Notes

Oleanane and Taraxerane Glycosides from the Roots of *Gomphrena macrocephala*

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Phytochemical screening of the roots of *Gomphrena macrocephala*, with particular attention to its triterpene glycoside constituents, has resulted in the isolation of two new oleanane glycosides (**1** and **2**) and a new taraxerane glycoside (**3**). The structures of **1**−**3** were determined as 11α,12α-epoxy-3β-[(*O*-β-D-glucuronopyranosyl)oxy]olean-28,13-olide (**1**), 11α,12α-epoxy-3β-[(*O*-β-D-galactopyranosyl-(1→3)-*O*-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl)-oxy]olean-28,13-olide (**2**), and 11α,12α-epoxy-3β-[(*O*-β-D-glucuronopyranosyl)oxy]taraxer-14-en-28-oic acid β-D-glucopyranosyl ester (**3**), respectively, on the basis of their spectroscopic data and the results of hydrolysis. The aglycones (**1a** and **3a**) of **1**−**3** with an epoxy group showed cytotoxic activity against HSC-2 human oral squamous carcinoma cells.

Gomphrena macrocephala St.-Hill. is a perennial herb belonging to the family Amaranthaceae and one of the well-known Brazilian medicinal plants. A decoction prepared from the roots of *G. macrocephala* has long been used as a tonic and stimulant in Brazil.[1] However, a literature survey showed that no systematic investigations have been done on *G. macrocephala* roots.[2] In connection with our work on bioactive secondary metabolites from traditional medicines, a chemical investigation has been done on the *n*-BuOH-soluble fraction of the roots of *G. macrocephala* 80% EtOH extract. This has resulted in the isolation of two new oleanane glycosides (**1** and **2**) and a new taraxerane glycoside (**3**). This paper deals with the structure elucidation of the three new triterpene glycosides on the basis of their spectroscopic data and the results of hydrolysis. The cytotoxic activity of **1**−**3** and their aglycones **1a** and **3a** against HSC-2 human oral squamous carcinoma cells is also described.

An 80% EtOH extract of the roots of *G. macrocephala* was suspended in H_2O and then successively partitioned with EtOAc and *n*-BuOH. The *n*-BuOH-soluble portion was repeatedly subjected to column chromatography over silica gel, octadecylsilanized (ODS) silica gel, and Sephadex LH-20 to give **1** (98.6 mg), **2** (83.3 mg), and **3** (41.3 mg).

Compound **1** was obtained as an amorphous solid. Its molecular formula was derived as $C_{36}H_{54}O_{10}$ by the HRESITOFMS data, showing an $[M + H]^+$ ion at *m*/*z* 647.3795, and ^{13}C NMR spectrum (36 carbon signals). The IR spectrum of **1** showed absorptions due to hydroxy groups at 3324 cm^{-1} and a five-membered lactone group at 1774 cm^{-1}. The ^1H NMR spectrum of **1** showed signals for seven tertiary methyl groups at δ 1.30, 1.26, 1.13, 0.98, 0.90, 0.88, and 0.81 (each s) and one anomeric proton at δ 5.03 (d, $J = 7.8$ Hz). Enzymatic hydrolysis of **1** with β-glucuronidase gave a known triterpenoid (**1a**), identified as 11α,12α-epoxy-3β-hydroxyolean-28,13-olide,[3] and D-glucuronic acid. D-Glucuronic acid, including its absolute configuration, was identified by direct HPLC analysis of the hydrolyzate, which was performed on an ion-exclusion column of sulfonated polystyrene with isocratic elution in 5 mM H_2SO_4, with detection carried out by using a combination of refractive index (RI) and optical rotation (OR) detectors. The linkage position of β-D-glucuronic acid was shown to be at C-3 of the aglycone by detecting a correlation between the anomeric proton at δ 5.03 and the C-3 carbon at δ 88.8 in the HMBC spectrum. From the above evidence, the structure of **1** was elucidated as 11α,-12α-epoxy-3β-[(*O*-β-D-glucuronopyranosyl)oxy]olean-28,13-olide.

