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Stryphnosides A–F, six new triterpene glycosides from the pericarps of *Stryphnodendron fissuratum*

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Abstract

Six new triterpene glycosides stryphnosides A–F (**1**–**6**) were isolated from the pericarps of *Stryphnodendron fissuratum* (Leguminosae). The structures of **1**–**6** were determined on the basis of extensive spectroscopic analysis, including two-dimensional (2D) NMR data, and the results of hydrolytic cleavage. The sugar moieties of **3**–**6** are very unique in structure having not only novel sugar sequences but also the terminal α-L-arabinopyranosyl unit with a $^1C_4$ conformation. Stryphnosides C (**3**)–F (**6**) are the first representative of naturally occurring glycosides with the $^1C_4$ terminal α-L-arabinopyranosyl group.

1. Introduction

Previously, we have carried out phytochemical examinations of plants native to Brazil, which have resulted in the isolation of triterpene saponins with selective cytotoxic activity against BAC1.2F5 mouse macrophages from *Enterolobium contortisiliquum*, and those with cytotoxic activity against HSC-2 human oral squamous cell carcinoma cells from *Gomphrena macrocephala*.[1–3] The genus *Stryphnodendron* (Leguminosae), with about 25 species, is mainly distributed in the tropical region of the continent of South America.[4] The barks of some *Stryphnodendron* species such as *Stryphnodendron adstringens*, *Stryphnodendron coriaceum*, and *Stryphnodendron polyphyllum* are rich in tannins, and have been used as medicinal plants for the treatment of hemorrhages, wounds, and diarrhea in Brazil.[4–6] An extract of the stem barks of *S. adstringens* was reported to show acute oral toxicity in mice.[7] In our continuing phytochemical studies on Brazilian plants, we have now investigated the triterpene constituents of *Stryphnodendron fissuratum* Mart., whose fruits are suggested to cause bovine death.[8] This has resulted in the isolation of six new triterpene glycosides on the basis of the oleanane skeleton, named stryphnosides A (**1**)–F (**6**). This paper deals with the structural determination of the six new glycosides on the basis of spectroscopic analysis, including various 2D NMR spectroscopic techniques, and of the results of hydrolytic cleavage.

2. Results and discussion

The concentrated *n*-BuOH-soluble phase of the EtOH extract of *S. fissuratum* pericarps was subjected to Diaion HP-20, silica gel, and ODS silica gel column chromatography to yield **1** (10.2 mg), **2** (83.6 mg), **3** (32.5 mg), **4** (59.9 mg), **5** (79.1 mg), and **6** (17.8 mg).

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{25}$ −56.3 (MeOH). The HRESITOFMS of **1** showed an accurate $[M+H]^+$ ion peak at *m/z* 1045.5618 in accordance with the empirical molecular formula of $C_{52}H_{84}O_{21}$, which was supported by the ^{13}C NMR spectrum showing a total of 52 signals and various DEPT data (C×8, CH×24, CH_2×12, and CH_3×8). The IR spectrum of **1** showed a broad absorption band for hydroxy groups at 3388 cm^{-1}, as well as strong absorption due to a carbonyl group at 1736 cm^{-1}. The ^1H

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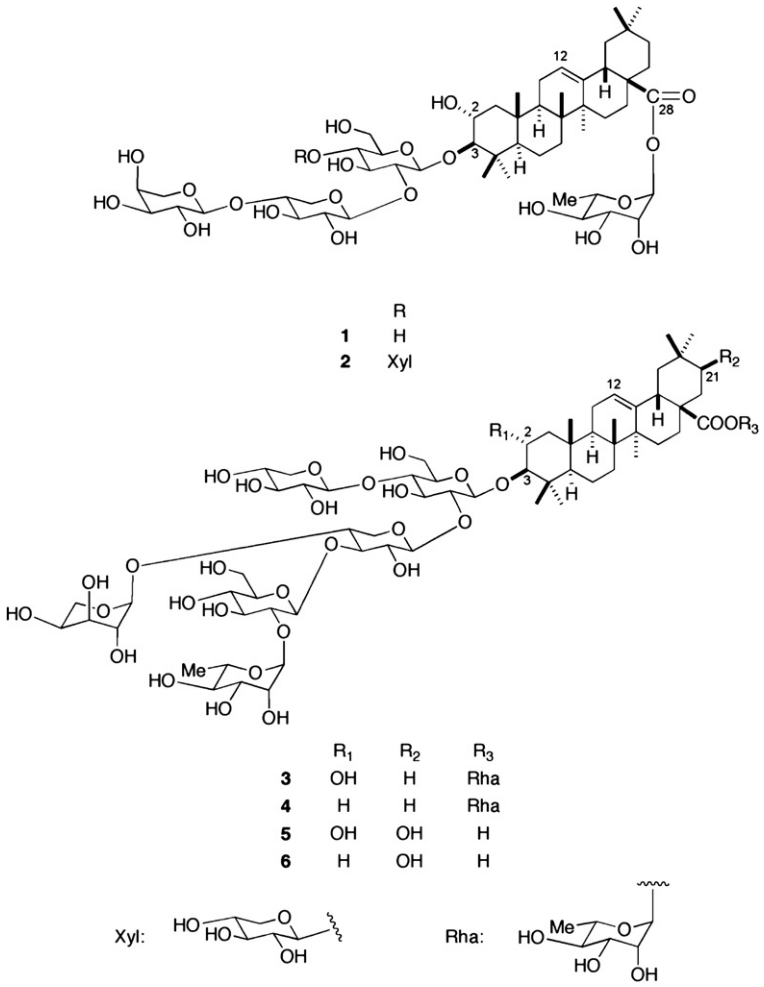

