CHAPTER III

RESULTS AND DISCUSSION

STRUCTURAL ELUCIDATION OF SUBSTANCES SEPARATED FROM CRUDE EXTRACT OF HEXANE, DICHLOROMETHANE AND METHANOL

Structural elucidation of BOV1

BOV1 was the bright white plate, m.p. 61-63 $^{\circ}$C and R_f = 0.81 (silica gel/dichloromethane: hexane ; 3:17). The IR spectrum is shown in Fig. 3 and absorption bands are exhibited in **Table 12.**

Table 12 The Infrared absorption band assignments of **BOV1**

Wavenumber (cm^{-1})	Intensity	Tentative assignment
2957, 2919, 2849	strong	C-H stretching vibration of CH_3-, -CH_2-
1473, 1464	moderate	C-H bending vibration of CH_3-, -CH_2-
1379	weak	C-H symmetric bending vibration of CH_3-
730, 720	moderate	C-H rocking vibration of -CH_2- (for carbon>4

From IR spectrum, C-H stretching vibration peaks of aliphatic substance were observed at 2957, 2919 and 2849 cm^{-1}. The absorption peaks at 1473 and

1464 cm^{-1} corresponded to C-H bending vibration mode of CH_3, CH_2 groups. In addition, the absorption peaks at 730 and 720 cm^{-1} indicated one or more saturated long chain of $(CH_2)n$; n>4. **BOV1** gave negative test to 2,4-dinitrophenylhydrazine (no yellow precipitate) reagent to exhibit no ketone and aldehyde. It gave negative test to Liebermann-Burchard reaction to exhibit no steroid and triterpenoid. From all data and from comparation with IR spectrum of standard long chain hydrocarbon (22),**BOV1** was long chain aliphatic hydrocarbon.

When **BOV1** was analyzed by GLC technique, the chromatogram exhibited **BOV1** as the mixture of substances having closely molecular weight(Fig. 5). Retention time of GLC chromatogram(**Table 13**) showed 8 peaks to exhibit **BOV1** as a mixture of eight long chain hydrocarbons. Comparison with GLC chromatogram of ten standard long chain aliphatic hydrocarbons(C = 24 - 33) (Fig. 4) using calibration curve of Log retention time with number of carbon(Fig. 6). Compared log retention times of **BOV1** with those of standard long chain aliphatic hydrocarbon, **BOV1** was the mixture of eight long chain hydrocarbons(C = 26 - 33)

Table 13 Retention time of **BOV1**

Retention time (minute)	Log retention time	Number of carbon
4.53	0.66	26
5.62	0.75	27
7.13	0.85	28
8.81	0.94	29
11.53	1.06	30
14.36	1.16	31
19.06	1.28	32
24.06	1.38	33

Note: Standard long chain aliphatic hydrocarbons were butacosane($C_{24}H_{50}$), pentacosane($C_{25}H_{52}$), hexacosane($C_{26}H_{54}$), heptacosane($C_{27}H_{56}$), octacosane($C_{28}H_{58}$), nonacosane($C_{29}H_{60}$), triacontane($C_{30}H_{62}$), hentriacontane($C_{31}H_{64}$), dotriacontane ($C_{32}H_{66}$) and tritriacontane($C_{33}H_{68}$).

Table 14 Names of long chain aliphatic hydrocarbons in **BOV1**

Molecular weight	Molecular formula	Structural formula	Name of substances	%composition
366	$C_{26}H_{54}$	CH_3-$(CH_2)_{24}$-CH_3	hexacosane	0.16
380	$C_{27}H_{56}$	CH_3-$(CH_2)_{25}$-CH_3	heptacosane	1.61
394	$C_{28}H_{58}$	CH_3-$(CH_2)_{26}$-CH_3	octacosane	1.28

Molecular weight	Molecular formula	Structural formula	Name of substances	%composition
408	$C_{29}H_{60}$	CH_3-$(CH_2)_{27}$-CH_3	nonacosane	43.50
422	$C_{30}H_{62}$	CH_3-$(CH_2)_{28}$-CH_3	triacontane	2.57
436	$C_{31}H_{64}$	CH_3-$(CH_2)_{29}$-CH_3	hentriacontane	44.46
450	$C_{32}H_{66}$	CH_3-$(CH_2)_{30}$-CH_3	dotriacontane	1.61
464	$C_{33}H_{68}$	CH_3-$(CH_2)_{31}$-CH_3	tritriacontane	4.82

From Table 14, **BOV1** was a mixture of 8 saturated long chain aliphatic hydrocarbons: hexacosane($C_{26}H_{54}$), heptacosane($C_{27}H_{56}$), octacosane($C_{28}H_{58}$), nonacosane($C_{29}H_{60}$), triacontane($C_{30}H_{62}$), hentriacontane($C_{31}H_{64}$), dotriacontane ($C_{32}H_{66}$) and tritriacontane($C_{33}H_{68}$). Nonacosane($C_{29}H_{60}$) and hentriacontane($C_{31}H_{64}$) were the main components, 43.50 % and 44.46 % respectively, while hexacosane ($C_{26}H_{54}$) was present as the trace component. The structure of **BOV1** is shown below:

$$CH_3 - (CH_2)_n - CH_3 \quad n = 24 - 31$$

Structural elucidation of **BOV2**

BOV2 was the white amorphous solid with of m.p. 77-78 °C. It was soluble in dichloromethane and was recrystallized from the mixture of hexane and ethylacetate. The Infrared spectrum is shown in Fig. 7 and absorption bands indicate in **Table 15.**

Table 15 The Infrared absorption band assignments of **BOV2**

Wavenumber (cm^{-1})	Intensity	Tentative assignment
2919, 2850	strong	C-H stretching vibration of CH_3-, -CH_2-
1737	strong	-C=O stretching vibration of ester
1466	moderate	C-H bending vibration of CH_3-, -CH_2-
1173	moderate	C-O stretching vibration of ester
723	moderate	C-H rocking vibration of -CH_2-(for carbon>4)

The IR spectrum indicated that this substance contained an ester functional group at 1737 cm^{-1} and 1173 cm^{-1}. Characteristic of long chain aliphatic moiety showed at 723 cm^{-1}. This substance gave negative results to 2,4-DNP, 0.1% $KMnO_4$, Br_2 in CCl_4, 1% $FeCl_3$ and Liebermann-Burchard reagents which indicated that it was not ketone or aldehyde, unsaturated substance, phenol, steroid or triterpenoid, respectively.

The ^{1}H-NMR spectrum (Fig. 8) exhibited two signals that belonged to protons of ester. The signal at δ 4.05 ppm which was triplet should be the chemical shift

O H

of α-proton in the alcoholic portion of the molecular(R-C-O-C-CH_2) and the signal at

H

δ 2.29 ppm which was also triplet was assigned for the chemical shift of α-proton

H O

in the acidic portion of (-CH_2-C-C-OR). Other signals around 1.60 to 0.88 ppm were

H

the signals of methyl, methylene and methine protons.

From all of spectroscopic data, **BOV2** was the mixture of long chain ester.

Structural elucidation of **BOV3**

BOV3, m.p. 82-86 °C, 0.78 g (equivalent to 0.23% wt. by wt. of Fraction I; 0.17% wt. by wt. of Fraction II) was eluted from the silica gel column of Fraction I (fraction no. 54-67) and Fraction II (fraction no. 31-35). After recrystallization from the mixture of hexane and ethylacetate for several times, **BOV3** was white amorphous solid. It revealed a single spot on TLC plate at R_f 0.60 (silica gel/dichloromethane). **BOV3** was neither triterpenoid, steroid nor unsaturated substance because it gave negative test to Liebermann-Bruchard and Br_2 in CCl_4 reagent.

