Two New Hopane-Triterpene Glycosides from a Fern, *Diplazium subsinuatum* (WALL. ex HOOK. *et* GREV.) TAGAWA

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From a whole plant of a fern, *Diplazium subsinuatum* (WALL. ex HOOK. *et* GREV.) TAGAWA, two new hopane-triterpene glycosides termed diplaziosides I and II were isolated together with a known hopane-triterpene glycoside named glycoside C (1). The structures of diplaziosides I and II were established as 24-*O*-[α-L-arabinofuranosyl-(1→2)]-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-hopan-28,22-olide (2) and (22*R*)-17-hydroxy-24-*O*-[α-L-arabinofuranosyl-(1→2)]-β-D-glucopyranosyl-28,22-carbonyloxy-hopan-30-oic acid (3), respectively, on the basis of spectral evidence. In addition, the full ^{1}H- and ^{13}C-NMR assignments for known 1 are also reported for the first time.

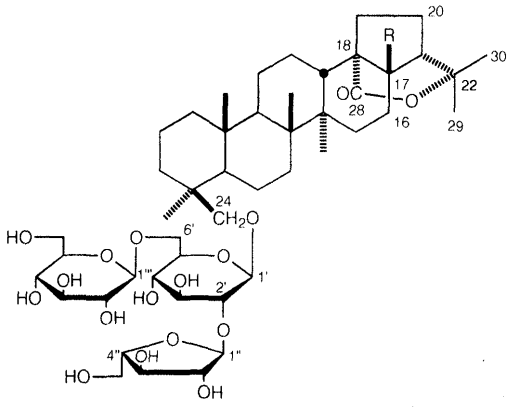
Key words *Diplazium subsinuatum*; fern; diplazioside I; diplazioside II; hopane-triterpene glycoside; triterpene lactone

The whole plant of a certain fern, *Diplazium subsinuatum* (WALL. ex HOOK. *et* GREV.) TAGAWA (Woodsiaceae) has been used only as folk medicine such as a diuretic, a hydragogue, *etc.* in China,[1] and from the fronds of this fern, a hopane-type triterpene (=hopane) glycoside termed glycoside C (**1**), along with its aglycone, mono- and di-glycosides of the same aglycone as **1**, have been identified by Tanaka *et al.*[2] In our preliminary search for new natural antiallergic agents, the 1-butanol fraction obtained from the hot-water extract of the fern was found to show comparatively potent inhibition on homologous cutaneous anaphylaxis (PCA) in rats.[3] This finding prompted us to investigate the chemical constituents[3] of the 1-butanol fraction and led us to isolate two new hopane glycosides named diplaziosides I (**2**) and II (**3**), together with known **1**.[2] In this paper we fully describe the structural elucidation of the new hopane glycosides (**2** and **3**). In this structural study, complete assignments for all the protons and carbons of **1** were required, and those established are also reported here for the first time.

The total 1-butanol fraction was subjected to precise separation by column chromatography and HPLC to isolated diplaziosides I (**2**) and II (**3**), together with a major component (**1**) from the fraction.

The mp, optical rotation, IR (KBr), electron impact (EI)-MS fragments, and ^{13}C-NMR chemical shift values of the isolated glycoside (**1**) were in agreement with those published for authentic glycoside C.[2] In addition, based on detailed 2D NMR [^{1}H–^{1}H and ^{13}C–^{1}H shift-correlation spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY), and heteronuclear multiple bond correlation spectroscopy (HMBC)] and distortionless enhancement by polarization transfer (DEPT) analyses, each proton and carbon of **1** was assigned as shown in Tables 1 and 2, respectively, for the first time, and the validity of the structure (**1**) reported for glycoside C[2] was confirmed.

Diplazioside I (**2**), colorless needles of mp 290—291 °C, $[\alpha]_D$ −17.8° (c=1.00, pyridine) showed a strong band at 1720 cm^{-1} in the IR spectrum due to a δ-lactone carbonyl together with a hydroxy absorption at 3370 cm^{-1}. In the FAB-MS (negative mode) spectrum, **2** gave the $[M-H]^-$ ion at m/z 911, and based on the high resolution (HR) spectrum in the same mode, **2** was formulated as $C_{47}H_{76}O_{17}$ which corresponds to that less one oxygen-atom unit compared with $C_{47}H_{76}O_{18}$ for **1**. Furthermore,



