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NEW DAMMARANE-TYPE GLYCOSIDES FROM *GYNOSTEMMA PENTAPHYLLUM*

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ABSTRACT.—Four novel dammarane glycosides were isolated from a MeOH extract of the aerial parts of *Gynostemma pentaphyllum*. Their structures were elucidated by 1D and 2D nmr experiments, including ^{1}H-^{1}H correlation spectroscopy (COSY, HOHAHA, NOESY) and ^{1}H-^{13}C heteronuclear correlation (HETCOR). The aglycone moieties are the new dammarane-type triterpenes (20*S*)-3β,20,23ξ-trihydroxydammar-24-en-21-oic acid-21,23-lactone for **1**, the corresponding epimer at C-20 (20*R*) for **2**, (20*S*)-dammar-23-ene-3β-20,25,26-tetraol for **3**, and (20*R*)-dammar-25-ene-3β,20,21,24ξ-tetraol for **4**.

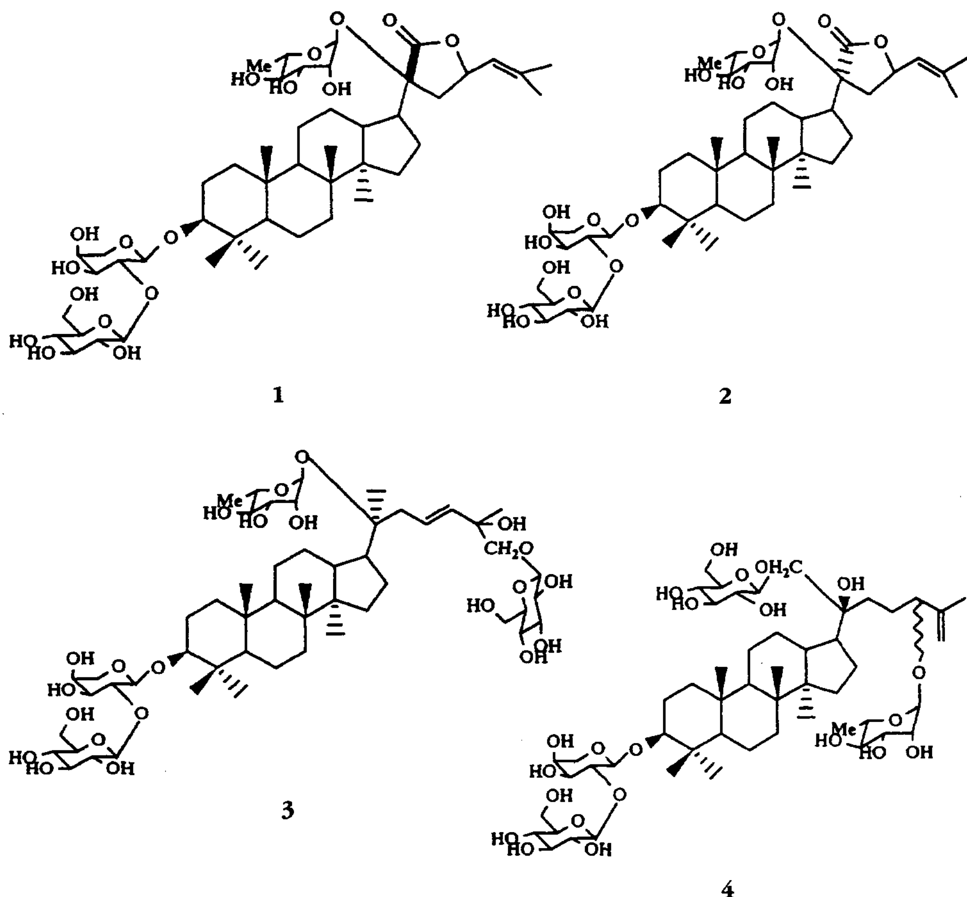
Gynostemma pentaphyllum Makino (Cucurbitaceae) is a perennial liana growing wild throughout Japan, China, and Korea, once used for its sweet properties (1). Previous investigations of this plant have shown the occurrence of dammarane-type glycosides structurally correlated to the ginseng saponins (2,3). Since ginsenosides are the well-known biologically active principles in Korean ginseng, *G. pentaphyllum* has received much attention. Continuing our studies on chemical constituents of medicinal plants, we have isolated four new triterpene oligosaccharides from *G. pentaphyllum* [**1**–**4**]. Structure elucidation was accomplished mainly on the basis of 2D nmr, ^{1}H-^{1}H, and ^{1}H-^{13}C shift correlation spectroscopy.

