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Seven new triterpene glycosides from the pericarps of *Stryphnodendron fissuratum*

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ABSTRACT

Seven new triterpene glycosides on the basis of the lupane skeleton (**1**–**7**) were isolated from the pericarps of *Stryphnodendron fissuratum* (Leguminosae). The structures of **1**–**7** were determined on the basis of extensive spectroscopic analysis, including two-dimensional NMR data, and the results of hydrolytic cleavage.

1. Introduction

Stryphnodendron fissuratum Mart. (Leguminosae) is mainly found in the tropical regions of the continent of South America (Hashimoto and Nishimoto, 1996). The fruits of this plant are poisonous and cause bovine death (Ferreira et al., 2009; Mendonça et al., 2010). Previously, we reported on the isolation and structural characterization of six new triterpene glycosides on the basis of the oleanane skeleton, named stryphnosides A–F, from the pericarps of *S. fissuratum* (Yokosuka et al., 2008a). Further phytochemical analysis of the plant suggested that it also contained triterpene glycosides with a lupane skeleton, but they remain to be isolated and identified (Haraguchi et al., 2006). Our detailed examination of the EtOH extract of *S. fissuratum* pericarps resulted in the isolation of seven new lupane-type triterpene glycosides (**1**–**7**). This paper deals with the structural determination of the new glycosides on the basis of spectroscopic analysis, including various two-dimensional (2D) NMR spectroscopic techniques, and the results of hydrolytic cleavage.

2. Results and discussion

Compound **1** was obtained as an amorphous powder. The HRESI-TOFMS of **1** showed an accurate $[M+Na]^+$ ion peak at m/z 935.4889 in accordance with the empirical molecular formula of $C_{47}H_{76}O_{17}$, which was supported by the ^{13}C NMR spectrum with a total of 47 signals and DEPT data. The IR spectrum of **1** showed a broad absorption band for hydroxy groups at 3395 cm^{-1}, as well as strong absorption due to a carbonyl group at 1725 cm^{-1}. The ^1H NMR spectrum of **1** showed signals for six triterpenoid methyl groups at δ 1.70, 1.08, 1.01, 0.94, 0.92, and 0.87 (each s), and an exomethylene group at δ 4.75 and 4.62 (each br s), which are characteristic of the lup-20(29)-en structure, as well as signals for three anomeric protons at δ 5.99 (br s), 4.59 (d, J = 7.6 Hz), and 4.40 (d, J = 7.5 Hz). The three-proton doublet signal at δ 1.27 (J = 6.3 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-dihydroxylup-20(29)-en-28-oic acid (2α-hydroxybetulinic acid) (Kumar et al., 1985), as well as L-rhamnose, D-xylose, and D-glucose as the carbohydrate components. 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 175.6, respectively, which suggested that **1** was a 3,28-bisdesmoside. The ^1H–^1H COSY experiment with **1** allowed the sequential assignments of the signals 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 a β-D-xylopyranosyl ($^4C_1$) unit (Xyl), a β-D-glucopyranosyl ($^4C_1$) unit (Glc), and an α-L-rhamnopyranosyl ($^1C_4$) unit (Rha). The proton resonances 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 Xyl and Rha groups were presented as the terminal units, whereas the Glc group was substituted at C-2 (Agrawal et al., 1985; Agrawal, 1992). The anomeric conformations of the Glc and Xyl groups were ascertained by the relatively large J values of their anomeric protons (7.5–7.6 Hz). For the Rha moiety,

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Table 1
^{1}H and ^{13}C NMR chemical shift assignments for the sugar moieties of compounds **1–7** in CD_3OD.[a]

