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SAPONINS FROM LEAVES OF *APHLOIA THEIFORMIS*

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Key Word Index—*Aphloia theiformis*; Flacourtiaceae, triterpene saponins; tormentic acid ester glucoside, 23-hydroxytormentic acid ester glucoside, 6β-hydroxytormentic acid ester glucoside

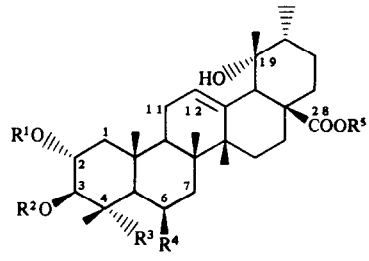
Abstract—Three triterpene glucosides have been isolated from the methanol extract of the leaves of *Aphloia theiformis*. The identities of the compounds were established by spectroscopic (^{1}H NMR, ^{13}C NMR, MS) and chemical methods (hydrolysis, acetylation). Two of the compounds were identified as tormentic acid ester glucoside and 23-hydroxytormentic acid ester glucoside, respectively. The structure of the third compound was established as 6β-hydroxytormentic acid ester glucoside, a new naturally occurring saponin.

INTRODUCTION

The leaves of *Aphloia theiformis* (Vahl.) Benn. (Flacourtiaceae) or *Bois Fandamane* are used in Mauritius, according to traditional healers, against rheumatism, jaundice, diabetes and bladder disorders, [1, 2] In the course of our systematic screening studies for biologically active compounds from medicinal plants, we observed that the methanolic extract of the leaves of *A. theiformis* was active within 24 hr against *Biomphalaria glabrata* snails, the intermediate host of *Schistosoma mansoni*, at a concentration of 400 ppm This prompted us to undertake a phytochemical investigation of the plant.

The Flacourtiaceae has not yet been extensively studied. However, the presence of saponins and alkaloids has been reported as part of a general screening of secondary metabolites [3]. A tetrahydroxyxanthone, aphloiol, has been isolated previously from the leaves of *A. theiformis* [4]

We now report on the isolation and identification of two known saponins, namely tormentic acid ester glucoside (**1**) and 23-hydroxytormentic acid ester glucoside (**2**), as well as the structure of the new compound, 6β-hydroxytormentic acid ester glucoside (**3**), which were obtained from the methanolic extract of the leaves.



1 $R^1 = R^2 = R^4 = H$, $R^3 = Me$, $R^5 = Glc$
1a $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Me$
2 $R^1 = R^2 = R^4 = H$, $R^3 = CH_2OH$, $R^5 = Glc$
2a $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = Me$
3 $R^1 = R^2 = H$, $R^3 = Me$, $R^4 = OH$, $R^5 = Glc$
3a $R^1 = R^2 = R^5 = H$, $R^3 = Me$, $R^4 = OH$
3b $R^1 = R^2 = Ac$, $R^3 = Me$, $R^4 = OH$, $R^5 = Glc(Ac)_4$

RESULTS AND DISCUSSION

The methanolic extract of the leaves of *A. theiformis* was subjected to different chromatographic techniques (see Experimental) yielding the pure saponins **1**–**3**. Saponin **1** presented a peak in the fast atom bombardment (FAB) mass spectrum (MS) (positive ion mode) at *m/z* 673 $[M+Na]^+$ suggesting a molecular weight of 650. Another peak at *m/z* 511 $[(M+Na)-162]^+$ indicated the presence of a hexose unit in the molecule. The ^{13}C NMR spectrum of **1** confirmed the presence of a sugar moiety and a triterpene aglycone with an ursolic acid type skeleton (C-12 and C-13 at δ128 24 and 139 26, respectively) Basic hydrolysis of **1** provided glucose and **1a**, meaning that the glycosidation site on the aglycone is at the carboxyl function. The identity of the aglycone was established as tormentic acid by comparison of its spectral data with those of the literature [5, 6] as well as by cochromatography with an authentic sample Further confirmation was obtained by preparation of the diacetylmethyl ester of **1a**, which also co-chromatographed with an authentic sample [5]. Thus, the structure of **1** is tormentic acid ester glucoside, a saponin previously isolated from *Rosa multiflora* (Rosaceae) [7]