RESULTS AND DISCUSSION

The aerial parts of *G. pentaphyllum* were extracted successively with petroleum ether, $CHCl_3$, and MeOH. The MeOH extract was partitioned into a mixture of *n*-BuOH and H_2O to afford the *n*-BuOH-soluble portion, which was subjected to Sephadex LH-20 cc, followed by dccc [$CHCl_3$-MeOH-H_2O (7:13:8), descending mode] and then reversed-phase hplc, to give four pure compounds [**1**–**4**].

Compound **1** had a molecular formula of $C_{47}H_{76}O_{17}$, as determined by ^{13}C, ^{13}C DEPT nmr, and fabms in negative-ion mode. The fabms spectrum of **1** showed the $[M-H]^-$ ion at *m/z* 911 and prominent fragments at *m/z* 749 $[(M-H)-162]^-$, *m/z* 765 $[(M-H)-146]^-$ (cleavage of a deoxyhexose unit with or without the glycosidic oxygen), and *m/z* 733 $[(M-H)-178]^-$ due to the loss of a hexose unit. The ^{13}C- and DEPT ^{13}C-nmr spectra showed 47 signals, of which 17 were assigned to the saccharide portion and 30 to a triterpenoid moiety. The ^{1}H-nmr spectrum of **1** showed, in addition to seven singlets assignable to tertiary methyls at δ 0.87–1.84 [two of which were diagnostic for methyls linked to sp^2 carbons (δ 1.81 and 1.84)], two signals at δ 1.86 (1H, dd, J=9.7 and 13 Hz) and δ 2.25 (1H, dd, J=5.3 and 13 Hz), ascribable to a methylene group. Further features were signals at δ 5.22 (1H, d, J=9.1 Hz) and δ 5.39 (1H, ddd, J=5.3, 9.1, and 9.7 Hz) indicating, respectively, the occurrence of an olefinic proton and a -CHOH group. Spin-decoupling experiments showed a CH_2-CHOH-CH=C sequence that was confirmed by 2D COSY experiments. A 3β-OH substitution was evident from the chemical shift and the J values of the proton ascribable to C-3 at δ 3.17 (dd, J=11 and 4.5 Hz).

The ^{13}C-nmr spectrum (Table 1) of **1** suggested a dammarane-type triterpene



skeleton with an unusual side-chain. A signal at δ 179.23 indicated the occurrence of a carboxylic group at C-21. HETCOR nmr experiments correlated the carbon resonances at δ 40.52 (C-22), 76.37 (C-23), and 123.63 (C-24) with those of the corresponding protons at δ 1.86 and 2.25, 5.39, and 5.22 in the ^{1}H-nmr spectrum; in addition, the signals at δ 1.84 and δ 1.81 were attributed to Me-26 and Me-27, allowing unambiguous assignment of the side-chain structure. The chemical shift of H-23 (δ 5.39) suggested that the alcoholic function was involved in a lactone ring with the C-21 carboxylic group. Glycosidation of the alcoholic function at C-3 and C-20 was indicated by the significant downfield shift observed for these carbon resonances in **1** (Table 1), relative to the corresponding signals in model compounds in the literature (4).