Position			**1**		Position			**2**		Position			**3**		Position			**4**	
Glc	1		4.40 d (7.5)	104.8	Glc	1		4.41 d (7.6)	104.7	Glc	1		4.45 d (7.7)	104.7	Glc	1		4.45 d (7.7)	104.7
	2		3.52 dd (8.8, 7.5)	82.2		2		3,51 dd (9.1,7.6)	82.4		2		3.62 dd (8.9, 7.7)	81.3		2		3.62 dd (8.9, 7.7)	81.3
	3		3.57 t (8.8)	78.5		3		3,57 t (9.1)	78.5		3		3.75 t (8.9)	76.7		3		3.74 t (8.9)	76.7
	4		3.36 t (8.8)	71.1		4		3.37 t (9.1)	71.0		4		3.61 t (8.9)	80,0		4		3.61 t (8.9)	80.0
	5		3.35 m	78.0		5		3.34 m	78.0		5		3.50 m	76.4		5		3.48 m	76.4
	6	a	3.84 dd (12.0, 1.8)	62.3		6	a	3.85 dd (11.8, 1.7)	62.3		6	(2H)	3.85 m	61.4		6	(2H)	3.85 m	61.4
		b	3.65 dd (12.0, 3.5)				b	3.65 dd (11.8, 3.5)											
Xyl	1		4.59 d (7.6)	105.9	Xyl	1		4.63 d (7.5)	105.8	Xyl	1		4.69 d (7.5)	105.4	Xyl	1		4.69 d (7.5)	105.4
	2		3.21 dd (9.0, 7.6)	76.1		2		3.27 dd (9.0, 7.5)	75.8		2		3.25 dd (8.7, 7.5)	75.8		2		3.25 dd (8.7, 7.5)	75.8
	3		3.30 t (9.0)	78.0		3		3.46 t (9.0)	76.1		3		3.46 t (8.7)	76.1		3		3.46 t (8.7)	76.1
	4		3.43 ddd	71.2		4		3.62 ddd	78.0		4		3.60 ddd	78.0		4		3.62 ddd	78.0
			(10.6, 9.0, 5.0)					(10.5, 9.0, 5.3)					(10.6, 8.7, 5.2)					(10.7, 8.7, 5.2)	
	5	a	3.78 dd (11.3, 5.0)	67.1		5	a	3.96 dd (11.6, 5.3)	64.7		5	a	3.96 dd (11.5, 5.2)	64.7		5	a	3.96 dd (11.5, 5.2)	64.7
		b	3.12 dd (11.3, 10.6)				b	3.24 dd (11.6, 10.5)				b	323 dd (11.5, 10.6)				b	323 dd (11.5, 10.7)	
Rha	1		5.99 br s	95.1	Ara	1		4,27 d (7.0)	103.8	Ara	1		4.27 d (7.0)	103.8	Ara	1		4.27 d (7.0)	103.8
	2		3,79 br d (3.5)	71.4		2		3.56 dd (8.8, 7.0)	72.0		2		3.56 dd (9.1, 7.0)	72.0		2		3.56 dd (9.0, 7.0)	72.0
	3		3.65 dd (9.6, 3.5)	72.5		3		3.51 dd (8.8, 3.2)	74.1		3		3.51 dd (9.1, 3.6)	74.2		3		3.50 dd (9.0, 3.6)	74.2
	4		3.45 t (9.6)	73.4		4		3.79 m	69.7		4		3.80 m	69.7		4		3.80 m	69.7
	5		3.67 dq (9.6, 6.3)	72.8		5	a	3.90 dd (12.5, 2.7)	67.3		5	a	3.90 dd (12.2, 2.7)	67.3		5	a	3.89 dd (11.6, 2.9)	67.3
	6		1.27 d (6.3)	18.2			b	3.58 dd (12.5, 2.8)				b	3.24 dd (12.2, 2.9)				b	3.58 dd (11.6, 4.1)	
					Rha	1		5.99 br s	95.0	Xyl′	1		4.34 d (7.7)	105.3	Xyl′	1		4.34 d (7.7)	105.3
						2		3.79 br d (3.3)	71.4		2		3.19 dd (8.9, 7.7)	74.9		2		3.19 dd (8.9, 7.7)	74.9
						3		3.66 dd (9.5, 3.3)	72.5		3		3.30 t (8.9)	77.9		3		3.30 t (8.9)	77.9
						4		3.45 t (9.5)	73.4		4		3.48 ddd	71.0		4		3.49 ddd	71.0
													(10.6, 8.9, 6.1)					(9.5, 8.9, 5.0)	
						5		3.66 dq (9.5, 6.1)	72.8		5	a	3.90 dd (11.2, 6.1)	67.1		5	a	3.89 dd (11.6, 5.0)	67.1
						6		1.27 d (6.1)	18.2			b	3.24 dd (11.2, 10.6)				b	3.24 dd (11.6, 9.5)	
										Rha	1		5.99 br s	95.1	Rha	1		4.64 br s	102.3
											2		3.79 br d (3.1)	71.4		2		3.79 br d (3.1)	72.4
											3		3.65 dd (9.5, 3.1)	72.5		3		3.62 dd (9.3, 3.1)	72.7
											4		3.45 t (9.5)	73.3		4		3.39 t (9.3)	73.9
											5		3.67 dq (9.5, 6.1)	72.8		5		3.57 m	70.0
											6		1.27 d (6.1)	18.2		6		1.27 d (6.3)	18.1

Position			**5**		Position			**6**		Position			**7**	
Glc	1		4.45 d (7.8)	104.7	Glc	1		4.43 d (7.7)	104.7	Glc	1		4.41 d (7.7)	105.1
	2		3.62 dd (8.8, 7.8)	81.3		2		3.60 dd (9.1, 7.7)	81.2		2		3.49 dd (9.2, 7.7)	82.4
	3		3.75 t (8.8)	76.7		3		3.72 t (9.1)	76.8		3		3.67 t (9.2)	76 6
	4		3.60 t (8.8)	80.0		4		3.59 t (9.1)	80.2		4		3.54 t (9.2)	80.7
	5		3.50 m	76.4		5		3.47	76.4		5		3.54 m	75.9
	6	(2H)	3.85 m	61.4		6	(2H)	3.85 m	61.3		6	(2H)	3.83 m	61.8
Xyl	1		4.69 d (7.7)	105.4	Xyl	1		4.67 d (7.7)	105.7	Xyl	1		4.57 d (7.7)	106.1
	2		3.25 dd (9.0, 7.7)	75.8		2		3.48 dd (8.8, 7.7)	76.5		2		3.20 dd (9.5, 7.7)	76.9
	1		3 46 t (9 0)	76.1		3		3 80 t (8.8)	79 9		3		134 t (9.5)	79 9
	4		3 62 ddd	78.0		4		3.78 ddd	71.8		4		3.49 ddd	71.8
			(10.5, 9.0, 5.3)					(9.3, 8.8, 3.7)					(9.5, 9.5, 4.2)	
	5	a	3.96 dd (11.6, 5.3)	64.7		5	a	4.00 dd (11.2, 3.7)	64.3		5	a	4.02 dd (11.5, 4.2)	64.4
		b	3.23 dd (11.6, 10.5)				b	3.20 dd (11.2, 9.3)				b	3.31 dd (11.5, 9.5)	

