CHAPTER IV

RESULTS AND DISCUSSION

Investigation of the chemical constituents of the dichloromethane and ethyl acetate extracts of the stem of *Polyalthia jucunda* (Pierre) Finet & Gagnep. by using chromatographic techniques led to the isolation of three compounds. The identification of these compounds were based on spectroscopic evidences (NMR and Mass Spectra) and also by comparison with previously reported data in the literatures. The details can be discussed as follows:

1. Identification of compound PJ1

Compound PJ1 was obtained from preparative TLC as whitish amorphous powder (0.00020%), The EIMS (Figure 3.) showed a molecular ion at *m/z* 190, which corresponded to the molecular formula $C_{12}H_{14}O_2$ whereas other prominent ions at *m/z* 175, 162 and 119 were also observed. The presence of 12 carbons and 14 protons were in agreement with signals observable in the ^{13}C-NMR (Figures 5a-5b) and ^{1}H-NMR (Figures 4a-4c) spectra, respectively.

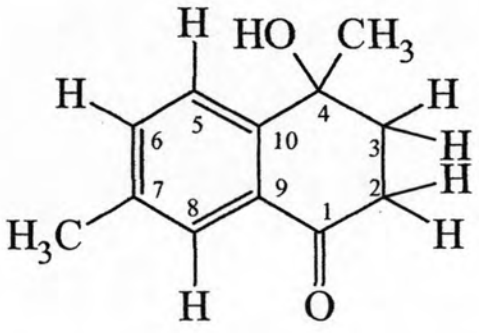
The DEPT experiments of PJ1 indicated that there were 5 quaternary carbons, 3 methine, 2 methylene and 2 methyl functions in the molecule (Figure 6). ^{1}H-NMR of PJ1 showed three typical aromatic proton signals appearing between δ 7-8 ppm. The multiplicity of these protons could be recognized as a pair of protons resided at ortho position to each other, one of which appeared as doublet (J = 8.0 Hz) whereas the coupled proton appeared as a doublet of doublets (J = 8.0, 2.0 Hz) at δ 7.44 ppm, indicating the presence of a third aromatic proton at meta position (J = 2.0 Hz) at δ 7.81 ppm to the latter (Figure 4b.). The remaining non-protonated aromatic position should be substituted by a methyl group which appeared as a singlet at δ 2.38 ppm in corresponding to a peak at δ 21.0 ppm in ^{13}C-NMR spectra.

Both proton and carbon of methyl function gave cross peaks correlation with the corresponded aromatic proton (2 and 3 bond near by nuclei) in HMBC experiment. The observation of connectivity among aromatic proton and carbon nuclei with those of the remaining part of the molecule would lead to the complete structural elucidation of PJ1.

In deed, it is found that, the aromatic proton signal δ 7.81 ppm (*d*, J = 2.0 Hz) showed cross peak with a carbonyl-carbon (δ 197.6 ppm) in HMBC spectrum (Figures 8a-8d.).

HMBC experiment revealed the linking of carbonyl carbon to some protons belonging to two methylene function 2 and 3 bonds away. The remaining function of PJ1 molecule, a hydroxyl, one carbon and one methyl group all of which could be placed perfectly through to from complete structure remaining HMBC evidences (Figures 8a.) observed between two methylene proton signal of H-2 (δ 2.26 ppm.), H-3 (δ 2.28 ppm) and C-4 (CH_3) (δ 29.0 ppm.), C-10 (δ 146.6 ppm.) and H-2 (δ 2.69 ppm) and C-10 (δ 146.6 ppm.), respectively

Compound PJ1 was therefore identified as the 4-hydroxy-4,7-dimethyl-α-tetralone. Comparison of its carbon and proton chemical shifts with those previously reported for 4-hydroxy-4,7-dimethyl-α-tetralone (Matsuoka, Machida, and Kikuchi, 2004.) was summarized in Table 11.



4-hydroxy-4,7-dimethyl-α-tetralone (PJ1).