Anhydrous acidic methanolysis of **1** gave methyl arabinoside, methyl rhamnoside, and methyl glucoside in a 1:1:1 ratio. The structure of the oligosaccharide unit was achieved using 2D nmr spectroscopy. Even at high field (500 MHz), the 1D nmr sugar spectral region of **1** was complex, as most of the shifts were found between δ 5.22 and 3.17 and were overlapped by the aglycone signals. 2D HOHAHA nmr spectroscopy experiments (5) allowed resolution of the overlapped spectra of oligosaccharides into a subset of individual monosaccharide spectra. In the 2D HOHAHA nmr spectrum of **1** the anomeric proton signal ascribable to an α-L-arabinose (H-1′, δ 4.52, J=5.2 Hz) unit showed connectivities to three methines (δ 4.08, 3.90, and 3.56). The coherence transfer to methylene H-5′ was not obtained because of the small coupling constants at H-4′ and H-5′ (6). As in the HOHAHA method, the cross-peaks represented both direct and

TABLE 1. ^{13}C-Nmr Data of the Aglycones of Compounds **1–4** in CD_3OD.

Carbon	Compound			
	1	**2**	**3**	**4**
C-1	39.09	40.71	39.75	40.09
C-2	27.23	27.36	27.39	27.24
C-3	89.46	89.37	89.68	89.46
C-4	40.43	40.65	40.61	40.09
C-5	57.63	57.88	57.78	57.61
C-6	19.07	19.30	19.26	19.07
C-7	36.36	36.59	36.49	36.38
C-8	41.53	41.47	41.74	41.59
C-9	52.09	52.00	52.01	51.98
C-10	37.94	37.61	38.06	38.01
C-11	22.39	22.56	22.60	22.48
C-12	26.49	26.03	24.90	24.95
C-13	45.48	43.20	42.63	42.44
C-14	50.92	51.02	51.97	51.05
C-15	32.25	32.36	31.98	32.02
C-16	28.29	27.74	28.60	28.38
C-17	45.58	46.50	46.85	46.58
C-18	16.26	16.13	16.55	16.60
C-19	16.48	16.86	16.81	16.79
C-20	81.83	79.60	82.00	77.41
C-21	179.23	180.67	24.64	75.20
C-22	40.52	41.35	40.49	32.50
C-23	76.37	75.46	127.26	26.00
C-24	123.63	124.74	138.35	90.94
C-25	141.13	140.42	78.00	146.56
C-26	25.73	25.73	75.16	114.06
C-27	18.27	18.24	24.85	17.06
C-28	28.39	28.68	28.37	28.38
C-29	15.84	15.92	16.04	15.97
C-30	16.93	17.10	16.95	16.91

relayed connectivities; we also recorded a 2D COSY-90 nmr spectrum, which established the proton sequence within this sugar fragment at H-1 (δ 4.52), H-2 (δ 3.90), H-3 (δ 3.56), and H-4 (δ 4.08) (Table 2).

Similar observations concerning the results of the HOHAHA and COSY experiments for all the other sugar residues (Table 2) allowed complete sequential assignments for all proton resonances starting from the well-isolated anomeric proton signals. HETCOR experiments which correlated all proton resonances with those of each corresponding carbon (Table 2) permitted assignments of the interglycosidic linkages by comparison of the carbon chemical shifts observed with those of the corresponding methyl pyranosides, taking into account the known effects of glycosidation (7).

The absence of any ^{13}C-nmr glycosidation shift for the glucopyranosyl and rhamnopyranosyl residues of **1** suggested that these sugars were terminal units, while a glycosidation shift at C-2 (+7 ppm) of arabinose indicated this monosaccharide to be glycosidated at C-2. To establish which of the two sugars (glucose or rhamnose) was linked to C-2 of the arabinopyranosyl unit and to determine unambigously the positions of the monosaccharidic residue and the disaccharidic chain, 2D NOESY experiments were performed. Data from the 2D NOESY experiment allowed us to establish an α-L-arabinopyranoside configuration in rapid conformational exchange.

Chemical shifts, the multiplicity of the signals, the absolute values of the coupling

TABLE 2. Nmr Data of the Sugar Units of Compound **1** (CD_3OD, 500 MHz).

