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GLYCOLIPIDS FROM *BYRSONIMA CRASSIFOLIA*

LUCA RASTRELLI, NUNZIATINA DE TOMMASI, INGEBORG BERGER,* ARMANDO CACERES,* AMARILLIS SARAVIA* and FRANCESCO DE SIMONE†

Dipartimento di Scienze Farmaceutiche, Università di Salerno, Piazza V. Emanuele 9 84084, Penta di Fisciano, Italy,* Facultad de Ciencias Quimicas y Farmacia, Universidad de San Carlos de Guatemala, Zona 12 01002 Guatemala C.A.

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Key Word Index—*Byrsonima crassifolia*; Malpighiaceae; 'nanche'; leaves; glycolipids; sulphonoglycolipids.

Abstract—From the leaves of *Byrsonima crassifolia* four new glycolipids, 1,2-di-*O*-miristoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol, 1,2-di-*O*-(8-hexadecenoyl)-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol, 1,2-di-*O*-palmitoyl-3-*O*-(β-D-glucopyranosyl)-glycerol and 1,2-di-*O*-(8-hexadecenoyl)-3-*O*-(β-D-glucopyranosyl)-glycerol, have been isolated. Nine known compounds were also found. The structures of new compounds were elucidated on the basis of chemical and spectral data.

INTRODUCTION

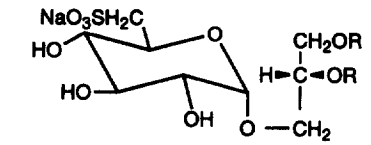
Byrsonima crassifolia Rich. ex Juss. is a tropical tree of the Malpighiaceae family widely distributed in several regions of Central and South America and popularly known as 'nanche'. The barks and leaves are used in folkloric medicine to treat coughs, gastrointestinal disorders, skin infections [1] and snake bites [2]. The fruits are edible and commonly sold in local markets. Leaf and bark extracts display spasmogenic effects on rat fundus [3] and antimicrobial activity [4, 5]. The genus *Byrsonima* has a small number of species and only a few have been examined chemically. In a previous study a new glycolipid derivative, 1,2-di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol, was isolated from leaves of *B. crassifolia* [6]. This paper deals with the isolation and structural elucidation of another four new glycolipids (**1–4**) from the leaves of *B. crassifolia*.

RESULTS AND DISCUSSION

The methanolic extract of the leaves of *B. crassifolia* afforded 14 compounds. The known compounds were identified as 1,2-di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol [6], oleanolic acid [7], 2-β-hydroxy-oleanolic acid [7], 2-α-hydroxy-oleanolic acid [7], lupeol [8], 2-β-hydroxy-lupeol [8], (+)-catechin [9], (−)-*epi*catechin [9], quercetin 3-*O*-β-D-galactopyranoside [10] and quercetin 3-*O*-β-D-glucopyranoside [10], by spectral data and direct comparison of their physical properties with those reported previously for these compounds. Four new compounds, **1–4**, were also isolated.

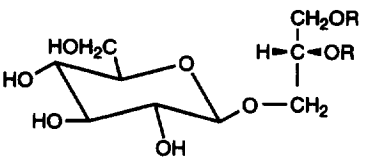
The molecular formulae ($C_{37}H_{69}O_{12}SNa$ for **1**, $C_{41}H_{73}O_{12}SNa$ for **2**, $C_{41}H_{78}O_{10}$ for **3** and $C_{41}H_{74}O_{10}$ for **4**) of compounds **1–4** were determined by ^{13}C NMR, ^{13}C DEPT NMR and negative ion FAB–mass spectrometry. The IR spectra of **1** and **2** indicated the presence of a sulphonyl group (1121, 1028, 792 cm^{-1}) in their structures. Compound **1** showed a molecular ion peak at *m/z* 759 $[M-H]^-$ in its FAB–mass spectrometry spectrum. The ^1H NMR spectrum exhibited two terminal methyl signals (δ 0.97, 6H, *t*), a broad methylene signal at δ 1.33 (40 H, fatty acid residues) and the signals due to two methylene protons linked to a carbonyl function (δ 2.39, 4H, *m*) and to two β-methylene protons (δ 1.78, 4H, *m*). From the ^1H-^1H COSY data, a spin system was easily assigned to an unsymmetrical and fully substituted glycerol moiety. The methylene protons at C-1 and C-3 were distinctly different and characteristic chemical shift (Table 1) reflecting the acyl versus glycosidic substitution at these two position [11]. Moreover, the ^1H-^1H COSY spectrum revealed that the coupling constants and splitting patterns of the protons in the sugar moiety of **1** were very similar to those of α-glucopyranoside, but the chemical shifts were in some ways different. Namely, observation of the C-6-methylene at δ 3.34 and 2.91 indicated the attachment of a sulphonyl group on the C-6 carbon, so the sugar moiety was sulphoquinovose. The ^{13}C NMR spectrum of **1** (Table 1), in which the C-6 carbon signal appeared at δ 54.6, also substantiated this conclusion. Treatment of **1** with sodium methoxide–methanol gave 6-sulpho-

†Author to whom correspondence should be addressed.



