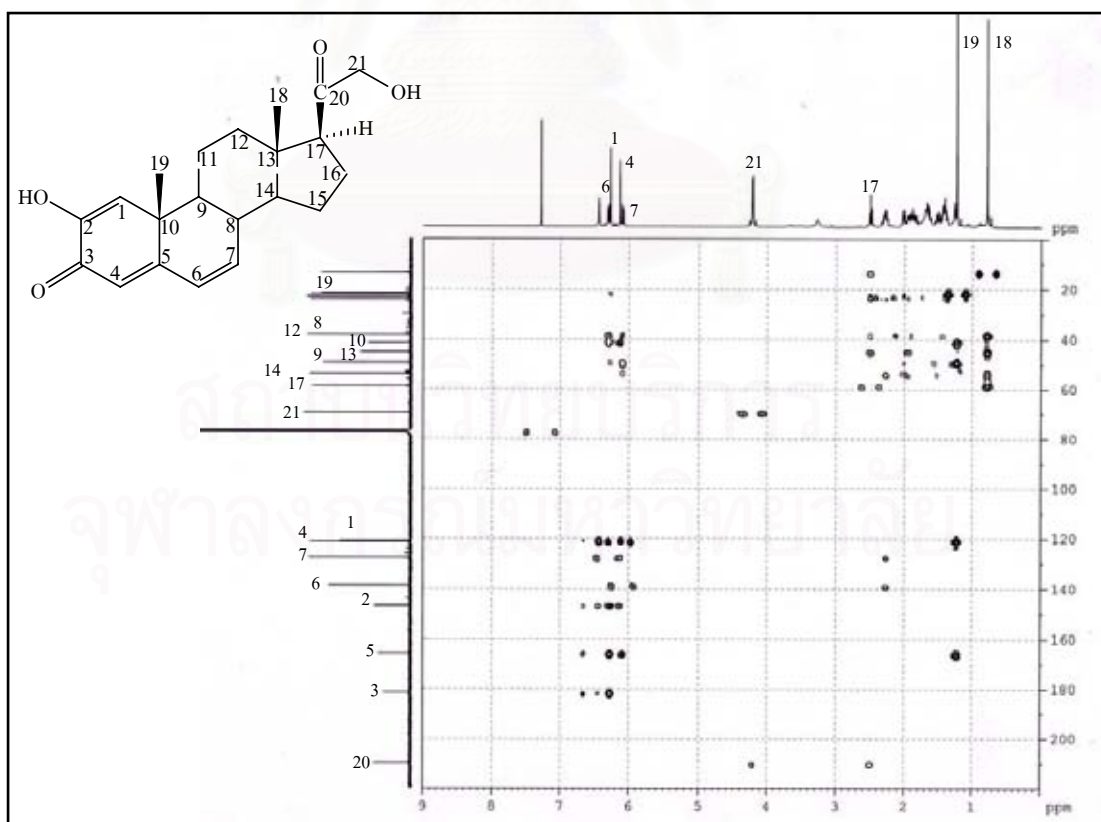


Figure 57. HMBC Spectrum of compound CB05

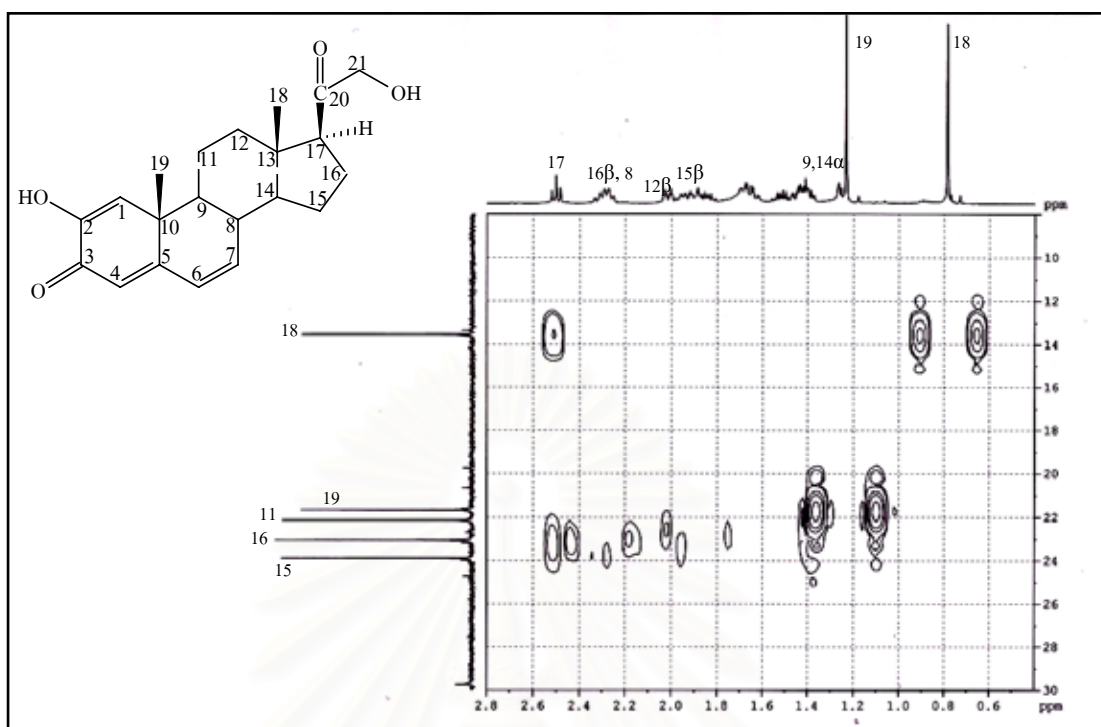


Figure 58. HMBC Spectrum of compound CB05 (expanded)

