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Triterpene glycosides from the aerial parts of *Larrea tridentata*

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ABSTRACT

Chemical study of the aerial parts of *Larrea tridentata* (Zygophyllaceae) resulted in the isolation of 25 triterpene glycosides, 13 of which were previously unknown. Their structures were determined on the basis of comprehensive spectroscopic analyses, including 2D NMR spectroscopy, and hydrolytic cleavage followed by chromatographic and spectroscopic analyses. This is the first systematic phytochemical study of *L. tridentata* with attention paid to its triterpene glycoside constituents. The isolated compounds were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells.

1. Introduction

Larrea tridentata (Sesse. & Moc. Ex DC.) Coville. (Zygophyllaceae) is an evergreen shrub that grows in the desert areas of the Americas. The aerial parts (leaves and stems) of this plant are called Chaparral and are an alternative herbal medicine used for the treatment of various cancers, tuberculosis, menstrual pains, and diabetes in the United States (Lambert et al., 2005). Fragmentary phytochemical studies have been carried out on *L. tridentata*, and lignans such as nordihydroguaiaretic acid, flavonoids, and triterpenes (Abou-Gazar et al., 2004) have been isolated and identified. The present investigation of the aerial parts of *L. tridentata*, with particular attention paid to its triterpene glycoside constituents, resulted in the isolation of 25 triterpene glycosides, 13 of which were found to be new compounds. This paper is a report on the structural determination of the new compounds on the basis of comprehensive spectroscopic analyses, including 2D NMR spectroscopy, and hydrolytic cleavage followed by chromatographic and spectroscopic analyses. The isolated compounds were evaluated for their cytotoxic activity against HL-60 human promyelocytic leukemia cells.

2. Results and discussion

2.1. Structure elucidation

The aerial parts of *L. tridentata* (3.0 kg) were extracted with MeOH under conditions of reflux. The concentrated MeOH extract (940 g) was subjected to porous-polymer polystyrene resin (Diaion HP-20) chromatography and successively eluted with MeOH in H_2O (3:7) MeOH–H_2O (1:1), MeOH, EtOH, and EtOAc. The MeOH eluate fraction (477 g), in which triterpene glycosides were enriched, was subjected to silica gel column chromatography and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, yielding compounds **1**–**25**. By comparison of the physical and spectroscopic data with literature values, **3**–**8**, **11**, and **21**–**25** were identified as 3-[(*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**3**; guaianin N) (Stolyarenko et al., 2000), 3-[(*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid β-D-glucopyranosyl ester (**4**; guaiacin B) (Ahmad et al., 1989), 3-[(*O*-β-D-xylopyranosyl-(1→3)-β-D-glucuronopyranosyl)oxy]olean-12-en-28-oic acid β-D-glucopyranosyl ester (**5**; quinoside D) (Mizui et al., 1990), 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid (**6**; patriniaglycoside B-II) (Shao et al., 1989), 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid β-D-glucopyranosyl ester (**7**; nudicaucin C) (Konishi et al., 1998), 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**8**; mateglycoside A) (Sugimoto et al., 2009), 3-[(β-D-xylopyranosyl)oxy]olean-12-ene-28,29-dioic acid (**11**) (Yu et al., 1995), 3β-hydroxy-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester (**21**) (Li et al., 2009), 3-[(*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester (**22**; guaiacin A) (Ahmad et al., 1989), 3-[(*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester (**23**; guaianin A2) (Ahmad

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Table 1
^{1}H and ^{13}C NMR chemical shift assignments for the sugar moieties of compounds **1**, **2**, **9**, **10**, **and 12–20** in C_5D_5N.

1				**2**				**9**				**10**				**12**				**13**				**14**			
		^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C
Ara	1	4.74 *d* (7.4)	107.4	Ara	1	5.24 *d* (3.8)	103.7	Xyl	1	4.83 *d* (7.5)	107.6	Xyl	1	4.78 *d* (7.5)	107.1	Ara	1	4.77 *d* (7.5)	107.4	Ara	1	4.79 *d* (7.4)	107.5	Ara	1	4.73 *d* (7.5)	107.5
	2	4.54 *dd* (9.1, 7.4)	71.8		2	5.54 *dd* (3.8, 3.8)	75.8		2	4.02 *dd* (8.7, 7.5)	75.5		2	4.04 *dd* (8.6, 7.5)	74.2		2	4.61 *dd* (9.0, 7.5)	72.0		2	4.40 *dd* (8.4, 7.4)	72.9		2	4.56 *dd* (9.2, 7.5)	71.8
	3	4.18 *dd* (9.1, 3.2)	84.1		3	4.71 *br s*	79.9		3	4.17 *dd* (8.7, 8.7)	78.6		3	4.14 *dd* (8.6, 8.6)	88.5		3	4.24 *m*	84.2		3	4.18 *m*	74.7		3	4.22 *dd* (9.2, 3.3)	83.9
	4	4.39 *br s*	69.3		4	4.62 *m*	67.0		4	4.24 *m*	71.3		4	4.11 *m*	69.6		4	4.45 *br s*	69.4		4	4.33 *br s*	69.6		4	4.44 *br s*	69.4
	5a	4.20 *br d* (11.5)	67.0		5a	4.30 *br d* (8.9)	63.3		5a	4.39 *br d* (11.5)	67.1		5a	4.33 *dd* (11.1, 4.9)	66.4		5a	4.23 *br d* (12.0)	67.0		5a	4.32 *br d* (11.1)	66.8		5a	4.20 *br d* (11.4)	67.1
	5b	3.77 *br d* (11.5)			5b	3.78 *br d* (8.9)			5b	3.78 *dd* (11.5, 10.9)			5b	3.69 *dd* (11.1, 10.5)			5b	3.76 *br d* (12.0)			5b	3.84 *br d* (11.1)			5b	3.75 *br d* (11.4)	
Glc	1	5.29 *d* (7.8)	105.9	Glc	1	5.21 *d* (7.7)	104.4	Glc	1	6.36 *d* (8.1)	95.8	Glc	1	5.32 *d* (7.8)	105.8	Glc	1	5.41 *d* (8.0)	106.4	Glc	1	6.30 *d* (8.1)	95.8	Glc	1	5.43 *d* (7.8)	106.1
	2	4.03 *dd* (8.7, 7.8)	75.6		2	4.00 *dd* (8.4, 7.7)	74.7		2	4.23 *dd* (8.9, 8.1)	74.1		2	4.09 *dd* (8.8, 7.8)	75.5		2	4.05 *dd* (8.3, 8.0)	75.8		2	4.20 *dd* (8.8, 8.1)	74.1		2	4.06 *dd* (9.5, 7.8)	73.5
	3	4.42 *dd* (9.4, 8.7)	76.8		3	4.18 *dd* (9.5, 8.4)	77.3		3	4.31 *dd* (8.9, 8.9)	78.9		3	4.26 *dd* (8.8, 8.8)	78.3		3	4.27 *dd* (8.5, 8.3)	78.4		3	4.28 *dd* (8.8, 8.8)	78.9		3	5.87 *dd* (9.5, 9.5)	79.3
	4	5.23 *dd* (9.4, 9.4)	75.8		4	4.16 *dd* (9.5, 8.9)	70.9		4	4.39 *dd* (9.3, 8.9)	71.1		4	4.22 *dd* (9.1, 8.8)	71.7		4	4.24 *dd* (8.5, 8.0)	71.6		4	4.34 *dd* (8.8, 9.2)	71.1		4	4.32 *dd* (9.5, 9.6)	69.3
	5	3.84 *br d* (9.4)	76.4		5	3.85 *m*	78.1		5	4.06 *m*	79.4		5	4.04 *m*	78.7		5	4.01 *m*	78.7		5	4.02 *m*	79.3		5	3.96 *br d* (9.6)	78.4
	6a	4.50 *dd* (11.7, 3.3)	61.6		6a	4.37 *dd* (11.2, 3.4)	61.7		6a	4.50 *dd* (11.9, 2.3)	62.2		6a	4.54 *dd* (11.6, 2.2)	62.5		6a	4.56 *dd* (12.0, 2.0)	62.7		6a	4.46 *br d* (11.7)	62.3		6a	4.49 *dd* (12.0, 2.1)	62.0
	6b	4.30 *dd* (11.7, 2.7)			6b	4.30 *dd* (11.2, 5.9)			6b	4.43 *dd* (11.9, 4.4)			6b	4.32 *dd* (11.6, 5.5)			6b	4.40 *dd* (12.0, 5.0)			6b	4.39 *br d* (11.7)			6b	4.40 *br d* (12.0)	
Glc′	1	6.34 *d* (8.0)	95.8	Glc′	1	6.31 *d* (8.1)	95.7																	Ac		1.95 s	21.2
	2	4.21 *dd* (9.0, 8.0)	74.1		2	4.20 *dd* (9.0, 8.1)	74.1																				170.8
	3	4.29 *dd* (9.0, 9.0)	78.9		3	4.28 *dd* (9.0, 9.0)	78.9																				
	4	4.37 *dd* (9.0, 9.0)	71.1		4	4.35 *dd* (9.0, 9.0)	71.1																	Glc′	1	6.29 *d* (8.2)	95.8
	5	4.04 *m*	79.3		5	4.03 *br d* (9.2)	79.3																		2	4.19 *dd* (8.8, 8.2)	74.1
	6a	4.48 *dd* (11.7, 2.1)	62.2		6a	4.46 *dd* (12.2, 2.2)	62.2																		3	4.28 *dd* (8.8, 8.8)	78.8
	6b	4.42 *dd* (11.7, 2.9)			6b	4.40 *dd* (12.2, 4.1)																			4	4.33 *dd* (9.1, 8.8)	71.2
																									5	4.02 *br d* (9.1)	79.3
																									6a	4.46 *dd* (12.2, 2.7)	62.3
																									6b	4.38 *br d* (12.2)	

