Fern Constituents: Two New Triterpenoid Hydrocarbons, Hop-16-ene and Isohop-22(29)-ene, Isolated from *Davallia mariesii*

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Two new triterpenoid hydrocarbons were isolated from the rhizomes of *Davallia mariesii* MOORE, together with neohop-13(18)-ene, hop-21-ene, hop-17(21)-ene, hydroxyhopane, cyclolaudenol and cyclobalanol. On the other hand, fern-9(11)-ene, ferna-7,9(11)-diene, fern-7-ene, hop-22(29)-ene, hydroxyhopane and dryocrassol were isolated from the leaves of the same fern. The structures of two new compounds, hop-16-ene (1) and isohop-22(29)-ene (2), were elucidated on the basis of spectral data and chemical correlations with known compounds.

Keywords fern; *Davallia mariesii*; triterpenoid; hydrocarbon; hopane; isohopane; migrated hopane; hop-16-ene; isohop-22(29)-ene

From the rhizomes of formosan ferns of genus *Davallia* (Davalliaceae), many kinds of triterpenoids belonging to the hopane and migrated hopane series have been isolated and their structures elucidated.[1] We have now investigated the chemical constituents of the only species in Japan, *Davallia mariesii* MOORE (shinobu in Japanese). This fern is a beautiful summer green species and has been used as an ornamental plant for a long time in Japan. The dried rhizomes of this fern were employed as one kind of the drug "Gu-sui-bu" in China.[2]

Results and Discussion

The dried rhizomes and the leaves of *D. mariesii* were extracted with methanol or *n*-hexane. The methanol extract of the rhizomes was separated by chromatography (see Experimental) to give two new triterpenoid hydrocarbons, namely hop-16-ene (**1**) and isohop-22(29)-ene (21αH-hop-22(29)-ene, **2**), together with several known compounds: neohop-13(18)-ene (**3**), hop-21-ene (**4**), hop-17(21)-ene (**5**) and hydroxyhopane (**6**). Cyclolaudenol (24-methylcycloart-25-en-3β-ol, **7**) and cyclobalanol (24,24-dimethylcycloart-25-en-3β-ol, **8**) were also isolated as their acetates. On the other hand, the *n*-hexane extract of the leaves was separated by chromatography to give several known compounds: fern-9(11)-ene (**9**), ferna-7,9(11)-diene (**10**), fern-7-ene (**11**), hop-22(29)-ene (**12**), **6** and dryocrassol (**13**). All triterpenoids isolated are listed in Table I together with their physical constants and yields.

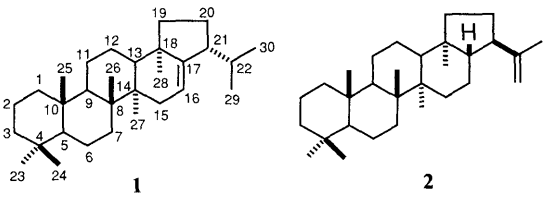

Chart 1

TABLE I. Triterpenoids Isolated from Rhizomes and Leaves of *Davallia mariesii*

	mp (°C)	$[\alpha]_D^{23}$	Yield (%) Rhizomes	Leaves
Hop-22(29)-ene (**12**)	211—212	+60.2	—	0.0003
Isohop-22(29)-ene (**2**)	212—214	+27.1	0.0024	—
Hop-21-ene (**4**)	194—195	+29.8	0.0002	—
Hop-17(21)-ene (**5**)	188—189	+50.0	0.0004	—
Hop-16-ene (**1**)	181—184	+26.8	0.0004	—
Neohop-13(18)-ene (**3**)	200—201	+2.9	0.0005	—
Fern-7-ene (**11**)	212—214	−27.8	—	0.0002
Fern-9(11)-ene (**9**)	170—171	−20.6	—	0.0364
Ferna-7,9(11)-diene (**10**)	201—202	−189.5	—	0.0002
Hydoxyhopane (**6**)	253—255	+44.0	0.0007	0.0003
Dryocrassol (**13**)	—	—	—	Trace
Cyclolaudenyl acetate (**7**)	127—128	+53.5	0.0060	—
Cyclobalanyl acetate (**8**)	176—179	+53.2	0.0049	—