The IR spectrum is shown in Fig. 9 and absorption bands indicate in **Table 16**.

Table 16 The Infrared absorption band assignments of **BOV3**

Wavenumber (cm^{-1})	Intensity	Tentative assignment
3672-3201	moderate	O-H stretching vibration
2919, 2849	strong	C-H stretching vibration of CH_3-, -CH_2-
1472, 1464	moderate	C-H bending vibration of CH_3-, -CH_2-
1123	weak	C-O stretching vibration and O-H bending vibration of 1°-R-OH
730, 720	weak	C-H rocking vibration of -CH_2-(for carbon>4)

The IR spectrum indicated that this substance contained 1°-alcohol functional group at 3672-3201 cm^{-1} and 1123 cm^{-1}. Absorption band at 730, 720 cm^{-1} revealed

that this substance was long chain aliphatic. **BOV3** gave negative tests for 2,4-DNP(no yellow precipitate), 0.1% $KMnO_4$, Br_2 in CCl_4, 1% $FeCl_3$ and Liebermann-Burchard reagents which revealed that it was not ketone or aldehyde, unsaturated substance, phenol, steroid or triterpenoid, respectively.

BOV3 ought to be long chain aliphatic alcohol when comparison with standard long chain aliphatic alcohol, they were similar.

When this substance was analyzed by GLC technique. The chromatogram (Fig. 11) showed 3 peaks. The comparison of the mentioned chromatogram with standard chromatogram of long chain aliphatic alcohols(C = 14, 16, 18, 20, 22) (Fig. 10) indicated that **BOV3** was a mixture of 3 long chain aliphatic alcohols. Number of carbons of **BOV3** were 23, 24, 32 by comparison with the calibration curve of log retention times and number of carbons of standard long chain aliphatic alcohols (Fig. 12). Retention time, log retention time and number of carbons of **BOV3** was shown in **Table 17** and the names of substances which contained in **BOV3** were displayed in **Table 18**.

Table 17 Retention times of **BOV3**

Retention time (minute)	Log retention time	Number of carbon
5.36	0.73	23
5.69	0.76	24
31.83	1.50	32

Note: Standard long chain aliphatic alcohols were tetradecanol($C_{14}H_{29}OH$), hexadecanol($C_{16}H_{33}OH$), octadecanol($C_{18}H_{38}OH$), eicosanol($C_{20}H_{41}OH$) and docosanol ($C_{22}H_{45}OH$).

Table 18 Names of long chain aliphatic alcohols in **BOV3**

Molecular weight	Molecular formula	Structural formula	Name of substances	% composition
340	$C_{23}H_{48}O$	CH_3-$(CH_2)_{21}$-CH_2-OH	triacosanol	2.42
354	$C_{24}H_{50}O$	CH_3-$(CH_2)_{22}$-CH_2-OH	tetracosanol	0.79
466	$C_{32}H_{66}O$	CH_3-$(CH_2)_{30}$-CH_2-OH	dotriacontanol	96.79

From **Table 18**, **BOV3** was the mixture of 3 saturated long chain aliphatic alcohols; triacosanol($C_{23}H_{48}O$), tetracosanol($C_{24}H_{50}O$) and dotriacontanol($C_{32}H_{66}O$). Dotriacontanol($C_{32}H_{66}O$) was the main component, 96.79% while triacosanol ($C_{23}H_{48}O$) and tetracosanol($C_{24}H_{50}O$) were the components. The structure **of BOV3** is shown below.

$$CH_3 - (CH_2)_n - CH_2 - OH \; ; \; n = 21, 22, 30$$

Structural elucidation of **BOV4**

BOV4 was white needle which was separated from **Mixture II** by preparative TLC technique. It was recrystallized by a mixture of methanol and dichloromethane for several times and weighted on 0.21 g(equivalent to 28% wt.

by wt. of Mixture II). Its melting point was 257-260 °C. R_f value was 0.82 (silica gel/dichloromethane).

The IR spectrum was shown in Fig.13 and absorption bands revealed in Table 19

Table 19 The Infrared absorption band assignments of **BOV4**

Wavenumber (cm^{-1})	Intensity	Tentative assignment
2900, 2850	strong	C-H stretching vibration of CH_3-, -CH_2-
1720	strong	O C=O stretching vibration of C-C-C
1460	moderate	C-H bending vibration of CH_3-, -CH_2-
1380	strong	C-H symmetric bending vibration of CH_3-

The IR absorption band at 1720 cm^{-1} revealed carbonyl functional group. **BOV4** gave positive test to 2,4-DNP (yellow precipitate). No absorption band at 2720 cm^{-1} revealed that **BOV4** was ketone. There were no absorption bands at > 3000 cm^{-1} and 1600 cm^{-1}, therefore **BOV4** was saturated substance which correspond to negative tests to Br_2 in CCl_4 and 0.1% $KMnO_4$ reagents). It gave a positive result to Liebermann-Burchard reagent(violet color) which indicate that **BOV4** was a triterpenoid.

From ^{1}H-NMR(Fig. 14) displayed the protons of CH_3 at 0.72, 0.86, 0.87, 0.95, 1.01, 1.02, 1.05, 1.18 ppm 24 protons and the protons of CH_2, CH at 0.93,

1.26-1.96 ppm 23 protons. This was the pattern of triterpenoid substance. The protons at 2.26-2.38 ppm, (multiplet, 3 protons) revealed α-proton of ketone corresponding to reaction tests and IR spectrum.

From ^{13}C-NMR(Fig. 15) displayed signals at 6.83-59.50 ppm of CH_3, CH_2, CH and C of triterpenoid and signal at the 207.50 ppm indicated a carbonyl function group of ketone. ^{1}H and ^{13}C-NMR spectrum of **BOV4** were similar to the NMR spectrum of friedelin(23,24) as **Table 20**.

Table 20 Comparison of ^{13}C-NMR of **BOV4** with friedelin(24)

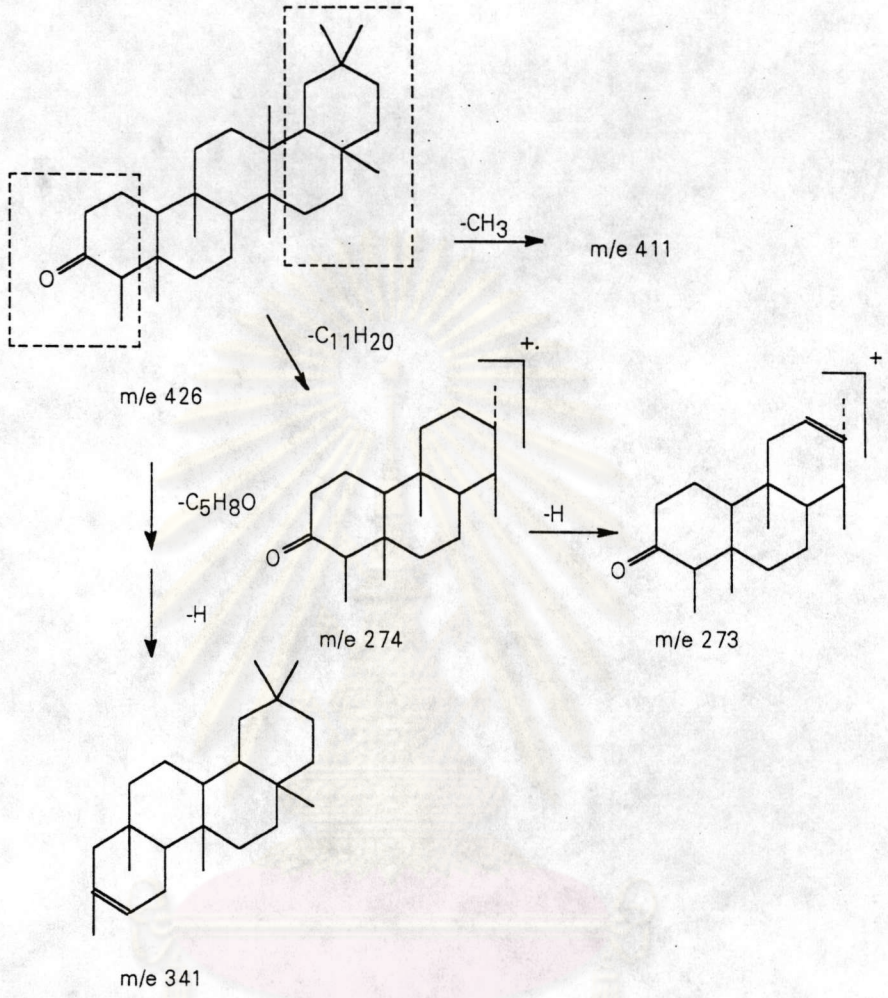
Position of carbon	Friedelin	BOV4
C_1	22.3	22.10
C_2	41.5	41.50
C_3	213.0	207.50
C_4	58.2	58.10
C_5	42.1	42.10
C_6	41.3	41.10
C_7	18.2	18.10
C_8	53.1	53.00
C_9	37.4	37.40
C_{10}	59.5	59.50
C_{11}	35.6	35.58
C_{12}	30.5	30.50
C_{13}	38.3	38.20
C_{14}	39.7	39.60
C_{15}	32.4	32.39