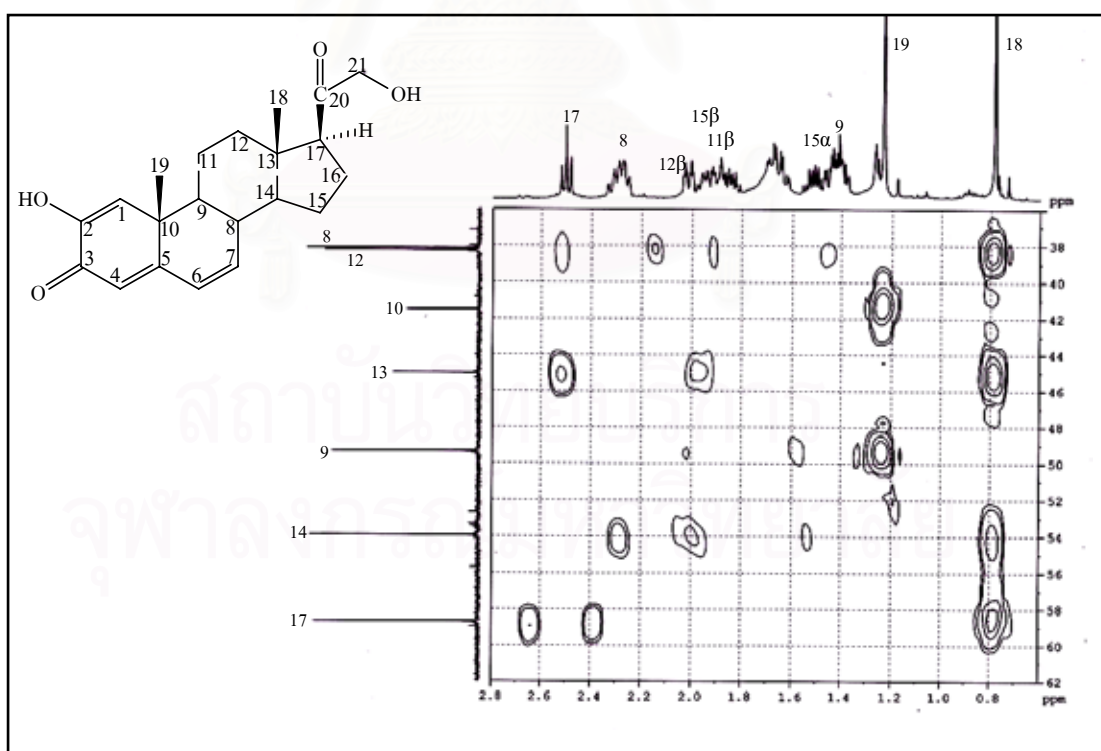


Figure 59. HMBC Spectrum of compound CB05 (expanded)

