Triterpenes and Sterol from *Pouteria campechiana*

Consolacion Y. Ragasa[a,*], Lynne M. Labaclado[a], and John A. Rideout[b]
[a]Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines and
[b]School of Chemical and Biomedical Sciences, Central Queensland University, Queensland 4701, Queensland, Australia
*Corresponding author: Tel/Fax: 062-5360230; E-mail: ragasac@dlsu.edu.ph

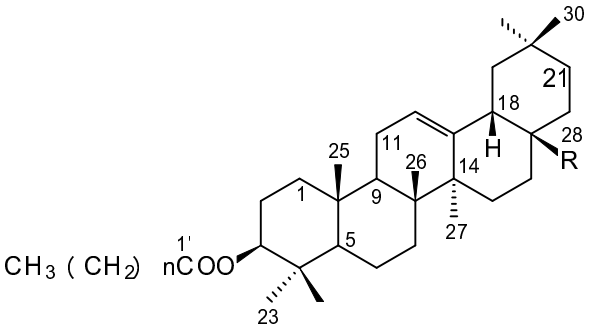
The ethyl acetate extract of the stem bark of *Pouteria campechiana* afforded 3β, 28-dihydroxy-olean-12-enyl fatty acid ester **1**, a mixture of a fatty acid ester of oleanolic acid **2a** and a fatty acid ester of betulinic acid **2b** in a 0.3:1 ratio, and spinasterol **3** by silica gel chromatography. The structures of **1**-**2b** were elucidated by extensive 1D and 2D NMR spectroscopy. The structure of **3** was identified by comparison of its ^{1}H NMR data with spinasterol. Antimicrobial tests on **1** and a mixture of **2a** and **2b** indicated that they are slightly active against the bacteria, *Escherichia coli* and *Pseudomonas areruginosa* and the fungi, *Candida albicans* and *Trichophyton mentagrophytes.* They are inactive against *Staphylococcus aureus, Bacillus subtilis,* and *Aspergillus niger*.

Key words: *Pouteria campechiana*, Sapotaceae, triterpene fatty acid esters

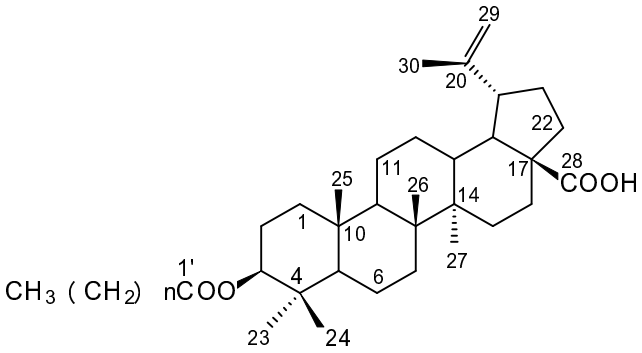
1. INTRODUCTION

Pouteria campechiana, commonly known as Tiessa is grown for its fruit which serves as an ingredient in sherbert and ice cream preparation (Coronel, Zuto, & Sotto, 1983). It is rich in carbohydrates, vitamin A, and minerals (Coronel, Zuto, & Sotto, 1983), while the seeds can be made into an instant beverage similar to coffee (Claustro, Madulid, & Gapo, 1997). To date, few studies have been conducted on this tree. The fruits of *P. campechiana* afford polyphenolic antioxidants, namely, gallic acid, gallocatechin, catechin, epicatechin, dihydromyricetin, catechin-3-O-gallate, and myricitrin (Ma, Yang, Basile, & Kennelly, 2004). A recent study reported the isolation of stilbenes and flavonoid glycosides from the ethyl acetate extract of *P. campechiana*. The distilbene, ampelopsin B exhibited antimitotic activity (Hernandez, Villasenor, Joseph, & Tolliday, 2008).