Compound **2** had the molecular formula $C_{48}H_{74}O_{20}$ on the basis of the HRESITOFMS, exhibiting an $[M + Na]^+$ peak at *m*/*z* 993.4672, and ^{13}C NMR spectrum (48 carbon signals). The ^1H NMR spectrum of **2** showed signals for three anomeric protons at δ 5.73 (d, $J = 7.8$ Hz), 5.31 (d, $J = 7.8$ Hz), and 4.91 (d, $J = 7.6$ Hz), along with seven tertiary methyl groups at δ 1.23, 1.21, 1.08, 1.05, 0.88, 0.81, and 0.78 (each s). Acid hydrolysis of **2** with 0.2 M HCl yielded D-glucuronic acid, D-glucose, and D-galactose as the carbohydrate moieties, whereas enzymatic hydrolysis of **2** with naringinase gave **1a**.[3] The results of the hydrolyses and the ^1H and ^{13}C NMR data indicated that the carbohydrate moiety of **2** was composed of D-glucuronic acid, D-glucose, and D-galactose. When the ^{13}C NMR spectrum of **2** was compared with that of **1**, the resonances due to C-2 and C-3 of the glucuronosyl moiety were displaced downfield by 3.2 and 8.9 ppm and were observed at δ 78.7 and 87.1, respectively. This suggests that the C-2 and C-3 hydroxy groups of the glucuronosyl moiety are the positions at which the additional D-glucose and D-galactose units are linked. In the HMBC spectrum, the anomeric proton resonances at δ 5.73 (H-1 of D-glucosyl), 5.31 (H-1 of D-galactosyl), and 4.91 (H-1 of D-glucuronosyl) exhibited correlations with the carbon signals at δ 78.7 (C-2 of glucuronic acid), 87.1 (C-3 of the glucuronic acid), and 89.4 (C-3 of aglycone), respectively. Accordingly, the structure of **2** was elucidated as 11α,12α-epoxy-3β-[(*O*-β-D-galactopyrano-

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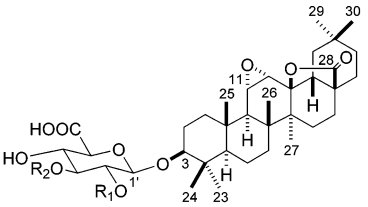
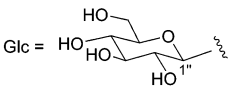
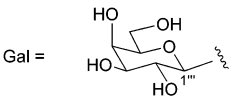
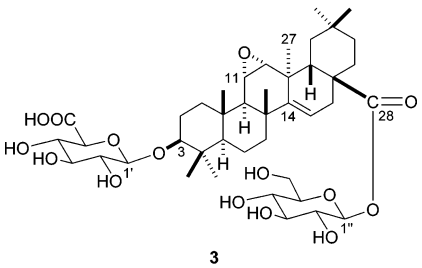


	R_1	R_2
1	H	H
2	Glc	Gal







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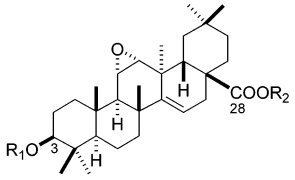

	R_1	R_2
3a	H	H
3b	Ac	H
3c	H	Me

syl-(1→3)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucuronopyranosyl)-oxy]olean-28,13-olide.

Compound **3**, obtained as an amorphous powder, has a molecular formula of $C_{42}H_{64}O_{15}$ on the basis of its HRESITOFMS (*m*/*z* 831.4066 $[M + Na]^+$) and ^{13}C NMR data (42 carbon signals). The NMR properties of **3** were suggestive of a bisdesmosidic triterpene glycoside. Acid hydrolysis of **3** with 0.2 M HCl gave D-glucuronic acid and D-glucose, and enzymatic hydrolysis of **3** with naringinase afforded **3a**. By the HRESITOFMS ($[M + H]^+$, *m*/*z* 471.3448) and ^{13}C NMR data (30 carbon signals), the molecular formula of **3a** was determined to be $C_{30}H_{46}O_4$, corresponding to eight degrees of unsaturation. The IR spectrum suggested the presence of hydroxy groups (3376 cm^{-1}) and a carboxyl group (1690 cm^{-1}). The ^1H NMR spectrum of **3a** displayed resonances for seven methyl groups at *δ* 1.20, 1.14, 1.13, 1.10, 1.06, 1.04, and 0.99 (each s) and an olefinic proton at *δ* 5.78 (dd, $J = 8.0$, 3.5 Hz). The ^{13}C NMR spectrum combined with various DEPT spectra indicated the presence of seven methyl groups (*δ* 32.5, 29.5, 28.5, 27.3, 20.9, 17.1, and 16.3), a trisubstituted double bond (*δ* 160.1 and 118.5), a carbonyl group (*δ* 179.9), six methines (*δ* 78.1, 58.7, 55.0, 54.2, 52.0, and 41.8), eight methylenes (*δ* 40.5, 39.0, 35.8, 34.4, 33.3, 31.6, 27.8, and 19.2), and six quaternary carbons (*δ* 50.8, 39.3, 39.2, 37.8, 37.0, and 29.3).

