CHAPTER IV

RESULTS AND DISCUSSION

1. Structure and Determination of the isolated Compounds

Part I : Heartwood of *X. xylocarpa* var. *kerrii* from Loei province

One kilogram of dried heartwood of *X. xylocarpa* var. *kerrii* was macerated with hexane, ethyl acetate and acetone, respectively. The hexane extract and ethyl acetate extract were separated by using the process described in chapter III. Compound A-1, A-2 and A-3 were obtained from hexane crude extract. Compound A-4 was obtained from ethyl acetate crude extract.

Spectroscopic data were used to determine the chemical structures of these compounds. The structure were also confirmed by comparative analysis using previous reports as references.

1. Identification of isolated compounds

1.1 Identification of compound A-1

Compound A-1 was obtained as white solid with a melting point of 152°C. The FT-IR spectrum (Figure 6) displayed bands as summarized in Table 10.

Table 10 The IR absorption band assignment of compound A-1

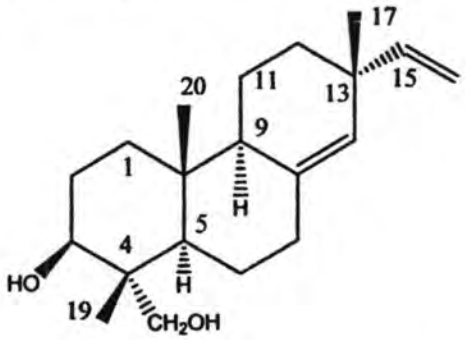
Wave number (cm^{-1})	Assignment
3412	O-H stretching
2948-2830	alkane C-H stretching
1448-1361	C-H bending
1090-996, 908	C=C stretching and bending

The ES TOF mass spectrum of compound A-1 displayed a molecular ion peak [M^+] at *m/z* 304. The molecular formula was process to be $C_{20}H_{32}O_2$ based on ^1H-^{13}C NMR spectra and mass spectral data.

The ^{1}H NMR spectrum of compound A-1 ($CDCl_3$, Figure 7) revealed the presence of three tertiary methyl groups at δ 0.82 (s, CH_3), 1.01 (s, CH_3), 0.91 (s, CH_3), an olefinic proton at δ 5.20 (s, 1H) and a vinyl proton from the mono-substituted double bond at δ 5.74 (1H, dd, J = 10.8, 17.1 Hz), 4.88 (1H, d, J = 17.1 Hz). These spectral patterns were corresponded with a pimaradiene-type skeleton containing the double bond at C-8-C-14 and C-15-C-16 position. The addition peaks observed at δ 3.67 (m) and 3.40 ppm (d, J = 10.5 Hz) could be assigned to a hydroxymethyl group attached to an asymmetric center. The chemical shifts for equatorial hydroxymethyl group should be at C-4 position (Feliciano *et. al.*, 1988). A multiplet at δ 3.67 ppm was belonged to the proton germinal to the secondary hydroxyl group and consistent with the reported value in the literature (Sittiwong, 2003).

The ^{13}C NMR spectrum ($CDCl_3$, Figure 8) showed twenty carbon resonances, four of which were olefinic carbons at δ 110.1, 128.9, 136.1, and 148.7, corresponding with C-16, C-14, C-8 and C-15, respectively. The carbon signals at δ 72.1 and 77.1 were attributed for primary and secondary alcohols, respectively, and those at δ 48.7, 50.4 could be possibly assigned for two tertiary carbons in a pimarane skeleton. Comparison of the ^{1}H and ^{13}C NMR spectrum in Table 11 with literature values (Sittiwong, 2003) indicated that the structure of this compound should be sandaraco-pimaradiene-3β,18-diol, which was previously identified in the heartwood of *X. dolabriformis.*

Moreover, the sign of optical rotation of this compound as $[\alpha]_D$ = -20.7° (c, 0.34 in MeOH at 20°C) was similar to that of sandaracopimaradiene-3β,18-diol which reported by Laidlaw and Morgan ($[\alpha]_D$ = -18.5°; c, 0.4 in $CHCl_3$) and by Sittiwong ($[\alpha]_D$ = -8°; c, 0.2 in $CHCl_3$ at 23°C). The structure is shown below.



sandaracopimaradiene-3β,18-diol

Table 11 Comparison of the ^{1}H, ^{13}C NMR chemical shift assignments of compound A-1 and sandaracopimaradiene-3β,18-diol (Sittiwong, 2003)

position	Chemical shift (ppm)			
	sandaracopimaradiene-3β,18-diol		Compound A-1	
	^{13}C	^{1}H .	^{13}C	^{1}H
1	37.0		37.0	
2	27.2		27.3	
3β	77.2	3.71 (dd, J = 4.7, 11.1 Hz)	77.1	3.67 (m)
4	42.2		42.3	
5	48.6		48.7	
6	22.4		22.6	
7	35.6		35.7	
8	136.3		136.1	
9	50.3		50.4	
10	38.0		38.1	
11	18.8		18.9	
12	34.4		34.6	
13	37.4		37.5	
14	129.0	5.26 (s)	128.9	5.21 (s)
15	148.9	5.80 (dd, J = 10.6, 17.6 Hz)	148.7	5.74 (dd, J = 10.5, 17.7 Hz)
16a	110.1	4.94 (d, J = 17.6 Hz)	110.1	4.89 (dd, J = 1.5, 17.7 Hz)
16b		4.92 (dd, J = 1.2, 10.6 Hz)		4.87 (dd, J = 1.5, 10.5 Hz)
17	26.0	0.88 (s)	26.1	0.82 (s)
18a	72.2	3.74 (d, J = 10.6 Hz)	72.1	3.67 (m)
18b		3.47 (d, J = 10.6 Hz)		3.40 (d, J = 10.5 Hz)
19	11.5	1.07 (s)	11.6	1.01 (s)
20	15.5	0.97 (s)	15.6	0.91 (s)