Table 20 (continue)

Position of carbon	Friedelin	BOV4
C_{16}	36.0	36.10
C_{17}	30.0	30.00
C_{18}	42.8	42.80
C_{19}	35.3	35.28
C_{20}	28.1	28.10
C_{21}	32.8	32.78
C_{22}	39.2	39.20
C_{23}	6.8	6.83
C_{24}	14.6	14.50
C_{25}	17.9	17.99
C_{26}	18.6	18.50
C_{27}	20.3	20.10
C_{28}	32.1	32.10
C_{29}	35.0	35.00
C_{30}	31.8	31.80

The mass spectrum(Fig.16) displayed the molecular ion peak at m/e 426 I revealed the fragmentation of molecule at m/e 411(M^+-CH_3), 341(M^+-C_5H_9O), 302 (M^+-C_9H_{16}), 274(M^+-$C_{11}H_{20}$), 273(M^+-$C_{11}H_{21}$), 247(M^+-$C_{13}H_{23}$), 218(M^+-$C_{14}H_{24}O$), 205 (M^+-$C_{16}H_{29}$), 191(M^+-$C_{16}H_{27}O$) and 163(M^+-$C_{19}H_{35}$). When the mass spectrum of **BOV4** was compared with friedelin(friedelan-3-one), they have similar fragmentation(25, 26).

The fragmentation pattern of **BOV4** is shown in **Scheme 4**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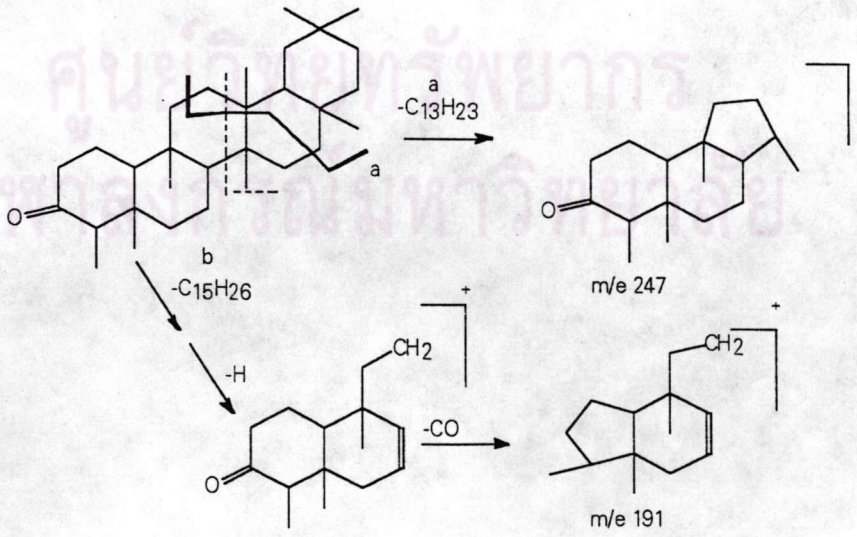
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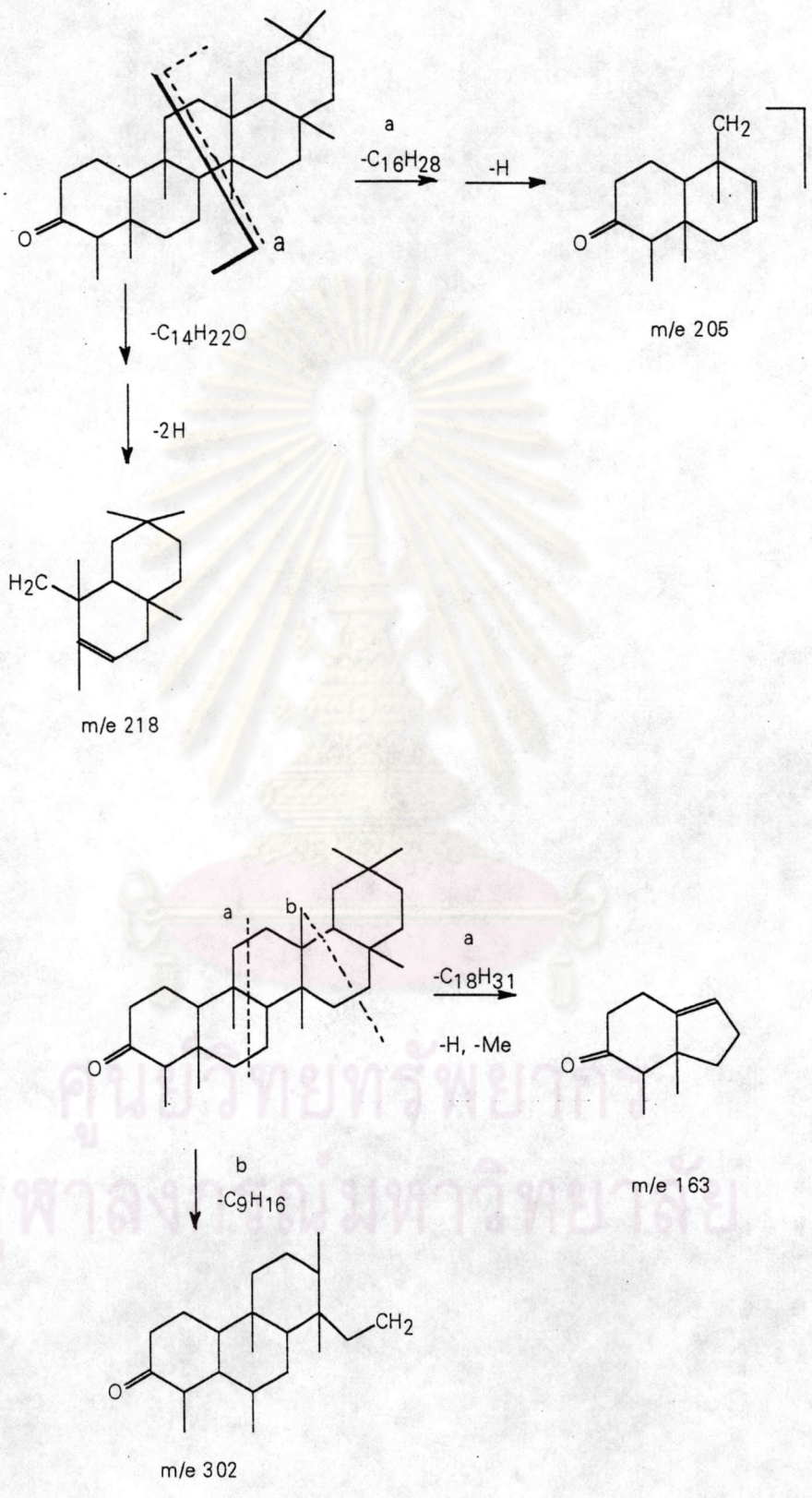


Schem 3 Fragmentation of BOV4

This structure of **BOV4** was confirmed by 2D-NMR analysis such as ^{13}C-^{1}H correlation(Fig. 17-18). The signals of carbons(δ, ppm) which were correlated with the signal of protons(δ, ppm) are shown in **Table 21**.

