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SESQUITERPENE GLYCOSIDES FROM *PITTOSPORUM PENTANDRUM*

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Key Word Index—*Pittosporum pentandrum*; Pittosporaceae; sesquiterpene glycosides; betulin.

Abstract—The chloroform extract of *Pittosporum pentandrum* afforded a mixture of two novel eudesmane-type sesquiterpene glycosides and the known triterpene betulin. The structures of these constituents were elucidated by spectroscopic methods. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

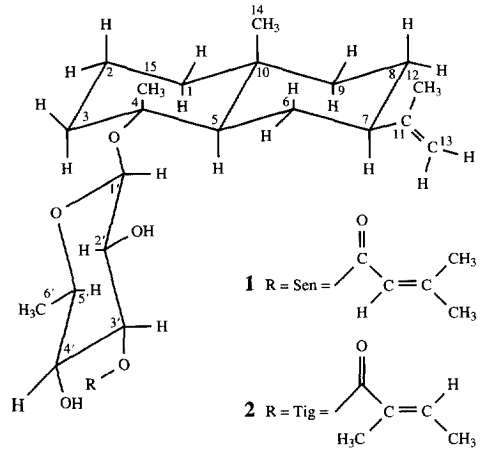
Pittosporum pentandrum is a small, slender tree found throughout the Philippines. In small doses, the powdered bark is used as a febrifuge. In larger doses, it is used as an antidote and is effective in treating bronchitis. The leaves are used by women in their baths following childbirth [1].

P. pentandrum has been reported to contain flavonoids, quercetin-3-6-*O*-caffeoyl-β-D glucoside, phytosteryl glycoside, methyl caffeate, potassium-4-β-D-glucosylproticachuate, emodin, sucrose and a volatile oil [2]. Other studies on the genus *Pittosporum* reported the isolation of an essential oil [4], triterpenoids [5] and terpene glycosides [6–8]. We now report on the isolation and structure elucidation of a mixture of two novel sesquiterpene glycosides (**1**, **2**) and the known triterpene betulin from this plant.

RESULTS AND DISCUSSION

The structure of betulin was elucidated by comparison of its ^{1}H NMR spectrum with that of betulin [9].

The ^{1}H NMR spectral data of a mixture of **1** and **2** (Table 1) contained resonances for a senecioyloxy substituent at δ 5.69 (1H, *s*, *br*), 2.15 (3H, *s*, *br*), and 1.90 (3H, *s*, *br*) [6, 7] for the major constituent (**1**) and a tigloyloxy substituent at δ 6.88 (1H, *m*), 1.82 (3H, *s*, *br*), and 1.78 (3H, *d*, J = 7.0 Hz) [8] for the minor one (**2**). This was supported by the ^{13}C NMR spectrum (Table 2) which showed stronger resonances for the



Scheme 1.

senecioyloxy substituent at δ 166.4, 158.1, 115.4, 27.5 and 20.4 [6, 7], and weaker resonances for the tigloyloxy substituent at δ 168.0, 138.4, 128.3, 14.5 and 12.1 [8]. The ester functionality was supported by the FT-IR absorptions at 1731 and 1157 cm^{-1}.

Another fragment deduced from the COSY spectrum was a glycoside. The anomeric H at δ 4.45 (1H, *d*, J = 7.7 Hz) was coupled to the carbinyl H at δ 3.39 (1H, *dd*, J = 7.7, 9.4 Hz), which was in turn coupled to another carbinyl H at δ 4.66 (1H, *t*, J = 9.4 Hz). The latter H was coupled to a carbinyl H at δ 3.62 (1H, *t*, J = 9.3 Hz), which was coupled to another carbinyl H at δ 3.42 (1H, *m*, J = 9.3, 6.2 Hz), in turn coupled to the methyl protons at δ 1.17 (3H, *d*, J = 6.2 Hz). It was evident from the ^{1}H NMR spectrum that the anomeric protons of **1** and **2** resonated with different chemical shift values as follows: δ 4.45 (1H, *d*, J = 7.7 Hz) for **1**, δ 4.44 (1H, *d*, J = 7.7 Hz) for **2**.

