Fern Constituents: *Adiantum cuneatum*. I. Three New Triterpenoids, Glaucanol B Acetate, 7β,25-Epoxyfern-8-ene and 25-Norfern-7-en-10β-yl Formate

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Sixteen triterpenoids including three new compounds, *viz.* glaucanol B acetate (1), 7β,25-epoxyfern-8-ene (2), and 25-norfern-7-en-10β-yl formate (3) were isolated from the fresh leaves of *Adiantum cuneatum*. Their structures were elucidated on the basis of spectral data.

Key words fern; *Adiantum cuneatum*; triterpenoid; glaucanol B acetate; 7β,25-epoxyfern-8-ene; 25-norfern-7-en-10β-yl formate

Adiantum cuneatum LANGSD. *et* FISCH. (*A. raddianum* PRESL, Adiantaceae) is a common fern widely distributed in South America. In Brazil, the dried whole plants have been used as a medicine for bronchitis and cough.[1] This fern is used as an ornamental plant in Japan and Europe. In a continuation of our chemotaxonomic studies[2] on the constituents of *Adiantum* ferns, we undertook a detailed chemical investigation on the fresh leaves of *A. cuneatum* (cultivated) and we isolated three new triterpenoids, *viz.* glaucanol B acetate (**1**), 7β,25-epoxyfern-8-ene (**2**), and 25-norfern-7-en-10β-yl formate (**3**), along with thirteen known triterpenoids. This paper deals with structure elucidation of these compounds.

Results and Discussion

The constituents of the crude hexane extract of the fresh leaves were purified by various chromatographic techniques (see Experimental) to give the triterpenoid compounds **1**—**16**, which are summarized in Table 1 along with their physical constants and yields.

Compound **1** was obtained as colorless needles, and its high-resolution MS (HR-MS) showed the molecular ion (M^+) at *m/z* 428.3633 (Calcd, 428.3654), suggesting the molecular formula to be $C_{29}H_{48}O_2$. Its IR spectrum indicated the presence of an *O*-acetyl group. The base peak at *m/z* 191 (a), and the other diagnostic peaks[3] at *m/z* 206 (11, b) and 147 (76, b−OAc) (Chart 2) in its low-resolution MS (LR-MS) indicated that **1** is a trisnorhopane derivative with an *O*-acetyl group in ring D or E of the molecule. The ^{1}H-NMR spectrum of **1** displayed signals for six tertiary methyl groups and one acetyl methyl group, and the chemical shifts of methyl protons were close to those of trisnorhopane (**4**) (Table 2).[4] The ^{13}C chemical shifts were also very close to those of **4**, except for those of C-16 to C-21. The down-field shift of C-17, C-20 and C-21 by 4, 8 and 50 ppm, respectively, and up-field shift of C-16, C-18 and C-19 by 1—2 ppm suggested that the OAc group of **1** was located at C-21. The heteronuclear multiple bond correlation (HMBC) data also fully corroborated the above observation (Fig. 1). The orientation of the OAc group was deduced to be β from the nuclear Overhauser effect spectroscopy (NOESY) spectrum of the compound, which exhibited an NOE correlation between the carbinyl proton and α-oriented H-28. Finally the structure of **1** was conformed by its preparation from glaucanone[4] (17βH-trisnorhopan-21-one) with $LiAlH_4$. The reaction yielded two products, *viz.* 17βH-trisnorhopan-21α-ol (less polar; glaucanol A), and 17βH-trisnorhopan-21β-ol (more polar; glaucanol B), of which the acetate of the latter was proved