15				**16**				**17**				**18**				**19**				**20**			
		^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C			^{1}H	^{13}C
Ara	1	4.77 *d* (7.5)	107.5	Ara	1	4.74 *d* (7.5)	107.3	Ara	1	4.81 *d* (6.0)	105.1	Ara	1	4.88 *d* (5.4)	104.7	Ara	1	4.87 *d* (5.4)	104.6	Ara	1	4.88 *d* (5.5)	104.6
	2	4.60 *dd* (8.3, 7.5)	71.8		2	4.53 *dd* (8.5, 7.5)	71.8		2	4.61 *dd* (6.0, 8.0)	74.8		2	4.65 *dd* (6.3, 5.4)	74.8		2	4.65 *dd* (6.3, 5.4)	74.7		2	4.66 *dd* (6.4, 5.5)	74.8
	3	4.22 *m*	84.1		3	4.19 *m*	84.0		3	4.21 *m*	82.6		3	4.34 *m*	81.9		3	4.34 *m*	81.9		3	4.34 *m*	82.0
	4	4.49 *br s*	69.4		4	4.37 *br s*	69.2		4	4.45 *br s*	68.6		4	4.53 *br s*	68.1		4	4.53 *br s*	68.1		4	4.54 *br s*	68.2
	5a	4.28 *br d* (11.0)	67.1		5a	3.77 *br d* (11.6)	67.0		5a	4.19 *br d* (10.5)	65.5		5a	3.75 *br d* (9.7)	64.7		5a	3.74 *dd* (11.5, 1.9)	64.7		5a	3.75 *dd* (9.7, 1.4)	64.8
	5b	3.83 *br d* (11.0)			5b	4.19 *br d* (11.6)			5b	3.73 *br d* (10.5)			5b	4.22 *dd* (9.7, 5.0)			5b	4.23 *br d* (11.5)			5b	4.24 *br d* (9.7)	

Glc	1	5.35 *d* (8.0)	106.2	Glc	1	5.29 *d* (7.8)	105.8	Rha	1	6.15 *s*	101.7	Rha	1	6.14 *s*	101.9	Rha	1	6.14 *s*	101.9	Rha	1	6.15 *s*	102.0
	2	4.00 *dd* (9.0, 8.0)	75.5		2	4.07 *dd* (8.6, 7.8)	75.4		2	4.74 *br d* (2.0)	72.3		2	4.73 *br s*	72.4		2	4.73 *br s*	72.4		2	4.73 *br s*	72.4
	3	4.22 *dd* (9.5, 9.0)	78.1		3	4.40 *dd* (9.1, 8.6)	76.6		3	4.60 *dd* (9.5, 2.0)	72.3		3	4.60 *dd* (9.4, 3.7)	72.5		3	4.60 *dd* (9.3, 3.3)	72.5		3	4.60 *dd* (9.2, 2.9)	72.6
	4	4.03 *dd* (9.5, 9.5)	75.3		4	5.24 *dd* (9.4, 9.1)	75.7		4	4.24 *dd* (10.0, 9.5)	73.9		4	4.28 *dd* (9.8, 9.4)	73.9		4	4.28 *dd* (9.4, 9.3)	73.9		4	4.28 *dd* (9.9, 9.2)	73.9
	5	4.04 *m*	75.5		5	3.81 *br d* (9.4)	76.3		5	4.60 *dq* (10.0, 6.0)	69.9		5	4.60 *dd* (9.8, 6.1)	70.1		5	4.59 *dq* (9.4, 6.1)	70.1		5	4.59 *dq* (9.9, 6.2)	70.1
	6a	4.92 *br d* (11.5)	64.7		6a	4.49 *dd* (12.6, 2.6)	61.4		6	1.60 *d* (6.0)	18.5		6	1.64 *d* (6.1)	18.6		6	1.64 *d* (6.1)	18.6		6	1.64 *d* (6.2)	18.6
	6b	4.75 *dd* (11.5, 5.5)			6b	4.27 *br d* (12.6)																	
								Glc	1	5.02 *d* (7.5)	104.5	Glc	1	5.11 *d* (7.7)	104.6	Glc	1	5.11 *d* (7.7)	104.6	Glc	1	5.12 *d* (7.8)	104.6
Ac		1.97 *s*	20.7	Glc′	1	6.28 *d* (8.1)	95.8		2	3.89 *dd* (9.0, 7.5)	74.8		2	3.96 *dd* (8.3, 7.7)	74.9		2	3.96 *dd* (8.3, 7.7)	75.0		2	3.96 *dd* (8.2, 7.8)	75.0
			170.8		2	4.19 *dd* (8.7, 8.1)	74.1		3	4.32 *dd* (9.0, 9.5)	76.5		3	4.16 *dd* (8.8, 8.3)	78.2		3	4.19 *dd* (8.8, 8.3)	78.2		3	4.18 *dd* (9.3, 8.2)	78.2
					3	4.27 *dd* (8.7, 8.7)	78.8		4	5.15 *dd* (9.5, 9.5)	75.7		4	4.19 *dd* (8.8, 6.5)	71.4		4	4.16 *dd* (8.8, 8.7)	71.4		4	4.17 *dd* (9.3, 8.7)	71.5
Glc′	1	6.30 *d* (8.0)	95.8		4	4.33 *dd* (9.1, 8.7)	71.2		5	3.79 *br d* (9.5)	76.3		5	3.91 *m*	78.6		5	3.93 *m*	78.6		5	3.93 *m*	78.6
	2	4.19 *dd* (8.8, 8.0)	74.1		5	4.01 *br d* (9.1)	79.3		6a	4.47 *br d* (13.0)	61.5		6a	4.47 *dd* (11.4, 1.9)	62.5		6a	4.50 *dd* (11.8, 2.2)	62.5		6a	4.50 *dd* (11.6, 2.2)	62.5
	3	4.28 *dd* (9.0, 8.8)	78.8		6a	4.45 *dd* (11.1, 2.5)	62.3		6b	4.28 *br d* (13.0)			6b	4.33 *br d* (11.4)			6b	4.33 *br d* (11.8)			6b	4.34 *br d* (11.6)	
	4	4.34 *dd* (9.0, 9.0)	71.2		6b	4.38 *dd* (11.1, 4.0)																	
	5	4.04 *m*	79.3					Glc′	1	6.30 *d* (8.0)	95.9	Glc′	1	6.22 *d* (8.1)	95.8	Glc′	1	6.34 *d* (8.1)	95.8	Glc′	1	6.35 *d* (8.1)	95.8
	6a	4.46 *br d* (11.0)	62.3						2	4.19 *dd* (9.0, 8.0)	74.1		2	4.12 *dd* (8.7, 8.1)	73.8		2	4.23 *dd* (8.5, 8.1)	74.1		2	4.22 *dd* (8.7, 8.1)	74.1
	6b	4.39 *dd* (11.0, 3.0)							3	4.28 *dd* (9.0, 9.0)	78.9		3	4.21 *dd* (8.7, 8.5)	78.7		3	4.30 *dd* (9.2, 8.5)	78.9		3	4.30 *dd* (9.1, 8.7)	78.9
									4	4.34 *dd* (9.0, 9.5)	71.2		4	4.32 *dd* (9.3, 8.5)	70.9		4	4.37 *dd* (9.2, 9.2)	71.1		4	4.37 *dd* (9.1, 9.0)	71.1
									5	4.02 *br d* (9.5)	79.3		5	4.09 *m*	77.9		5	4.03 *m*	79.4		5	4.06 *m*	79.3
									6a	4.46 *dd* (12.0, 2.5)	62.3		6a	4.70 *br d* (10.5)	69.5		6a	4.45 *dd* (11.9, 2.3)	62.1		6a	4.49 *dd* (11.9, 2.5)	62.3
									6b	4.39 *dd* (12.0, 4.5)			6b	4.31 *br d* (10.5)			6b	4.39 *dd* (11.9, 4.1)			6b	4.42 *dd* (11.9, 4.4)	
												Glc″	1	5.01 *d* (7.8)	105.3								
													2	4.01 *dd* (8.2, 7.8)	75.1								
													3	4.19 *dd* (8.3, 8.2)	78.5								
													4	4.22 *dd* (8.3, 8.2)	71.5								
													5	3.90 *m*	78.5								
													6a	4.49 *dd* (11.8, 2.4)	62.7								
													6b	4.36 *dd* (11.8, 5.2)									

et al., 1988), 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid (**24**; guaiacin C) (Ahmad et al., 1990), and 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester (**25**; guaiacin D) (Ahmad et al., 1990), respectively.

