Constituents of a Fern, *Diplazium subsinuatum*. II.[1] Structure Elucidation of Five New Hopane-Triterpene Glycosides

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From whole fern, *Diplazium subsinuatum* (WALL. *ex* HOOK. *et* GREV.) TAGAWA, two new hopane-triterpene glycosides named diplaziosides III and IV were isolated, together with three new hopane glycosides with acetylated sugars. The structures of diplaziosides III and IV were established as (22*S*)-24-*O*-α-L-arabinofuranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-20α-*O*-β-D-glucopyranosyl-30-hydroxyhopan-28-oic acid (1) and (22*R*)-24-*O*-α-L-arabinofuranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-30-carboxy-17-hydroxy-hopano-28,22-lactone (2), respectively, on the basis of spectral evidence. Diplazioside III (1) is novel not only in its glycoside structure, but also in its triterpene structure and moreover, 1 provides the first instance of a naturally occurring bisdesmoside with a hopane aglycone. The structures (3a, 3b, and 4a) of the acetylated glycosides were also elucidated, and this is the first report of naturally occurring acetates of hopane glycosides.

Key words *Diplazium subsinuatum*; fern; diplazioside III; diplazioside IV; hopane-triterpene glycoside; acetyl hopane-triterpene glycoside

The whole plant of the fern *Diplazium subsinuatum* (WALL. *ex* HOOK. *et* GREV.) TAGAWA (Herashida in Japanese; Woodsiaceae) has been used as a folk medicine (diuretic, hydragogue, *etc.*) in China.[2] From the fronds of the fern, Tanaka *et al.* have identified a hopane-type triterpene together with a series of its glycosides [mono-, di- (**3**), and triglycosides (**4**)][3] and from the whole plant, we identified two new hopane glycosides, diplaziosides I and II.[1] Upon continued chemical study of the 1-butanol fraction obtained from the hot-water extract of the fern, we isolated five new hopane glycosides, that is, diplaziosides III (**1**) and IV (**2**), and three acetates (**3a**, **3b**, and **4a**). This paper describes the isolation and the structural elucidation of these five new compounds.

The 1-butanol fraction was separated on silica gel and octadecyl silica (ODS) gel column chromatography successively, followed by repeated HPLC separation to afford the five glycosides (**1**, **2**, **3a**, **3b**, and **4a**).

In the structural elucidation of each compound, the ^{1}H- and ^{13}C-NMR assignments were performed with the aid of two dimensional (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)] experiments.

Diplazioside III (**1**), colorless needles of mp 203—205 °C, $[\alpha]_D$ −12.7° (c = 1.00, pyridine) showed a carboxyl C=O band at 1690 cm^{-1} along with a hydroxyl absorption at 3380 cm^{-1} in the IR spectrum. In the FAB-MS (negative mode), **1** gave the $[M-H]^-$ ion peak at m/z 1107, and the high-resolution (HR) spectrum in the same mode revealed the formula to be $C_{53}H_{88}O_{24}$. Furthermore, the

