Fern Constituents: Dryocrassy Formate, Sitostanyl Formate and 12α-Hydroxyfern-9(11)-ene from *Cyathea podophylla*

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Dryocrassyl formate, sitostanyl formate, and 12α-hydroxyfern-9(11)-ene were isolated from the fresh fronds of *Cyathea podophylla*. Their structures were elucidated by spectroscopic techniques and synthesis. Ten known triterpenoids, three derivatives of phytol, a stanol, and β-tocopherol were also identified from this fern.

Key words *Cyathea podophylla*; Cyatheaceae; triterpenoid; dryocrassyl formate; sitastanyl formate; 12α-hydroxyfern-9(11)-ene

In the course of chemotaxonomic studies of Cyatheaceae ferns, we have reported many kind of triterpenoids belonging to hopane and migrated hopane skeletons.[1,2] In a continuation of this study, we have now investigated *Cyathea podophylla*. (Cultivated), resulting in the isolation of three new triterpenoids, characterized as dryocrassyl formate (**1**), sitostanyl formate (**2**) and 12α-hydroxyfern-9(11)-ene (**3**), along with ten known triterpenoids. We report herein the isolation of the constituents and structural elucidation of **1**—**3**.

The dried fronds of *Cyathea podophylla* (Kurohego in Japanese) were extracted with *n*-hexane and the extract was chromatographed through silica gel to give ten fractions. The individual fractions were subjected to rechromatography and preparative HPLC to obtain a total of nineteen compounds: new compounds **1**—**3**, and known compounds: trisnorhopane (**4**),[3] neohop-13(18)-ene (**5**),[4] fern-9(11)-ene (**6**),[4] ferna-7,9(11)-diene (**7**),[4] neohopa-11,13(18)-diene (**8**),[4] squalene (**9**),[5] 9α,11α-epoxyfernane (**10**),[6] 9β,11β-epoxyfernane (**11**),[6] friedelin (**12**),[7] hydroxyhopane (**13**),[3] and esters of phytol[8] (**14**—**16**), sitostanol (**17**),[2] β-tocopherol (**18**) and phytoic acid (3,7,11,15-tetramethyl-2-haxadecen-1-oic acid **19**).[8] These known triterpenoids were identified with the authentic samples by ^{1}H-NMR.

Compound **1**, mp 66—67 °C, $[\alpha]_D$ −21.3° (c=0.4, $CHCl_3$) was obtained as colorless plates, and its IR spectrum suggested the presence of an ester group in the molecule. Its molecular formula was found to be $C_{31}H_{52}O_2$ by high resolution electron impact MS (HR-EI-MS, M^+ at *m/z* 456.3999, Calcd 456.3967). The ^{1}H-NMR spectrum of **1** indicated the presence of six tertiary and one secondary methyl groups and a formoxyl proton (δ 8.077). The ^{1}H-NMR data of **1** were similar to those of dryocrassol (**20**)[4] except for the signals of Me-29 and Me-30. That a –O–COH group is attached to C-30 of a dryocrassyl skeleton was clearly revealed by connectivity between a proton of the formoxyl group and the carbon at C-30 (δ 68.88) in the heteronuclear multiple bond connectivity (HMBC) spectrum of **1**: *viz.* 4.181 (H-30) with δ $^1J_{C,H}$ and δ 8.077 (CHO) with 68.88 (C-30). From the above HMBC and nuclear Overhauser effects (NOEs) from nuclear Overhauser effect spectroscopy (NOESY) spectrum, the structure of **1** was proved to be dryocrassyl formate.

Compound **2**, $C_{30}H_{52}O_2$ (HR-EI-MS; M^+ at *m/z* 444.3946, Calcd 444.3967), mp 105—107 °C, $[\alpha]_D$ −9.9° (c=0.3, $CHCl_3$) showed an ester in the molecule in its IR spectrum. Its ^{1}H-NMR data displayed the signals for two tertiary and three secondary methyls, a methyl proton of an ethyl group, and a formoxyl proton (δ 8.028). The chemical shifts of the methyl groups of **2** resemble those of sitostanol (**17**)[2] except for H-19 (δ 0.827) and H-3 (δ 4.821 dddd). That a –O–COH group is attached to C-3 of a sitostanol was clearly elucidated by connectivity between a proton of the formoxyl group and the carbon at C-3 (δ 43.80) in the HMBC spectrum of **2**: *viz.* δ 4.821 (H-3) with 160.84 (CHO) and δ 8.028 (CHO) with 73.80 (C-3). Finally, the relative stereochemistry of **2** was deduced from its NOESY spectrum. Based on the above evidence, the structure of **2** can be represented as sitostanyl formate.