NMR spectrum of **1** measured in CD_3OD showed signals for seven tertiary methyl groups at δ 1.17, 1.11, 1.01, 0.96, 0.92, 0.89, and 0.79 (each s), and a trisubstituted olefinic proton at δ 5.33 (br s), which are typical of the oleanolic acid skeleton. In addition, signals for four anomeric protons were observed at δ 5.92 (d, J=1.9 Hz), 4.63 (d, J=7.6 Hz), 4.42 (d, J=7.5 Hz), and 4.27 (d, J=7.0 Hz). The three-proton doublet signal at δ 1.23 (J=6.1 Hz) indicated the presence of one deoxyhexopyranosyl unit in **1**. Acid hydrolysis of **1** with 1.0 M HCl in dioxane–H_2O (1:1) resulted in the production of an aglycone (**1a**), identified as 2α,3β-dihydroxyolean-12-en-28-oic acid,[9] as well as L-rhamnose, L-arabinose, D-xylose, and D-glucose as the carbohydrate components. The monosaccharides were identified by direct HPLC analysis of the hydrolyzate, which was performed on an aminopropyl bonded silica gel column using MeCN–H_2O (17:3) as a solvent system. Detection was carried out using a combination of refractive index (RI) and optical rotation (OR). In the ^{13}C NMR spectrum of **1**, the C-3 and C-28 carbons of the aglycone moiety were observed at δ 96.4 and 177.1, respectively, which suggested that **1** is a 3,28-bisdesmoside. The ^1H–^1H COSY experiment with **1** allowed the sequential assignments from H-1 to H_2-6, H_2-5, and Me-6 of the monosaccharides. Their signal multiplet patterns and coupling constants (Table 1) indicated the presence of an α-L-arabinopyranosyl ($^4C_1$) unit (Ara), a β-D-glucopyranosyl ($^4C_1$) unit (Glc), an α-L-rhamnopyranosyl ($^1C_4$) unit (Rha), and a β-D-xylopyranosyl ($^4C_1$) unit (Xyl). The proton resonances were correlated with those of the one-bond coupled carbons using the HMQC spectrum. Comparison of the carbon chemical shifts thus assigned with those of the reference methyl glycosides suggested that the Ara and Rha groups are presented as the terminal units, whereas the Glc and Xyl groups are substituted at C-2 and C-4, respectively.[10,11] The anomeric conformation of the Ara, Glc, and Xyl groups was ascertained by the relatively large J values of their anomeric protons (7.0–7.6 Hz).[12] For the Rha moiety, the large $^1J_{C,H}$ value (172.7 Hz) indicated that the anomeric proton was equatorial thus possessing an α-pyranoid anomeric form.[13] In the HMBC spectrum of **1**, long-range correlations were observed between δ 5.92 (H-1 of Rha) and 177.1 (C-28 of aglycone), 4.63 (H-1 of Xyl) and 82.4 (C-2 of Glc), 4.42 (H-1 of Glc) and 96.4 (C-3 of aglycone), and 4.27 (H-1 of Ara) and 78.0 (C-4 of Xyl). Accordingly, the structure of **1** was established as 3β-[(*O*-α-L-arabinopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-2α-hydroxyolean-12-en-28-oic acid α-L-rhamnopyranosyl ester.

Compound **2** was analyzed as $C_{57}H_{92}O_{25}$ on the basis of HRESITOFMS (m/z 1177.6044 $[M+H]^+$). The deduced

molecular formula was higher than that of **1** by $C_5H_8O_4$, corresponding to one pentose unit. The ^{1}H NMR spectrum of **2** contained signals for five anomeric protons at δ 5.93 (br s), 4.69 (d, J=7.6 Hz), 4.46 (d, J=7.7 Hz), 4.34 (d, J=7.7 Hz), and 4.27 (d, J=7.0 Hz), as well as signals for seven tertiary methyl groups at δ 1.17, 1.11, 1.01, 0.96, 0.92, 0.89, and 0.79, and an olefinic proton at δ 5.33 (br s) derived from an oleanolic acid skeleton. Acid hydrolysis of **2** with 1 M HCl gave **1a**, L-rhamnose, L-arabinose, D-xylose, and D-glucose. On comparison of the ^{13}C NMR spectrum of **2** with that of **1**, a set of five additional signals corresponding to a terminal β-D-xylopyranosyl unit (Xyl′) could be observed at δ 105.3, 74.8, 77.8, 70.9, and 67.1, and the signal due to C-4 of the Glc moiety and its neighboring carbons varied, while all other signals remained almost unaffected. In the HMBC spectrum, the anomeric proton of the Xyl′ at δ 4.34 showed a correlation peak with C-4 of Glc at δ 80.0, of which the anomeric proton at δ 4.46 exhibited a correlation with C-3 of the aglycone at δ 96.6, giving evidence for a linkage of the Xyl′ group to C-4 of the Glc unit. HMBC correlations between δ 5.93 (H-1 of Rha) and 177.1 (C-28 of aglycone), 4.69 (H-1 of Xyl) and 81.3 (C-2 of Glc), and 4.27 (H-1 of Ara) and 78.0 (C-4 of Xyl) were also noted.

Table 1
^{1}H NMR chemical shift assignments for sugar moieties of compounds **1**–**6** in CD_3OD[a]

