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TRITERPENOID SAPONINS FROM THE ROOT OF *SIDEROXYLON FOETIDISSIMUM*

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Key Work Index—*Sideroxylon foetidissimum*; Sapotaceae; saponins; sideroxyloside B; sideroxyloside C; electrospray mass spectrometry.

Abstract—Two novel triterpenoid saponins named sideroxyloside B and sideroxyloside C were isolated from the root of *Sideroxylon foetidissimum*. On the basis of spectroscopic and chemical methods, their structures were established as 3-*O*-β-D-glucopyranosyl-28-*O*{[β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)] [β-D-apiofuranosyl(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} protobassic acid and 28-*O*{[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)] [β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} protobassic acid, respectively.

INTRODUCTION

Sideroxylon foetidissimum Jacq. is a small to medium-sized evergreen tree found in the West Indies from Cuba to Grenada [1]. As part of our continuing interest in saponins from plants [2, 3], we studied the root of this species. Here, we report the isolation and identification from this material of two new triterpenoid saponins named sideroxyloside B (**1**) and sideroxyloside C (**2**).

RESULTS AND DISCUSSION

The electrospray mass spectrum of **1** displayed a molecular peak at *m/z* 1510 $[MH+Na]^+$ together with the multicharged peak at *m/z* 766 $[M + 2Na]^{2+}$ indicating the molecular formula $C_{68}H_{110}O_{35}$. The ^{13}C NMR spectrum of **1** showed signals for seven sugar residues (Table 1). Two of them could rapidly be identified by their characteristic quaternary resonances at δ 77.5 and 77.2 as β-D-apiose (Api) [δ 2 × H-1, 5.37)]. Two other sugar moieties were identified as α-L-rhamnose (Rha) [δ 2 × H-1, 5.13 and 5.20] from their typical pattern in the COSY spectrum. Alkaline hydrolysis of **1** led to the known monodesmoside **3** [2], allowing simultaneous identification of the aglycone of **1** as protobassic acid and of β-D-glucose (Glc) [δH-1, 4.51] as a single C-3 substituting sugar. Once ^{13}C resonances corresponding to these five sugars were assigned, it became obvious that the two missing sugars were β-D-xylose (Xyl) [δH-1, 4.72] and α-L-arabinose (Ara) [δH-1, 5.76], this latter being attached to the aglycone at C-28 via an ester bond. Thus, the side chain was constituted of two molecules of apiose, one molecular of xylose and two molecules of rhamnose attached to the aglycone via an arabinose moiety. ^1H, ^{13}C, 2D homonuclear and heteronuclear NMR experiments allowed localization of the proton signals of the sugar residues and full attributions of their carbon resonances. Analysis of the chemical shifts of these latter and comparison with lit. data [4–6], established that **1** was composed of one glucose and two apiose terminal residues, one xylose residue substituted at position 3, one rhamnose residue substituted at position 4, one rhamnose residue substituted at positions 3 and 4, and one arabinose residue substituted at position 2. Interglycosidic linkage of **1** was deduced from analysis of both 2D NOESY and long-range ^{13}C–^1H correlation spectra. From the latter, correlation peaks were observed between glucose C-1 and aglycone H-3, and aglycone C-28 and arabinose H-1. The 2D NOESY spectrum showed correlation peaks, respectively, between 3,4-disubstituted rhamnose H-1, H-3, H-4 and 2-substituted arabinose H-2, apiose H-1 and 4-substituted rhamnose H-1; 4-substituted rhamnose H-4 and 3-substituted xylose H-1; 3-substituted xylose H-3 and apiose′ H-1 (Fig. 1). Thus, **1** was identified as 3-*O*-β-D-glucopyranosyl-28-*O*-{[β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)][β-D-apiofuranosyl-(1→3)}-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} protobassic acid, and designated as sideroxyloside B.

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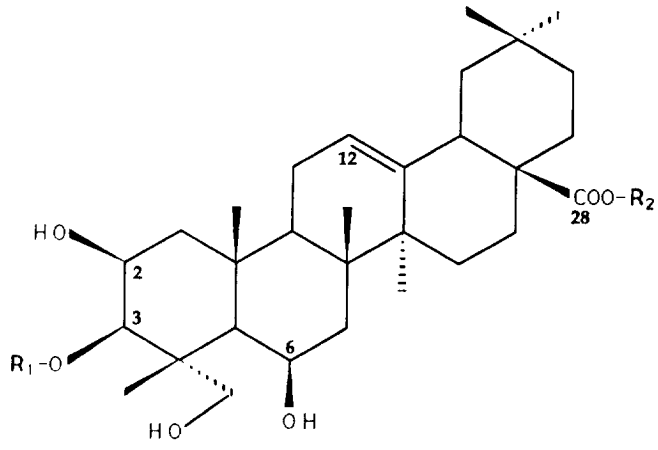

	R_1	R_2
1	Glc	-Ara(2<-1)Rha[(4<-1)Rha(4<-1)Xyl(3<-1)Api][(3<-1)Api]
2	H	-Ara(2<-1)Rha[(4<-1)Rha(4<-1) Xyl][(3<-1)Api]
3	Glc	H