Compound **3**, mp 110—113 °C; ν_{max} cm^{-1}: 3356, 1019, 862; $C_{30}H_{48}O$ (HR-EI-MS; M^+ at *m/z* 424.3715, Calcd 424.3705) showed besides the signals for six tertiary and two secondary methyl groups, one proton at δ 3.817 of carbon bearing a hydroxyl group and one olefin proton at δ 5.188 (dd J=2.1, 2.1 Hz) in its ^{1}H-NMR spectrum (Table 1). Detailed analysis of the correlations observed in the HMBC spectrum of **3**: *viz.* δ 0.851 (Me-23) with δ $^1J_{C,H}$, 33.65 (C-4), 42.30 (C-3) and 44.60 (C-5); δ 0.891 (Me-24) with δ $^1J_{C,H}$, 32.75 (C-23), 33.65 (C-4), 42.30 (C-3) and 44.60 (C-5); δ 1.062 (Me-25) with δ $^1J_{C,H}$, 37.81 (C-10), 41.17 (C-1), 44.60 (C-5) and 154.02 (C-9); δ 0.807 (Me-26) with δ $^1J_{C,H}$, 29.45 (C-15) and 39.79 (C-8); δ 0.859 (Me-27) with δ $^1J_{C,H}$, 40.51 (C-14), 42.30 (C-13), 51.44 (C-18) and 75.96 (C-12); δ 0.763 (Me-28) with δ $^1J_{C,H}$, 35.91 (C-16), 43.58 (C-17),

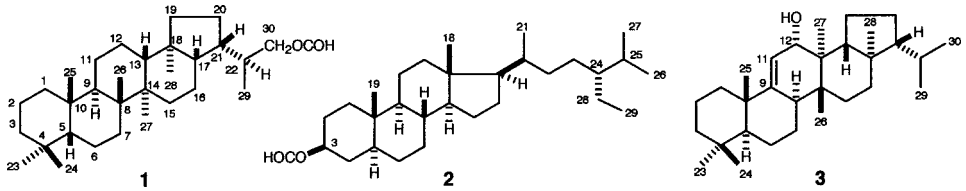

Chart 1

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Table 1. ^{1}H-NMR Chemical Shifts for **3** (500 MHz, $CDCl_3$, δ)

Proton	**3**	Proton	**3**
23	0.851 s	29	0.905 d
24	0.891 s		(6.4)
25	1.062 s	30	0.843 d
26	0.812 s		(6.4)
27	0.859 s	11	5.188 dd
28	0.763 s		(2.1, 2.1)
		12	3.817 bs

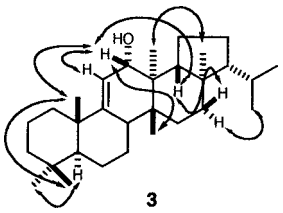
Multiplicity and coupling constant (J, Hz) are shown in parentheses.

Table 2. ^{13}C-NMR Chemical Shifts[a] (125 MHz, $CDCl_3$, δ)

Carbon	**1**	**2**	**3**
1	44.30	36.70	41.17
2	18.70	27.48	19.40
3	42.11	73.80	42.30
4	33.26	33.99	33.65
5	56.14	54.21	44.60
6	18.70	28.58	19.32
7	33.29	31.97	17.72
8	41.83	35.47	39.79
9	50.42	44.67	154.02
10	37.41	35.45	37.81
11	20.93	21.21	120.42
12	23.98	39.97	75.96
13	49.26	42.59	42.30
14	41.71	56.41	40.51
15	33.62	24.22	29.45
16	22.60	28.26	35.91
17	54.18	56.17	43.58
18	44.42	12.08	51.44
19	41.58	12.22	23.53
20	27.24	36.18	28.63
21	42.80	18.74	58.90
22	36.62	33.92	30.71
23	33.41	26.08	32.75
24	21.59	45.84	21.64
25	15.91	29.15	24.82
26	16.61	19.82	15.56
27	16.53	19.03	11.20
28	15.74	23.06	14.09
29	18.49	11.98	23.06
30	68.88		22.16
–COH	161.43	160.84	

a) Assigments were made on the basis of DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectra.