1:R=OH
2:R=H

3

Chart 1

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Table 1. ^{1}H-NMR Data of **1**, **2**, and **3** in C_5D_5N (600 MHz)[a]

Proton No.	**1**	**2**	**3**
Aglycone moiety			
1α (ax)	0.76 (ddd, 13.0, 13.0, 3.5)	0.75 (ddd, 13.2, 13.2, 3.6)	0.81 (m)
β (eq)	1.61 (br d, 13.0)	1.60 (br d, 13.2)	1.63 (br d, 13.2)
2α (eq)	1.40 (m)	1.38[b]	1.44 (m)
β (ax)	1.76[b]	1.74[b]	1.74 (m)
3α (ax)	1.01 (ddd, 13.0, 13.0, 3.5)	1.00 (ddd, 13.2, 13.2, 3.6)	1.05 (ddd, 13.2, 13.2, 3.5)
β (eq)	2.28 (br d, 13.0)	2.22 (br d, 13.2)	2.38 (br d, 13.2)
5α (ax)	0.89 (br d, 10.5)	0.86 (br d, 11.0)	0.91 (br d, 12.0)
6α (eq)	1.70 (m)	1.72[b]	1.58 (m)
β (ax)	1.46 (m)	1.47 (ddd, 13.2, 11.0, 3.6)	1.29 (m)
7α (ax)	1.30 (m)	1.26[b,c]	1.31 (m)
β (eq)	1.31 (m)	1.36 (m)[c]	1.46[b]
9α (ax)	1.42 (br d, 9.0)	1.38[b]	1.46[b]
11α (eq)	1.58 (br d, 13.0)	1.50 (m)	1.59 (m)
β (ax)	1.24 (m)	1.15 (m)	1.27 (m)
12α (ax)	2.83 (dddd, 13.0, 13.0, 13.0, 4.5)	2.71 (dddd, 13.2, 13.2, 13.2, 4.8)	2.87 (m)
β (eq)	1.76[b]	1.72[b]	1.79 (br d, 13.2)
13β (ax)	2.62 (dd, 13.0, 3.5)	1.54[b]	2.70 (dd, 13.2, 3.5)
15α (eq)	1.05 (br d, 13.0)	1.26[b]	1.14 (br d, 13.2)
β (ax)	2.38 (ddd, 13.0, 13.0, 4.5)		2.46 (ddd, 13.2, 13.2, 4.0)
16α (ax)	2.24 (ddd, 13.0, 13.0, 4.5)	1.68 (m)	2.35 (ddd, 13.2, 13.2, 4.0)
β (eq)	2.01 (br d, 13.0)		2.12 (m)
17β (ax)		1.26[b]	
β (ax)-OH	5.78 (br s)		[d]
19	2.10 (ddd, 13.0, 13.0, 3.5)	1.54[b]	2.14 (m)
	1.90 (m)	1.84 (m)	
20	2.40 (m)	1.54[b]	2.67 (m)
	1.89 (m)	1.81 (m)	2.00 (m)
21β	2.24 (d, 6.5)	1.96 (br dd, 4.2, 4.2)	2.89 (d, 7.0)
23	1.23 (s)	1.21 (s)	1.22 (s)
24	4.12 (d, 9.5)	4.24 (d, 9.6)	4.05 (d, 9.5)
	3.85 (d, 9.5)	3.81 (d, 9.6)	3.92 (d, 9.5)
25	0.86 (s)	0.89 (s)	0.80 (s)
26	1.13 (s)	0.94 (s)	1.12 (s)
27	1.14 (s)	1.05 (s)	1.21 (s)
29	1.49 (s)	1.39 (s)	1.99 (s)
30	1.26 (s)	1.20 (s)	
Sugar moiety			
Inner Glc			
1′	4.82 (d, 7.8)	4.79 (d, 7.8)	4.93 (d, 7.8)
2′	4.01 (dd, 9.0, 7.8)	3.99 (dd, 9.0, 7.8)	4.12 (dd, 9.1, 7.8)
3′	4.18 (dd, 9.0, 9.0)	4.15 (dd, 9.0, 9.0)	4.29 (dd, 9.1, 9.1)
4′	4.05 (dd, 9.0, 9.0)	4.00 (dd, 9.0, 9.0)	4.18 (dd, 9.1, 9.1)
5′	4.00[b]	3.99[b]	3.91 (ddd, 9.1, 5.2, 2.5)
6′	4.76 (dd, 10.8, 1.5)	4.73 (d, 10.8)	4.53 (dd, 11.8, 2.5)
	4.29 (dd, 10.8, 5.3)	4.28 (dd, 10.8, 5.4)	4.37 (dd, 11.8, 5.2)
Ara			
1″	6.31 (s)	6.28 (s)	6.39 (s)
2″	4.97 (d, 1.5)	4.93 (d, 1.8)	5.05 (d, 2.2)
3″	4.83[b]	4.80[b]	4.88 (dd, 4.1, 2.2)
4″	4.94 (ddd, 4.2, 4.2, 4.2)	4.91 (ddd, 4.2, 4.2, 4.2)	4.97 (ddd, 4.1, 4.1, 4.1)
5″	4.30 (dd, 11.5, 4.2)	4.28 (dd, 11.5, 4.2)	4.33 (dd, 11.3, 4.1)
	4.23 (dd, 11.5, 4.2)	4.20 (dd, 11.5, 4.2)	4.25 (dd, 11.3, 4.1)
Terminal Glc			
1‴	5.04 (d, 7.8)	5.04 (d, 7.8)	
2‴	4.00 (dd, 9.0, 7.8)	3.98 (dd, 9.0, 7.8)	
3‴	4.19 (dd, 9.0, 9.0)	4.17 (dd, 9.0, 9.0)	
4‴	4.16 (dd, 9.0, 9.0)	4.13 (dd, 9.0, 9.0)	
5‴	3.90 (ddd, 9.0, 5.4, 2.4)	3.88 (ddd, 9.0, 5.4, 2.4)	
6‴	4.48 (dd, 12.0, 2.4)	4.46 (dd, 12.0, 2.4)	
	4.31 (dd, 12.0, 5.4)	4.29 (dd, 12.0, 5.4)	