The new compound, **1**, was obtained as colorless plates. The mass spectrum (MS) of **1** showed a molecular ion, *m/z* 410.3883 ($C_{30}H_{50}$) and many significant fragments at *m/z* (rel. int.): 395 (53; M^+-CH_3), 367 (11; $M^+-C_3H_7$), 221 (22; a), 204 (17; b), 203 (9; b'), 191 (100; c), 189 (47; d), 187 (15; e), 150 (22; f), 145 (34; g) and 107 (50; h) (Chart 2). The fragment ions of *m/z* 367, 191 and 189, and also *m/z* 187, 150 and 107 suggested the hopane skeleton and the Δ^{16} double bond for the structure of **1**,[3] respectively.

The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** indicated the presence of six tertiary and two secondary methyl groups, and a trisubstituted double bond (Table II). The methyl protons of H-27 (δ 1.041) and H-28 (δ 0.897) of **1** were observed at lower fields than those of hopane (**14**), and the proton of the trisubstituted double bond appeared at δ 5.202 (dd, J=2.5, 3.6). The ^{13}C-NMR signals of **1** were assigned by the off-resonance decoupling technique as well as by comparison of the signals with those of various compounds of the hopane group. The chemical shift values of the carbons of **1** were similar to those of the corresponding carbons of **14**, except for the olefinic carbons and neighbors (Table III). To confirm the carbon skeleton,

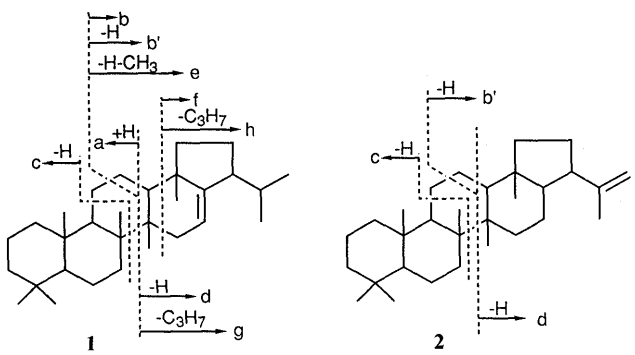

Chart 2

TABLE II. ^{1}H-NMR Spectral Data for Triterpenoids of Hopane and Isohopane Groups (100/270 MHz, $CDCl_3$, δ)

	1	2	4	5	12	14	15
H-23	0.857	0.845	0.850	0.845	0.845	0.845	0.848
H-24	0.801	0.794	0.796	0.794	0.794	0.791	0.794
H-25	0.840	0.821	0.818	0.835	0.818	0.817	0.821
H-26	0.924	0.948	0.970	0.938	0.948	0.951	0.948
H-27	1.041	0.978	0.970	1.044	0.963	0.951	0.978
H-28	0.897	0.683 (d, 1.0)	0.587 (d, 1.0)	0.845	0.727	0.704 (d, 1.0)	0.649 (d, 1.0)
H-29	0.866[a] (d, 6.5)	4.674 (2H, br s)	1.571[a]	0.917[a] (d, 6.9)	4.777 (2H, br s)	0.788[a] (d, 6.6)	0.787[a] (d, 6.6)
H-30	0.932[a] (d, 6.5)	1.671 (d, 1.0)	1.728[a]	0.978[a] (d, 6.9)	1.750	0.866[a] (d, 6.6)	0.888[a] (d, 6.6)
H-16	5.202 (1H, dd, 2.3, 3.6)						

Signals, unless otherwise stated, are 3H, singlet. br s, broad singlet; d, doublet; dd, double doublet. Coupling constants (J) are also shown in parentheses. *a*) Signals assigned to H-29 and H-30 may be interchanged.