Position			**1**		Position			**2**		Position			**3**	
Glc	1		4.42 d (7.5)	104.7	Glc	1		4.46 d (7.7)	104.7	Glc	1		4.44 d (7.7)	104.7
	2		3.53 dd (8.9, 7.5)	82.4		2		3.63 dd (9.0, 7.7)	81.3		2		3.62 dd (9.0, 7.7)	81.3
	3		3.57 dd (8.9, 8.9)	78.5		3		3.75 dd (9.0, 9.0)	76.7		3		3.73 dd (9.0, 9.0)	76.8
	4		3.37 dd (8.9, 8.9)	71.0		4		3.61 dd (9.0, 9.0)	80.0		4		3.60 dd (9.0, 9.0)	80.3
	5		3.32 m	78.0		5		3.49 m	76.4		5		3.48 m	76.4
	6	a	3.84 dd (11.8, 1.9)	62.3		6		3.86 m (2H)	61.4		6		3.85 m (2H)	61.4
		b	3.65 dd (11.8,3.5)											
Xyl	1		4.63 d (7.6)	105.8	Xyl	1		4.69 d (7.6)	105.4	Xyl	1		4.68 d (7.6)	105.7
	2		3.27 dd (9.0, 7.6)	75.8		2		3.26 dd (9.1, 7.6)	75.8		2		3.48 dd (9.1, 7.6)	76.5
	3		3.46 dd (9.0, 9.0)	76.1		3		3.47 dd (9.1, 9.1)	76.1		3		3.80 dd (9.1, 9.1)	79.9
	4		3.62 ddd (10.6, 9.0, 5.3)	78.0		4		3.62 ddd (10.4, 9.1, 4.9)	78.0		4		3.79 ddd (10.1, 9.1, 4.7)	71.8
	5	a	3.96 dd (11.7, 5.3)	64.7		5	a	3.96 dd (11.2, 4.9)	64.7		5	a	4.01 dd (11.7, 4.7)	64.3
		b	3.24 dd (11.7, 10.6)				b	3.24 dd (11.2, 10.4)				b	3.20 dd (11.7, 10.1)	
Ara	1		4.27 d (7.0)	103.8	Ara	1		4.27 d (7.0)	103.8	Glc′	1		4.90 d (7.3)	102.6
	2		3.55 dd (9.1, 7.0)	72.0		2		3.57 dd (9.2, 7.0)	72.0		2		3.42 dd (9.1, 7.3)	80.4
	3		3.46 dd (9.1, 3.4)	74.1		3		3.51 dd (9.2, 3.4)	74.1		3		3.44 dd (9.1, 9.1)	79.1
	4		3.75 m	69.7		4		3.80 m	69.7		4		3.47 dd (9.1, 9.1)	70.8
	5	a	3.89 dd (12.5, 2.8)	67.3		5	a	3.90 dd (12.6, 2.6)	67.3		5		3.20 m	78.1
		b	3.57 dd (12.5, 4.1)				b	3.59 dd (12.6, 4.2)			6	a	3.82 br d (12.3)	62.1
												b	3.73 br d (12.3)	
Rha	1		5.92 d (1.9)	95.0	Xyl′	1		4.34 d (7.7)	105.3	Rha	1		5.12 br s	102.7
	2		3.75 dd (3.3, 1.9)	71.4		2		3.20 dd (9.2, 7.7)	74.8		2		3.95 br d (3.9)	72.2
	3		3.67 dd (9.5, 3.3)	72.5		3		3.31 dd (9.2, 9.2)	77.8		3		3.72 dd (9.5, 3.9)	72.3
	4		3.44 dd (9.5, 9.5)	73.4		4		3.49 ddd (10.3, 9.2, 5.3)	70.9		4		3.40 dd (9.5, 9.5)	74.0
	5		3.66 dq (9.5, 6.1)	72.5		5	a	3.90 dd (11.3, 5.3)	67.1		5		4.09 dq (9.5, 6.2)	69.7
	6		1.23 d (6.1)	18.2			b	3.23 dd (11.3, 10.3)			6		1.27 d (6.2)	18.1
					Rha	1		5.93 br s	95.0	Ara	1		4.63 d (2.9)	99.5
						2		3.75 br d (3.3)	71.4		2		3.77 dd (4.0, 2.9)	70.7
						3		3.67 dd (9.5, 3.3)	72.5		3		3.69 dd (4.0, 4.0)	73.0
						4		3.44 dd (9.5, 9.5)	73.4		4		3.93 ddd (10.2, 4.0, 3.0)	66.0
						5		3.68 dq (9.5, 6.1)	72.5		5	a	4.09 dd (12.1, 10.2)	62.3
						6		1.23 d (6.1)	18.2			b	3.50 dd (12.1, 3.0)	
										Xyl′	1		4.34 d (7.7)	105.4
											2		3.19 dd (9.1, 7.7)	74.9
											3		3.31 dd (9.1, 9.1)	77.9
											4		3.50 ddd (10.2, 9.1, 4.8)	71.0
											5	a	3.93 dd (11.4, 4.8)	67.1
												b	3.25 dd (11.4, 10.2)	
										Rha′	1		5.92 br s	95.0
											2		3.75 br d (4.2)	71.4
											3		3.67 dd (9.1, 4.2)	72.3
											4		3.44 dd (9.1, 9.1)	73.4
											5		3.66 dq (9.1, 6.1)	72.5
											6		1.23 d (6.1)	18.2

(*continued on next page*)

Table 1. (*continued*)