Detailed analysis of the $^1H-^1H$ COSY, HOHAHA, and HMQC spectra of **3a** allowed the sequential assignments of the ^1H NMR signals and the one-bond coupled ^{13}C NMR signals as shown in Table 1, giving rise to the following six structural fragments, a: $-C_{(1)}H_2-C_{(2)}H_2-C_{(3)}H(-O-)-$; b: $-C_{(5)}H-C_{(6)}H_2-C_{(7)}H_2-$; c: $-C_{(9)}H-C_{(11)}H(-O-)-C_{(12)}H(-O-)-$; d: $-C_{(15)}H-C_{(16)}H_2-$; e: $-C_{(18)}H-C_{(19)}H_2-$; f: $-C_{(21)}H_2-C_{(22)}H_2-$, as well as seven methyl groups and a carbonyl group (Figure 1 and Figure 2). In the HMBC spectrum of **3a**, a pair of the geminal methyl protons of Me-23 (*δ* 1.20) and Me-24 (*δ* 1.04) showed long-range correlations not only with their attached C-4 (*δ* 39.2) but also with C-3 (*δ* 78.1) and C-5 (*δ* 55.0), whereas the angular Me-25 protons (*δ* 1.06) were correlated with the C-1 (*δ* 39.0), C-5, and C-10 (*δ* 37.0) carbons. These correlations gave evidence for a connection of fragments a and b via the C-4 and C-10 quaternary carbons and linkages of Me-23 and Me-24 to C-4, and Me-25 to C-10. Long-range correlations between a methyl singlet at *δ* 1.14 attributable to Me-26 and C-8 (*δ* 39.3)/C-9 (*δ* 54.2)/C-14 (160.1) and between another methyl singlet at *δ* 1.13 (Me-27) and C-12 (*δ* 58.7)/C-13 (*δ* 37.8)/C-14, as well as between an olefinic proton at *δ* 5.78 assigned to H-15 and C-14, implied a linkage of fragments c and d through the C-13 and C-14 quaternary carbons and the locations of Me-26 and Me-27 at C-8 and C-13, respectively. Likewise, long-range correlations between a pair of the geminal methyl protons of Me-29 (*δ* 0.99) and Me-30 (*δ* 1.10) and C-19 (*δ* 35.8)/C-20 (*δ* 29.3)/C-21 (*δ* 34.4), as well as long-range cross-peaks observed from H-18 (*δ* 3.16) and H_2-22 (*δ* 2.08 and 1.63) to the C-17 quaternary carbon (*δ* 50.8) and C-28 carboxyl carbon (*δ* 179.9), confirmed a linkage of fragments e and f through the C-17 and C-20 quaternary carbons and the locus of a carboxyl group at C-17. Finally, the partially connected structural fragments, a/b, c/d, and e/f, were combined by detecting HMBC correlations between H-11 (*δ* 3.27) and C-10, Me-25 and C-9, H-7ax (*δ* 1.30) and C-14, Me-26 and C-7 (*δ* 40.5), H-18 and C-12, H_2-19 (*δ* 1.57 and 1.40) and C-13, Me-27 and C-18, and H_2-16 (*δ* 2.90 and 2.22) and C-17, allowing construction of a modified taraxerane skeleton (Figure 1). Acetylation of **3a** with Ac_2O in pyridine gave a monoacetate (**3b**; $C_{32}H_{48}O_5$). When the ^1H NMR spectrum of **3b** was compared with that of **3a**, the signal due to H-3 was shifted downfield by 1.24 ppm through *O*-acetylation and was observed at *δ* 4.69; however, the signals attributable to H-11 and H-12 (*δ* 3.23) were almost unaffected. On the other hand, treatment of **3a** with CH_2N_2 in MeOH yielded a methyl ester (**3c**; $C_{31}H_{48}O_4$). These findings indicate that **3a** has a hydroxy group at C-3, an epoxy ring between C-11 and C-12, and a free carboxyl group at C-17. Thus, the planar structure of **3a** was established.

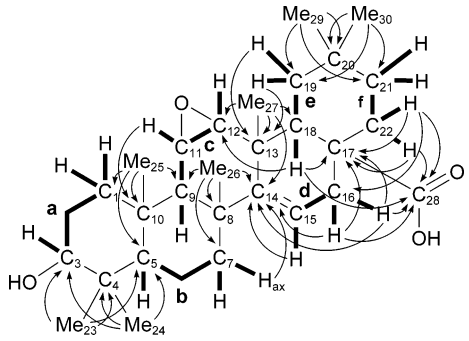

Figure 1. HMBC correlations of **3a**.

