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MELANDRIOSIDE A, A SAPONIN FROM *MELANDRIUM FIRMUM*

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ABSTRACT.—A new nortriterpene glycoside named melandrioside A [**1**] was isolated from *Melandrium firmum*, and its structure was elucidated as 3-*O*- {β-D-galactopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucuronopyranosyl} -melandrigenin by use of modern nmr spectroscopic techniques.

Melandrium firmum Rohrb. (Caryophyllaceae) is widely distributed in Korea and has been used for the treatment of various ailments (1). In pharmacological experiments the *n*-BuOH-soluble fraction exhibited hepatotoxic activities (2). In previous investigations it yielded a number of sapogenins (3,4). We present here the isolation and structure determination of a new nortriterpenoid glycoside, melandrioside A.

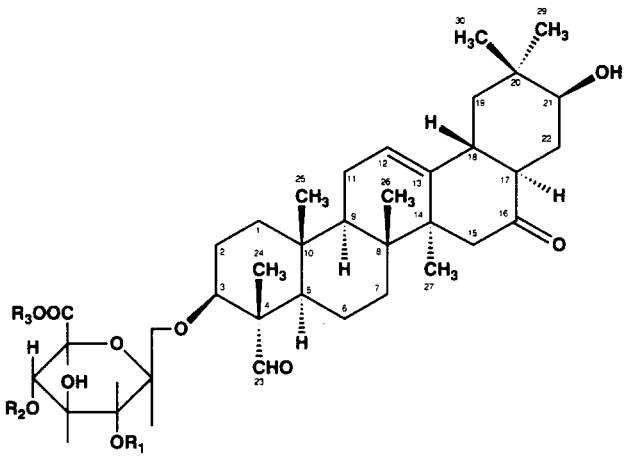
RESULTS AND DISCUSSION

The *n*-BuOH-soluble fraction of the MeOH extracts from the whole plants of *M. firmum*, after repeated chromatographic purification on Si gel columns, afforded melandrioside A [**1**]. Compound **1** gave a positive Molisch test but was negative in Liebermann-Burchard test (pale yellow).

The glycosidic nature of **1** was indicated by the broad absorption bands at 3340 and 1000–1100 cm^{-1} in its ir spectrum and many resonances in the region of δ 3.1–4.6 ppm in its ^{1}H-nmr spectrum and δ 67–85 ppm in its ^{13}C-nmr spectrum.

The ^{13}C-nmr spectrum of **1** (Table 1) showed 47 carbon resonances, including the trisubstituted double bond resonances at δ 117.03 and 141.75, which indicated the presence of an aglycone having 17α-hydrogen-trans-D/E ring junction (4,5).

^{1}H nmr exhibited six singlets at δ 0.78, 0.81, 0.91, 0.94, 0.95, and 1.08, corresponding to six tertiary methyl groups, and one doublet (J = 6.0 Hz) at δ 1.12 attrib-



1 R_1=gal, R_2=rha, R_3=H
2 R_1=R_3=H, R_2=rha
3 R_1=R_2=H, R_3=Me

TABLE 1. ^{13}C-nmr Chemical Shifts of Aglycone Moieties of Compounds **1**–**3** (DMSO-d_6).

Carbon	Compound		
	1 (125 MHz)	**2** (125 MHz)	**3** (20 MHz)
C-1	37.14	37.24	37.1
C-2	24.05	24.04	24.0
C-3	82.02	80.33	80.7
C-4	53.90	54.49	54.2
C-5	47.32	46.54	46.6
C-6	19.72	19.77	19.6
C-7	31.72	31.68	31.6
C-8	38.36	38.41	38.4
C-9	46.36	46.47	46.3
C-10	35.82	35.73	35.6
C-11	22.70	22.76	22.6
C-12	117.03	117.08	116.9
C-13	141.75	141.76	141.8
C-14	42.30	42.36	42.2
C-15	43.67	43.74	43.4
C-16	212.91	213.09	212.7
C-17	47.45	47.51	47.7
C-18	35.17	35.21	35.6
C-19	41.82	41.83	42.2
C-20	35.35	35.42	35.0
C-21	73.86	73.97	74.8
C-22	29.21	29.28	31.2
C-23	209.32	206.98	206.4
C-24	10.07	9.62	9.4
C-25	15.28	15.26	15.0
C-26	16.50	16.54	16.3
C-27	24.95	25.02	24.9
C-29	28.92	28.97	29.1
C-30	18.93	19.01	18.2