Ara	1		4.27 d (7.0)	103.8	Glc′	1		4.90 d (7.3)	102.6	Glc′	1		4.89 d (7.3)	102.7
	2		3.56 dd (9.1, 7.0)	72.0		2		3.41 dd (9.5, 7.3)	80.4		2		3.40 dd (9.4, 7.3)	80.0
	3		3.51 dd (9.1, 3.7)	74.1		3		3.43 t (9.5)	79.1		3		3.47 t (9.4)	79.1
	4		3.79 m	69.7		4		3.46 t (9.5)	70.7		4		3.49 t (9.4)	70.7
	5	a	3.90 dd (12.7, 3.0)	67.3		5		3.19 m	78.1		5		3.22 m	7S.1
		b	3.58 dd (12.7, 3.5)			6	a	3.82 br d (12.5)	62.1		6	a	3.82 br d (12.1)	62.1
							b	3.72 br d (12.5)				b	3.73 br d (12.1)	
Xyl′	1		4.34 (1(7.7)	105.3	Rha	1		5.11 br s	102.7	Rha	1		5.13 br s	102.6
	2		3.19 dd (9.0, 7.7)	74 .9		2		3.95 br d (3.6)	72.2		2		3.96 br d (4.0)	72.2
	3		3.30 t (9.0)	77.9		3		3.71 dd (9.2, 3.6)	72.3		3		3.72 dd (9.1, 4.0)	72,.1
	4		3.49 ddd (10.5, 9.0, 4.7)	71.0		4		3.39 t (9.2)	74.0		4		3.34 t (9.1)	73.9
	5	a	3.90 dd (11.4, 4.7)	67.1		5		4.11 dq (9.2, 7.4)	69.7		5		4.12 dq (9.1, 6.2)	69.7
		b	3.23 dd (11.4, 10.5)			6		1 29 d (7.4)	18.1		6		1.27 d (6.2)	18.0
					Ara	1		4.63 d (2.9)	99.4	Ara	l		4.61 d (3.7)	99.4
						2		3.76 dd (3.7, 2,9)	70.7		2		3.74 dd (4.2, 3.7)	70.7
						3		3.69 t (3.7)	73.0		3		3.68 t (4.2)	73.0
						4		3.91 ddd (8.9, 3.7, 2.6)	65.9		4		3.92 ddd (8.8, 4.2, 3.5)	66.0
						5	a	4.09 dd (11.5, 8.9)	62 1		5	a	4.07 dd (11.6, 8.8)	62.1
							b	3.50 dd (11.5, 2.6)				b	3.51 dd (11.6, 3.5)	
					Xyl′	1		4.33 d (7.7)	105.4	Xyl′	1		4.33 d (7.7)	105.3
						2		3.19 dd (9.1,7.7)	74.9		2		3.19 dd (9.2, 7.7)	74.9
						3		3.31 t (9.1)	77.9		3		3.37 t (9.2)	77 9
						4		3 49 ddd (10.3, 9.1, 4.1)	71.0		5		3.50 ddd (10.8, 9.2, 4.8)	71.0
						5	a	3.92 dd (11.0, 4.1)	67.1			a	3.93 dd (11.5, 4.8)	67.1
							b	3.24 dd (11.0, 10.3)				b	3.24 dd (11.5, 10.8)	
					Rha′	1		4.62 br s	102.3	Rha′	1		4.62 br s	105.3
						2		3.79 br d (3.8)	72.4		2		3.80 br d (4.1)	72.3
						3		3.62 dd (9.4, 3.8)	72.6		3		3 63 dd (9.2, 4.1)	72 6
						4		3.39 t (9.4)	73.9		4		3.34 t (9.2)	74.0
						5		3.57 dq (9.4, 7,4)	70.0		5		3.56 dq (9.2, 6.2)	70.0
						6		l.29 d (7.4)	18.1		6		1.27 d (6.2)	18.1

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

the large $^{1}J_{C\text{-}1,H\text{-}1}$ value (173.0 Hz) indicated that the anomeric proton was equatorial thus possessing an α-pyranoid anomeric form (Jia et al., 1998). In the HMBC spectrum of **1**, long-range correlations were observed between H-1 of Rha at δ 5.99 and C-28 of aglycone at δ 175.6, H-1 of Xyl at δ 4.59 and C-2 of Glc at δ 82.2, and between H-1 of Glc at δ 4.40 and C-3 of aglycone at δ 96.4. Thus, **1** was determined to be 2α-hydroxy-3β-[(*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl)oxy]lup-20(29)-en-28-oic acid α-L-rhamnopyranosyl ester (Fig. 1).