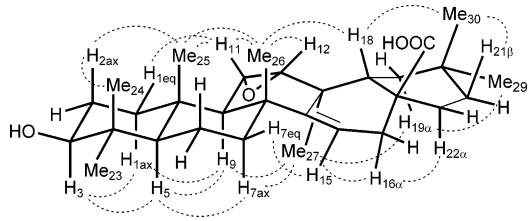

Figure 2. NOE correlations of **3a**.

In the phase-sensitive NOESY spectrum of **3a**, NOE correlations between Me-25 and H-2ax (*δ* 1.94)/Me-26, H-5 (*δ* 0.83) and H-3 (*δ* 3.45)/H-7ax/H-9, H-18 and Me-26/Me-30, Me-27 and H-9 (*δ* 1.19)/H-16α (*δ* 2.22)/H-19α (*δ* 1.57), and H-16α and H-22α (*δ*

Table 1. ^{1}H NMR Data for **1** and **3** in $C_5D_5N-D_2O$ (5:1) and **3a** in C_5D_5N

	1			**3**			**3a**		
position	^{1}H		*J* (Hz)	^{1}H		*J* (Hz)	^{1}H		*J* (Hz)
1 eq	1.68	m	1.74	br d	13.4	1.94	br d	13.3	
ax	1.05	t-like	11.9	1.16	m	1.34	br dd	13.3, 12.1	
2 eq	2.27	br dd	13.7, 4.4	2.26	dd	13.4, 4.2	1.87	m	
ax	1.91	br ddd	13.7, 13.7, 11.7	1.90	br ddd	13.4, 13.4, 11.6	1.94	br ddd	13.1, 13.1, 11.0
3	3.37	dd	11.7, 4.4	3.36	dd	11.6, 4.2	3.45	dd	11.0, 4.8
4									
5	0.74	br d	11.8	0.72	br d	11.2	0.83	dd	11.8, 1.6
6 eq	1.48	br d	16.1	1.45	m		1.59	br d	13.4
ax	1.35	br dd	16.1, 13.2	1.28	br dd	12.2, 11.2	1.43	m	
7 eq	1.04	br d	12.5	2.11	br d	11.8	1.99	br dd	13.0, 3.5
ax	1.25	m		1.25	m		1.30	br dd	13.0, 4.4
8									
9	1.65	br s		1.07	d	5.0	1.19	d	4.9
10									
11	3.03	br d	3.7	3.12	dd	5.0, 4.5	3.27	dd	4.9, 4.6
12	3.22	d	3.7	3.10	d	4.5	3.23	d	4.6
13									
14									
15 α	1.02	br dd	11.4, 5.5	5.98	dd	7.9, 3.3	5.78	dd	8.0, 3.5
β	1.70	ddd	13.1, 11.4, 4.2						
16 α	2.17	ddd	13.1, 13.1, 5.5	2.18	dd	14.6, 3.3	2.22	dd	14.7, 3.5
β	1.29	m		2.93	dd	14.6, 7.9	2.90	dd	14.7, 8.0
17									
18	2.53	dd	13.7, 2.9	2.98	dd	14.1, 3.2	3.16	dd	14.0, 3.4
19 α	1.99	dd	15.6, 13.7	1.50	dd	14.1, 13.3	1.57	dd	14.0, 13.4
β	1.73	br d	15.6	1.33	dd	13.3, 3.2	1.40	dd	13.4, 3.4
20									
21 α	1.37	ddd	14.0, 14.0, 4.8	1.14	m		1.33	br d	13.4
β	1.17	br d	14.0	1.33	m		1.45	br dd	13.4, 2.9
22 α	1.69	br dd	14.0, 3.6	1.54	ddd	13.9, 11.2, 2.9	1.63	ddd	13.8, 13.8, 3.2
β	1.81	ddd	14.0, 14.0, 4.2	1.98	br d	13.9	2.08	br dd	13.8, 3.2
23	1.30	s		1.25	s		1.20	s	
24	0.98	s		0.98	s		1.04	s	
25	0.88	s		0.93	s		1.06	s	
26	1.13	s		1.15	s		1.14	s	
27	1.26	s		1.10	s		1.13	s	
28									
29	0.90	s		0.91	s		0.99	s	
30	0.81	s		0.99	s		1.10	s	
1′	5.03	d	7.8	5.00	d	7.8			
2′	4.16	dd	8.7, 7.8	4.12	dd	8.9, 7.8			
3′	4.34	dd	9.0, 8.7	4.32	dd	9.2. 8.9			
4′	4.63	dd	9.7, 9.0	4.58	dd	9.7, 9.2			
5′	4.71	d	9.7	4.67	d	9.7			
6′									
1″				6.17	d	8.1			
2″				4.16	dd	9.0, 8.1			
3″				4.25	dd	9.3, 9.0			
4″				4.32	dd	9.3, 9.3			
5″				3.98	ddd	9.3, 4.6, 2.2			
6″ a				4.38	dd	11.9, 2.2			
b				4.32	dd	11.9, 4.6			

