Triterpene Alcohols and Sterols from the Fern *Gleichenia japonica* SPR.

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Isolation and identification of five triterpene alcohols, twelve 4α-methylsterols, and seventeen sterols as acetyl derivatives from the unsaponifiable lipid of the methanol extract of *Gleichenia japonica* were described. This is the first report on the identification of (22*E*,24*R*)-4α,23-dimethyl-5α-ergosta-7,22-dien-3β-ol (7-dehydrodinosterol) and (22*E*,24*R*)-4α-methyl-5α-ergosta-7,22-dien-3β-ol in a terrestrial plant.

Keywords *Gleichenia japonica*; Gleicheniaceae; 4α-methylsterol; fern; triterpene alcohol; sterol

Gleichenia japonica SPR. (Gleicheniaceae) (urajiro in Japanese) is widely distributed in Japan. Decoction of this plant is used as a diuretic.[1] This paper describes the results of our study on the triterpene alcohol and sterol constituents of the fronds of *G. japonica*.

The fronds of *G. japonica* were extracted with methanol and the extract was then saponified to give an unsaponifiable lipid. Column chromatography of the unsaponifiable lipid yielded triterpene alcohol, 4α-methylsterol, and sterol fractions. These were acetylated and the resulting acetate fractions were subjected to argentation TLC and HPLC, which enabled the isolation of individual components. Table I shows the five triterpene alcohols, 12 4α-methylsterols, and 17 sterols isolated and identified as acetyl derivatives from the fronds of *G. japonica*. Table I also gives the abundance (% of whole) and the relative retention times (Rt_R) of each compound in HPLC and GLC. Identification of all compounds, with some exceptions, was performed by direct comparison of the chromatographic (HPLC and GLC) and spectral (MS and ^{1}H-NMR) data with respective authentic specimens. Compounds **1**, **2**, and **3**, for which the authentic specimens were unavailable, were identified based on their spectral (MS and ^{1}H-NMR) data.

(22*E*,24*R*)-4α,23-Dimethyl-5α-ergosta-7,22-dien-3β-ol (7-dehydrodinosterol, **1**) possesses a unique side chain doubly alkylated at C-23 and C-24. This sterol has, so far, only been isolated from the dinoflagellate algal symbionts of marine gorgonians.[2] It has been stated in several papers that the multiply alkylated side chain is one of the typical features of marine sterols.[3] However, we have recently found "marine-type" sterols (acetylenic[4] and *cis*-Δ^{22}-unsaturated sterols,[5] and sterols with a C-24 *gem* dimethyl group[6]) in some higher plants as well, which led us to conclude that the terrestrial plants also will prove to be treasure houses of sterols and that the distinction between marine and terrestrial sterols will gradually fade. The isolation of the "dinoflagellate sterol" **1** in *G. japonica* supports this conclusion.

The occurrence of (22*E*,24*R*)-4α-methyl-5α-ergosta-7,22-dien-3β-ol (**2**) has previously been reported in dinoflagellate algae,[2] the tunicate *Ascidia nigra*,[7] and in the protozoan *Naegleria gruberi*.[8] This study is the first report on its identification in a terrestrial plant. There has been no report, so far, on the occurrence of a 4α-methylsterol possessing a Δ^{22}-side chain in higher plants.[6]

Hop-22(29)-en-3β-ol (hopenol-b, **3**) has been reported as a metabolite of 2,3-epoxy-2,3-dihydrosqualene in a cell-free system of the bacterium *Acetobacter pasteurianum*.[9] It has also been isolated from the flowering plant *Euphorbia supina*.[10] Its 3-deoxy derivative, hop-22(29)-ene (diploptene), has been found in several pteridophytes.[11]

This is the first report on the isolation of sterols possessing a $\Delta^{9(11)}$-double bond, *viz.*, **4**, **5**, **6**, **7**, and **8**, in a pteridophyte.[6]

