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Fern Constituents: Triterpenoids Isolated from the Leaves of *Adiantum edgeworthii*. Structures of 19α-Hydroxyadiantone and Fern-9(11)-en-25-oic Acid

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From the leaves of *Adiantum edgeworthii*, two new triterpenoids, 19α-hydroxyadiantone (1) and fern-9(11)-en-25-oic acid (2) were isolated, together with fern-9(11)-ene (3), fern-7-ene (4), neohop-12-ene (5), filic-3-ene (6), hop-22(29)-ene (7), adiantone (8), hydroxyhopane (9) and zeorin (10). The structures of 1 and 2 were elucidated on the basis of spectral data and chemical correlations with a known compound.

Keywords fern; *Adiantum edgeworthii*; triterpenoid; 19α-hydroxyadiantone; fern-9(11)-en-25-oic acid

Adiantum edgeworthii HOOK. ("otome-kujaku" in Japanese, Pteridaceae) is only distributed over Ooita Prefecture in Japan, though it is widely distributed throughout China and India. This species is very characteristic because it forms runners at the top of leaves and reproduce individuals. As a part of our chemotaxonomic investigation of *Adiantum* ferns,[1] we have isolated two new triterpenoids, 19α-hydroxyadiantone (**1**) and fern-9(11)-en-25-oic acid (**2**) together with eight known compounds. This paper deals with the structural elucidation of these compounds.

Results and Discussion

The fresh leaves of *A. edgeworthii* were extracted with hexane, and the concentrated extract was refluxed with benzene. It was left to stand for 3 d, then the insoluble material was filtered off, and the benzene-soluble material was separated by chromatography to give two new compounds, **1** and **2**, and eight known compounds, fern-9(11)-ene (**3**), fern-7-ene (**4**), neohop-12-ene (**5**), filic-3-ene (**6**), hop-22(29)-ene (**7**), adiantone (**8**), hydroxyhopane (**9**) and zeorin (**10**). The insoluble material was also purified to give **1** and **10** (see Experimental). Compound **10** is a well-known lichen product, and this is the second isolation from ferns.[2]

A new compound, **1**, was obtained as colorless needles, mp 281—283 °C, $[\alpha]_D$ +56.8°. The IR spectrum of **1** indicated the presence of a hydroxyl group and an acetyl group. The MS of **1** showed the molecular ion at *m/z* 428.3678 ($C_{29}H_{48}O_2$) and many significant fragment ions at *m/z* (rel. int.): 410 (15, M^+-H_2O), 367 (44, $M^+-H_2O-COCH_3$), 328 (28, a), 191 (98, b) and 161 (100, c) (Chart 2). These fragment ions suggested that **1** is a hopane derivative with functional groups in the E-ring of the molecule. The ^{1}H-NMR spectrum of **1** indicated the presence of six tertiary and one acetylmethyl groups. The methyl protons of H-23, 24, 25, 26 and 27 were observed at almost the same positions, but those of H-28 appeared at lower fields compared with those of **8**, as shown in Table I. Treatment of **1** with Al_2O_3 overnight gave an isomer, an isoadiantone derivative (**11**), the H-28 methyl protons of which were also observed at lower field than those of isoadiantone (**12**).[3] The α orientation of the 19-hydroxyl group of **11** was also indicated by nuclear Overhauser effect spectroscopy (NOESY), because cross peaks were observed between H-19β (δ 3.81) and H-13β (δ 1.54), and H-19β and H-20β (δ 2.21).[4] Acetylation of **11** gave an acetate (**11a**), and oxidation of **11** with CrO_3–pyridine afforded a diketone (**13**). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **13** with those of **12** clearly showed that **13** has the second carbonyl group at C-19 (Tables I and II). Compound **13** was reduced