1.63) provided evidence for the triterpenoid ring fusions of A/B *trans*, B/C *trans*, and D/E *cis*, and the 13*S* configuration. The *β*-equatorial orientation of the hydroxy group at C-3 was revealed by the coupling constants of H-3 (dd, $J = 11.0$, 4.8 Hz) and was supported by NOEs between H-3 and H-1ax (δ 1.34)/H-5. Further NOE correlations between H-11 and Me-25/Me-26 and between H-12 and Me-26 revealed the α-configuration of the epoxy ring. The structure of the new triterpene **3a** may thus be defined as 11α,-12α-epoxy-3*β*-hydroxytaraxer-14-en-28-oic acid.

In the HMBC spectrum of **3**, the anomeric proton signals at δ 5.00 and 6.17, which were assigned to H-1 of *β*-D-glucuronosyl and H-1 of *β*-D-glucosyl, respectively, exhibited three-bond-coupled carbon signals at δ 89.1 (C-3 of aglycone) and 176.5 (C-28 of aglycone).

From the data described above, the structure of **3** was elucidated as 11α,12α-epoxy-3*β*-[(*O*-*β*-D-glucuronopyranosyl)oxy]taraxer-14-en-28-oic acid *β*-D-glucopyranosyl ester.

Compounds **1** and **2** are new oleanane glycosides, and **3** is a glycoside of a new taraxerane derivative (**3a**). Triterpenes with the taraxerane skeleton have been rarely found in natural sources,[4−6] and this is the first isolation of a taraxerane glycoside. Compounds **1**−**3** and their aglycones **1a** and **3a** were evaluated for their cytotoxic activity against HSC-2 cells. Although **1**−**3** did not show apparent cytotoxicity at a sample concentration of 100 μM, **1a** and **3a** were cytotoxic to HSC-2 cells (IC_{50} 21 μM) as potent as etoposide (IC_{50} 24 μM) used as a positive control.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ-values

with reference to TMS as an internal standard. ESIMS data were obtained on a Micromass LCT mass spectrometer (Manchester, U.K.). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh) and a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port with a 20 μL sample loop. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS); penicillin and streptomycin sulfate (Meiji-Seika, Tokyo, Japan); and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (Sigma, St. Louis, MO). All other chemicals used were of biochemical reagent grade.

Plant Material. The roots of *G. macrocephala* were collected in the fields of the Brazilian Cerrado in São Paulo, Brazil. This plant was identified by comparison with a specimen deposited in the Herbarium Maria Eneida P. K. Fidalgo of Instituto de Botanica de São Paulo, Brazil, by one of the authors (M.H.) (voucher #GM-92-12).

Extraction and Isolation. The plant material (dry weight 4.0 kg) was extracted with 80% EtOH at room temperature for 30 days. The extract was concentrated under reduced pressure, and the viscous concentrate was partitioned between H_2O and EtOAc and then between H_2O and *n*-BuOH. A 58.5 g portion of the *n*-BuOH extract (148 g) was subjected to silica gel column chromatography (85 mm i.d. × 200 mm) eluted with a stepwise gradient mixture of $CHCl_3$−MeOH−H_2O (20:10:1; 7:4:1; 6:4:1; 5:4:1) and finally with MeOH alone, giving seven fractions (I, II, III, IV, V, VI, and VII). Fraction II was subjected to a silica gel column (60 mm i.d. × 200 mm) eluted with $CHCl_3$−MeOH−H_2O (80:10:1; 30:10:1; 20:10:1) and a Sephadex LH-20 column (65 mm i.d. × 250 mm) with MeOH to give **1** (98.6 mg). Fraction IV was chromatographed on ODS silica gel (45 mm i.d. × 230 mm) eluted with MeOH−H_2O (6:4; 7:3) and MeCN−H_2O (1:2) to yield **2** (83.3 mg) and **3** (41.3 mg).