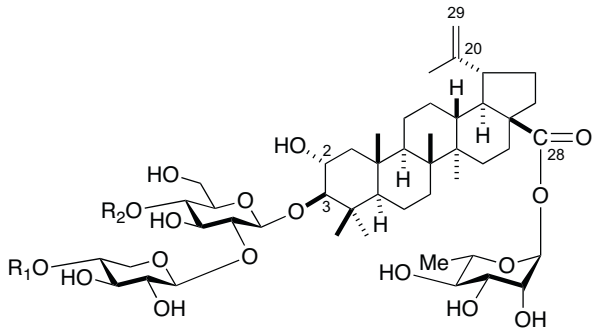
Compound **2** was shown to have the molecular formula $C_{52}H_{84}O_{21}$ on the basis of HRESI-TOFMS (*m*/*z* 1045.5582 $[M+H]^+$). The deduced molecular formula was higher than that of **1** by $C_5H_8O_4$, corresponding to one pentose unit. The ^{1}H NMR spectrum of **2** exhibited signals for four anomeric protons at δ 5.99 (br s), 4.63 (d, *J* = 7.5 Hz), 4.41 (d, *J* = 7.6 Hz), and 4.27 (d, *J* = 7.0 Hz), as well as signals for six triterpenoid methyl groups at δ 1.70, 1.08, 1.01, 0.94, 0.92, and 0.86 (each s), and an exomethylene group at δ 4.75 and 4.62 (each br s). 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 α-L-arabinopyranosyl unit (Ara) were observed at δ 103.8, 72.0, 74.1, 69.7, and 67.3, and the signals due to C-4 of the Xyl moiety and its neighboring carbons varied, while all other signals remained almost unaffected. In the HMBC spectrum, long-range correlations were observed between H-1 of Rha and C-28 of the aglycone, H-1 of Ara and C-4 of Xyl, H-1 of Xyl and C-2 of Glc, and between H-1 of Glc and C-3 of the aglycone. Thus, **2** was formulated as 3β-[(*O*-α-L-arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl)oxy]-2α-hydroxylup-20(29) -en-28-oic acid α-L-rhamnopyranosyl ester.

Compound **3** was analyzed for $C_{57}H_{92}O_{25}$ by HRESI-TOFMS (*m*/*z* 1177.5925 $[M+H]^+$), higher than that of **2** by $C_5H_8O_4$. On the basis of the spectral properties of **3** and the results of acid hydrolysis, giving **1a**, L-rhamnose, L-arabinose, D-xylose, and D-glucose, **3** was shown to be a triterpene glycoside closely related to **2**; however the ^{1}H NMR spectrum of **3** contained resonances for five anomeric protons at δ 5.99 (br s), 4.69 (d, *J* = 7.5 Hz), 4.45 (d, *J* = 7.7 Hz), 4.34 (d, *J* = 7.7 Hz), and 4.27 (d, *J* = 7.0 Hz). On comparison of the ^{13}C NMR spectrum of **3** with that of **2**, a set of five additional signals corresponding to a terminal β-D-xylopyranosyl unit (Xyl′) [δ 105.3, 74.9, 77.9, 71.0, and 67.1] were observed, and C-4 of Glc was significantly shifted downfield. In the HMBC spectrum of **3**, long-range correlations were detected between H-1 of Rha and C-28 of the aglycone, H-1 of Ara and C-4 of Xyl, H-1 of Xyl and C-2 of Glc, H-1 of Xyl′ and C-4 of Glc, and between H-1 of Glc and C-3 of the aglycone. Thus, **3** 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-hydroxylup-20(29)-en-28-oic acid α-L-rhamnopyranosyl ester.

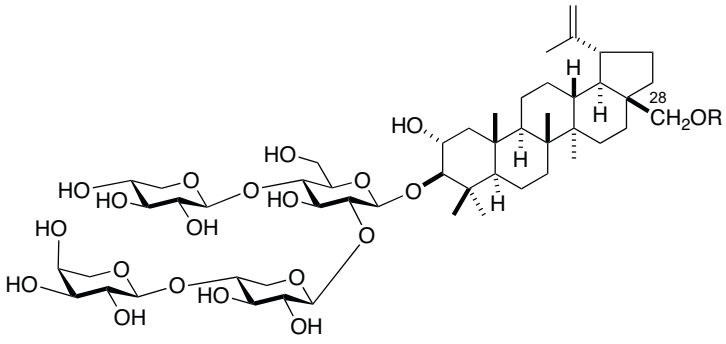
Compound **4** was deduced as $C_{57}H_{94}O_{24}$ on the basis of HRESI-TOFMS (*m*/*z* 1163.6212 $[M+H]^+$). The ^{1}H NMR spectrum of **4** showed signals for five anomeric protons at δ 4.69 (d, *J* = 7.5 Hz), 4.64 (br s), 4.45 (d, *J* = 7.7 Hz), 4.34 (d, *J* = 7.7 Hz), and 4.27 (d, *J* = 7.0 Hz), along with signals for six triterpenoid methyl groups at δ 1.69, 1.08, 1.07, 1.01, 0.92, and 0.87 (each s), and an exomethylene group at δ 4.71 and 4.59 (each br s). Acid hydrolysis **4** gave L-rhamnose, L-arabinose, D-xylose, and D-glucose, while the aglycone was decomposed under acidic conditions. The ^{1}H and ^{13}C NMR spectral properties of **4** ware essentially analogues to those of **3** except for the signals arising from the ring D and E portions of the aglycone. However, the ester carbonyl group attached to C-28 of the aglycone could not be observed in the IR and ^{13}C NMR spectra of **4**. The signals for an oxymethylene group [δ_H 3.41 and 3.22 (*ABq*, *J* = 9.4 Hz)/δ_C 68.0] newly appeared in the ^{1}H and ^{13}C NMR spectra of **4**. In the HMBC spectrum, the oxymethylene protons, which were assigned to H_2-28, showed long-range correlations with C-16 (δ_C 30.4), C-17 (δ_C 48.5), and C-22 (δ_C 35.1). A long-range correlation was also observed between H-1 of Rha at δ 4.64 and C-28 of aglycone at δ 67.4. Accordingly, the structure of the aglycone moiety of **4** was determined to be lup-20(29)-en-2α,3β, 28-triol (Schmidt et al., 1995), and **4** to be 2α-hydroxy-28-[(α-L-rhamnopyranosyl)oxy]lup-20(29)-en-3β-yl *O*-α-L-arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside.