Table 21 ^{13}C-^{1}H correlation of **BOV4**(500 MHz, $CDCl_3$, δ)

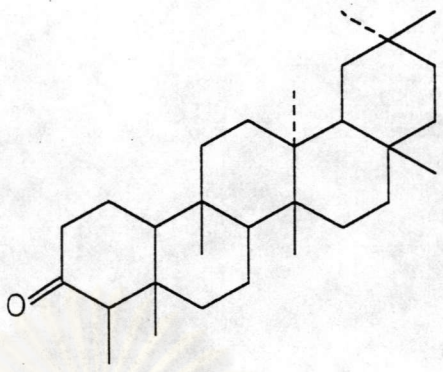
Position		Chemical shift of correlation	
Proton	Carbon-13	Proton	Carbon-13
H_1	C_1	1.96	22.10
H_2	C_2	2.38	41.50
H_3	C_3	-	207.50
H_4	C_4	2.26	58.10
H_5	C_5	-	42.10
H_6	C_6	1.76	41.10
H_7	C_7	1.39, 1.50	18.10
H_8	C_8	1.38	53.00
H_9	C_9	-	37.40
H_{10}	C_{10}	1.54	59.50
H_{11}	C_{11}	1.45	35.58
H_{12}	C_{12}	1.34	30.50
H_{13}	C_{13}	-	38.20
H_{14}	C_{14}	-	39.60
H_{15}	C_{15}	1.49	32.39
H_{16}	C_{16}	1.59	36.10
H_{17}	C_{17}	-	30.00

Table 21 (continue)

Position		Chemical shift of correlation	
Proton	Carbon-13	Proton	Carbon-13
H_{18}	C_{18}	1.55	42.80
H_{19}	C_{19}	1.26	35.28
H_{20}	C_{20}	-	28.10
H_{21}	C_{21}	1.29	32.78
H_{22}	C_{22}	0.93, 1.51	39.20
H_{23}	C_{23}	0.86	6.83
H_{24}	C_{24}	0.72	14.50
H_{25}	C_{25}	0.87	17.99
H_{26}	C_{26}	1.05	18.50
H_{27}	C_{27}	1.02	20.10
H_{28}	C_{28}	1.18	32.10
H_{29}	C_{29}	0.95	35,00
H_{30}	C_{30}	1.01	31.80

From ^{1}H-^{1}H COSY (Fig. 19-20) and ^{1}H-^{1}H NOESY (Fig. 21-22), the signal of proton at 2.38 ppm(H_2) coupled with the signal of proton at 1.96 ppm(H_1). The signal of proton at 2.26 ppm(H_4) coupled with the signal of proton at 0.86 ppm(H_{23}). The signal at 2.26 ppm(H_4) coupled long range with the signal at 1.54 ppm(H_{10}).

From spectroscopic data and comparison of **BOV4** with compound 5 which was the chemical constituent of the branches of Makaa, it can conclude that **BOV4** was friedelin($C_{30}H_{50}O$). The structure is shown below.



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

STRUCTURAL ELUCIDATION OF **BOV5**

BOV5 was the bright white plate, m.p. 279-280 °C approximately 0.26 g (equivalent to 34.67% wt. by wt. of Mixture II). It was obtained from the separation of Mixture II by preparative TLC technique and recrystallization from the mixture of dichloromethane and methanol for several times. R_f was 0.68 (silica gel/dichloromethane).

The IR spectrum is exhibited in Fig. 23 and absorption bands indicate in **Table 22**

Table 22 The Infrared absorption band assignments of **BOV5**

Wavenumber (cm^{-1})	Intensity	Tentative assignment
3600-3400	moderate	O-H stretching vibration of R-OH
2950, 2850	strong	C-H stretching vibration of CH_3-, -CH_2
1460	moderate	C-H bending vibration of CH_3-, -CH_2-
1390	strong	C-H symmetric bending vibration of CH_3-
1030	moderate	C-O stretching and O-H bending vibration

Absorption band at 3600-3400 cm^{-1} revealed an alcohol functional group. **BOV5** gave negative test to 2,4-DNP(no yellow precipitate) and had no absorption band at 1710 cm^{-1}, so **BOV5** was not ketone or aldehyde. It gave violet color to Liebermann-Burchard reagent, therefore **BOV5** was triterpenoid which has an alcoholic pattern.

From ^{1}H-NMR spectrum(Fig. 24), signals at 0.86, 0.94, 0.95, 0.99, 1.00, 1.01, 1.51, and 1.17 ppm(24 protons) indicated CH_3 group. Signals of CH_2, CH of triterpenoid substance displayed at 0.89, 0.91, 0.97,1.26-1.91 ppm(27 protons). Other signals at 3.74 ppm(1 proton, broad singlet) displayed -C<u>H</u>-OH group.

From ^{13}C-NMR spectrum(Fig. 25), signals at 61.05 ppm exhibited OH functional group linked with carbon. ^{1}H and ^{13}C-NMR spectra of **BOV5** were similar to those of friedelan-3β-ol. Therefore other signals at 11.60-72.20 ppm were assigned as **Table 23**.

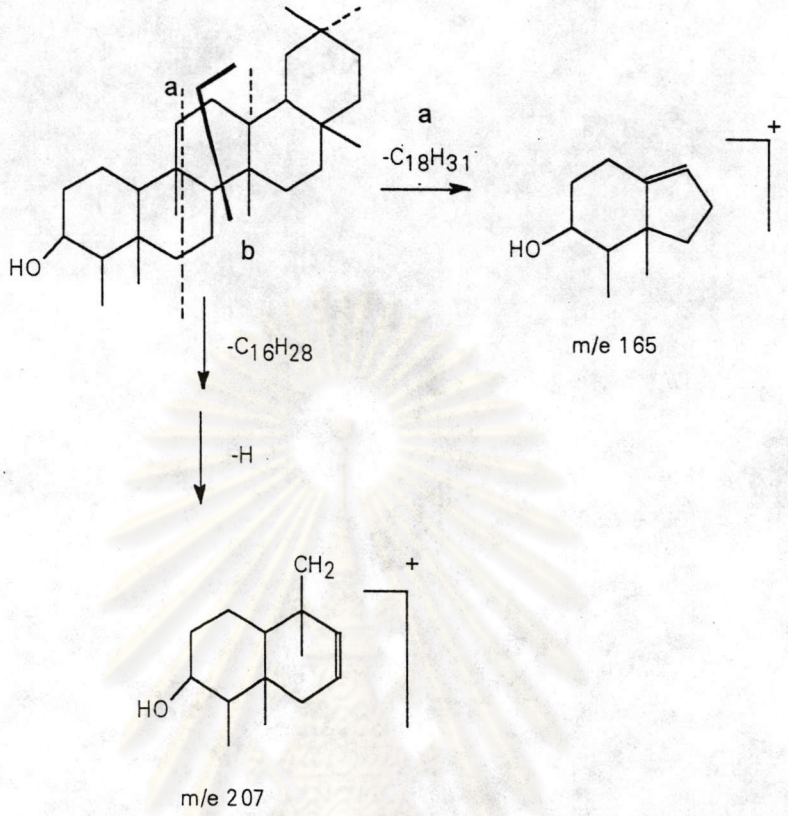
Table 23 ^{13}C-NMR spectrum of **BOV5**