Compound 1: amorphous solid; $[\alpha]_D^{24}$ +28.0 (*c* 0.10, MeOH); IR (film) ν_{max} 3324 (OH), 2933 and 2870 (CH), 1774 (C=O), 1508, 873 (epoxy) cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 647.3795 $[M + H]^+$ (calcd for $C_{36}H_{55}O_{10}$, 647.3820).

Enzymatic Hydrolysis of 1. Compound **1** (9.9 mg) was treated with β-glucuronidase (Sigma, EC 3.2.1.31; 9.5 mg) in a phosphate buffer (pH 6.9, 2.5 mL) at room temperature for 48 h. The reaction mixture was passed through a Sep-Pak C18 cartridge (Waters, Milford, MA) eluted with 30% MeOH (6.0 mL) followed by MeOH (6.0 mL). Column chromatography of the MeOH eluate fraction on silica gel (25 mm i.d. × 100 mm) was eluted with $CHCl_3$−MeOH (9:1) to give **1a** (3.0 mg).[3] The 30% MeOH eluate fraction was passed through a Sephadex LH-20 column (18 mm i.d. × 160 mm) using MeOH to yield a sugar fraction (1.1 mg). The sugar fraction was analyzed by HPLC under the following conditions: column, Aminex HPX-87H (7.8 mm i.d. × 300 mm, 5 mm, Bio-Rad Laboratories, Hercules, CA); solvent, 5 mM H_2SO_4; flow rate, 0.6 mL/min; detection, RI and OR. Identification of D-glucuronic acid present in the sugar fraction was carried out by comparison of its retention time and polarity with those of an authentic sample: t_R (min), 8.16 (D-glucuronic acid, positive polarity).

Compound 2: amorphous solid; $[\alpha]_D^{22}$ +21.7 (*c* 0.12, MeOH); IR (film) ν_{max} 3376 (OH), 2927 and 2873 (CH), 1777 and 1615 (C=O), 871 (epoxy); ^1H NMR (C_5D_5N−D_2O, 5:1) δ 5.73 (1H, d, J = 7.8 Hz, H-1″), 5.31 (1H, d, J = 7.8 Hz, H-1‴), 4.91 (1H, d, J = 7.6 Hz, H-1′), 4.54 (1H, d, J = 9.5 Hz, H-5′), 4.49 (1H, dd, J = 10.8, 3.1 Hz, H-6″a), 4.48 (1H, dd, J = 9.2, 7.8 Hz, H-2‴), 4.47 (1H, dd, J = 9.5, 8.7 Hz, H-4′), 4.45 (1H, dd, J = 8.7, 7.6 Hz, H-2′), 4.44 (1H, dd, J = 10.6, 6.0 Hz, H-6‴a), 4.43 (1H, m, H-4‴), 4.38 (1H, dd, J = 8.7, 8.7 Hz, H-3′), 4.32 (1H, dd, J = 9.1, 4.5 Hz, H-6″b), 4.31 (1H, dd, J = 10.6, 3.8 Hz, H-6‴b), 4.26 (1H, dd, J = 9.0, 9.0 Hz, H-3″), 4.13 (1H, m, H-5‴), 4.12 (1H, dd, J = 9.0, 9.0 Hz, H-4″), 4.11 (1H, dd, J = 9.1, 3.2 Hz, H-3‴), 4.07 (1H, dd, J = 9.0, 7.8 Hz, H-2″), 3.85 (1H, ddd, J = 9.0, 4.5, 3.1 Hz, H-5″), 3.22 (1H, dd, J = 12.5, 4.4 Hz, H-3), 3.20 (1H, d, J = 3.9 Hz, H-12), 2.99 (1H, br d, J = 3.9 Hz, H-11), 2.50 (1H, dd, J = 13.5, 2.4 Hz, H-18), 2.16 (1H, ddd, J = 13.2, 13.2, 5.6 Hz, H-16α), 2.09 (1H, br dd, J = 13.2, 4.4 Hz, H-2eq), 1.97 (1H, dd, J = 13.5, 13.5 Hz, H-19α), 1.82 (1H, br ddd, J = 13.2, 13.2, 12.5 Hz, H-2ax), 1.78 (1H, ddd, J = 15.2, 14.1, 4.3 Hz, H-22β), 1.71 (1H, dd, J = 13.5, 2.4 Hz, H-19β), 1.68 (1H, br d, J = 14.1 Hz, H-22α), 1.46 (1H, br d, J = 10.1 Hz, H-6eq), 1.23 (3H, s, Me-27), 1.21 (3H, s, Me-23), 1.15 (1H, br d, J = 15.2 Hz, H-21β), 1.08 (3H, s, Me-26), 1.05 (3H, s, Me-24), 1.01 (1H, br d, J = 13.2 Hz, H-7eq), 0.88 (3H, s, Me-29), 0.81 (3H, s, Me-25), 0.78 (3H, s, Me-30), 0.65 (1H, br d, J = 10.1 Hz, H-5); ^{13}C NMR, see Table 2; HRESITOFMS *m/z* 993.4672 $[M + Na]^+$ (calcd for $C_{48}H_{74}O_{20}Na$, 993.4671).