Position			**4**		Position			**5**		Position			**6**	
Glc	1		4.42 d (7.7)	105.2	Glc	1		4.45 d (7.7)	104.7	Glc	1		4.42 d (7.7)	105.2
	2		3.49 dd (9.2, 7.7)	82.5		2		3.63 dd (9.0, 7.7)	81.3		2		3.50 dd (9.1, 7.7)	82.5
	3		3.67 dd (9.2, 9.2)	76.6		3		3.74 dd (9.0, 9.0)	76.7		3		3.67 dd (9.1, 9.1)	76.6
	4		3.54 dd (9.2, 9.2)	80.8		4		3.62 dd (9.0, 9.0)	80.2		4		3.54 dd (9.1, 9.1)	80.8
	5		3.37 m	75.9		5		3.49 m	76.3		5		3.36 m	75.9
	6		3.83 m (2H)	61.9		6		3.87 m (2H)	61.3		6		3.84 m (2H)	61.9
Xyl	1		4.57 d (7.6)	106.2	Xyl	1		4.68 d (7.6)	105.7	Xyl	1		4.57 d (7.5)	106.2
	2		3.49 dd (8.9, 7.6)	76.9		2		3.49 dd (9.2, 7.6)	76.5		2		3.50 dd (9.1, 7.5)	76.9
	3		3.78 dd (9.2, 9.2)	80.0		3		3.81 dd (9.2, 9.2)	79.9		3		3.78 dd (9.1, 9.1)	79.9
	4		3.77 ddd (10.2, 9.2, 3.4)	71.8		4		3.79 ddd (10.1, 9.2, 4.7)	71.8		4		3.77 ddd (10.1, 9.1, 4.7)	71.8
	5	a	4.00 dd (11.5, 3.4)	64.5		5	a	4.02 dd (11.5, 4.7)	64.3		5	a	4.00 dd (11.7, 4.7)	64.5
		b	3.20 dd (11.5, 10.2)				b	3.21 dd (11.5, 10.1)				b	3.19 dd (11.7, 10.1)	
Glc′	1		4.90 d (7.3)	102.7	Glc′	1		4.91 d (7.4)	102.6	Glc′	1		4.89 d (7.6)	102.7
	2		3.41 dd (9.1, 7.3)	80.0		2		3.42 dd (9.0, 7.4)	80.3		2		3.41 dd (8.8, 7.6)	80.0
	3		3.44 dd (9.1, 9.1)	79.1		3		3.46 dd (9.0, 9.0)	79.1		3		3.44 dd (8.8, 8.8)	79.1
	4		3.48 dd (9.1, 9.1)	70.6		4		3.50 dd (9.0, 9.0)	70.7		4		3.48 dd (8.8, 8.8)	70.7
	5		3.20 m	78.0		5		3.22 m	78.0		5		3.19 m	78.1
	6	a	3.82 br d (12.7)	62.1		6	a	3.84 br d (11.8)	62.1		6	a	3.82 br d (12.2)	62.1
		b	3.72 br d (12.7)				b	3.74 br d (11.8)				b	3.71 br d (12.2)	
Rha	1		5.15 br s	102.5	Rha	1		5.12 br s	102.7	Rha	1		5.14 br s	102.6
	2		3.95 br d (3.8)	72.2		2		3.97 br d (3.4)	72.1		2		3.94 br d (3.3)	72.2
	3		3.72 dd (9.4, 3.8)	72.3		3		3.73 dd (9.5, 3.4)	72.3		3		3.72 dd (9.5, 3.3)	72.3
	4		3.39 dd (9.4, 9.4)	74.0		4		3.40 dd (9.5, 9.5)	74.0		4		3.40 dd (9.5, 9.5)	73.9
	5		4.13 dq (9.4, 6.2)	69.7		5		4.13 dq (9.5, 6.2)	69.7		5		4.11 dq (9.5, 6.3)	69.7
	6		1.28 d (6.2)	18.1		6		1.28 d (6.2)	18.1		6		1.27 d (6.3)	18.1
Ara	1		4.63 d (3.1)	99.5	Ara	1		4.64 d (2.7)	99.4	Ara	1		4.62 d (3.1)	99.5
	2		3.76 dd (4.1, 3.1)	70.7		2		3.78 dd (4.2, 2.7)	70.7		2		3.75 dd (4.0, 3.1)	70.7
	3		3.69 dd (4.1, 4.1)	73.0		3		3.70 dd (4.2, 4.2)	73.0		3		3.69 dd (4.2, 4.0)	73.0
	4		3.92 ddd (8.1, 4.1, 2.9)	66.0		4		3.93 ddd (10.4, 4.2, 3.3)	66.0		4		3.92 ddd (10.2, 4.1, 3.0)	66.0
	5	a	4.09 dd (11.9, 8.1)	62.2		5	a	4.10 dd (12.2, 10.4)	62.1		5	a	4.09 dd (11.5, 8.6)	62.2
		b	3.50 dd (11.9, 2.9)				b	3.51 dd (12.2, 3.3)				b	3.50 dd (11.5, 3.0)	
Xyl′	1		4.34 d (7.7)	105.4	Xyl′	1		4.34 d (7.7)	105.4	Xyl′	1		4.34 d (7.7)	105.4
	2		3.19 dd (9.0, 7.7)	74.9		2		3.21 dd (9.0, 7.7)	74.9		2		3.19 dd (9.0, 7.7)	75.0
	3		3.31 dd (9.0, 9.0)	77.9		3		3.33 dd (9.4, 9.0)	77.9		3		3.31 dd (9.0, 9.0)	77.9
	4		3.50 ddd (10.8, 9.0, 3.8)	71.0		4		3.52 ddd (10.8, 9.4, 4.0)	70.9		4		3.49 ddd (10.8, 9.0, 3.7)	71.0
	5	a	3.93 dd (11.3, 3.8)	67.1		5	a	3.94 dd (11.0, 4.0)	67.1		5	a	3.92 dd (11.2, 3.7)	67.1
		b	3.25 dd (11.3, 10.8)				b	3.26 dd (11.0, 11.0)				b	3.25 dd (11.2, 10.8)	
Rha′	1		5.93 br s	94.9										
	2		3.76 br d (3.0)	71.4										
	3		3.67 dd (9.4, 3.0)	72.4										
	4		3.44 dd (9.4, 9.4)	73.4										
	5		3.67 dq (9.4, 6.2)	72.4										
	6		1.23 d (6.2)	18.2										

[a] Values in parentheses are coupling constants in hertz.

Thus, the structure of **2** was characterized as 3β-[(*O*-α-L-arabinopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-2α-hydroxyolean-12-en-28-oic acid α-L-rhamnopyranosyl ester.

Compound **3** was obtained as an amorphous powder. The HRESITOFMS of **3** showed an $[M+H]^+$ peak at *m/z* 1485.7100, corresponding to the empirical molecular formula of $C_{69}H_{112}O_{34}$. The ^1H NMR spectrum of **3** showed signals for seven tertiary methyl groups at δ 1.16, 1.11, 1.01, 0.96, 0.92, 0.88, and 0.79 (each s), and an olefinic proton at δ 5.33 (br s), along with signals for seven anomeric protons at δ 5.92 (br s), 5.12 (br s), 4.90 (d, *J*=7.3 Hz), 4.68 (d, *J*=7.6 Hz), 4.63 (d, *J*=2.9 Hz), 4.44 (d, *J*=7.7 Hz), and 4.34 (d, *J*=7.7 Hz). Acid hydrolysis of **3** gave **1a**, L-rhamnose, L-arabinose, D-xylose, and D-glucose. The above data and the carbon chemical shifts of C-3 (δ 96.6) and C-28 (δ 177.2) suggested that **3** is a **1a** 3,28-bisdesmoside, whose sugar moieties were composed of a total of seven monosaccharides. The severe overlapping of the proton signals for the sugar moieties excluded the possibility of a complete assignment in a straightforward way using conventional 2D NMR methods, such as the $^1H-^1H$ COSY, 2D TOCSY, and HMQC spectra. Analysis of the 1D TOCSY spectra followed by interpretation of the $^1H-^1H$ COSY, HSQC-TOCSY, or HMQC spectra allowed the exact sugar sequences of the sugars to be determined. The ^1H NMR subspectra of the individual monosaccharide units were obtained by using selective irradiation of easily identifiable anomeric proton signals at δ 5.92, 5.12, 4.90, 4.68, 4.63, 4.44, and 4.34 as