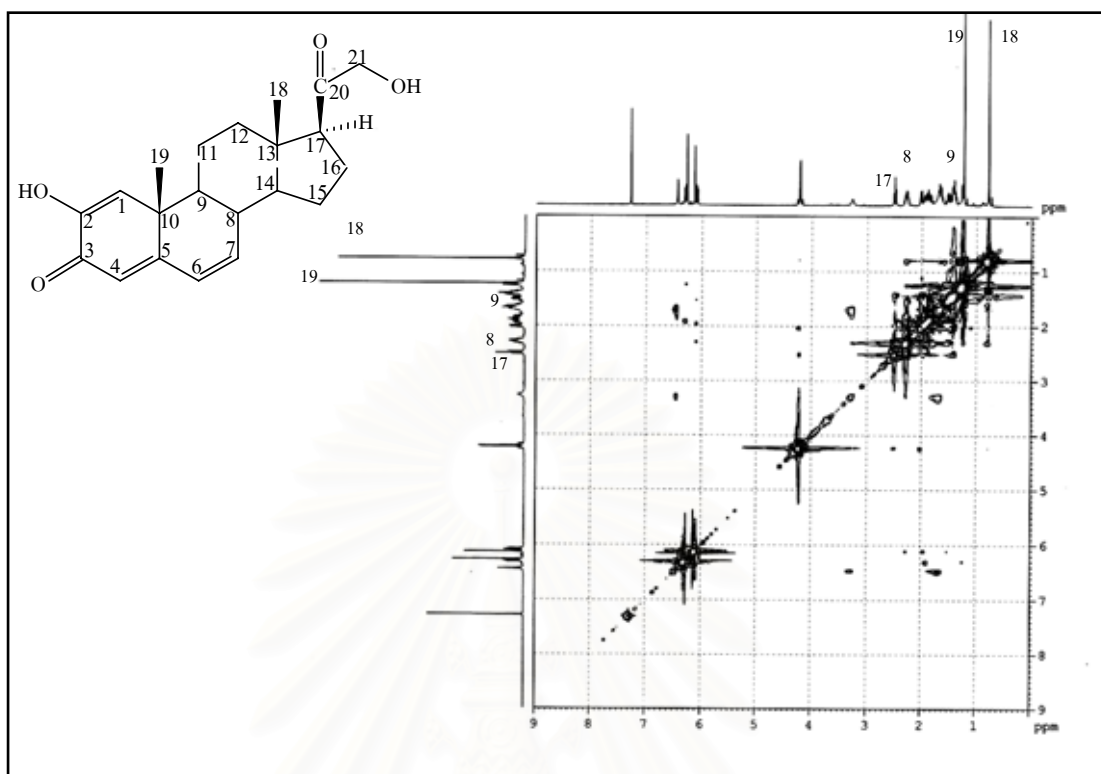


Figure 60. NOESY Spectrum of compound CB05

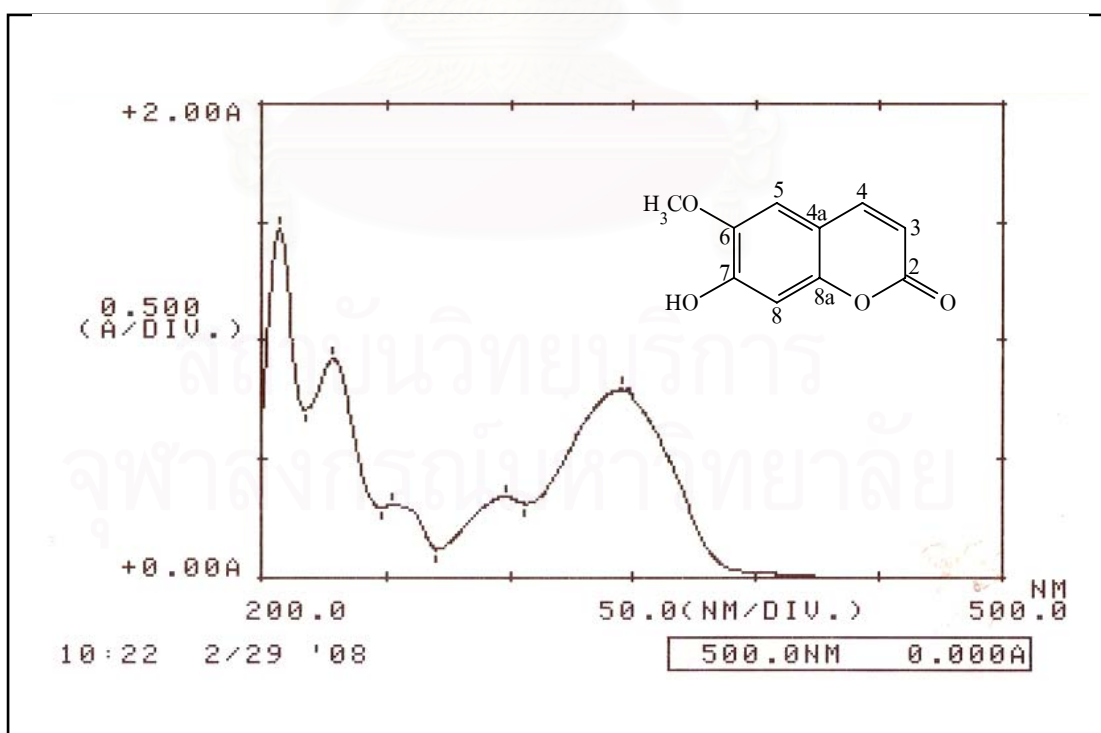


Figure 61. UV Spectrum of compound CB06 (in MeOH)



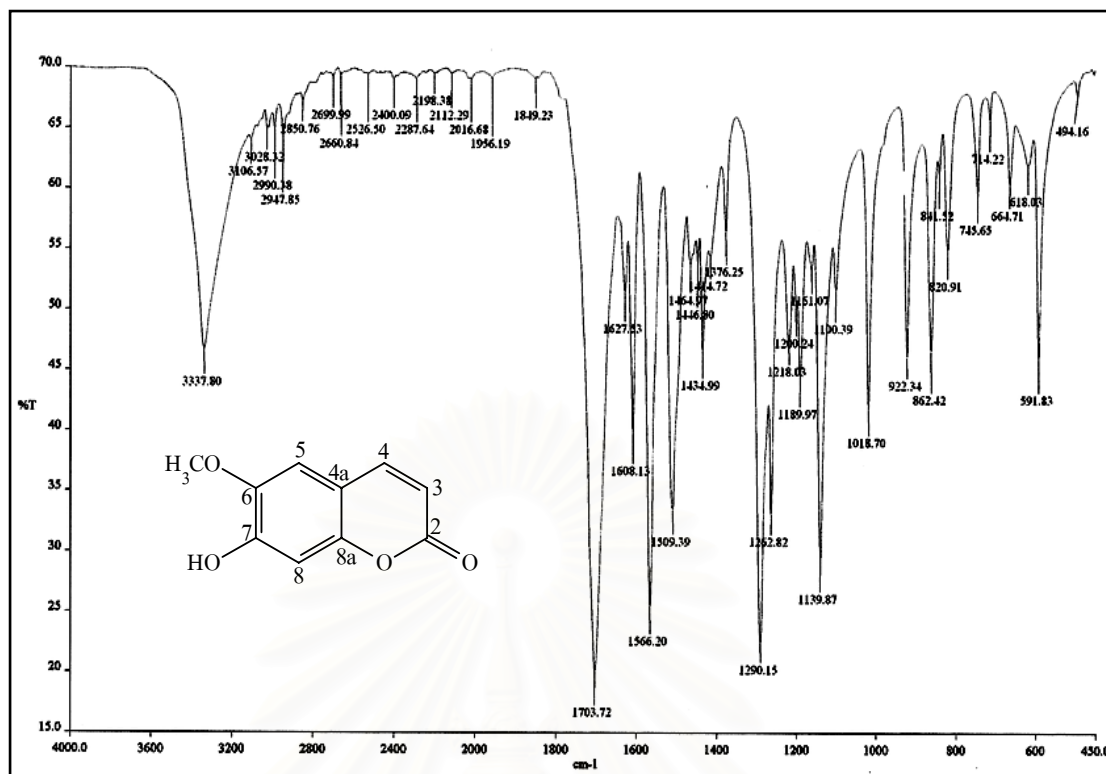


Figure 62. IR Spectrum of compound CB06 (KBr disc)