The ^{1}H NMR spectrum of compound **5** ($C_{51}H_{84}O_{20}$) exhibited signals for four anomeric protons at δ 4.69 (d, *J* = 7.7 Hz), 4.45 (d, *J* = 7.8 Hz), 4.34 (dd, *J* = 7.7 Hz), and 4.27 (d, *J* = 7.0 Hz), together with signals for six triterpenoid methyl groups at δ 1.68, 1.09, 1.07, 1.00, 0.92, and 0.87 (each s), and an exomethylene group at δ 4.68 and 4.57 (each br s). Acid hydrolysis **5** gave L-rhamnose, L-arabinose, D-xylose, and D-glucose. When the ^{1}H and ^{13}C NMR signals of **5** were compared with those of **4**, the two compounds were in complete agreement as to the signals arising from the tetraglycoside group attached to C-3 of the aglycone. However, the signals assignable to the α-L-rhamnopyranosyl residue bonded to C-28 of the aglycone could not be observed in the ^{1}H and ^{13}C NMR spectra of **5**, and the C-28 carbon signal of **5** was shifted upfield by 7.0 ppm in comparison with that of **4**. The above chemical and spectral data implied that **5** was the C-28 derhamnosyl derivative of **4** and allowed for the structural determination of **5** to be made as 2α,28-dihydroxylup-20(29)-en-3β-yl *O*-α-L-arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside.

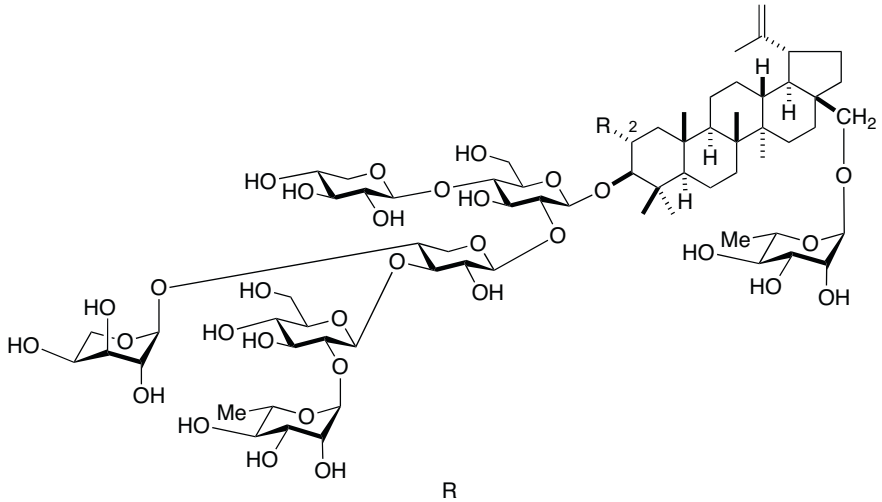
Compound **6** had the molecular formula $C_{69}H_{114}O_{33}$ on the basis of HRESI-TOFMS (*m*/*z*: 1493.7139 $[M+Na]^+$). The ^{1}H NMR spectrum of **6** contained signals for six quaternary methyl groups at δ 1.69, 1.09, 1.07, 1.00, 0.93, and 0.86 (each s), an exomethylene group at δ 4.71 and 4.58 (each br s), and seven anomeric protons at δ 5.11 (br s), 4.90 (d, *J* = 7.3 Hz), 4.67 (d, *J* = 7.7 Hz), 4.63 (d, *J* = 2.9 Hz), 4.62 (br s), 4.43 (d, *J* = 7.7 Hz), and 4.33 (d, *J* = 7.7 Hz). Acid hydrolysis of **6** yielded L-rhamnose, L-arabinose, D-xylose, and D-glucose. In comparison of ^{13}C NMR spectrum of **6** with that of **4**, the signals due to the aglycone moiety and an α-L-rhamnopyranosyl group linked to C-28 of the aglycone were observed at almost the same positions for each of the compounds. However, differences were recognized in the glycoside moiety attached to C-3 of the aglycone. Detailed analysis of the 1D TOCSY and 2D NMR spectra resulted in the assignments of all the proton resonances due to the seven glycosyl units, including identification of their multiplet patterns and coupling constants, and the corresponding one-bond coupled carbons (Table 1). The carbon chemical shifts thus assigned indicated that **6** contained a C-2 and C-4 disubstituted β-D-glucopyranosyl moiety (Glc), a C-3 and C-4 disubstituted β-D-xylopyranosyl moiety (Xyl), a C-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 $^{3}J_{H\text{-}1,H\text{-}2}$ values of their anomeric protons (7.3–7.7 Hz) and $^{1}J_{H\text{-}1,C\text{-}1}$ values (Glc: 158.4 Hz; Xyl: 156.6 Hz; Glc′: 157.8 Hz; Xyl′: 154.9 Hz). For the Rha and Rha′ moieties, the large $^{1}J_{H\text{-}1,C\text{-}1}$ values (Rha: 171.8 Hz; Rha′: 168.0 Hz) indicated that each anomeic proton possessed an α-pyranoid anomeric form. The proton chemical shifts and spin-coupling constants of the Ara moiety of **6** were different from those of **2**–**5**. The coupling constants assigned by the 1D TOCSY spectra, the large $^{1}J_{H\text{-}1,C\text{-}1}$ value (172.8 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 $^{1}C_4$ with an α-orientation of the anomeric center. This phenomenon has been observed in the case of stryphnosides C–F isolated from the title plant (Yokosuka et al., 2008a). In the HMBC