Table 11. Comparison between the ^{13}C-NMR and ^{1}H-NMR data of hydroxy-4,7-dimethyl-α-tetralone and compound PJ1 (in $CDCl_3$)

Position	Chemical shift (δ) ppm			
	hydroxy-4,7-dimethyl-α-tetralone		PJ1	
	C	H	C	H
1	197.7	-	197.6	-
2	36.0	(2a) 2.88, *ddd* J = 17.8, 5.2, 5.2 Hz (2b) 2.70, *ddd* J = 17.8, 9.1, 7.1 Hz	35.9	(2a) 2.88, *ddd* J = 17.8, 5.2, 5.2 Hz (2b) 2.69, *ddd* J = 17.8, 9.2, 7.1 Hz
3	38.3	(3a) 2.27, *d* J = 5.3 Hz (3b) 2.26, *dd* J = 5.3, 2.0 Hz	38.4	(3a) 2.28, *d* J = 5.3 Hz (3b) 2.26, *dd* J = 5.3, 2.0 Hz
4	70.2	-	70.2	-
4 -CH_3	28.9	1.63, *s*	29.0	1.63, *s*
5	125.3	7.60, *d* J = 8.0 Hz	125.2	7.60, *d* J = 8.0 Hz
6	135.5	7.45, *dd* J = 8.0, 2.1 Hz	135.2	7.44, *dd* J = 8.0, 2.0 Hz
7	138.1	-	137.8	-
7-CH_3	21.3	2.37, *s*	21.0	2.38, *s*
8	127.5	7.81, *d* J = 2.1 Hz	127.2	7.81, *d* J = 2.0 Hz
9	130.3	-	130.3	-
10	146.7	-	146.6	-

Matsuoka, Machida, and Kikuchi, 2004.

2. Identification of compound PJ2

Compound PJ2 was obtained from preparative TLC as whitish amorphous powder (0.00018%), The EIMS (Figure 3.) showed a molecular ion at *m/z* 226, which corresponded to the molecular formula $C_{13}H_{22}O_3$ whereas other prominent ions at *m/z* 208, 177, 165, 128, 109 and 71 were also observed. The presence of 13 carbons and 22 protons were in agreement with signals observable in the ^{13}C-NMR (Figures 11a-11b) and ^{1}H-NMR (Figures 10a-10b) spectra, respectively.

The DEPT experiments of PJ2 indicated that there were 3 quaternary carbons, 4 methine, 2 methylene and 4 methyl functions in the molecule (Figure 12).

^{1}H-NMR COSY spectrum of PJ2 gave cross peak signals of two olefinic protons (-CH=CH-), one of which was observed at δ 5.70 ppm (*dd*, *J* = 15.7, 1.0 Hz), whereas the other was found at δ 5.84 ppm (*dd*, *J* = 15.7, 5.6 Hz). The latter exhibited a cross peaks to a methine (-CH) proton at δ 4.44 ppm (*m*)

Protons of positions H-7 and H-8 were *trans* according to their observable of a large coupling constant (*J* = 15.7 Hz). A methyl proton could be placed next to methine carbon at δ 68.3 ppm, as they gave HMBC correlation with each other. The remaining substitution (R-group) at methine function was given as a hydroxyl group, which made carbon signal shift rather downfield of position at δ 68.3 ppm.

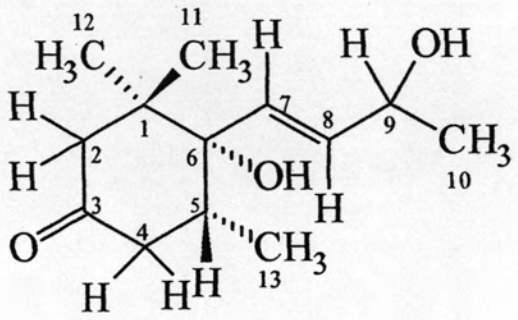
The other part of PJ2 molecule consists of the presence of a carbonyl function (δ C = 211.4 ppm) situated between 2 methylene groups. Methylene protons of each group resonated at different chemical shifts and with large geminal coupling (*J* = 13.6 Hz) due to the anisotopic effect from the carbonyl group.

Protons at δ1.92 and 2.84 ppm of a methylene group gave cross peaks in HMBC experiments with two methyl carbon signal at δ 24.4 ppm and δ 24.5 ppm and a quaternary carbon signal at δ 42.5 ppm and position H-2b at δ 1.92 ppm was slightly long-range coupled with position H-4b δ 2.25 ppm, as a consequence of the geometry (H-2b-C-2-C-3-C-4-H-4b) in compound PJ2 being a chair conformation distorted by the presence of a carbonyl system in the ring.