Method[a]	Sugar[b]	ppm (J_{HH}, Hz)[c]						
^{1}H nmr (from H-1′ to H-5′)	Ara[d]	4.52 (5.2) d	3.90 (5.2, 8.2) dd	3.56 (8.2, 3) dd	4.08 m	3.92 (2, 12) dd	3.52 (12, 2.5) dd	
^{13}C nmr (from C-1′ to C-5′)		105.00	82.06	73.66	68.41	64.53		
^{1}H nmr (from H-1″ to H-6″)	Glc	4.57 (7.5) d	3.33 (7.5, 9.5) dd	3.45 (9.5, 9.5) dd	3.31 (9.5, 9.5) dd	3.37 m	3.87 (12, 3.5) dd	3.72 (12, 5) dd
^{13}C nmr (from C-1″ to C-6″)		105.14	75.08	77.82	71.00	77.82	62.18	
^{1}H nmr (from H-1‴ to H-6‴)	Rha	5.22 (1.5) d	3.93 (1.5, 2.5) dd	3.73 (2.5, 9) dd	3.42 (9, 9) dd	3.86 (9, 6.5) dd	1.25 (6.5) d	
^{13}C nmr (from C-1‴ to C-6‴)		101.92	71.94	71.94	74.87	70.08	17.86	

[a]Assignments confirmed by HOHAHA, COSY-90, and HETCOR nmr experiments.
[b]Ara=arabinose, glc=glucose, rha=rhamnose.
[c]^{1}H-^{1}H coupling constants in the sugar spin-system were measured from COSY and HOHAHA nmr spectra.
[d]Sugar bound to C-3 of the aglycone.

constants, and their magnitude in the ^{1}H-nmr spectrum, as well as the ^{13}C-nmr data (Table 2), indicated a β-configuration at the anomeric positions for the glucopyranosyl units ($J_{H1\text{-}H2}$=7.5 Hz) and an α-configuration for the rhamnopyranosyl unit (Table 2) ($J_{H1\text{-}H2}$=1.5 Hz). ^{13}C-Nmr data allowed assignment of the pyranose form to L-arabinose (8), but no further support for the anomeric configuration of the L-arabinopyranose unit could be drawn from the ^{1}H- and ^{13}C-nmr data. In fact, as we also observed in previous work (9), the value of its $J_{H1\text{-}H2}$ coupling constant (5.2 Hz), midway between that observed for methyl-β-L-arabinopyranoside (4 Hz) and methyl-α-L-arabinopyranoside (8 Hz) (10,11), has been reported not to be diagnostic on its own owing to the high conformational mobility of arabinopyranosides ($^4C_1$<—> $^1C_4$).

Nuclear Overhauser effects were observed from C_{ara}-1 to C_{ara}-2 and from C_{ara}-1 to C_{ara}-3 as expected for the $^1C_4$ and $^4C_1$ conformations, respectively. The nOe C_{ara}-1 to C_{ara}-3 would not be expected for both $^1C_4$ and $^4C_1$ β-L-arabinopyranosides. An nOe was also observed between C_{ara}-1 and C_{ara}-5 as expected for an α-L-arabinopyranoside in a $^4C_1$ conformation. In addition, the 2D NOESY nmr spectrum displayed correlations between the signals at δ 3.17 (H-3 of the aglycone) and δ 4.52 (H-1_{ara}) and between the resonances at δ 4.57 (H-1_{glc}) and δ 3.90 (H-2_{ara}) allowing us to establish the linkage of the arabinopyranosyl unit at C-3 of the aglycone moiety, which in turn was substituted at C-2 with a glucopyranosyl unit; thus, the rhamnopyranosyl unit should be linked to C-20 of the aglycone moiety.