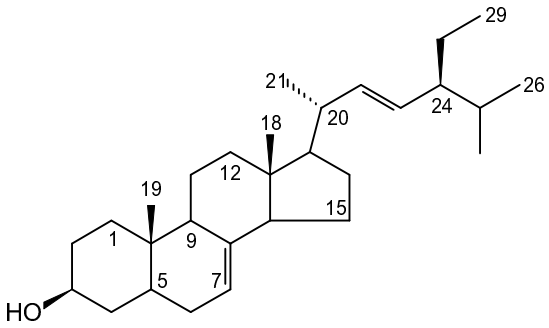
We now report the isolation, structure elucidation, and antimicrobial test results of three triterpene fatty acid esters (**1**-**2a**) from the stem bark of *Pouteria campechiana*. Spinasterol (**3**) was also isolated from the stem bark. To the best of our knowledge this is the first report on the isolation of these compounds from *P. campechiana.*



1 R = CH_2OH
2a R = COOH



2b



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2. MATERIALS AND METHODS

2.1. General Experimental Procedure

NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C. Column chromatography was performed with silica gel 60 (70-230 mesh), while the TLC was performed with plastic backed plates coated with silica gel F_{254}. The plates were visualized by spraying with vanillin-H_2SO_4, followed by warming.

2.2. Sample Collection

Three branches of the Tiessa sample was collected from the quadrangle of Epifanio delos Santos Elementary School, Singalong, Manila on September 13, 2005. It was identified as *Pouteria campechiana* (H.B.K.) at the Botany Division of the National Museum.

2.3 Isolation of Secondary Metabolites

The ground and air-dried stem bark of *P. campechiana* (479.2 g) was soaked in ethyl acetate for three days, then filtered. The filtrate was concentrated under vacuum to afford the crude extract (11.2 g) which was subjected to silica gel chromatography and eluted with increasing proportions of acetone in dicloromethane at 10% increment. The dichloromethane fraction was rechromatographed in 2.5% ethyl acetate in petroleum ether to afford **1** (13.7 mg). The 10% acetone in dichloromethane fraction was rechromatographed in 2.5% ethyl acetate in petroleum ether (2x) to afford a mixture of **2a** and **2b** (10.5 mg). The 40% acetone in dichloromethane fraction was rechromatographed in dichloromethane to afford **3** (65.3 mg).

2.4 Antimicrobial Tests

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Aspergillus niger* UPCC 4219, *Candida albicans* UPCC 2168, *Bacillus subtilis* UPCC 1295, *Pseudomonas aeruginosa* UPCC 1244, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, and *Trichophyton mentagrophyte* UPCC 4193. The test compound was dissolved in 95% ethanol. The antimicrobial assay procedure reported by Guevara and Recio (1985) was employed. The activity index was computed by subtracting the diameter of the well from the diameter of the clearing zone divided by the diameter of the well.

3. RESULTS AND DISCUSSION

The dichloromethane extract of the stem of *Pouteria campechiana* afforded three triterpene fatty acid esters (**1**-**2b**) by silica gel chromatography. Their structures were elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR spectrum of **1** gave resonances for an olefinic proton at δ 5.19, methylene carbinyl protons at δ 3.22 and 3.55, and seven methyl singlets at δ 0.90, 0.875, 1.17, 0.94, 0.96, 0.869, and 0.866. The resonances attributed to the long chain fatty acid esters attached to C-3 were the methyl group at δ 0.88 (t, J = 6.6 Hz), the methylene alpha to the carbonyl at δ 2.29 (t, J = 7.2 Hz), and the short chain of methylene protons which integrated for about 12 centered at δ 1.25. The ^{13}C NMR spectrum gave resonances for 30 carbons for the triterpene with the following functionalities: a methylene carbinyl carbon at δ 69.71, a methine carbinyl carbon at δ 80.54, olefinic carbons at δ 144.21 and 122.29, seven methyl groups, 10 methylene, three methine, and six quaternary carbons. The fatty acid chain gave resonances for a carbonyl carbon of an ester at δ 173.69, a methylene carbon α to a carbonyl at δ 34.86, overlapping methylene carbons of the fatty acid at around δ 29.7, and a methyl carbon at δ 14.07. The ^{1}H and ^{13}C connectivities in **1** (Table 1) were verified by HMQC.