Compound **1** was obtained as an amorphous solid. Its molecular formula was determined to be $C_{47}H_{75}NaO_{20}S$ by HRESI-TOFMS (*m*/*z* 1037.4329 [M + Na]$^+$). The ^{1}H NMR spectrum of **1** was typical of a triterpene triglycoside based upon 3β-hydroxyolean-12-en-28-oic acid, exhibiting seven three-proton singlet signals at δ 1.29, 1.27, 1.11, 0.96, 0.92, 0.90, and 0.87, an olefinic proton signal at δ 5.44 (*br s*), and three anomeric proton (H-1) signals at δ 6.34 (*d*, *J* = 8.0 Hz), 5.29 (*d*, *J* = 7.8 Hz), and 4.74 (*d*, *J* = 7.4 Hz). Acid hydrolysis of **1** with 1.0 M HCl in dioxane–H_2O (1:1) gave 3β-hydroxyolean-12-en-28-oic acid (**1a**) as the aglycone, and L-arabinose and D-glucose as the carbohydrate moieties. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate using an optical rotation (OR) detector. Furthermore, when 0.5 M $BaCl_2$ aqueous solution was added to the hydrolysate, it gave white precipitate, indicating the presence of sulfate ion. From the above spectroscopic and chemical data, together with the information from the IR spectrum of **1**, exhibiting the strong absorption at 1259 cm^{-1} (S = O), **1** was deduced to be an olean-12-en-28-oic acid triglycoside with a sodium sulfate group. Analyses of the ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) suggested that the sugar moieties and their linkage positions to the aglycone of **1** were the same as those of the concomitantly isolated compound (**4**) in this study. This was ascertained by HMBC correlations between H-1 of one β-D-glucopyranosyl unit (Glc) at δ_H 5.29 and C-3 of α-L-arabinopyranosyl unit (Ara) at δ_C 84.1, H-1 of the other β-D-glucopyranosyl unit (Glc′) at δ_H 6.34 and C-28 of the aglycone at δ_C 176.5, and between H-1 of Ara at δ_H 4.74 and C-3 of the aglycone at δ_C 88.7. The sulfate group was shown to be involved in a linkage at C-4 of Glc because the signals assignable to H-4 and C-4 were markedly displaced downfield at δ_H 5.23 (*dd*, *J* = 9.4, 9.4 Hz; +0.86 ppm) and δ_C 75.8 (+4.2 ppm), respectively, when the ^{1}H and ^{13}C NMR spectra of **1** were compared with those of **4**. Accordingly, the structure of **1** was determined to be 3-[(*O*-(4-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid β-D-glucopyranosyl ester sodium salt.

Compound **2** was shown to have a molecular formula of $C_{47}H_{75}NaO_{20}S$ on the basis of HRESI-TOFMS, which was the same as that of **1**. The spectroscopic properties of **2** were essentially analogous to those of **1**, and acid hydrolysis of **2** furnished **1a**, L-arabinose, and D-glucose, together with sulfuric acid. These data implied that **2** was a positional isomer of **1** in regard to the sulfate group. When the ^{1}H and ^{13}C NMR spectra of **2** were compared with those of **4**, the signals attributable to H-2 and C-2 were moved downfield at δ_H 5.54 (*dd*, *J* = 3.8, 3.8 Hz; +0.94 ppm) and δ_C 75.8 (+3.9 ppm), respectively. Accordingly, the structure of **2** was determined to be 3-[(*O*-β-D-glucopyranosyl-(1→3)-2-*O*-sulfo-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid β-D-glucopyranosyl ester sodium salt. The small $^3J_{H\text{-}1,H\text{-}2}$ coupling constant (3.8 Hz), as well as HMBC correlations between H-1 at δ_H 5.24 and C-3 at δ_C 79.9/C-5 at δ_C 63.3 suggested that the arabinopyranosyl moiety of **2** had a $^1C_4$ conformation to reduce the steric hindrance caused by the C-2 sulfate group. However, in the NOESY spectrum of **2**, H-1 showed NOE correlations with H-2, H-3 at δ 4.71 (*br s*), and H-5ax (H-5a) at δ 3.78 (*br d*, *J* = 8.9 Hz) (Fig. 1). These data indicated that the arabinopyranosyl group is present as the $^1C_4$ and $^4C_1$ forms in equilibrium with rapid conformational exchange (Kuroda et al., 2002).

Compounds **9** and **10** had the same molecular formula of $C_{41}H_{64}O_{14}$ as determined by their HRESI-TOFMS data, and gave the common hydrolysates 3β-hydroxyolean-12-ene-28,29-dioic acid (**9a**; seratagenic acid) (Yu et al., 1995), D-glucose, and D-xylose on acid hydrolysis. These results, as well as ^{1}H and ^{13}C NMR

Table 2
^{13}C NMR chemical shift assignments for the aglycone moiety of compounds **1**, **2**, **9**, **10**, **and 12–20** in C_5D_5N.

Position	1	2	9	10	12	13	14	15	16	17	18	19	20
1	38.8	38.9	38.8	38.7	38.8	38.8	38.8	38.8	38.8	39.1	39.0	39.0	39.0
2	26.7	26.4	26.7	26.7	26.7	26.6	26.7	26.7	26.7	26.7	26.6	26.6	26.6
3	88.7	88.4	88.6	88.7	88.7	88.7	88.8	88.7	88.7	88.3	88.2	88.1	88.2
4	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6	39.6
5	55.8	55.7	55.8	55.8	55.9	55.8	55.8	55.9	55.8	56.0	56.0	56.0	56.0
6	18.5	18.4	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5
7	32.5	32.5	33.1	33.1	33.2	33.1	33.1	33.1	33.1	33.1	33.1	33.1	33.1
8	39.9	39.9	39.9	39.8	39.8	39.9	39.9	39.9	39.9	39.9	39.9	39.9	39.9
9	48.0	48.0	48.0	48.0	48.0	48.0	48.0	48.0	48.0	48.0	48.0	48.1	48.0
10	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0
11	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.7	23.8	23.7
12	122.9	122.8	123.1	123.1	123.0	123.1	123.1	123.1	123.1	123.1	123.1	122.5	123.1
13	144.1	144.1	143.6	144.3	144.1	143.4	143.4	143.4	143.4	143.4	143.4	144.3	143.6
14	42.1	42.1	42.1	42.2	42.1	42.1	42.1	42.1	42.1	42.1	42.1	42.2	42.1
15	28.3	28.2	28.2	28.3	28.3	28.2	28.1	28.2	28.2	28.2	28.2	28.4	28.2
16	23.4	23.4	23.5	23.8	23.8	23.5	23.5	23.5	23.5	23.6	23.5	23.3	23.6
17	47.0	47.0	46.9	46.6	47.0	47.3	47.3	47.3	47.3	47.3	47.3	46.8	47.1
18	41.7	41.7	40.8	41.1	47.9	47.6	47.6	47.6	47.6	47.6	47.5	41.1	44.1
19	46.2	46.2	40.8	41.1	42.0	41.7	41.7	41.7	41.7	41.7	41.7	46.2	47.8
20	30.8	30.7	42.3	42.6	149.1	148.5	148.5	148.5	148.4	148.5	148.4	67.9	69.6
21	34.0	34.0	29.1	29.3	30.4	30.1	30.1	30.1	30.1	30.1	30.1	34.2	35.9
22	33.1	33.1	31.7	32.4	38.4	37.6	37.6	37.6	37.6	37.6	37.6	31.9	34.5
23	28.1	28.3	28.2	28.0	28.2	28.2	28.1	28.1	28.1	28.1	28.1	28.1	28.1
24	16.9	17.0	17.0	17.0	17.0	16.9	16.9	17.0	16.9	17.0	17.0	17.0	17.0
25	15.6	15.6	15.6	15.5	15.5	15.6	15.5	15.6	15.6	15.7	15.7	15.6	15.7
26	17.5	17.4	17.5	17.4	17.3	17.5	17.4	17.5	17.4	17.5	17.5	17.5	17.5
27	26.1	26.1	26.0	26.1	26.1	26.0	26.0	26.0	26.0	26.0	26.0	26.1	26.0
28	176.5	176.5	176.2	179.9	179.4	175.7	175.7	175.7	175.8	175.7	175.8	176.5	176.3
29	33.1	33.1	180.9	181.0	107.1	107.3	107.3	107.3	107.3	107.3	107.3	32.0	
30	23.7	23.6	19.9	20.0									25.6