Position of carbon	Friedelan-3β-ol	BOV5
C_1	16.43	16.20
C_2	35.28	34.10
C_3	72.77	72.00
C_4	49.28	48.00
C_5	37.90	37.15
C_6	41.81	41.80
C_7	17.60	17.10
C_8	53.25	52.00
C_9	35.17	36.80
C_{10}	61.44	59.99
C_{11}	35.63	35.00
C_{12}	30.69	29.99
C_{13}	38.35	38.10
C_{14}	39.72	40.05
C_{15}	32.40	31.50
C_{16}	36.15	36.00
C_{17}	30.08	29.00
C_{18}	42.91	42.00
C_{19}	35.39	34.80
C_{20}	28.22	27.80
C_{21}	32.90	31.99
C_{22}	39.31	38.80

Table 23 (continue)

Position of carbon	Friedelan-3β-ol	BOV5
C_{23}	11.59	11.60
C_{24}	15.84	15.82
C_{25}	18.27	18.15
C_{26}	18.66	18.50
C_{27}	20.13	19.99
C_{28}	32.12	31.00
C_{29}	35.04	34.00
C_{30}	31.81	30.90

Mass spectrum(Fig. 26) displayed molecular ion at m/e 428 and others fragmentation at m/e 413(M^+-CH_3), 304(M^+-C_9H_{16}), 275(M^+-$C_{11}H_{20}$-H), 273(M^+-$C_{10}H_{18}O$), 249(M^+-$C_{13}H_{23}$), 233(M^+-$C_{13}H_{23}$-CH_3-H), 218(M^+-$C_{14}H_{22}O$-2H), 207(M^+-$C_{16}H_{28}$-H), 205(M^+-$C_{15}H_{26}O$), 195(M^+-$C_{17}H_{30}$), 193(M^+-$C_{17}H_{30}$-H) and 165(M^+-$C_{18}H_{31}$-H-CH_3). Fragmentation of **BOV5** was similar to that of friedelan-3β-ol(25) and is shown in **Scheme 4**.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+
HO
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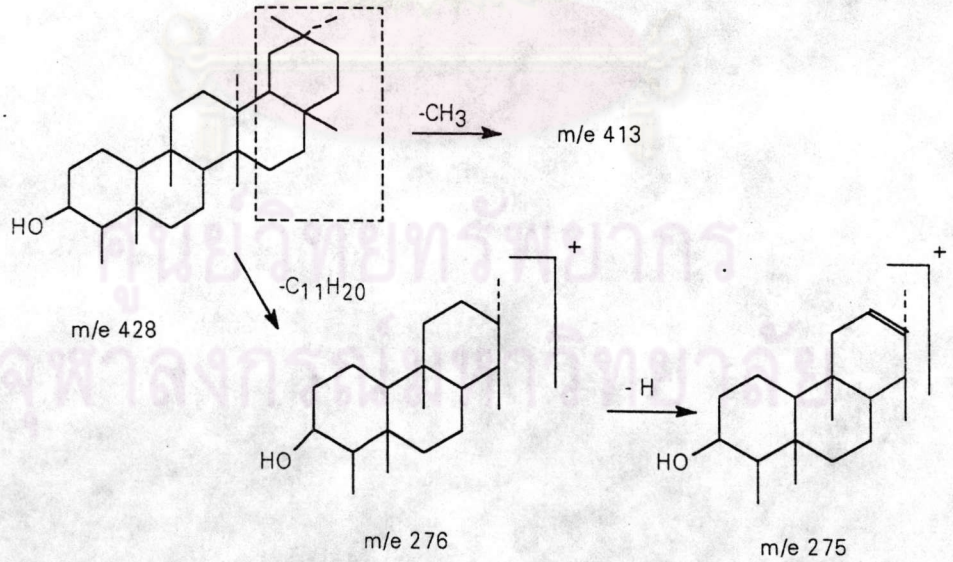
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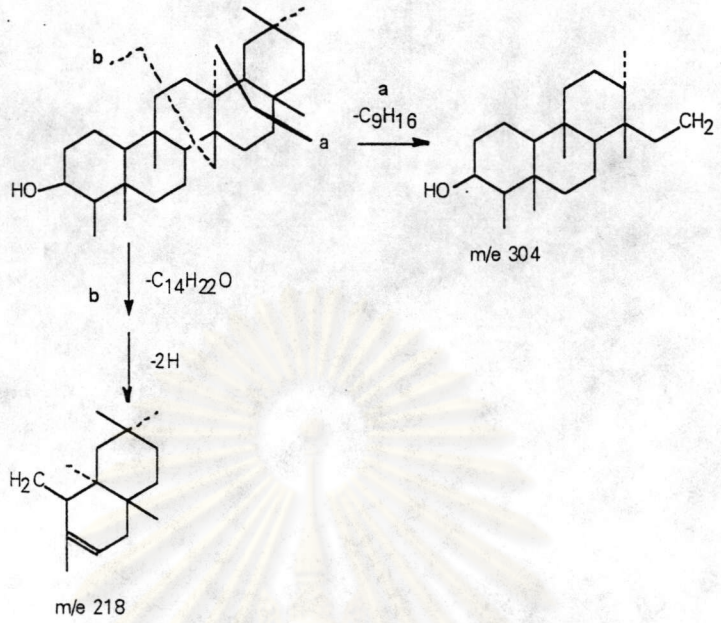


Scheme 4 Fragmentation of BOV5

The structure of **BOV5** was confirmed by 2D-NMR analysis such as ^{13}C-^{1}H correlation (Fig. 27-29). The signals of carbons(δ, ppm) which were correlated with the signals of protons(δ, ppm) are shown in **Table 24.**

Table 24 ^{13}C-^{1}H correlation of **BOV5**(500 MHz, $CDCl_3$, δ)

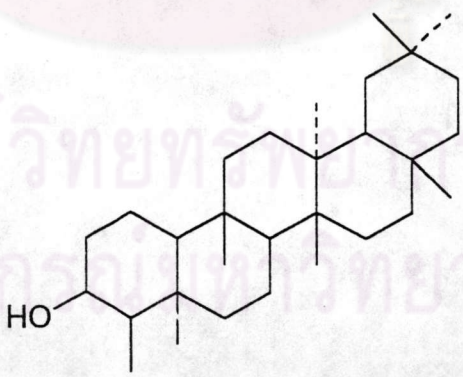
Position		Chemical shift of correlation	
Proton	Carbon-13	Proton	Carbon-13
H_1	C_1	0.97	16.40
H_2	C_2	1.89, 1.91	35.20
H_3	C_3	3.74	72.20
H_4	C_4	1.26	49.25
H_5	C_5	-	37.90