uted to the secondary methyl of the rhamnopyranosyl unit. The anomeric proton signals were observed at δ 4.15, 4.24, and 4.57 and were correlated with ^{13}C resonances at δ 101.53, 104.71, and 102.50 (Table 2), respectively, in the HMQC, thus confirming the triglycosidic nature of **1**.

Acid hydrolysis of **1** afforded an aglycone, $[M]^+$ *m/z* 456, identified as melandrigenin (earlier called genin A) from its mass spectral data and by ^{1}H and ^{13}C nmr of its acetate (4). The structure of **2** determined by nmr contained the aglycone moiety melandrigenin. The sugars detected in the hydrolysate by tlc were glucuronic acid, galactose, and rhamnose.

Positive ion fabms of **1**, in agreement with its molecular formula $C_{47}H_{72}O_{19}$, gave rise to a peak at *m/z* 947 corresponding to the quasi-molecular ion $[M + Li]^+$.

The interglycosidic linkages as well as the position of attachment of the sugar chain to the aglycone were established by ^{1}H- and ^{13}C-nmr spectra, interpreted with the aid of COSY and HMQC spectra. The presence of rhamnose and galactose as the terminal sugar moieties was deduced from the carbon chemical shifts by a comparison with those of methyl-*O*-glycosides (6), and was confirmed by the detection of these monosaccharides on partial hydrolysis of **1** on tlc in an HCl atmosphere (7).

These results showed that the sugar chain was branched and that the glucuronic acid moiety was linked with galactose and rhamnose. The shifts of the C-2 and C-4 ^{13}C resonances of glucuronic acid, at δ 81.34 and 84.32, respectively, in addition to the ap-

TABLE 2. ^{13}C-nmr Chemical Shifts of Sugar Moieties of Compounds **1–3** (DMSO-d_6).

Carbon	Compound		
	1 (125 MHz)	**2** (125 MHz)	**3** (20 MHz)
Glucuronic acid			
1	101.53	102.91	103.2
2	81.57	73.65	73.2
3	76.32	76.74	75.8
4	84.76	84.85	71.3
5	72.49	72.07	75.3
6	173.34	172.87	169.2
Rhamnose			
1	102.50	102.51	
2	70.69	70.73	
3	70.83	70.86	
4	72.05	72.07	
5	68.55	68.61	
6	17.65	17.73	
Galactose			
1	104.71		
2	71.80		
3	72.93		
4	67.74		
5	75.17		
6	59.80		