The COSY spectrum indicated seven isolated spin systems as follows: H_2-1/H_2-2/H-3; H-5/H_2-6/H_2-7; H-9/H_2-11/H-12; H_2-15/H_2-16; H-18/H_2-19; H_2-21/H_2-22; H_2-25 (Fig. 1).

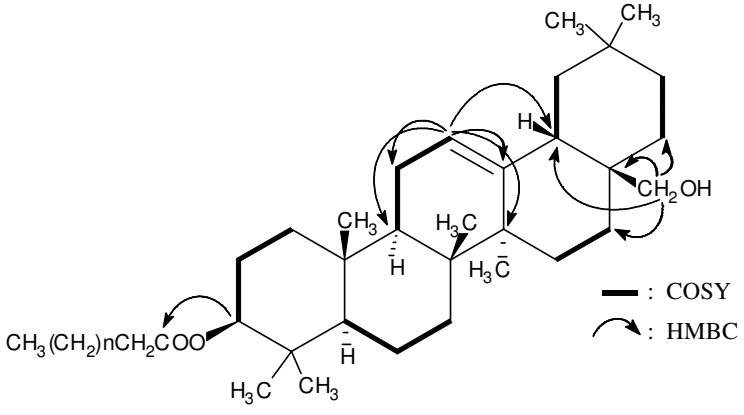

Figure 1. ^{1}H-^{1}H COSY and Key ^{1}H-^{13}C long-range correlations for **1**

The structure of **1** was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 1. The methylene carbinyl was assigned to C-25 due to

long-range correlation between the methylene carbinyl protons at δ 3.22 and 3.55 (H_2-25) and the carbons at C-16, C-17, C-18, and C-22. The olefinic carbon was placed at C-12 since long-range correlations were observed between the proton at δ 5.19 (H-12) and the carbons at C-9, C-11, C-13, C-14, and C-18. The fatty acid was esterified to C-3 since long-range correlations were observed between the carbinyl proton (H-3) and the carbonyl carbon of the fatty acid ester at δ 173.69. All long-range correlations observed are consistent with the structure of **1**.

The relative stereochemistry of **1** was deduced from the NOESY spectrum as follows: the carbinyl proton (H-3) was close in space to the methyl protons (H_3-29) and methine proton (H-5), which was in turn close to H-9, which was finally close to the methyl singlet (H-27). This indicated that they are on the same face of **1**. The methyl singlet (H-25) was close in space to another methyl singlet (H-26), which was in turn close to the methine proton (H-18), which was finally close to the methylene hydroxy protons (H_2-28). Thus, **1** is 3β, 28-dihydroxy-olean-12-enyl fatty acid ester (Barreiros, David, Pereira, & Gueden, 2002). The ^{13}C NMR data of **1** and 3β, 28-dihydroxy-olean-12-enyl palmitate match in all essential respects. The difference is in the chain length of the fatty acid. Whereas, **1** has a short fatty acid chain, that of 3β, 28-dihydroxy-olean-12-enyl palmitate from the literature has a long fatty acid chain.