The D/CIMS of saponin **2** gave peaks at *m/z* 684 $[M+NH_4]^+$ and 666 $[M^+]$ suggesting a molecular formula of $C_{36}H_{58}O_{11}$ Other peaks at *m/z* 522 $[(M+NH_4)-162]^+$ and 505 $[(M-162)+H]^+$ indicated the loss of a hexose sugar moiety. The ^{13}C NMR spectrum of **2** was quite similar to that of **1** with the only major difference arising from the appearance of a $-CH_2OH$ carbon signal at δ66.74 in **2** together with the absence of one of the

signals corresponding to a methyl group in **1** Saponin **2** underwent basic hydrolysis to yield glucose, indicating that the sugar is attached to the aglycone through an ester linkage The site of attachment for the $-CH_2OH$ group was established by comparison of the ^{13}C NMR spectral data of **2** with those reported in the literature for 23-hydroxytormentic acid glucoside [8] which were found to match each other Saponin **2** is therefore identified as 23-hydroxytormentic acid glucoside, a compound previously isolated from *Desfontainia spinosa* (Loganiaceae) [9] and *Geum japonicum* (Rosaceae) [8]

Saponin **3** exhibited signals in the FABMS (positive ion mode) at m/z 689 $[M+Na]^+$ and 527 $[(M+Na)-162]^+$. These indicated a M_r of 666 accompanied by the loss of a hexose unit The presence and identity of the sugar molecule was confirmed when basic hydrolysis of **3** yielded glucose and the aglycone **3a**. The D/CIMS of **3a** showed peaks at m/z 522 $[M+NH_4]^+$ and 505 $[M+H]^+$ which correspond to a M_r of 504 and a molecular formula of $C_{30}H_{48}O_6$ Comparison of the ^{13}C NMR spectra of **1** and **3** showed great similarities with one major difference being the disappearance of a $-CH_2$ group signal at δ 19 10 in **1** and the appearance of a –CH–OH signal at δ 67.75 in **3** The problem that remained was to determine the position of attachment for the third hydroxyl group to the aglycone moiety This was established as follows the presence in the EIMS of **3a** of a peak at m/z 264, which arises from the typical RDA fragmentation of urs-12-ene triterpenes [10], suggests that the third hydroxyl group is not found in rings C, D or E. This left only three possibilities, namely C-1, C-6 and C-7 Saponin **3** was acetylated at room temperature providing the hexaacetate **3b** D/CIMS of **3b**, which presented peaks at m/z 937 $[M+NH_4]^+$ and 919 $[M+H]^+$ for a M_r of 918, confirmed the identity of the hexaacetate. The ^1H NMR spectrum of **3b** showed that, in addition to the four acetate groups on the glucose portion, only the –OH groups at C-2 and C-3 of the aglycone were acetylated, with chemical shifts of δ 5 10 and 4 68, respectively. These results clearly suggest that some steric hindrance does not allow the third secondary –OH group to react Therefore, only positions C-6 and C-7 can now be envisaged as possible places for hydroxylation since an –OH at C-1 would have been easily acetylated Molecular models showed that 6β- and 7α-configurations are the ones which present some steric hindrance, as was also noted previously [12] Acetylation of **3** under reflux conditions failed to yield the heptaacetate, the expected product if the third secondary –OH were at C-7α, as has been observed earlier [9] Furthermore, when **3a** was chromatographed with an authentic sample of 7α-hydroxytormentic acid, it exhibited different behaviour both on silica gel and on reversed phase TLC

The possible substitution at C-6 was given further support by considering the ^{13}C NMR spectrum of **3** When ursenes are unsubstituted at C-6 and C-7, the signals for these carbons are found at δ 18 3 and 32.6, respectively [13] The 6β-hydroxyursenes have been shown to give shifts of about δ 67 for C-6 and δ 41 for C-7 [14]. This seems to be exactly the case for **3** since we observed a down field shift for C-7 from δ 33 51 in **1** to 41 85 in **3** while C-6 undergoes a shift from δ 18.10 to 67 75. Based on all the information discussed above, we propose the structure of **3** as 6β-hydroxytormentic acid ester glucoside, to our knowledge a new naturally occurring saponin

Table 1 ^{13}C NMR spectral data* for compounds **1**, **3** and **3a** (pyridine-d_5)