a) Chemical shifts are in δ-values from internal TMS and are followed by multiplicities and *J*-values (in Hz). *b*) Overlapping with other signal(s) and hence, its multiplicity and *J*-value are both obscure. *c*) Signal assignments with the same symbol in each column may be interchangeable with each other. *d*) Not observed under the present conditions.

Table 2. ^{13}C-NMR Data of **1**, **2**, and **3** in C_5D_5N (150 MHz)[a]

Carbon No.	1	2	3
Aglycone moiety			
1	40.7	40.7	40.7
2	18.9	18.8	18.9
3	36.7	36.7	36.5
4	38.3	38.2	38.4
5	57.5	57.4	57.5
6	19.3	19.3	19.1
7	34.7	34.5	34.7
8	42.3[b]	42.8[b]	42.3
9	51.4	51.4	51.4
10	37.7	37.6	37.7
11	22.4	22.1	22.4
12	25.4	25.9	25.3
13	41.0	49.8	41.1
14	42.2[b]	41.7[b]	42.3
15	27.6	32.7	27.4
16	31.4	24.9	31.1
17	77.9	50.1	77.5
18	55.7	50.6	56.7
19	33.8	35.5	34.3
20	23.5	25.1	27.1
21	53.3	45.7	51.2
22	80.1	81.7	84.3
23	28.4	28.3	28.3
24	73.1	73.1	72.9
25	16.9	17.0	16.9
26	16.8	16.4	16.8
27	15.8	16.1	15.9
28	176.7	175.8	175.9
29	29.3	29.4	25.5
30	30.5	29.8	175.6
Sugar moiety			
Inner Glc			
1′	104.0	103.9	104.4
2′	77.4	77.4	77.8
3′	78.0	77.9	78.3
4′	71.5	71.4	71.7
5′	77.0	77.0	78.4
6′	69.8	69.7	62.7
Ara			
1″	109.5	109.4	109.6
2″	81.0	81.0	81.1
3″	78.9	78.8	79.0
4″	88.3	88.1	88.4
5″	62.7	62.6	62.8
Terminal Glc			
1‴	105.3	105.1	
2‴	75.2	75.1	
3‴	78.4	78.2	
4‴	71.7	71.6	
5‴	78.3	78.2	
6‴	62.8	62.7	

a) Assignments were determined based on ^{1}H–^{13}C COSY, DEPT, and HMBC experiments. b) Signal assignments may be interchanged in each column.