Compound **2** ($C_{47}H_{76}O_{17}$) showed a fabms fragmentation pattern superimposable on that of **1**; comparison of the ^{1}H- and ^{13}C-nmr data of the two compounds indicated an identical saccharide chain and structural similarity in the aglycone moiety. The main differences were the chemical shifts of H-22 (δ 1.95 and 2.55 in **2** vs. δ 1.86 and 2.25 in **1**), H-23 (δ 5.24 in **2** vs. δ 5.39 in **1**), H-24 (δ 5.33 in **2** vs. δ 5.22 in **1**) in the ^{1}H-nmr spectra, and the resonances of C-13 and C-17 in the ^{13}C-nmr spectra (Table 1). Analysis of the observed shifts and comparison with some dammarane model compounds from the literature (12–14) promped us to hypothesize that the difference between the two compounds should be confined to the stereochemistry at C-20. Previously Asakawa *et al.* determined the absolute configuration at C-20 in ginseng saponins using shift reagent techniques (12). In this way it has been found that the differences of the C-13,

C-17, and C-21 chemical shifts between pairs of C-20 isomers are remarkable. The ^{13}C-nmr resonances of C-13 (δ 45.48) and C-17 (45.58) in **1** were in good agreement with published data for 20*R* epimers (13), whereas in **2** the chemical shifts of C-13 (δ 43.20) and C-17 (δ 46.50) exhibited, respectively, an upfield shift and a downfield shift, as expected for 20*S* epimers (14). Because of the modification in priority due to the substitution of Me-21 in model compounds with a carboxylic group in **1** and **2**, the stereochemistry at C-20 was established as *S* in **1** and *R* in **2**.

Several attempts were made to establish the stereochemistry of the -OH group at C-23 in compounds **1** and **2**, both by chemical transformation and by nmr analysis. Regrettably, these efforts were unsuccessful due to both decomposition of the compounds on attempted chemical transformation and ambiguities in the nmr analysis.

On the basis of the foregoing data, the structures of compounds **1** and **2** are proposed to be, in turn, (20*S*)-3β,20,23ξ-trihydroxydammar-24-en-21-oic acid-21,23-lactone-3-*O*[(β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-20-*O*-β-D-rhamnopyranoside [**1**], and its epimer at C-20 (20*R*) [**2**].

The fabms of **3** ($C_{53}H_{90}O_{22}$) showed a quasi-molecular anion [$(M-H)^-$] at *m/z* 1077 and peaks due to the fragmentation of the oligosaccharide portion at *m/z* 915 [$(M-H)-162]^-$, *m/z* 899 [$(M-H)-178]^-$, *m/z* 737 [$(M-H)-(178+162)^-$] and *m/z* 591 [$(M-H)-(178+146+162)^-$]. Comparison of the ^{1}H- and ^{13}C-nmr data with those of **1** and **2** suggested that **3** had a different aglycone with an additional sugar unit (Table 3) that was found to be a glucose unit by gc analysis, which gave methyl arabinoside, methyl rhamnoside, and methyl glucoside in a 1:1:2 ratio. The ^{1}H-nmr spectrum of **3** displayed resonances for seven methyls (δ 0.88–1.32), two of which were linked to oxygenated carbons (δ 1.31 and 1.32). In addition, it was possible to observe olefin

TABLE 3. ^{13}C-Nmr Data for the Oligosaccharide Moieties of Compounds **2**–**4** in CD_3OD.

Sugar		Compound		
		2	**3**	**4**
Ara	C-1	104.87	104.87	104.81
	C-2	81.67	82.10	82.17
	C-3	74.00	73.83	73.65
	C-4	68.20	68.50	68.19
	C-5	64.29	64.62	64.40
Glc	C-1	104.22	105.00[a]	104.75
	C-2	75.11	75.27[b]	75.13[a]
	C-3	78.08[a]	78.03[c]	77.81
	C-4	71.44	71.75[d]	71.55[b]
	C-5	78.14[a]	77.98[c]	77.81
	C-6	62.58	62.39[e]	62.22[c]
Rha	C-1	101.82	101.98	101.82
	C-2	72.20[b]	72.11[f]	72.35[d]
	C-3	72.32[b]	72.13[f]	72.81[d]
	C-4	75.11	75.06[b]	74.89[a]
	C-5	70.35	70.28	70.14
	C-6	17.95	18.00	17.84
Glc	C-1		105.10[a]	105.02
	C-2		75.33[b]	75.05[a]
	C-3		78.00[c]	77.81
	C-4		71.19[d]	71.00[b]
	C-5		77.98[c]	77.81
	C-6		62.86[e]	62.67[c]