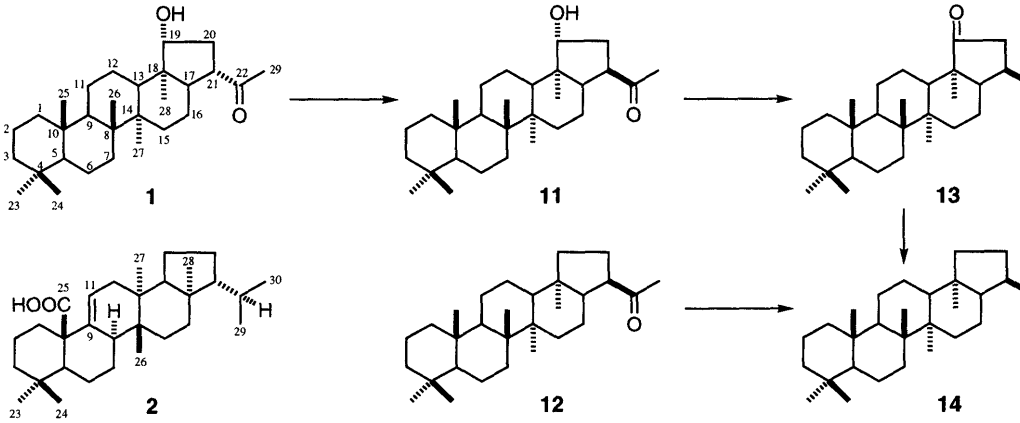

Chart 1

by the Wolff–Kishner–Barton method to give a saturated hydrocarbon, which was identical with a synthetic sample of isoadiantane (**14**) derived from **12**. As the position of the hydroxyl group of **11** as well as **1** was concluded to be 19α from the evidence described above, compound **1** is 19α-hydroxyadiantone.

Compound **2** was obtained as colorless needles, mp 289—291 °C, $[\alpha]_D$ −19.9°. The IR spectrum indicated the presence of a carboxyl group. The MS of **2** showed the molecular ion, m/z 440.3681 ($C_{30}H_{48}O_2$) and many significant fragment ions at m/z (rel. int.): 425 (18, M^+-CH_3), 397 (6, $M^+-C_3H_7$), 394 (100, $M^+-HCOOH$), 379 (85, $M^+-HCOOH-CH_3$), 287 (3, d), 273 (11, e), 261 (4, f), 241 (33, d−HCOOH), 227 (22, e−HCOOH) and 215 (17, f−HCOOH) (Chart 2). These fragment ions suggested that **2** is a fernene derivative with a carboxyl group in the left-hand part of the molecule.[5] As the fragment ion (M^+-COOH) appeared as a base peak, the carboxyl group was assumed to be at the allylic position with respect to the double bond.[6] The ^{1}H-NMR spectrum of **1** indicated the presence of five tertiary and two secondary methyl groups. The chemical shift and splitting pattern of an olefinic proton were very similar to those of **3**. The H-24 methyl signal of **2** was observed at higher field than that of **3**. Furthermore, the H-24 methyl signal of methyl fern-9(11)-en-25-oate (**15**), which was prepared from **2** by diazomethane methylation, appeared at higher field than that of **2** (Table I). The ^{13}C-NMR spectrum of **15** clearly indicated that **15** has a carbomethoxy group at C-25 instead of the methyl group of **3** (Table II). Thus, the structure of **2** was established as fern-9(11)-en-25-oic acid (Chart 1). Recently this compound was isolated from *Adiantum venustum* Don var. *monoclamys* (Eaton)[7] of Western Himalayan origin, and the structure was elucidated by ^{13}C-NMR analyses.[8] Assignments of the ^{1}H- and ^{13}C-signals are in good agreement, although the assignments for H-29 and H-30, as well as those for C-2 and C-6 should be reversed.[9]

It is noteworthy that all triterpenoids obtained from the leaves of *Adiantum edgeworthii* are pentacyclic and belong to the hopane and migrated hopane groups. The isolation of a large quantity of the nor-compounds is also a characteristic feature of this fern, as well as other *Adiantum* ferns.[1f,g]