1.2 Identification of compound A-2

Compound A-2 was obtained as white solid. The FT-IR spectrum (Figure 11) displayed bands as shown below:

Table 12 The IR absorption band assignment of compound A-2

Wave number (cm^{-1})	Assignment
2988-2826	alkane C-H stretching
1701	C=O stretching
1461-1427, 1385, 1369	C-H bending
1001, 912	C=C stretching and bending

The ES TOF mass spectrum displayed for compound A-2 a molecular ion peak [M^+] at *m/z* 286. The molecular formula was proposed to be $C_{20}H_{30}O$ based on ^1H, ^{13}C NMR spectra and mass spectral data.

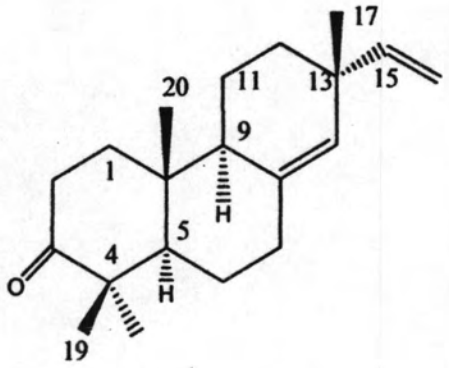
The ^1H NMR spectrum of compound A-2 ($CDCl_3$, Figure 12) suggested the presence of four tertiary methyl groups at δ 1.05 (s, CH_3), 1.07 (s, CH_3), 1.05 (s, CH_3), 0.98 (s, CH_3), an olefinic proton at δ 5.27 (s, 1H), which could be placed at C-14 in association with a C-8-C-14 double bond and the pattern of three vinyl protons for the sandaracopimaradiene type.

The ^{13}C NMR spectrum ($CDCl_3$, Figure 13) showed twenty carbon resonances, four of which were olefinic carbons at δ 110.4, 129.6, 135.8, and 148.6 corresponding with C-16, C-14, C-8 and C-15, respectively and a carbon signal at δ 216.8 which could be assigned to one carbonyl group. Comparing the ^{13}C NMR spectrum (Table 13) of this compound with reported values (Sittiwong, 2003) indicated that the structure of this compound should be sandaracopimaradiene-3-one, which was previously identified in the heartwood of *X. dolabriformis.*

Table 13 Comparison of the ^{13}C NMR chemical shift assignments of compound A-2 and sandaracopimaradiene-3-one

position	^{13}C	Chemical shift (ppm)	
		sandaracopimaradiene-3-one	Compound A-2
1	CH_2	37.7	37.7
2	CH_2	34.8	34.8
3	C=O	216.8	216.8
4	C	47.9	47.8
5	CH	55.3	55.4
6	CH_2	23.3	23.3
7	CH_2	35.6	35.6
8	C	135.8	135.8
9	CH	49.5	49.5
10	C	38.1	38.0
11	CH_2	18.9	19.0
12	CH_2	34.4	34.4
13	C	37.5	37.5
14	CH	129.6	129.6
15	CH	148.6	148.6
16	CH_2	110.4	110.4
17	CH_3	26.1	26.1
18	CH_3	25.8	25.8
19	CH_3	22.3	22.3
20	CH_3	14.7	14.7

In addition, the sign of optical rotation value of this compound was $[\alpha]_D = -30.9°$ (c, 0.10 in MeOH at 20°C), similar to that of sandaracopimaradiene-3-one which was reported as $[\alpha]_D = -56°$ (c, 2 in $CHCl_3$) (Laidlaw and Morgan, 1963) and as $[\alpha]_D = -46°$ (c, 1 in $CHCl_3$ at 23.4°C) (Sittiwong, 2003).



sandaracopimaradiene-3-one

1.3 Identification of compound A-3

Compound A-3 was obtained as white solid, m.p. 106-110°C. The FT-IR spectrum (Figure 16) indicated functional groups as shown below:

Table 14 The IR absorption band assignment of compound A-3

Wave number (cm^{-1})	Assignment
3388	O-H stretching
2941-2869	C-H stretching
1706-1639	C=C stretching of alkene
1458,1382	C-H bending
1090, 1032, 997, 911	C=C stretching and bending

The ES TOF mass spectrum of compound A-3 showed a molecular ion peak [M^+] at *m/z* 288. The molecular formula was determined to be $C_{20}H_{32}O$ based on ^1H, ^{13}C NMR spectra and mass spectral data.

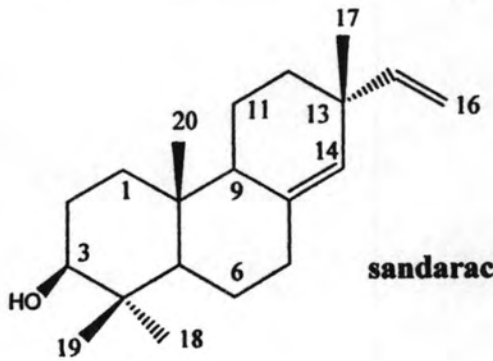
The ^1H NMR spectrum of compound A-3 ($CDCl_3$, Figure 17) suggested the presence of four tertiary methyl groups at δ 1.02 (s, CH_3), 0.99 (s, CH_3), 0.80 (s, CH_3), 0.78 (s, CH_3), an olefinic proton at δ 5.21 (s, 1H), which could be placed at C-14 in association with a C-8-C-14 double bond and three vinyl protons from the mono-substituted double bond at δ 5.75 (dd, J = 10.5, 17.7 Hz).