51.44 (C-18) and 58.90 (C-21); δ 0.905 (Me-29) with δ $^1J_{C,H}$, 22.16 (C-30), 30.71 (C-22) and 58.90 (C-21); δ 0.843 (Me-30) with δ $^1J_{C,H}$, 23.06 (C-29), 30.71 (C-22) and 58.90 (C-21); δ 5.188 (H-11) with 120.42 (C-11), 37.81 (C-10), 39.79 (C-8), 42.30 (C-13) and 154.02 (C-9); δ 3.817 (H-12) with δ $^1J_{C,H}$, 11.20 (C-27), 42.30 (C-13), 51.44 (C-18), 120.42 (C-11) and 154.02 (C-9) revealed the presence of the partial structure of 12-hydroxyfern-9(11)-ene. A structure suggestive of 12-hydroxyfern-9(11)-ene was isolated from *Adiantum capillus-veneris* as 12β-hydroxyfern-9(11)-ene[9] (**21**). However, the physical data including ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) of **3** differ from those of **21**. Finally, the structure of **3** was determined based on the NOE correlations on the NOESY spectrum (Fig. 1). NOEs between δ 0.891 (H_3-24β)–1.062 (H_3-25β)–3.817 (H-12)–0.807 (H_3-26β), 3.817 (H-12)–5.188 (H-11)–1.780 (H-18β)–1.40 (H-16β),



3

Fig. 1. NOEs Interactions Observed in the NOESY Spectrum of Compound **3**

0.859 (H_3-27α)–0.763 (H_3-28α), 1.65 (H-16α)–0.843 (H_3-29) and 0.905 (H_3-30)–1.21 (H-20α) revealed the hydroxyl group of **3** to be α (Fig. 1). Thus, the structure of **3** was concluded to be 12α-hydroxyfern-9(11)-ene. Additionally, its structure was confirmed by a derivative from fern-9(11)-en-12-one (**22**) with Na/*n*-PrOH.

Of these three new triterpenoids, **1** seems to be an interesting compound, since formate of triterpenoid is the second example from fern species.[10]

Especially, it was suggested that identification of **2** and **17** characterize Cyathea in fern because for **2** was isolated from other Cyathea[1,2] remarkably. 12α-Hydroxyfern-9(11)-ene (**3**) is interesting also from the point of detection of the most instable compound, which was reduced easily to fern-7,9(11)-diene.

Experimental

General Melting points: uncorr.; EI-MS: 30 eV; TLC: on precoated Kiesel gel 60. ^{1}H-NMR (270 MHz for identification and 500 MHz for structure determination) and ^{13}C-NMR (125 MHz) spectra: in $CDCl_3$ (TMS as int. standard); HPLC: reverse phase C_{18} column, RI detector, CH_3CN : $CHCl_3$ (9 : 1); CC: SiO_2 60 (230—400 mesh, Merck) and 20% $AgNO_3$-impregnated silica gel (Mallinkrodt); GC: 1.4% SE-30 on Chromosorb G, Oven: 260 °C (cholestane as int. standard).

Plant Material The material was collected on Ishigaki island in Okinawa prefecture. The voucher specimen has been deposited in the herbarium of Showa Pharmaceutical University, Tokyo.

Extraction and Separation The freshly cut fronds (2.56 kg) were extracted with *n*-C_6H_{14} three times by a modified Soxhlet extraction method to give an extract (6.4 g). The extract was chromatographed over silica gel to give ten fractions: Fr. 1 (205 mg, eluted with *n*-C_6H_{14}), Fr. 2 [106 mg, *n*-C_6H_{14}–C_6H_6 (9 : 1)], Fr. 3 [8 mg, *n*-C_6H_{14}–C_6H_6 (9 : 1)], Fr. 4 [33 mg, *n*-C_6H_{14}–C_6H_6 (7 : 3)], Fr. 5 [302 mg, *n*-C_6H_{14}–C_6H_6 (7 : 3)], Fr. 6 [168 mg, *n*-C_6H_{14}–C_6H_6 (1 : 1)], Fr. 7 [61 mg, *n*-C_6H_{14}–C_6H_6 (1 : 1)], Fr. 8 [70 mg, *n*-C_6H_{14}–C_6H_6 (1 : 1)], Fr. 9 (403 mg, C_6H_6), and Fr. 10 (3.6 g, Et_2O). Rechromatography of Fr. 1 and 2 with silica gel followed by recrystallization from Me_2CO gave five triterpenoid hydrocarbons: *viz.* **4** (2 mg, mp 161—163 °C),[3] **5** (3 mg, mp 210—212 °C),[4] **6** (40 mg, mp 170—171 °C),[4] **7** (2 mg, mp 200—202 °C),[4] **8** (15 mg, mp 214—215 °C),[4] and **9** (45 mg).[5] Fraction 3 was chromatographed on Al_2O_3 followed by preparative HPLC to give **10** (3 mg, mp 155—160 °C)[4] and **11** (3 mg, mp 254—256 °C).[4] Fraction 4 was recrystallized from MeOH for dewax, tha filtrate were followed by preparative HPLC to give **14** (5 mg), **15** (7 mg) and **16** (9 mg). Fractions 8 and 9 were chromatographed on Al_2O_3 to give **12** (4 mg, mp 267—269 °C)[7] and **13** (5 mg, mp 255—257 °C)[3] from Fr. 8, and **17** (12 mg),[2] **18** (14 mg) and **19** (14 mg)[8] from Fr. 9.

