Fern Constituents: Triterpenoids Isolated from Leaflets of *Cyathea lepifera*

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Received April 21, 1995; accepted July 14, 1995.

Four new minor triterpenoids, 9α,11α-epoxyfernane (1), 9β,11β-epoxyfernane (2), 3α,10α-epoxyfilic-4(23)-ene (3) and 20α,22-dihydroxyhopane (4) were isolated from fresh leaflets of *Cyathea lepifera*, together with known triterpenoids, fern-9(11)-ene (5), fern-7-ene (6), filic-3-ene (7), adiantone (8), glaucanone (9), fern-9(11)-en-12-one (10), hydroxyhopane (11), hopan-30-oic acid (12) and cycloartanoid esters (13). The structures of the new compounds were elucidated on the basis of spectral data.

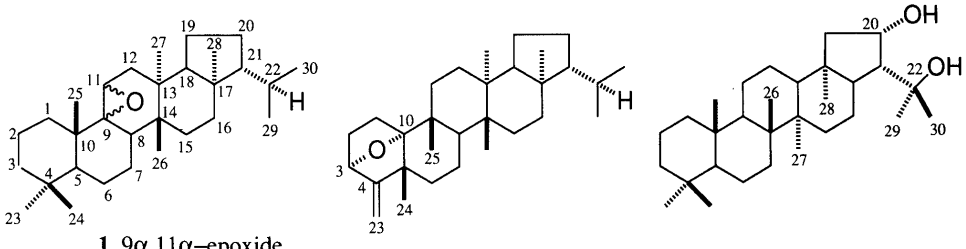
Key words *Cyathea lepifera*; triterpenoid; 9α,11α-epoxyfernane; 9β,11β-epoxyfernane; 3α,10α-epoxyfilic-4(23)-ene; 20α,22-dihydroxyhopane

Cyathea lepifera COPEL. (Cyatheaceae, "morihego" or "hikagehego" in Japanese) is a tall tree fern (up to 7 m high), that is commonly distributed in the subtropical or tropical areas of Japan, Taiwan and the Philippines. This paper describes triterpenoid constituents isolated from fresh leaflets of *C. lepifera*, in comparison with the components of *C. spinulosa*.[1] The hexane extract of the plant materials was separated by the same method as before[1] to give three new triterpenoid epoxides (**1**, **2**, **3**), and a new diol (**4**), together with the known compounds, fern-9(11)-ene (**5**),[2] fern-7-ene (**6**),[2] filic-3-ene (**7**),[2] adiantone (**8**),[3] glaucanone (**9**),[3] fern-9(11)-en-12-one (**10**),[3] hydroxyhopane (**11**),[4] hopan-30-oic acid (**12**),[5] cycloartanoid esters (**13**),[6] sitosteryl palmitate (**14**), α-tocopherol (**15**), a sitosterol mixture (**16**) and saturated fatty acids.

Results and Discussion

Compounds **1**, **2** and **3** were isolated from the same fraction of chromatography (see Experimental) by HPLC. Compounds **1**, mp 155—160 °C, and **2**, mp 254—256 °C, did not show characteristic absorptions in the IR spectra. The high-resolution mass spectra (HR-MS) of both compounds showed molecular ion peaks corresponding to the triterpenoid epoxide of $C_{30}H_{50}O$ (*m/z* 426.3908 for **1**, *m/z* 426.3842 for **2**). The EI-MS of **1** and **2** showed similar fragment ion peaks, *m/z* 365 ($M^+ - C_3H_7 - H_2O$), 301 (cleavage of B ring), 255 (cleavage of D ring) and 205 (cleavage of C ring), which indicated that **1** and **2** were triterpenoids having an epoxide ring on the B or C ring of the fernane skeleton (Fig. 1).[7] The ^{1}H-NMR spectra of **1** and **2** afforded signals of six tertiary and two secondary methyl protons, and one proton on carbon bearing an oxygen function (Table 1). The above data and consideration of the chemical shift of the proton on carbon bearing an oxygen function suggested **1** and **2** to be 9,11-epoxyfernane, which has been derived from fern-9(11)-ene by O_3 oxidation.[8] The H-11 proton signal appears at δ 2.982 (d, J=5.8) in **1**, and δ 3.056 (dd, J=1.9, 1.9) in **2**. By comparing these chemical shifts and splitting patterns with those of authentic samples,[8] the structures of **1** and **2** were established as 9α,11α-epoxyfernane and 9β,11β-epoxyfernane, respectively.

