Fern Constituent: A New Triterpenoid Hydrocarbon, Trisnorhopane, Isolated from the Leaves of *Dryopteris crassirhizoma* and *Gleichenia japonica*

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From the leaflets of *Dryopteris crassirhizoma*, a new triterpenoid hydrocarbon, trisnorhopane (1) was isolated together with fern-7-ene (2), neohop-12-ene (3), fern-9(11)-ene (4) and hop-22(29)-ene (5). Compound 1 was also isolated from the leaflets of *Gleichenia japonica*, together with 4, 5 and neohop-13(18)-ene (6). The structure of 1 was elucidated on the basis of spectral data.

Keywords fern; *Dryopteris crassirhizoma*; *Gleichenia japonica*; triterpenoid; trisnorhopane

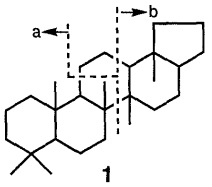
Further investigation of minor triterpenoid hydrocarbons of the fresh leaflets of *Dryopteris crassirhizoma* NAKAI ("oshida" in Japanese) resulted in the isolation of trisnorhopane (**1**), fern-7-ene (**2**), neohop-12-ene (**3**), fern-9(11)-ene (**4**) and hop-22(29)-ene (**5**). We have already reported **4**, **5**, neohop-13(18)-ene (**6**), dryocrassyl acetate (**7**), fern-9(11)-en-12-one (**8**), isoadiantone (**9**), 17α*H*-trisnorhopanone (**10**), hydroxyhopane (**11**) and dryocrassol (**12**) from this fern.[1] On the other hand, **1**, **4**, **5** and **6** were also isolated from the fresh leaflets of *Gleichenia japonica* SPR. ("urajiro" in Japanese), in addition to a reported compound **11**.[2] The known compounds **2**, **3**, **4**, **5** and **6** were identified by direct comparison with authentic samples.[3]

The new compound **1** was obtained as colorless plates. The MS of **1** showed a molecular ion, m/z 370.3575 ($C_{27}H_{46}$) and some significant fragments at m/z (relative intensity): 355 (15), 191 (83, a), 149 (100, b) (Chart 1). The fragment ions suggested that **1** was a hopane derivative lacking the side chain. The ^{1}H-NMR spectrum of **1** supported this view, because it showed the presence of six tertiary methyl groups on the nucleus; the chemical shifts of H-23, 24, 25, 26 and 27 were similar to those of hopane (**13**), while the H-28 signal appeared at higher field.[4] To establish the stereochemistry of the DE rings without ambiguity, the ^{1}H- and ^{13}C-NMR spectra of **1** were compared with those of trisnorhopan-21-one (**14**),[1] isomeric trisnorhopan-21-one (**10**)[1] and isomeric trisnorhopane (**15**) derived from **10**. Assignments of the signals were confirmed by modern NMR techniques[4] and are shown in Tables I and II. The signals of H-17, H-28 and C-28 of **15** and **10** are found at remarkably lower field than those of **1** and **14**, indicating that the former have DE-*cis* (17α*H*) and the latter have DE-*trans* (17β*H*). The fact that steric energies[5] of **15** (78.289 kcal/mol) and **10** (76.000 kcal/mol) are lower than those of **1** (81.120 kcal/mol) and **14** (80.940 kcal/mol), respectively, also supports this conclusion. Thus, compounds **1** and **14** were confirmed to be trisnorhopane and trisnorhopan-21-one having 17β*H* structure, and **15** and **10** to be 17α*H*-trisnorhopane and 17α*H*-trisnorhopan-21-one, respectively (Chart 2).

This is the first time that **1** has been found in living organisms, and is of interest because **1** is supposed to be derived from hop-21-ene by reductive degradation. On the other hand, compound **15**, isolated from Baltic sea sediment by Ourisson *et al.*,[6] might be derived from the stable ketone, **10**.

Experimental

Melting points were measured on a Yanagimoto micro apparatus



1

Chart 1

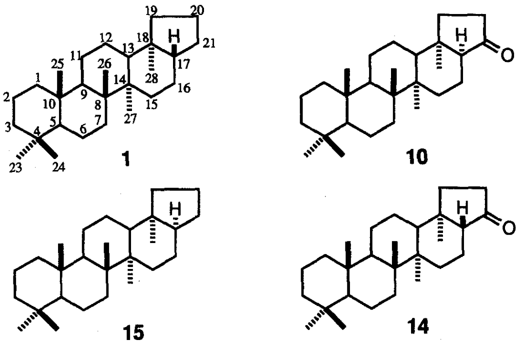


Chart 2

TABLE I. ^{1}H-NMR Spectral Data (500 MHz, $CDCl_3$, δ)

	1	14	10	15
H-23	0.848	0.855	0.838	0.846
H-24	0.794	0.799	0.781	0.793
H-25	0.818	0.829	0.803	0.823
H-26	0.965	0.981	0.838	0.965
H-27	0.951	0.975	1.024	0.992
H-28	0.599	0.716	1.153	0.929
H-17 (1H)	1.00	1.71	1.86	1.57
H-20 (2H)	1.58; 1.56	2.16; 2.22	2.23; 2.23	1.26; 1.58
H-21 (2H)	1.35; 1.21	—	—	1.36; 1.25

Signals, unless otherwise stated, are 3H, singlet. 2H signals are shown as α-H; β-H.