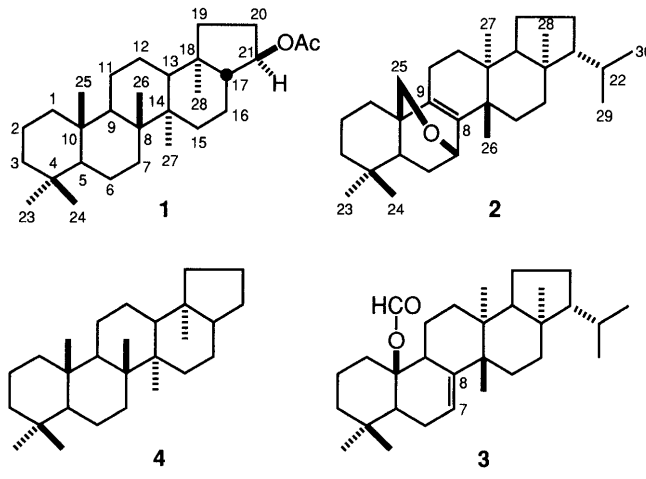

Chart 1

Table 1. Triterpenoids Isolated from *Adiantum cuneatum*

	mp (°C)	$[\alpha]_D^{23}$ (°)	Yield (%)[a]	Ref.
Glaucanol B acetate (**1**)	288—289	+11.6	0.0169	2*b*
7β,25-Epoxyfern-8-ene (**2**)	172—173	−86.6	0.0012	—
25-Norfern-7-en-10β-yl formate (**3**)	154—155	+21.6	0.0002	—
Trisnorhopane (**4**)	161—163	+35.5	0.0006	4
Fern-9(11)-ene (**5**)	171—172	−18.3	0.0514	5
Neohop-13(18)-ene (**6**)	199—201	+2.1	0.0139	5
Ferna-7,9(11)-diene (**7**)	202—203	−180.4	0.0039	
Fern-7-ene (**8**)	212.5—214.0	−29.0	0.0481	5
Neohop-12-ene (**9**)	210—212	+41.6	0.0796	5
Filic-3-ene (**10**)	232—234	+58.0	0.1778	5
Neohopa-11,13(18)-diene (**11**)	214.5—215.5	+25.7	0.0039	
Adiantone (**12**)	227—230	+79.9	0.4200	2*b*
Isoadiantone (**13**)	236—238	+3.6	0.0030	2*b*
Isoglaucanone (**14**)	243—245	+140.1	0.0009	2*b*
Filicenal (**15**)	272	+74.0	0.0080	2*b*
Hydroxyadiantone (**16**)	270—275	+50.0	0.0115	2*a*

a) Yield from the dried materials after removal of water by azeotropic distillation.

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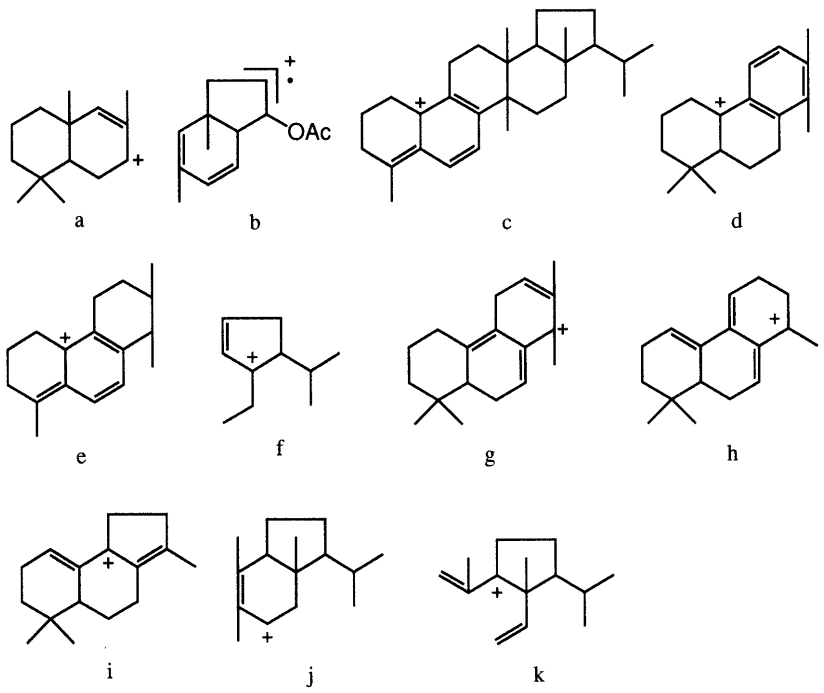