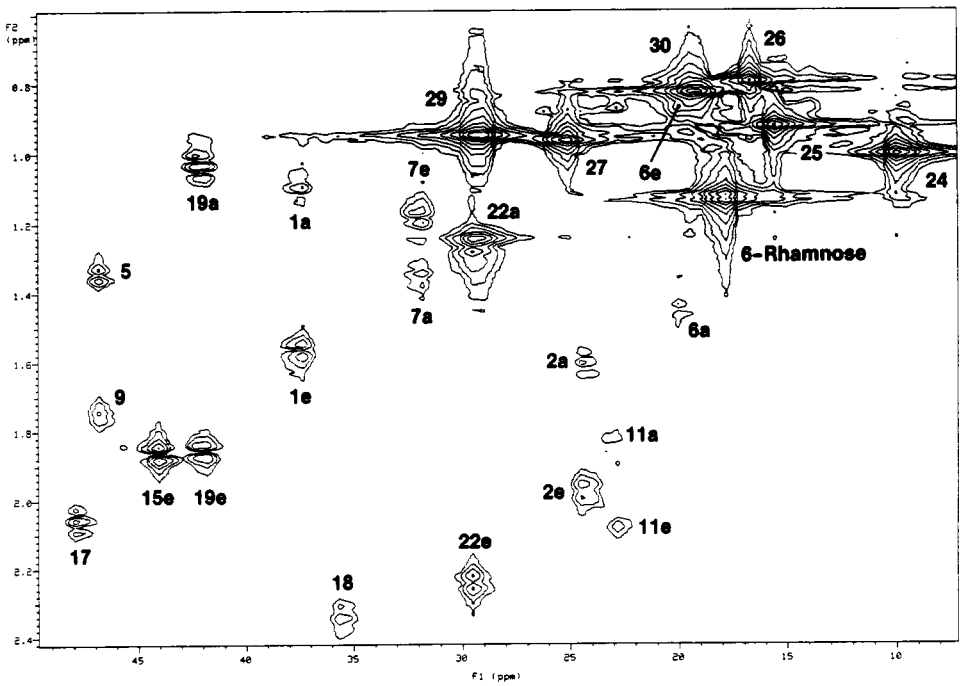


FIGURE 1. Expansion of the high field region of the ^{1}H-^{13}C HMQC spectrum of **2**. The F2 or vertical dimension is the proton shift and the F1 or horizontal dimension is the carbon shift. The crosspeak assigned to 5 (extreme left) must be a carbon with a single proton attached, while the crosspeaks assigned to 7ax and 7eq (center top) reveal a methylene pair. The 6ax crosspeak can be assigned independently from the proton and TOCSY experiments. Finally the HOM2DJ and TOCSY results together indicate that the 6eq must fall as indicated (upper right).

pearance of these resonances in an H_2O/D_2O ^{13}C experiment, indicated their involvement in glycosidic linkage formation (8).

Partial hydrolysis of **1** with acid afforded a prosapogenin, **2**, which had two anomeric resonances at δ 4.02 and 4.61 (^1H nmr) (Table 3) and at δ 102.91 and 102.51 (^{13}C nmr). The absence of resonances for galactose and the detection of rhamnose by tlc in a partial hydrolysis experiment with **2** supported the loss of galactose. The C-2 of the glucuronic acid moiety in **2** was shifted by 7.92 ppm to higher field relative to **1**; this was attributed to lack of glycosylation, whereas the remaining ^{13}C shielding data closely resembled those of **1**, indicating that the attachment point of galactose was C-2 of the glucuronic acid. The H_2O/D_2O experiment also showed that this carbon is C-OH in **2**.

The process of making nmr assignments is shown explicitly for the ring B portion of the aglycone of **2** (Table 4). The only readily visible ring B proton in the 1D nmr was that at 1.45 ppm, which exhibited three large couplings, one geminal and two trans, hence it was assigned to H_{ax}-6. The HMQC spectrum of the high field region (Figure 1) showed the cross peak from H_{ax}-6. The TOCSY spectrum showed this entire proton system, and showed that one proton is found at 0.86 ppm (Figure 2) even though it is obscured by low mol wt impurities in the 1D spectrum. This proton is seen clearly in the 2D *J* spectrum as a doublet of 15.6 Hz, a geminal coupling constant. Since the HMQC shows that the two other protons (H-7) are attached to C-7, this must be the 6 equatorial proton. In Figure 1 this proton can be seen, albeit only as a slight bulge at the side of a large methyl resonance. The upfield shift of H_{eq}-6 vs. H_{ax}-6 is unusual; however, this can be rationalized using known empirical effects. The three 1,3 6ax-methyl interactions cause a downfield shift expected to be about 0.6–0.9 ppm for H_{ax}-6 (9). A

TABLE 3. ^1H-nmr Chemical Shifts of Sugar Moieties of Compounds **1** and **2** (500 MHz, DMSO-d_6).[a]