The ^{13}C NMR spectrum ($CDCl_3$, Figure 18) showed twenty carbon resonances, four of which were olefinic carbons at δ 110.1, 128.9, 136.7 and 149.0 corresponding with C-16, C-14, C-8 and C-15, respectively and the carbon signals at δ 79.2 which could be assigned to one hydroxyl group. Comparing the ^{13}C NMR spectrum (Table 15) of this compound with reported values (Sittiwong, 2003) indicated that the structure of this compound should be sandaracopimaradiene-3β-ol, which was previously identified in the heartwood of *X. dolabriformis.*

Moreover, the sign of optical rotation $[\alpha]_D$ = -32.5° (c, 0.02 in MeOH at 20°C) was similar with that of sandaracopimaradiene-3β-ol (Sittiwong, 2003).

Table 15 Comparison of the ^{13}C NMR chemical shift assignments of compound A-3 and sandaracopimaradiene-3β-ol

position	^{13}C	Chemical shift (ppm)	
		sandaracopimaradiene-3-ol	Compound A-3
1	CH_2	37.3	37.3
2	CH_2	27.6	27.6
3	CH	79.2	79.2
4	C	39.0	39.0
5	CH	54.1	54.2
6	CH_2	22.2	22.3
7	CH_2	35.9	35.9
8	C	136.7	136.7
9	CH	50.4	50.4
10	C	38.1	38.9
11	CH_2	18.8	18.8
12	CH_2	34.5	34.5
13	C	37.5	37.4
14	CH	128.8	128.9
15	CH	149.0	149.0
16	CH_2	110.0	110.1
17	CH_3	26.0	26.0
18	CH_3	28.5	28.5
19	CH_3	15.8	15.7
20	CH_3	15.0	15.0



sandaracopimaradiene-3β-ol

1.4 Identification of compound A-4

Compound A-4 was obtained as white solid. The FT-IR spectrum (Figure 21) displayed bands as summarized below:

Table 16 The IR absorption band assignment of compound A-4

Wave number (cm^{-1})	Assignment
3482	O-H stretching
2968, 2936-2869	C-H stretching
1692	C=O stretching
1013, 909	C=C stretching and bending

The ES TOF mass spectrum of compound A-4 displayed a molecular ion peak [M^+] at *m/z* 302. The molecular formula was determined to be $C_{20}H_{30}O_2$ based on ^1H-^{13}C NMR spectra and mass spectral data.

The ^1H NMR spectrum of compound A-4 showed signals at δ 0.70-1.10 ppm ($CDCl_3$, Figures 22-23) suggesting the presence of four tertiary methyl groups at δ 1.07 (s, CH_3), 1.10 (s, CH_3), 1.11 (s, CH_3) and 1.44 (s, CH_3) and three signals of an ABX system corresponding to three vinyl protons from a mono-substituted double bond at δ 5.71 (dd, J = 11.1, 17.7 Hz) 4.95 (dd, J = 1.2, 11.1 Hz) and 4.98 (dd, J = 1.2, 17.7 Hz) that was well matched with the skeleton of pimarane type, The presence of the fourth methyl group was expected for isopimarane carbon skeleton compounds, as shown by the ^1H and ^{13}C NMR spectral data together with 2D NMR data including COSY (Figure 26), HSQC (Figures 27-28) and HMBC (Figures 29-31) spectral correlations. In addition, the stereochemistry at C-13 was established by comparison of the ^{13}C NMR chemical shifts of C-15, C-16 and C-17 with those of isopimarane diterpenoids, indicating a β-equatorial position for the C-17 methyl group and an α-axial position for the vinyl group.

The ^{13}C NMR spectrum ($CDCl_3$, Figure 24) showed twenty carbon resonances, four of which were olefinic carbons at δ 112.7, 128.7, 139.3 and 143.7. The carbon signals at δ 217.5 could be assigned to one carbonyl group. In a DEPT experiment (Figure 25) the chemical shifts at 128.7 and 139.3 indicated that C-C double bond was

two tetrasubstituted sp carbons, suggesting a fully substituted olefinic double bond at C-8 and C-9 position.

The downfield at δ 74.4 signal should be attached to an oxygen atom in the molecule. The IR spectra band a hydroxyl group, together with one-proton signal of NMR spectra at δ 3.48, indicated that the proton should also be α-axial and a β-equatorial hydroxyl group at C-14 position. The HMBC correlations of C-14 to δ H-17 (1.07 ppm), H-12 (1.44 , 1.58 ppm) and H-15 (5.71 ppm) confirmed the structure.

The combination of HSQC and HMBC data was indeed confirmed the pimarane skeleton having the ketone group at position 3. The C-3 (δ 217.5 ppm) showed correlation with H-1a (1.58 ppm), H-1b (1.99 ppm), H-2a (2.44 ppm), H-2b (2.56 ppm) and H-18, H-19 of tertiary methyl groups at δ 1.07, 1.11 ppm, respectively. The ketone group at position 3 was also confirmed by comparison of the recorded data with previous reported values (Sittiwong, 2003).