It was found that a proton at δ 1.92 ppm of a methylene group of H-2b as well as 2 olefinic protons of position H-7 and H-8 (δ 5.70 ppm and δ 5.84 ppm as mentioned earilier) gave HMBC cross peak relations to the same quaternary carbon of position C-6 at δ 77.3 ppm.

This information enable to connect 2 major parts of PJ2 molecule together and the transformation of methyl singlet into doublet compared with 4,5-dihydroblumenol A, led to conclude that compound PJ2.

Thus, the structure of PJ2 was identified as 4,5-dihydroblumenol A a norsesquiterpene previously isolated from *Perrottertia multiflora* (González *et al*, 1994.).



4,5-dihydroblumenol A (PJ2)

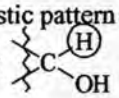
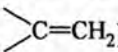
Table 12. Comparison between the ^{13}C-NMR and ^{1}H-NMR data of 4,5-dihydroblumenol A and compound PJ2

Position	Chemical shift (δ) ppm			
	4,5-dihydroblumenol A		PJ2	
	C	H	C	H
1	42.9	-	42.5	-
2	51.9	(2a) 2.84, *d* *J* = 13.6 Hz (2b) 1.92, *dd* *J* = 13.6, 1.0 Hz	51.4	(2a) 2.84, *d* *J* = 13.6 Hz (2b) 1.92, *dd* *J* = 13.6, 1.0 Hz
3	211.7	-	211.4	-
4	45.6	(4a) 2.42, *m* (4b) 2.23, *m*	45.1	(4a) 2.42, *t* *J*=12.1 Hz (4b) 2.15-2.25, *m*
5	36.8	2.27, *m*	36.4	2.27, *m*
6	77.3	-	77.3	-
7	132.3	5.71, *d* *J* = 15.8 Hz	131.8	5.70, *dd* *J* = 15.7, 1.0 Hz
8	135.6	5.84, *dd* *J* = 15.8, 5.7 Hz	135.1	5.84, *dd* *J* = 15.7, 5.6 Hz
9	68.7	4.44, *m*	68.3	4.44, *m*
10	24.3	1.33, *d* *J* = 6.3 Hz	23.9	1.33, *d* *J* = 6.4 Hz
11	24.9	0.95, *s*	24.4	0.94, *s*
12	24.8	0.97, *s*	24.5	0.97, *s*
13	16.3	0.88, *d* *J* = 6.3 Hz	15.9	0.87, *d* *J* = 6.4 Hz

González *et al*, 1994.

3. Identification of compound PJ3

Compound PJ1 was obtained from preparative TLC as whitish amorphous powder (0.00024%), The FABMS (Figure 3.) showed a molecular ion at *m/z* 454, which corresponded to the molecular formula $C_{31}H_{50}O_2$ whereas other prominent ions at *m/z* 437, 421, 367 and 327 were also observed. The presence of 31 carbons and 50 protons were in agreement with signals observable in the ^{13}C-NMR (Figures 19a-19b) and ^{1}H-NMR (Figures 18a-18c) spectra, respectively.

The ^{1}H-NMR spectrum of PJ3 displayed characteristic pattern of triterpene or sterol compound, 2 isolated protons, each of which attach to carbo-hydroxy () moieties individually at δ 3.25 ppm (H-3) and δ 4.28 ppm (H-15), 2 isolated olefenic protons at δ 5.31 ppm (H-11) and δ 5.85 ppm (H- 7) and one exomethylene () function. There were exomethylene protons each of which appeared as downfield singlet at δ 4.66 ppm and 4.72 ppm (H-24")

Molecular formular of PJ3 suggested 7 rings equivalent, three of which were double bonds, then PJ3 should have a tetracyclic moiety.