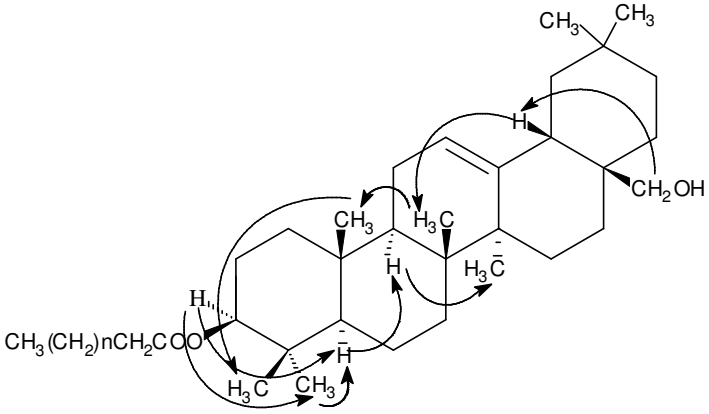

Figure 2. Key NOESY correlations for **1**

A mixture of **2a** and **2b** in a 0.3:1 ratio based on integrals and disparity in single hydrogen peaks. Compound **2a** gave similar resonances to **1** (Table 1), except for the non-appearance of the resonances for the methylene carbinyl protons (H_2-28) at δ 3.22 and 3.55 in **2a**, and the long fatty acid chain in **2a** as deduced from the resonance centered at δ 1.25 which integrated for about 36 protons. In the ^{13}C NMR spectrum of **2a**, instead of the carbinyl carbon resonance at δ 69.71 (C-28) in **1**, a carboxylic acid resonance at δ 181.0 (C-28) was detected in **2a**. Thus, **2a** is a fatty acid ester of oleanolic acid. Confirmatory evidences are the ^{13}C NMR resonances of **2a** and oleanolic acid (Ragasa & Lim, 2005a) for the triterpene part and 3β, 28-dihydroxy-olean-12-enyl fatty acid ester (Barreiros et al., 2002) for the fatty acid part which match in all essential respects.

Compound **2b** gave similar resonances to **2a** (Table 1). It contained a carboxylic acid resonance at δ 181.0. However, the olefinic proton at δ 5.28 (H-12) in **2a** was no longer present in **2b**. Furthermore, olefinic methylene protons at δ 4.61 and 4.74 were found in **2b**. Instead of the seven methyl singlets in **2a**, five methyl singlets and an allylic methyl were found in **2b**. This suggested that one of the methyl groups was converted to olefinic methylene, with one methyl allylic to this group. Thus, **2b** is a fatty acid ester of betulinic

acid. Confirmatory evidences are the ^{13}C NMR resonances of **2b** and betulinic acid (Su et al., 2002) for the triterpene part and 3β, 28-dihydroxy-olean-12-enyl fatty acid ester (Barreiros et al., 2002) for the fatty acid part which match in all essential respects.

The **3** was identified by comparison of its ^{1}H NMR data those of spinasterol found in the literature (Ragasa & Lim, 2005b). The antimicrobial activities of spinasterol isolated from *Cucurbita maxima* has been reported (Ragasa & Lim, 2005b).

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, **1** and a mixture of **2a** and **b** were tested for their antimicrobial potentials against seven microorganisms. Results of the study (Table 2) indicated that **1** and a mixture of **2a** and **2b** are slightly active against the bacteria, *Escherichia coli* and *Pseudomonas areruginosa* and the fungi, *Candida albicans* and *Trichophyton mentagrophytes.* They are inactive against *Staphylococcus aureus, Bacillus subtilis,* and *Aspergillus niger*.

Table 1
*400 MHz ^{1}H NMR and 100MHz ^{13}C NMR Data of **1**, **2a** and **2b** in $CDCl_3$*

Position	δ_C **1**	δ_H mult. (J Hz) **1**	δ_C **2a**	δ_H mult. (J Hz) **2a**	δ_C **2b**	δ_H mult. (J Hz) **2b**
1	38.23	1.60, 1.03	38.38	1.60, 1.03	38.42	1.65
2	23.53	1.60, 1.60	25.2	1.60, 1.60	25.2	1.58
3	80.54	4.49 (dd, 5, 7.2)	80.59	4.47 (dd, 5, 7.2)	80.59	4.49
4	37.75	-	37.83	-	37.75	
5	55.22	0.82	55.42	0.80	55.2	0.80
6	18.23	1.42, 1.57	18.16	1.40, 1.50	18.27	
7	32.51	1.35, 1.53	34.3	1.37, 1.37	32.5	1.4
8	39.8	-	40.70	-	39.2	
9	47.5	1.52	50.40	1.33	47.6	1.60
10	36.83	-	37.13		37.05	
11	23.6	1.60, 1.85	20.9	1.55, 1.55	23.5	1.60
12	122.29	5.19 (t, 3.6)	25.5	1.65, 1.65	122.6	5.28
13	144.21	-	38.42	2.20 (br t)	143.4	
14	41.73	-	42.43	-	41.6	
15	25.54	1.60, 1.60	30.6	1.43, 2.00	27.76	1.60
16	23.90	1.10, 1.10	32.2	1.40, 2.25	22.7	1.60
17	31.91	-	56.38	-	46.5	
18	42.34	1.98 (ddd, 3, 3, 9.4)	49.27	1.58	41.0	2.81
19	46.42	1.03, 1.72	46.94	3.00 (br t)	45.8	
20	34.08	-	150.36	-	30.7	
21	30.94	1.35, 1.50	31.9	1.42, 1.42	33.8	1.65
22	38.23	1.60, 1.60	37.1	1.45, 1.98	32.5	1.4
23	16.74	0.869 (Me, s)	16.53	0.83 (Me, s)	15.52	
24	28.04	0.866 (Me, s)	28.0	0.837 (Me, s)	28.08	
25	15.55	0.958 (Me, s)	16.1	0.85 (Me, s)	15.3	