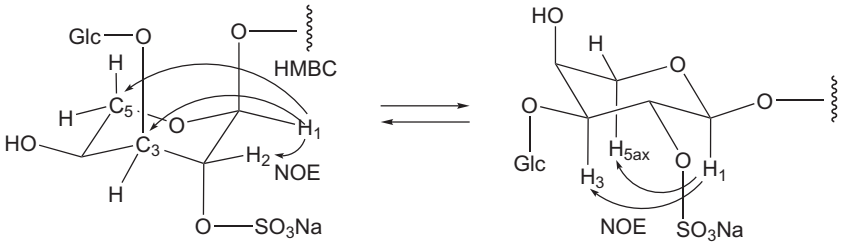
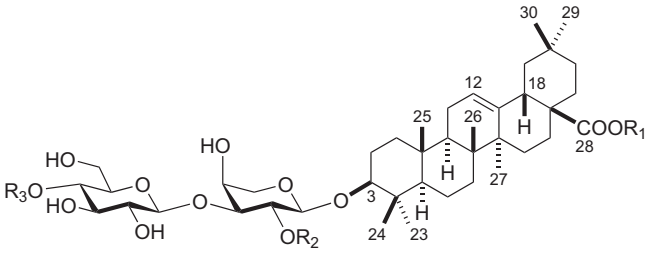


Fig. 1. HMBC and NOE correlations of the arabinopyranosyl moiety of **2**.

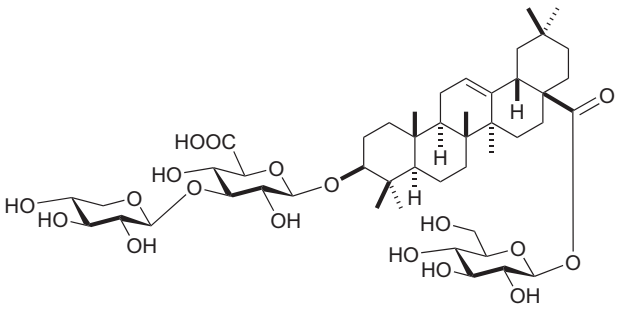
spectroscopic data, indicated that **9** and **10** were closely related triterpene diglycoside and had a β-D-glucopyranosyl unit (Glc) [$\delta_{H\text{-}1}$ 6.36 (*d*, *J* = 8.1 Hz) in **9**; $\delta_{H\text{-}1}$ 5.32 (*d*, *J* = 7.8 Hz) in **10**] and a β-D-xylopyranosyl unit (Xyl) [$\delta_{H\text{-}1}$ 4.83 (*d*, *J* = 7.5 Hz) in **9**; $\delta_{H\text{-}1}$ 4.78 (*d*, *J* = 7.5 Hz) in **10**] in each molecule. The C-3 oxymethine carbon and C-28 carbonyl carbon were observed at δ 88.6 and 176.2, respectively, in the ^{13}C NMR spectrum of **9**, which suggested that **9** was a bisdesmosidic triterpene. In the HMBC spectrum of **9**, long-range correlations were observed between H-1 of Glc at δ_H 6.36 and C-28 of the aglycone at δ_C 176.2, and between H-1 of Xyl at δ_H 4.83 and C-3 of the aglycone at δ_C 88.6. On the other hand, the HMBC spectrum of **10** showed long-range correlations between H-1 of Glc at δ_H 5.32 and C-3 of Xyl at δ_C 88.5, and between H-1 of Xyl at δ_H 4.78 and C-3 of the aglycone at δ_C 88.7. The structures of **9** and **10** were assigned as 3-[(β-D-xylopyranosyl)oxy]olean-12-ene-28,29-dioic acid 28-β-D-glucopyranosyl ester and 3-[(*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl)oxy]olean-12-ene-28,29-dioic acid, respectively.

Compounds **12** and **13** had the same molecular formula of $C_{40}H_{62}O_{12}$ on the basis of HRESI-TOFMS, and gave D-glucose and L-arabinose on acid hydrolysis. The ^{1}H NMR spectrum of **12** showed signals for an exomethylene group at δ 4.82 and 4.76 (each *s*), as well as signals for five tertiary methyl groups at δ 1.33, 1.29, 0.99 × 2, and 0.84 (each *s*), an olefinic proton at δ 5.50 (*br s*), and two anomeric protons at δ 5.41 (*d*, *J* = 8.0 Hz) and 4.77 (*d*, *J* = 7.5 Hz), which were characteristic of a triterpene glycoside with the 3β-hydroxy-30-norolean-12-en-28-oic acid framework. The molecular formula of **12** was less than that of the known 30-nortriterpene bisdesmoside (**22**) by $C_6H_{10}O_5$, corresponding to the lack of one hexose. Alkaline treatment of **22** with 4% KOH in EtOH yielded **12**. Thus, the structure of **12** was elucidated as 3-[(*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid. Compound **13** was an isomer of **12** in regard to the monosaccharide linkage positions. In the HMBC spectrum of **13**, H-1 of an α-L-arabinopyranosyl unit at δ_H 4.79 (*d*, *J* = 7.4 Hz) showed a long-range correlation with C-3 of the 30-nortriterpene aglycone at δ_C 88.7, whereas H-1 of a β-D-glucopyranosyl unit at δ_H 6.30 (*d*, *J* = 8.1 Hz) had a correlation with C-28 of the aglycone at δ_C 175.7. The structure of **13** was identified as 3-[(α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester.

Compounds **14** and **15**, which had the same molecular formula of $C_{48}H_{74}O_{18}$, were established to contain an acetyl group in each molecule by examination of the ^{1}H and ^{13}C NMR spectra [δ_H 1.95 (3H, *s*)/δ_C 170.8 (C = O) and 21.2 (Me) in **14**; δ_H 1.97 (3H, *s*)/δ_C 170.8 (C = O) and 20.7 (Me) in **15**]. Alkaline hydrolysis of **14** and **15** gave the same hydrolysate (**22**). When the ^{1}H NMR spectra of **14** and **15** were compared with that of **22**, H-3 of the β-D-glucopyranosyl unit (Glc) attached to C-3 of the α-L-arabinopyranosyl unit was shifted to a lower field by 1.6 ppm in **14**, while H-6a and H-6b of Glc were moved downfield by 0.46 and 0.36 ppm, respectively, in **15**. Furthermore, the carbonyl carbon of the acetyl group of **14** at δ_C 170.8 showed a long-range correlation with H-3 of Glc at



	R_1	R_2	R_3
1	Glc	H	SO_3Na
2	Glc	SO_3Na	H
3	H	H	H
4	Glc	H	H
6	H	Rha	H
7	Glc	Rha	H
8	Glc-(1→6)-Glc	Rha	H



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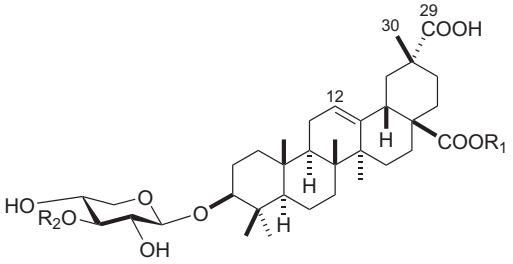


	R_1	R_2
9	Glc	H
10	H	Glc
11	H	H

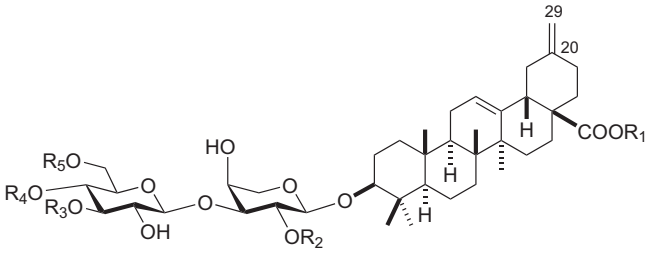
δ_H 5.87 (*dd*, *J* = 9.5, 9.5 Hz), and that of **15** at δ_C 170.8 had correlations with H_2-6 at δ_H 4.92 (*br d*, *J* = 11.5 Hz) and 4.75 (*dd*, *J* = 11.5,

5.5 Hz) in the HMBC spectra of **14** and **15**. Thus, the structures of **14** and **15** were formulated as 3-[(*O*-(3-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester and 3-[(*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester, respectively.