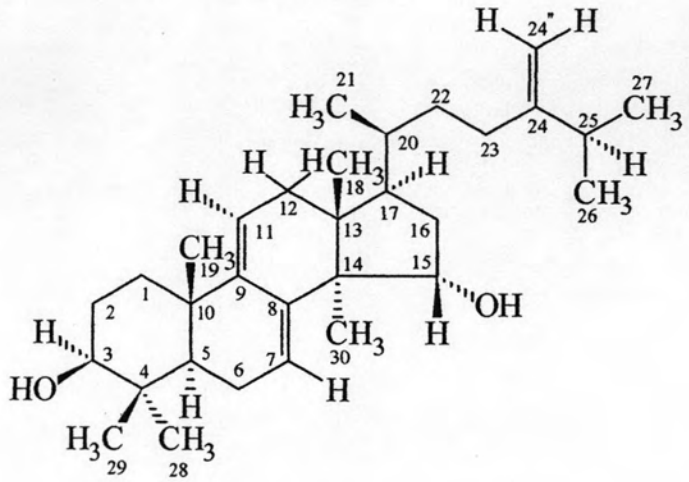
The DEPT experiments of PJ3 indicated that there were 7 quaternary carbons, 8 methine, 8 methylene and 8 methyl functions in the molecule (Figure 20a-20c).

The ^{13}C-NMR exhibited thirty-one carbon resonance, showing the existence of eight methyl groups, two oxygen-bearing methine groups, two double bonds and exomethylene group. Taking into account the presence of a transoid heroannular diene group in PJ3, the comparison of the ^{13}C-NMR resonances with those of tetracyclic triterpenoids suggested that PJ3 was a $\Delta^{7,9(11)}$ lanostane type triterpenoid.

The hydroxy-bearing methine proton signal at δ 3.25 ppm (H-3) and the β configuration of the C-3 hydroxyl group was confirmed by as large coupling constant (*dd*, J = 10.0, 4.6 Hz). The COSY spectrum, as well as the ^{1}H-^{13}C long-range correlation between proton signal at δ 0.95 ppm (H-30) and the hydroxy-bearing methine carbon resonances at δ 74.7 ppm (C-15), indicated that the other hydroxyl group was presence at C-15. Furturemore, observation of the NOESY between proton signal at δ 0.61 ppm (H-18) and δ 4.28 ppm (H-15) confirm the configuration of hydroxyl group at methine carbon at δ 74.7 ppm (C-15) to be α.

The position of the exomethylene function provided connection through HMBC cross peaks inspection with other long-range (2-3 bonds) nuclei between the exomethylene proton at δ 4.66 ppm and 4.72 ppm (H-24") and the carbon resonances at δ 31.2 ppm (C-23) and 4.72 ppm (C-25). Moreover, the ^{13}C-NMR resonances of PJ3 were in good accord with those of structurally related triterpenoid, which possess the same partial structure. On the basis of the spectroscopic evidence, the structure of PJ3 was found to be previously as 24-methylenelanosta-7,9(11)-dien-3β,15α-diol isolated

from *Polyalthia lancilimba*. (Lue *et al*, 1998.) and *Polyalthia suberosa* was found to showed significant anti-HIV activity (Li *et al*, 1993.).



24-methylenelanosta-7,9(11)-dien-3β,15α-diol (PJ3)

Table 13. Comparison of ^{13}C-NMR and ^{1}H-NMR data of 24-methylenelanosta-7,9(11)-dien-3β,15α-diol and compound PJ3

Position	Chemical shift (δ) ppm			
	24-methylenelanosta-7,9(11)-dien-3β,15α-diol		PJ3	
	C	H	C	H
1	35.8	2.01, *br*	35.7	1.97, *dd* *J* = 13.0, 3.1 Hz 1.43, *dd* *J* = 13.0, 4.7 Hz
2	27.8	1.68, *m*	27.7	1.63-1.80, *m*
3	78.9	3.22, *dd* *J* = 10.9, 5.0 Hz	78.9	3.25, *dd* *J* = 10.0, 4.6 Hz
4	38.7	-	38.6	-
5	49.1	1.07, *m*	48.9	1.10, *m*
6	23.0	2.11, *br*	22.9	2.02-2.18, *m*
7	121.3	5.82, *d* *J* = 6.9 Hz	121.3	5.85, *d* *J* = 6.3 Hz
8	140.9	-	140.8	-
9	146.2	-	146.0	-
10	37.5	-	37.4	-
11	116.0	5.28, *d* *J* = 6.0 Hz	116.0	5.31, *d* *J* = 5.9 Hz
12	38.6	2.05, *br* 2.24, *br*	38.5	2.08, *dd* *J* = 17.1, 6.3 Hz 2.30, *d* *J* = 1.2 Hz
13	44.4	-	44.3	-
14	52.0	-	51.9	-
15	74.9	4.25, *dd* *J* = 9.6, 5.8 Hz	74.7	4.28, *dd* *J* = 9.7, 5.2 Hz
16	40.1	1.72, *m* 1.95, *m*	40.1	1.70-1.80, *m* 2.10, *m*

