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Biologically Active Novel Clerodane-type Diterpene Glycosides from the Root-stalks of *Gleichenia japonica*

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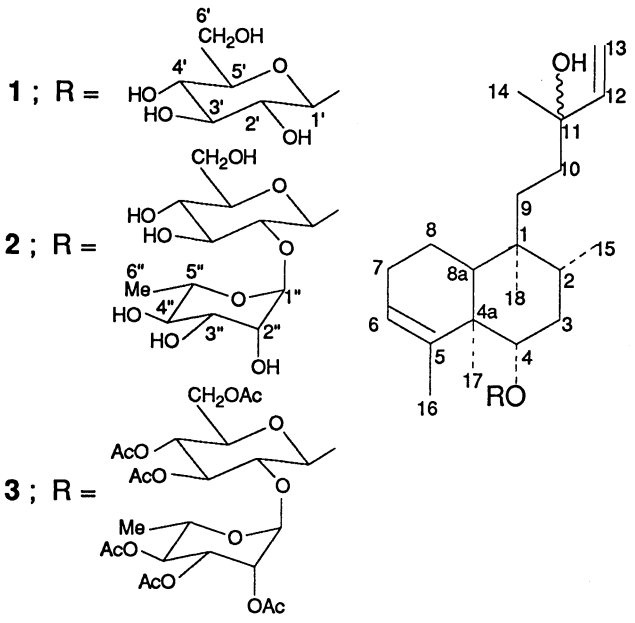
Two glycosides of a novel clerodane-type diterpene alcohol were isolated from the root-stalks of *Gleichenia japonica* (a fern; Urajiro in Japanese). Their structures were determined to be α-vinyl-1,2,3,4,4a,7,8,8a-octahydro-α,1,2,4a,5-pentamethyl-1-naphthalene-propanol 4-O-β-glucopyranoside and α-vinyl-1,2,3,4,4a,7,8,8a-octahydro-α,1,2,4a,5-pentamethyl-1-naphthalenepropanol 4-O-α-rhamnopyranosyl-(1→2)-β-glucopyranoside, respectively, by physicochemical methods. The former glycoside inhibited the growth of lettuce, while the latter one accelerated the growth.

Many researches[1] have been reported on the chemistry and chemotaxanomy of the ferns including the families Aspidiaceae, Botrychiaceae, Cyatheaceae, Cheiropleuriaceae, Dennstaedtiaceae, Dipteridaceae, Davalliaceae, Plagiogyriaceae, Polypodiaceae, Pteridaceae, and Thelypteridaceae. This review article describes the isolation of a series of terpenoid constituents including pterosine-type sesquiterpenes, pterosine-type dinorsesquiterpene dimer, ent-kaurane-type diterpenes, nor-ent-kaurane type diterpenes, ent-pimarane-type diterpenes and its glycosides, and hopane-type triterpenes from the aerial parts of the above described ferns. However, the chemical constituents present in the root-stalks of ferns have not been reported yet. *Gleichenia japonica* Spreng (Urajiro in Japanese) makes a large community containing no other species of plant. In the course of the study on allelopathic substances, we have now examined the water-soluble constituents in the root-stalks of *G. japonica* and isolated two novel clerodane-type diterpene glycosides.

Fresh root-stalks (5.05 kg) of *G. japonica* were extracted with distilled water, followed by a mixed solvent of water and methanol (1:1, v/v) to give a water-soluble fraction. To remove less polar materials this fraction was then treated with chloroform. The chloroform insoluble fraction was suspended in methanol to give a

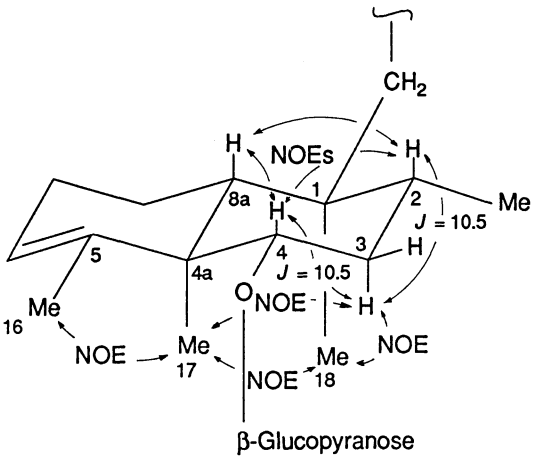
methanol-soluble fraction. Repeated column chromatography of the latter fraction on silica gel with $CHCl_3$-MeOH-water (9:1:0.1, v/v) gave two compounds (**1**, R_f 0.54 on TLC) and (**2**, R_f 0.3). The two compounds, each was finally purified by reversed phase HPLC with MeOH-H_2O (4:1, v/v) to afford **1** (16 mg) and **2** (70 mg).