TABLE III. ^{13}C-NMR Spectral Data for Triterpenoids of Hopane and Isohopane Groups (68 MHz, $CDCl_3$, δ)

	1	2	4	5	12	14	15
C-1	40.3	40.4	40.4	40.4	40.4	40.4	40.4
C-2	18.7	18.8	18.7	18.7	18.7	18.8	18.8
C-3	42.1	42.2	42.2	42.1	42.2	42.2	42.2
C-4	33.2	33.3	33.3	33.2	33.3	33.3	33.3
C-5	56.1	56.2	56.2	56.2	56.2	56.2	56.2
C-6	18.5	18.7	18.8	18.7	18.7	18.8	18.8
C-7	33.5	33.4	33.3	33.3	33.3	33.4	33.4
C-8	41.4	42.3	41.9	41.8	41.9	41.8	42.0
C-9	50.6	50.5	50.5	50.9	50.4	50.6	50.5
C-10	37.4	37.5	37.4	37.4	37.4	37.5	37.5
C-11	20.9	21.0	21.0	21.2	21.0	21.1	21.0
C-12	24.2	24.0	23.8	24.0	24.0	24.1	22.8
C-13	44.8	48.7	48.1	49.2	49.5	49.4	48.6
C-14	40.5	42.0	41.5	41.9	42.1	41.7	42.4
C-15	34.0	32.7	32.9	31.8	33.7	33.8	32.8
C-16	119.4	20.9	23.3	19.8	21.7	22.7	21.6
C-17	147.6	53.9	56.0	135.7	54.9	54.7	53.3
C-18	43.6	44.3	44.4	49.7	44.8	44.4	44.5
C-19	43.2	40.2	39.1	41.6	41.9	41.9	39.9
C-20	27.0	27.4	28.4	27.4	27.4	27.6	23.9
C-21	51.3	48.0	135.6	139.8	46.5	48.0	45.5
C-22	35.0	148.2	120.6	26.3	148.7	32.0	28.9
C-23	33.4	33.4	33.4	33.3	33.4	33.4	33.4
C-24	21.6	21.6	21.6	21.6	21.6	21.6	21.6
C-25	16.0	15.9	15.9	16.2	15.9	15.9	15.9
C-26	16.9	16.7	16.7	16.3	16.7	16.6	16.8
C-27	18.0	16.8	16.6	14.9	16.8	16.6	16.8
C-28	19.3	15.1	14.7	19.1	16.1	15.9	15.2
C-29	21.5	109.4	19.4	21.2	110.7	22.8	17.5
C-30	22.0	19.7	22.8	21.9	25.0	23.9	22.1

1 was treated with sulfuric acid in acetic acid–benzene to give a single crystalline product, which was proved to be identical with **5**. On the basis of the above spectral data and the result of acid treatment, the structure of **1** was established as hop-16-ene, a new member of the hopenes (Chart 1).

The new compound, **2**, was obtained as colorless needles. The MS of **2** exhibited the molecular ion at m/z 410.3912 ($C_{30}H_{50}$) and some significant fragments at m/z (rel. int.): 395 (10; $M^+ - CH_3$), 203 (12; i), 191 (100; c), 189 (78; d). These fragment ions suggested the hopane skeleton for **2**. The ^{1}H-NMR spectrum of **2** indicated the presence of seven tertiary methyl groups and a terminal methylene group (Table II). The signals of methyl groups, H-28 (δ 0.683) and H-30 (δ 1.671), of **2** were observed at higher fields than those of **12**, and the protons of the terminal methylene group appeared at δ 4.674 as a broad singlet. As shown in Table III, the ^{13}C-NMR spectrum of **2** revealed that the carbon signals were very similar to those of **12** and isohopane (21αH-hopane, **15**) except for the terminal methylene carbon signals. Compound **2** was found to be identical with the specimen of isohop-22(29)-ene derived from isoadiantone (**16**).[4]

Hop-16-ene (**1**) and isohop-22(29)-ene (**2**) are monoene hydrocarbons of the hopane series triterpenoids, joining the fourteen known compounds.[5-7] The occurrence of gammacer-16-en-3β-yl acetate, a pentacyclic triterpenoid having a Δ^{16} double bond, in a composite plant, *Picris hieracioides* LINNÉ subsp. *japonica* (THUNB.) KRYLOV., has been reported from our laboratory.[3] On the other hand, the discovery of **2** as a natural product is very important because this compound is probably a direct precursor of so-called migrated hopenes. The components of the rhizomes and the leaves of *Davallia mariesii* were very different from each other in the composition of the triterpenoid hydrocarbons. Besides some hopane triterpenoids, isohop-22(29)-ene (**2**), which is the starting material of migrated derivatives, was obtained from the rhizomes as a principal component, while the migrated hopane, fern-9(11)-ene (**9**), which is widely distributed among ferns, was obtained from the leaves in a considerable amount. This finding is very interesting from the biogenetic point of view.