1 R = —CO $(CH_2)_{12}$ CH_3

2 R = —CO $(CH_2)_6$ $(CH_2)_6$ CH_3



3 R = —CO $(CH_2)_{14}$ CH_3

4 R = —CO $(CH_2)_6$ $(CH_2)_6$ CH_3

Table 1. ^{1}H NMR chemical shifts of glycerol glycoside moiety for compounds **1–4** (in CD_3OD)*

H	1	2	3	4
1a	4.50*dd* (12, 3)	4.51*dd* (12, 3)	4.38*dd* (12, 3)	4.40*dd* (12, 3)
1b	4.18*dd* (12, 7)	4.19*dd* (12, 7)	4.19*dd* (12, 7)	4.20*dd* (12, 7)
2	5.33*m*	5.33*m*	5.34*m*	5.33*m*
3a	3.57*dd* (11, 6)	3.57*dd* (11, 6)	3.62*dd* (11, 6)	3.62*dd* (11, 6)
3b	4.10*dd* (11, 5)	4.11*dd* (11, 5)	3.96*dd* (11, 5)	3.98*dd* (11, 5)
1′	4.76*d* (3.7)	4.75*d* (3.7)	4.42*d* (7.5)	4.42*d* (7.5)
2′	3.40*dd* (9.5, 3.7)	3.39*dd* (9.5, 3.7)	3.32*dd* (7.5, 9)	3.33*d* (7.5, 9)
3′	3.08*dd* (9.0, 9.5)	3.08*dd* (9.0, 9.5)	3.18*dd* (9.5, 9.5)	3.20*dd* (9.5, 9.5)
4′	3.64*dd* (9.0, 9.5)	3.63*dd* (9.0, 9.5)	3.43*dd* (9.5, 9.5)	3.44*dd* (9.5, 9.5)
5′	4.07*ddd* (2, 9.2, 9.5)	4.07*ddd* (2, 9.2, 9.5)	3.98*m*	3.98*m*
6′a	3.34*dd* (2, 14.3)	3.33*dd* (2, 14.3)	3.86*dd* (12, 3)	3.86*dd* (12, 3)
6′b	2.91*dd* (9.2, 14.3)	2.91*dd* (9.2, 14.3)	3.72*dd* (12, 5)	2.73*dd* (12, 5)

*J values in Hz presented in parentheses.

quinovopyranosyl glycerol (**1a**), identified by ^{1}H, ^{13}C and ^{13}C DEPT NMR spectra and by comparison with literature data [12], and methyl myristate (analysed by GC mass spectrometry). The stereochemistry of *sn*-2 in the glycerol portion was determined to be *S* by comparing the specific rotation of **1a** with that reported previously [12, 13]. Based on these findings, the structure of the glycolipid **1** was determined as (2*S*)-1,2-di-*O*-miristoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol.

Compound **2** shows a molecular ion peak at *m/z* 811 $[M-H]^-$ in its FAB-MS spectrum, and its ^{1}H NMR and ^{13}C NMR spectra closely resembled those of **1** except for the signals ascribable to the unsaturated fatty acid residues (Tables 1 and 2). Treatment of **2** with sodium methoxide–methanol as carried out for **1** furnished the same glyceryl sulphoquinovopyranoside (**1a**) and methyl 8-hexadecenoate (analysed by GCMS). Since signals ascribable to olefinic carbons of the 8-hexadecenoyl residues of **2** were observed at δ_C 130.2 and 129.8 (C-8″, C-9″) and allylic carbons at δ_C 27.4 (C-7″, C-10″), the unsaturated fatty acid residues were identified as two *cis*-8-hexadecenoyl groups [14]. It was, therefore, proposed that two molecules of 8-hexadecenoyc acid were attached on the glycerol moiety. The above data were consistent with a structure of (2*S*)-1,2-di-*O*-(8-hexadecenoyl)-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol for compound **2**.