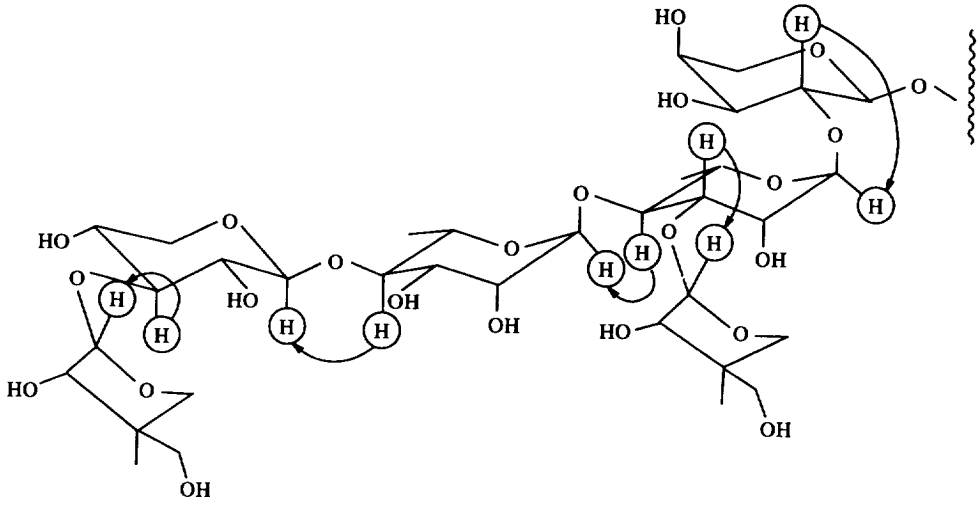

Fig. 1. Essential NOEs observed for the sugar component on C-28 of **1**.

After identification of **1**, the structure determination of **2** was straightforward. Its FAB mass spectrum displayed a molecular peak at *m/z* 1215 $[M+Na]^+$ indicating the molecular formula $C_{57}H_{92}O_{26}$. Its NMR spectra, compared to those of **1**, presented four characteristic features (Tables 1 and 2): the presence of five anomeric signals; carbons at δ93.8, 101.4, 102.6, 105.1 and 111.8; protons at δ4.73 (Xyl H-1), 5.13 (Rha H-1), 5.20 (Rha H-1), 5.37 (Api H-1) and 5.76 (Ara H-1), the lack of signals corresponding to a glucose moiety, the presence of only one apiose

Table 1. ^{13}C NMR chemical shifts of sugar moieties of **1** and **2** in CD_3OD (ppm)

C	1	2
3-*O*-Glucose-1	105.0	
2	75.3	
3	77.3	
4	73.9	
5	77.5	
6	62.1	
28-*O*-Arabinose-1	93.8	93.8
2	75.9	76.0
3	68.9	69.0
4	67.0	67.1
5	63.6	63.6
Rhamnose-1	101.3	101.4
2	72.1	72.2
3	81.7	81.8
4	78.4	78.5
5	70.0	70.0
6	17.9	17.9
Apiose-1	111.7	111.8
2	78.0	78.1
3	80.4	80.1
4	74.9	74.8
5	65.0	64.7
Rhamnose′-1	102.6	102.6
2	72.1	72.1
3	72.1	72.4
4	84.1	84.1
5	70.0	70.0
6	19.2	19.3
Xylose-1	104.9	105.1
2	75.9	76.0
3	85.7	78.5
4	71.0	74.0
5	66.9	67.0
Apiose′-1	111.2	
2	77.8	
3	80.0	
4	74.9	
5	64.7	

Table 2. ^{13}C NMR spectral data of the aglycone moieties of **1** and **2** in CD_3OD (ppm)

C	1	2	C	1	2
1	46.6	47.1	16	23.7	23.7
2	71.2	71.2	17	49.6	49.2
3	83.7	73.5	18	42.7	42.7
4	43.6	43.6	19	47.1	47.6
5	49.8	49.7	20	31.5	31.6
6	68.4	68.6	21	34.9	35.0
7	41.2	41.3	22	33.3	33.4
8	39.7	39.9	23	65.4	65.1
9	48.8	48.9	24	16.3	15.6
10	37.0	37.4	25	18.9	18.9
11	24.6	24.6	26	18.2	18.2
12	124.6	124.6	27	26.4	26.4
13	144.1	144.2	28	177.7	177.7
14	43.9	43.6	29	33.5	33.5
15	28.9	28.9	30	24.0	24.0

residue and the shielding of the resonances corresponding to C-3 and xylose C-3. Based on these observations, the structure of **2** was established as 28-*O*-{β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)][β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} protobassic acid, and designated as sideroxyloside C.

EXPERIMENTAL

^1H NMR (200 and 300 MHz) and ^{13}C NMR (50 and 75 MHz): CD_3OD. COSY, NOESY, COLOC and ^{13}C–^1H correlations spectra were performed with the use of Bruker microprograms. Chemical shifts (δ) are reported in ppm by setting the ref. on CD_3OD residual solvent peak at δ3.50 and 49.0 for ^1H and ^{13}C, respectively. Coupling constants (*J*) are given in Hz.