FAB-MS (negative mode) afforded significant fragments at *m/z* 773 $[M-H-132$ (pentose unit)$]^-$ and 749 $[M-H-162$ (hexose unit)$]^-$, and the EI-MS gave a fragment peak at *m/z* 456, ascribed to the $[aglycone]^+$ ion, suggesting that **2** is a triglycoside carrying a sugar part comprised of two hexosyl and one pentosyl units. Detailed NMR studies for **2** were also performed with the aid of 2D (^{1}H–^{1}H and ^{13}C–^{1}H COSY, NOESY, and HMBC) and DEPT methods, and the full assignments for all protons and carbons were achieved as shown in Tables 1 and 2.

The saccharide component-structure of **2** was established as follows. The configurations of the component glucose and arabinose were determined to be D and L, respectively (see Experimental), according to the reported procedure.[5] The ^{1}H- and ^{13}C-NMR data (chemical shifts, multiplicities, and/or coupling constants) for the sugar part were superimposable on those for the trisaccharide part of **1** (see Tables 1 and 2), indicating that **2** carries a trisaccharide moiety the same as **1**. This established trisaccharide structure was further confirmed by additional NMR (NOESY, HMBC, *etc.*) evidence.[6] On the other hand, the molecular formula ($C_{30}H_{48}O_3$) for the aglycone part of **2** was revealed to be less by one oxygen-atom unit compared with that ($C_{30}H_{48}O_4$) for the aglycone part of **1** based on the EI- and FAB-MS spectral proofs described previously. A detailed comparison of ^{1}H-NMR data for the aglycone of **2** with those for the aglycone of **1** was indicative of the presence of a 17β-H signal (δ 1.26) in **2** instead of the 17β-OH signal (δ 5.78) in **1** (Table 1); this suggested that the aglycone of **2** must correspond to the 17-deoxy compound of the aglycone of **1**. This inferred structure for the aglycone of **2** was confirmed by a detailed comparison of **2** with **1** in the ^{13}C-NMR data between their aglycone parts (Table 2). Atom C-17 of **2** resonated at δ 50.08, at a largely upfield-shifted position (by -27.82 ppm) from the corresponding signal (δ 77.90) for **1** and the neighboring carbons to C-17, *i.e.*, C-16 (δ 24.89), C-18 (50.60), and C-21 (45.66) of **2** also resonated upfield (by *ca.* -5.1—-7.7 ppm) from the corresponding signals (C-16, δ 31.43; C-18, 55.74; C-21, 53.27) for **1**. Contrary to this, C-13 (δ 49.79), C-15 (32.74), C-19 (35.48), C-20 (25.14), and C-22 (81.68), all of these being in a 1,3-relation to C-17, resonated downfield (by *ca.* $+1.5$—$+8.8$ ppm) compared with the corresponding carbon signals (see Table 2) of **1**, suggesting that the γ-effect ascribed to the 17βax.-hydroxy substituent observed in **1** disappears in **2**. The chemical shifts of all carbons of the aglycone part of **2**, except the carbons (C-13 and from C-15 to C-22) around C-17, coincided with those of the corresponding carbons of the aglycone part of **1** (Table 2). These lines of evidence led us to establish the aglycone of **2** as 24-hydroxyhopan-28,22-olide.[7] Finally, the location of the sugar moiety on the aglycone was determined to be at β-axial hydroxymethylene (C-24) by the following evidence: i) the chemical shift of C-24 was identical with that of C-24 of **1**; ii) the NOESY and HMBC correlations were observed between H_2-24/H-1′ and between H-1′/C-24, respectively. Based on the accumulated proofs, the structure of diplazioside I is defined as 24-*O*-[α-L-arabinofuranosyl-(1→2)]-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-hopan-28,22-olide (**2**).

Diplazioside II (**3**), colorless needles, mp >300 °C, $[\alpha]_D$ +16.5° (c=1.00, pyridine), gave two strong carbonyl stretching bands due to a δ-lactone at 1720 cm^{-1} and a carboxy group at 1700 cm^{-1} along with a hydroxy absorption at 3380 cm^{-1} in the IR spectrum. The FAB-MS (negative mode) afforded the $[M-H]^-$ peak at *m/z* 795, and based on the HR-FAB-MS in the same mode, the molecular formula was determined to be $C_{41}H_{64}O_{15}$. This FAB-MS also gave a series of significant fragments at 663 $[M-H-132$ (a pentosyl unit)$]^-$ and at 501 $[M-H-$