Proton	Compound	
	1	**2**
Glucuronic acid[b]		
1	4.15 d (7.5)	4.02 d (7.9)
2	3.19 dd (7.5, 9.0)	2.84 dd (7.9, 8.2)
3	3.33 dd (9.0, 9.0)	3.07 dd (8.2, 9.0)
4	3.18 dd (8.5, 9.0)	3.18 dd (9.0, 8.0)
5	3.27 d (8.5)	3.07 d (8.0)
Rhamnose		
1	4.57 d (3.5)	4.61 d (1.5)
2	3.61 dd (3.3, 3.5)	3.63 dd (1.5, 3.1)
3	3.40 dd (3.3, 9.4)	3.40 dd (9.4, 3.1)
4	3.19 dd (9.3, 9.4)	3.19 dd (9.3, 9.4)
5	3.49 dq (6.1, 9.3)	3.48 dq (6.1, 9.3)
6	1.12 d (6.1)	1.12 d (6.1)
Galactose		
1	4.24 d (7.24)	
2	3.31 dd (7.24, 9.0)	
3	3.27 dd (3.0, 9.0)	
4	3.65 dd (1.0, 3.0)	
5	3.35 ddd (1.0, 5.9, 7.0)	
6a	3.51 dd (5.9, 10.6)	
6b	3.56 dd (7.0, 10.6)	

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

[b]The coupling constants connecting Glucuronic Acid 2–5 protons are rather imprecise ±1 Hz.

TABLE 4. ^{1}H-nmr Chemical Shifts of Aglycone Moiety of Compound **2** (500 MHz, DMSO-d_6).

Proton	δ, ppm multiplicity (*J*, Hz)
H_{ax}-1	1.09 t (13, 13)
H_{eq}-1	1.56 d (13)
H_{ax}-2	1.62 t (12, 12, 11.6)
H_{eq}-2	1.97 d (12)
H-3	3.84 dd (11.6, 4.1)
H-5	1.36 m (12.1)
H_{ax}-6	1.45 ddd (15.6, 12.1, 12.1)
H_{eq}-6	0.86 d (15.6)
H_{ax}-7	1.34 dd (13, 12.1)
H_{eq}-7	1.18 d (13)
H-9	1.75 t (9.1, 7.6)
H_{ax}-11	1.82 dd (13, 9.1)
H_{eq}-11	2.08 dd (13, 7.6)
H-12	5.42 s, br
H_{ax}-15	2.62 d (15.6)
H_{eq}-15	1.89 d (15.6)
H-17	2.06 td (12.4, 12.3, 3.6)
H-18	2.35 td (12.4, 11.6, 3.6)
H_{ax}-19	1.04 t (12.5, 11.6)
H_{eq}-19	1.86 dd (12.5, 3.6)
H-21	3.19 dd (12.3, 4.2)
H_{ax}-22	1.28 q (12, 12, 12)
H_{eq}-22	2.23 dt (12.3, 4.2, 3.6)
CHO-23	9.36 s
Me-24	1.00 s
Me-25	0.92 s
Me-26	0.79 s
Me-27	0.96 s
Me-29	0.94 s
Me-30	0.82 s

recent report of a similar sapogenin structure shows a similar qualitative effect (H_{eq}-6 upfield of H_{ax}-6) (10); differences in substitutents at C-4 lead to differences in the chemical shifts for H-5 and H_{eq}-6 (10).

The relative stereochemistry of the aglycone of compound **2** was confirmed by obtaining 2D rotating frame Overhauser spectra (ROESY) and using them in conjunction with proton coupling constant data. In several cases precise coupling constants could not be obtained, but it was possible to observe the number of large (geminal or axial-axial) couplings present at a particular resonance. H-3, at 3.84 ppm, shows an 11.6 Hz coupling; thus the glucuronic acid is attached in an equatorial manner. The presence of an nOe between the aldehyde proton (H-23) and H-3, and the lack of any nOe between H-3 and Me-24, then shows that Me-24 is axial. Strong nOe's between Me-24, 25, and 26 and H_{ax}-6 at 1.45 ppm show that all of these methyls are axial. Me-26 shows a strong nOe to H-15 at 2.62 ppm, which was assigned axial; a weak nOe cross peak to H-18 (2.32 ppm) confirms this. Large couplings around the ring E system indicated that H-17, H-18, H_{ax}-19, and H_{ax}-22 are all mutually diaxial. Then nOe data connecting the H-30 to H-18 and H_{ax}-22, and corresponding nOe's connecting H-29 to axial protons H_{ax}-19, H-17, and H-21, show that H-30 is axial while H-29 is equatorial. A summary of observed nOe cross peaks is shown in Table 5. Key nOe connections and the complete structure are shown in Figure 3 for the aglycone of compound **2**.