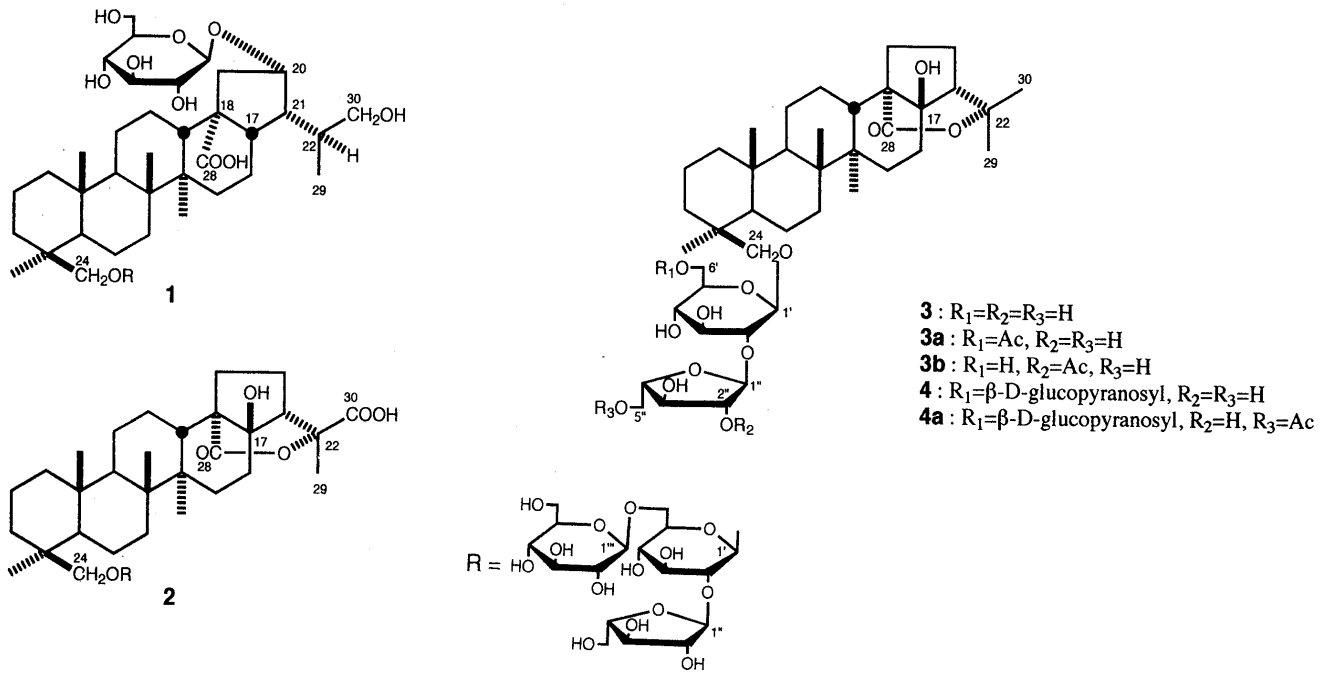

Chart 1

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Table 1. ^{1}H-NMR Data for **1** in C_5D_5N (600 MHz)[a]

Proton No.	Aglycone moiety of **1**	Proton No.	Sugar moiety of **1**
1α (ax)	0.72 (ddd, 13.2, 13.2, 3.5)	Trisaccharide at C-24	
1β (eq)	1.59[b]	Inner Glc	
2α (eq)	1.40[b]	1′	4.84 (d, 7.8)
2β (ax)	1.77[b]	2′	4.03 (dd, 9.0, 7.8)
3α (ax)	1.00 (ddd, 13.2, 13.2, 3.5)	3′	4.20 (dd, 9.0, 9.0)
3β (eq)	2.25 (br d, 13.2)	4′	4.04 (dd, 9.0, 9.0)
5α (ax)	0.86 (br d, 12.6)	5′	4.03[b]
6α (eq)	1.71 (br d, 13.2)	6′	4.78 (d, 10.8)
6β (ax)	1.51[b]		4.32 (dd, 10.8, 5.4)
7α (ax)	1.33[b]	Ara	
7β (eq)		1″	6.34 (s)
9α (ax)	1.30 (br d, 12.0)	2″	4.99 (d, 1.8)
11α (eq)	1.44 (br d, 13.2)	3″	4.85 (dd, 4.1, 1.8)
11β (ax)	1.16 (ddd, 13.2, 13.2, 3.5)	4″	4.96 (ddd, 4.1, 4.1, 4.1)
12α (ax)	2.16 (dddd, 13.2, 13.2, 13.2, 4.0)	5″	4.32 (dd, 11.5, 4.1)
12β (eq)	1.88[b]		4.24 (dd, 11.5, 4.1)
13β (ax)	1.56[b]	Terminal Glc	
15α (eq)	1.36[b]	1‴	5.09 (d, 7.8)
15β (ax)		2‴	4.03 (dd, 9.0, 7.8)
16α (ax)	2.93 (ddd, 13.2, 13.2, 4.0)	3‴	4.21 (dd, 9.0, 9.0)
16β (eq)	1.86[b]	4‴	4.20 (dd, 9.0, 9.0)
17β (ax)	1.53[b]	5‴	3.93 (ddd, 9.0, 5.4, 2.4)
19α	3.43 (d, 12.6)	6‴	4.51 (dd, 12.0, 2.4)
19β	1.57[b]		4.35 (dd, 12.0, 5.4)
20β	4.58 (dd, 7.2, 7.2)	Glc at C-20	
21β	2.36 (ddd, 10.8, 10.8, 7.2)	1	4.81 (d, 7.8)
22	3.05 (m)	2	3.83 (dd, 9.0, 7.8)
23	1.23 (s)	3	4.12 (dd, 9.0, 9.0)
24	4.18 (d, 9.6)	4	3.98 (dd, 9.0, 9.0)
	3.85 (d, 9.6)	5	3.89 (ddd, 9.0, 5.3, 2.4)
25	0.91 (s)	6	4.47 (dd, 12.0, 2.4)
26	0.98 (s)		4.22[b]
27	1.20 (s)		
29	1.53 (d, 6.0)		
30	3.98 (dd, 10.5, 6.6)		
	3.67 (dd, 10.5, 2.5)		

a) Chemical shifts are in δ values from internal tetramethylsilane (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.

FAB-MS also afforded a series of fragment ions at m/z 975 [M−H−132 (pentose unit)]$^-$, 945 [M−H−162 (hexose unit)]$^-$, 813 [M−H−294 (=132+162)]$^-$, and 651 [M−H−456 (=132+2×162)]$^-$ and the EI-MS gave a significant fragment at m/z 472, ascribed to the [aglycone−H_2O]$^+$ ion. The ^{1}H-NMR spectrum (Table 1) showed the presence of four *tert* methyls, a *sec* methyl, two hydroxymethylenes [δ 3.85 (d, J=9.6 Hz), 4.18 (d, J=9.6 Hz); δ 3.67 (dd, J=10.5, 6.6 Hz), 3.98 (dd, J=10.5, 2.5 Hz)], and a hydroxymethine [δ 4.58 (dd, J=7.2, 7.2 Hz)] in the aglycone part and four anomeric protons [δ 4.81 (d, J=7.8 Hz), 4.84 (d, J=7.8 Hz), 5.09 (d, J=7.8 Hz), 6.34 (s)] in the sugar part. These spectral data indicated **1** to be a tetraglycoside of a triterpene.