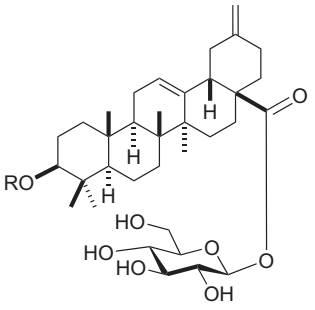
Compound **16** exhibited a molecular formula of $C_{46}H_{71}NaO_{20}S$ on the basis of HRESI-TOFMS. The ^{1}H NMR spectroscopic features of **16** were essentially analogous to those of **22**, showing signals for an exomethylene group at δ 4.77 and 4.70 (each *s*), as well as resonances for five tertiary methyl groups at δ 1.29, 1.25, 1.09, 0.95, and 0.86 (each *s*), an olefinic proton at δ 5.45 (*br s*), and three anomeric protons at δ 6.28 (*d*, *J* = 8.1 Hz), 5.29 (*d*, *J* = 7.8 Hz), and 4.74 (*d*, *J* = 7.5 Hz). The presence of a sodium sulfate group in **16** was shown by not only the deduced molecular formula, but also the prominent absorption at 1259 cm^{-1} in the IR spectrum and the results of acid hydrolysis, giving sulfuric acid, as well as D-glucose and L-arabinose. HMBC correlations between H-1 of one β-D-glucopyranosyl unit (Glc) at δ_H 5.29 and C-3 of α-L-arabinopyranosyl unit (Ara) at δ_C 84.0, H-1 of the other β-D-glucopyranosyl unit (Glc′) at δ_H 6.28 and C-28 of the aglycone at at δ_C 175.8, and between H-1 of Ara at δ_H 4.74 and C-3 of the aglycone at δ_C 88.7 gave evidence that the sugar moieties of **16** were the same as those of **22**. When the ^{1}H and ^{13}C NMR spectra of **16** were compared with those of **22**, the signals assignable to H-4 and C-4 of Glc were markedly displaced downfield at δ_H 5.24 (*dd*, *J* = 9.4, 9.1 Hz; +0.91 ppm) and δ_C 75.7 (+4.1 ppm), respectively, allowing a sodium sulfate group to be located at the C-4 hydroxy group of Glc in **16**. The structure of **16** was determined to be 3-[(*O*-(4-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester sodium salt.

Compound **17** was found to have a molecular formula of $C_{52}H_{81}NaO_{24}S$ as determined by HRESI-TOFMS, which was higher than that of **16**, with the difference corresponding to a $C_6H_{10}O_4$. The ^{1}H NMR spectrum showed signals for four anomeric protons at δ 6.30 (*d*, *J* = 8.0 Hz), 6.15 (*s*), 5.02 (*d*, *J* = 7.5 Hz), and 4.81 (*d*, *J* = 6.0 Hz), together with signals for the 30-nortriterpene aglycone. On the basis of the spectral properties of **17** and the results of acid hydrolysis experiments, in which **17** yielded D-glucose, L-arabinose, L-rhamnose, and sulfuric acid, **17** was shown to be a triterpene glycoside with a sodium sulfate group closely related to **16**; however, the sugar chain attached to C-3 of the aglycone was made up of three monosaccharides and differed from that of **16** by the presence of an additional α-L-rhamnopyranosyl unit (Rha). In the HMBC spectrum of **17**, H-1 of Rha at δ_H 6.15 exhibited a long-range correlation with C-2 of Ara at δ_C 74.8. HMBC correlations were also observed between H-1 of Glc at δ_H 5.02 and C-3 of Ara at δ_C 82.6, H-1 of Glc′ at δ_H 6.30 and C-28 at δ_C 175.7, and between H-1 of Ara δ_H 4.81 and C-3 of the aglycone at δ_C 88.3. The H-4 and C-4 signals of Glc appeared at δ_H 5.15 (*dd*, *J* = 9.5, 9.5 Hz) and δ_C 75.7, respectively. The structure of **17** was characterized as 3-[(*O*-(4-*O*-sulfo-β-D-glucopyranosyl)-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester sodium salt.

Compound **18** was deduced to be $C_{58}H_{92}O_{26}$ on the basis of HRESI-TOFMS, which was higher than that of **25** by $C_6H_{10}O_5$, corresponding to a hexose unit. The ^{1}H NMR spectrum contained signals for five anomeric protons at δ 6.22 (*d*, *J* = 8.1 Hz), 6.14 (*s*), 5.11 (*d*, *J* = 7.7 Hz), 5.01 (*d*, *J* = 7.8 Hz), and 4.88 (*d*, *J* = 5.4 Hz), together with signals for the 30-nortriterpene aglycone. Acid hydrolysis of **18** gave D-glucose, L-arabinose, and L-rhamnose. These data and comparison of the ^{13}C NMR spectrum of **18** with that of **25** suggested that **18** was related to **25** with an additional β-D-glucopyranosyl unit (Glc″). In the HMBC spectrum of **18**, H-1 of Glc″ at δ_H 5.01 showed a long-range correlation with C-6 of the inner Glc′ at δ_C 69.5, of which H-1 at δ_H 6.22 exhibited a correlation with C-28 of the aglycone at δ_C 175.8. HMBC correlations were also observed between H-1 of Rha at δ_H 6.14 and C-2 of Ara at δ_C 74.8, H-1 of Glc at δ_H 5.11 and C-3 of Ara at δ_C 81.9, and between H-1 of Ara at δ_H 4.88 and C-3 of the aglycone at δ_C 88.2. The structure of **18** was determined to be 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.



	R_1	R_2	R_3	R_4	R_5
12	H	H	H	H	H
14	Glc	H	Ac	H	H
15	Glc	H	H	H	Ac
16	Glc	H	H	SO_3Na	H
17	Glc	Rha	H	SO_3Na	H
18	Glc-(1→6)-Glc	Rha	H	H	H
22	Glc	H	H	H	H
24	H	Rha	H	H	H
25	Glc	Rha	H	H	H

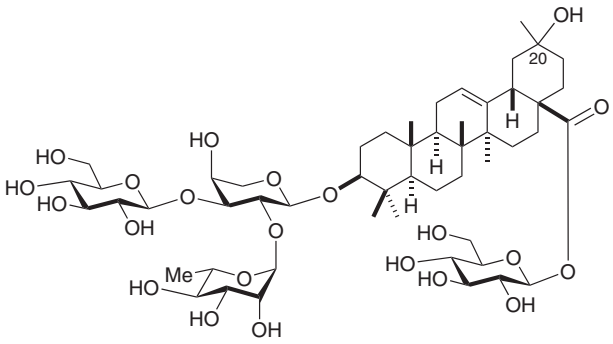


	R
13	Ara
21	H

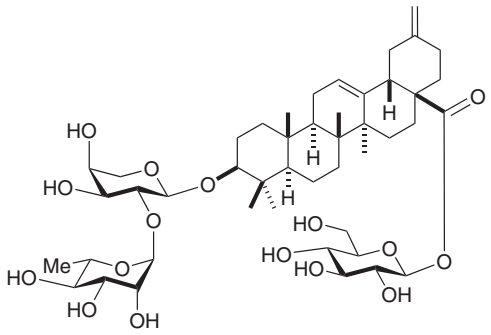
Compound **19** had a molecular formula of $C_{52}H_{84}O_{22}$ on the basis of HRESI-TOFMS. It was shown to be a 30-nortriterpene derivative related to **25**, having the same sugar moieties at C-3 and C-28 of the aglycone as **25** by the ^{1}H and ^{13}C NMR spectra. However, differences were recognized in the NMR signals arising from the E-ring moiety of the hexacyclic skeleton. Instead of the signals for the C-20(29) exomethylene group, signals assignable to a methyl group at δ_H 1.43 (3H, *s*) and δ_C 32.0, and a quaternary carbon bearing an oxygen atom at δ 67.9 were observed. Long-range correlations between δ_H 1.43 (Me-29) and δ_C 46.2 (C-19)/δ_C 67.9 (C-20)/δ_C 34.2 (C-21) in the HMBC spectrum indicated that **19** was a 20(29)-saturated derivative with the introduction of a hydroxy group to C-20. The C-20β configuration of the hydroxy group was determined by an NOE correlation between Me-29 and H-19ax at δ 2.04 (*dd*, *J* = 13.3, 13.3 Hz) in the NOESY spectrum of **19**. The significant downfield shifts of the ^{1}H NMR signals for H-18 and

H-22ax, which were observed at δ 3.80 (*dd*, *J* = 13.4, 4.2 Hz) and 2.57 (*m*), respectively, were ascribed to the 1,3-diaxial interactions with the C-20β hydroxy group. The structure of **19** was assigned as 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-20β-hydroxy-30-norolean-12-en-28-oic acid β-D-glucopyranosyl ester.

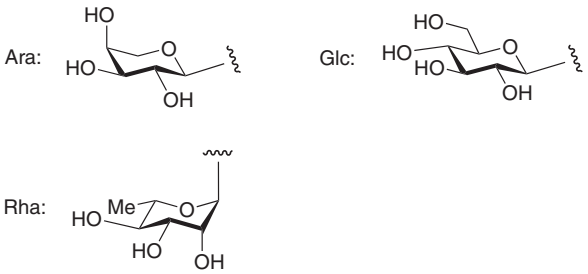
Compound **20** exhibited a molecular formula of $C_{52}H_{84}O_{22}$ on the basis of HRESI-TOFMS, which was the same as that of **19**. The spectroscopic data of **20** was very similar to those of **19** except for the signals arising from C-20 and its neighboring protons and carbons, suggesting that **20** was a stereoisomer of **19** in regard to the C-20 hydroxy group. In the NOESY spectrum of **20**, NOE correlations were detected between Me-30 at δ 1.46 (*s*) and H-18 at δ 3.25 (*dd*, *J* = 13.7, 4.1 Hz)/H-22ax at δ 1.85 (*m*). The structure of **20** was elucidated as 3-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-20α-hydroxy-29-norolean-12-en-28-oic acid β-D-glucopyranosyl ester.