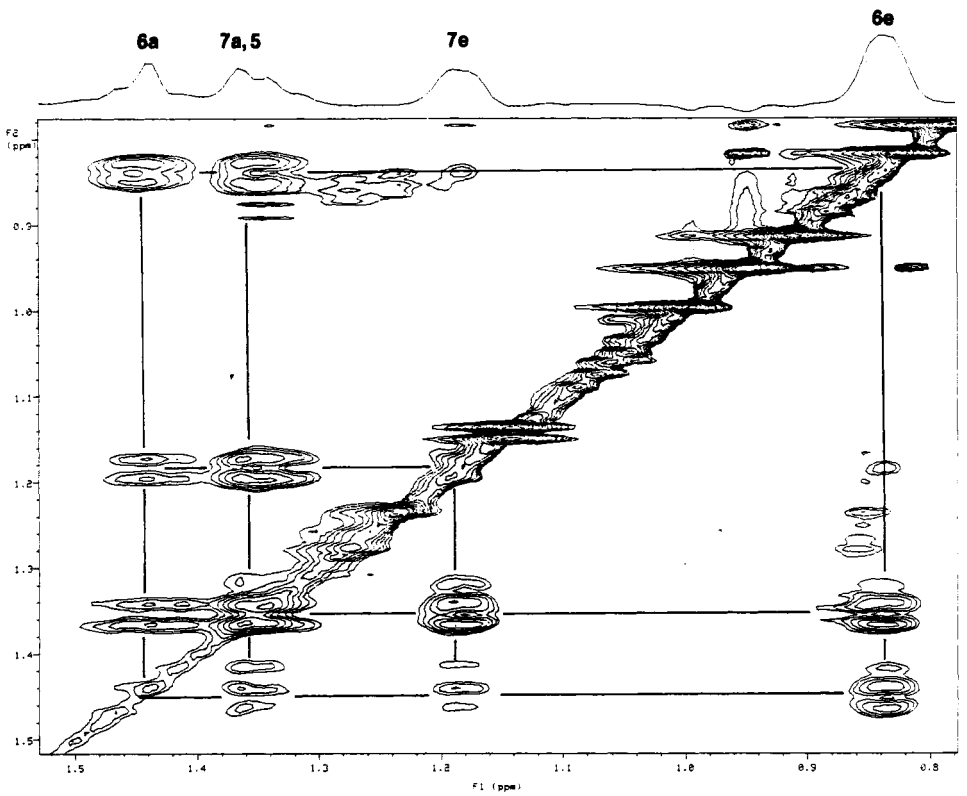


FIGURE 2. Expansion of the TOCSY spectrum of **2** showing connections between the 5, 6, and 7 protons of **2**. These resonances are labelled on the trace spectrum (taken at 1.37 ppm) shown at the top.

Methanolysis of compound **2** gave 3-*O*-β-D-glucuronopyranosyl melandrigenin methyl ester [**3**], identified by a comparison with an authentic sample (5). Further comparison of the ^{13}C-nmr signals of compound **2** with those of **3** showed significant glycosidation shift of the signal for C-4 of the glucuronic acid moiety.

Considering the coupling constants of the anomeric protons and the chemical shifts of the anomeric carbons, C-1 atoms of glucuronic acid and galactose were assigned as β configuration, and that of rhamnose as α configuration.

On the basis of the above evidence, the structure of melandrioside A was elucidated to be 3-*O*- { β-D-galactopyranosyl-(1↦2)-[α-L-rhamnopyranosyl-(1↦4)]-β-D-glucuronopyranosyl } -melandrigenin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. Ir measurements were recorded on a Perkin-Elmer 283B spectrophotometer using KBr. Tlc used Si gel GF-254. Optical rotations were measured on a Rudulph Autopol III polarimeter. ^{1}H (499.843 MHz) and high field ^{13}C (125.697 MHz) spectra were obtained on a Varian VXR-500S spectrometer equipped with a SUN 4/110 work station. Low field ^{13}C nmr (20 MHz) of compound **3** was performed on a Varian FT-80A nmr spectrometer. D_2O exchange experiments were used to identify nonexchangeable proton resonances. H_2O-D_2O (50:50) (approximately 100 μl) was added to the samples to obtain carbon spectra distinguishing COH carbons from COR carbons (12,13). Proton-proton *J* connectivities were established using 2D homonuclear experiments, namely COSY (14) and TOCSY (15). Carbon-proton *J* connectivities were established using a one-bond heteronuclear inverse detection 2D experiment, HMQC (16). Spin simulation, without iteration, and selective proton decoupling experiments were

TABLE 5. ROESY Cross Peaks of the Aglycone Moiety of **2**.