	R_1	R_2
1	H	H
2	Ara	H
3	Ara	Xyl



	R
4	Rha
5	H



	R
6	OH
7	H

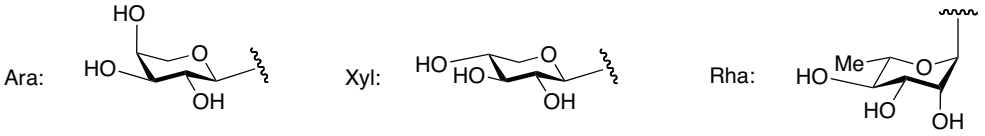


Fig. 1. Structures of 1–7.

spectrum of **6**, long-range correlations were observed between H-1 of Rha at δ_H 5.11 and C-2 of Glc′ at δ_C 80.4, H-1 of Glc′ at δ_H 4.90 and C-3 of Xyl at δ_C 79.9, H-1 of Xyl at δ_H 4.67 and C-2 of Glc at δ_C 81.2, H-1 of Ara at δ_H 4.63 and C-4 of Xyl at δ_C 71.8, H-1 of Xyl′ at δ_H 4.33 and C-4 of Glc at δ_C 80.2, H-1 of Glc at δ_H 4.43 and C-3 of the aglycone at δ_C 96.6, and between H-1 of Rha′ at δ_H 4.62 and C-28 of the aglycone at δ_C 67.3. Accordingly, the structure of **6** was elucidated as 2α-hydroxy-28-[(α-L-rhamnopyranosyl)oxy]lup-

20(29)-en-3β-yl *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-glucopyranoside.

Compound **7** exhibited a molecular formula of $C_{69}H_{114}O_{32}$ on the basis of HRESI-TOFMS (*m*/*z* 1477.7146 $[M+Na]^+$). Comparison of the ^1H and ^{13}C NMR spectra of **7** with those of **6** showed considerable structural similarities. However, the molecular formula of **7** was lower by one oxygen atom than that of **6**, implying the lack of one hydroxy group. When the ^{13}C NMR spectrum of **7** was compared with that of **6**, the signal due to the C-2 hydroxymethine carbon, which was observed at δ 68.2 in **6**, was displaced by a methylene carbon signal at δ 27.3 in **7**. In addition, the carbon signals due to C-1 and C-3 were shifted upfield by 7.7 and 5.5 ppm, respectively. All other NMR signals of **7**, which were assigned by the 1D-TOCSY, ^1H–^1H COSY, HMQC, and HSQC-TOCSY spectra, were almost superimposable with those of **6**. Acid hydrolysis of **7** gave L-rhamnose, L-arabinose, D-xylose, and D-glucose, while the aglycon was decomposed under acidic conditions. Analysis of the HMBC spectrum of **7** indicated that the hexaglycoside attached to C-3 of the aglycone is the same as that of **6**, and that an α-L-rhamnopyranosyl group forms a linkage with the C-28 of the aglycone. Thus, the structure of **7** was formulated as 28-[(α-L-rhamnopyranosyl)oxy]lup-20(29)-en-3β-yl *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-glucopyranoside.

Compounds **1**–**7** are new lupane-type triterpene glycosides with up to seven monosaccharides. This is the first report of the isolation of lupane-type triterpene glycosides from the genus *Stryphnodendron.* Compound **1** contains an *O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl group at the C-3 hydroxy group of the aglycone. Triterpene 3-*O*-diglycoside is first isolated from *S. fissuratum.* Compounds **4**–**6** have the common aglycone of lup-20(29)-ene-2α,3β,28-triol, among which **4** and **6** are bisdesmosides with the sugar unit at the C-3 and C-28 hydroxy groups whereas **5** is a monodesmoside with the sugar unit at the C-3 hydroxy group. To the best of our knowledge, these types of triterpene glycosides have not been isolated from natural sources. The structure of the aglycone moiety of **7** is lup-20(29)-ene-3β,28-diol (betulin). Betulin glycosids are mainly synthesized (Gauthier et al., 2006) and have been isolated only from *Oplopanax elatus* Nakai (Araliaceae) (Wang and Xu, 1993).

3. Experimental

3.1. General

The instruments and experimental conditions were the same as described in the previous papers (Yokosuka and Mimaki, 2008b; Yokosuka et al., 2009).

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 the 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). Column chromatography (CC) of the 80% MeOH eluate portion on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (30:10:1; 20:10:1; 10:10:1), and finally with MeOH alone, gave seven fractions (A–H). Fraction C was subjected to CC on ODS silica gel eluted with MeCN–H_2O (1:2) and MeOH–H_2O (2:1) to give **1** (9.1 mg). Fraction E was chromatographed on ODS silica gel eluted with MeCN–H_2O (1:2) and MeOH–H_2O (2:1) and silica gel with $CHCl_3$–MeOH–H_2O (20:10:1) to give **2** (10.0 mg) and **5** (10.3 mg). Fraction F was subjected to CC on ODS silica gel eluted with MeCN–H_2O (1:2) and MeOH–H_2O (3:2; 2:1) and silica gel with $CHCl_3$–MeOH–H_2O (20:10:1) to give **7** (7.0 mg). Fraction G was chromatographed on ODS silica gel eluted with MeOH–H_2O (2:1) and MeCN–H_2O (1:2) to give **3** (53.2 mg) and **4** (10.7 mg). Fraction H was subjected to CC on ODS silica gel eluted with MeOH–H_2O (4:1) and MeCN–H_2O (1:2), and preparative HPLC using MeCN–H_2O (1:2) to give **6** (9.3 mg).