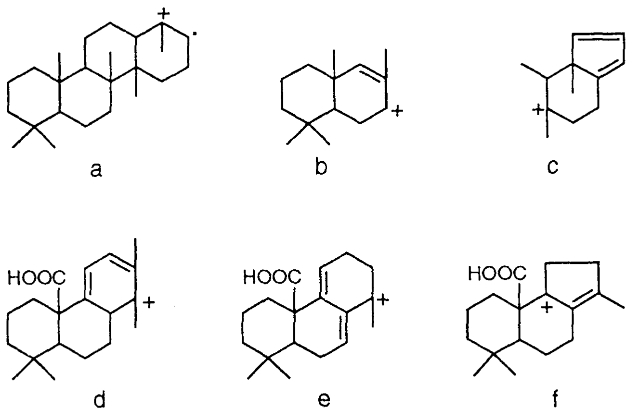

Chart 2

Experimental

Melting points were measured on a Yanagimoto microapparatus and were corrected. Specific rotation was observed in $CHCl_3$ solution (c=0.1—0.6) at 22—24 °C. ^{1}H- and ^{13}C-NMR spectra were taken at 500 and 68/125 MHz by the Fourier transform (FT) method with tetramethylsilane as an internal standard. MS were recorded (direct inlet) at 30 eV and relative intensities of peaks were reported with reference to the most intense peak higher than m/z 100. Gas liquid chromatography (GLC) was performed on a 1 m glass column containing Chromosorb G HP with 1.4% SE-30 at 260 °C under N_2 using cholestane as an internal standard (its retention time was set at 3.0 min). Silica gel 60, 230—400 mesh (Merck), Al_2O_3 (Woelm or Wako, neutral or basic, grade 1 or 3) and 20% $AgNO_3$-impregnated silica gel were used for column chromatography (CC). Precoated Silica gel 60 plates (Merck) were used for thin layer chromatography (TLC), and spots were detected by spraying with concentrated H_2SO_4 followed by heating.

Plant Materials The leaves of *Adiantum edgeworthii* were collected in October 1979, at Hazama-cho, Ooita Prefecture. A voucher specimen has been deposited in the Herbarium of Shōwa College of Pharmaceutical

Table I. ^{1}H-NMR Spectral Data for Triterpenoids of Adiantane and Fernane Groups (500 MHz, $CDCl_3$, δ)

	1	8	11	11a	12	13	2[a]	3	15
H-23	0.843	0.845	0.844	0.842	0.845	0.842	0.868	0.847	0.858
H-24	0.790	0.790	0.791	0.788	0.791	0.790	0.794	0.888	0.691
H-25	0.813	0.813	0.815	0.805	0.815	0.815	—	1.053	—
H-26	0.965	0.963	0.963	0.952	0.965	0.965	0.702	0.733	0.672
H-27	0.949	0.932	0.973	0.967	0.948	0.993	0.816	0.822	0.810
H-28	0.652	0.580 (d, 2.0)	0.791	0.846	0.690 (d, 1.0)	0.891	0.762	0.759	0.758
H-29	2.125	2.117	2.198	2.132	2.148	2.217	0.888 (d, 6.3)	0.890 (d, 6.4)	0.888 (d, 6.1)
H-30	—	—	—	—	—	—	0.826 (d, 6.3)	0.830 (d, 6.4)	0.827 (d, 6.1)
CH=C							5.632 (1H, ddd, 5.2, 2.5, 2.5)	5.286 (1H, ddd, 5.1, 2.4, 2.4)	5.559 (1H, ddd, 5.1, 2.4, 2.4)
CH–O	3.677 (1H, dd, 16.5, 8.5)		3.811 (1H, dd, 14.3, 8.0)	4.605 (1H, dd, 9.5, 6.7)					
CH_3-OCO									3.617
CH_3-CO				2.013					

Signals, unless otherwise stated, are 3H, singlet. Multiplicity and coupling constants (J) are shown in parentheses. *a*) Measured at 100 MHz.

TABLE II. ^{13}C-NMR Spectral Data for Triterpenoids of Adiantane and Fernane Groups (125 MHz, $CDCl_3$, δ)

	1	**8**	**11**	**11a**	**12**	**13**	**3**	**15**
C-1	40.3	40.3	40.3	40.3	40.3	40.3	41.5	40.5
C-2	18.7	18.7	18.7	18.7	18.7	18.7	19.6	20.8
C-3	42.1	42.1	42.1	42.1	42.1	42.1	42.4	42.2
C-4	33.3	33.4	33.2	33.4	33.3	33.2	33.6	34.1
C-5	56.1	56.1	56.1	56.1	56.1	56.1	44.9	46.0
C-6	18.7	18.7	18.7	18.7	18.7	18.7	19.5	20.6
C-7	33.3	33.2	33.2	33.4	33.3	33.3	17.9	18.7
C-8	41.9[a]	41.9[a]	42.0[a]	42.0[a]	41.9[a]	42.1[a]	40.0	39.5
C-9	50.2	50.3	50.2	50.2	50.4	50.4	151.7	142.9
C-10	37.4	37.4	37.4	37.3	37.4	37.4	38.1	48.5
C-11	20.6	20.9	20.6	20.9	20.9	20.8	115.6	121.2
C-12	23.4	24.1	23.9	23.4	24.1	22.2	36.8	36.6
C-13	49.0	48.9	48.1	47.8	48.2	41.8	36.7	36.7
C-14	42.2[a]	40.3[a]	42.2[a]	42.1[a]	42.0[a]	42.7[a]	37.7	38.0
C-15	33.1	33.4	32.2	32.1	32.4	32.0	29.3	29.4
C-16	22.1	22.2	21.4	21.6	21.6	20.5	36.2	36.0
C-17	50.3	53.9	51.0	50.2	54.2	51.4	43.0	42.9
C-18	45.6	44.8	46.4	45.7	45.0	50.0	52.0	51.9
C-19	83.8	41.3	82.3	83.3	40.1	215.5	20.2	20.1
C-20	33.1	22.9	35.5	32.8	25.5	38.7	28.2	28.2
C-21	50.5	53.6	51.1	51.3	53.8	48.5	59.7	59.6
C-22	209.4	210.7	211.7	210.7	212.8	209.7	30.8	30.8
C-23	33.4	33.4	33.4	33.4	33.4	33.4	32.8	31.4
C-24	21.6	21.6	21.6	21.6	21.6	21.6	21.7	19.9
C-25	15.8	15.9	15.8	15.8	15.9	15.8	25.1	176.2
C-26	16.6	16.7	16.6	16.6	16.7	16.6	15.8	15.6
C-27	16.8	16.6	16.9	16.9	16.7	17.1	15.4	15.5
C-28	9.3	14.6	9.6	10.7	14.9	11.0	14.0	14.0
C-29	31.0	30.9	30.6	30.0	30.2	31.1	22.1	22.1
C-30	—	—	—	—	—	—	23.0	23.0
OCH_3	—	—	—	21.3	—	—	—	51.1
CO	—	—	—	170.8	—	—	—	—