[a–f]Values in any column bearing the same superscript may be reversed.

proton signals at δ 5.68 (1H, d, J=15.5 Hz) and δ 5.73 (1H, ddd, J=15.5, 8.5, and 5.5 Hz) indicating the presence of a trans double bond connected to a methylene group at δ 2.27 (1H, dd, J=5.5 and 13 Hz) and δ 2.48 (1H, dd J=8.5 and 13 Hz) as suggested by spin-decoupling experiments. The ^{13}C-nmr spectrum (Table 1) showed the resonances ascribable to two olefinic carbons (δ 127.26, C-23 and 138.35, C-24), as well as signals indicating the occurrence of two tertiary hydroxyl groups (δ 82.00 and 78.00) and a CH_2OH group (δ 75.16) that were located, respectively, at C-20, C-25, and C-26 by comparison with published data for isofouquierol, isolated from *Fouquieria splendens* (15), which differed from the aglycone of **3** by the absence of the hydroxy group at C-26. The downfield shift (δ 78.00 vs. 70.62, β-effect) and the upfield shift (δ 24.85 vs. 29.88, γ-effect) exhibited, respectively, by C-25 and Me-27, when compared to the corresponding carbons in isofouquierol, were in fair agreement with the location of the -CH_2OH group at C-26.

The stereochemistry at C-20 in **3** was established as *S* by comparison with model compounds, in which the diagnostic resonances of C-13, C-17, and Me-21 were considered (12–14). The chemical shifts of C-3 (δ 89.68) and C-20 (δ 82.00) indicated that also in **3** both alcoholic functions were glycosylated (4). The additional glucose unit was located at C-26 by comparison of this carbon shift with the analogous shift in unglycosylated model compounds (16). Thus, compound **3** is (20*S*)-dammar-23-ene-3β-20,25,26-tetraol-3-*O*-[(β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-20-*O*-β-D-rhamnopyranosyl-26-*O*-glucopyranoside.

Compound **4** showed a quasi-molecular anion [$(M-H)^-$] at *m/z* 1077 and a fragmentation pattern very similar to that of compound **3**. In the ^{1}H-nmr spectrum, notable features for the aglycone moiety were the occurrence of five methyl signals (δ 0.88–1.04) and one further signal due to a methyl group linked to an olefinic carbon (δ 1.74). The chemical shift of H-3 (δ 3.20, dd, J=11.0 and 4.5 Hz) was found at almost the same position as those of **1**–**3**, and an additional resonance ascribable to a -CHOH group linked to an olefinic carbon was evident at δ 4.18 (1H, t, J=7 Hz). Comparison of the chemical shifts of C-22–C-27 (Table 1) with those of the corresponding atoms in chikusetsusaponin-L9bc from *Panax ginseng* (17) indicated **4** to possess an acyclic side-chain having a hydroxy group at C-24 (δ 90.94), a tertiary hydroxyl group on C-20 (δ 77.41), and an isopropenyl moiety (δ 146.56, 114.06, 17.06). One other signal at δ 75.00 suggested the occurrence of a -CH_2OH group that was located at C-21, on the basis of the absence of a signal due to a methyl group linked to an oxygenated carbon in the ^{1}H-nmr spectrum, and by comparison with model compounds (18). Glycosylation at C-3, C-21, and C-24 was indicated by the significant downfield shifts exhibited by these carbon resonances in **4**, relative to the corresponding signals in model compounds (4,18,17). Glc analysis gave methyl arabinoside, methyl rhamnoside, and methyl glucoside in a 1:1:2 ratio.