Chart 2

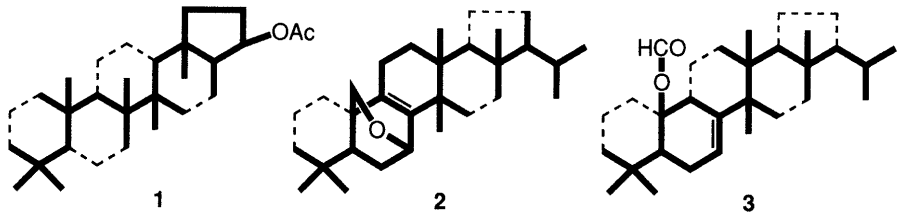

Fig. 1. Partial Structures of **1**, **2** and **3**, Based on the HMBC Spectra

Table 2. ^{1}H-NMR Spectral Data[a] (500 MHz, $CDCl_3$, δ)

	1	2	3	4
H-23	0.849	0.830	0.891	0.848
H-24	0.794	1.043	0.914	0.794
H-25	0.819	2.687 (dd, 7.7, 1.8)	—	0.818
		4.085 (d, 7.7)		
H-26	0.974	1.080	1.006	0.965
H-27	0.950	0.734	0.884	0.951
H-28	0.698	0.779	0.734	0.599
H-29	—	0.900 (d, 6.7)	0.898 (d, 6.7)	—
H-30	—	0.837 (d, 6.4)	0.829 (d, 6.7)	—
H-7α		4.476 (dd, 6.8, 4.5)	—	
H-21α	4.812 (ddd, 9.5, 9.5, 4.6)	—	—	—
>C=C<u>H</u>–	—	—	5.505 (ddd, 3.4, 3.4, 3.4)	—
–O–C<u>H</u>O	—	—	8.398	—

Multiplicity and coupling constants (J, Hz) are shown in parentheses. *a*) Assignments have been done on the basis of distortionless enhancement by polarization transfer (DEPT), ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C COSY, HMBC and NOESY spectra.

to be identical (melting point, IR and ^{1}H-NMR comparisons) with **1**.[2b] Thus, the structure of **1** was established as glaucanol B acetate (trisnorhopan-21β-yl acetate).

Compound **2** was obtained as colorless needles, and its molecular formula was found to be $C_{30}H_{48}O$ by HR-MS (M^+ at m/z 424.3699, Calcd, 424.3705). The ^{1}H-NMR spectra of **2** indicated the presence of five tertiary methyl groups, two secondary methyl groups, a methylene group and a methine group attached to oxygen (Table 2). As the ^{1}H- (Table 2) and ^{13}C- (Table 3) NMR data were not conclusive in the determination of the structure of the compound, the HMBC spectrum of **2** was recorded. Detailed analysis of the HMBCs (partial structure shown in Fig. 1) revealed that the compound has a fern-8-ene skeleton with an unusual ether linkage between C-25 and C-7. The mass spectral fragmentation also supported the assigned structure. Thus, the spectrum showed intense peaks at m/z 394 (90, $M^+ - HCHO$), 377(75, c), 241 (77, d), 227 (100, e), 225 (45, e$-$2H) and 137 (76, f) (Chart 2).[3] The relative stereochemistry at most of the chiral centers of **2** was established by the NOESY spectrum, which showed NOE interactions connecting H-24–H-25–H-26–H-18β (δ, 1.58)–H-21β (δ, 1.01), and H-27–H-15α (δ, 1.63)–H-28 (Table 2). Thus, the structure of **2** was established as 7β, 25-epoxyfern-8-ene.