19 20β-OH
20 20α-OH



23



2.2. Cytotoxic activity

The isolated compounds were evaluated for their cytotoxic activity against HL-60 cells (Table 3). Compounds **3**, **6**, **13**, **21**, and **23** showed moderate cytotoxicity with respective IC_{50} values of 18.6, 6.9, 6.0, 10.1, and 4.1 μM, when etoposide and cisplatin (positive controls) had IC_{50} values of 0.33 and 1.1 μM, respectively. The other compounds (**1**, **2**, **4**, **5**, **7**–**12**, **14**–**20**, **22**, **24**, and **25**) were not cytotoxic to HL-60 cells at sample concentrations of 20–100 μM. In the glycosides of 3β-hydroxyolean-12-en-28-oic acid (oleanolic acid), only the monodesmosides with a glucosyl-(1→3)-arabinosyl and a glucosyl-(1→3)-[rhamnosyl-(1→2)]-arabinosyl unit at C-3 of the aglycone (**3** and **6**) exhibited cytotoxic activity against HL-60 cells; however, the corresponding 30-nor-12,20(29)-diene derivatives (akebonoic acid derivatives) (**12** and **24**) were not cytotoxic. On the other hand, akebonoic acid 28-*O*-glucoside (monodesmoside) (**21**), and its 3-*O*-arabinoside (**13**) and 3-*O*-[rhamnosyl-(1→2)-arabinoside] (bisdesmosides) (**23**) showed cytotoxicity against HL-60 cells. The above results suggested that the structures of both the aglycone and sugar moieties contributed to the appearance of cytotoxicity in these glycosides.

Table 3
Cytotoxic activity of compounds **1**–**25** against HL-60 leukemia cells.

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)
1	–[b]	15	–
2	–	16	–
3	18.6 ± 0.12	17	–
4	–	18	–
5	–	19	–
6	6.9 ± 0.11	20	–
7	–	21	10.1 ± 0.20
8	–	22	–
9	–	23	4.1 ± 0.37
10	–	24	–
11	–	25	–
12	–		
13	6.0 ± 1.47	Etoposide	0.33 ± 0.01
14	–	Cisplatin	1.1 ± 0.03

[a] Data represent the mean value ± SEM of three experiments performed in triplicate.
[b] Means IC_{50} >20 μM.

2.3. Conclusions

Fragmentary phytochemical studies of *L. tridentata* (Zygophyllaceae) have been carried out, and lignans, flavonoids, and triterpenes have been isolated and identified (Abou-Gazar et al., 2004). The present investigation of the aerial part of *L. tridentata*, with attention paid to its triterpene glycoside constituents, resulted in the isolation of a total of 25 triterpene glycosides including 13 new compounds. The new compounds (**1**, **2**, **16**, and **17**) are triterpene glycosides with a sulfate group at their sugar moieties. Triterpene glycosides with a sulfate group have been also isolated from other plants belonging to the Zygophyllaceae family such as *Fagonia indica* (Shaker et al., 2000), *F. arabica* (Perrone et al., 2007), and *Zygophyllum fabago* (Feng et al., 2009). Among the 25 compounds, five showed cytotoxic activity, which may partially account for the alternative use of *L. tridentata* to treat cancers.

3. Experimental

3.1. General

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR and CD spectra were recorded on a JASCO FT-IR 620 and a JASCO V-520 spectrophotometer, respectively. NMR spectra were recorded on either a Bruker DPX-400 spectrometer (400 MHz for ^{1}H NMR, Karlsruhe, Germany) or a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR) using standard Bruker pulse programs. Chemical shifts are given as δ

values with reference to tetramethylsilane (TMS) as an internal standard. ESIMS data were obtained on a Waters-Micromass LCT mass spectrometer (Manchester, UK). 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 Silica gel 60 F_{254} (0.25 or 0.5 mm thick, Merck, Darmstadt, Germany) and RP-18 F_{254S} (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 in H_2O followed by heating. HPLC was performed with a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a CCP 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 C18 AQ column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC. The following materials and reagents were used for cell culture assay; microplate reader, Spectra Classic, Tecan (Salzburg, Austria); 96-well flat-bottom plate, Iwaki Glass (Chiba, Japan); HL-60 cells, Human Science Research Resources Bank (JCRB 0085, Osaka, Japan); fetal bovine serum (FBS), Bio-Whittaker (Walkersville, MD, USA); RPMI 1640 medium, etoposide, cisplatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), and 0.25% Tripsin–EDTA solution, Sigma (St. Louis, MO, USA); Dulbecco's modified Eagle medium (DMEM) medium, penicillin G sodium salt, and streptomycin sulfate, Gibco (Grand Island, NY, USA). All other chemicals used were of biochemical reagent grade.

3.2. Plant material

The aerial parts of *L. tridentata* were obtained from Richters, Ontario, Canada. A small amount of the sample is preserved in our laboratory (07-003-LR).

3.3. Extraction and isolation

The aerial parts of *L. tridentata* (3.0 kg of dry weight) were extracted with hot MeOH (77 l). After removing the solvent, the MeOH extract (940 g), which showed cytotoxic activity against HL-60 cells (IC_{50} 6.8 μg/ml), was passed through a Diaion HP-20 column and successively eluted with MeOH–H_2O (3:7), MeOH–H_2O (1:1), MeOH, EtOH, and EtOAc. The MeOH, EtOH, and EtOAc eluate fractions exhibited cytotoxic activity against HL-60 cells (IC_{50} 2.7, 2.4, and 11.0 μg/ml, respectively). Column chromatography (CC) of the MeOH eluate portion (477 g) on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH–H_2O (19:1:0; 9:1:0; 40:10:1), and finally with MeOH alone, gave six fractions (I–VI). Fraction V was subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (9:1:0; 40:10:1) to collect 11 subfractions (V-1–11). Fraction V-2 was applied to ODS silica gel CC eluted with MeCN–H_2O (2:5; 1:2; 1:1) and MeOH–H_2O (2:1), and then silica gel CC with $CHCl_3$–MeOH (9:1) to afford **11** (15.3 mg). Fraction V-9 was subjected to CC on ODS silica gel eluted with MeCN–H_2O (2:5; 1:1) and MeOH–H_2O (3:2), and then silica gel with $CHCl_3$–MeOH–H_2O (40:10:1) to afford **9** (14.0 mg), **10** (13.4 mg), **12** (6.2 mg), **13** (12.6 mg), and **15** (9.4 mg) as pure compounds, and **3** with few impurities. Compound **3** (12.0 mg) was purified by preparative HPLC using MeOH–H_2O (9:1). Fraction VI was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (40:10:1; 20:10:1; 7:4:1) to collect 10 subfractions (VI-1–10). Fraction VI-2 was applied to ODS silica gel CC eluted with MeOH–H_2O (2:1; 4:1) and silica gel CC with $CHCl_3$–MeOH–H_2O (50:10:1; 40:10:1) to afford **14** (28.2 mg) and **23** (4.5 mg). Fraction VI-4 was subjected to CC on ODS silica gel eluted with MeOH–H_2O (7:3; 4:1), and then silica gel with $CHCl_3$–MeOH–H_2O (30:10:1) to afford **4** (71.1 mg), **15** (5.2 mg), **22** (75.4 mg), and **24** (226 mg) as pure compounds, and **6** with few impurities. Compound **6** (2.0 mg) was purified by preparative HPLC using MeOH–H_2O (17:3). Fraction VI-7 was applied to ODS silica gel CC eluted with MeOH–H_2O (11:9; 2:1; 4:1) and MeCN–H_2O (2:5; 1:2; 2:3), and then silica gel CC with $CHCl_3$–MeOH–H_2O (40:10:1; 30:10:1) to give a mixture of **1** and **5**, **7** (23.6 mg), **19** (9.3 mg), **20** (5.0 mg), **21** (7.4 mg), and **25** (8.5 mg). Compounds **1** and **5** were separated by preparative TLC using $CHCl_3$–MeOH–H_2O (7:4:1) to furnish **1** (9.7 mg) and **5** (16.5 mg). Fraction VI-9 was applied to an ODS silica gel column eluted with MeOH–H_2O (1:1; 2:1) and silica gel with $CHCl_3$–MeOH–H_2O (14:6:1; 75:40:7) to yield **16** (36.4 mg) and **17** with few impurities. Compound **17** (8.3 mg) was purified by preparative HPLC using MeCN–MeOH–H_2O (1:1:3). Fraction VI-10 was subjected to CC on ODS silica gel eluted with MeOH–H_2O (1:1; 2:1), and then silica gel with $CHCl_3$–MeOH–H_2O (14:6:1; 7:4:1) to gave **8** (8.7 mg) and **18** (92.3 mg) as pure compounds, and **2** with few impurities. Compound **2** (19.5 mg) was refined by preparative TLC using $CHCl_3$–MeOH–H_2O (20:10:1).