Position		Chemical shift of correlation	
Proton	Carbon-13	Proton	Carbon-13
H_6	C_6	1.72, 1.75	41.80
H_7	C_7	1.38, 1.39	17.59
H_8	C_8	1.29	53.25
H_9	C_9	-	35.15
H_{10}	C_{10}	0.89	61,40
H_{11}	C_{11}	1,41, 1.44	35.60
H_{12}	C_{12}	1.31	30.68
H_{13}	C_{13}	-	38.40
H_{14}	C_{14}	-	39.70
H_{15}	C_{15}	1.28, 1.46	32.40
H_{16}	C_{16}	1.36	36.10
H_{17}	C_{17}	-	30.02
H_{18}	C_{18}	1.55	42.91
H_{19}	C_{19}	1.36, 1.39	35.38
H_{20}	C_{20}	-	28.20
H_{21}	C_{21}	1.42, 1.46	32.90
H_{22}	C_{22}	0.91, 0.95	39.30
H_{23}	C_{23}	0.94	11.60
H_{24}	C_{24}	1.51	15.82
H_{25}	C_{25}	0.86	18.26
H_{26}	C_{26}	1.01	18.60

Position		Chemical shift of correlation	
Proton	Carbon-13	Proton	Carbon-13
H_{27}	C_{27}	0.99	20.10
H_{28}	C_{28}	1.17	32.10
H_{29}	C_{29}	0.95	35.01
H_{30}	C_{30}	1.00	31.80

From ^{1}H-^{1}H COSY (Fig. 30-31) and ^{1}H-^{1}H NOESY (Fig. 32), the signal of proton at 3.74 ppm(H_3) coupled with the signals of protons at 1.89 and 1.91 ppm (H_2) and the signal at 1.26 ppm(H_4). The signal at 3.74 ppm(H_3) coupled long range with the signal at 0.94 ppm(H_{23}).

From the spectroscopic data and comparison of **BOV5** with compound 5ก which was the chemical constituent of the branches of Makaa(27), they can be concluded that **BOV5** was friedelan-3β-ol($C_{30}H_{52}O$). The structure is shown below.



Friedelan-3β-ol($C_{30}H_{52}O$)

Structural elucidation of **BOV6**

BOV6 was bright white needle which was separated by column chromatography of Fraction I and III using silica gel as an adsorbent and of Fraction IV using aluminium oxide as an adsorbent. It was recrystallized by a mixture of dichloromethane and methanol for several times to obtain approximately 2.79 g (equivalent to 0.56% of Fraction I, 0.40% of Fraction III and 0.64% of Fraction IV). Its melting point was 160-162 $^{\circ}$C and R_f = 0.46(silica gel/dichloromethane). **BOV6** dissolved in dichloromethane.

The IR spectrum is shown in Fig. 33 and absorption bands indicate in **Table 25.**

Table 25 The IR absorption band assignments of **BOV6**

Wavenumber (cm-1)	Intensity	Tentative assignment
3712-3146	strong	O-H stretching vibration of R-OH
2937, 2866	strong	C-H stretching vibration of CH_3-, -CH_2-
1641	weak	C=C stretching vibration of alkene
1460	strong	C-H bending vibration of CH_3-, -CH_2-
1381, 1370	moderate	C-H symmetric bending vibration of CH_3-
1157	moderate	C-O stretching vibration and O-H bending vibration of R-OH

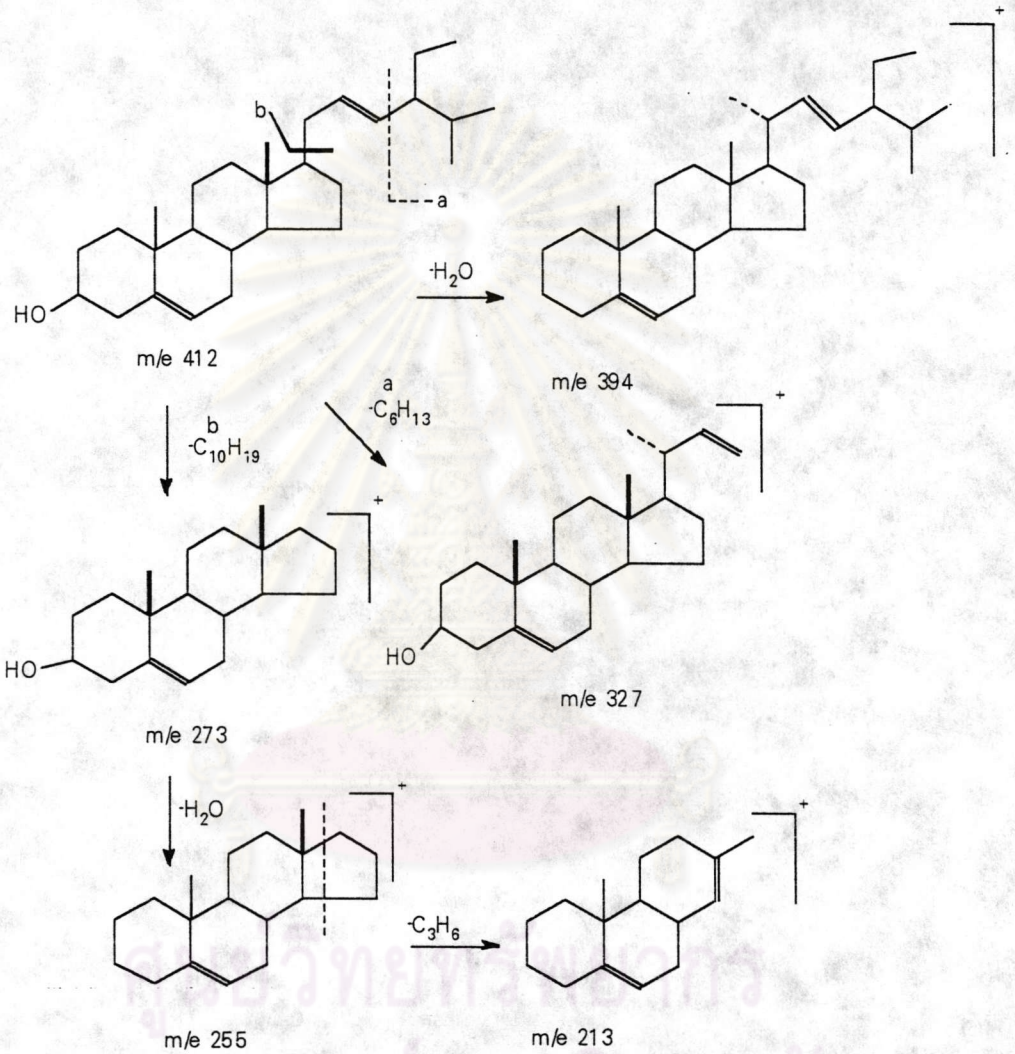
From IR spectrum, absorption band of alcoholic functional group displayed at 3712-3146 cm^{-1} and absorption band of unsaturation displayed at 1641, 970 and 800 cm^{-1}. It corresponded to the reactions such as positive test to 0.1% $KMnO_4$ and Br_2 in CCl_4 reagents. **BOV6** gave negative result to 2,4-DNP(no yellow precipitate) and 1% $FeCl_3$ reagents. It exhibited that **BOV6** was not aldehyde, ketone or phenol. **BOV6** gave green color with Liebermann-Burchard reagent, this showed that **BOV6** was steroid.