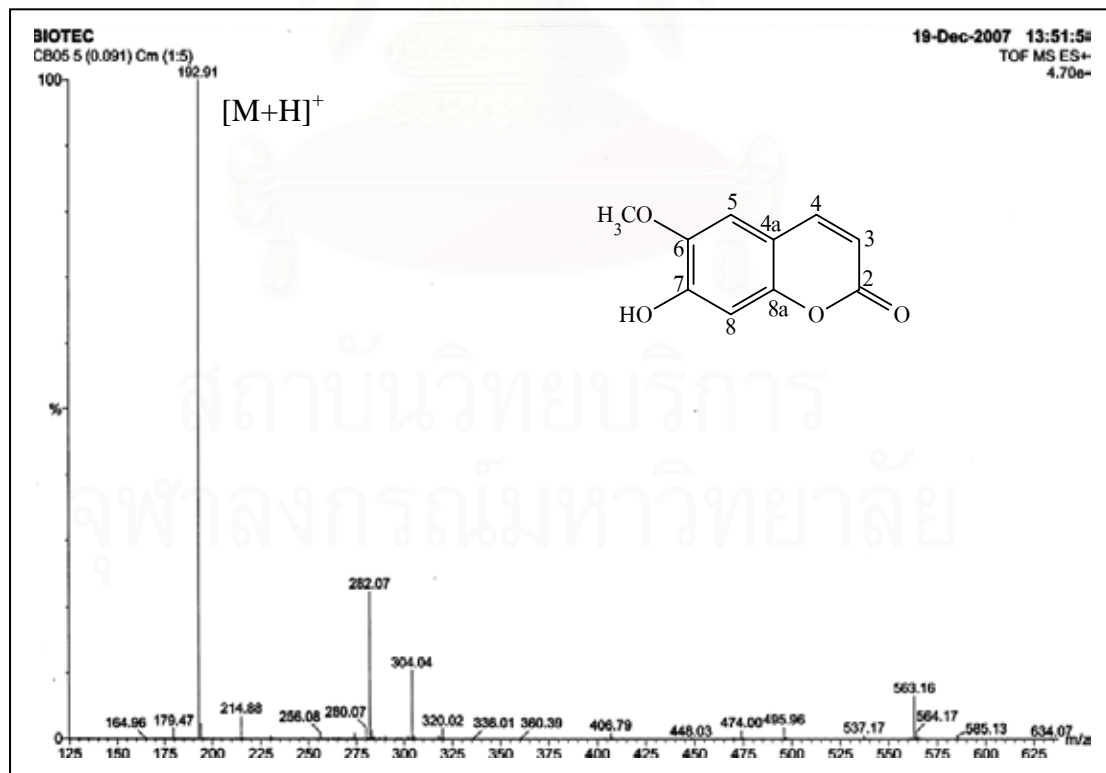


Figure 63. ESI TOF Mass spectrum of compound CB06

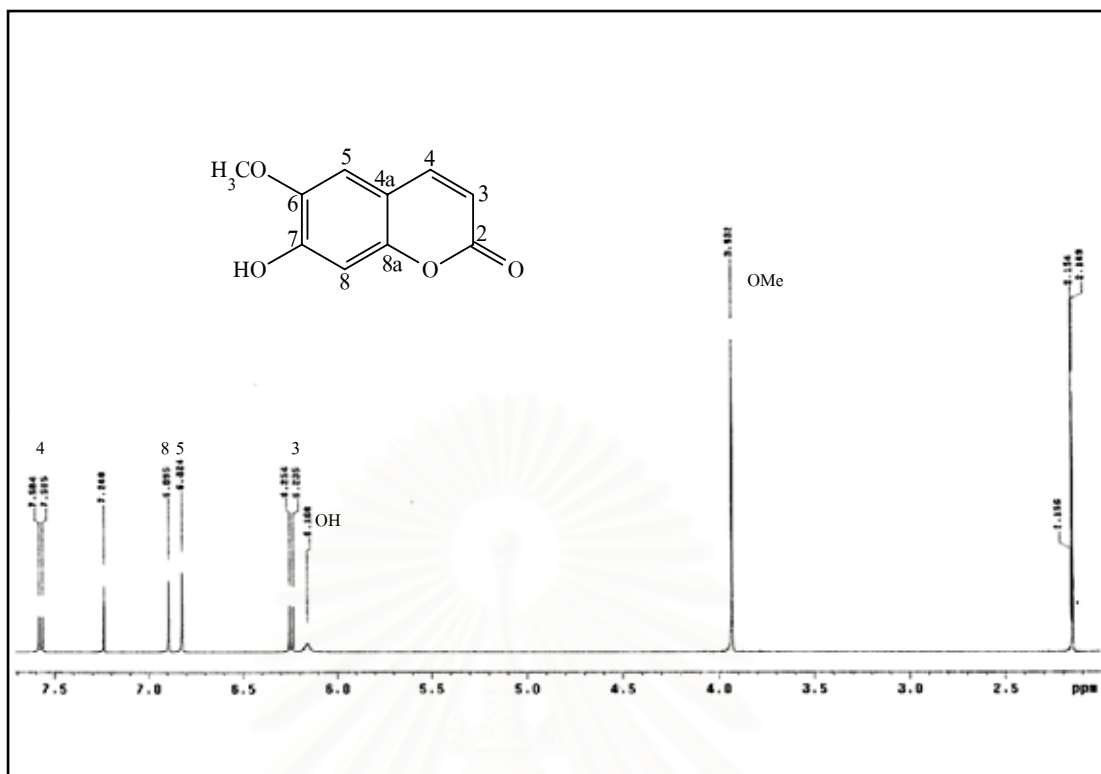


Figure 64.  $^1\text{H-NMR}$  (500 MHz) Spectrum of compound CB06 (in  $\text{CDCl}_3$ )