Glycolipids **3** and **4** showed molecular ion peaks at *m/z* 729 $[M-H]^-$ and 725 $[M-H]^-$ respectively, in their FAB–mass spectrometry spectra. Alkaline hydrolysis of **3** and **4** furnished glycerol glycoside and fatty acid methyl esters. The composition of fatty acid residues was shown to be methylpalmitate for **3** and methyl 8-hexadecenoate for **4** by GC mass spectrometry analysis. The glycerol glycoside was shown to be glyceryl β-D-glucopyranoside (**3a**) by assignment of the ^{1}H and ^{13}C NMR signals with the aid of ^{1}H-^{1}H COSY and HETCOR experiments (Tables 1 and 2). The chemical shifts of the methylene protons at C-1 and C-3 (Table 1) were in agreement with the acyl vs glycosidic substitution at these two positions [7]. From these data the structures of the glycolipids was concluded to be 1,2-di-*O*-palmitoyl-3-*O*-(β-D-glucopyranosyl)-glycerol (**3**) and 1,2-di-*O*-(8-hexadecenoyl)-3-*O*-(β-D-glucopyranosyl)-glycerol (**4**). Monoglycosyl diacylglycerols including such as sugars 6-sulpho-α-D-quinovopyranosyl and -β-D-glucopyranosyl have been isolated previously from other natural sources [12, 15, 16], the novelty of our compounds resides in the different combination of these sugars within the fatty acid residues.

Table 2. ^{13}C NMR chemical shifts of glycerol glycoside moiety of compounds **1–4** and related compounds (in CD_3OD)*

C	DEPT	1	1a	2	3	3a	4
1	CH2	64.4	63.7	64.3	64.4	63.6	64.2
2	CH	71.6	71.2	71.5	72.0	71.8	71.9
3	CH2	67.3	70.0	67.4	68.8	72.2	68.7
1′	CH	100.1	98.6	98.8	104.9	104.8	104.9
2′	CH	72.5	72.2	72.6	74.4	74.4	74.5
3′	CH	73.5	73.6	73.8	78.6	78.5	78.6
4′	CH	74.9	74.4	74.8	71.2	71.0	71.3
5′	CH	69.9	68.8	69.0	78.2	78.2	78.4
6′	CH2	54.6	53.8	54.5	64.2	64.3	64.4

*All signals were assigned by ^1H-^1H COSY and HETCOR experiments.

EXPERIMENTAL

NMR: 500 (^1H) and 139 (^{13}C) MHz, CD_3OD; negative ion FAB MS, DEPT, COSY and HETCOR experiments were performed as described earlier [17].

Plant material. The leaves of *B. crassifolia* were collected in Quiché Department, Guatemala, in July 1995 and identified by J. Castillo. A voucher sample is deposited at the Herbario of the Facultad de Agronomia, Universidad de San Carlos de Guatemala.

Extraction and isolation. The leaves of *B. crassifolia* (550 kg) were defatted with *n*-hexane and $CHCl_3$ in a Soxhlet apparatus and then extracted at room temp with MeOH (25 g). Part of the MeOH extract (12 g) was partitioned between *n*-BuOH and H_2O to afford a *n*-BuOH-soluble portion (6.0 g) which was chromatographed on a Sephadex LH-20 column using MeOH as eluent and collecting frs of 8 ml. Frs 6–14 (270 mg), containing the unresolved glycolipids, were combined according to TLC (silica gel, *n*-BuOH–HOAc–H_2O, 60 : 15 : 25). Fractionation was achieved by HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 ml min^{-1}) using MeOH–H_2O (9 : 1) to yield pure compounds **1** (16.0 mg), **2** (18.7 mg), **3** (20.4 mg) and **4** (25.3 mg).

Alkaline hydrolysis and GC analysis. Compounds **1–4** (5.0 mg) in dry MeOH (1 ml) were separately treated with 5% NaOMe–MeOH (0.5 ml) at room temp for 10 min. The reaction mixt. was neutralised with Dowex 50 W × 8 and the resin removed by filtration. The filtrate was extracted with hexane and the hexane layer was concentrated to yield fatty acid methyl esters. These were analysed by GC-MS: Hewlett-Packard 5890 GC equipped with mass-selective detector MSD 5970 MS, a split/splitness injector and a fused-silica column HP-5 (25 m × 0.2 mm; i.d. 0.33 mm film); column temp. 230°, carrier N_2, flow rate 30 ml min^{-1}. R_t (min): methyl myristate 12.5, methyl 8-hexadecenoate 14.6, methyl palmitate 15.2. Retention times were identical to those of the authentic standard mixture. Removal of the solvent from the MeOH layer under reduced pressure gave a residue which was purified by silica gel CC ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1) to furnish **1a** (2 mg) from compounds **1** and **2** and **3a** (1.7 mg) from compounds **3** and **4**.