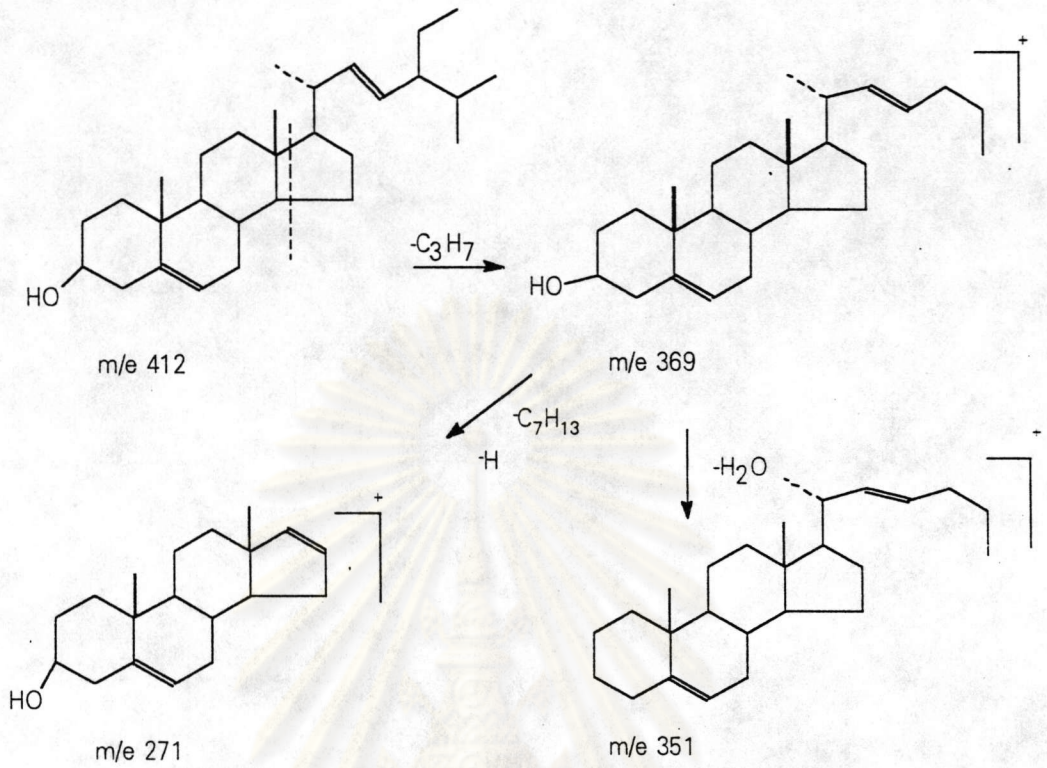
When **BOV6** was analyzed by GLC technique and compared with chromatogram of the mixture of three standard steroids(Fig. 34) such as campesterol, stigmasterol and β-sitosterol. The retention time of this chromatogram(Fig. 35) indicated that **BOV6** was stigmasterol. Retention times of **BOV6** and three standard steroids are shown in **Table 26**

Table 26 Retention times of BOV6 and those of 3 standard steroids

Steroid	Retention time of standard steroid	Retention time of BOV6
campesterol	18.57	-
stigmasterol	19.73	19.42
β-sitosterol	22.03	-

The mass spectrum(Fig. 36), it displayed the molecular ion peak at m/e 412. It revealed the fragmentation of molecule at m/e 394(M^+-H_2O), 369(M^+-C_3H_7), 327 (M^+-C_6H_{13}), 271(M^+-$C_{10}H_{20}$-H), 255(M^+-$C_{10}H_{19}$-H_2O). The mass spectrum of stigmasterol, fragmentation of **BOV6** is similar to that of stigmasterol and is shown in **Scheme 5.**


b
a
HO
m/e 412
-H2O
m/e 394
a
-C6H13
b
-C10H19
HO
m/e 273
HO
m/e 327
-H2O
-C3H6
m/e 255
m/e 213



Scheme 5 Fragmentation of BOV6

From ^{1}H-NMR spectrum(Fig. 37), the signals of protons in CH_3-, CH_2-, CH- groups of steroid indicated at 0.69-2.28 ppm, a signal at 3.52 ppm exhibited the proton linked to carbon having hydroxy group. Signal of proton at 5.09 ppm revealed -CH=CH- group and the signal of proton at 5.36 ppm exhibited C=CH group. This ^{1}H-NMR spectrum was similar to that of stigmasterol and spectra of campesterol and β-sitosterol had no signal of proton at 5.09 ppm.

The ^{13}C-NMR spectrum(Fig. 38) of **BOV6** appeared 29 peaks signals of carbon in $\underline{C}H_3$-, $\underline{C}H_2$-, $\underline{C}H$-, $\underline{C}$ at 12.04-56.86 ppm exhibited steroid substance. The signal of carbon at 71.80 revealed carbon linked to hydroxy group. The signal of carbon in $\underline{C}H$=C , CH=$\underline{C}$, -$\underline{C}$H=CH- and -CH=$\underline{C}$H- patterns were indicated at 121.71, 140.74, 129.27 and 138.31 ppm, respectively.

When **BOV6** was analyzed by DEPT-90 ^{13}C-NMR(Fig. 39) technique, CH appeared 11 signals at 31.89(two signals), 40.49, 50.15, 51.23, 55.95, 56.86, 71.80, 121.71, 129.27 and 138.31 ppm. CH_3 and CH (up phase) from DEPT-135 ^{13}C-NMR spectrum(Fig. 40) appeared 17 signals at 12.04, 12.24, 18.98, 19.39, 21.08, 21.21, 31.89(two signals), 40.49, 50.15, 51.23, 55.95, 56.86, 71.80, 121.71, 129.27 and 138.31 ppm. CH_2 from DEPT-135 ^{13}C-NMR spectrum appeared 9 signals at 21.08, 24.36, 25.40, 28.91, 31.65, 31.89, 37.25, 39.68 and 42.29 ppm.

The spectrum was similar to that of stigmasterol. The ^{13}C-NMR spectrum of **BOV6** and stigmasterol are shown in **Table 27**.

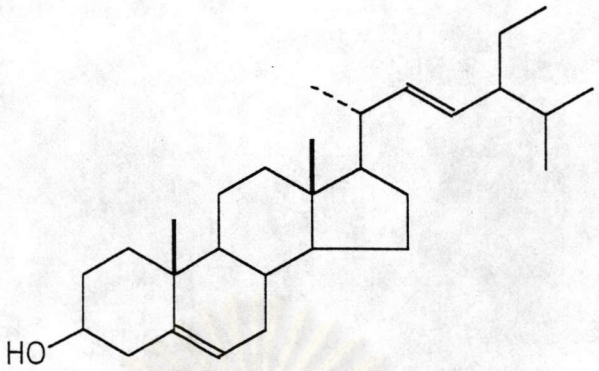
Table 27 Comparison of ^{13}C-NMR of BOV6 with stigmasterol

Position of carbon	Stigmasterol	BOV6
C_1	37.4	37.25
C_2	31.7	31.65
C_3	71.8	71.80
C_4	42.4	42.29
C_5	140.9	140.74
C_6	121.7	121.71
C_7	31.9	31.89
C_8	31.9	31.89
C_9	50.3	50.15
C_{10}	36.6	36.51
C_{11}	21.1	21.08

Table 27 (continue)

Position of carbon	Stigmasterol	BOV6
C_{12}	39.8	39.68
C_{13}	42.4	42.29
C_{14}	57.0	56.86
C_{15}	24.4	24.36
C_{16}	28.9	28.91
C_{17}	56.0	55.95
C_{18}	12.2	12.24
C_{19}	19.4	19.39
C_{20}	40.5	40.49
C_{21}	21.1	21.08
C_{22}	138.4	138.31
C_{23}	129.4	129.27
C_{24}	51.2	51.23
C_{25}	31.9	31.89
C_{26}	19.0	18.98
C_{27}	21.1	21.21
C_{28}	25.4	25.40
C_{29}	12.0	12.04

From all data, it was concluded that **BOV6** was stigmasterol($C_{29}H_{48}O$). The structure is shown below.



Stigmasterol($C_{29}H_{48}O$)

Structural elucidation of BOV7

BOV7 was bright violet amorphous which was obtained from preparative TLC and HPLC technique of fraction 45-70. It was recrystallized from the mixture of dichloromethane and hexane for several times to obtain approximately 0.020 g (equivalent to 0.01% wt.by wt. of Fraction III). Its melting point was 205-207 °C and R_f = 0.88 (silica gel/methanol:dichloromethane = 1:9).