Table 2. ^{13}C NMR Data for **1**−**3** in C_5D_5N−D_2O (5:1) and **3a** in C_5D_5N

carbon	1	2	3	3a
1	38.4	38.2	38.6	39.0
2	26.3	26.0	26.2	27.8
3	88.8	89.4	89.1	78.1
4	39.6	39.5	39.3	39.2
5	54.9	54.7	54.9	55.0
6	17.7	17.6	18.8	19.2
7	31.3	31.2	40.3	40.5
8	41.6	41.5	39.3	39.3
9	51.1	50.9	54.0	54.2
10	36.4	36.2	36.5	37.0
11	52.7	52.7	51.9	52.0
12	57.3	57.2	58.4	58.7
13	87.6	87.6	37.6	37.8
14	40.9	40.8	159.7	160.1
15	27.0	26.9	118.9	118.5
16	21.6	21.5	32.4	33.3
17	44.1	44.0	51.4	50.8
18	49.8	49.7	42.0	41.8
19	38.0	37.9	35.5	35.8
20	31.5	31.4	29.1	29.3
21	34.4	34.3	33.9	34.4
22	27.6	27.5	30.6	31.6
23	27.8	27.5	27.8	28.5
24	16.4	16.0	16.6	16.3
25	17.2	17.1	17.0	17.1
26	20.3	20.3	26.9	27.3
27	18.9	18.8	20.8	20.9
28	178.9	179.0	176.5	179.9
29	33.1	33.0	32.2	32.5
30	23.4	23.4	29.1	29.5
1′	107.3	105.0	107.1	
2′	75.5	78.7	75.2	
3′	78.2	87.1	77.8	
4′	73.4	71.7	73.2	
5′	77.9	76.9	77.6	
6′	172.9	171.6	172.5	
1″		103.6	96.2	
2″		76.2	73.9	
3″		78.3	78.5	
4″		72.3	70.8	
5″		77.9	79.0	
6″		63.2	61.9	
1‴		104.9		
2‴		72.7		
3‴		75.0		
4‴		59.9		
5‴		77.1		
6‴		62.0		

Enzymatic Hydrolysis of 2. Compound **2** (27.3 mg) was subjected to enzymatic hydrolysis of naringinase (Sigma, EC 232-962-4; 48.1 mg) in an HOAc/KOAc buffer (pH 4.3, 10.0 mL) at room temperature for 408 h. The reaction mixture was passed through a Diaion HP-20 column (20 mm i.d. × 160 mm) eluted with 20% MeOH followed by EtOH−Me_2CO (1:1). The EtOH−Me_2CO (1:1) eluate fraction was purified by silica gel column chromatography (30 mm i.d. × 100 mm) eluted with $CHCl_3$−MeOH−H_2O (20:10:1) and ODS silica gel column chromatography (20 mm i.d. × 160 mm) with MeOH−H_2O (13:2) to yield **1a** (6.6 mg).[3]

Acid Hydrolysis of 2. A solution of **2** (5.3 mg) in 0.2 M HCl (dioxane−H_2O, 1:1, 2.0 mL) was heated at 95 °C for 1 h under an Ar atmosphere. The reaction mixture was diluted with H_2O and then

neutralized by adding $AgCO_3$. The resulting precipitate was filtered off, and the filtrate was passed through a Sep-Pak C18 cartridge eluted with H_2O (6.0 mL) followed by EtOH (6.0 mL), giving a sugar fraction (2.3 mg) and an aglycone fraction (2.1 mg). TLC analysis of the aglycone fraction showed that it contained several unidentified artifacts. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucuronic acid, D-galactose, and D-glucose; t_R (min) 8.07 (D-glucuronic acid, positive polarity), 8.99 (D-glucose, positive polarity), 9.63 (D-galactose, positive polarity).

Compound 3: amorphous solid; $[\alpha]_D^{20}$ −40.0 (*c* 0.01, MeOH); IR (film) ν_{max} 3388 (OH), 2926 and 2867 (CH), 1741 and 1604 (C═O), 874 (epoxy); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m*/*z* 831.4066 $[M + Na]^+$ (calcd for $C_{42}H_{64}O_{15}Na$, 831.4143).