The structures of the two compounds were determined as structure **1** and **2**, mainly by NMR and mass spectral measurements. Compound **1**, $[\alpha]_D^{25}$ -10.7° (*c* 0.24, MeOH), showed a molecular ion at m/z 491 $(M + Na)^+$ and a characteristic fragment ion at m/z 289 $(M - O\text{-Hexose})^+$ in the SIMS spectrum. The hexose part of **1** was determined to be β-glucopyranose[2] in view of the ^1H and ^{13}C NMR spectra as given in Table 1. The planar structure of the aglycone part of **1** was characterized as follows. The ^1H and ^{13}C-DEPT NMR spectra of **1** exhibited the signals characteristic of the methyl vinyl carbinol terminus of the C-1 side chain of manool[3] and sagittariol.[4,5] Further, the chemical shifts of the other carbon signals for **1** were similar to those of the signals due to the A and B rings of kolavenic acid having a A/B trans clerodane-type skeleton,[6] except for the signal due to an oxygenated CH group at δ85.7. In the ^{13}C-^1H COSY spectrum of **1**, the signal at δ85.7 showed a cross peak to a proton signal at δ3.78 (4-H). This proton signal showed cross peaks to the methylene signals at δ1.80 (3-H*ax*) and δ2.48 (3-H*eq*) in the ^1H-^1H COSY spectrum. The 3-H signals showed a cross peak to a methine signal at δ1.56 (2-H) and the latter showed a cross peak to a methyl signal at δ0.80 (15-Me). From these findings, the planar structure of the aglycone part of **1** was determined to be 4-hydroxy-α-vinyl-1,2,3,4,4a,7,8,8a-octahydro-α,1,2,4a,5-pentamethyl-1-naphthalene propanol, i.e., 6,13-dihydroxy-cleroda-3,14-diene. The position of the glycosidic linkage in **1** was determined by the NOE experiment. In the difference NOE spectrum of **1**, the anomeric proton signal at δ5.03 of β-glucopyranose showed an NOE to the 4-H signal. Thus, the planar structure of **1** was characterized as α-vinyl-1,2,3,4,4a,7,8,8a-octahydro-α,1,2,4a,5-pentamethyl-1-naphthalene propanol 4-O-β-glucopyranoside.

On the basis of the ^1H and ^{13}C NMR (Table 1) and the difference NOE spectra, the relative configuration of the A and B rings in the aglycone part of **1** was elucidated as

shown in partial structure of **1**. Two vicinal couplings with 10.5 Hz ($J_{2\text{-}3}$ and $J_{3\text{-}4}$) in the ^{1}H NMR spectrum of **1** indicated that 2-H and 4-H are axially oriented. A trans fused system in **1** was assigned by appearance of 17-Me carbon signal at δ18.2.[6] This was supported by the fact that the proton signal at δ 3.78 due to 4-H showed NOEs to the signals at δ1.56 (2-H) and δ1.45 (8a-H), while the proton signal at δ 0.70 due to 17-Me showed NOEs to the signals at δ1.80 due to 3-H *axial* proton and δ 2.40 due to 16-Me.