The assignments of the other carbons and protons of this compound are displayed in the Table 17. The correlations of HMBC are as follows:

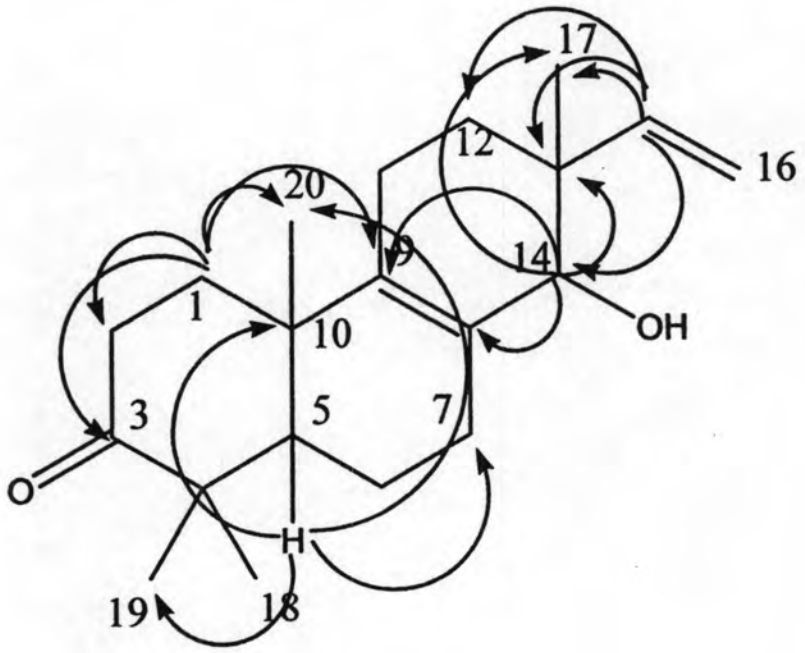


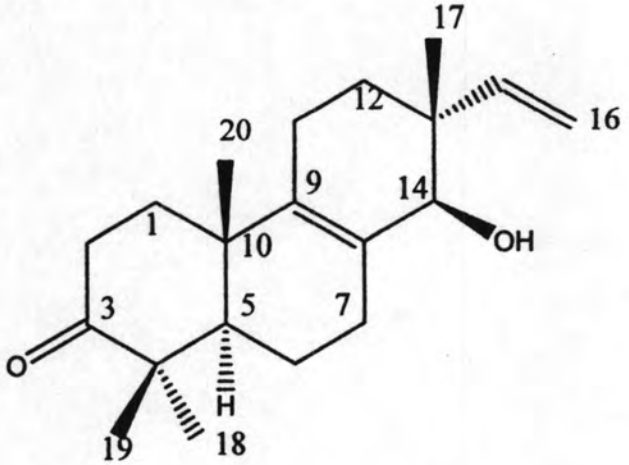
Table 17 The ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMBC spectral data of compound A-4

position	DEPT	Chemical shift (ppm)		H-HCOSY	HMBC
		^{13}C	^{1}H		
1a	CH_2	34.8	1.58 (m)	H1b, H2a, H2b	C-9,C-20
1b			1.99 (dd, J = 3.5, 15.9 Hz)	H2a, H2b	C-2,C-3,C-9
2a	CH_2	34.3	2.44 (ddd, J = 3.5, 7.3, 15.9 Hz)	H1a, H1b	C-1,C-3,C-4,C-10
2b			2.56 (m)	H1a, H1b, H2a	C-1,C-3,C-5,C-10
3	C	217.5	-		-
4	C	47.3	-		-
5	CH	51.1	1.68 (m)		C-7,C-8,C-10, C-11, C-19,C-20
6a	CH_2	21.4	1.20 (m)		C-13,C-18,C-19
6b			1.96 (m)	H7a, H7b	C-5,C-8,C-9
7a	CH_2	19.9	1.60 (m)	H6a, H7b	C-6,C-8
7b			1.68 (m)	H7a	C-8,C-18,C-20
8	C	128.7	-		-
9	C	139.3	-		-
10	C	37.2	-		-
11a	CH_2	29.7	1.98 (m)	H12a, H12b	C-10,C-13
11b			2.60 (m)	H12b	C-8,C-9
12a	CH_2	29.0	1.44 (m)	H11b, H12b	C-9,C-13,C-15
12b			1.54 (m)	H11a, H11b, H12b	C-9,C-13,C-15
13	C	39.5	-		-
14	CH	74.4	3.48 (br s)		C-8, C-9,C-11, C-12,C-13,C-15, C-17

position	DEPT	Chemical shift (ppm)		H-HCOSY	HMBC
		^{13}C	^{1}H		
15	CH	143.7	5.71 (dd, J = 11.1, 17.7 Hz)	H16a, H16b	C-12,C-13,C-14, C-17
16a	CH_2	112.7	4.98 (dd, J = 1.2, 17.7 Hz)	H16b	C-13,C-17
16b			4.95 (dd, J = 1.2, 11.1 Hz)	H16a	C13,C-15
17	CH_3	23.1	1.06 (s)		C-12,C-13,C-15
18	CH_3	26.5	1.11 (s)		C-3,C-4,C-5,C-19
19	CH_3	21.2	1.07 (s),		C-3,C-4,C-5, C-6,C-18
20	CH_3	19.2	1.10 (s)		C-1,C-9,C-10

In addition, the sign of optical rotation value of this compound was recorded $[\alpha]_D$ = +77.2° (c, 0.10 in MeOH at 20°C)

Thus, this compound was determined as 8(9),15-isopimaradiene-3-one,14β-ol which had not found in the previous reports.



8(9),15-isopimaradiene-3-one,14β-ol

Part II : Heartwood of *X. xylocarpa* var. *kerrii* from Mae Hong Son province

One kilogram of dried heartwood of *X. xylocarpa* var. *kerrii* was macerated with hexane, ethyl acetate and acetone, respectively. The hexane extract and ethyl acetate extract were separated using the process described in chapter III. Compounds B-1- B-5 were obtained from hexane crude extract.

Spectroscopic data were examined to determine the chemical structures of these compounds. The structures were also confirmed by comparative analysis using previous reports as references.