well as irradiation of other non-overlapping proton signals at δ 1.27 and 1.23 in a series of 1D TOCSY experiments.[14,15] Subsequent analysis of the ^{1}H–^{1}H COSY spectrum resulted in the sequential assignments of all the proton resonances due to the seven monosaccharide units, including identification of their signal multiplet patterns and coupling constants (Table 1). The proton resonances were correlated with those of the corresponding one-bond coupled carbons using the HMQC and HSQC-TOCSY spectra, leading to unambiguous assignments of the carbon shifts. Comparison of the carbon chemical shifts thus assigned with those of the reference methyl glycosides, taking into account the known effects of the O-glycosylation shift, indicated that **1** contains a 2,4-disubstituted β-D-glucopyranosyl moiety (Glc), 3,4-disubstituted β-D-xylopyranosyl moiety (Xyl), 2-substituted β-D-glucopyranosyl moiety (Glc′), two terminal α-L-rhamnopyranosyl moieties (Rha, Rha′), a terminal α-L-arabinopyranosyl moiety (Ara), and a terminal β-D-xylopyranosyl moiety (Xyl′). The β-orientations of the anomeric centers of the Glc, Glc′, Xyl, Xyl′ moieties were supported by the relatively large $^3J_{H\text{-}1,H\text{-}2}$ values of their anomeric protons (7.3–7.7 Hz) and $^1J_{H\text{-}1,C\text{-}1}$ values (Glc: 158.2 Hz; Xyl: 159.9 Hz; Glc′: 159.9 Hz; Xyl′: 160.0 Hz). For the Rha and Rha′ moieties, the large $^1J_{C-H}$ values (Rha: 169.0 Hz; Rha′: 172.6 Hz) and three-bond coupled strong HMBC correlations from the anomeric protons to the C-3 and C-5 carbons (the dihedral angles between H-1 and C-3 and between H-1 and C-5 about 180°) indicated that each anomeic proton is equatorial thus possessing an α-pyranoid anomeric form. The proton chemical shifts and spin-coupling constants of the Ara moiety of **3** were different from those of **1** and **2**. The coupling constants assigned by the 1D-selective-TOCSY spectra, the large $^1J_{H\text{-}1,C\text{-}1}$ value (171.9 Hz), and three-bond coupled strong HMBC correlations from the anomeric proton to the C-3 and C-5 carbons indicated that the conformation of the Ara group is present as $^1C_4$ (Fig. 1) with an α-orientation of the anomeric center. In the HMBC spectrum, correlation peaks were observed from δ 5.92 (H-1 of Rha′) to 177.2 (C-28 of the aglycone), 5.12 (H-1 of Rha) to 80.4 (C-2 of Glc′), 4.90 (H-1 of Glc′) to 79.9 (C-3 of Xyl), 4.68 (H-1 of Xyl) to 81.3 (C-2 of Glc), 4.63 (H-1 of Ara) to 71.8 (C-4 of Xyl), 4.34 (H-1 of Xyl′) to 80.3 (C-4 of Glc), and 4.44 (H-1 of Glc) to 96.6 (C-3 of the aglycone) (Fig. 2). Accordingly, the structure of **3** was elucidated as 3β-[(*O*-α-L-arabinopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-2α-hydroxyolean-12-en-28-oic acid α-L-rhamnopyranosyl ester.

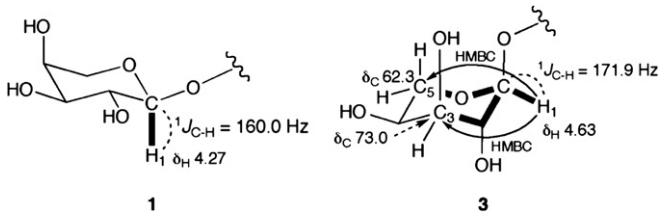

Figure 1. The $^1J_{C-H}$ values and HMBC correlations of the arabinosyl moieties of **1** and **3**

Compound **4** had the molecular formula $C_{69}H_{112}O_{33}$ on the basis of HRESITOFMS (*m/z* 1469.7120 $[M+H]^+$). Comparison of the ^{1}H and ^{13}C NMR spectra of **4** with those of **3** showed their considerable structural similarities. However, the molecular formula of **4** was lower by one oxygen atom than that of **3**, implying the lack of one hydroxy group. When the ^{13}C NMR spectrum of **4** was compared with that of **3**, the signal due to the C-2 hydroxymethine carbon, which was observed at δ 67.9 in **3**, was displaced by a methylene carbon signal at δ 27.1 in **4**. In addition, the carbon signals due to C-1 and C-3 were shifted upfield by 7.7 and 5.6 ppm, respectively. All other NMR signals of **4**, which were assigned by the 1D TOCSY, ^{1}H–^{1}H COSY, HMQC, and HSQC-TOCSY spectra, were very similar to those of **3**. Acid hydrolysis of **4** gave 3β-hydroxyolean-12-en-28-oic acid (**4a**),[16] L-rhamnose, L-arabinose, D-xylose, and D-glucose. Analysis of the HMBC spectrum of **4** indicated that the hexaglycoside attached to C-3 of the aglycone is the same as that of **3**, and that an α-L-rhamnopyranosyl group forms an ester linkage with the C-28 carboxyl group. On the basis of these data, the structure of **4** was determined to be 3β-[(*O*-α-L-arabinopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]olean-12-en-28-oic acid α-L-rhamnopyranosyl ester.

Compound **5** was obtained as an amorphous powder and its molecular formula $C_{63}H_{102}O_{31}$ was deduced from HRESITOFMS (*m/z* 1355.6528 $[M+H]^+$). The ^{1}H NMR spectrum of **5** showed six anomeric proton signals at δ 5.12 (br s), 4.91 (d, *J*=7.4 Hz), 4.68 (d, *J*=7.6 Hz), 4.64 (d, *J*=2.7 Hz), 4.45 (d, *J*=7.7 Hz), and 4.34 (d, *J*=7.7 Hz) and seven triterpene methyl signals at δ 1.15, 1.12, 1.01, 0.95, 0.90, 0.89, and 0.81 (each s). The C-28 carbon signal was observed at δ 180.6 in the ^{13}C NMR spectrum of **5**, which suggested that the C-28 carboxyl group has no sugar unit. Comparison of the ^{1}H and ^{13}C NMR assignments of **5**, which were established by analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra, with those of **3** revealed that the structure of the ring A–D portion of the aglycone and the hexaglycoside moiety attatched to C-3 of the aglycone was identical to that of **3**. However, the ^{1}H and ^{13}C NMR signals assignable to the ring E part of **5** were different from those of **3**, and suggested the presence of a hydroxy group at C-21 (δ_H 3.47; δ_C 73.8). The stereostructure of the C-21 hydroxy group was determined to be β on the basis of its relative proton spin-coupling constants (*J*=12.0, 5.5 Hz) and NOE correlations between H-21 and H-16α (δ 1.98)/H-19α (δ 1.79). Acid hydrolysis of **5** with 1.0 M HCl gave 2α,3β,21β-trihydroxyolean-12-en-28-oic acid (**5a**),[17] L-rhamnose, L-arabinose, D-xylose, and D-glucose. The hexaglycoside attached to C-3 of the aglycone was confirmed to be the same as that of **3** by analysis of the HMBC spectrum of **5**. Thus, the structure of **5** was determined to be 3β-[(*O*-α-L-arabinopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-2α,21β-dihydroxyolean-12-en-28-oic acid.