The chemical shifts of the signals of the sugar moiety of **4** (Table 3) were in fair agreement with those of **3**, indicating the presence of an arabinopyranosyl residue linked to C-3 of the aglycone and branched, in turn, at C-2 with a glucopyranosyl moiety. The location of another glucopyranosyl unit was postulated at C-21 on the basis of the chemical shift of the anomeric proton (δ 4.28), which indicated that this sugar was linked to a secondary alcoholic carbon (19). Thus, the rhamnose unit should be linked to C-24.

The 20*R* configuration was deduced by comparison with **1** and **2**. Because of the small quantity (4 mg), it was not possible to establish the stereochemistry at C-24; consequently **4** was defined as (20*R*)-dammar-25-ene-3β,20,21,24ξ-tetraol-3-*O*-[(β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-21-*O*-β-D-glucopyranosyl-24-*O*-rhamnopyranoside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: for nmr, a Bruker WH-250 Spectroscopin or a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer using the UXNMR software package. Two-dimensional homonuclear proton chemical shift correlation (COSY) experiments were measured by employing the conventional pulse sequence. The COSY spectrum was obtained using a data set (t1×t2) of 1024×1024 points for a spectral width of 1165 Hz (relaxation delay 1 sec). The data matrix was processed using an unshifted sine bell window function, followed by transformation to give a magnitude spectrum with symmetrization (digital resolution in both F2 and F1 dimensions 1.13 Hz/pt). The 2D-HOHAHA experiment was performed in the phase-sensitive mode (TPPI) using an MLEV-17 sequence for mixing (16). The spectral width (t2) was 1002 Hz; 512 experiments of 40 scans each (relaxation delay 1.5 sec, mixing time 100 msec) were acquired in both dimensions before transformation. The resulting digital resolution in F2 was 0.48 Hz/pt. The NOESY experiment was performed in the phase-sensitive mode (TPPI). The spectral width (t2) was 1002 Hz; 512 experiments of 80 scans each (relaxation delay 1.5 sec, mixing time 200–300 msec) were acquired with 2K data points. For processing, a sine bell window function was applied in both dimensions before transformation. The HETCOR experiment was performed on a data matrix of 512×1024, using a CH coupling of 135 Hz and relaxation delay of 1.5 sec. The data matrix was processed using a q sine window function.

Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. Fabms were recorded in a glycerol matrix in the negative-ion mode on a VG ZAB instrument (Xe atoms of energy of 2–6 kV). Droplet-counter current chromatography (dccc) was performed on an apparatus manufactured by Buchi, equipped with 300 tubes. Glc analyses were performed on a Supelco SP200 capillary column (30 m, i.d. 0.32 mm, film thickness 0.25 mm, carrier gas He, 5 ml/min^{-1}, 156°).

PLANT MATERIAL.—*Gynostemma pentaphyllum* was collected at Suzhou, Jiang-Sou province, People's Republic of China. A voucher sample of the plant is deposited at the herbarium of the Dipartimento di Chimica della Sostanze Naturali, University degli Studi di Napoli "Federico II."

EXTRACTION AND ISOLATION.—The air-dried leaves (270 g) were defatted with petroleum ether and $CHCl_3$ and then extracted with MeOH to give 18 g of residue. Part of the MeOH extract (7 g) was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (4 g) which was chromatographed on a Sephadex LH-20 column (100×5 cm), with MeOH as eluent. Fractions (8 ml) were collected and checked by tlc [Si gel plates, *n*-BuOH-HOAc-H_2O (60:15:25)]. Fractions 16–24 (600 mg) containing the crude glycosidic mixture were further purified by dccc with $CHCl_3$-MeOH-H_2O (7:13:8) in which the stationary phase consisted of the upper phase (descending mode, flow 10 ml/h). Fractionation of each glycoside was achieved by hplc on a C_{18} μ-Bondapak column (30 cm×7.8 mm i.d.) eluting with MeOH-H_2O (7:3), flow 3 ml/min, to yield pure **1** (28 mg, R_t 46 min) and **2** (20 mg, R_t 30 min) and with MeOH-H_2O (3:2) to give **3** (7 mg, R_t 44 min) and **4** (4 mg, R_t 37 min).