Experimental

Crystallizations were performed from acetone–MeOH. Melting points (mp) were determined on a Yanagimoto micro melting point apparatus and are uncorrected. TLC plates (Kieselgel 60G, Merck) were developed with hexane–EtOAc (4:1, v/v). Preparative argentation TLC plates (silica gel–$AgNO_3$, 4:1, w/w) were developed with CCl_4–CH_2Cl_2 (2:1, v/v). Preparative HPLC was carried out on an octadecyl silica column (Beckman Ultrasphere ODS 5 μ column, 25 cm × 10 mm i.d.; Beckman Instruments, Inc., San Ramon, California) with MeOH (4 ml/min) using an SSC Flow System 3100K (Senshu Scientific Co.) and an ERC-7520 refractive index detector (Erma Optical Works, Ltd.). GLC was performed on a Shimadzu GC-14A instrument using a DB-17 fused silica capillary column (30 cm × 0.3 mm i.d., column temp. 275 °C). Rt_R in HPLC and GLC were expressed relative to cholesteryl acetate. MS and high-resolution (HR) MS were obtained using a Hitachi M-80B double focusing gas chromatograph-mass spectrometer (70 eV, direct inlet system). NMR spectra were recorded using a JEOL JNM GSX-400 spectrometer at 400 MHz (^{1}H-NMR) and 100.62 MHz (^{13}C-NMR) in $CDCl_3$ with tetramethylsilane as an internal standard; chemical shifts are δ values. Acetyl derivatives of the sterols and triterpene alcohols used as the authentic specimens were described previously[6] with the exception of **1**, **2**, and **3** for which the authentic specimens were unavailable. *Gleichenia japonica* was collected in the mountain area near Hamamatsu (Shizuoka Pref., Japan).

Isolation of Triterpene Alcohols and Sterols Sliced fresh fronds of *G. japonica* (5.7 kg) were extracted with cold MeOH (40 l) for 2 d. The MeOH extract was concentrated to 1.02 kg *in vacuo* and was then saponified by refluxing it with 5% KOH in 90% MeOH for 3 h. The unsaponifiable lipid (10 g) extracted by diisopropyl ether was chromatographed over silica gel (300 g; Wakogel C-300; Wako Pure Chemical Industries, Co.). Eluting solvents were hexane (1.6 l) and a mixture of hexane–EtOAc [4:1 (2.4 l)]. The hexane–EtOAc (4:1) eluate yielded triterpene alcohol (fraction A; *Rf* 0.42 on TLC; 2.66 g), 4α-methylsterol (fr. B; *Rf* 0.30; 192 mg), and sterol (fr. C; *Rf* 0.21; 2.27 g) fractions which, upon acetylation followed by further purification over silica gel, afforded acetylated fractions A (1.37 g), B (85 mg) and C (1.30 g), respectively. Isolation of individual components (as the acetates) was carried out by argentation TLC and HPLC. Spectroscopic evidence for the identifica-

TABLE I. Abundance (%) and Chromatographic Data of Acetates of Triterpene Alcohols, 4α-Methylsterols, and Sterols from *Gleichenia japonica*