1. Identification of isolated compounds

1.1 Identification of compound B-1

Compound B-1 were identified to be sandaracopimaradiene-3β-ol (compound A-3)

1.2 Identification of component B-2

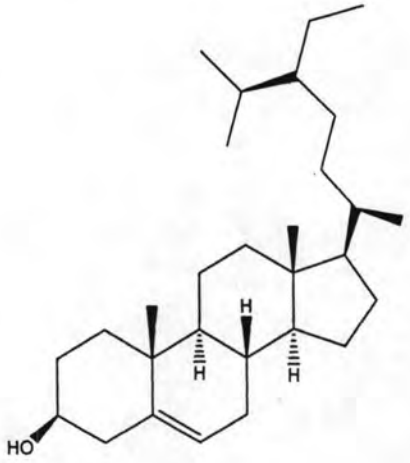
Compound B-2 was obtained as white solid with a melting point of 134-137°C.

The ^{1}H NMR spectrum of this compound ($CDCl_3$, Figure 34) showed the signals at δ 0.65-1.01 ppm which were the signals of methyl protons that were located at C-18, C-19 and at side chain of the steroidal compounds. The signals at δ 1.12-2.3 indicated the presence of methylene and methine proton of steroids and that at δ 3.50 ppm (m) was the signal of proton at C-3. The olefinic protons at δ 5.13 ppm (dd, J = 8.4, 15.2 Hz) and 5.05 (dd, J = 8.4, 15.2 Hz) were *trans*-disubstituted vinyl protons (H-22 and H-23) and that at δ 5.33 (br d J = 4.7 Hz) could be assigned to H-6 which was a trisubstituted vinyl proton.

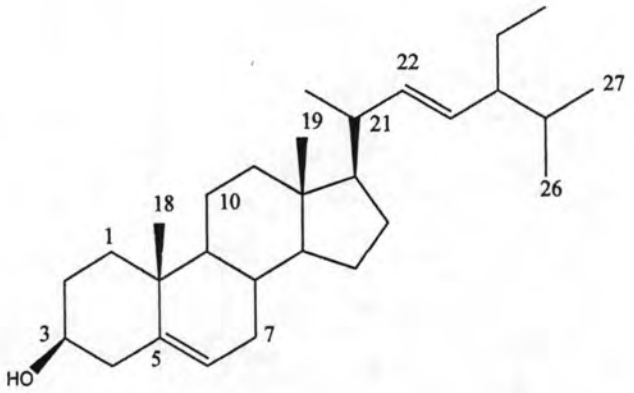
The ^{13}C NMR spectrum ($CDCl_3$, Figure 35) showed carbon signals that were close to the signals from β-sitosterol and stigmasterol which were similar to literature values (Ingkaninan, 1994) as shown in Table 18. The structure of this component should be a mixture of β-sitosterol and stigmasterol which was previously identified in the heartwood of *X. dolabriformis*. In addition, the sign of optical rotation value of this compound was found to be $[\alpha]_D$ = -50.7° (c, 0.04 in MeOH at 25°C).

Table 18 Comparison of the ^{13}C NMR chemical shift assignments of compound B-2 and β-sitosterol and stigmasterol (Ingkaninan, 1994).

Position Carbon	Chemical shift		
	β-sitosterol	stigmasterol	Compound B-2
1	37.1	37.4	37.3
2	31.8	31.7	30.3
3	71.9	71.8	71.8
4	42.4	42.4	45.9
5	140.9	140.0	140.8
6	121.8	121.7	121.7
7	32.0	31.9	31.9, 31.9
8	32.0	31.9	31.9, 31.9
9	50.3	50.3	50.2
10	36.6	36.6	36.5
11	21.1	21.1	21.2, 21.1
12	39.9	39.8	39.8, 39.7
13	42.4	42.4	42.3
14	56.8	57.0	56.8, 56.9
15	24.3	24.4	24.3, 24.4
16	28.2	28.9	28.2, 28.9
17	56.2	56.0	56.1, 56.0
18	11.9	12.2	12.0, 12.2
19	19.4	19.4	19.4
20	36.2	40.5	36.2, 40.5
21	19.1	21.1	19.0, 21.1
22	34.0	138.4	34.0, 138.3
23	29.3	129.4	29.2, 129.3
24	50.3	51.3	51.24
25	26.2	31.9	26.1, 29.2
26	18.8	19.0	18.8, 19.0
27	19.8	21.1	19.8, 21.1
28	23.1	25.4	23.1, 25.4
29	11.9	12.0	11.9, 12.0



β-sitosterol



stigmasterol

1.2 Identification of compound B-3

Compound B-3 was obtained as white solid with a melting point of 143-144°C. The FT-IR spectrum (Figure 38) displayed bands as shown below:

Table 19 The IR absorption band assignment of compound B-3

Wave number (cm^{-1})	Assignment
3429	O-H stretching
3050-2832	C-H stretching
1694	C=O stretching
1276	C=C stretching and bending

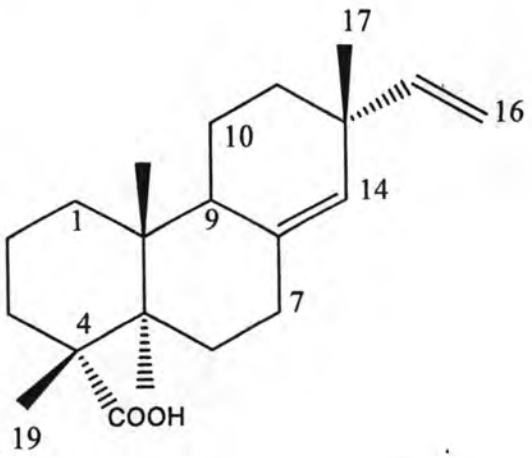
The ES TOF mass spectrum displayed for compound B-3, a molecular ion peak [M^+] at *m/z* 302. The molecular formula was determined to be $C_{20}H_{30}O_2$ based on ^1H-^{13}C NMR spectra and mass spectral data.