Compound **3** was obtained as colorless needles, and its

Table 3. ^{13}C-NMR Spectral Data[a] (125 MHz, $CDCl_3$, δ)

	1	2	3	4
C-1	40.31	28.26	33.23	40.33
C-2	18.69	18.98	17.79	18.71
C-3	42.08	41.34	41.54	42.12
C-4	33.26	33.40	33.20	33.27
C-5	56.24	41.98	49.44	56.11
C-6	18.69	29.96	23.82	18.73
C-7	33.26	66.21	117.22	33.30
C-8	41.97	141.34	142.90	41.93
C-9	50.38	137.56	44.88	50.40
C-10	37.41	38.95	84.75	37.42
C-11	20.76	18.32	16.84	20.95
C-12	23.19	29.59	32.10	24.14
C-13	48.52	36.93	42.01	48.24
C-14	42.16	39.51	36.06	42.22
C-15	32.15	26.94	30.28	32.66
C-16	20.25	35.65	36.09	22.65
C-17	55.09	43.23	42.83	51.03
C-18	42.50	51.77	53.93	43.59
C-19	38.96	20.38	19.97	40.75
C-20	28.84	28.01	28.17	20.23
C-21	78.03	59.78	59.44	27.60
C-22	—	30.74	30.66	—
C-23	33.40	33.13	31.65	33.43
C-24	21.59	20.69	20.87	21.61
C-25	15.85	67.28	—	15.85
C-26	16.68	24.02	24.33	16.73
C-27	16.62	15.58	21.21	16.73
C-28	15.06	14.90	14.09	14.01
C-29	—	22.08	22.09	—
C-30	—	22.97	22.96	—
–OCHO			164.14	

a) Assignments have been done on the basis of DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and HMBC spectra.

IR spectrum suggested the presence of an ester group in the molecule. Its molecular formula was deduced to be $C_{30}H_{48}O_2$ by HR-MS (M^+ at m/z 440.3689, Calcd, 440.3654). The ^{1}H-NMR spectrum of **3** indicated the presence of five tertiary methyl groups, two secondary methyl groups, and a formyl proton (Table 2). The ^{13}C-NMR spectrum of **3** was similar to that of fern-7-ene (**8**) except for the signals of C-1, C-9, C-10 and C-25 (Table 3).[5] That a –O–COH group is attached to C-10 of a 25-norfern-7-ene skeleton was clearly revealed by connectivity between a proton of the formoxyl group and carbon at C-10 (δ, 84.75) in the HMBC spectrum of **3** (Fig. 1). Its LR-MS also supported the above structural assignment. Thus, it exhibited the base peak at m/z 394 afforded by the elimination of HCOOH from the molecular ion. The formation of other prominent peaks at m/z 241 (47, g), 227 (72, h), 215 (23, i), 205 (25, j) and 191 (34, k) could also be rationalized on the basis of the assigned structure **3** for the compound.[3] Finally, the relative stereochemistry of **3** was deduced from its NOESY spectrum which showed NOEs connecting H-24–10β-formyloxy proton (δ, 8.398)–H-26–H-18β (δ, 1.47)–H-21β (δ, 0.95), and H-9α (δ, 2.76)–H-27–H-28 (Table 2). Based on the above evidence, the structure of **3** can as represented by 25-norfern-7-en-10β-yl formate.

Experimental

Melting points were measured on a Yanagimoto micro melting point apparatus without correction. Specific rotations were observed in $CHCl_3$ solution (c=0.1—0.5) at 22—24 °C. ^{1}H- and ^{13}C-NMR spectra were taken at 500 and 125 MHz, respectively, by the Fourier-transform (FT) method in $CDCl_3$ solution with tetramethylsilane as an internal standard. MS was recorded (direct inlet) at 30 eV and the relative intensities of peaks were reported with reference to the most intense peak higher than m/z 100. HPLC was performed on a C18 reverse-phase column (8 × 250 mm, 5 μ, RI detector) with CH_3CN–$CHCl_3$ (19 : 1) as a mobile phase. Silica gel 60, 230—400 mesh (Merck), and 20% $AgNO_3$–impregnated silica gel were used for column chromatography (CC).