C	1	3	3a
1	48 04	50 41	50 34
2	68 62	68 50	69 57
3	83 82	84 18	84 72
4	38 51	38 37	38 81
5	55 96	56 65	57 40
6	19 10	67 75	68 82
7	33 51	41 85	41 84
8	40 63	40 80	41 16
9	47 87	48 46	49 05
10	39 88	40 04	40 31
11	24 21	24 33	24 70
12	128 24	128 66	129 56
13	139 47	138 47	139 38
14	42 29	42 80	42 12
15	29 25	29 23	29 54
16	26 75	26 78	27 76
17	48 63	48 66	49 72
18	54 42	54 33	55 13
19	72 59	72 68	73 61
20	42 22	42 16	43 10
21	26 14	24 63	26 63
22	37 77	37 65	38 99
23	29 46	29 30	28 96
24	16.77	16 75	16 58
25	17 06	18 69	18 49
26	17 76	19 41	18 77
27	24 62	26 34	24 82
28	176 85	176 76	182 22
29	27 03	27 08	27 08
30	17 51	18 74	18 54
1′	95 82	95 88	
2′	74 04	74 03	
3′	79 96	78 71	
4′	71 14	71 25	
5′	79 31	79 15	
6′	62 25	62 26	

*Spectra were run at 50 1 MHz Carbon multiplicities were determined using DEPT experiments

The pure saponins **1–3** were individually tested for their molluscicidal properties against *B glabrata* snails and were found to be inactive. This is not surprising since we are dealing with esterified saponins and, as it has already been pointed out, bidesmosidic saponins (e g. sugar chains at C-3 and C-28) are usually inactive against schistosomiasis-transmitting snails while their monodesmosidic derivatives (e g. sugar at C-3) are active [15] We also observed that removal of tannin material from the crude methanol extract by the use of hide powder rendered it inactive against the snails [16]. Thus, it appears that the molluscicidal properties of the methanolic extract are due to the presence of tannins in this plant

EXPERIMENTAL

General TLC was carried out on silica gel precoated Al-sheets (Merck). For normal phase CC, silica gel 60, 40–63 μm (Merck)

was used Reversed-phase chromatography was performed on a Lobar LiChroprep RP-8 column (40–63 μm, i d 2 5 × 27 cm) (Merck) equipped with a Duramat 80 pump (Chemie and Filter) DCCC separations were performed on a Buchi 670 DCC chromatograph (294 tubes of 2.7 mm internal diameter). D/CIMS was done on a Ribermag R10-1013 quadrupole instrument with NH_3 as reactant gas FABMS were measured on a ZAB 1S spectrometer using a thioglycerol matrix and bombarded using 5 keV Xe atoms EIMS were measured at 70 eV NMR spectra were recorded at 50 1 MHz for ^{13}C and 200 MHz for ^1H

Extraction and isolation The leaves of *Aphloia theiformis* were collected in the forests Le Petrin and Mare Longue, Mauritius. A voucher specimen was deposited at the herbarium of the Mauritius Sugar Industry Research Institute The dried plant material (50 g) was extracted with solvents of increasing polarity and afforded a CH_2Cl_2 extract (1 2 g) and a methanolic extract (10 g). Only the methanolic extract exhibited molluscicidal activity at 400 ppm within 24 hr against *Biomphalaria glabrata* snails This extract (10 g) was partitioned between *n*-BuOH and H_2O The organic portion was evapd to dryness to provide 5 g of syrup which was further pptd with Et_2O The ppt (3 5 g) was separated by DCCC, ascending mode with $CHCl_3$–MeOH–*i*-PrOH–H_2O (5 6 1 4), the separation was monitored by TLC and 9 fractions were obtained (I–IX) Fraction IV (300 mg) was purified (3 × 100 mg) by low-pressure liquid chromatography on a Lobar RP-8 column with MeOH–H_2O (8 2) to yield 60 mg of pure **3** and 20 mg of a mixture containing **2** The latter was further purified on a Lobar silica gel column with $CHCl_3$–MeOH (8·2) and afforded saponin **2** (10 mg) Fraction VI (250 mg) was purified in the same manner providing 50 mg of **1**. All compounds underwent a final purification step on Sephadex LH-20 with MeOH

Basic hydrolysis was performed using 0.5 N aq. KOH. Work-up, aglycone and sugar analysis were carried out as previously described [17].