The ^{13}C-NMR chemical shift values (Table 2) of **1** were compared with those of known hopane 24-*O*-triglycosides: the signals of carbons (from C-1 to C-12 and from C-23 to C-26) ascribed to the A, B, and C rings of the triterpene part and the carbons due to the trisaccharide part linked to the C-24-OH group were in agreement with those of the corresponding carbons of glycoside C[1,3] and diplazioside I.[1] The validity of the trisaccharide part structure[4] was confirmed by ^{1}H- and ^{13}C-2D-NMR evidence similar to that found in the structural study of diplazioside I[1] and the position of the trisaccharide on the triterpene was confirmed to be at C-24-OH by the presence of the respective cross peaks in NOESY (between H_2-24/H-1′) and HMBC (between H-1′/C-24). The plane structure for the D and E ring part carrying a side chain attached at C-21, a carboxyl group (δ_C 179.4) at C-18 (δ_C 53.3), and a *tert* methyl at C-14, was established to be that shown by the formula **1** on the basis of detailed ^{1}H- and ^{13}C-NMR analyses (Tables 1 and 2) with the aid of ^{1}H–^{1}H and ^{1}H–^{13}C COSY and HMBC (Fig. 1) experiments. This structure is consistent with hopane (or isohopane) triterpenes, but not with a lupane skeleton. The NOESY networks[5] (Fig. 2) observed from the aglycone part suggested that the aglycone of **1** is a hopane-type triterpene[6,7] and not an isohopane-type one. The location and configuration of the secondary hydroxyl group on the triterpene aglycone were decided to be at C-20 and α-oriented based on the J values of the H-20β (dd, $J_{20\beta,21\beta}=J_{20\beta,19\beta}=7.2$ Hz) and the H-21β (ddd, $J_{21\beta,17\beta}=J_{21\beta,22}=10.8$ Hz, $J_{21\beta,20\beta}=7.2$ Hz) signals in the ^{1}H-NMR (Table 1).[8] Furthermore, the coupling constant between H-17 and H-21 ($J_{21\beta,17\beta}$) revealed that these protons are both β-oriented and in the eclipsed conformation.[9] These J values of H-20 and H-21, and

Table 2. ^{13}C-NMR Data for **1**, **2**, **3a**, **3b** and **4a** in C_5D_5N[a]

Carbon No.	1	2	3a	3b	4a
Aglycone moiety					
1	40.7	40.7	40.7	40.6	40.7
2	18.8	18.9	18.8	18.8	18.9
3	36.7	36.7	36.8	36.6	36.6
4	38.2	38.3	38.2	38.3	38.3
5	57.4	57.5	57.4	57.4	57.5
6	19.3	19.3	19.3	19.2	19.3
7	34.6	34.7	34.7	34.6	34.7
8	41.9	42.3	42.3	42.3[b]	42.3[b]
9	51.2	51.4	51.4	51.4	51.4
10	37.5	37.7	37.7	37.7	37.7
11	21.9	22.4	22.5	22.4	22.4
12	26.8	25.3	25.4	25.4	25.4
13	51.3	41.0	41.1	41.1	41.1
14	42.5	42.3	42.3	42.2[b]	42.2[b]
15	34.2	27.4	27.6	27.6	27.6
16	23.8	31.0	31.5	31.5	31.5
17	54.3	77.4	77.9	77.9	77.9
18	53.3	56.7	55.8	55.7	55.8
19	49.8	34.2	33.8	33.8	33.8
20	83.8	27.1	23.5	23.5	23.5
21	48.3	51.1	53.4	53.3	53.3
22	36.9	84.2	80.1	80.1	80.1
23	28.3	28.4	28.2	28.3	28.3
24	73.0	73.1	72.7	72.9	73.0
25	17.0	16.9	16.8	16.8	16.9
26	16.5	16.8	16.8	16.8	16.8
27	14.5	15.9	15.9	15.9	15.9
28	179.4	175.8	176.6	176.6	176.7
29	18.7	25.5	30.5	30.5	30.5
30	70.3	175.5	29.3	29.3	29.3
Sugar moiety at C-24					
Inner Glc					
1′	103.9	104.0	103.8	104.0	103.8
2′	77.7	77.5	77.3	77.9	77.9
3′	78.0	78.0	78.1	78.3	78.4
4′	71.5	71.6	71.4	71.6	71.7
5′	77.1	77.1	75.0	78.3	77.0
6′	69.7	69.8	64.4	62.7	69.9
Ac at C-6′			170.7		
			20.8		
Ara					
1″	109.4	109.5	109.6	106.6	109.5
2″	81.0	81.0	81.3	84.6	82.4
3″	78.9	78.9	79.0	76.4	79.5
4″	88.2	88.3	88.2	87.4	83.4
5″	62.7	62.7	62.7	62.2	65.3
Ac at C-2″				170.3	
				20.7	
Ac at C-5″					170.7
					20.7
Terminal Glc					
1‴	105.2	105.3			105.3
2‴	75.2	75.2			75.2
3‴	78.3	78.4			78.4
4‴	71.7	71.7			71.7
5‴	78.3	78.4			78.4
6‴	62.7	62.8			62.8
Glucosyl moiety at C-20					
1	106.3				
2	75.4				
3	78.5				
4	71.9				
5	77.4				
6	63.1				