The ^1H NMR spectrum of compound B-3 showing signals at δ 0.80-1.21 ppm ($CDCl_3$, Figure 39), suggested the presence of three tertiary methyl groups (δ 1.04 (s), 1.21 (s) and 0.84 (s), indicating that one methyl group was replaced with a carboxylic group. The position of carboxylic group was assigned at C-18 position from the chemical shift of C-20 (0.84 ppm) based upon the previous report (Wenkert and Buckwalter, 1972). This indicated that the carboxylic group should be placed at C-18 position. The typical signals of 8(14), 15-isopimaradiene were shown by the vinyl

protons at δ 5.75 (1H, dd, J = 10.5, 17.5 Hz), 4.90 (1H, d, J = 17.5 Hz), 4.85 (1H, d, J = 10.5 Hz). An olefinic proton at δ 5.20 (1H, s) could only be placed at C14 in association with a C-8-C-14 double bond in a normal pimarane type skeleton.

The ^{13}C NMR spectrum ($CDCl_3$, Figure 40.) showed three methyl signals (δ 26.0 (s, CH_3), 16.8 (s, CH_3), 15.2 (s, CH_3)), the olefinic carbons at δ 129.2, 136.6, and vinyl carbons at δ 110.2, 148.9 corresponding to C-14, C-8 and C-16, C-15, respectively, and the carbon signals at δ 184.4 could be assigned to carboxylic group. Other signals were detected and consistent with the reported values for sandaraco-pimaric acid as shown in Table 20.

These the ^{1}H and ^{13}C NMR data indicated that compound B-3 was sandaraco-pimaric acid. In addition, the sign of optical rotation value of this compound measure as $[\alpha]_D$ = -30.3° (c, 0.10 in MeOH at 25°C) was similar to that of sandaracopimaric acid ($[\alpha]_D$ = -9° (c, 0.5 in $CHCl_3$ at 23°C)) which was identified in previous reports (Laidlaw and Morgan, 1963, Sittiwong, 2003).



sandaracopimaric acid

Table 20 Comparison of the ^{13}C NMR chemical shift assignments of compound B-3 and sandaracopimaric acid (Sittiwong, 2003)

position		Chemical shift (ppm)	
		Sandaracopimaric acid	Compound B-3
		^{13}C	^{13}C
1	CH_2	38.3	38.3
2	CH_2	18.1	18.2
3	CH_2	37.0	37.1
4	C	47.3	47.3
5	CH	48.8	48.9
6	CH_2	24.9	24.9
7	CH_2	35.5	35.5
8	C	136.6	136.6
9	CH	50.5	50.6
10	C	37.7	37.8
11	CH_2	18.5	18.6
12	CH_2	34.4	34.5
13	C	37.4	37.4
14	CH	129.1	129.2
15	CH	148.9	148.9
16	CH_2	110.2	110.2
17	CH_3	26.0	26.1
18	C	185.4	184.4
19	CH_3	16.8	16.8
20	CH_3	15.2	15.2

1.3 Identification of compound B-4

Compound B-4 was obtained as white solid. The FT-IR spectrum (Figure 43) displayed bands as shown in the Table 21.

Table 21 The IR absorption band assignment of compound B-4

Wave number (cm^{-1})	Assignment
3395	O-H stretching
2965, 2942, 2870, 2852	C-H stretching
1456, 1093, 1057, 995	C=C stretching and bending

The ES TOF mass spectrum displayed for compound B-4, a molecular ion peak [M^+] at *m/z* 304. The molecular formula was determined to be $C_{20}H_{32}O_2$ based on ^1H ^{13}C NMR spectra and mass spectral data.

The ^1H NMR spectrum of compound B-4 showed signals at δ 0.80-1.05 ppm ($CDCl_3$, Figures 44-45) indicating the presence of four methyl groups and an olefinic proton at δ 5.25 (br s, 1H), which could only be placed at C-14 in association with a C-8-C-14 double bond in a normal pimarane type skeleton and three vinyl protons from the mono-substituted double bond at δ 5.70 (1H dd, J=10.5, 17.4 Hz), 4.86 (1H dd, J=1.5, 10.5 Hz) and 4.89 (1H dd, J=1.5, 17.4 Hz)

The ^{13}C NMR spectrum ($CDCl_3$, Figure 46.) showed twenty carbon resonances, four of which were olefinic carbons at δ 110.2, 129.5, 136.0, and 148.8 corresponding to C-16, C-14, C-8 and C-15, respectively, as expected for an isopimarane skeleton. It was further supported by the complete NMR spectral data including DEPT (Figures 47-48), COSY (Figure 49), HSQC (Figures 50-51) and HMBC (Figures 52-54) spectral correlations.

The two downfield signals at δ 68.8 and 83.8 should be carbons attached to oxygen atom. IR bands for hydroxyl groups together with two proton signals at δ 3.64 and 3.05 indicated that should be an α-equatorial and a β-equatorial hydroxyl group at C-2 and C-3 position, respectively. The HMBC correlations for C-2 and C-3 as shown in Table 22, which confirmed this proposed structure.