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Table 1. ^{1}H NMR (300 MHz) spectral data of compounds **1** and **2** in $CDCl_3$

H	1	2
1′	4.45 (1H, *d*, *J* = 7.7 Hz)	4.44 (1H, *d*, *J* = 7.7 Hz)
2′	3.39 (1H, *dd*, *J* = 7.7, 9.4 Hz)	3.39 (1H, *dd*, *J* = 7.7, 9.4 Hz)
3′	4.66 (1H, *t*, *J* = 9.4 Hz)	4.66 (1H, *t*, *J* = 9.4 Hz)
4′	3.62 (1H, *t*, *J* = 9.3 Hz)	3.62 (1H, *t*, *J* = 9.3 Hz)
5′	3.42 (1H, *m*, *J* = 9.3, 6.2 Hz)	3.42 (1H, *m*, *J* = 9.3, 6.2 Hz)
6′	1.17 (3H, *d*, 6.2Hz)	1.17 (3H, *d*, 6.2 Hz)
7	1.90 (1H, *m*)	1.90 (1H, *m*)
12	1.72 (3H, *s*, *br*)	1.72 (3H, *s*, *br*)
13b	4.66 (1H, *s*, *br*)	4.66 (1H, *s*, *br*)
13a	4.67 (1H, *s*, *br*)	4.67 (1H, *s*, *br*)
14	0.90 (3H, *s*)	0.90 (3H, *s*)
15	1.18 (3H, *s*)	1.18 (3H, *s*)
Osen	5.69 (1H, *s*, *br*)	
	2.15 (3H, *s*, *br*)	
	1.90 (3H, *s*, *br*)	
OTig		6.88 (1H, *m*)
		1.82 (3H, *s*, *br*)
		1.78 (3H, *d*, 7.0 Hz)
OH	2.74 (1H, *s*, *br*)	2.74 (1H, *s*, *br*)
	3.11 (1H, *s*, *br*)	3.11 (1H, *s*, *br*)

Table 2. ^{13}C NMR (75 MHz) and DEPT spectral data of compounds **1** and **2** in $CDCl_3$

1	2	Functionalities
166.4	168.0	C=O
158.1, 150.8	150.8, 128.3	-C=
115.4	138.4	=CH
108.2	108.2	$=CH_2$
95.6	95.6	O-CH-O
80.0	80.0	C-O
69.85, 74.57, 75.07, 75.15	69.78, 74.61, 75.1, 76.21	CH-O
52.1, 46.3	52.1, 46.3	CH
45.0, 40.8, 39.2, 26.9, 26.1, 19.8	45.0, 40.8, 39.2, 26.9, 26.1, 19.8	CH_2
27.5, 21.0, 20.4, 19.8, 19.2, 17.8	20.73, 20.74, 19.8, 17.82, 12.1, 14.5	CH_3
34.8	34.8	-C-

Based on integral values, the ratio of **1**:**2** is 2.3:1.0. The other carbinyl protons were overlapping resonances. The ^{1}H NMR spectrum further revealed two hydroxyl groups at δ 2.74 (1H, *s*, *br*) and 3.11 (1H, *s*, *br*), which was supported by the IR spectrum with absorptions at 3400, 1085 and 1060 cm^{-1}. On chemical shift grounds, the hydroxyl groups were attached to C-2 and C-4, while the senecioyloxy substituent for **1** and the tigloyloxy substituent for **2** were attached to C-3. The positions of the hydroxyl groups were confirmed by acetylation of the mixture which showed the following changes in chemical shifts: appearance of two acetates at δ 1.95 and 1.98, and deshielding of H-2 (δ 3.39), H-3 (δ 4.66) and H-4 (δ 3.62) to δ 4.87, 5.16 and 4.88, respectively.

The COSY spectrum of **1** and **2** indicated coupling between the olefinic protons at δ 4.67 (1H, *s*, *br*) and 4.66 (1H, *s*, *br*) and the allylic protons at δ 1.72 (3H, *s*, *br*) and 1.90 (1H, *m*). The latter H was coupled to the methylene protons at δ 1.16, 1.18, 1.39 and 1.40. Because of overlapping resonances, it was not possible to construct fragments of **1** and **2** containing the remaining methine and methylene groups as indicated by the ^{13}C and DEPT NMR spectral data (Table 2). Subtraction of the resonances for the glycoside portion of **1** and **2** gave 3 CH_3, 6 CH_2, 1 C, 1 C-O, 2 CH, 1 -C= and 1 $=CH_2$, indicating a sesquiterpene moiety. A literature search revealed that the sesquiterpene is eudesm-11-en-4-α-ol [10, 11], whose spectral data are similar to **1** and **2**, except for the slight deshielding of H-15 due to the glycoside. This was supported by the FT-IR spectrum which showed absorptions at 1659 and 880 cm^{-1} due to a vinylidene group [10, 11].