Compound	Abundance (%)[a]	Acetate	
		Rt_R[b] (HPLC)	Rt_R[b] (GLC)
Triterpene alcohol fraction (fr. A)			
Hop-22(29)-enol (hopenol-b) (**3**)	2.1	1.25	1.97
9β,19-Cyclolanost-24-enol (cycloart-24-enol;cycloartenol)	25.9	0.99	1.81
24-Methylcycloart-24(28)-enol (24-methylenecycloartanol)	20.1	1.08	1.99
(24*S*/β)-24-Methylcycloart-25-enol (cyclolaudenol)	21.7	1.07	1.94
Lup-20(29)-enol (lupeol)	12.9	0.81	1.99
Others, unidentified	17.3		
4α-Methylsterol fraction (fr. B)			
(24*R*/β)-4α,23-Dimethylergosta-7,22-dienol (7-dehydrodinosterol) (**1**)	2.2	1.21	1.75
(24*R*/β)-4α-Methylergosta-7,22-dienol (**2**)	2.6	1.08	1.47
24-Methyl-31-norlanosta-9(11), 24(28)-dienol (**4**)	3.7	0.87	1.66
24-Methyl-31-norcycloart-24(28)-enol (cycloeucalenol)	5.0	0.97	1.72
(24*S*/β)-24-Methyl-31-norcycloart-25-enol (31-norcyclolaudenol)	5.9	0.95	1.69
24-Methyl-31-norlanosta-8,24(28)-dienol (obtusifoliol)	19.4	0.87	1.46
4α-Methylcholest-7-enol (lophenol)	1.5	1.14	1.30
(24*R*/α)-4α-Methylergost-7-enol[c]	0.6	1.33	1.65
(24*S*/β)-4α-Methylergost-7-enol[c]	2.0	1.33	1.65
4α-Methylergosta-7,24(28)-dienol (gramisterol)	25.1	0.97	1.72
[24(28)*Z*]-4α-Methylstigmasta-7,24(28)-dienol (citrostadienol)	23.1	1.18	2.22
[24(28)*E*]-4α-Methylstigmasta-7,24(28)-dienol (isocitrostadienol)	1.3	1.18	2.12
Others, unidentified	6.9		
Sterol fraction (fr. C)			
(24*R*/α)-14α-Methylergost-9(11)-enol (**5**)[c]	0.5	1.02	1.46
(24*S*/β)-14α-Methylergost-9(11)-enol (**6**)[c]	0.2	1.02	1.46
14α-Methylergosta-9(11),24(28)-dienol (**7**)	0.1	0.77	1.55
(24*R*/α)-14α-Methylstigmast-9(11)-enol (**8**)	0.3	1.16	1.76
Cholestanol	Trace	1.18	1.01
(24*R*/α)-Ergostanol[c]	0.2	1.32	1.28
(24*S*/β)-Ergostanol[c]	0.1	1.32	1.28
(24*R*/α)-Stigmastanol	0.9	1.48	1.55
Cholest-5-enol (cholesterol)	37.8	1.00	1.00
(24*R*/α)-Ergost-5-enol (campesterol)[c]	6.4	1.14	1.27
(24*S*/β)-Ergost-5-enol (22-dihyrobrassicasterol)[c]	3.4	1.14	1.27
(24*S*/α)-Ergosta-5,22-dienol (crinosterol)	Trace	0.87	1.13
(24*R*/β)-Ergosta-5,22-dienol (brassicasterol)	Trace	0.91	1.13
Ergosta-5,24(28)-dienol (24-methylenecholesterol)	1.1	0.84	1.33
(24*R*/α)-Stigmast-5-enol (sitosterol)	43.8	1.27	1.54
(24*S*/α)-Stigmasta-5,22-dienol (stigmasterol)	1.7	1.09	1.37
[24(28)*Z*]-Stigmasta-5,24(28)-dienol (isofucosterol)	1.8	1.02	1.71
Others, unidentified	1.7		

a) Abundance in each fraction. Fractions A and B contained large amounts of compounds which eluted faster than cholesteryl acetate on GLC. These were considered to be aliphatic alcohols and were excluded from the calculation of sterol compositions. *b*) Standard: cholesteryl acetate (Rt_R: 1.00; actual retention times: 48 min in HPLC and 24 min in GLC). *c*) Isolated as the C-24 epimeric mixture.

tion of the acetates of **1**, **2**, and **3** is given below.