132−162 (a hexosyl unit)]$^-$, indicating that **3** carries a pentosyl-hexosyl residue as the sugar part and the molecular weight of the aglycone part corresponds to 502. Detailed ^{1}H- and ^{13}C-NMR studies of **3** were made with the aid of DEPT and 2D [^{1}H–^{1}H and ^{13}C–^{1}H COSY, rotating-frame Overhauser enhancement spectroscopy (ROESY), and HMBC] methods, and each proton and carbon was assigned as shown in Tables 1 and 2, respectively. The ^{1}H- and ^{13}C-assignments established for the sugar part, in conjunction with the presence of ROESY (between H-2′/H-1″) and HMBC (between H-2′/C-1″ and between H-1″/C-4″) correlations, were indicative of the presence of an α-L-arabinofuranosyl-(1→2)-β-D-glucopyranosyl (^{4}C$_1$) moiety as the disaccharide group in **3**. On the other hand, the presence of a lactone carbonyl (δ_C 175.91), a carboxy (δ_C 175.55), and a hydroxy methylene (δ_C 72.87) group, along with five *tert*-methyls in the aglycone part revealed from the ^{1}H- and ^{13}C-NMR spectra, led us to infer a triterpene aglycone, the precise structure of which was established as follows. A comparison of the molecular formula ($C_{30}H_{46}O_6$) of the aglycone of **3** with that ($C_{30}H_{48}O_4$) of the aglycone of **1** suggested that any one of the six *tert*-methyls in the aglycone of **1** must be oxidized to a carboxy group in the aglycone of **3**. The ^{13}C-NMR data for the aglycone part of **3** were compared with those for the aglycone part of **1** in detail (Table 2), and it was deduced from the following lines of spectral evidence that the aglycone-structure of **3** differs from that of **1** only in a substituent (C-30) attached to C-22, *i.e.*, the aglycone of **3** carries a carboxy (C-30) group (δ_C 175.55) instead of a *tert*-methyl (C-30) (δ_C 30.46) in **1**. According to the change of the C-30 substituent from a *tert*-methyl (in **1**) to a carboxy group (in **3**), C-22 and C-20 (being in a 1,3-relation to C-22) of **3** resonated at δ 84.33 and 27.17, respectively, downfield (by *ca.* 4.2 and 3.6, respectively) from the corresponding signals (δ 80.14 and 23.48, respectively) of **1**, and contrary to this, C-29 (*tert*-methyl) of **3** at δ 25.51, upfield (by *ca.* 3.8 ppm) compared with that (*tert*-methyl; δ 29.28) of **1**. Each carbon ascribed to the aglycone of **3**, except the above-mentioned carbons (C-20, C-22, C-29, and C-30), were in agreement with the corresponding carbons of **1** in their chemical shifts (Table 2). In addition, HMBC (two- and three-bonds) correlations were observed between H_3-29/each of C-21, C-22, and C-30, and between H-21/each of C-17, C-18, C-19, and C-29. The absolute configuration of C-22 on the aglycone was indicated by the following ROESY experiment of **3**: strong cross peaks were observed between H_3-29/each of H-16α and H-21β, suggesting that the methyl group (C-29) attached at C-22 is oriented toward C-16 and not toward C-20,[8] *i.e.*, C-22 is of an *R*-configuration.[9] Thus, the aglycone of **3** was assigned to (22*R*)-17,24-dihydroxy-28,22-carbonyloxy-hopan-30-oic acid.[7] Finally, the location of the disaccharide on the aglycone was determined as follows: i) the C-24 chemical shift (δ 72.87) of **3** coincided with that (δ 73.07) of **1** and ii) both ROESY (between H_2-24/H-1′) and HMBC (between H-1′/C-24) correlations were observed. In conclusion, diplazioside II is defined as (22*R*)-17-hydroxy-24-*O*-[α-L-arabinofuranosyl-(1→2)]-β-D-glucopyranosyl-28,22-carbonyloxy-hopan-30-oic acid, as shown in the formula (**3**).

Both **2** and **3** are new not only in their glycoside structures but also in their aglycone structures and, to our knowledge, a hopane-type triterpene (**3**) bearing both of a carboxy and lactone groups in the molecule was found for the first time in the nature.