Assignment	δ, ppm	Connections
23	9.36	5_{ax}, 3, 24, 6_{eq}
12	5.42	19_{eq}, 26, 19_{ax}, 18_{ax}, 9_{ax}, 11_{ax}
3	3.89	2_{eq}, 1_{ax}, 23
21	3.19	29, 19_{ax}, 22_{ax}, 30
15_{ax}	2.62	26, 15_{eq}, 18_{ax}
18	2.35	29, 30, 12, 22_{ax}, 19_{ax}, 26, 15_{ax}
22_{eq}	2.23	22_{ax}, 29, 19_{ax}
17	2.06	22_{ax}, 19_{ax}, 30, 29
11_{eq}	2.08	11_{ax}, 1_{ax}, 1_{eq}, 25, 26
2_{eq}	1.97	2_{ax}, 1_{ax}, 3
15_{eq}	1.89	15_{ax}, 7_{ax}, 7_{eq}
11_{ax}	1.82	11_{eq}, 25, 26, 12
19_{eq}	1.86	30, 19_{ax}, 29, 12
9	1.75	27, 26, 25, 1_{ax}, 7_{ax}, 12
2_{ax}	1.62	2_{eq}, 24, 25
1_{eq}	1.56	1_{ax}, 11_{eq}
6_{ax}	1.45	26, 7_{eq}, 6_{eq}, 24, 25
5	1.36	24, 25, 26, 9_{ax}, 6_{eq}, 23
7_{ax}	1.34	27, 6_{eq}, 7_{eq}, 9_{ax}
22_{ax}	1.28	22_{eq}, 30_{ax}, 17_{ax}, 21, 18_{ax}
7_{eq}	1.19	7_{ax}, 6_{ax}, 15_{eq}
1_{ax}	1.09	3, 1_{eq}, 2_{eq}, 9_{ax}, 11_{eq}
19_{ax}	1.04	19_{eq}, 29, 21, 17_{ax}, 12, 18_{ax}, 22_{eq}
24	1.00	2_{ax}, 6_{ax}, 25, 5_{ax}, 23
27	0.96	9_{ax}, 6_{ax}
29	0.94	30, 19_{ax}, 21, 22_{eq}, 18_{ax}, 19_{eq}, 17_{ax}
25	0.92	24, 25, 6_{ax}, 5_{ax}, 2_{ax}, 9_{ax}, 11_{ax}, 11_{eq}
6_{eq}	0.86	6_{ax}, 5_{ax}, 7_{ax}, 23
30	0.82	19_{eq}, 29, 22_{ax}, 21, 18_{ax}, 17_{ax}
26	0.79	25, 15_{ax}, 6_{ax}, 11_{ax}, 9_{ax}, 12, 18_{ax}, 11_{eq}

also used to confirm proton assignments and to allow measurement of coupling constants. Some proton-proton coupling constants were estimated by examining the 2D cross peaks of COSY, TOCSY or HMQC. A homonuclear 2D *J* spectrum of compound **2** provided additional coupling constant information. Nuclear Overhauser connectivities were obtained using ROESY (17). Typical experimental parameters for the 2D spectra included 512 fid's acquired with 2K data points zero-filled to 2K × 2K, using sine bell or cosine apodization, and an overall recycle time of 1.3 sec, including an acquisition time of 0.3 sec. The typical ^{1}H 90° pulse width was 8.5 μsec, ^{13}C 90° pulse width was 14.0 μsec, and ^{13}C indirect detection 90° pulse width was 27 μsec. All experiments were performed non-spinning at 27°. ^{1}H-nmr shifts are expressed in ppm downfield from TMS; ^{13}C-nmr shifts in ppm vs. DMSO-d_6 solvent = 39.50 ppm relative to TMS. Coupling constants are in Hz.