a) Assignments of the two signals might be reversed.

Sciences, Tokyo.

Extraction of Fresh Leaves of *Adiantum edgeworthii* and Separation The fresh leaves (68 g) were extracted three times with hexane (200 ml each). The extract was evaporated and the residue (1.1 g) was refluxed with benzene for 1 h. After three days, the insoluble material was filtered off (fraction A), and the solution was evaporated to dryness. The residue was chromatographed on silica gel with hexane (fr. B), hexane–benzene (9 : 1) (fr. C), benzene (fr. D, E, F, G), benzene–Et_2O (9 : 1) (fr. H) and Et_2O (fr. I) to give nine fractions.

Fern-9(11)-ene (3), Fern-7-ene (4), Neohop-12-ene (5), Filic-3-ene (6), and Hop-22(29)-ene (7) Fraction B was chromatographed on 20% $AgNO_3$-impregnated silica gel to give the following products in order of elution (recrystallized from acetone to obtain pure specimens): **3** (3 mg), mp 170—171 °C; **4** (0.5 mg), mp 212—214 °C; **5** (0.5 mg), mp 210—211 °C; **6** (0.5 mg), mp 232—234 °C and **7** (3.5 mg), mp 210—212 °C. These compounds were identified by comparison (GC, MS) with authentic samples.[1f]

Adiantone (8) Fraction E was chromatographed on silica gel, and then the crystalline product obtained from the benzene eluate was recrystallized from methanol to give **8** (1 mg), mp 227—230 °C, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1705. Compound **8** was identified by comparison (IR, TLC) with an authentic sample.[1f]

19α-Hydroxyadiantone (1) and Zeorin (10) a) Fraction I was recrystallized twice from acetone to give **1** (16 mg), mp 281—283 °C, $[\alpha]_D$ +56.8°, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3573, 1695, 1175, 1055. The filtrate was evaporated to dryness, and the crystalline residue was chromatographed on Al_2O_3. The crystalline product (28 mg) obtained from the benzene eluate was recrystallized from EtOH to give **10**, mp 222—224 °C, $[\alpha]_D$ +56.0°, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3350, 1150, 1038, 1020. Compound **10** was identified by comparison (IR, TLC) with an authentic sample.[2] b) Fraction A was refluxed with acetone. After cooling, the insoluble material was filtered off (273 mg) (fr. L), and the solution was evaporated to dryness (65 mg) (fr. M). Fraction L was chromatographed on silica gel, and the crystalline product (218 mg) obtained from the benzene–Et_2O (9 : 1) eluate was recrystallized from acetone to give **1**. Fraction M was chromatographed on Al_2O_3, and the crystalline product (39 mg) obtained from the benzene eluate was recrystallized from acetone to give **10**.