Although only a few species of *Davallia* have been examined for triterpenoid constituents, each species contains quite specific components of the hopane series. The rhizomes of *Davallia divalicata* BLUME contains davallic acid[1a] and 24-norferna-4(23),9(11)-diene,[1b] and the rhizomes of *D. solida* SW. ferna-9(11),18-diene, ferna-7,18-diene, filica-3,18-diene, filica-3,18,20-triene, 23-hydroxyfern-9(11)-ene, 19α-hydroxyfern-9(11)-ene, 19α-hydroxyfern-7-ene and 19α-hydroxyfilic-3-ene.[1b] Not all of these compounds could be isolated from the rhizomes of *D. mariesii*. The results suggest that species of the genus *Davallia* differentiated a long time ago, and the components of each species have varied independently. This situation is quite different from that of many species of ferns belonging to Aspidiaceae, the triterpenoid constituents of whose species in a given and related genus are very similar.

Experimental

Melting points were measured with a Yanagimoto microapparatus and were corrected. Specific rotations were observed in $CHCl_3$ solutions (c = 0.4—1.1) at 22—24 °C. ^{1}H- and ^{13}C-NMR spectra were taken at 100 or 270 MHz and 68 MHz, respectively, by the FT method with tetramethylsilane as an internal standard. MS were recorded (direct inlet) at 70 eV and the 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 AW DMCS 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), silica gel for dry-column (Woelm) and Al_2O_3 (Woelm, neutral or basic, grade 1 or 3) 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 conc.

H_2SO_4 followed by heating.

Plant Materials The leaves and rhizomes of *Davallia mariesii* were collected in August at Tomisawa-cho, Yamanashi Prefecture. A voucher specimen has been deposited in the Herbarium of Showa College of Pharmaceutical Sciences, Tokyo.

Extraction of Dried Rhizomes The dried rhizomes (4.8 kg) was extracted five times with methanol (25 l each) and the extract was concentrated to 10 l in total. This was allowed to stand overnight, a waxy substance was filtered off, and the solution was evaporated to dryness. The resultant solid was extracted with Et_2O and water, and the ether solution was dried and evaporated to give a residue (48 g). The residue was subjected to CC on silica gel with *n*-hexane (fraction A), benzene (B), benzene–Et_2O (1 : 1) (C) and methanol (D) to give four fractions.

Neohop-13(18)-ene (3), Hop-16-ene (1), Hop-21-ene (4), Hop-17(21)-ene (5) and Isohop-22(29)-ene (2) Fraction A was chromatographed repeatedly on Al_2O_3 and 20% $AgNO_3$ impregnated silica gel with *n*-hexane to give the following crystalline solids (weight) in order of elution, and these were recrystallized from acetone giving pure **3** (25 mg). **1** (20 mg), **4** (11 mg), **5** (18 mg), and **2** (115 mg). Infrared (IR) ν_{max}^{KBr} cm^{-1}: 793 (**1**); 3049, 1643, 882 (**2**). Compounds **3**, **4** and **5** were identified by comparison (GLC, IR) with the authentic samples.[6]

Hydroxyhopane (6) Fraction C was chromatographed on silica gel with *n*-hexane–EtOAc (9.5 : 0.5) to give two fractions (E, F). Fraction E was chromatographed three times on Al_2O_3. Elution with *n*-hexane–benzene (1 : 1) afforded a crystalline solid (33 mg), which was recrystallized from acetone to give **6**, ν_{max}^{KBr} cm^{-1}: 3440, 1150. Compound **6** was identified by comparison (IR, TLC) with the authentic sample.[8]