TABLE II. ^{13}C-NMR Spectral Data (125 MHz, $CDCl_3$, δ)

	1	**14**	**10**	**15**
C-1	40.33	40.31	40.35	40.33
C-2	18.71	18.66	18.63	18.71
C-3	42.12	42.05	42.08	42.14
C-4	33.27	33.25	33.25	33.27
C-5	56.11	56.04	56.28	56.31
C-6	18.73	18.66	18.68	18.71
C-7	33.30	33.16	32.98	33.12
C-8	41.93[a)]	42.12[a)]	41.59[a)]	42.11[a)]
C-9	50.40	50.31	50.81	50.87
C-10	37.42	37.40	37.45	37.47
C-11	20.95	20.60	21.34	21.84
C-12	24.14	22.56	23.53	23.51
C-13	48.24	49.03	38.17	37.90
C-14	42.22[a)]	42.62[a)]	40.89[a)]	41.47[a)]
C-15	32.66	31.60	26.90	25.36
C-16	22.65	16.76	17.53	21.61
C-17	51.03	61.91	57.48	46.22
C-18	43.59	41.65	41.98	43.16
C-19	40.75	36.42	34.62	42.70
C-20	20.23	34.56	35.65	28.78
C-21	27.60	217.07	221.46	29.70
C-23	33.43	33.40	33.36	33.40
C-24	21.61	21.58	21.54	21.58
C-25	15.85	15.80	16.16	16.12
C-26	16.73	16.71[b)]	16.00	16.27
C-27	16.73	16.74[b)]	15.29	15.95
C-28	14.01	14.61	24.42	23.78

a, b) Assignments of signals with the same superscript might be reversed.

without correction. Specific rotation was observed in $CHCl_3$ solution ($c=0.2$) 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 were 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 C-18 reverse-phase column (detected by refraction index) with MeOH–$CHCl_3$ (8 : 2) as the eluent. Silica gel 60, 230—400 mesh (Merck), and 20% $AgNO_3$-impregnated silica gel were used for column chromatography (CC).

Plant Material The leaflets of *Dryopteris crassirhizoma* were collected in September, 1989, at Mt. Nyūgasa, Fujimi, Nagano Prefecture, and those of *Gleichenia japonica* in December, 1986, at Miyagahara, Nishiizu, Shizuoka Prefecture, Japan. Voucher specimens have been deposited in the Herbarium of Shōwa College of Pharmaceutical Sciences, Tokyo.

Extraction of Fresh Leaflets of *D. crassirhizoma* and Separation The fresh leaflets (4.9 kg) were extracted twice with *n*-hexane. The extract was evaporated and the residue (30 g) was chromatographed on silica gel with *n*-hexane (fr. A), *n*-hexane–benzene (8 : 2) (frs. B and C), *n*-hexane–benzene (7 : 3) (frs. D, E and F), *n*-hexane–benzene (1 : 1) (frs. G and H), benzene (fr. I) and benzene–ether (9 : 1) (fr. J) to give ten fractions.

Trisnorhopane (1), Fern-7-ene (2), Neohop-12-ene (3), Fern-9(11)-ene (4) and Hop-22(29)-ene (5) Fraction A was chromatographed on 20% $AgNO_3$-impregnated silica gel followed by HPLC to give the following crystalline solids (weight) in order of elution (recrystallized from acetone to obtain pure specimens): **1**: 4 mg, mp 161—163 °C, $[\alpha]_D$ +35.5°; **4**: 306 mg, mp 171—172 °C; **2**: 3 mg, mp 211—213 °C; **3**: 1 mg, mp 209—211 °C; **5**: 66 mg, mp 211—212 °C. **2**, **3**, **4** and **5** were identical (mp, ^{1}H-NMR) with authentic samples.[3,4)]

Extraction of Fresh Leaflets of *G. japonica* and Separation The fresh leaflets (1.6 kg) were extracted twice with *n*-hexane. The extract was evaporated and the residue (13 g) was chromatographed on silica gel with *n*-hexane (fr. A), *n*-hexane–benzene (8 : 2) (fr. B), *n*-hexane–benzene (7 : 3) (frs. C, D and E), *n*-hexane–benzene (1 : 1) (frs. F and G), benzene (frs. H and I) and benzene–ether (9 : 1) (fr. J) to give ten fractions.

Trisnorhopane (1), Fern-9(11)-ene (4), Hop-22(29)-ene (5) and Neohop-13(18)-ene (6) Fraction A was chromatographed on 20% $AgNO_3$-impregnated silica gel followed by HPLC to give the following crystalline solids (weight) in order of elution (recrystallized from acetone to obtain pure specimens): **1**, 2 mg; **6**, 3 mg, 198—200 °C; **4**, 1 mg; **5**, 32 mg. **4**, **5** and **6** were identical (mp, ^{1}H-NMR) with authentic samples.[3,4)]

Reduction of 17α*H*-Trisnorhopanone (9) Compound **9** (25 mg) was reduced by the Wolff–Kishner–Barton method. The product was chromatographed on Al_2O_3, and the crystalline product (21 mg) obtained from the *n*-hexane eluate was recrystallized from acetone to give 17α*H*-trisnorhopane (**14**), mp 218—220 °C.

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References and Notes

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