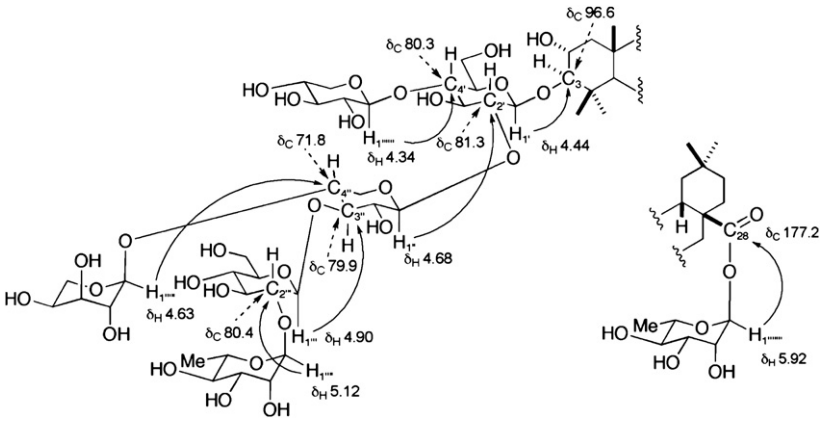

Figure 2. HMBC correlations of the sugar moieties of **3**

Compound **6** was assigned the molecular formula $C_{63}H_{102}O_{30}$ using HRESITOFMS. The molecular formula of **6** was lower by one oxygen atom than that of **5**. The ^1H and ^{13}C NMR spectra of **6** were almost superimposable with those of **5** except for the signals due to the ring A portion. The lack of the 2α-hydroxy group in **6** was shown by comparison of the ^1H and ^{13}C NMR spectra of **6** with those of **5**; the C-2 hydroxymethine carbon signal at δ 68.0 in **5** was displaced by the methylene carbon signal at δ 27.1 in **6**, and the carbon signals due to C-1 and C-3 were shifted upfield by 7.6 and 5.5 ppm, respectively, in **6**. Acid hydrolysis of **6** with 1.0 M HCl gave 3β,21β-dihydroxyolean-12-en-28-oic acid (**6a**),[18] L-rhamnose, L-arabinose, D-xylose, and D-glucose. The hexaglycoside attached to C-3 of the aglycone was confirmed to be the same as that of **5** by analysis of the HMBC spectrum of **6**. Thus, the structure of **6** was formulated as 3β-[(*O*-α-L-arabinopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-21β-hydroxyolean-12-en-28-oic acid.

Compounds **1**–**6** are new triterpene glycosides with up to seven monosaccharides. α-L-Arabinopyranosyl groups are stable in a $^4C_1$ conformation in glycosides. However, the arabinopyranosyl group is present as a $^1C_4$ conformation when its C-2 position is substituted by the $^4C_1$ glycosyl group such as D-glucose, D-galactose, and D-xylose.[19,20] To the best of our knowledge, **3**–**6** are the first representatives of naturally occurring glycosides having a terminal arabinosyl group with a $^1C_4$ conformation. This phenomenon is assumed to be due to the steric hindrance between the terminal arabinosyl group and the bulky rhamnosyl-(1→2)-glucosyl group attached to C-3 of the inner xylosyl group.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^1H NMR, Karlsruhe, Germany) and a Bruker DRX-600 (600 MHz for ^1H NMR) spectrophotometers using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HRESITOFMS were recorded on a Micromass LCT mass spectrometer (Manchester, U.K.). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP_{18} F_{254} S plates (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed by using a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a PX-8010 controller (Tosoh), a RI-8010 (Tosoh) or a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port. A Capcell Pak C_{18} UG80 column (10 mm i.d.×250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC.

3.2. Plant material

The pericarps of *S. fissuratum* Mart. were collected in the fields of Água Boa ward, Mato Grosso State, Brazil, in May and June 2003. The plant was identified by Dr. Heleno Dias Ferreira (Department of Morphology, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiás State, Brazil). A voucher specimen has been deposited in Instituto Biológico with the number 26796.

3.3. Extraction and isolation

The pericarps of *S. fissuratum* (2.0 kg) were macerated and extracted with EtOH, and the EtOH extract (353 g) was partitioned between *n*-BuOH and H_2O. The *n*-BuOH soluble portion was concentrated under reduced pressure, and the viscous concentrate (70 g) was passed through a Diaion HP-20 column and successively eluted with 20% MeOH, 40% MeOH, 80% MeOH, MeOH, and EtOAc (each 5 L). The 80% MeOH eluate portion (21.1 g) was chromatographed on silica gel (58 mm i.d.×380 mm) eluted with a stepwise

gradient mixture of $CHCl_3$–MeOH–H_2O (30:10:1; 20:10:1; 10:10:1) and finally with MeOH, giving seven fractions (A–H). Fraction D was subjected to column chromatography on ODS silica gel (47 mm i.d.×290 mm) eluted with MeOH–H_2O (2:1) and MeCN–H_2O (1:2), and silica gel (30 mm i.d.×160 mm) with $CHCl_3$–MeOH–H_2O (20:10:1) to give **1** (10.2 mg). Fraction F was subjected to column chromatography on ODS silica gel (45 mm i.d.×290 mm) eluted with MeOH–H_2O (2:1) and MeCN–H_2O (1:2), and silica gel (42 mm i.d.×210 mm) with $CHCl_3$–MeOH–H_2O (20:10:1) to give **2** (83.6 mg) and **6** (17.8 mg). Fraction K was subjected to column chromatography on ODS silica gel (58 mm i.d.× 230 mm) eluted with MeOH–H_2O (2:1) and MeCN–H_2O (1:2), and silica gel (22 mm i.d.×260 mm) with $CHCl_3$–MeOH–H_2O (7:4:1) to give **3** (32.5 mg), **4** (59.9 mg), and **5** (79.1 mg).

