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Prieurianoside, a protolimonoid glucoside from the leaves of *Trichilia prieuriana*

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Abstract

The ubiquitous glycolipid 1,2-dilinolenoyl-3-galactopyranosylglycerol and a new protolimonoid glucoside, named prieurianoside, were isolated from the leaves of *Trichilia prieuriana*. The structure of the latter was established, by spectroscopic techniques, as 12β,21-diacetoxy-29-β-D-glucopyranosyloxy-23ξ-hydroxytirucalla-7,24-dien-3-one.

Keywords: *Trichilia prieuriana*; Meliaceae; Protolimonoid glucoside; Prieurianoside; Monogalactosyl diglyceride

1. Introduction

Previously, two protolimonoids named prieurone and 29-hydroxyprieurone were reported from the leaves of *Trichilia prieuriana* (Olugbade, 1991). In continuation of this work, we have examined further the plant leaves for new triterpenoids. We now report the isolation and characterisation of a new protolimonoid glucoside from the leaves.

2. Results and discussion

Prieurianoside (**1**) is a major constituent of the chloroform extract of the leaves. Its ^{13}C-NMR spectrum showed a close resemblance to prieurone (12β-acetoxy-21,23-epoxytirucalla-7,24-dien-3-one) and even a closer one to hydroxyprieurone (Olugbade, 1991). Thus, the 12-acetyloxy and C-4 oxymethyl features of these two compounds appear conserved in **1**. However, the presence of an additional acetyloxy function and a hexose unit (^{13}C-NMR) accounts for the major difference. Peracetylation of the glycoside gave five additional acetyl groups (^{1}H-NMR and ^{13}C-NMR) consistent with the presence of a hexose sugar and an alcohol function in the natural glycoside. Although, C-21 and C-23 appear to be also oxygenated (^{13}C-NMR) as in earlier compounds (Olugbade, 1991), an ether linkage to give the tetrahydrofuran ring could not account for all the NMR features. A hypothetical biogenetic precursor with uncyclised C-21, C-23 dioxygenated side chain, similar to that of an epoxytriol from *Entandrophragma angolense* (Okorie and Taylor, 1977), is suggested. The CIMS gave an abundant quasi-molecular ion at m/z 752 $[M+NH_4]^+$ (63) consistent with the formula $C_{40}H_{62}O_{12}$ for the glycoside. The peracetate (**2**) gave the ion at m/z 902 $[M+NH_4\text{-}60]^+$ as base peak. The significant downfield shift of H-23 ($\Delta\delta$1.2) after peracetylation located the free OH of the original glycoside at C-23. Consistent with this was the observation of a correlation (HMBC) between C-25 and H-23. It does remain to locate the positions of glycosidation and the extra acetyl group between C-28 (or C-29) and C-21 of the glycoside. The facile loss of two acetic acid molecules in the CIMS and HMBC correlation suggest that the acetyloxy function is on C-21. The position of glycosidation and the relative stereochemistry at C-4 and C-12 were established by 2D-NOESY experiments (MeOD, 400 MHz). Cross connectivity between H-12 (δ4.75) and Me-18 (δ0.88) enabled the stereochemistry of the former as α. The

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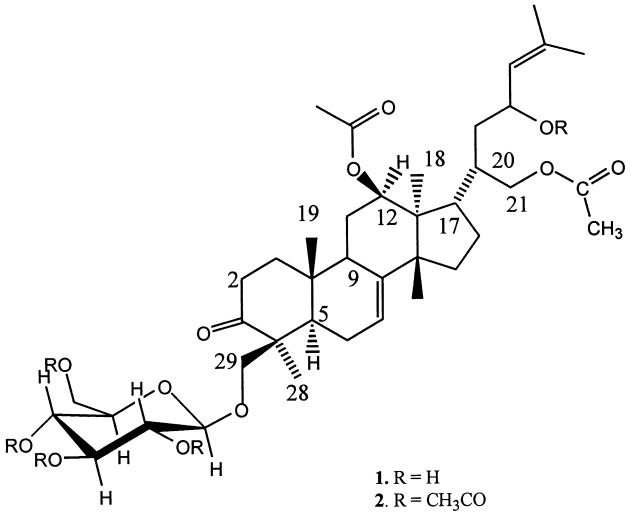

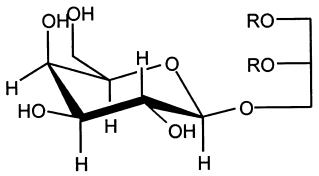

stereochemistry at C-4 was apparent from the observation that H-2β (δ2.92, *m*) correlated to both Me-19 (δ1.10) and H-29a (δ4.08, *d*, part of an ABq, $J = 9.5$ Hz). Correlation between glucose H-1′ (δ4.18, *d*, $J = 7.3$ Hz) and H-29b (δ3.96) confirm the position of glycosidation as C-29. Further correlations include H-24 (δ5.10, *d*, $J = 7.3$ Hz)/Me-26 (δ1.74) and H-23 (δ4.44, *m*)/Me-27 (δ1.70).