Enzymatic Hydrolysis of 3. Compound **3** (13.8 mg) was subjected to enzymatic hydrolysis with naringinase (30.0 mg) in an HOAc/KOAc buffer (pH 4.3, 10.0 mL) at room temperature for 240 h. The reaction mixture was purified by silica gel column chromatography (20 mm i.d. × 100 mm) using $CHCl_3$−MeOH−H_2O (7:4:1; 30:10:1) to yield **3a** (7.0 mg).

Compound 3a: amorphous solid; $[\alpha]_D^{23}$ −6.0 (*c* 0.10, $CHCl_3$); IR (film) ν_{max} 3376 (OH), 2933 and 2857 (CH), 1690 (C═O), 871 (epoxy); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESITOFMS *m*/*z* 471.3448 $[M + H]^+$ (calcd for $C_{30}H_{47}O_4$, 471.3474).

Acid Hydrolysis of 3. A solution of **3** (4.6 mg) was subjected to acid hydrolysis as described for **2** to give a sugar fraction (1.8 mg) and an aglycone fraction (2.4 mg). TLC analysis of the aglycone fraction showed that it contained several unidentified artifacts. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucuronic acid and D-glucose.

Acetylation of 3a. Compound **3a** (1.3 mg) was treated with Ac_2O (1.0 mL) and pyridine (1.0 mL) at room temperature for 20 h. After the addition of H_2O (3.0 mL) into the reaction mixture, followed by evaporation to dryness, it was chromatographed on silica gel (10 mm i.d. × 135 mm) eluted with hexane−Me_2CO (5:1) to yield **3b** (1.1 mg).

Compound 3b: amorphous solid; IR (film) ν_{max} 2930 and 2861 (CH), 1733 and 1691 (C═O), 865 (epoxy); ^{1}H NMR (C_5D_5N) δ 5.76 (1H, dd, J = 8.0, 3.3 Hz, H-15), 4.69 (1H, dd, J = 11.0, 5.3 Hz, H-3), 3.23 (1H, d, J = 4.1 Hz, H-12), 3.22 (1H, t-like, J = 4.1 Hz, H-11), 3.17 (1H, dd, J = 14.6, 8.1 Hz, H-18), 2.22 (1H, dd, J = 14.7, 3.4 Hz, H-16α), 2.07 (3H, s, Ac), 1.14, 1.11 × 2, 1.01, 1.00, 0.89, 0.87 (each 3H, s, tertiary methyls); HRESITOFMS *m*/*z* 513.3604 $[M + H]^+$ (calcd for $C_{32}H_{49}O_5$, 513.3580).

Methylation of 3a. Compound **3a** (3.1 mg) was dissolved in MeOH (3.0 mL) and cooled at 0 °C. A large excess of CH_2N_2 in Et_2O was added to the sample solution and allowed to stand at room temperature for 12 h. The reaction mixture was chromatographed on silica gel (10 mm i.d. × 160 mm) eluted with hexane−Me_2CO (5:1) to yield **3c** (1.9 mg).

Compound 3c: amorphous solid; IR (film) ν_{max} 3325 (OH), 2925 and 2859 (CH), 1724 (C═O), 866 (epoxy); ^{1}H NMR (C_5D_5N) δ 5.62 (1H, dd, J = 8.0, 3.6 Hz, H-15), 3.67 (3H, s, OMe), 3.45 (1H, dd, J = 11.1, 4.6 Hz, H-3), 3.24 (1H, dd, J = 5.0, 4.6 Hz, H-11), 3.13 (1H, d, J = 4.6 Hz, H-12), 2.94 (1H, dd, J = 14.0, 3.4 Hz, H-18), 2.63 (1H, dd, J = 14.8, 8.0 Hz, H-16β), 2.12 (1H, dd, J = 14.8, 3.6 Hz, H-16α), 1.22, 1.08, 1.07 × 2, 1.03 × 2, 0.96 (each 3H, s, tertiary methyls); HRESITOFMS *m*/*z* 485.3631 $[M + H]^+$ (calcd for $C_{31}H_{49}O_4$, 485.3631).

Cell Culture and Assay for Cytotoxic Activity. HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. Cells were trypsinized and inoculated at 6×10^3 to 1.2×10^4 per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA) and incubated for 24 h. After washing once with PBS, they were treated for 24 h without or with test compounds. The cells were then washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL of DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10.[7,8] The IC_{50} value, which reduces the viable cell number by 50%, was determined from the dose−response curve.

References and Notes

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