(+)-Catechin $[\alpha]_D^{25} = +17°$ (MeOH; *c* 1).
(−)-Epicatechin $[\alpha]_D^{25} = -68°$ (MeOH; *c* 1).

1,2-*Di*-O-*miristoyl*-3-O-(6-*sulpho*-α-D-*quinovopyranosyl*)-*glycerol* (**1**). FAB-MS, *m/z*: 759 $[M-H]^-$; ^1H NMR (CD_3OD): δ 0.97 (6H, *t*, $J = 7$ Hz, 14″-Me), 1.33 (40 H, *br s*), 1.78 (4H, *m*, 3″-CH_2), 2.39 (4H, *m*, 2″-CH_2), glycerol glycoside moiety see Table 1; ^{13}C NMR (CD_3OD): δ 173.1 (C-1″), 34.9 (C-2″), 25.7 (C-3″), 30.5 (C-4″–C-6″), 31.0 (C-7″–C-10″), 30.0 (C-11″), 32.9 (C-12″), 23.6 (C-13″), 14.8 (C-14″), glycerol glycoside moiety see Table 2. $[\alpha]_D^{25} = +43°$ (MeOH; *c* 1).

1,2-*Di*-O-(8-*hexadecenoyl*)-3-O-(6-*sulpho*-α-D-*quinovopyranosyl*)-*glycerol* (**2**). FAB-MS, *m/z*: 811 $[M-H]^-$; ^1HNMR(CD_3OD):δ0.97(6H,*t*,J = 7Hz,14″-Me),1.33 (40 H, *br s*), 1.78 (4H, *m*, 3″-CH_2), 2.39 (4H, *m*, 2″-CH_2), 5.36 (4H, *t*, H-8″, H-9″), glycerol glycoside moiety see Table 1; ^{13}C NMR (CD_3OD): δ 173.0 (C-1″), 34.8 (C-2″), 25.6 (C-3″), 30.5 (C-4″), 30.6 (C-5″), 30.7 (C-6″), 27.4 (C-7″, 10″), 130.2 (C-8″), 129.8 (C-9″), 31.0 (C-11″,12″),30.3(C-13″),32.9(C-14″),23.9(C-15″),14.8 (C-16″), glycerol glycoside moiety see Table 2. $[\alpha]_D^{25} = +50°$(MeOH;*c*1).

1,2-*Di*-O-*palmitoyl*-3-O-(β-D-*glucopyranosyl*)-*glycerol* (**3**). FAB-MS, *m/z*: 729 $[M-H]^-$; ^1H NMR (CD_3OD): δ 0.98 (6H, *t*, $J = 7$ Hz, 16″-Me), 1.35 (48 H, *br s*), 1.77 (4H, *m*, 3″-CH_2), 2.36 (4H, *m*, 2″-CH_2); glycerol glycoside moiety see Table 2; ^{13}C NMR (CD_3OD): δ 173.4 (C-1″), 34.7 (C-2″), 25.8 (C-3″), 30.5 (C-4″-C6″), 31.0 (C-7″-C12″), 30.3 (C-13″), 33.1 (C-14″), 23.6 (C-15″), 14.7 (C-16″), glycerol glycoside moiety see Table 2. $[\alpha]_D^{25} = -4°$ (MeOH; *c* 1).

1,2-*Di*-O-(8-*hexadecenoyl*)-3-O-(β-D-*glucopyranosyl*)-*glycerol* (**4**). FAB-MS, *m/z*: 725 $[M-H]^-$; ^1H NMR (CD_3OD): δ 0.97 (6H, *t*, $J = 7$ Hz, 14″-Me), 1.33 (40 H, *br s*), 1.74 (4H, *m*, 3″-CH_2), 2.33 (4H, *m*, 2″-CH_2), 5.34 (4H, *t*, H-8″, H-9″), glycerol glycoside

moiety see Table 1; ^{13}C NMR (CD_3OD): δ 173.0 (C-1″), 34.8 (C-2″), 25.6 (C-3″), 30.5 (C-4″), 30.6 (C-5″), 30.7 (C-6″), 28.3 (C-7″, 10″), 130.7 (C-8″), 129.9 (C-9″), 31.0 (C-11″, 12″), 30.3 (C-13″), 32.9 (C-14″), 23.9 (C-15″), 14.8 (C-16″), glycerol glycoside moiety see Table 2. $[\alpha]_D^{25} = -8°$ (MeOH; *c* 1).

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