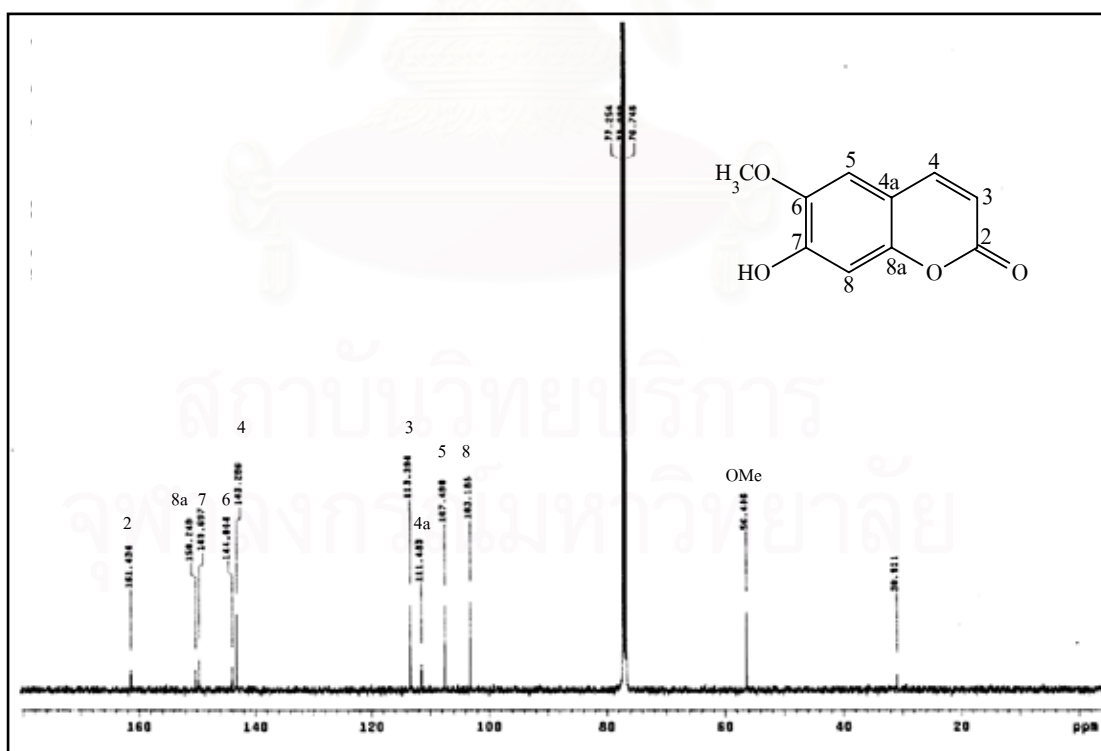


Figure 65.  $^{13}\text{C-NMR}$  (125 MHz) Spectrum of compound CB06 (in  $\text{CDCl}_3$ )