3.3.1. Compound *1*

Amorphous powder; $[\alpha]_D^{25}$ −56.3 (*c* 0.10, MeOH); IR (film) ν_{max} 3388 (OH), 2926 (CH), 1736 (C=O) cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.33 (1H, br s, H-12), 3.72 (1H, ddd, *J*=12.7, 9.4, 4.5 Hz, H-2), 2.97 (1H, d, *J*=9.4 Hz, H-3), 1.17 (3H, s, Me-27), 1.11 (3H, s, Me-23), 1.01 (3H, s, Me-25), 0.96 (3H, s, Me-30), 0.92 (3H, s, Me-29), 0.89 (3H, s, Me-24), 0.79 (3H, s, Me-26); ^{13}C NMR (125 MHz, CD_3OD): see Table 2; signals for sugar moieties: see Table 1; HRESITOFMS *m/z*: 1045.5618 $[M+H]^+$ (calcd for $C_{52}H_{85}O_{21}$: 1045.5642).

Table 2
^{13}C NMR chemical shift assignments for compounds **1**–**6** in CD_3OD

Position	1	2	3	4	5	6
1	47.5	47.5	47.5	39.8	47.5	39.9
2	67.9	67.9	67.9	27.1	68.0	27.1
3	96.4	96.6	96.6	91.0	96.6	91.1
4	41.7	41.7	41.7	40.3	41.7	40.3
5	56.8	56.8	56.8	57.1	56.8	57.1
6	19.3	19.3	19.3	19.3	19.3	19.3
7	34.0	34.0	34.0	34.1	33.9	34.1
8	40.7	40.7	40.7	40.7	40.5	40.6
9	49.2	49.2	48.8	49.0	49.0	49.0
10	38.8	38.8	38.8	37.9	38.8	37.9
11	24.7	24.7	24.7	24.6	24.6	24.6
12	124.2	124.2	124.2	124.4	123.8	123.9
13	144.9	144.9	144.9	144.8	144.4	144.4
14	43.0	43.0	43.0	42.9	42.9	42.9
15	28.6	28.6	28.6	28.6	28.9	29.0
16	23.9	23.9	24.0	23.9	25.4	25.4
17	49.3	49.3	49.3	49.3	49.3	49.3
18	43.0	43.0	43.0	43.0	42.3	42.4
19	46.9	46.9	46.9	46.9	48.1	48.2
20	31.6	31.6	31.6	31.6	37.2	37.2
21	34.7	34.7	34.7	34.7	73.8	74.0
22	33.8	33.8	33.8	33.8	41.3	41.3
23	28.6	28.6	28.8	28.4	28.8	28.4
24	17.6	17.6	17.6	16.7	17.6	16.7
25	17.1	17.2	17.1	16.0	17.2	16.0
26	18.1	18.2	18.1	18.1	17.8	17.8
27	26.4	26.4	26.4	26.4	26.3	26.3
28	177.1	177.1	177.2	177.1	180.6	180.7
29	33.4	33.4	33.4	33.5	29.6	29.6
30	24.0	24.0	23.9	24.0	17.6	17.6

3.3.2. Acid hydrolysis of *1*

A solution of **1** (6.0 mg) in 1.0 M HCl (dioxane–H_2O, 1:1, 2 mL) was heated at 95 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-96SB (Organo, Tokyo, Japan) column (10 mm i.d.×100 mm) and chromatographed on Diaion HP-20 (10 mm i.d.×100 mm), eluted with H_2O–MeOH (3:2) followed by EtOH–Me_2CO (1:1), to yield an aglycone fraction and a sugar fraction (2.5 mg). The aglycone fraction was chromatographed on silica gel (12 mm i.d.×120 mm) and eluted with hexane–Me_2CO (1:1) to give 2α,3β-dihydroxyolean-12-en-28-oic acid (**1a**, 2.2 mg). The sugar fraction was dissolved in H_2O (1 mL) and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, U.S.A.), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH2 SG80 (4.6 mm i.d.×250 mm, 5 mm, Shiseido); solvent, MeCN–H_2O (17:3); flow rate, 1.0 mL/min; detection, RI and OR. Identification of D-glucose, D-xylose, and L-rhamnose present in the sugar fraction was carried out by comparison of their retention times and optical rotations with those of authentic samples; t_R (min) 6.86 (L-rhamnose, negative optical rotation), 8.10 (D-arabinose, positive optical rotation), 8.71 (D-xylose, positive optical rotation), 13.50 (D-glucose, positive optical rotation).

3.3.3. Compound *2*

Amorphous powder; $[\alpha]_D^{25}$ −44.8 (*c* 0.10, MeOH); IR (film) ν_{max} 3389 (OH), 2931 (CH), 1728 (C=O) cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.33 (1H, br s, H-12), 3.70 (1H, ddd, *J*=12.5, 9.3, 4.1 Hz, H-2), 2.97 (1H, d, *J*=9.3 Hz, H-3), 1.17 (3H, s, Me-27), 1.11 (3H, s, Me-23), 1.01 (3H, s, Me-25), 0.96 (3H, s, Me-30), 0.92 (3H, s, Me-29), 0.89 (3H, s, Me-24), 0.79 (3H, s, Me-26); ^{13}C NMR (125 MHz, CD_3OD): see Table 2; signals for sugar moieties: see Table 1; HRESITOFMS *m/z*: 1177.6044 $[M+H]^+$ (calcd for $C_{57}H_{93}O_{25}$: 1177.6006).

3.3.4. Acid hydrolysis of *2*

A solution of **2** (15.7 mg) was subjected to acid hydrolysis as described for **1** to give **1a** (1.4 mg) and a sugar fraction (3.7 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose, D-xylose, L-arabinose, and L-rhamnose; t_R (min) 6.69 (L-rhamnose, negative optical rotation), 7.78 (D-arabinose, positive optical rotation), 8.44 (D-xylose, positive optical rotation), 13.00 (D-glucose, positive optical rotation).