Extraction and isolation. Root material of *S. foetidissimum* Jacq. was collected in the Dominican Republic during 1993. A voucher specimen (R. García No. 4815) was deposited at the Herbarium of the National Botanical Garden of Santo Domingo. Dried and powdered roots (136 g) were extracted with MeOH to afford 27 g of extract. This extract was partitioned between *n*-BuOH and H_2O. The BuOH layer was purified first by vacuum liquid chromatography. The residue was dissolved in MeOH (30 ml) and poured into Et_2O (150 ml). The pellet obtained after centrifugation was subjected to silica gel CC using CH_2Cl_2–MeOH–H_2O as solvent. Some frs were collected and again subjected to chromatography using a Lichroprep RP-8 column with MeOH–H_2O (13:7) to yield 100 mg of **1**. Others frs were chromatographed using both silica gel CC and Lichroprep RP-8 CC to yield 60 mg of **2**.

Saponin **1**. Powder from MeOH, $[\alpha]_D^{24}$ −49.4 (MeOH; *c* 1.6). Electrospray MS *m/z*: 1510 $[MH+Na]^+$, 766 $[M+2Na]^{2+}$; ^1H NMR: δ1.00, 1.04, 1.16, 1.23, 1.40 and 1.71 (6 × 3H, *s*, Me-29, Me-30, Me-27, Me-26, Me-24 and Me25, respectively), 1.34 (6H, Rha 2 × Me), 1.82 (1H, *m*, H-19), 3.05 (1H, *dd*, *J* = 13.5 and 3 Hz, H-18), 4.42 (1H, *m*, H-2), 4.54 (2H, *m*, Glc-1, H-6), 4.72 (1H, *d*, *J* = 7.7 Hz, Xyl H-1), 5.13 (1H, *br s*, Rha H-1), 5.20 (1H, *br s*, Rha H-1), 5.37 (2H, 2 × Api H-1), 5.45 (1H, *br t*, H-12), 5.76 (1H, *d*, *J* = 3.3 Hz, Ara H-1); ^{13}C NMR: (Tables 1 and 2).

Saponin 2. Powder from MeOH, $[\alpha]_D^{24}$ −42.8 (MeOH; *c* 0.6). FAB MS (positive) *m/z*: 1215 $[M+Na]^+$; ^1H NMR: δ1.00, 1.04, 1.16, 1.23, 1.40 and 1.70 (6 × 3H, *s*, Me-29, Me-30, Me-27, Me-26, Me-24 and Me-25, respectively), 1.33 and 1.34 (6H, Rha 2 × Me), 1.82 (1H, *m*, H-19), 3.03 (1H, *dd*, *J* = 12.6 and 3.8 Hz, H-18), 4.53 (1H, *m*, H-6), 4.73 (1H, *d*, *J* = 7.7 Hz, Xyl H-1), 5.13 (1H, *br s*, Rha H-1), 5.20 (1H, *br s*, Rha H-1), 5.37 (1H, *d*, *J* = 4.0 Hz, Api H-1), 5.45 (1H, *br t*, H-12), 5.76 (1H, *d*, *J* = 3.3 Hz, Ara H-1); ^{13}C NMR: (Tables 1 and 2).

Alkaline hydrolysis of **1**. A soln of saponin **1** (10 mg) in 0.5 M KOH (10 ml) was refluxed for 3 hr. The pH of the reaction mixt. was adjusted to 6 with 10% HCl before being extracted with *n*-BuOH satd with H_2O. The BuOH layer was evapd to dryness and the residue subjected to silica gel CC using CH_2Cl_2–MeOH–H_2O (14:6:1) as solvent to give **3**.

Acid hydrolysis of **1**. A soln of saponin **1** (2 mg) in 10% HCl (1 ml) was refluxed for 3 hr and then partitioned with Et_2O. The acidic aq. layer was neutralized with Ag_2CO_3, filtered and taken to dryness. The residue was analysed by TLC using MeCOEt–HOAc–MeOH (3:1:1) as solvent, and authentic samples of glc, xyl, ara, rha and api.

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REFERENCES

1. Liogier, A. H. (1974) in *Diccionario Botánico de Nombers Vulgares de la Española*, p. 255. Universidad P. H. Ureña, Sto, Domingo, Dominican Republic.
2. Jiang, Y., Oulad-Ali, A., Guillaume, D., Weniger, B. and Anton, R. (1994) *Phytochemistry* **35**, 1013.
3. Oulad-Ali, A., Guillaume, D., Weniger, B., Jiang, Y. and Anton, R. (1994) *Phytochemistry* **36**, 445.
4. Nigam, S. K., Li, X. C., Wang, D. Z., Misra, G. and Yang, C. R. (1992) *Phytochemistry* **31**, 3169.
5. Charrouf, Z., Wieruszeski, J. M., Fkih-Tetouani, S., Leroy, Y., Charrouf, M. and Fournet, B. (1992) *Phytochemistry* **31**, 2079.
6. Bader, G., Tuja, D., Wray, V. and Hiller, K. (1994) *Pharmazie* **49**, 209.