Cyclolaudenyl Acetate (7) and Cyclobalanyl Acetate (8) Fraction F was treated with pyridine–Ac_2O overnight at room temperature. The reaction product was chromatographed on Silica gel. Elution with *n*-hexane–EtOAc (9.5 : 0.5) afforded a crystalline solid (470 mg), which was proved to be a mixture of **7** (52%) and **8** (48%) by GLC. The mixture was separated by preparative GLC followed by recrystallization from methanol to give **7**, Rt_R 4.55, ν_{max}^{KBr} cm^{-1}: 1732, 1250, 1645, 885 and **8**, Rt_R 5.70, ν_{max}^{KBr} cm^{-1}: 1735, 1245, 1640, 887. Compounds **7** and **8** were identified by comparison (GLC, ^{1}H-NMR) with the authentic samples.[9]

Extraction of Dried Leaves The dried leaves (1.1 kg) was extracted three times with *n*-hexane (25 l each). The extract was evaporated and the residue was refluxed with methanol (5 l) for 2 h. After standing overnight a waxy substance was filtered off, and the solution was evaporated to dryness. Th residue was chromatographed on silica gel with *n*-hexane (fraction G), *n*-hexane–benzene (1 : 1) (H), benzene (I), benzene–Et_2O (9 : 1) (J), and Et_2O (K) to give five fractions.

Fern-9(11)-ene (9), Ferna-7,9(11)-diene (10), Fern-7-ene (11) and Hop-22(29)-ene (12) Fraction G was chromatographed repeatedly on Al_2O_3 and 20% $AgNO_3$ impregnated silica gel to give the following crystalline solids (weight) in order of elution (recrystallized from acetone to obtain pure specimens): **9** (400 mg). **10** (2 mg). **11** (2 mg). **12** (3 mg). Compounds **9**, **10**, **11**, and **12** were identified by comparison (GLC and IR) with the authentic samples.[6]

Hydroxyhopane (6) and Dryocrassol (13) Fractions I and J were chromatographed several times on Al_2O_3. Elution with *n*-hexane–benzene (1 : 1) afforded a crystalline solid (4 mg), which was recrystallized from acetone to give **6**, ν_{max}^{KBr} cm^{-1}: 3440, 1150. Compound **6** was identified by comparison (IR, TLC) with the authentic sample.[8] The product subsequently eluted with benzene was identified as **13** by comparison (TLC) with the authentic sample.[10]

Hydroxyisohopane (17) Compound **16** (500 mg) in absolute benzene (20 ml) and ether (15 ml) was added to the Grignard reagent derived from Mg (1.0 g) and methyl iodide (4 ml) in absolute Et_2O (40 ml) under N_2 gas. The mixture was refluxed for 5 h, and excess regents were resolved with methanol. Acidification followed by extraction with Et_2O gave a product which was chromatographed on Al_2O_3. Elution with *n*-hexane–benzene (1 : 1) afforded a crystalline solid (470 mg), which was recrystallized from *n*-hexane to give **17**, mp 225—228 °C, ν_{max}^{KBr} cm^{-1}: 3480, 1135. ^{1}H-NMR δ: 0.847 (H-23), 0.793 (H-24), 0.819 (H-25), 0.977 (H-26), 0.947 (H-27), 0.629 (d, J = 1.0 Hz, H-28), 1.183, 1.188 (H-29, 30). MS *m/z* (rel. int.): 428 (M^+; 1), 410 (23), 395 (18), 367 (13), 221 (14), 203 (14), 191 (100), 189 (80), 175 (12), 161 (18).

Hop-21-ene (4) and Isohop-22(29)-ene (2) Compound **17** (100 mg) in Ac_2O (20 ml) and anhydrous K_2CO_3 (2.0 g) was refluxed for 1 h. After cooling, the reaction mixture was added to water and the whole was extracted with *n*-hexane–Et_2O. The organic solution was washed with water, dried, and evaporated to give a yellow crystalline solid, which was chromatographed on Al_2O_3 and 20% $AgNO_3$ impregnated silica gel with *n*-hexane. The first fraction (3 mg) was recrystallized from acetone to give **4**, mp 193—195 °C, and the second fraction (78 mg) was recrystallized from acetone to give **2**, mp 212—214 °C.

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References and Notes

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