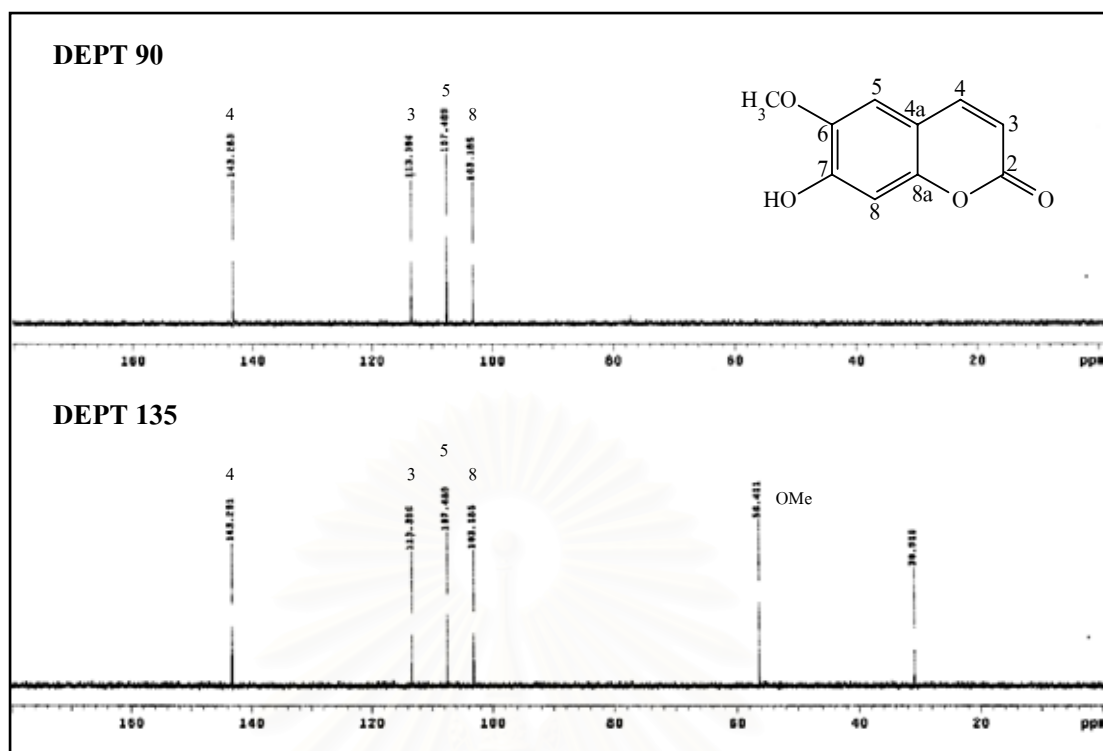


Figure 66. DEPT 90 and 135 Spectrum of compound CB06

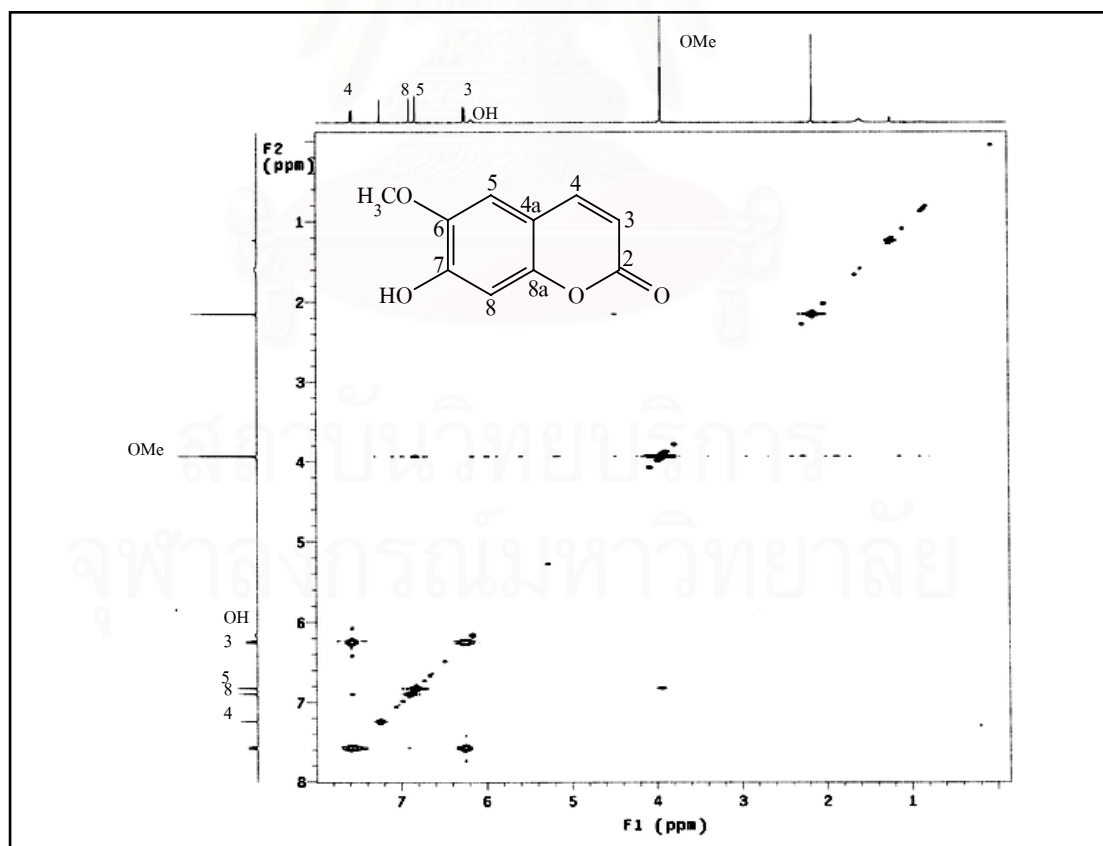


Figure 67.  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of compound CB06

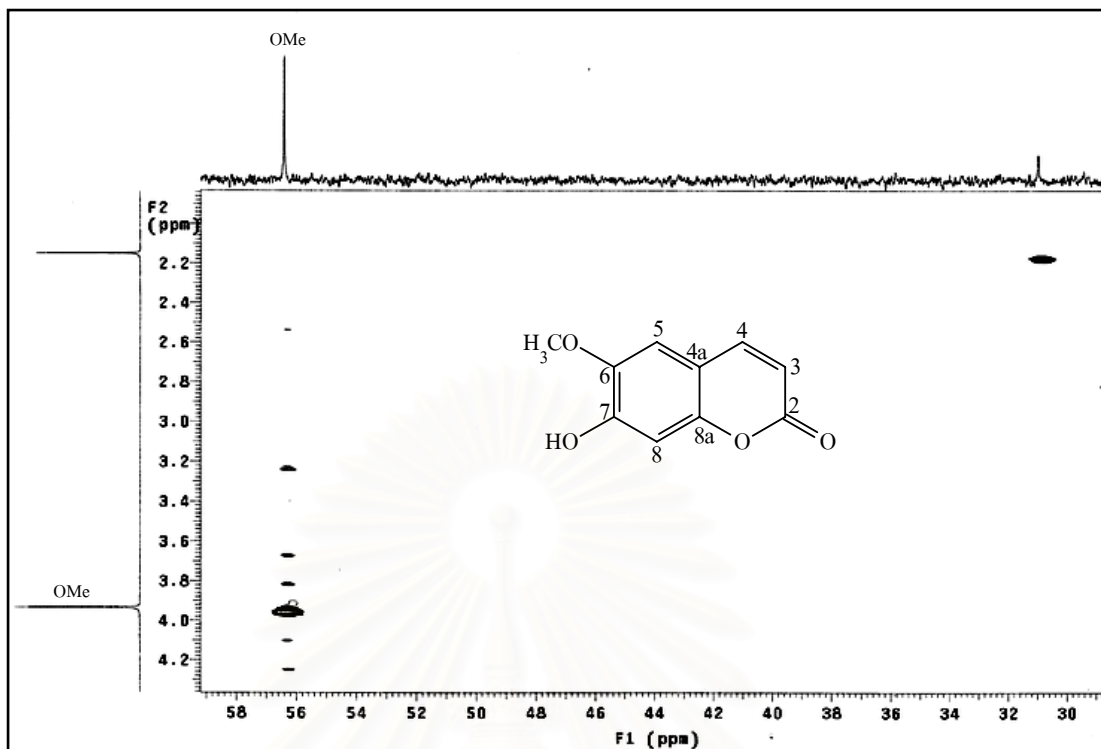


Figure 68. HSQC Spectrum of compound CB06

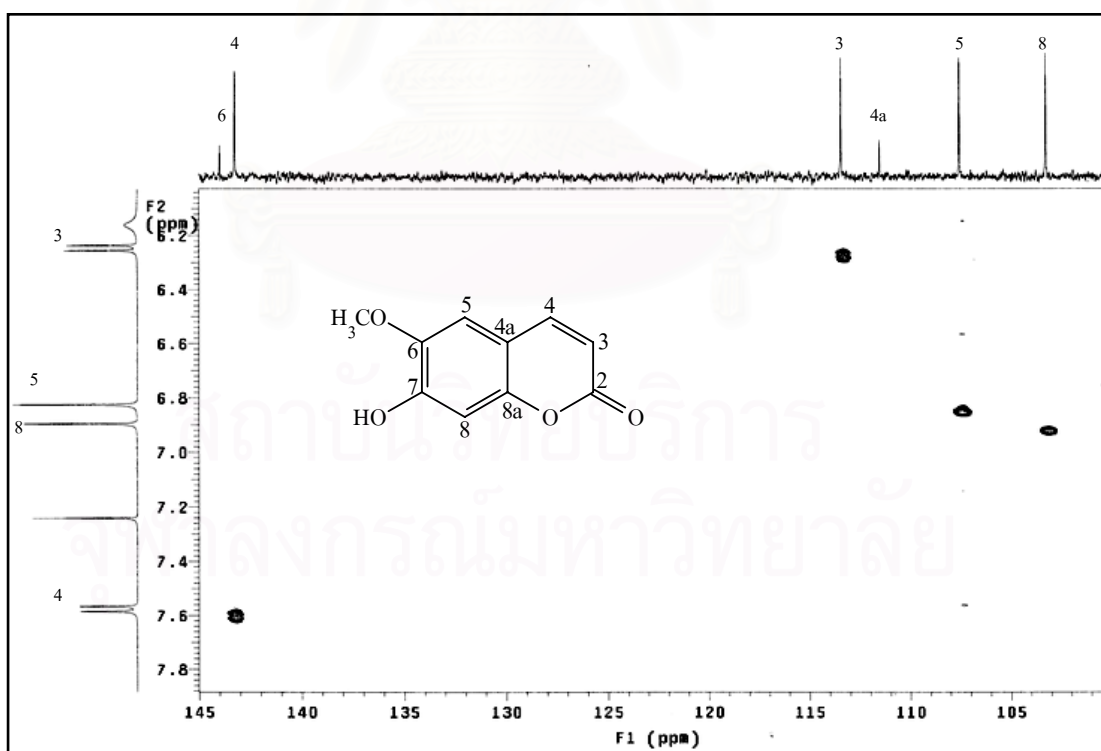


Figure 69. HSQC Spectrum of compound CB06 (expanded)