The protons of the sugar portion of the peracetate **2** were well resolved at 360 MHz to establish the natural compound **1** as a β-glucoside. The aqueous portion of the product of acid hydrolysis also indicated glucose (TLC). To the best of our knowledge, prieurianoside (**1**) is the first protolimonoid glycoside to be reported from Meliaceae.

Neither **1** nor **2** demonstrated any appreciable cytotoxicity against KB human buccal carcinoma cells ($ED_{50} > 10$ μg/ml) (Williams et al., 1983).

The ^{13}C-NMR of **3** revealed three oxymethylene carbon signals (δ68.2, 62.9, 62.1) and six oxymethine carbon signals (δ104.1, 74.6, 73.5, 71.4, 70.3, 69.3) ascribable to one hexose unit and a glyceryl residue. The NMR also revealed diacylation (carbon signals at δ173.5, 173.9) and polyunsaturation (multiple vinyl protons at δ5.35 and CH carbon signals at δ127.2, 127.9, 128.3, 128.4, 130.3, 132.0). One methyl signal (δ0.95, *t*, ^{1}H-NMR) (δ14.3, ^{13}C-NMR) and multiple methylene signals characteristic of polyunsaturated fatty acyl units (Murakami et al., 1991; Arai et al., 1991) were observed. Acid hydrolysis revealed galactose as the only sugar in the aqueous portion (TLC) and essentially linolenic acid in the organic phase (GCMS). The monogalactosyldiglyceride was thus identified as the ubiquitous 1,2-dilinolenoyl-3-galactopyranosylglycerol (**3**) (Murakami et al., 1991).

3. Experimental

3.1. Extraction and isolation

Dried plant leaves (1.2 kg) obtained from the previous source (Olugbade, 1991) were extracted with chloroform in a Soxhlet apparatus. The resulting extract (114 g) was chromatographed on charcoal (400 g) eluting successively with *n*-hexane, *n*-hexane-EtOAc (8:2). *n*-hexane-EtOAc (1:1), EtOAc and methanol to give a combined *n*-hexane-EtOAc fraction (210 g) and methanol fraction (6.6 g). A portion of the methanol fraction (4.0 g) was then subjected to CC over silica gel eluting with EtOAc–MeOH in increasing polarity. The EtOAc–MeOH (95:5) eluent, after purification by preparation. Preparative TLC (Silica gel, CH_2Cl_2–MeOH, 8:2), gave the glycolipid **3** as a slightly tinted grease-like material (100 mg); light brown with 10% H_2SO_4 after heating; rf 0.4 on silica gel TLC, EtOAc–MeOH (9:1) (system A). The EtOAc:MeOH (9:1) column eluent gave prieurianoside (**1**).

3.1.1. Prieurianoside (*1*)

(1.5 g), microcrystals; mp 113–115°; red to purple with H_2SO_4 after heating, rf 0.2 (system A); $[\alpha]_D^{20}$: -73.8 (MeOH); IR ν_{max}^{KBr} cm^{-1}:3400 (*br*) and 1710–1750 (*br*); DCIMS (NH_3) *m*/*z* (relative intensity): 752 [M + NH_4]+ (63), 734 [M + NH_4–H_2O]+ (32), 692 [M + NH_4-60]+ (58), 572 [M + H-163]+ (26) 495 [M + NH_4-179-60-H_2O]+ (56), 453 [M + NH_4-179-60-60]+ (100), 435 [M + NH_4-179-60-60-H_2O]+ (82). NMR data are provided in Table 1.

3.1.2. Acetylation of *1*

1 (170 mg) was stirred in a 1:1 mixture (6 ml) of acetic anhydride and pyridine overnight. After the usual work-up, a whitish solid which gels on cooling from hot diisopropyl ether was obtained. Alternative purification by precipitation with addition of *n*-hexane to a solution in diethyl ether gave an amorphous white solid (130 mg) **2**; mp 110–111°; DCIMS (NH_3) *m*/*z* (relative intensity): 902 [M + NH_4-60] (100), 331 [tetra-acetylhexosyl]+ (40). NMR data of the peracetate **2** are provided in Table 1.