The assignments of the other carbons and protons of this compound are displayed in the Table 22. The HMBC correlations are illustrated as follows:

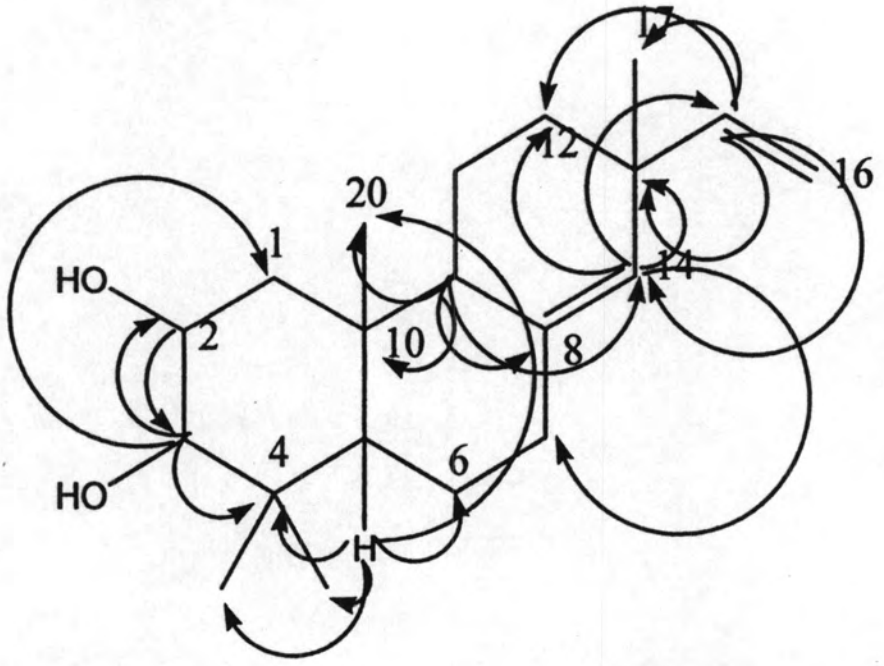
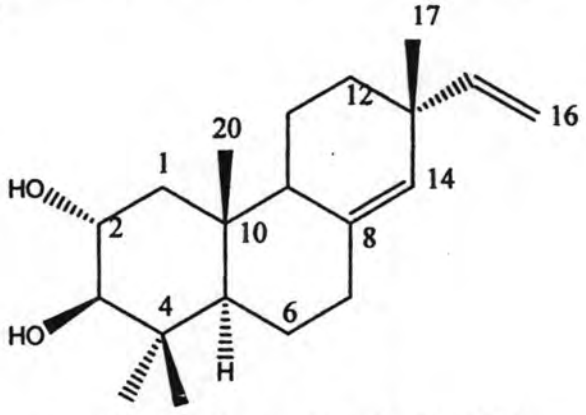


Table 22 The ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMBC spectral data of compound B-4

position	DEPT	Chemical shift (ppm)		H-HCOSY	HMBC
		^{13}C	^{1}H		
1a	CH_2	45.2	1.13 (t, J = 12.2 Hz)		C-2,C-3,C-6, C-9,C-10,C-20
1b			2.03 (dd, J =4.3,12.2 Hz)	H2	C-2,C-3,C-5, C-6,C-10,C-20
2	CH	68.8	3.64 (dt, J =4.3,10.1 Hz)	H3	C-3
3	CH	83.8	3.05 (d, J =10.1 Hz)	H2	C-1,C-2,C-4
4	C	39.3	-		-
5	CH	54.1	1.11 (dd, J =2.6, 12.4 Hz)	H6a	C-4,C-6,C-18, C-19, C-20
6a	CH_2	22.1	1.37 (m)	H7b, H5,H7a	C-5,C-7,C-10
6b			1.57 (dd, J =2.6, 4.3 Hz)	H7a	C-7,C-8,C-10
7a	CH_2	35.7	2.05 (dd, J =4.3, 12.2 Hz)	H6b	C-5,C-6,C-8, C-10, C-14
7b			2.27 (ddd, J =2.1, 4.3, 14.4 Hz)	H6a	C-4,C-5,C-6, C-8,C-9, C-14
8	C	136.0	-		-
9	CH	50.4	1.73 (m)	H14	C-8,C-10, C-14,C-20
10	C	39.0	-		-
11a	CH_2	18.9	1.52 (dd, J =3.2, 6.4 Hz)	H12b	C-9
11b			1.62 (dd, J =1.8,13.7 Hz)	H12a	C-8,C-10,C-12, C-13,C-14
12a	CH_2	34.4	1.33 (m)	H11b, H12b	C-9,C-10,C-11 C-13,C-14, C-15,C-17
12b			1.44 (m)	H12a	C-9,C-11, C-13,C-17
13	C	37.5	-		-

position	DEPT	Chemical shift (ppm)		H-HCOSY	HMBC
		^{13}C	^{1}H		
14	CH	129.5	5.25 (br s)	H7a, H9	C-7,C-12,C-13, C-15
15	CH	148.8	5.70 (dd, J = 10.5, 17.4 Hz)	H16	C-12,C-13, C-14,C-17
16a	CH_2	110.2	4.89 (dd, J = 1.5, 17.4 Hz)	H15	C-15
16b			4.86 (dd, J = 1.5, 10.5 Hz),	H15	C-14,C-15
17	CH_3	26.0	1.04 (s)		C-12,C-13, C-14,C-15,C-16
18	CH_3	29.0	1.04 (s)		C-2,C-3,C-4, C-5
19	CH_3	16.9	0.86 (s),		C-5,C-18
20	CH_3	15.9	0.87 (s)		C-1,C-4,C-9, C-10

In addition, the sign of optical rotation value of this compound was found to be $[\alpha]_D$ = -52.3° (c, 0.10 in MeOH at 25°C) Thus, these results of the ^{1}H and ^{13}C NMR indicated that this compound was sandaracopimaradiene-2α,3β-ol which had not found in the previous reports.



sandaracopimaradiene-2α,3β-diol

1.5 Identification of compound B-5

Compound B-5 were identified to be sandaracopimaradiene-3β,18-diol (compound A-1).