a) Assignments were determined based on ^{1}H–^{13}C COSY and HMBC experiments. The spectra of **1**, **2**, and **4a** were measured at 150 MHz and those of **3a** and **3b** at 125 MHz. *b*) Signal assignments may be interchanged in each column.

accordingly, the inferred E-ring stereostructure, were consistent with a hopane skeleton[6,7] and not with an isohopane-type skeleton. Next, the absolute configuration at C-22 on the side chain was decided as follows; the significant NOESY cross peaks (Fig. 2) observed between H_3-29 and each of H-16α, H-16β, and H-21β and between H_2-30/H-21β revealed that the *tert* methyl (C-29) is oriented to the β-side and toward C-16, and the hydroxymethylene (C-30) to the β-side (assumed to be toward C-20).[10] Furthermore, the *J* value (10.8 Hz) between H-21β and H-22 suggested that these protons are in a *trans*-diaxial relation to each other and thus, H-22 is oriented to the α-side. This steric feature around C-22 is consistent with a hopane skeleton and with the *S*-configuration at C-22, considering the absolute configuration of a hopane-type triterpene.[6,7] Thus, the aglycone of **1** was assigned as (22*S*)-20α,24,30-trihydroxyhopan-28-oic acid, a new hopane triterpene. Finally, the location of another β-D-glucopyranosyl moiety was determined from the following HMBC and NOESY experiments on **1**; an HMBC cross peak between the anomeric proton of the glucose (δ 4.81, d, J=7.8 Hz) and C-20 (δ_C 83.76) of the aglycone together with NOESY cross peaks between the glucosyl H-1 and each of the terpenic H-20β and H-19α suggested that the glucosyl residue is connected with the 20α-OH on the aglycone *via* a glycosidic linkage. In conclusion, diplazioside III is defined as (22*S*)-24-*O*-α-L-arabinofuranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-20α-*O*-β-D-glucopyranosyl-30-hydroxyhopan-28-oic acid, as shown in the formula **1**. Diplazioside III (**1**) is new not only in its glycoside structure, but also in its aglycone structure. In addition, **1** provides the first instance of a naturally occurring bisdesmoside of a hopane triterpene.

Diplazioside IV (**2**), colorless needles, mp >300 °C, $[\alpha]_D$ −20.1° (c=1.00, pyridine), showed two strong C=O bands due to a δ-lactone at 1720 cm^{-1} and a carboxyl group at 1700 cm^{-1}, along with a hydroxyl absorption band at 3380 cm^{-1} in the IR spectrum. The FAB-MS (negative mode) afforded the $[M-H]^-$ peak at m/z 957 and the negative ion HR-FAB-MS indicated the molecular formula of $C_{47}H_{74}O_{20}$. The FAB-MS also gave a series of significant fragments at 825 [M−H−132 (a pentose unit)]$^-$, 795 [M−H−162 (a hexose unit)]$^-$, 663 [M−H−294 (both pentose and hexose units)]$^-$ and electron impact (EI)-MS gave a fragment at m/z 502 due to the $[aglycone]^+$ ion, suggesting that **2** is a triglycoside carrying a sugar part consisting of a pentosyl and two hexosyl units. Furthermore, these data led us to assume that **2** must be a hexosyl derivative of diplazioside II.[1] Each ^{13}C-NMR signal of **2** (Table 2) was compared with the corresponding signal of diplazioside II[1] and it became clear that in **2** an additional β-D-glucopyranosyl moiety (H-1‴: δ 5.07, d, J=7.8 Hz) is located at 6-OH of the glucosyl residue of diplazioside II *via* a glycosidic linkage, that is, **2** corresponds to the 6′-*O*-β-D-glucopyranosyl derivative of diplazioside II. In addition, the trisaccharide part attached at C-24 of the aglycone was revealed to be identical with that in glycoside C[1,3] and diplaziosides I[1] and III (**1**) by comparison of the ^{1}H- (Tables 1 and 3) and ^{13}C-NMR (Table 2) data. The established structure

Table 3. ^{1}H-NMR Data for the Sugar Moieties of **2**, **3a**, **3b**, and **4a** in C_5D_5N[a]