Table 13. Comparison of ^{13}C-NMR and ^{1}H-NMR data of 24-methylenelanosta-7,9(11)-dien-3β,15α-diol and compound PJ3 (continued)

Position	Chemical shift (δ) ppm			
	24-methylenelanosta-7,9(11)-dien-3β,15α-diol		PJ3	
	C	H	C	H
17	48.9	1.68, *m*	48.8	1.07, *d* *J* = 4.2 Hz
18	15.8	0.58, *s*	15.8	0.61, *s*
19	22.8	0.91, *s*	22.8	0.91, *s*
20	36.0	1.32, *m*	36.0	1.30-1.40, *m*
21	18.5	0.82, *d* *J* = 6.5 Hz	18.4	0.90, *d* *J* = 6.5 Hz
22	35.0	1.11, *m* 1.50, *m*	34.8	1.10-1.18, *m* 1.49-1.59, *m*
23	31.3	1.85, *m* 2.10, *m*	31.2	2.05-2.12, *m* 1.88, *ddd* *J* = 14.7, 10.2, 4.9 Hz
24	156.5	-	156.5	-
25	33.8	2.19, *hept* *J* = 6.4 Hz	33.8	2.21, *dd* *J* = 14.2, 7.2 Hz
26	21.9	1.00, *d* *J* = 6.9 Hz	22.0	1.03, *d* *J* = 6.8 Hz
27	22.0	0.99, *d* *J* = 6.8 Hz	21.8	1.02, *d* *J* = 6.9 Hz
28	28.2	0.97, *s*	28.1	0.98, *s*
29	16.0	0.85, *s*	15.9	0.88, *s*
30	17.2	0.95, *s*	17.1	0.95, *s*
24"	106.2	4.62, *s* 4.69, *s*	106.1	4.72, *s* 4.66, *s*

Lue *et al*, 1998.

4. Effect of compounds on the growth of human tumor and non-tumor cell lines

Compounds PJ1, PJ2 and PJ3 were evaluated for their ability to inhibit the *in vitro* growth of four human tumor cell lines, namely the estrogen-dependent ER(+) MCF-7 (human breast adenocarcinoma), the estrogen-independent ER(-) MDA-MB-231 (human breast adenocarcinoma), SF-268 (CNS cancer) and NCI-H460 (non-small cell lung cancer). The effect of there compounds on the non-tumor cell line MRC-5 (human fetal lung) was also evaluated using the same procedure. Results are summarized in Table 14. Doxorubicin was used as positive control.

Table 14. Effect of compounds PJ1-PJ3 on the growth of human tumor and non-tumor cell lines

Compounds	GI_{50} (μM)				
	MCF-7	MDA-MB-231	SF-268	NCI-H460	MRC-5
PJ1	> 150	> 150	> 150	> 150	not done
PJ2	> 150	> 150	> 150	> 150	> 150
PJ3	19.3 ±1.2	18.8 ± 2.0	21.8 ± 0.6	23.0 ± 1.7	40.3 ± 3.4

Results expressed as GI_{50} (concentration of that cause 50% inhibition of cell growth) are mean ± SEM of 3-5 independent experiments performed in duplicate.

GI_{50} of doxorubicin : MCF-7 = 42.8 ± 8.2 nM; SF-268 = 93.0 ± 7.0 nM; NCI-H460 = 94.0 ± 8.7 nM

Compounds PJ1 and PJ2 were found to be ineffective as cell growth inhibitors even when tested at concentrations of 150 μM. However, after a continuous exposure of 48 hr. period, compound PJ3 exhibited an interesting dose-dependent growth inhibitory effect extensive not only against the tumor cell lines but also against the non-tumor MRC-5 cells. Curiously, a more potent effect was found against the tumor cells suggesting a high sensitivity of these cells to this compound.