26	16.14	0.944 (Me, s)	16.2	0.93 (Me, s)	17.0	0.94 (Me, s)
27	25.90	1.165 (Me, s)	14.7	0.97 (Me, s)	25.90	1.17 (Me, s)
28	69.67	3.22 (d, 11), 3.55 (d, 11)	181.0	-	181.0	-
29	23.58	0.90 (Me, s)	109.69	4.61 (br s), 4.74 (br s)	33.05	
30	33.17	0.875 (Me, s)	19.34	1.69 (Me, br s)	23.66	
1'	173.69		173.73		173.73	
2'	34.86	2.29 (t, 7.2)	34.85	2.28 (t, 7.2)	34.85	2.28 (t, 7.2)
3'	25.17	1.63	25.16		25.16	
$(CH_2)_n$	22.00, 28.04, -29.17-29.66, 31.03	1.2-1.35	22.67, 28.00, 29.17-29.69, 31.91	1.25 (br s)	22.67, 28.00, 29.17-29.69, 31.91	1.25 (br s)
CH_3	14.10	0.88 (t, 6.8)	14.1	0.88, (t, 6.8)	14.1	0.88 (t, 6.8)

Table 2
*Antimicrobial Test Results on **1** and a Mixture of **2a** and **2b***

Organism	**Sample (30 µg)**	**Clearing Zone (mm)**			**Activity Index (AI)**
		Replicate 1	**Replicate 2**	**Replicate 3**	
Escherichia coli	**1**	12	12	12	0.2
	2a and **2b**	11	11	11	0.1
	Chloramphenicol	23			2.8
Pseudomonas aeruginosa	**1**	11	11	11	0.1
	2a and **2b**	11	11	11	0.1
	Chloramphenicol	14			1.3
Staphylococcus aureus	**1**	11	11	11	0.1
	2a and **2b**	-	-	-	0
	Chloramphenicol	25			3.2
Bacillus subtilis	**1**	-	-	-	0
	2a and **2b**	-	-	-	0
	Chloramphenicol	20			2.3
Candida albicans	**1**	12	12	12	0.2
	2a and **2b**	12	12	12	0.2
	Canesten, 0.2 g[a]	18			0.8
Trichophyton mentagrophytes	**1**	12	12	12	0.2
	2a and **2b**	11	11	11	0.1
	Canesten, 0.2 g[a]	55			4.5
Aspergillus niger	**1**	-	-	-	0
	2a and **2b**	-	-	-	0
	Canesten, 0.2 g[a]	23			1.3

[a] Contains 1% chlotrimazole

4. CONCLUSION

The ethyl acetate extract of the stem bark of *Pouteria campechiana* afforded the triterpenes, **1**, **2a** and **2b** and the sterol, **3**. Their structures were elucidated by NMR spectroscopy. To the best of our knowledge this is the first report on the isolation of these compounds from *P. campechiana.*

The triterpenes exhibited slight activity against the bacteria, *Escherichia coli* and *Pseudomonas areruginosa* and the fungi, *Candida albicans* and *Trichophyton mentagrophytes.*

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