Partial structure of **1**

Table 1. ^{1}H and ^{13}C NMR data (C_5D_5N) and coupling constants (Hz) of **1** and **2**

	1		**2**			**1**		**2**	
No.	H	C	H	C	No.	H	C	H	C
1	—	44.6	—	44.6	15	0.80d (4.0,6.6)	16.0	0.78d (6.6)	15.9
2	1.56ddd (4.0,6.6,11.4)	34.5	1.68ddd (4.0,6.6,11.4)	34.8	16	2.40s	24.0	2.18s	22.7
3*ax*	1.80dd (4.0,11.4)	35.8	1.80dd (4.0,11.4)	35.8	17	0.70s	18.1	0.72s	18.3
					18	1.29s	16.8	1.44s	16.1
3*eq*	2.48brd (11.4)		2.48brd (11.4)		1'	5.03d (7.7)	104.4	5.10d (7.1)	102.7
4	3.78dd (4.0,11.4)	85.7	3.78dd (4.0,11.4)	84.0	2'	4.07dd (7.7,8.1)	75.7	4.31dd (7.1,9.2)	77.9
4a	—	38.2	—	38.3	3'	4.25dd (8.1,8.7)	79.2	4.29dd (8.3,9.2)	79.6
5	—	145.1	—	144.4					
6	5.28brs	123.1	5.14brs	122.5	4'	4.25dd (8.1,8.7)	71.8	4.19dd (8.3,9.2)	72.4
7	1.98brs	27.1	1.97brs	27.1					
8	1.74m	18.3	1.58m	18.3	5'	3.99m	78.3	3.96m	78.4
8a	1.45dd (3.7,12.0)	46.1	1.38dd (3.7,12.0)	46.4	6'a	4.40dd (4.7,11.0)	62.9	4.40dd (4.6,11.0)	62.9
9	1.52m	32.6	1.52m	32.6	6'b	4.60d (11.0)		4.51d (11.0)	
	1.66m		1.66m						
10	1.78m	38.9	1.78m	37.3	1"			6.54s	101.9
11	—	72.6	—	72.4	2"			4.83d (2.3)	72.6
12	6.18dd (10.6,17.2)	147.3	6.16dd (11.0,16.5)	149.4	3"			4.66dd (2.3,9.2)	72.5
13a	5.19brd (10.6)	111.3	5.17brd (11.0)	111.2	4"			4.34t (9.2)	74.1
13b	5.60brd (17.2)		5.56brd (16.5)		5"			4.86m	69.5
14	1.52s	28.7	1.50s	28.5	6"			1.74d (5.5)	18.6

Compound **2**, $[\alpha]_D^{25}$ -21.8° (*c* 0.07, MeOH), showed a molecular ion peak at m/z 637 $(M + Na)^+$ and two fragment ions at m/z 309 $(Hexose\text{-}O\text{-}6\text{-}deoxy\ hexose)^+$ and m/z 147 $(6\text{-}Deoxy\ hexose)^+$ in the SIMS spectrum. The ^1H and ^{13}C NMR spectra were similar to those of **1**, except for the signals due to an additional sugar moiety (Table 1). The ^1H and ^{13}C NMR spectra due to the sugar part were identical with those of ophiogenin 3-O-α-L-rhamnosyl-(1→2)-β-D-glucopyranoside.[7] The compound **2**, on acid hydrolysis, gave a glucopyranose and a rhamnopyranose. Further, the positions of the glycosidic linkages in **2** were confirmed by the fact that the proton signals due to H-4 in the aglycone part and H-2' in the glucopyranose part, appeared at δ3.48 (dd, J =6.4 and 9.2 Hz) and δ3.81 (dd, J =6.4 and 9.2 Hz), respectively, in the ^1H NMR spectrum of the hexaacetate (**3**) obtained from **2**. Thus, the structure of compound **2** was determined to be α-vinyl-1,2,3,4,4a,7,8,8a-octahydro-α,1,2,4a,5-pentamethyl-1-naphthalenepropanol 4-O-α-rhamnopyranosyl-(1→2)-β-glucopyranoside, i.e., 13-hydroxycleroda-3,14-diene 6-O-α-rhamnopyranosyl-(1→2)- β-glucopyranoside.

The biological activity of compounds **1** and **2** towards the seeds of *Lactuca sativa* (lettuce) was tested at 1, 10, and 100 ppm in the dark (25 °C). After 3 days, the lengths of the roots and stems of the germinated seeds were measured. It was found that the compound **1** showed a tendency to inhibit the growth of the both parts at 100 ppm (relative value to control; 85% for roots, 77% for stems) while the compound **2** accelerated the growth at the same concentration (123% for roots, 142% for stems).

The absolute configurations of **1** and **2** are now under investigation.

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