7-Dehydrodinosterol (1) Acetate mp 172—174 °C (fine needles). HR-MS *m/z*: 468.3982 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3965]. ^{1}H-NMR δ: 0.56 (3H, s, H-18), 0.78 (3H, d, J=6.6 Hz) and 0.84 (3H, d, J=6.8 Hz) (H-26, H-27), 0.84 (3H, s, H-19), 0.85 (3H, d, J=6.8 Hz, H-30), 0.94 (6H, d, J=6.8 Hz, H-21, H-28), 1.50 (3H, s, H-29), 1.52 (1H, m, H-25), 1.65 (1H, m, H-24), 2.06 (3H, s, 3β-OAc), 2.34 (1H, m, H-20), 4.41 (1H, dt, J=4.4, 11.0 Hz, H-3α), 4.90 (1H, d, J=9.6 Hz, H-22), 5.17 (1H, br d, J=2.8 Hz, H-7). ^{1}H-NMR and MS data of **1** (free sterol) are in the literature.[2]

(22*E*,24*R*)-4α-Methyl-5α-ergosta-7,22-dien-3β-ol (2) Acetate mp 160—163 °C (fine needles). HR-MS *m/z*: 454.3798 [Calcd for $C_{31}H_{50}O_2$ (M^+): 454.3808]. ^{1}H-NMR δ: 0.54 (3H, s, H-18), 0.82 (3H, d, J=6.3 Hz, H-27), 0.84 (3H, s, H-19), 0.84 (3H, d, J=5.5 Hz, H-26), 0.85 (3H, d, J=6.6 Hz, H-30), 0.91 (3H, d, J=6.9 Hz, H-28), 1.02 (3H, d, J=6.6 Hz, H-21), 2.06 (3H, s, 3β-OAc), 4.40 (1H, dt, J=4.1, 10.8 Hz, H-3α), 5.16 (1H, dd, J=7.1, 15.2 Hz, H-22), 5.18 (1H, m, H-7), 5.22 (1H, dd, J=7.1, 15.1 Hz, H-23). The ^{1}H-NMR data were consistent with the literature data of the same[2,8] and structurally related compounds.[12] ^{13}C-NMR δ (assignment): 171.0 (acetate C=O at C-3), 139.2 (C-8), 135.7 (C-22), 131.9 (C-23), 117.3 (C-7), 78.5 (C-3), 55.9 (C-17), 55.0 (C-14), 49.4 (C-9), 46.7 (C-5), 43.3 (C-13), 42.4 (C-24), 40.1 (C-20), 39.4 (C-12), 37.0 (C-4), 36.6 (C-1), 34.7 (C-10), 33.1 (C-25), 28.1 (C-16), 27.1 (C-2), 26.6 (C-6), 21.4 (2×C; C-11, acetate methyl at C-3), 21.1 (C-21), 20.0 (C-26), 19.7 (C-27), 17.6 (C-28), 15.2 (C-30), 14.1 (C-19), 12.1 (C-18). The ^{13}C signal assignment was done by comparison with the literature data for relevant compounds,[13] and the ^{13}C-NMR data confirmed the (24*R*/β)-chirality at C-24 of **2**-acetate.

Hop-22(29)-en-3β-ol (3) Acetate mp 230—232 °C (fine needles) (lit.,[10] mp 233—235 °C). HR-MS *m/z*: 468.3977 [Calcd for $C_{32}H_{52}O_2$ (M^+): 468.3965. ^{1}H-NMR δ: 0.72 (3H, s, H-28), 0.84 (6H) and 0.85 (3H) (each s, H-23, H-24, H-25), 0.93 (3H, s, H-26), 0.96 (3H, s, H-27), 1.75 (3H, s, H-30), 2.04 (3H, s, 3β-OAc), 2.68 (1H, m, H-22), 4.48 (1H, dd, J=6.3, 10.2 Hz, H-3α), 4.78 (2H, br s, H-29). The ^{1}H-NMR data were consistent with the literature data.[9] The ^{1}H signals were assigned by comparison with the literature data for **3**-acetate[9,10] and hop-22(29)-ene.[11b]

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