Dryacrassyl Formate (1) and Sitostanyl Formate (2) Fraction 5 was chromatographed over silica gel with C_6H_6 and then subjected to preparative HPLC followed by crystallization from Ac_2O to give **1** (3 mg) and **2** (6 mg). **1**, mp 67 °C. $[\alpha]_D$ +21.3° (c=0.3, $CHCl_3$). ν_{max} cm^{-1}; 2849, 1720. EI-MS *m/z* (rel. int.): 456 $[M]^+$ (25), 422 $[M-H_2O]^+$ (29), 267 (100), 235 (40), 199 (45), 183 (74). HR-EI-MS; M^+ *m/z*: 456.3999 (Calcd for $C_{31}H_{52}O_2$: 456.3967). ^{1}H-NMR δ: 0.846 (3H, s, H-23), 0.791 (3H, s, H-24), 0.815 (3H, s, H-25), 0.953 (3H, s, H-26), 0.953 (3H, s, H-27), 0.724 (3H, s, H-28), 1.039 (3H, d, J=5.8, H-29), 3.893 (1H, dd, J=10.6, 6.4, H-30), 4.184 (1H, dd, J=10.6, 6.1, H-30), 8.077 (1H, s, OCOH). ^{13}C-NMR: Table 2. **2**, mp 107 °C. $[\alpha]_D$ 9.9° (c=0.3, $CHCl_3$). ν_{max} cm^{-1}; 2866, 1637. EI-MS *m/z* (rel.

int.): 444 $[M]^+$ (100), 429 $[M-CH_3]^+$ (15), 398 $[M-COOH]^+$, 383 $[M-COOH-CH_3]^+$ (14), 261 (56), 251 (44). HR-EI-MS; M^+ *m/z*: 444.3946 (Calcd for $C_{30}H_{52}O_2$: 444.4586). ^{1}H-NMR δ: 0.650 (3H, s, H-18), 0.827 (3H, s, H-19), 0.905 (3H, d, *J*=6.4, H-21), 0.811 (3H, d, *J*=7.0, H-26), 0.833 (3H, d, *J*=6.4, H-27), 0.843 (3H, dd, *J*=7.9, 7.9, H-29), 8.028 (1H, s, OCOH), 4.821 (1H, dddd, *J*=11.0, 11.0, 5.1, 5.1, H-3). ^{13}C-NMR: Table 2.

12α-Hydroxyfern-9(11)-ene (3) Fraction 7 was recrystallized for dewax with MeOH and the filtrates were chromatographed on silica gel, and then subjected to preparative HPLC to give **3** (5 mg), mp 110—113 °C. ν_{max} cm^{-1}; 3474, 1015, 855. EI-MS *m/z* (rel. int.): 426 $[M]^+$ (100), 411 $[M-CH_3]^+$ (18), 408 $[M-H_2O]^+$ (61), 393 $[M-CH_3-H_2O]^+$ (63), 273 (30), 255 (89), 135 (22). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Synthesis of 3 Compound **22** (100 mg) derived from **6** with $CrO_3/H_2O/CH_3COOH$ in the usual manner was reduced with sodium (300 mg) in *n*-PrOH (50 ml) for 1 h. The reaction mixture was followed up in the usual manner to give **3** (20 mg) through SiO_2 CC and HPLC.

Acknowledgements We thank Mr. Y. Takase and Dr. H. Suzuki of the Central Analysis Center of our university for conducting the MS and NMR measurements.

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