Methanolysis of compounds **1–4**.—A solution of each compound (2 mg) in anhydrous 2N HCl/MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 12 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged, and the supernatant evaporated to dryness under N_2. The residue was reacted with TRISIL-Z (Pierce) and analyzed by glc. Retention times were identical to those of the authentic methyl sugars.

Compound **1**.—$[\alpha]^{25}D$ +2.5°, (c=1, MeOH); ^1H nmr for aglycone (500 MHz) Me-18 (δ 1.02), Me-19 (δ 1.03), Me-26 (δ 1.84), Me-27 (δ 1.81), Me-28 (δ 0.87), Me-29 (δ 0.92), Me-30 (δ 0.97), H-3 (δ 3.17, dd, J=11 and 4.5 Hz), H-22a (δ 1.86, dd, J=9.7 and 13 Hz), H-22b (δ 2.25, dd, J=5.3 and 13 Hz), H-23 (δ 5.39, ddd, J=5.3, 9.1, and 9.7 Hz), H-24 (δ 5.22, d, J=9.1 Hz); for ^1H-nmr data of the sugar portion, see Table 2; ^{13}C-nmr data, see Tables 1 and 2; fabms m/z 911 $[M-H]^-$.

Compound **2**.—$[\alpha]^{25}D$ −9.3° (c=1, MeOH); ^1H nmr for aglycone (500 MHz) Me-18 (δ 1.02), Me-19 (δ 1.03), Me-26 (δ 1.82), Me-27 (δ 1.80), Me-28 (δ 0.87), Me-29 (δ 0.92), Me-30 (δ 0.96), H-3 (δ 3.17, dd, J=11 and 4.5 Hz), H-22a (δ 1.95, dd, J=9.7 and 13 Hz), H-22b (δ 2.55, dd, J=5.3 and 13 Hz), H-23 (δ 5.24, ddd, J=5.3, 9.1, and 9.7 Hz), H-24 (δ 5.34, d, J=9.1 Hz); sugar signals were superimposable with those of **1** (Table 2); ^{13}C-nmr data, see Tables 1–3; fabms m/z 911 $[M-H]^-$.

Compound **3**.—$[\alpha]^{25}D$ −8.5° (c=1, MeOH); ^1H nmr for aglycone (500 MHz) Me-18 (δ 1.04), Me-19 (δ 1.04), Me-21 (δ 1.31), Me-26 (δ 1.32), Me-28 (δ 0.88), Me-29 (δ 0.91), Me-30 (δ 0.92), H-3 (δ 3.17, dd, J=11 and 4.5 Hz), H-22a (δ 2.27, dd, J=5.5 and 13 Hz), H-22b (δ 2.48, dd, J=8.5 and 13 Hz), H-23 (δ 5.73, ddd, J=5.5, 8.5, and 15.5 Hz), H-24 (δ 5.68, d, J=15.5 Hz); sugar signals were identical to those of **1** to +/− 0.02 ppm (Table 2), for the additional glucose residue the anomeric signal was centered at δ 4.28, the remaining protons were overlapping signals; ^{13}C-nmr data, see Tables 1–3; fabms m/z 1077 $[M-H]^-$.

Compound **4**.—$[\alpha]^{25}D$ $-33.6°$ (c=1, MeOH); ^1H nmr for aglycone (500 MHz), Me-18 (δ 1.03), Me-19 (δ 1.04), Me-26 (δ 1.75), Me-28 (δ 0.88), Me-29 (δ 0.92), Me-30 (δ 0.92), H-3 (δ 3.20, dd, J=11 and 4.5 Hz), H-24 (δ 4.18, t, J=7 Hz); sugar signals as for **1** with an additional doublet at δ 4.28 (J=7.5 Hz) for a glucose unit; fabms m/z 1077 $[M-H]^-$.

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