3.4. Compound **1**

3-[(*O*-(4-*O*-Sulfo-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid β-D-glucopyranosyl ester sodium salt (**1**); amorphous solid; $[\alpha]_D^{25}$ 20.1 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3389 (OH), 2927 and 2878 (CH), 1746 (C = O), 1651 (C = C), 1259 (S = O), 1078 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.44 (1H, *br s*, H-12), 1.29 (3H, *s*, Me-23), 1.27 (3H, *s*, Me-27), 1.11 (3H, *s*, Me-26), 0.96 (3H, *s*, Me-24), 0.92 (3H, *s*, Me-29), 0.90 (3H, *s*, Me-30), 0.87 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1037.4329 $[M + Na]^+$ (Calcd for $C_{47}H_{75}Na_2O_{20}S$: 1037.4368).

3.5. Acid hydrolysis of **1**

A solution of **1** (7.1 mg) in 1 M HCl (dioxane–H_2O, 1:1; 2 ml) was heated at 95 °C for 1 h under Ar atmosphere. After the reaction mixture was diluted with H_2O, it was extracted with EtOAc saturated with H_2O (10 ml × 3). The EtOAc extract was subjected to silica gel CC eluted with hexane–Me_2CO (4:1) to yield oleanolic acid (1.9 mg). The H_2O residue was neutralized by passage through an Amberlite IRA-96SB (Organo, Tokyo, Japan) column and passed through a Sep-Pak C18 cartridge (Waters, Milford, MA, USA), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG 80 (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. The identification of L-arabinose and D-glucose 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): 8.53 (L-arabinose, positive optical rotation), 14.04 (D-glucose, positive optical rotation).

3.6. Compound **2**

3-[(*O*-β-D-Glucopyranosyl-(1→3)-2-*O*-sulfo-α-L-arabinopyranosyl)oxy]olean-12-en-28-oic acid β-D-glucopyranosyl ester sodium salt (**2**); amorphous solid; $[\alpha]_D^{25}$ 2.6 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3418 (OH), 2944 and 2880 (CH), 1733 (C = O), 1645 (C = C), 1259 (S = O), 1075 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.42 (1H, *br s*, H-12), 1.33 (3H, *s*, Me-23), 1.25 (3H, *s*, Me-27), 1.14 (3H, *s*, Me-26), 1.07 (3H, *s*, Me-24), 0.91 (3H, *s*, Me-29), 0.88 (3H, *s*, Me-30), 0.83 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1037.4353 $[M + Na]^+$ (Calcd for $C_{47}H_{75}Na_2O_{20}S$: 1037.4368).

3.7. Acid hydrolysis of **2**

A solution of **2** (6.3 mg) was subjected to acid hydrolysis as described for **1** to give oleanolic acid (2.3 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose and D-glucose. t_R (min): 9.45 (L-arabinose, positive optical rotation), 16.27 (D-glucose, positive optical rotation).

3.8. Compound **9**

3-[(β-D-Xylopyranosyl)oxy]olean-12-ene-28,29-dioic acid 28-β-D-glucopyranosyl ester (**9**); amorphous solid; $[\alpha]_D^{25}$ −2.1 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3390 (OH), 2939 and 2877 (CH), 1730 (C = O), 1651 (C = C), 1074 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.50 (1H, *br s*, H-12), 1.46 (3H, *s*, Me-30), 1.29 (3H, *s*, Me-23), 1.27 (3H, *s*, Me-27), 1.12 (3H, *s*, Me-26), 0.99 (3H, *s*, Me-24), 0.88 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 803.4208 $[M + Na]^+$ (Calcd for $C_{41}H_{64}NaO_{14}$: 803.4194).

3.9. Acid hydrolysis of **9**

A solution of **9** (4.9 mg) in 0.2 M HCl (dioxane–H_2O, 1:1; 2 ml) was heated at 95 °C for 5 h under Ar atmosphere. After the reaction mixture was diluted with H_2O, it was extracted with EtOAc saturated with H_2O (10 ml × 3). The EtOAc extract was applied to silica gel CC eluted with $CHCl_3$–MeOH (19:1) to yield serratagenic acid (2.2 mg) (Yu et al., 1995). The H_2O residue was neutralized by passage through an Amberlite IRA-96SB column and passed through a Sep-Pak C18 cartridge to give a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-xylose and D-glucose. t_R (min): 10.08 (D-xylose, positive optical rotation), 16.39 (D-glucose, positive optical rotation).

3.10. Compound **10**

3-[(*O*-β-D-Glucopyranosyl-(1→3)-β-D-xylopyranosyl)oxy]olean-12-ene-28,29-dioic acid (**10**); amorphous solid; $[\alpha]_D^{25}$ −13.2 (*c* 0.043; MeOH); IR ν_{max} (film) cm^{-1}: 3390 (OH), 2928 and 2877 (CH), 1691 (C = O), 1647 (C = C), 1075 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.55 (1H, *br s*, H-12), 1.60 (3H, *s*, Me-30), 1.29 (3H, *s*, Me-23), 1.31 (3H, *s*, Me-27), 1.01 (3H, *s*, Me-26), 0.99 (3H, *s*, Me-24), 0.85 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 803.4159 $[M + Na]^+$ (Calcd for $C_{41}H_{64}NaO_{14}$: 803.4194).

3.11. Acid hydrolysis of **10**

A solution of **10** (3.1 mg) was subjected to acid hydrolysis as described for **9** to give serratagenic acid (0.7 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-xylose and D-glucose. t_R (min): 10.02 (D-xylose, positive optical rotation), 16.19 (D-glucose, positive optical rotation).

3.12. Compound **12**

3-[(*O*-β-D-Glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid (**12**); amorphous solid; $[\alpha]_D^{25}$ 15.9 (*c* 0.033; MeOH); IR ν_{max} (film) cm^{-1}: 3365 (OH), 2927 and 2876 (CH), 1683 (C = O), 1647 (C = C), 1077 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.50 (1H, *br s*, H-12), 4.82 and 4.76 (each 1H, *s*, H-29), 1.33 (3H, *s*, Me-23), 1.29 (3H, *s*, Me-27), 0.99 (3H, *s*, Me-26), 0.99 (3H, *s*, Me-24), 0.84 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 757.4138 $[M + Na]^+$ (Calcd for $C_{40}H_{62}NaO_{12}$: 757.4139).

3.13. Acid hydrolysis of **12**

A solution of **12** (3.2 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose and D-glucose. t_R (min): 9.43 (L-arabinose, positive optical rotation), 16.22 (D-glucose, positive optical rotation).

3.14. Preparation of **12** from **22**

Compound **22** (11.2 mg) was treated with 4% KOH in EtOH (10.0 ml) at 80 °C for 2 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Oregano, Tokyo, Japan) and purified by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (50:10:1) to give **12** (7.3 mg).

3.15. Compound **13**

3-[(α-L-Arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester (**13**); amorphous solid; $[\alpha]_D^{25}$ 73.3 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3390 (OH), 2941 and 2879 (CH), 1733 (C = O), 1651 (C = C), 1074 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.45 (1H, *br s*, H-12), 4.77 and 4.70 (each 1H, *s*, H_2-29), 1.29 (3H, *s*, Me-23), 1.24 (3H, *s*, Me-27), 1.09 (3H, *s*, Me-26), 0.96 (3H, *s*, Me-24), 0.87 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 757.4103 $[M + Na]^+$ (Calcd for $C_{40}H_{62}NaO_{12}$: 757.4139).

3.16. Acid hydrolysis of **13**

A solution of **13** (3.2 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose and D-glucose. t_R (min): 9.41 (L-arabinose, positive optical rotation), 16.19 (D-glucose, positive optical rotation).

3.17. Compound **14**

3-[(*O*-(3-*O*-Acetyl-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester (**14**); amorphous solid; $[\alpha]_D^{25}$ 16.3 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3389 (OH), 2932 and 2878 (CH), 1732 (C = O), 1651 (C = C), 1076 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.44 (1H, *br s*, H-12), 4.77 and 4.70 (each 1H, *s*, H_2-29), 1.31 (3H, *s*, Me-23), 1.24 (3H, *s*, Me-27), 1.09 (3H, *s*, Me-26), 0.96 (3H, *s*, Me-24), 0.85 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 961.4713 $[M + Na]^+$ (Calcd for $C_{48}H_{74}NaO_{18}$: 961.4773).