3.3.5. Compound *3*

Amorphous powder; $[\alpha]_D^{25}$ −37.2 (*c* 0.10, MeOH); IR (film) ν_{max} 3376 (OH), 2926 (CH), 1729 (C=O) cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.33 (1H, br s, H-12), 3.71 (1H, ddd, *J*=12.5, 9.3, 4.3 Hz, H-2), 2.96 (1H, d, *J*=9.3 Hz, H-3), 1.16 (3H, s, Me-27), 1.11 (3H, s, Me-23), 1.01 (3H, s, Me-25), 0.96 (3H, s, Me-30), 0.92 (3H, s, Me-29), 0.88 (3H, s, Me-24), 0.79 (3H, s, Me-26); ^{13}C NMR (125 MHz, CD_3OD): see Table 2; signals for sugar moieties: see Table 1; HRESITOFMS *m/z*: 1485.7100 $[M+H]^+$ (calcd for $C_{69}H_{113}O_{34}$: 1485.7113).

*3.3.6. Acid hydrolysis of **3***

A solution of **3** (15.2 mg) was subjected to acid hydrolysis as described for **1** to give **1a** (1.4 mg) and a sugar fraction (3.6 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose, D-xylose, L-arabinose, and L-rhamnose; t_R (min) 6.67 (L-rhamnose, negative optical rotation), 7.75 (L-arabinose, positive optical rotation), 8.42 (D-xylose, positive optical rotation), 13.02 (D-glucose, positive optical rotation).

*3.3.7. Compound **4***

Amorphous powder; $[\alpha]_D^{25}$ −30.0 (*c* 0.15, MeOH); IR (film) ν_{max} 3393 (OH), 2937 (CH), 1725 (C=O) cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.31 (1H, br s, H-12), 3.13 (1H, dd, *J*=11.6, 4.7 Hz, H-3), 1.16 (3H, s, Me-27), 1.05 (3H, s, Me-23), 0.96 (3H, s, Me-30), 0.95 (3H, s, Me-25), 0.92 (3H, s, Me-29), 0.83 (3H, s, Me-24), 0.79 (3H, s, Me-26); ^{13}C NMR (125 MHz, CD_3OD): see Table 2; signals for sugar moieties: see Table 1; HRESITOFMS *m/z*: 1469.7120 $[M+H]^+$ (calcd for $C_{69}H_{113}O_{33}$: 1469.7164).

*3.3.8. Acid hydrolysis of **4***

A solution of **4** (13.3 mg) was subjected to acid hydrolysis as described for **1** to give 3β-hydroxyolean-12-en-28-oic acid (**4a**, 1.3 mg) and a sugar fraction (4.1 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose, D-xylose, L-arabinose, and L-rhamnose; t_R (min) 6.71 (L-rhamnose, negative optical rotation), 7.77 (L-arabinose, positive optical rotation), 8.43 (D-xylose, positive optical rotation), 13.21 (D-glucose, positive optical rotation).

*3.3.9. Compound **5***

Amorphous powder; $[\alpha]_D^{25}$ −44.0 (*c* 0.10, MeOH); IR (film) ν_{max} 3375 (OH), 1697 (C=O) cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.28 (1H, br s, H-12), 3.73 (1H, ddd, *J*=12.4, 10.9, 3.5 Hz, H-2), 3.47 (1H, dd, *J*=12.0, 5.5 Hz, H-21), 2.89 (1H, d, *J*=10.9 Hz, H-3), 1.98 (1H, m, H-16α), 1.79 (1H, dd, *J*=14.1, 14.1 Hz, H-19α), 1.74 (1H, m, H-16β), 1.21 (1H, dd, *J*=14.1, 5.3 Hz, H-19β), 1.15 (3H, s, Me-27), 1.12 (3H, s, Me-23), 1.01 (3H, s, Me-25), 0.95 (3H, s, Me-29), 0.90 (3H, s, Me-30), 0.89 (3H, s, Me-24), 0.81 (3H, s, Me-26); ^{13}C NMR (125 MHz, CD_3OD): see Table 2; signals for sugar moieties: see Table 1; HRESITOFMS *m/z*: 1355.6528 $[M+H]^+$ (calcd for $C_{63}H_{103}O_{31}$: 1355.6483).

*3.3.10. Acid hydrolysis of **5***

A solution of **5** (14.9 mg) was subjected to acid hydrolysis as described for **1** to give 2α,3β,21β-trihydroxyolean-12-en-28-oic acid (**5a**, 1.0 mg) and a sugar fraction (4.7 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose, D-xylose, L-arabinose, and L-rhamnose; t_R (min) 6.72 (L-rhamnose, negative optical rotation), 8.00 (L-arabinose, positive optical rotation), 8.62 (D-xylose, positive optical rotation), 13.72 (D-glucose, positive optical rotation).

*3.3.11. Compound **6***

Amorphous powder; $[\alpha]_D^{25}$ −24.2 (*c* 0.10, MeOH); IR (film) ν_{max} 3375 (OH), 2930 (CH), 1697 (C=O) cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.25 (1H, br s, H-12), 3.45 (1H, dd, *J*=12.3, 5.4 Hz, H-21), 3.12 (1H, dd, *J*=11.6, 4.3 Hz, H-3), 1.90 (1H, m, H-2a), 1.68 (1H, m, H-2b), 1.14 (3H, s, Me-27), 1.05 (3H, s, Me-23), 0.94 (3H, s, Me-25), 0.94 (3H, s, Me-29), 0.89 (3H, s, Me-30), 0.82 (3H, s, Me-24), 0.80 (3H, s, Me-26); ^{13}C NMR (125 MHz, CD_3OD): see Table 2; signals for sugar moieties: see Table 1; HRESITOFMS *m/z*: 1339.6581 $[M+H]^+$ (calcd for $C_{63}H_{103}O_{30}$: 1339.6534).

*3.3.12. Acid hydrolysis of **6***

A solution of **6** (10.6 mg) was subjected to acid hydrolysis as described for **1** to give 3β,21β-dihydroxyolean-12-en-28-oic acid (**6a**, 1.1 mg) and a sugar fraction (4.6 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose, D-xylose, L-arabinose, and L-rhamnose; t_R (min) 6.81 (L-rhamnose, negative optical rotation), 8.07 (L-arabinose, positive optical rotation), 8.67 (D-xylose, positive optical rotation), 13.60 (D-glucose, positive optical rotation).

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