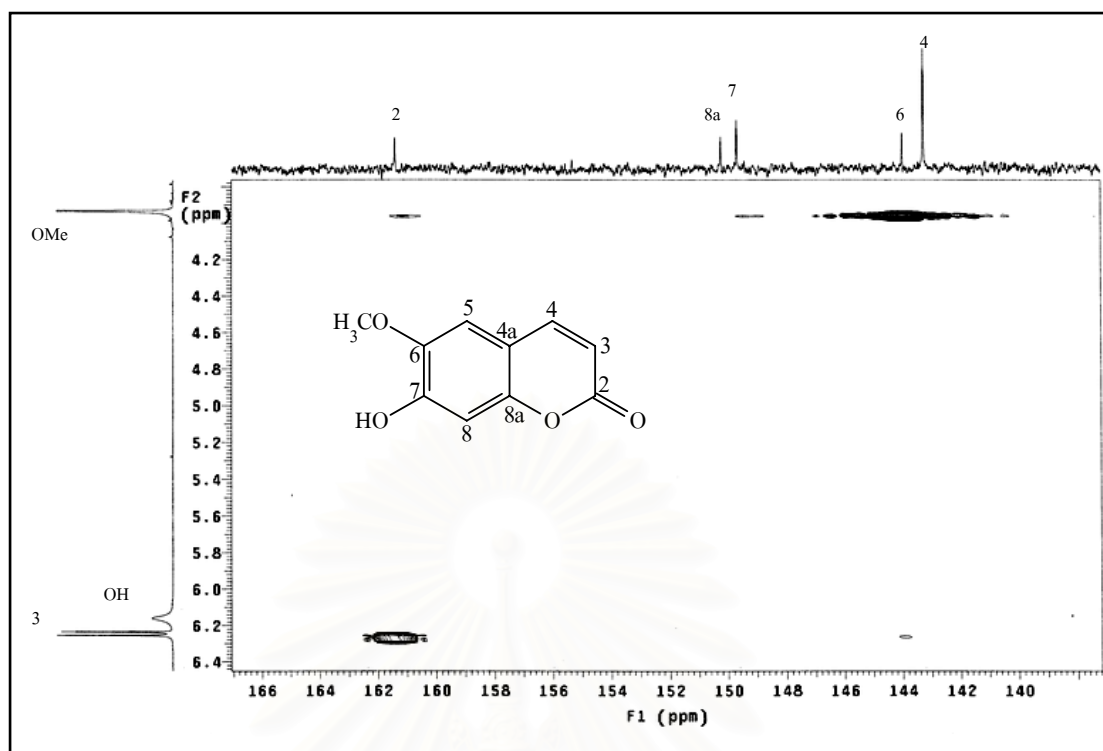


Figure 70. HMBC Spectrum of compound CB06

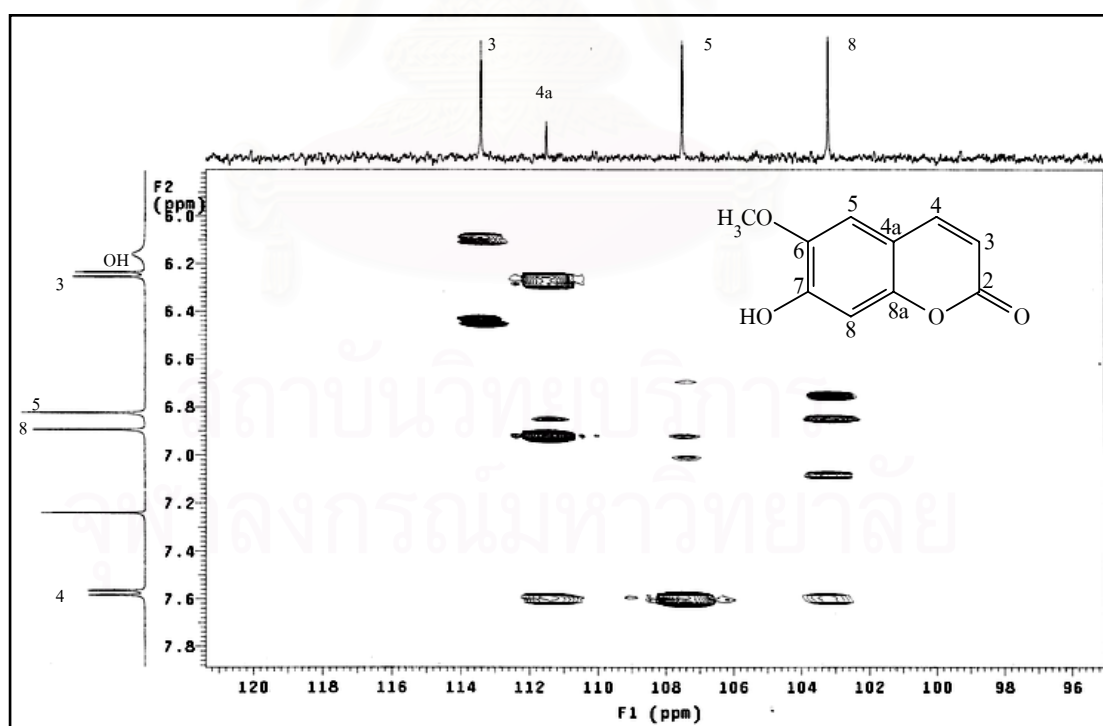


Figure 71. HMBC Spectrum of compound CB06 (expanded)