3.18. Alkaline hydrolysis of **14**

A solution of **14** (4.3 mg) was treated with 10% KOH (dioxane–H_2O, 1:1; 2 ml) at room temperature for 4 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and purified by silica gel CC eluted with $CHCl_3$–MeOH–H_2O (30:10:1) to yield **22** (1.1 mg).

3.19. Compound **15**

3-[(*O*-(6-*O*-Acetyl-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester (**15**); amorphous solid; $[\alpha]_D^{25}$ 36.5 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3389 (OH), 2927 and 2879 (CH), 1739 (C = O), 1651 (C = C), 1076 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.45 (1H, *br s*, H-12), 4.77 and 4.70 (each 1H, *s*, H_2-29), 1.31 (3H, *s*, Me-23), 1.25 (3H, *s*, Me-27), 1.09 (3H, *s*, Me-26), 0.98 (3H, *s*, Me-24), 0.86 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 961.4722 $[M + Na]^+$ (Calcd for $C_{48}H_{74}NaO_{18}$: 961.4773).

3.20. Alkaline hydrolysis of **15**

Compound **15** (3.8 mg) was subjected to alkaline hydrolysis as described for **14** to give **22** (1.1 mg).

3.21. Compound **16**

3-[(*O*-(4-*O*-Sulfo-β-D-glucopyranosyl)-(1→3)-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid β-D-glucopyranosyl ester sodium salt (**16**); amorphous solid; $[\alpha]_D^{25}$ 49.5 (*c* 0.095; MeOH); IR ν_{max} (film) cm^{-1}: 3409 (OH), 2937 and 2877 (CH), 1732 (C = O), 1651 (C = C), 1259 (S = O), 1076 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.45 (1H, *br s*, H-12), 4.77 and 4.70 (each 1H, *s*, H_2-29), 1.29 (3H, *s*, Me-23), 1.25 (3H, *s*, Me-27), 1.09 (3H, *s*, Me-26), 0.95 (3H, *s*, Me-24), 0.86 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1021.4078 $[M + Na]^+$ (Calcd for $C_{46}H_{71}Na_2O_{20}S$: 1021.4055).

3.22. Acid hydrolysis of **16**

A solution of **16** (6.4 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-arabinose and D-glucose. t_R (min): 9.61 (L-arabinose, positive optical rotation), 16.55 (D-glucose, positive optical rotation).

3.23. Compound **17**

3-[(*O*-(4-*O*-Sulfo-β-D-glucopyranosyl)-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-dioic acid β-D-glucopyranosyl ester sodium salt (**17**); amorphous solid; $[\alpha]_D^{25}$ 26.7 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3417 (OH), 2937 and 2882 (CH), 1748 (C = O), 1651 (C = C), 1261 (S = O), 1074 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.44 (1H, *br s*, H-12), 4.77 and 4.70 (each 1H, *s*, H_2-29), 1.24 (3H, *s*, Me-27), 1.21 (3H, *s*, Me-23), 1.11 (3H, *s*, Me-24), 1.08 (3H, *s*, Me-26), 0.86 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1167.4736 $[M + Na]^+$ (Calcd for $C_{52}H_{81}Na_2O_{24}S$: 1167.4634).

3.24. Acid hydrolysis of **17**

A solution of **17** (6.1 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose. t_R (min): 8.09 (L-rhamnose, negative optical rotation), 9.67 (L-arabinose, positive optical rotation), 16.63 (D-glucose, positive optical rotation).

3.25. Compound **18**

3-[(*O*-β-D-Glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-30-noroleana-12,20(29)-dien-28-oic acid *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**18**); amorphous solid; $[\alpha]_D^{25}$ 6.9 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3390 (OH), 2935 and 2877 (CH), 1733 (C = O), 1651 (C = C), 1074 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.42 (1H, *br s*, H-12), 4.73 and 4.70 (each 1H, *s*, H_2-29), 1.23 (3H, *s*, Me-27), 1.21 (3H, *s*, Me-23), 1.12 (3H, *s*, Me-24), 1.08 (3H, *s*, Me-26), 0.87 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1227.5760 $[M + Na]^+$ (Calcd for $C_{58}H_{92}NaO_{26}$: 1227.5775).

3.26. Acid hydrolysis of **18**

A solution of **18** (8.1 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose. t_R (min): 8.10 (L-rhamnose, negative optical rotation), 9.49 (L-arabinose, positive optical rotation), 16.27 (D-glucose, positive optical rotation).

3.27. Compound **19**

3-[(*O*-β-D-Glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-20β-hydroxy-30-norolean-12-en-28-oic acid β-D-glucopyranosyl ester (**19**); amorphous solid; $[\alpha]_D^{25}$ −10.2 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3364 (OH), 2925 and 2854 (CH), 1731 (C = O), 1652 (C = C), 1076 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.48 (1H, *br s*, H-12), 3.80 (1H, *dd*, *J* = 13.4, 4.2 Hz, H-18), 2.57 (1H, *m*, H-22ax), 2.04 (1H, *dd*, *J* = 13.3, 13.3 Hz, H-19ax), 1.89 (1H, *br d*, *J* = 16.0 Hz, H-22eq), 1.84 (1H, *dd*, *J* = 13.3, 4.2 Hz, H-19eq), 1.43 (3H, *s*, Me-29), 1.30 (3H, *s*, Me-27), 1.20 (3H, *s*, Me-23), 1.12 (3H, *s*, Me-24), 1.12 (3H, *s*, Me-26), 0.85 (3H, *s*, Me-25): For ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1083.5345 $[M + Na]^+$ (Calcd for $C_{52}H_{84}NaO_{22}$: 1083.5352).

3.28. Acid hydrolysis of **19**

A solution of **19** (7.0 mg) in 0.2 M HCl (dioxane–H_2O, 1:1; 2 ml) was heated at 95 °C for 1 h under Ar atmosphere. After the reaction mixture was diluted with H_2O, it was extracted with EtOAc saturated with H_2O (10 ml × 3). The H_2O residue was neutralized by passage through an Amberlite IRA-96SB column and passed through a Sep-Pak C18 cartridge to give a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose. t_R (min): 7.25 (L-rhamnose, negative optical rotation), 8.59 (L-arabinose, positive optical rotation), 14.06 (D-glucose, positive optical rotation).

3.29. Compound **20**

3-[(*O*-β-D-Glucopyranosyl-(1→3)-*O*-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl)oxy]-20α-hydroxy-29-norolean-12-en-28-oic acid β-D-glucopyranosyl ester. (**20**); amorphous solid; $[\alpha]_D^{25}$ −2.7 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3364 (OH), 2925 and 2878 (CH), 1731 (C = O), 1651 (C = C), 1075 (C–O); ^1H NMR (500 MHz, C_5D_5N): δ 5.49 (1H, *br s*, H-12), 3.25 (1H, *dd*, *J* = 13.7, 4.1 Hz, H-18), 1.96 (1H, *br d*, *J* = 13.5 Hz, H-22eq), 1.85 (1H, *m*, H-22ax), 1.46 (3H, *s*, Me-30), 1.23 (3H, *s*, Me-27), 1.20 (3H, *s*, Me-23), 1.12 (3H, *s*, Me-24), 1.09 (3H, *s*, Me-26), 0.85 (3H, *s*, Me-25):

For ^{1}H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data, see Tables 1 and 2; HRESI-TOFMS *m*/*z*: 1083.5345 $[M + Na]^+$ (Calcd for $C_{52}H_{84}NaO_{22}$: 1083.5352).

3.30. Acid hydrolysis of **20**

A solution of **20** (5.0 mg) was subjected to acid hydrolysis as described for **19** to give 3β,20α-dihydroxy-29-norolean-12-en-28-oic acid (1.1 mg) (Fu et al., 2001) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of L-rhamnose, L-arabinose, and D-glucose. t_R (min): 7.26 (L-rhamnose, negative optical rotation), 8.57 (L-arabinose, positive optical rotation), 14.09 (D-glucose, positive optical rotation).

3.31. Detection of sulfate ion

A few drops of 0.5 M $BaCl_2$ aqueous solution was added to each H_2O residue (12 mL) of **1**, **2**, **16**, and **17** to give white precipitate ($BaSO_4$); the qualitative test for sulfate ion.

3.32. HL-60 cell culture assay

The cell growth was measured with an MTT reduction assay as described in a previous paper (Jitsuno et al., 2009). Briefly, HL-60 cells were maintained in RPMI 1640 medium containing heat-inactivated 10% (v/v) FBS supplemented with L-glutamine, 100 unit/ml penicillin G sodium salt, and 100 μg/ml streptomycin sulfate. The cells (4×10^4 cells/ml) were continuously treated with each compound for 72 h, and cell growth was measured with an MTT reduction assay procedure. A dose–response curve was plotted for **3**, **6**, **13**, **21**, and **23**, and the concentration giving 50% growth inhibition (IC_{50}) was calculated.

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