3.4. Compound 1

Amorphous powder; $[\alpha]_D^{25}$ −84.5° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3395 (OH), 2931 (CH), 1725 (C=O); ^1H NMR (500 MHz, CD_3OD): δ 4.75 and 4.62 (each 1H, br s, H_2-29), 3.69 (1H, ddd, *J* = 11.8, 9.3, 4.4 Hz, H-2), 2.94 (1H, d, *J* = 9.3 Hz, H-3), 1.70 (3H, s, Me-30), 1.08 (3H, s, Me-23), 1.01 (3H, s, Me-27), 0.94 (3H, s, Me-26), 0.92 (3H, s, Me-25), 0.87 (3H, s, Me-24); For ^1H NMR data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 935.4889 $[M+Na]^+$ (calculated for $C_{47}H_{76}O_{17}Na$, 935.4980).

3.5. Acid hydrolysis of 1

A solution of **1** (8.4 mg) in 1 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) eluted with hexane–Me_2CO (1:1) to give 2α,3β-dihydroxylup-20(29)-en-28-oic acid (**1a**, 2.1 mg). The sugar fraction was dissolved in H_2O (1 mL) and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 SG80 (4.6 mm i.d. × 250 mm, 5 μm, 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) 13.6 (D-glucose, positive optical rotation), 8.7 (D-xylose, positive optical rotation), 6.9 (L-rhamnose, negative optical rotation).

3.6. Compound 1a

Amorphous powder; $[\alpha]_D^{25}$ +1.1° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3365 (OH), 2944 (CH), 1696 (C=O); ^1H NMR (500 MHz, CD_3OD): δ 4.70 and 4.59 (each 1H, br s, H_2-29), 3.60 (1H, ddd, *J* = 11.3, 9.6, 4.6 Hz, H-2), 2.88 (1H, d, *J* = 9.6 Hz, H-3), 1.69 (3H, s, Me-30), 1.00 (3H, s, Me-27), 0.98 (3H, s, Me-23), 0.96 (3H, s, Me-26), 0.91 (3H, s, Me-25), 0.77 (3H, s, Me-24); for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Table 2; HRESI-TOFMS *m*/*z*: 473.3643 $[M+H]^+$ (calculated for $C_{30}H_{49}O_4$, 473.3631).

Table 2
^{13}C NMR chemical shift assignments for the aglycone moieties of compounds **1**–**7** in CD_3OD.

Position	1	Ia	2	3	4	5	6	7
1	47.7	48.7	47.7	47.7	47.7	47.7	47.7	40.0
2	68.2	69.8	68.2	68.2	68.2	68.2	68.2	27.3
3	96.4	84.4	96.4	96.6	96.6	96.6	96.6	91.1
4	41.8	40.5	41.8	41.8	41.8	41.8	41.8	40.4
5	56.9	56.8	56.9	56.9	56.8	56.8	56.8	57.1
6	19.3	19.5	19.3	19.3	19.4	19.3	19.4	19.3
7	35.4	35.5	35.4	35.5	35.3	35.1	35.4	35.5
8	42.0	42.0	42.0	42.0	42.1	42.2	42.1	42.1
9	51.9	52.0	51.9	51.9	51.8	51.8	51.8	51.8
10	39.0	39.5	39.0	39.0	39.0	39.0	38.9	38.0
11	22.2	22.2	22.2	22.3	22.1	22.1	22.1	22.0
12	26.8	26.8	26.8	26.8	26.5	26.6	26.5	26.6
13	40.0	39.6	40.0	40.0	39.0	38.7	38.9	39.0
14	43.7	43.6	43.7	43.7	43.9	43.9	43.9	43.8
15	30.8	30.8	30.8	30.8	28.3	28.2	28.3	28.3
16	33.1	33.4	33.1	33.1	30.7	30.4	30.8	30.7
17	58.2	57.5	58.2	58.2	48.5	48.5	48.1	48.1
18	50.5	50.5	50.5	50.5	50.0	50.0	50.0	50.0
19	48.7	48.7	48.9	48.7	49.3	49.0	49.3	49.3
20	151.4	152.0	151.4	151.4	151.7	151.9	151.7	151.7
21	31.7	31.7	31.7	31.7	31.2	30.9	31.2	31.2
22	37.9	38.2	37.9	37.9	36.1	35.1	36.2	36.1
23	28.4	29.1	28.4	28.4	28.4	28.4	28.7	28.3
24	17.4	17.2	17.3	17.4	17.4	17.4	17.4	16.5
25	17.9	17.9	17.9	17.9	17.9	17.9	17.9	16.6
26	16.8	16.7	16.7	16.8	16.6	16.5	16.6	16.7
27	15.1	15.1	15.1	15.1	15.2	15.2	15.2	15.2
28	175.6	180.0	175.6	175.6	67.4	60.4	67.3	67.3
29	110.6	110.1	110.6	110.6	110.5	110.3	110.5	110.4
30	19.5	19.5	19.5	19.5	19.4	19.4	19.3	19.2

3.7. Compound 2

Amorphous powder; $[\alpha]_D^{25}$ −1.1° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3390 (OH), 2947 (CH), 1727 (C=O); ^{1}H NMR (500 MHz, CD_3OD): δ 4.75 and 4.62 (each 1H, br s, H_2-29), 3.70 (1H, ddd, *J* = 12.7, 9.4, 4.5 Hz, H-2), 2.95 (1H, d, *J* = 9.4 Hz, H-3), 1.70 (3H, s, Me-30), 1.08 (3H, s, Me-23), 1.01 (3H, s, Me-27), 0.94 (3H, s, Me-26), 0.92 (3H, s, Me-25), 0.86 (3H, s, Me-24); For ^{1}H NMR data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1045.5582 $[M+H]^+$ (calculated for $C_{52}H_{85}O_{21}$, 1045.5583).