Fern-9(11)-en-25-oic Acid (2) and Hydroxyhopane (9) Fraction G was refluxed with hexane. After cooling, the insoluble material was filtered off (fr. J), and the solution was evaporated to dryness (fr. K). Fraction J was chromatographed on silica gel, and the crystalline product obtained from the benzene eluate was recrystallized from acetone to give **2** (5 mg), mp 289—291 °C, $[\alpha]_D$ −19.9°, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1675. Fraction K was chromatographed on Al_2O_3, and the crystalline product obtained from the benzene eluate was recrystallized from acetone to give **9** (3 mg), mp 253—255 °C, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3610, 3460, 1152. Compound **9** was identified by comparison (IR, TLC) with an authentic sample.[3]

19α-Hydroxyisoadiantone (11) **1** (80 mg) was adsorbed on Al_2O_3 in benzene. After standing overnight, elution with Et_2O afforded a crystalline solid, which was recrystallized from acetone to give **11**, mp 267—268 °C, $[\alpha]_D$ −5.64°, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3535, 1695, 1145, 1055.

19α-Acetoxyisoadiantone (11a) **11** (20 mg) was treated with Ac_2O–pyridine overnight at room temperature, and the product was chromatographed on silica gel. The crystalline product obtained from the benzene–Et_2O (9 : 1) eluate was recrystallized from MeOH to give **11a**, mp 183—184 °C, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1730, 1710.

Isoadiantane-19,22-dione (13) A solution of **11** (20 mg) in pyridine (5 ml) was combined with CrO_3 in pyridine (5 ml), and the mixture was stirred at room temperature for 3 h, then chromatographed on Al_2O_3. The crystalline product obtained from the benzene eluate was recrystallized twice from MeOH and hexane to give **13**, mp 264—265 °C, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1732, 1705. MS M^+ *m/z*: 426.3515 ($C_{29}H_{46}O_2$).

Reduction of 13 **13** (5 mg) was reduced by the Wolff–Kishner–Barton method. The product was chromatographed on Al_2O_3, and the crystalline product obtained from the hexane eluate was recrystallized from acetone to give a hydrocarbon (**14**), mp 180—182 °C, which was identified as isoadiantane.

Methyl Fern-(11)-en-25-oate (15) A solution of **2** (4 mg) in Et_2O was treated with CH_2N_2 and the Et_2O was evaporated off. The crystalline material was chromatographed on a dry column of silica gel. The crystalline product obtained from the hexane–EtOAc (9.5 : 0.5) eluate was recrystallized from acetone to give **15**, mp 212—214 °C.

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References and Notes

1) *a*) H. Ageta, K. Iwata, S. Natori, *Tetrahedron Lett.*, **1964**, 3413; *b*) H. Ageta, K. Iwata, Y. Arai, Y. Tsuda, K. Isobe, S. Fukushima, *ibid.*, **1966**, 5679; *c*) H. Ageta, K. Iwata, *ibid.*, **1966**, 6069; *d*) H. Ageta, K. Shiojima, Y. Arai, *Chem. Commun.*, **1968**, 1105; *e*) H. Ageta, K. Shiojima, R. Kamaya, K. Masuda, *Tetrahedron Lett.*, **1978**, 899; *f*) K. Shiojima, Y. Arai, T. Kasama, H. Ageta, *Chem. Pharm. Bull.*, **41**, 262 (1993); *g*) K. Shiojima, Y. Sasaki, H. Ageta, *ibid.*, **41**, 268 (1993).
2) H. Ageta, K. Masuda, Y. Tanaka, *Shoyakugaku Zasshi*, **35**, 259 (1981).
3) K. Shiojima, Y. Arai, H. Ageta, *Phytochemistry*, **29**, 1079 (1990).
4) H. Ageta, K. Shiojima, H. Suzuki, S. Nakamura, *Chem. Pharm. Bull.*, **41**, 1939 (1993).
5) K. Shiojima, Y. Arai, K. Masuda, Y. Takase, T. Ageta, H. Ageta, *Chem. Pharm. Bull.*, **40**, 1683 (1992).
6) R. Takahashi, H.-C. Chang, O. Tanaka, S. Shibata, *Tetrahedron Lett.*, **1972**, 2039.
7) We consider that this scientific name is not valid. *Adiantum monochlamys* was first applied to the Japanese specimens ("Hakone-shida" in Japanese) of Shizuoka Prefecture by Eaton. The results of our chemical studies on this Japanese fern have clearly indicated it contains quite different triterpenoids compared with those reported from the Indian species.
8) J. Banerjee, G. Datta, C. P. Dutta, T. Eguchi, Y. Fujimoto, K. Kakinuma, *Phytochemistry*, **30**, 3478 (1991).
9) H. Ageta, K. Shiojima, Y. Arai, H. Suzuki, T. Kiyotani, *Chem. Pharm. Bull.*, **42**, 39 (1994).