3.1.3. Acid hydrolysis of *1* and *3*

The glycoside **1** (100 mg) or glycolipid **3** (40 mg)

Table 1
^{13}C- NMR and correlated ^{1}H-NMR ($CDCl_3$) data of **1** and **2** coupling constants (Hz) provided in parentheses where discernible

Position	Carbon correlated proton				
	1			**2**	
	Carbon	Proton (HMQC[a])	HMBC	Carbon	Proton
1	38.9	1.4, 2.1	Me-19β	38.5	1.45; 1.95
2	35.8	2.76 *m*; 2.2		35.5	2.65, *ddd* (14, 14, 6); 2.2
3	215.9	–	Me-28α, Ha-29β	212.9	–
4	52.7	–	Me-28α	52.4	–
5	53.9	1.7	Me-19β	53.5	1.7
6	25.0	2.0, 2.2		25.1	2.1
7	119.3	5.34 *br.s*		119.5	5.35 *br.s*
8	144.6	–	Me-30β	144.3	–
9	47.5	2.25	Me-19β	47.3	2.25
10	35.2	–	Me-19β	35.0	–
11	27.9	2.45, 3		27.9	2.45 *m*; 1.3
12	76.3	4.83, *br.t*	MeCO	76.3	4.81, *dd* (9.3, 6.7)
13	47.0	–	Me-18α, Me-30β	47.0	–
14	51.3	–	Me-30β, Me-18α, H-12α	51.4	–
15	34.6	1.5		34.5	1.5
16	27.2	1.35, 1.5		27.3	2.05; 1.3
17	41.6	2.3	H-12α	41.0	2.2
18	20.3	0.8		20.1	0.82
19	13.8	1.0		13.6	0.96
20	37.0	1.7		36.7	1.7
21	67.2	3.70, *dd* (8.3, 11.2); 4.0	MeCO	66.4	3.73; 3.99 *m*
22	39.1	1.5		36.0	1.7
23	67.6	4.48,m		70.1	5.56, *m*
24	128.4	5.15, *br.d*		124.0	5.07, *br.d*
25	135.4	–	H-23	137.8	–
26	25.8	1.74	H-24	25.8	1.71[b]
27	18.3	1.70	H-24	18.5	1.71[b]
28	20.3	1.13		20.3	1.07
29	72.9	3.85, 4.0		72.1	3.83, 3.95, ABq (9.7)
30	28.4	1.10		28.4	1.07
1′	104.0	4.17, *d* (8.3)		100.8	4.41, *d* (8.0)
2′	73.2	3.20		70.7	4.94, *dd* (9.5, 8.0)
3′[c]	76.3	3.35		72.7	5.16, *dd* (9.5, 9.5)
4′[c]	69.6	3.4		68.5	5.08, *dd* (9.5, 9.5)
5′	75.7	3.2		71.9	3.66, *m*
6′	61.7	3.75		61.9	4.13, *dd* (12.3, 2.);4.26, *dd* (12.3, 4.4)
CH_3–C=O	21.1, 21.5	2.05, 2.09		21.4 × 2, 21.0, 20.8, 20.6 × 3	1.97, 1.98, 1.99, 2.01, 2.04, 2.05, 2.06
CH_3–C=O	169.9, 170.9	–		170.8, 170.6, 170.2, 170.1, 169.9, 169.4, 169.3	–

[a] Assignment of proton signals reinforced by COSY experiment.
[b] Resolved of δ1.60 and δ1.70 in dilute solution(10 mg/ml at 360 MHz).
[c] Signals interchangeable.

was refluxed in a 1:1 mixture of dioxane and 2N HCI (5 ml) for 1.5 h. The reaction mixture was evaporated to complete dryness and then partitioned between water (2 ml) and CH_2Cl_2 (10 ml). The aqueous portion, in either case, was analysed for sugars on silica gel TLC (Arai et al., 1991) using EtOAc–H_2O–MeOH–HOAc (65:15:15:25) as solvent system and aniline hydrogen phthalate as detecting reagent to reveal glucose and galactose for the glycoside **1** and glycolipid **3**, respectively. The organic phase of the hydrolysate from the glycoside indicated a multicomponent mixture on TLC and was not investigated further. The organic extract of the hydrolysate from the glycolipid was methylated with methanolic HCI and analysed by GCMS, comparing with computer MS library and GCMS of an authentic commercial fatty acid methyl ester mixture, both indicating methyl linolenate as the essential component of the methylated hydrolysate.

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