Plant Material Cultivated *Adiantum cuneatum* was collected in March, 1994, at a farm near Katsuura city, Chiba Prefecture, Japan. Voucher specimens have been deposited in the Herbarium of Showa College of Pharmaceutical Sciences, Tokyo.

Extraction and Separation of Triterpenoids The fresh leaves (4.7 kg) were extracted with hexane three times to give the extract (51 g) and separated H_2O (3820 g). The extract was refluxed with benzene for 1 h and kept for 2 d. The insoluble materials were filtered off (fraction A), and the filtrate was evaporated to dryness to afford a gummy residue, which was chromatographed on silica gel to give seven fractions: fr. B [eluted with hexane], fr. C [hexane–benzene (8 : 2)], fr. D [hexane–benzene (1 : 1)], fr. E, F [benzene], fr. G [benzene–Et_2O (9 : 1)] and fr. H [Et_2O].

Trisnorhopane (4), Fern-9(11)-ene (5), Neohop-13(18)-ene (6), Ferna-7,9(11)-diene (7), Fern-7-ene (8), Neohop-12-ene (9), Filic-3-ene (10) and Neohopa-11,13(18)-diene (11) Chromatography of fr. B on 20% $AgNO_3$-impregnated silica gel followed by recrystallization from acetone to give nine triterpenoid hydrocarbons in the following order of elution, in pure form: **4**, 5 mg, **5**, 394 mg, **6**, 99 mg, **7**, 30 mg, **8**, 368 mg, **9**, 610 mg, **10**, 1361 mg, **11**, 9 mg. These compounds were identified by direct comparison (GC, MS, ^{1}H-NMR, IR) with authentic samples.[2a,5]

Glaucanol B Acetate (1), and 7β,25-Epoxyfern-8-ene (2) Fraction D was chromatographed on silica gel with hexane–benzene (7 : 3), followed by HPLC to give the following crystalline solids (recrystallized from MeOH–$CHCl_3$ or acetone to give the pure compounds). **1**, 130 mg, IR ν_{max}^{KBr} cm^{-1}: 1741, 1242. **2**, 9 mg.

25-Norfern-7-en-10β-yl Formate (3), Adiantone (12), Isoadiantone (13), Isoglaucanone (14), and Filicenal (15) Repeated recrystallization of fr. E from MeOH–$CHCl_3$ to give **12**, 4.05 g, IR ν_{max}^{KBr} cm^{-1}: 1705. The filtrate was evaporated and the residue was chromatographed on silica gel with hexane–benzene (1 : 1) and then subjected to HPLC followed by recrystallization from MeOH–$CHCl_3$ or acetone to give **3**, **12**, **13**, **14** and **15** in pure form. **3**, 2 mg, IR ν_{max}^{KBr} cm^{-1}: 1723, 1193, 1165. **13**, 225 mg, IR ν_{max}^{KBr} cm^{-1}: 1704. **14**, 62 mg, IR ν_{max}^{KBr} cm^{-1}: 2720, 1682, 1630. **15**, 9 mg, IR ν_{max}^{KBr} cm^{-1}: 1732. Compounds **12**—**15** were identified by direct comparison (melting point and IR) with authentic samples.[2b]

Hydroxyadiantone (16) Fraction A was repeatedly recrystallized from MeOH–$CHCl_3$ to give **16**, 112 mg, IR ν_{max}^{KBr} cm^{-1}: 3430, 1695, 1082. Compound **16** was identified by direct comparison (melting point and IR) with an authentic sample.[2a]

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