From the ^{1}H-NMR spectrum(Fig. 42), the signal in CH_3-, CH_2- appeared at 1.21-1.80 ppm. The signals of protons in CH_2-Ar and CH_2-C=O revealed at 2.10-2.80 ppm and CH2-O indicated at 3.10-3.90 ppm. There are signals of proton in CH_2=CH_2 of alkene and aromatic ring at 6.10-8.80 ppm.

The ^{13}C-NMR spectrum(Fig. 43) of **BOV7** appeared 38 peaks to find signals of carbon in $\underline{C}H_3$-, $\underline{C}H_2$-, $\underline{C}H$-, $\underline{C}$- at 11.00-189.50 ppm. The signal of carbon at 189.50 ppm revealed carbonyl functional group(C=O) of ester and the signal of carbon at 63.00 ppm revealed carbon linked to hydroxy group(CH_2OH).

When ^{13}C-NMR spectrum of **BOV7** was compared with **BOV8**, the spectrum were similar but **BOV7** but no signal of carbon at 187.50 ppm.

DEPT-90 ^{13}C-NMR(Fig. 44) experiment of **BOV7** revealed 11 signals of CH- at 129.00, 106.50, 104.60, 101.50, 97.90, 93.00, 92.90, 53.80, 53.50, 52.10 and 50.10 ppm. From DEPT-135 ^{13}C-NMR spectrum, CH_3 and CH(up phase) (Fig. 45) appeared 15 signals at 129.00, 106.50, 104.60, 101.50, 97.90, 93.00, 92.90, 53.80, 53.50, 52.10, 50.10, 23.50, 17.50, 13.10 and 11.00 ppm and CH_2(down phase) appeared 7 signals at 123.00, 122.00, 63.00, 31.00, 29.50, 19.10 and 18.50 ppm.

From all data of NMR spectrum, **BOV7** was composed of CH_3 4 groups, CH_2 7 groups, CH 11 groups, quarternary carbon(-C-) 16 groups and carbonyl(C=O) signal at 189.50 ppm.

The mass spectrum(scan at 70 eV and scan at 30 eV, Fig. 41) dissociated so that molecular ion was not indicated. From this data, **BOV7** was unstable substance. Therefore molecular weight of **BOV7** was not known, it could not exhibit molecular formula.

Unfortunately, this substance was obtained in such a limited amount that its structure cannot be further elucidated. This study in **BOV7** will increase data for mass spectrum by using Fast Atomic Bombardment Technique. The other method was analysed by Elemental Analysis for molecular formula.

Structural elucidation of **BOV8**

BOV8 was black amorphous solid which was obtained from preparative TLC and HPLC technique of fraction 45-70. It was recrystallized from a mixture of dichloromethane and hexane for several times to obtain approximately 0.025 g (equivalent to 0.02% wt.by wt. of Fraction III). Its melting point was 194-196 $^{\circ}$C and R_f = 0.90(silica gel/methanol: dichloromethane = 1:9).

The IR spectrum of **BOV8** is shown in Fig. 46 and absorption band assignments In **Table 28**.

Table 28 The IR absorption band assignments of **BOV8**

Wavenumber(cm^{-1})	Intensity	Tentative assignment
3600-3080	moderate	O-H stretching vibration
2990, 2950	weak	C-H stretching vibration of CH_3-, -CH_2-
1725, 1700	strong	C=O stretching vibration
1675	weak	C=C stretching vibration of alkene
1600	moderate	C=C stretching vibration of aromatic ring
1450	moderate	C-H bending vibration of CH_3-, CH_2-
1390	moderate	O-H bending vibration
1210	weak	C-O stretching vibration

From IR spectrum(Fig. 46), absorption band of alcoholic functional group displayed at 3600-3080 cm^{-1} and absorption band of carbonyl functional group of ester or carboxylic displayed at 1725 and 1700 cm^{-1}.

From ^{1}H-NMR spectrum(Fig. 48), the signals of proton in CH_3-, CH_2- appeared at 1.60-1.90 ppm. The signals of proton in CH_2-O, CH_2-Ar and CH_2-C=O indicated at 2.10-3.85 ppm and there are signals of protons in alkene(CH_2=CH_2) and aromatic ring at 6.10-8.00 ppm. The signals of proton in -CHO indicated at 9.30 and 9.45 ppm.

The ^{13}C-NMR spectrum(Fig. 49) of **BOV8** appeared 40 peaks to find signals of carbon in CH_3-, CH_2-, CH-, C- at 11.00-189.50 ppm. The signal of carbon at

189.50 ppm revealed carbonyl functional group(C=O). The signal of carbon in -C=CH-OH pattern was indicated at 187.50 ppm and the signal of carbon at 65.00 ppm revealed carbon linked to hydroxy group.

When **BOV8** was analysed by DEPT-90 ^{13}C-NMR(Fig. 50) technique, CH- appeared 14 signals at 187.50, 129.00, 128.00, 106.50, 104.60, 101.50, 97.90, 93.00, 92.90, 65.00, 53.80, 53.50, 52.10 and 50.10 ppm. CH_3 and CH(up phase) from DEPT-135 ^{13}C-NMR spectrum(Fig. 51) appeared 18 signals at 187.50, 129.00, 128.00, 106.50, 104.60, 101.50, 97.90, 93.00, 92.90, 65.00, 53.50, 52.10, 50.10, 23.50, 17.50, 13.10 and 11.00 ppm. CH_2 from DEPT-135 ^{13}C-NMR spectrum(down phase) appeared 6 signals at 123.00, 122.00, 31.00, 29.50, 19.10 and 18.50 ppm.

From all data of ^{13}C-NMR, DEPT-90 ^{13}C-NMR and DEPT-135 ^{13}C-NMR spectra ,**BOV8** was composed of CH_3 4 groups, CH_2 6 groups, CH 14 groups and 16 quaternary carbon(-C-). Because of the IR spectrum, absorption bands of carbonyl and alcoholic functional group, it is apparent that **BOV8** was composed of oxygen.

Because of **BOV8** was unstable, mass spectra(scan at 70 eV and scan at 30 eV, Fig. 47) of this substance were dissociated so that molecular ion was not indicated. Molecular weight of **BOV8** was not known therefore it could no exhibit molecular formula.

Unfortunately, this substance was obtained in such a limited amount that its structure cannot be further elucidated. Increasing data of **BOV8** for mass spectrum was analysed by Fast Atomic Bombardment Technique and the other method was Elemental Analysis for molecular formula.

When the chemical constituents in the leaves and the branches of B. ovata were compared, they are summarized in **Table 29**.

Table 29 Comparison of chemical constituents in the leaves and the branches(27) of B. ovata

Chemical constituents in	
Branches	Leaves
- the mixture of seven long chain aliphatic hydrocarbon(C_{27}-C_{33})	- the mixture of eight long chain aliphatic hydrocarbon(C_{26}-C_{33})
- the mixture of long chain aliphatic esters	- the mixture of long chain aliphatic esters
- the mixture of three steroid esters	
- friedelin	- friedelin
- friedelan-3β-ol	- friedelan-3β-ol
- the mixture of three long chain aliphatic alcohols (C_{30}-C_{32})	- the mixture of three long chain aliphatic alcohols(C_{23}, C_{24} and C_{32})
- the mixture of thirteen aliphatic carboxylic acid(C_{23}, C_{24}, C_{26}-C_{36})	
- *trans*-triacontyl-4-hydroxy-3-methoxy cinnamate	
- the mixture of three steroids	
- the mixture of three steroid glycosides	
- the mixture of two triterpenoids	
	- the bright violet amorphous
	- the black amorphous solid