Proton No.	**2**	**3a**	**3b**	**4a**
Inner Glc				
1′	4.84 (d, 7.8)	4.88 (d, 7.8)	4.92 (d, 7.8)	4.83 (d, 7.8)
2′	4.03 (dd, 9.0, 7.8)	4.15 (dd, 9.0, 7.8)	4.23 (dd, 9.0, 7.8)	4.09 (dd, 9.0, 7.8)
3′	4.19 (dd, 9.0, 9.0)	4.25 (dd, 9.0, 9.0)	4.30 (dd, 9.0, 9.0)	4.20[b]
4′	4.05 (dd, 9.0, 9.0)	4.00 (dd, 9.0, 9.0)	4.18 (dd, 9.0, 9.0)	4.02[b]
5′	4.02[b]	3.96 (ddd, 9.0, 5.5, 2.5)	3.91 (ddd, 9.0, 4.8, 2.4)	4.02[b]
6′	4.77 (d, 10.8)	4.89 (dd, 12.0, 2.5)	4.53 (dd, 12.0, 2.4)	4.77 (d, 10.8)
	4.31 (dd, 10.8, 5.4)	4.77 (dd, 12.0, 5.5)	4.36 (dd, 12.0, 4.8)	4.29 (dd, 10.8, 5.4)
Ac at C-6′		2.00 (s)		
Ara				
1″	6.33 (s)	6.41 (s)	6.32 (s)	6.38 (s)
2″	4.98 (d, 1.8)	5.06 (br s)	5.92 (d, 1.8)	5.01 (d, 3.0)
3″	4.84[b]	4.90[b]	4.91[b]	4.62 (dd, 5.4, 3.0)
4″	4.96 (ddd, 4.2, 4.2, 4.2)	5.00 (ddd, 4.2, 4.2, 4.2)	5.03 (ddd, 4.2, 4.2, 4.2)	4.97 (ddd, 6.0, 5.4, 3.6)
5″	4.32 (dd, 11.5, 4.2)	4.36 (dd, 11.5, 4.2)	4.34 (dd, 11.5, 4.2)	4.80 (dd, 11.4, 3.6)
	4.24 (dd, 11.5, 4.2)	4.28 (dd, 11.5, 4.2)	4.32 (dd, 11.5, 4.2)	4.65 (dd, 11.4, 6.0)
Ac at C-2″			1.88 (s)	
Ac at C-5″				1.98 (s)
Terminal Glc				
1‴	5.07 (d, 7.8)			5.05 (d, 7.8)
2‴	4.01 (dd, 9.0, 7.8)			4.02[b]
3‴	4.20 (dd, 9.0, 9.0)			4.20[b]
4‴	4.18 (dd, 9.0, 9.0)			4.20[b]
5‴	3.91 (ddd, 9.0, 5.4, 2.4)			3.91 (ddd, 9.0, 5.4, 2.4)
6‴	4.49 (dd, 12.0, 2.4)			4.49 (dd, 12.0, 2.4)
	4.33 (dd, 12.0, 5.4)			4.34 (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). The spectra of **2** and **4a** were measured at 600 MHz and those of **3a** and **3b** at 500 MHz. b) Overlapping with other signal(s) and hence, the multiplicity and J value are both obscure.

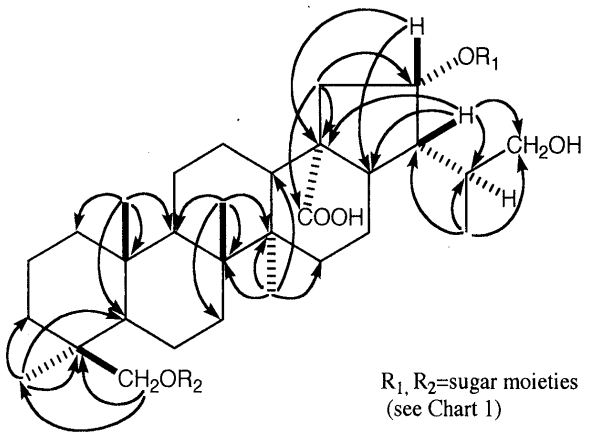

Fig. 1. HMBC Correlations of the Aglycone Part of **1**

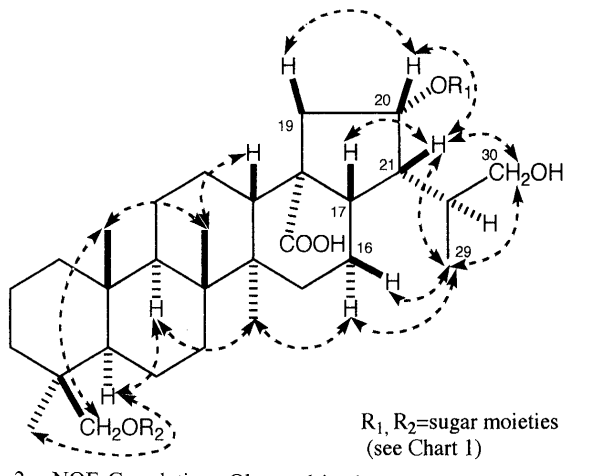

Fig. 2. NOE Correlations Observed in the NOESY Spectrum of the Aglycone Part of **1**

(**2**), (22*R*)-24-*O*-α-L-arabinofuranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-30-carboxy-17-hydroxyhopano-28,22-lactone, was further corroborated by additional NMR (^{1}H, ^{1}H–^{1}H and ^{1}H–^{13}C COSY, NOESY, and HMBC) evidence.