2. Cytotoxicity

The chemical constituents were tested *in vitro* for their cytotoxicity against 5 human cancer cell lines; KATO-3 (human gastric carcinoma), SW620 (human colon adenocarcinoma), BT474 (human breast ductal carcinoma), HEP-G2 (human liver hepatoblastoma), CHAGO (human undifferentiated lung carcinoma), are reported in Table 23

Table 23 Cytotoxic activity of compounds from *X. xylocarpa* var. *kerrii*

Compounds	PS (%)					
(10 (μg/ml)	CH-Liver	KATO-3	SW620	BT474	HEP-G2	CHAGO
CH1	20.96	22.38	18.04	41.90	39.08	18.77
CH2	20.21	29.11	19.13	54.40	35.94	20.65
CE1	27.18	39.09	19.43	41.67	82.48	95.61
CE2	21.39	22.66	15.49	77.98	34.14	23.65
CT1	126.12	105.75	101.59	118.93	103.32	101.65
CT2	68.22	77.06	85.17	96.67	110.78	100.38
A-1	17.60	26.54	22.73	59.40	37.11	18.81
A-2	31.59	38.88	25.82	118.45	77.27	99.29
A-3	20.15	32.78	16.38	65.00	42.41	15.73
A-4	34.83	48.16	46.07	124.29	90.30	97.64
B-2	87.56	67.29	83.35	155.36	157.14	98.01
B-3	24.19	37.14	21.02	25.12	39.08	58.86
B-4	74.69	93.76	83.97	153.21	115.54	99.14

PS = percent survival of the cancer cell lines

A-1 = sandaracopimaradiene-3β,18-diol

A-2 = sandaracopimaradiene-3β-one

A-3 = sandaracopimaradiene-3β-ol

A-4 = 8(9),15-isopimaradiene-3-one,14β-ol (new compound)

B-2 = β-sitosterol and stigmasterol

B-3 = sandaracopimaric acid

B-4 = sandaracopimaradiene-2α,3β-diol (new compound)

CH1 = crude hexane extract of *X. xylocarpa* var. *kerrii* from Loei

CH2 = crude hexane extract of *X. xylocarpa* var. *kerrii* from Mae Hong Son

CE1 = crude ethyl acetate extract of *X. xylocarpa* var. *kerrii* from Loei

CE2 = crude ethyl acetate extract of *X. xylocarpa* var. *kerrii* from Mae Hong Son

CT1 = crude acetone extract of *X. xylocarpa* var. *kerrii* from Loei

CT2 = crude acetone extract of *X. xylocarpa* var. *kerrii* from Mae Hong Son

According to above the result, the hexane, ethyl acetate extract from Loei and Mae Hong Son, compound A-1, A-2, A-3, A-4 and B-3 exhibited cytotoxic activity as indicated by the percent survival of cancer cell lines less than 50% in KATO-3, SW620. They were CH1, CE2 and B-3 against BT474, CH1, CH2, CE2, A-1, A-3, and B-3 against HEP-G2, and CH1, CH2, CE2, A-1 and A-3 against CHAGO. The acetone crude extract, B-2 and B-4 were devoided of cytotoxic activity in all cancer cell lines. But these compound also exibited cytotoxic activity to normal cell (CH-liver), except acetone crude extracts, B-2 and B-4.

The five isolated compounds exhibited cytotoxic activity against human cancer cell lines with at IC_{50} values as shown below:

Table 24 IC_{50} of active compounds from *X. xylocarpa* var. *kerrii*

compounds	IC_{50} (μg/ml)					
	CH-Liver	KATO-3	SW620	BT474	HEP-G2	CHAGO
A-1	7.66	8.19	6.71	5.45	6.93	>10
A-2	7.19	5.17	6.73	>10	>10	>10
A-3	4.92	3.67	5.50	7.04	3.84	6.21
A-4	7.36	6.83	8.51	>10	7.49	>10
B-3	5.56	0.33	7.07	7.28	5.02	6.76

All of them exhibited moderate cytotoxic activity (IC_{50} < 10 μg/ml) against KATO-3 and SW620 cancer cell lines except B-3 which showed strong activity against KATO-3. But these compounds also inhibited CH-liver (normal cell). A-1, A-3 and B-3 showed moderate cytotoxic activity against BT474. A-2 and A-4 showed less activity (IC_{50} >10 μg/ml). A-1, A-3, A-4, B-3 had moderate cytotoxic activity but A-2 were less cytotoxic against HEP-G2. A-1, A-2 and A-4 showed less cytotoxic activity against CHAGO or could be considered as inactive (IC_{50} = >10 μg/ml).

Comparing the structure activity relationship of the tested compounds were shown that A-1 and A-3 which have hydroxyl group at position C3, but having hydroxyl group at position C18 of A-1 that was less activity than A-3. The absence of a free hydroxyl group at position C14 in A-2 that exhibited moderate cytotoxic activity against all cell lines as same as A-4 which also has a ketone at C3. Compound B-3 showed strong cytotoxic activity against KATO-3 but the others exhibited moderate cytotoxic activity against this cell line because it has a carboxyl group at position C18. B-3 may be selective to human gastric carcinoma cell line. However, the data was not sufficient to conclude and relationship between activity and hydroxyl or ketone or carboxyl group substitutions on the tricyclic ring of pimarane-type diterpenoids.