The relative stereochemistry of **1** and **2** was determined by a combination of coupling constants analyses and NOESY. The anomeric configuration was deduced to be β based on the axial H-1′ at δ 4.45 (1H, *d*, *J* = 7.7 Hz). For H-1′ and H-2′ (*J* = 7.7 Hz); H-2′ and H-3′ (*J* = 9.4 Hz); H-3′ and H-4′ (*J* = 9.3 Hz);

and H-4′ and H-5′ ($J = 9.3$ Hz), the coupling constants are within the axial–axial coupling range of 6–14 Hz. Thus, H-1′ to H-5′ were in the axial positions and the glycoside was a 6-deoxyglucoside. The NOESY established the relative stereochemistry of **1** and **2**, particularly the point of attachment of the glycoside and the positions of the carbinyl protons. It indicated that the methyl groups (C-14 and C-15) of the sesquiterpene moiety lie close together in the molecule. Since the C-14 of eudesm-11-en-4-α-ol is in the axial position [10, 11], then C-15 should be in the axial position and the glycoside should be in the equatorial position. The bulkier substituent in the equatorial position reduces steric strain. The NOESY spectral data supported results of the coupling constants analyses. The anomeric H (δ 4.45) in the axial position was found to be close in space with the protons oriented in the axial positions of C-7 at δ 1.90, C-6 at δ 1.20 and C-5 at δ 1.40 of the sesquiterpene moiety, and with the carbinyl H oriented in the axial position of C-5′ at δ 3.42 of the glycoside portion.

The mass spectrum did not show a $[M]^+$ peak. However, spectral data revealed the highest mass at *m/z* 229 due to $C_{26}H_{42}O_6$–$C_{15}H_{25}O$. The base peak at *m/z* 83 (100%) is due to C_5H_7O from the fragmentation of the senecioyloxy and tigloyloxy substituents of the glycoside.

A formula index search of **1** and **2** with molecular formula $C_{26}H_{42}O_6$ revealed that they have not been reported in literature. Therefore, **1** and **2** with the following proposed names: eudesm-11-en-4-α-*O*-β-D-3-senecioyloxy-6-deoxyglucopyranoside (**1**) and eudesm-11-en-4-α-*O*-β-D-3-tigloyloxy-6-deoxyglucopyranoside (**2**) are novel compounds.

EXPERIMENTAL

IR: $CHCl_3$ solns; NMR: 300 (^1H) and 75 (^{13}C) Hz, $CDCl_3$; CC: Silica gel 60 (70–230 mesh); TLC: plastic backed plates coated with Silica gel F_{254}. Plates were visualized by spraying with vanillin–H_2SO_4 and warming.

P. pentandrum was collected from Miagao, Iloilo in February 1995, and a voucher specimen is held at the Chemistry Department of De La Salle University. Air-dried leaves (3.8 kg) of this species were soaked in 12 l of $CHCl_3$ for 3 days, then filtered. The filtrate was concd under vacuum to afford a crude extract which was treated with 4% $Pb(OAc)_2$ to precipitate the pigments [12]. The treated extract (6.15 g) was subjected to gravity CC (dry packing). The solvent systems used were based on the step gradient technique: starting with 200 ml $CHCl_3$, then 100 ml each of Me_2CO in $CHCl_3$ (10% increment). Frs 4–6 were rechromatographed using DCM–Et_2O–Me_3CN (18:1:1) as eluent. Frs 5–12 from the rechromatography of F4–6 afforded betulin (15 mg) after recrystallization from Et_2O, while F13–20 afforded a mixture of compounds which was rechromatographed using DCM–Et_2O–Me_3CN (18:1:1) as eluent. The less polar fractions afforded **1**–**2** (10 mg) after recrystallization from Et_2O.

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