Compound **3** was obtained as colorless needles of mp 174—178 °C from the peak between those of **1** and **2**, and showed absorptions at 1664 and 875 cm^{-1} due to exocyclic methylene in the IR spectrum. The HR-MS of **3** exhibited the molecular ion peak at *m/z* 424.3719 ($C_{30}H_{48}O$). Characteristic fragment ions of **3** in the EI-MS were related to those of filic-4(23)-ene (**17**),[1] a triterpenoid with an exo-methylene moiety on the A ring isolated from *Polypodiodes formosana*. (Fig. 2). The fragment ions *m/z* 245 and 189 indicated the presence of an epoxy ring and an exo-methylene moiety on the A ring. The ^{1}H-NMR spectrum of **3** showed proton signals due to five tertiary and two secondary methyls, and an exocyclic methylene (Table 1). The ^{1}H-signals of two tertiary and two secondary methyls resembled those of H-26, H-28, H-29 and H-30 of **17**, while those of three other methyls differed from those of H-24, H-25 and H-27 of **17**. In addition, the protons of the exocyclic methylene were observed as two singlets in **3**, while these signals of



1 9α,11α-epoxide
2 9β,11β-epoxide

3

4

Chart 1

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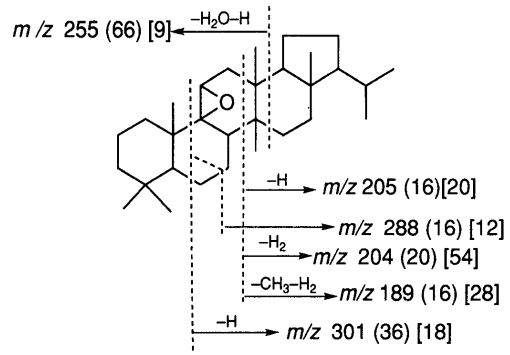

Fig. 1. Mass Fragments of **1** (α-Epoxide, Relative Intensities in Parentheses) and **2** (β-Epoxide, Relative Intensities in Square Brackets)

Table 1. Selected ^{1}H-NMR Spectral Data for **1**, **2**, **3** and **4**

Proton No.	1	2	3	4
H-23	0.907	0.892	4.675, 4.477	0.897
H-24	0.864	0.833	1.181	0.830
H-25	0.743	1.045	1.053	0.830
H-26	0.931	1.094	0.912	0.997
H-27	0.974	0.924	1.014	1.032
H-28	0.733	0.720	0.780	1.392
H-29	0.875	0.873	0.882	1.682
	(d, J=6.7 Hz)	(d, J=6.7 Hz)	(d, J=6.7 Hz)	
H-30	0.816	0.822	0.823	1.692
	(d, J=6.7 Hz)	(d, J=6.7 Hz)	(d, J=6.7 Hz)	
H-11	2.982	3.056		
	(1H, d, J=5.8 Hz)	(1H, dd, J=1.9, 1.9 Hz)		
H-20				4.995 (1H, m)
H-3			4.704 (1H, d, J=4.8 Hz)	

Signals are 3H, singlet unless otherwise indicated. Multiplicity and coupling constants are shown in parentheses.

Table 2. ^{13}C-NMR Spectral Data for **3** and **4**

Carbon No.	3	4	Carbon No.	3	4
C-1	23.56	40.45	C-16	35.66	22.24
C-2	32.40	18.98	C-17	42.72	54.67
C-3	80.48	42.26	C-18	51.77	44.39
C-4	165.05	33.39	C-19	19.93	52.17
C-5	47.50	56.31	C-20	29.70	75.76
C-6	39.42	18.98	C-21	60.15	55.80
C-7	18.67	33.43	C-22	30.78	73.85
C-8	42.45	42.16	C-23	98.10	33.56
C-9	38.41	50.64	C-24	22.02	21.80
C-10	90.61	37.57	C-25	21.72	16.02
C-11	28.40	21.22	C-26	16.22	16.96
C-12	28.12	24.59	C-27	15.32	17.27
C-13	38.79	50.29	C-28	16.25	16.88
C-14	40.12	40.45	C-29	21.95	34.23
C-15	29.36	34.47	C-30	29.91	31.27

17 were observed as a broad singlet. Thus, it was suggested that the epoxy ring affects the methyl groups at the α side and the exocyclic methylene. As cross peaks in the heteronuclear multiple bond correlation (HMBC) spectrum showed **3** to be a filicane derivative having the epoxide between C-3 and C-10 (Fig. 3), the exocyclic methylene must be at C-4(23), explaining the lower field shifts and