3.8. Compound 3

Amorphous powder; $[\alpha]_D^{25}$ −1.6° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3389 (OH), 2940 (CH), 1737 (C=O); ^{1}H NMR (500 MHz, CD_3OD): δ 4.75 and 4.62 (each 1H, br s, H_2-29), 3.70 (1H, ddd, *J* = 11.8, 9.3, 4.4 Hz, H-2), 2.94 (1H, d, *J* = 9.3 Hz, H-3), 1.70 (3H, s, Me-30), 1.09 (3H, s, Me-23), 1.01 (3H, s, Me-27), 0.94 (3H, s, Me-26), 0.92 (3H, s, Me-25), 0.86 (3H, s, Me-24); For ^{1}H NMR data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1177.5925 $[M+H]^+$ (calculated for $C_{57}H_{93}O_{25}$, 1177.6006).

3.9. Compound 4

Amorphous powder; $[\alpha]_D^{25}$ −26.3° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3375 (OH), 2935 (CH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.71 and 4.59 (each 1H, br s, H_2-29), 3.71 (1H, ddd, *J* = 11.9, 9.4, 4.3 Hz, H-2), 3.55 and 3.41 (each 1H, *ABq*, *J* = 9.4 Hz, H_2-28), 2.94 (1H, d, *J* = 9.4 Hz, H-3), 1.69 (3H, s, Me-30), 1.08 (3H, s, Me-23), 1.07 (3H, s, Me-26), 1.01 (3H, s, Me-27), 0.92 (3H, s, Me-25), 0.87 (3H, s, Me-24); For ^{1}H NMR data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1163.6212 $[M+H]^+$ (calculated for $C_{57}H_{95}O_{24}$, 1163.6213).

3.10. Compound 5

Amorphous powder; $[\alpha]_D^{25}$ −1.7° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3388 (OH), 2943 (CH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.68 and 4.57 (each 1H, br s, H_2-29), 3.73 and 3.28 (each 1H, *ABq*, *J* = 10.9 Hz, H_2-28), 3.70 (1H, ddd, *J* = 11.3, 9.4, 4.6 Hz, H-2), 2.74 (1H, d, *J* = 9.4 Hz, H-3), 1.68 (3H, s, Me-30), 1.09 (3H, s, Me-23), 1.07 (3H, s, Me-26), 1.00 (3H, s, Me-27), 0.92 (3H, s, Me-25), 0.87 (3H, s, Me-24); For ^{1}H NMR data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1017.5709 $[M+H]^+$ (calculated for $C_{51}H_{85}O_{20}$, 1017.5634).

3.11. Compound 6

Amorphous powder; $[\alpha]_D^{25}$ −31.6° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3365 (OH), 2925 (CH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.71 and 4.58 (each 1H, br s, H_2-29), 3.70 (1H, *m*, H-2), 3.55 and 3.38 (each 1H, *ABq*, *J* = 9.2 Hz, H_2-28), 2.93 (1H, d, *J* = 9.3 Hz, H-3), 1.69 (3H, s, Me-30), 1.09 (3H, s, Me-23), 1.07 (3H, s, Me-26), 1.00 (3H, s, Me-27), 0.93 (3H, s, Me-25), 0.86 (3H, s, Me-24); For ^{1}H NMR data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1493.7139 $[M+Na]^+$ (calculated for $C_{69}H_{114}O_{33}Na$, 1493.7140).

3.12. Compound 7

Amorphous powder; $[\alpha]_D^{25}$ −29.2° (*c* 0.10, MeOH); IR ν_{max} (film) cm^{-1}: 3367 (OH), 2927 (CH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.70 and 4.57 (each 1H, br s, H_2-29), 3.54 and 3.42 (each 1H, *ABq*, *J* = 13.7 Hz, H_2-28), 3.11 (1H, dd, *J* = 11.2, 5.3 Hz, H-3), 1.68 (3H, s, Me-30), 1.03 (3H, s, Me-23), 1.07 (3H, s, Me-26), 1.00 (3H, s, Me-27), 0.86 (3H, s, Me-25), 0.80 (3H, s, Me-24); For ^{1}H NMR data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, CD_3OD) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1477.7146 $[M+Na]^+$ (calculated for $C_{69}H_{114}O_{32}$ Na, 1477.7191).

3.13. Acid hydrolysis of 2–7

Compounds **2** (6.1 mg), **3** (14.2 mg), **4** (10.4 mg), **5** (6.8 mg), **6** (8.0 mg), and **7** (6.0 mg) were independently subjected to acid hydrolysis as described for **1** to give aglycones (**1a**: 0.7 and 0.8 mg from **2** and **3**, respectively) and sugar fractions (**2**: 3.5 mg, **3**: 6.1 mg, **4**: 5.4 mg, **5**: 2.1 mg, **6**: 2.4 mg, and **7**: 3.2 mg). The aglycons of **4**–**7** were decomposed under acidic conditions. HPLC analysis of the sugar fractions under the same conditions as in the case of **1** showed the presence of L-rhamnose, L-arabinose, D-xylose, and D-glucose in those of **2**–**7**.

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