Experimental

General Remarks All melting points were recorded on a Yanagimoto micro melting point apparatus without correction. IR spectra were measured with a JASCO A-302 in KBr discs, and ^{1}H- and ^{13}C-NMR spectra with a GE-OMEGA 600 spectrometer operating at 600 (^{1}H) and 150 (^{13}C) MHz, respectively, with pyridine-d_5 as a solvent and tetramethylsilane as the internal standard. EI-MS (at 30 eV) and FAB-MS (negative mode; matrix, triethanolamine) spectra were obtained from a JEOL JMS-D 300 spectrometer and HR-FAB-MS (negative mode) from a JEOL JMS-HX 110/110 spectrometer. Optical rotations were determined for solutions in pyridine on a JASCO DIP-140 polarimeter. GLC was carried out on a Shimadzu GC-7AG under the following conditions: capillary column, TC-1 (0.32 mm i.d. × 30 m, GL Sciences Inc.; a column similar to SE-30); detector, hydrogen flame ionization detector; column temperature, 229 °C; injection temperature, 250 °C; carrier N_2 gas, 2.0 ml/min; split ratio, 1/70. Preparative HPLC was performed on a Waters 600E instrument with a Shodex RI SE-31 differential refractometer and a Waters μ-Bondapak C_{18} column (7.8 mm i.d. × 30 cm), and also on a JAI LC-908 instrument with a JAIGel-GS310 column (20 mm i.d. × 50 cm). For column chromatography and TLC, Merck Kieselgel 60 and precoated Silicagel 60 plates were used, respectively.

Extraction and Isolation Wild *Diplazium subsinuatum* was collected in Awa District, Chiba Prefecture, Japan in 1992. The whole plants (20 g), air-dried and cut, were extracted twice with boiling water (550 ml) for 1 h. After filtration, the resulting aqueous solution was extracted three times with 1-butanol (700 ml) and the solvent was taken off *in vacuo* to give a butanol extract. Repetition of this extraction procedure afforded a total of 29.9 g of the butanol extract from 820 g of the plant material. The butanol extract (16.5 g) was chromatographed on silica gel, eluting successively with a lower phase of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) and a mixture solvent of $CHCl_3$–MeOH–H_2O (6 : 4 : 1) to get the 11 separated fractions (Nos. 1 to 11). The former eluent eluted each of the fractions from No. 1 to No. 10 in this order and the latter eluted fraction No. 11. Fraction No. 5 (2.50 g) was recrystallized from MeOH to afford **1** (1.55 g). Fraction No. 4 (1.05 g) was further purified by preparative HPLC (μ-Bondapak C_{18}, MeOH : H_2O = 75 : 25) to give **2** (232 mg). Fraction No. 10 (253 mg) was separated into two fractions by preparative HPLC (JAIGel-GS310, MeOH) and the resulting latter fraction was further purified by HPLC separation (μ-Bondapak C_{18}, MeOH : H_2O = 65 : 35) to yield **3** (44.8 mg).

Glycoside C (**1**): Colorless needles, mp 278—280 °C (lit.[2] mp 260—268 °C), $[\alpha]_D$ −14.9° (c = 1.00, pyridine) [lit.[2] $[\alpha]_D$ −12.7° (c = 0.55, pyridine)]. IR (KBr) and EI-MS: essentially the same as the reported data.[2] As ^{1}H-NMR data, only methyl proton chemical shifts have been reported in the lit.[2] and thus, the full assignments for all protons are reported here (Table 1) for the first time. The ^{13}C-chemical shifts of the isolated sample (150 MHz, pyridine-d_5) were in agreement with those published for an authentic specimen (25 MHz, pyridine-d_5),[2] and the fully-assigned data are also reported here (Table 2). FAB-MS (negative mode) *m/z*: 927 $[M-H]^-$, 795 $[M-H-Ara]^-$, 765 $[M-H-Glc]^-$, 633 $[M-H-Ara-Glc]^-$.

Diplazioside I (**2**): Colorless needles from MeOH, mp 290—291 °C, $[\alpha]_D$ −17.8° (c = 1.00, pyridine). FAB- and HR-FAB-MS (negative mode) *m/z*: 911.5016 (Calcd for $C_{47}H_{75}O_{17}$, $[M-H]^-$: 911.5004), 779 $[M-H-Ara]^-$, 749 $[M-H-Glc]^-$, 617 $[M-H-Ara-Glc]^-$. EI-MS *m/z* (%): 456 (17, [aglycone]$^+$), 438 (24), 425 (37), 207 (100), 189 (44), 176 (37), 147 (58), 109 (48). IR cm^{-1}: 3370 (OH), 2930, 1720 (δ-lactone), 1060, 1040. ^{1}H- and ^{13}C-NMR: Given in Tables 1 and 2, respectively.