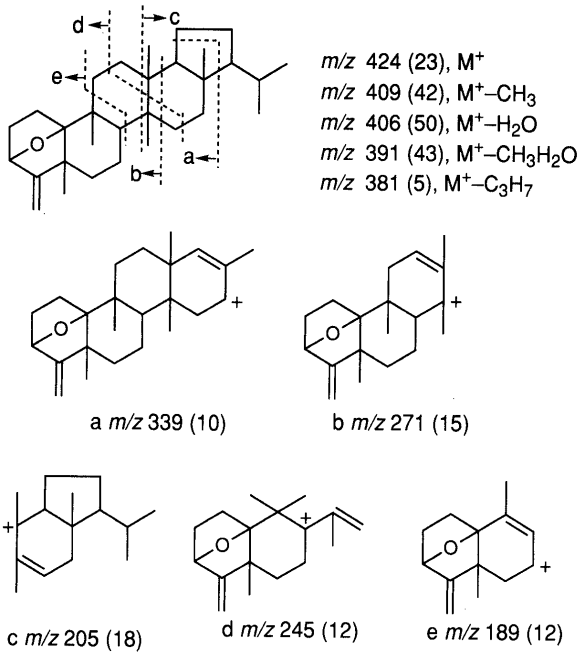

Fig. 2. Mass Fragment Ions of **3**

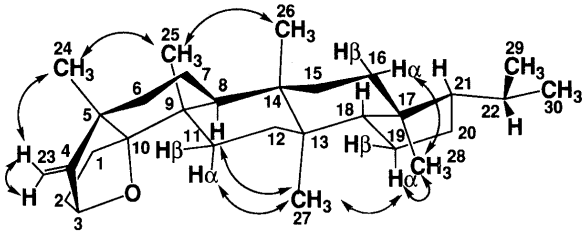

Fig. 3. NOEs of **3**

splitting pattern of the exocyclic methylene protons. The configuration of the epoxy ring was determined from the correlated spectroscopy (COSY) (^{1}H–^{1}H, ^{1}H–^{13}C) spectrum and nuclear Overhauser effects (NOEs) in the nuclear Overhauser enhancement spectroscopy (NOESY) spectrum. As the signals of H-8α (δ 2.08), H-11α (δ 2.16), and H-6α (δ 1.65) were observed at lower field, the epoxy ring must be on the same side (α). The NOEs between H-23β (δ 4.477) and H-24, H-24 and H-25, H-25 and H-26; H-8α and H-27; and H-11α and H-27, firmly supported the epoxide linkage between C-3α and C-10α. The ^{13}C-chemical shifts at rings C, D and E, including the side chain (Table 2) were very similar to those of filic-3-ene.[4] Therefore, the structure of **3** was established as 3α,10α-epoxyfilic-4(23)-ene (Fig. 3).

Compound **4** was obtained as colorless needles of mp 222—224 °C, and was suggested to be a triterpenoid diol of $C_{30}H_{52}O_2$ on the basis of the HR-MS (*m/z* 444.3968) and its strong IR absorptions (3320, 1022 cm^{-1}). The characteristic fragment ion peak at *m/z* 191 in the EI-MS of **4** suggested that **4** was a derivative of hydroxyhopane (**11**)[7] with an extra hydroxyl at the D or E ring (Fig. 4). Among eight methyl signals observed in the ^{1}H-NMR spectrum of **4**, three were assigned to H-23, H-24 and H-25 by comparison with those of **11**,[4] and other methyl signals including C-28 appeared at lower fields owing to the influence of the oxygen function (Table 1). This