Acetylation was done using Ac_2O–pyridine for 24 hr at room temp.

TLC on silica gel of compounds **1**–**3** and **3a** was carried out with $CHCl_3$–MeOH (7 3) to give R_f values of 0.33, 0.25, 0.25 and 0 88, respectively Detection with Godin reagent [18] showed blue coloration for compounds **1** and **2** and purple colour for **3** and **3a**

Tormentic acid ester glucoside (**1**) Amorphous white powder, $C_{36}H_{58}O_{10}$, (M_r=650). FABMS· *m/z* 673 $[M+Na]^+$, 511 $[(M+Na)-162]^+$ D/CIMS 668 $[M+NH_4]^+$, 506 $[(M+NH_4)-162]^+$, 489 $[(M-162)+H]^+$, 443 $[(M-162)-COOH]^+$

Tormentic acid ester glucoside hexaacetate Amorphous solid, $C_{48}H_{70}O_{16}$, (M_r=902) D/CIMS *m/z* 920 $[M+NH_4]^+$, 903 $[M+H]^+$, 544, 527, 509, 391, 366, 331.

23-Hydroxytormentic acid ester glucoside (**2**). Amorphous white powder, $C_{36}H_{58}O_{11}$, (M_r 666) D/CIMS *m/z* 684 $[(M+NH_4]^+$, 656 $[M]^+$, 522 $[(M+NH_4)-162]^+$, 505 $[(M-162)+H]^+$, 180 $[C_6H_{10}O_5+NH_4]^+$, 162 $[C_6H_{10}O_5]^+$ ^1H NMR (pyridine-d_5)· δ 1.06 (*d*, 3H, H_3–30), 1.09, 1 15, 1 25, 1.37 and 1 62 (each 3H, Me-24 to 29), 2.92 (*s*, H-18), 3 71 (*m*, H-3), 4 07 (*m*, H-2), δ4.23 (*s*, 2H, H-23 a and b), 4 12–4 51 (sugar protons), 5.53 (*br s*, H-12), 6 33 (*d*, H-1′)

6β-Hydroxytormentic acid ester glucoside (**3**). Amorphous white powder, $C_{36}H_{58}O_{11}$, (M_r 666) FABMS *m/z* 689 $[M+Na]^+$, 589 $[(M+Na)-162]^+$ ^1H NMR (pyridine-d_5) δ1.06 (*d*, 3H, H_3–30), 1 18, 1 41, 1 45, 1 70, 1.77 and 1 80 (3H each, Me-23 to 29), 2.95 (*s*, H-18 a), 3 40 (*d*, H-3a), 5.62 (*br s*, H-6b), 5.62 (*br s*, H-12), 6 27 (*d*, H-1′)

6β-Hydroxytormentic acid (**3a**) Amorphous white powder, $C_{30}H_{48}O_5$, (M_r 504) EIMS *m/z* (rel int): 504 (1 27) $[M]^+$, 486 (3 36) $[M-H_2O]^+$, 468 (2.91) $[M-2H_2O]^+$, 458 (15 97) $[M-HCOOH]^+$, 440 (8 58) $[M-HCOOH-H_2O]^+$, 386 (11 12), 264 (13 06), 246 (23 73), 222 (19.10), 201 (35 00), 185 (41 87), 173 (32.69), 159 (29.10), 146 (100.00), 131 (31.79), 105 (38 66), 91 (29 48), 55 (47 16), 43 (82 54) D/CIMS 522 $[M+NH_4]^+$, 505 $[M+H]^+$ 487 $[(M+H)-H_2O]^+$, 180 $[C_6H_{10}O_5+NH_4]^+$ ^1H NMR (CD_3OD) δ 0 92 (*d*, 3H, H_3–30), 1.08, 1 18, 1 20, 1 29, 1 31 and 1 36 (3H each H_3-23, H_3-24, H_3-25, H_3-26, H_3-27, H_3-28, H_3-29), 2 08 (*m*, H-11a), 2 52 (*br s*, H-18), 2 57 (*m*, H-11b), 2.85 (*d*, H-3a), 3 68 (*m*, H-2b), 4 44 (*br s*, H-6b), 5 33 (coarse *t*, H-12).

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