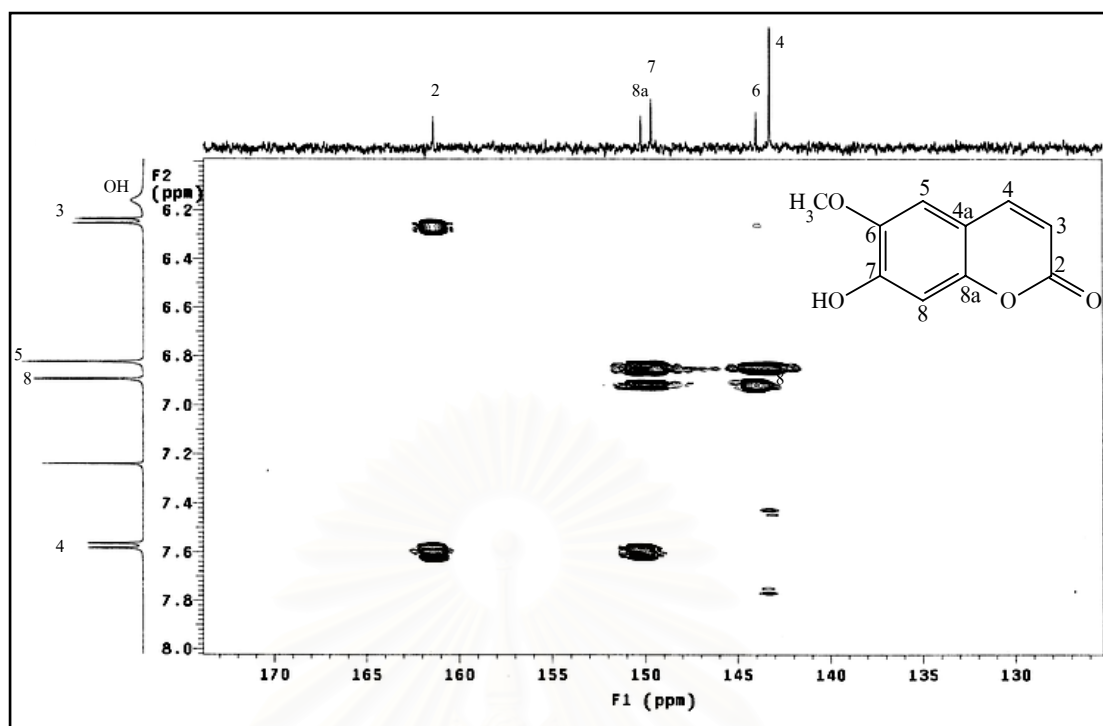


Figure 72. HMBC Spectrum of compound CB06 (expanded)

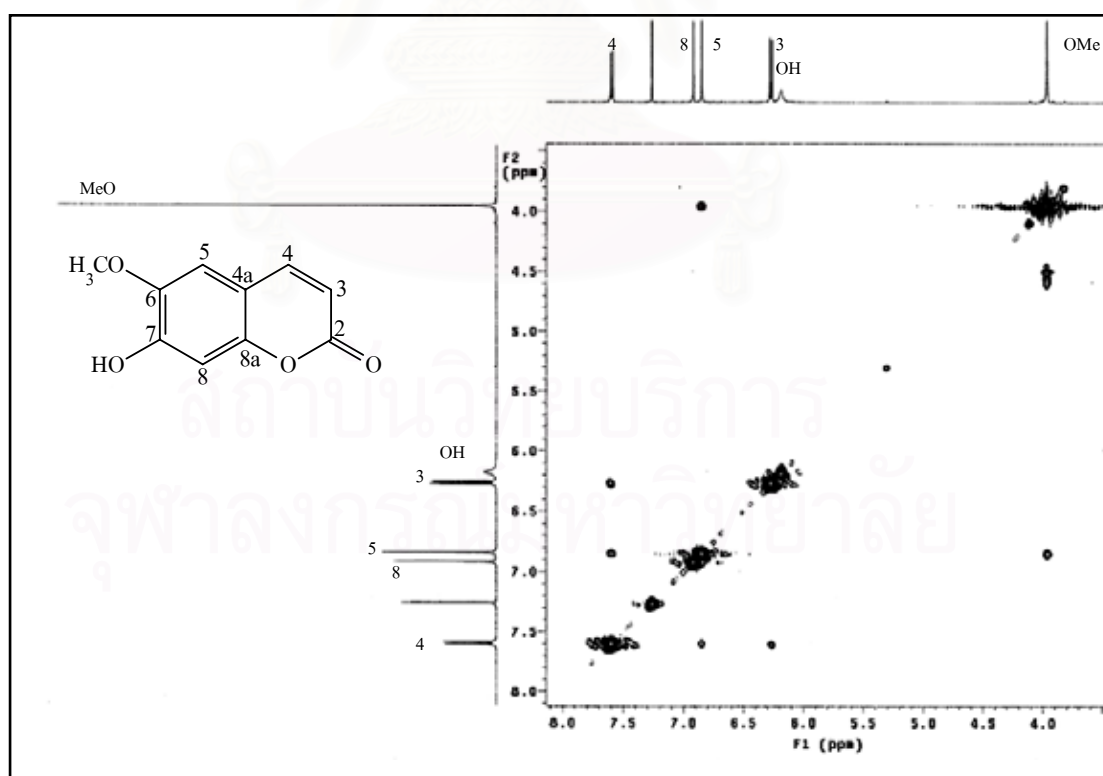


Figure 73. NOESY Spectrum of compound CB06

**VITA**

Miss Amornthip Somsook was born on May 16, 1978 in Songkhla, Thailand. She received her Bachelor degree in Pharmaceutical Sciences in 2002 from the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hatyai, Thailand. She is now working as a hospital pharmacist at Satun hospital, Satun, Thailand.



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