The structures of the three new acetylated glycosides (**3a**, **3b**, and **4a**) were elucidated in the following way. Based on HR-FAB-MS (negative or positive mode) measurements, the molecular formulas of these glycosides were decided as $C_{43}H_{68}O_{14}$ for **3a** and **3b** and $C_{48}H_{78}O_{19}$ for **4a**, and based on ^{1}H- and ^{13}C-NMR analyses, it was suggested that each of these compounds carries one acetyl group [**3a**: δ2.00 (s), δ_C 170.7 and 20.8; **3b**: δ1.88 (s), δ_C 170.3 and 20.7; **4a**: δ1.98, δ_C 170.7 and 20.7]. The above-mentioned structural evidence in conjunction with further detailed analyses of the FAB (negative mode)- and EI-MS fragment data (see Experimental) let us presume that **3a** and **3b** correspond to mono-acetates of glycoside B (**3**),[3] and **4a** to a mono-acetate of glycoside C (**4**).[1,3] By comparison of the ^{13}C-chemical shifts of **3a** and **3b** (Table 2) with those of glycoside B (**3**),[3] **3a** was assigned as the 6-*O*-acetate of the glucosyl residue of glycoside B (=6′-*O*-acetyl glycoside B) and **3b** as the 2-*O*-acetate of the arabinosyl moiety of glycoside B (=2″-*O*-acetyl glycoside B), respectively. Analogously, a detailed comparison of the ^{13}C-NMR data (Table 2) of **4a** with those of glycoside C (**4**)[1,3] showed that **4a** corresponds to the 5-*O*-acetate of the arabinosyl residue of glycoside C (=5″-*O*-acetyl glycoside C). The established structures (the formulas **3a**, **3b**, and **4a**) for these three acetyl derivatives of hopane glycosides were further corroborated by additional ^{1}H-NMR, NOESY, and HMBC data (see

Experimental). To our knowledge, naturally occurring acetates of hopane glycosides have not previously been reported.

Experimental

General Remarks All melting points were recorded on a Yanagimoto melting points apparatus without correction. IR spectra were measured with a JASCO A-302 in KBr discs, and ^{1}H- and ^{13}C-NMR spectra with GE-Omega 600 (^{1}H at 600 and ^{13}C at 150 MHz, respectively) and Varian VXR-500 (^{1}H at 500 and ^{13}C at 125 MHz, respectively) spectrometers with pyridine-d_5 as a solvent and tetramethylsilane as an internal standard. EI-MS (at 30 eV) and FAB-MS (negative mode matrix, triethanolamine) spectra were obtained with a JEOL JMS-DX300 spectrometer and HR-FAB-MS (negative mode matrix, triethanolamine; positive mode matrix, dithiothreitol and thioglycerol) with JEOL JMS-HX 110/110 and JEOL JMS-700 spectrometers. Optical rotations were determined for solutions in pyridine on a JASCO DIP-140 polarimeter. For column chromatography, Kieselgel 60 (Merck) and Chromatorex ODS DM1020T (Fuji Silysia) were used. Preparative HPLC was performed on a JAI LC-908 instrument with a JAIGEL-ODS, S-343-15 column (20 i.d. × 250 mm).

Extraction and Isolation Wild *Diplazium subsinuatum* was collected in Awa District, Chiba Prefecture, Japan in 1994. The whole plant (30 g) was air-dried, cut, and extracted twice with boiling water (700 ml) for 1 h. After filtration, the resulting aqueous solution was extracted three times with 1-butanol (1.2 l) and the solvent was taken off *in vacuo* to give a butanol extract. Repetition of a similar extraction procedure afforded a total of 82.2 g of the butanol extract from 2.73 kg of the plant material. The whole of the obtained butanol extract was chromatographed on silica gel, eluting successively with the lower phase of $CHCl_3$–MeOH–H_2O (9 : 3 : 1 and 7 : 3 : 1) and a mixture solvent of $CHCl_3$–MeOH–H_2O (6 : 4 : 1) to give 13 fractions (Nos. 1 to 13). Fraction No. 2 (1.59 g) was subjected to reversed-phase (ODS) column chromatography (eluting with 80% MeOH) followed by preparative HPLC (80% MeOH) to afford the acetyl compounds **3b** (53.5 mg) and **3a** (21.7 mg) in that order. Fraction No. 9 (2.16 g) was further column-chromatographed over silica gel [the lower phase of $CHCl_3$–MeOH–H_2O (65 : 35 : 10)] followed by preparative HPLC (70% MeOH) to give the third acetyl compound **4a** (120.0 mg). Fraction No. 12 (5.52 g) was further purified on a silica gel column [the lower phase of $CHCl_3$–MeOH–H_2O (65 : 35 : 10)] and an ODS column (70% MeOH), and subjected to preparative HPLC separation (60% MeOH) to give **1** (43.2 mg). Fraction No. 13 (8.41 g) was further separated by ODS column chromatography (60% MeOH) and preparative HPLC (50% MeOH) to give **2** (46.0 mg).