EXTRACTION AND ISOLATION.—The plant material and the extraction procedure were described earlier (4). The MeOH extract was partitioned with hexane, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The *n*-BuOH-soluble fraction (15 g) was subjected to SiO_2 cc using $CHCl_3$-MeOH (25:6, 25:7, 25:8, 25:9). The eluates were collected in 250-ml portions and combined to give nine main fractions. Fraction 7 was chromatographed first over Sephadex LH-20 [MeOH-H_2O (7:3)] and then SiO_2 [$CHCl_2$-MeOH-H_2O (13:10:2)] to obtain pure **1** as an amorphous powder (300 mg): mp >360°; $[\alpha]_D$ −74.3 (c = 0.105, pyridine); ir ν max cm^{-1} 3440 (OH), 1720, 1712, 1698 (C=O), 1100–1000 (glycoside); ^{13}C nmr see Tables 1 and 2; ^{1}H nmr see Table 3.

ACID HYDROLYSIS OF COMPOUND **1**.—Compound **1** (100 mg) was hydrolyzed with 5% H_2SO_4 for 5 h. The precipitate was filtered, washed with H_2O, and crystallized from MeOH as amorphous powder: mp 294°; ms (70 eV) *m/z* (rel. int.) $[M]^+$ 456 (30.5), 234 (D/E ring) (65.2), 221 (A/B ring) (12.9). Acetate: mp 267–268°, ms (70 eV) *m/z* (rel. int.) $[M - HOAc]^+$ 480 (59.4), 276 (D/E ring) (29.5), 216 (276-HOAc) (100). It was identified as melandrigenin by direct comparison with an authentic sample (mmp,

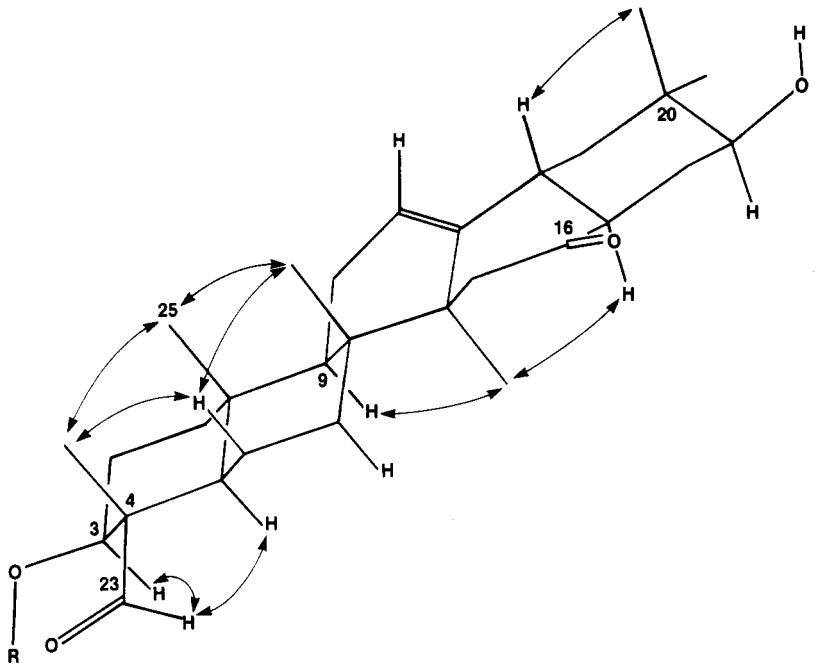


FIGURE 3. Projection of the 3D structure of the aglycone of melandioside A [**1**] rotated to display the juxtaposition of protons on the "top" and "bottom" sides of the molecule. Arrows connecting protons reflect key nOe cross-peaks from the ROESY spectrum, in particular, the H_{ax}-6–Me-24–Me-25–Me-26 connections; the CHO-23–H_{ax}-3–H_{ax}-5 connections; the H_{ax}-9–Me-27–H_{ax}-17 connections; and the H_{ax}-18–Me-30 connection.

co-tlc, ^{13}C nmr). The filtrate was neutralized with $BaCO_3$, filtered and concentrated in vacuo. Rhamnose, galactose, and glucuronic acid were detected by tlc [precoated cellulose plate, pyridine-EtOAc-HOAc-H_2O (36:36:7:21)].