Diplazioside II (**3**): Colorless needles from MeOH, mp >300 °C, $[\alpha]_D$ +16.5° (c = 1.00, pyridine). FAB- and HR-FAB-MS (negative mode) *m/z*: 795.4156 (Calcd for $C_{41}H_{63}O_{15}$, $[M-H]^-$: 795.4167), 663 $[M-H-Ara]^-$, 501 $[M-H-Ara-Glc]^-$. IR cm^{-1}: 3380 (OH), 2910, 1720 (δ-lactone), 1700 (COOH), 1080, 1030. ^{1}H- and ^{13}C-NMR: Given

in Tables 1 and 2, respectively.

Determination of Configurations of Glucosyl and Arabinosyl Moieties in 2[5] A solution of **2** (5 mg) in 1 N HCl (0.7 ml) was heated at 90 °C for 2 h. The precipitate deposited on cooling was removed by centrifugation. The supernatant was neutralized with Ag_2CO_3. After centrifugation of the inorganic precipitate, the supernatant was concentrated *in vacuo* to afford a residue containing glucose and arabinose. The residual mixture of glucose and arabinose was subjected to the preparation of the respective thiazolidine derivatives, the trimethylsilylation, and then, the GLC analysis, according to the reported procedure.[5] The D- and L-configurations for glucose and arabinose were confirmed, respectively, based on direct comparisons with the D- and L-standards of both sugars (t_R: D-Glc, 13 min 55 s; L-Glc, 15 min 16 s; L-Ara, 7 min 56 s; D-Ara, 8 min 48 s). The glucosyl and arabinosyl components in **3** must be of the same configurations (D and L, respectively) to those in **2** from the co-occurrence of **2** and **3** in the plant.

References and Notes

1) Hotta M., *et al.* (ed), "Useful Plants of the World," Heibonsha, Tokyo, 1989, p. 390; Fang Ding *et al.* (ed), "Dictionary of Guangxi Medicinal Plants," Guangxi People's Publishing House, Guilin, China, 1986, p. 20.
2) Tanaka N., Yamauchi K., Murakami T., Saiki Y., Chen C.-M., *Chem. Pharm. Bull.*, **30**, 3632—3639 (1982).
3) By an ordinary biological test,[4] the 1-butanol fraction showed a comparatively potent inhibitory effect (63%) at a concentration of 100 mg/kg (rat), though weaker than that [69% at 10 mg/kg(rat)] of a positive control, ketotifen. Detailed biological studies on the butanol fraction and its components are now in progress and the results will be published elsewhere in the future.
4) Aibara S., Mori M., Tsubokawa M., Iwamoto T., Tsukada W., *Int. Arch. Allergy Immunol.*, **98**, 146—152 (1992).
5) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **34**, 1843—1845 (1986).
6) The additional lines of evidence for the sugar-part structure in **2** are as follows: i) on the sugar sequence : from the NOESY (between H-2′/H-1″ and between H_2-6′/H-1‴) and HMBC (between H-2′/C-1″ and between H_2-6′/C-1‴) correlations; ii) on the β-configuration and $^4C_1$ pyranosyl form of both glucosyl moieties: from the coupling constants of all glucosyl protons (Table 1), the chemical shifts of all glucosyl carbons (Table 2), and NOESY correlations (between H-1/each of H-3 and H-5) in each glucosyl molecule; iii) on the furanosyl form of the arabinosyl moiety: from the HMBC correlation (between H-1/C-4) in the arabinosyl molecule; iv) on the α-configuration of the L-arabinosyl residue: from the chemical shifts of the arabinosyl C-1 and C-2.
7) Besides being proven by reasonable ^{13}C-chemical shift values, the β-axial configuration of the hydroxy-methylene (C-24) in both **2** and **3** was also substantiated by the presence of NOESY cross peaks between H_2-24/H_3-25 in both **2** and **3**.
8) In the NOESY experiment of **1**, two series of significant cross peaks were observed between H_3-29/each of H-21β, H-16α, and H-16β and between H_3-30/each of H-21β and H_2-20, suggesting that one (C-29) of the methyls attached at C-22 is oriented toward C-16 and the other (C-30) toward C-20.
9) Atoms C-29 and C-30 in **3** were numbered according to the following literature: Ageta H., Shiojima K., Arai Y., Kasama T., Kajii K., *Tetrahedron Lett.*, **1975**, 3297—3298.