Diplazioside III (**1**): Colorless needles from MeOH, mp 203—205 °C, $[\alpha]_D$ −12.7° (c = 1.00, pyridine). FAB- and HR-FAB-MS m/z: 1107.5574 (Calcd for $C_{53}H_{87}O_{24}$, $[M-H]^-$: 1107.5588), 975 $[M-H-Ara]^-$, 945 $[M-H-Glc]^-$, 813 $[M-H-Ara-Glc]^-$, 651 $[M-H-Ara-2\times Glc]^-$. EI-MS m/z (%): 472 (9, [aglycone (=Ag.)−H_2O]$^+$), 441 (31), 427 (25), 426 (54), 369 (13), 207 (100), 189 (38). IR cm^{-1}: 3380 (OH), 2920, 1690 (COOH), 1080, 1040. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Diplazioside IV (**2**): Colorless needles from MeOH, mp > 300 °C, $[\alpha]_D$ −20.1° (c = 1.00, pyridine). FAB- and HR-FAB-MS (negative mode) m/z: 957.4686 (Calcd for $C_{47}H_{73}O_{20}$, $[M-H]^-$: 957.4695), 825 $[M-H-Ara]^-$, 795 $[M-H-Glc]^-$, 663 $[M-H-Ara-Glc]^-$. EI-MS m/z (%): 502 (9, [Ag.]$^+$), 484 (29), 472 (57), 471 (48), 410 (16), 396 (78), 207 (100). IR cm^{-1}: 3380 (OH), 2910, 1720 (δ-lactone), 1700 (COOH), 1080, 1030. ^{1}H-NMR: aglycone moiety δ: 0.88 (3H, s, H_3-25), 1.16 (3H, s, H_3-26), 1.21 (3H, s, H_3-27), 1.25 (3H, s, H_3-23), 1.98 (3H, s, H_3-29), 3.86 (1H, d, J = 9.6 Hz), 4.15 (1H, d, J = 9.6 Hz) (H_2-24); sugar moiety: Table 3. ^{13}C-NMR: Table 2.

6′-*O*-Acetyl Glycoside B (**3a**): Colorless needles from MeOH, mp 278—280 °C, $[\alpha]_D$ −4.8° (c = 1.00, pyridine). FAB- and HR-FAB-MS (negative mode) m/z: 807.4573 (Calcd for $C_{43}H_{67}O_{14}$, $[M-H]^-$: 807.4530), 675 $[M-H-Ara]^-$. HR-FAB-MS (positive mode) m/z: 809.4695 (Calcd for $C_{43}H_{69}O_{14}$, $[M+H]^+$: 809.4688). EI-MS m/z (%): 472 (10, [Ag.]$^+$), 454 (19), 441 (21), 410 (16), 385 (10), 207 (100), 189 (77), 109 (64). IR cm^{-1}: 3380 (OH), 2910, 1720 (δ-lactone, acetyl), 1070, 1040. ^{1}H-NMR: aglycone moiety δ: 0.88 (3H, s, H_3-25), 1.17 (3H, s, H_3-26), 1.18 (3H, s, H_3-27), 1.21 (3H, s, H_3-23), 1.26 (3H, s, H_3-30), 1.50 (3H, s, H_3-29), 3.88 (1H, d, J = 9.5 Hz), 4.07 (1H, d, J = 9.5 Hz) (H_2-24); sugar moiety: Table 3. HMBC: a cross peak between H_2-6′/acetyl carbonyl C.

2″-*O*-Acetyl Glycoside B (**3b**): Colorless needles from MeOH, mp 274—276 °C, $[\alpha]_D$ −2.1° (c = 1.00, pyridine). FAB- and HR-FAB-MS (negative mode) m/z: 807.4532 (Calcd for $C_{43}H_{67}O_{14}$, $[M-H]^-$: 807.4530), 765 $[M-H-CH_2CO]^-$, 633 $[M-H-CH_2CO-Ara]^-$. EI-MS m/z (%): 472 (27, [Ag.]$^+$), 454 (30), 441 (37), 410 (24), 385 (17), 207 (100), 189 (98), 109 (78). IR cm^{-1}: 3380 (OH), 2910, 1720 (δ-lactone, acetyl), 1070, 1040. ^{1}H-NMR: aglycone moiety δ: 0.79 (3H, s, H_3-25), 1.12 (3H, s, H_3-26), 1.17 (3H, s, H_3-27), 1.23 (3H, s, H_3-23), 1.26 (3H, s, H_3-30), 1.50 (3H, s, H_3-29), 3.87 (1H, d, J = 9.5 Hz), 4.06 (1H, d, J = 9.5 Hz) (H_2-24); sugar moiety: Table 3. HMBC: a cross peak between H-2″/acetyl carbonyl C.