PARTIAL ACID HYDROLYSIS OF COMPOUND **1**.—Compound **1** (100 mg) in 0.5% HCl (7 ml) was refluxed for 1 h and added to ice-H_2O. The precipitate was filtered and chromatographed over SiO_2. Elution with $CHCl_3$-MeOH-H_2O (7:3.5:0.6) gave the prosapogenin **2** as amorphous powder (20 mg): mp 269–270°; $[\alpha]D$ −37.0 ($c = 0.1$, pyridine); ir ν max (KBr) cm^{-1} 3440 (OH), 1715, 1705, 1690 (CO), 1100–1000 (glycoside); ^{13}C nmr see Tables 1 and 2, ^{1}H nmr see Tables 3 and 4.

IDENTIFICATION OF TERMINAL SUGARS.—Compounds **1** and **2** were run on Si gel tlc and left in an HCl atmosphere at room temperature for 1 h. HCl vapor was eliminated under hot ventilation, and authentic sugar samples were applied to the plate. The plate was developed with $CHCl_3$-MeOH-H_2O (8:5:1) and spots detected by spraying with aniline hydrogenphthalate reagent followed by heating. Rhamnose and galactose were detected as the terminal sugars of **1**, whereas galactose was detected for that of **2**.

METHANOLYSIS OF COMPOUND **2**.—Compound **2** (100 mg) in 1% methanolic HCl (5 ml) was refluxed for 30 min, added to crushed ice, and extracted with EtOAc. The EtOAc extract was chromatographed on an SiO_2 column with $CHCl_3$-MeOH (95:5) to give melandrigenin and 3-*O*-β-glucuronopyranosyl melandrigenin methylester [**3**], mp 132–133°, identified by comparison with authentic samples (mmp, co-tlc, ^{13}C nmr) (4,11).

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LITERATURE CITED

1. L.M. Perry, "Medicinal Plants of East and Southeast Asia," MIT, Massachusetts, 1980, p. 74.
2. K.H. Shin, W.S. Woo, and C.K. Lee, *Kor. J. Pharmacogn.*, **16**, 1 (1985).
3. W.S. Woo, J.S. Choi, and H.S. Chang, *Arch. Pharm. Res.*, **8**, 133 (1985).
4. H.S. Chang, Y.B. Han, W.S. Woo, S.S. Kang, H. Lotter, and H. Wagner, *Planta Med.*, **55**, 544 (1989).
5. W.S. Woo, *Arch. Pharm. Sci.*, **14**, 160 (1991).
6. P.A.J. Gorin and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975).
7. M. Amoros and R.L. Girre, *Phytochemistry*, **26**, 787 (1987).
8. P.K. Agrawal, D.C. Jain, R.K. Gupta, and R.S. Thakur, *Phytochemistry*, **24**, 2479 (1985).
9. L.M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed., Pergamon Press, New York, 1969, p. 237.
10. R.L. Baxter, K.R. Price, and G.R. Fenwick, *J. Nat. Prod.*, **53**, 298 (1990).
11. E.H. Woo and W.S. Woo, *Kor. J. Pharmacogn.*, **22**, 211 (1991).
12. S. Ho, H.J. Koch, and R.S. Stuart, *Carbohydr. Res.*, **64**, 251 (1978).
13. D. Gagnaire and M. Vincendon, *J. Chem. Soc., Chem. Commun.*, **15**, 509 (1977).
14. L. Muller, A. Kumar, and R.R. Ernst, *J. Chem. Phys.*, **63**, 5490 (1975).
15. L. Braunschweiler and R.R. Ernst, *J. Magn. Reson.*, **53**, 521 (1983).
16. A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).
17. H. Kessler, C. Griesinger, R. Kerssebaum, K. Wagner, and R.R. Ernst, *J. Am. Chem. Soc.*, **109**, 607 (1987).

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