5″-*O*-Acetyl Glycoside C (**4a**): Colorless needles from MeOH, mp 271—273 °C, $[\alpha]_D$ +14.1° (c = 1.00, pyridine). FAB- and HR-FAB-MS (negative mode) m/z: 969.5085 (Calcd for $C_{49}H_{77}O_{19}$, $[M-H]^-$: 969.5059), 927 $[M-H-CH_2CO]^-$, 807 $[M-H-Glc]^-$, 795 $[M-H-CH_2CO-Ara]^-$, 765 $[M-H-CH_2CO-Glc]^-$, 633 $[M-H-CH_2CO-Ara-Glc]^-$. EI-MS m/z (%): 472 (9, [Ag.]$^+$), 454 (15), 441 (18), 410 (16), 358 (10), 207 (100), 189 (63), 109 (48). IR cm^{-1}: 3380 (OH), 2910, 1720 (δ-lactone, acetyl), 1070, 1040. ^{1}H-NMR: aglycone moiety δ: 0.86 (3H, s, H_3-25), 1.15 (3H, s, H_3-26), 1.17 (3H, s, H_3-27), 1.24 (3H, s, H_3-23), 1.26 (3H, s, H_3-30), 1.49 (3H, s, H_3-29), 3.86 (1H, d, J = 9.5 Hz), 4.12 (1H, d, J = 9.5 Hz) (H_2-24); sugar moiety: Given in Table 3. HMBC: a cross peak between H_2-5″/acetyl carbonyl C.

References and Notes

1) Nakanishi T., Inatomi Y., Nishi M., Murata H., Inada A., Aibara S., *Chem. Pharm. Bull.*, **43**, 2256—2260 (1995).
2) Hotta M., Ogata K., Nitta A., Hoshikawa K., Yanagi M., Yamasaki K. (ed.), "Useful Plants of the World," Heibonsha, Tokyo, 1989, p. 390; Fang D., Chen S. S., Rou G. Y. (ed.), "Dictionary of Guangxi Medicinal Plants," Guangxi People's Publishing House, Guilin, China, 1986, p. 20.
3) Tanaka N., Yamauchi K., Murakami T., Saiki Y., Chen C.-M., *Chem. Pharm. Bull.*, **30**, 3632—3639 (1982).
4) Regarding the absolute configurations of the component monosaccharides of the hopane glycosides contained in the fern *Diplazium subsinuatum*, the D- and L-configurations for the component glucose and arabinose have been established, respectively, by the previous investigations of glycoside B[3] and diplazioside I,[1] and hence, the absolute configurations of the component glucose and arabinose in the present hopane glycosides (**1**, **2**, **3a**, **3b**, and **4a**) were also inferred to be D and L, respectively, in view of the co-occurrence of these glycosides with glycoside B and diplazioside I in the fern.
5) Ageta H., Shiojima K., Suzuki H., Nakamura S., *Chem. Pharm. Bull.*, **41**, 1939—1943 (1993).
6) As the absolute configuration of a hopane framework, the enantiomer shown in the present formulas has been generally accepted based on X-ray evidence,[7] *etc.*, and to our knowledge, its antipode, that is, an *ent*-hopane-type triterpene has not so far been isolated from nature.
7) Nakanishi T., Fujiwara T., Tomita K., *Tetrahedron Lett.*, **1968**, 1491—1495; Nakanishi T., Yamauchi H., Fujiwara T., Tomita K., *ibid.*, **1971**, 1157—1160.
8) Based on Dreiding model inspection, the dihedral angles between H-20β and H-21β and between H-20β and H-19β were estimated to be both about 30°, consistent with the observed coupling constant (J = 7.2 Hz). No coupling between H-20β and H-19α was observed, which may be due to a dihedral angle of about 90°. For the dihedral angle-coupling constant relationship, see: Pretsch E., Clerc T., Seibe J., Simon W., "Tables of Spectral Data for Structure Determination of Organic Compounds," second English edition, ed. by Fresenius W., Huber J. F. K., Pungor E., Rechnitz G. A., Simon W., West Th. S., Springer-Verlag, Tokyo, 1989, p. H25.
9) Based on Dreiding model inspection, the dihedral angle between H-17β and H-21β is estimated to be near 0°, in agreement with the observed J value (10.8 Hz). This suggests the view that these vicinal protons are in the eclipsed conformation, and thus, the aglycone of **1** is a hopane-type triterpene.
10) Atoms C-29 and C-30 of the hopane glycosides in this work were numbered according to Ageta H., Shiojima K., Arai Y., Kasama T., Kajii K., *Tetrahedron Lett.*, **1975**, 3297—3298.