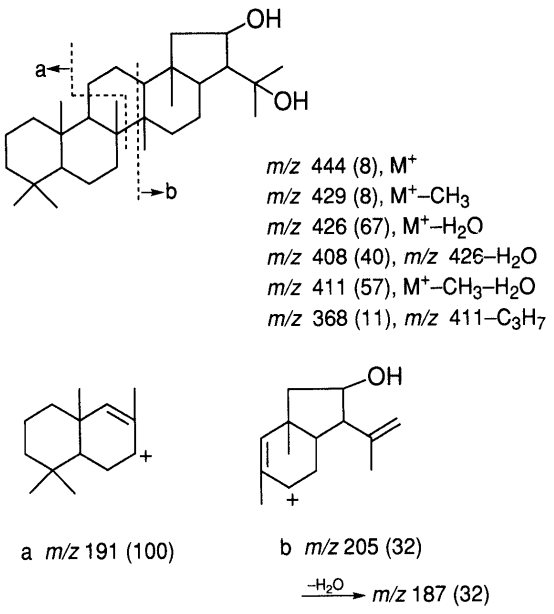

Fig. 4. Mass Fragment Ions of **4**

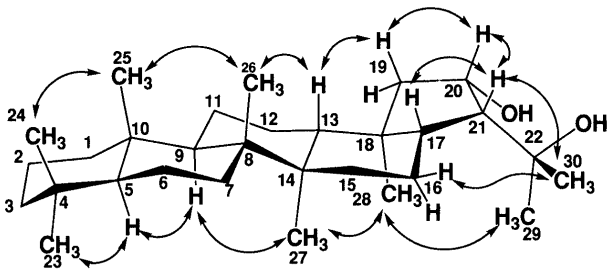

Fig. 5. NOEs of **4**

evidence suggested that the extra hydroxyl group is located at C-19α or C-20α of the E ring. The ^{13}C-NMR spectrum of **4** showed thirty signals, which were assigned using the distortionless enhancement by polarization transfer (DEPT), HMBC and COSY spectra as shown in Table 2. In addition, the carbon skeleton and location of the hydroxyl group on the E ring were confirmed by the HMBC spectrum. Cross peaks were observed between H-19 (δ 2.064, d) and C-28, 18, 17 and 21, and between H-21 (δ 2.674, d) and C-30, 29, 18, 17 and 22. As the carbon signal of C-19 in the ^{13}C-NMR of **4** was obviously secondary, the hydroxyl group of **4** was suggested to be at C-20 (Fig. 4). The configuration of the hydroxyl group was confirmed by the NOESY spectrum. The NOEs were observed between H-24 and H-25, H-25 and H-26 (β-side); H-27 and H-28, H-28 and H-29 (α-side), and H-19β (δ 2.064) and H-20β (δ 4.995), H-20β and H-21β (δ 2.674) (Fig. 5). Thus, the structure of **4** was established as 20α,22-dihydroxyhopane.

All triterpenoids isolated from *C. lepifera* were simply oxidized components of the hopane, fernane and filicane groups. Compound **3** is related to 3α-hydroxyfilic-4(23)-ene isolated from *C. spinulosa*,[1] and may be biosynthesized from 10α-hydroxyfilic-3-ene. Compounds **1** and **2** are related to the main hydrocarbon **6**. This is the first report of isolation of the epoxides **1**, **2** and **3** from a natural source, and they seem to be characteristic components of this fern. Compound **12** is the second report from the fern plant next to *Nephrolepis tuberosa*.[5]

Experimental

General Procedures Melting points were measured with a Yanagimoto micro apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were taken at 270 MHz (400 MHz for NOEs and 500 MHz for HMBC and NOESY) and 68 MHz, respectively, in $CDCl_3$ solution. Tetramethylsilane (TMS) was used as an internal standard and chemical shifts are given as δ values (ppm). EI-MS were obtained by a direct inlet system at 70 eV unless otherwise stated, and the relative intensities of peaks are reported with reference to the most intense peak higher than *m/z* 100. TLC was carried out on SiO_2 gel (Merck 5721) with a hexane–EtOAc solvent system, the spray reagent being H_2SO_4. GC was performed on a 1 m glass column containing Chromosorb G HP with 1.4% SE-30 with N_2 at 260 °C. Cholestane was used as an internal reference and its Rt_R was set at 3.0 min. HPLC was performed on a C18 reverse-phase column (8 mm × 250 mm) (detected by refractive index) with $CHCl_3$–MeOH–H_2O (76 : 14 : 10) as the eluent.

Plant Materials The leaflets of *Cyathea lepifera*, originally native to Okinawa, were collected at the greenhouse of this college in May. A voucher specimen has been deposited in the Herbarium of Shôwa College of Pharmaceutical Sciences, Tokyo.

Extraction and Isolation The fresh leaflets (1.25 kg) were extracted with hexane to give the extract (7.5 g), which was separated by SiO_2 gel column chromatography (CC) into fraction 1 (fr. 1) (solvent: hexane; 0.67 g), fr. 2 (hexane (9): benzene (1); 0.05 g), fr. 3 (hexane (7): benzene (3); 0.54 g), fr. 4 (hexane (1): benzene (1); 0.25 g), fr. 5 (hexane (1): benzene (1); 0.31 g), fr. 6 (hexane (3): benzene (7); 1.22 g), fr. 7 (benzene; 3.08 g), fr. 8 (benzene (9): ether (1); 0.36 g), fr. 9 (ether; 0.97 g).

Triterpenoid Hydrocarbons Fraction 1 was chromatographed on 20% $AgNO_3$–SiO_2 followed by HPLC to give triterpenoid hydrocarbons. Fern-9(11)-ene (**5**, 250 mg), colorless plates (Me_2CO), mp 169—171 °C. IR ν_{max}^{KBr} cm^{-1}: 811, 796. Fern-7-ene (**6**, 10 mg), colorless plates (Me_2CO), mp 209—211 °C. IR ν_{max}^{KBr} cm^{-1}: 827, 818. Filic-3-ene (**7**, 2 mg), colorless plates (Me_2CO), mp 234—239 °C. IR ν_{max}^{KBr} cm^{-1}: 851, 821. ^{1}H-NMR data of each compound have been reported.[1]

9α,11α-Epoxyhopane (1), 9β,11β-Epoxyhopane (2) and 3α,10α-Epoxyfilic-4(23)-ene (3) Fraction 2 was subjected to HPLC to give **1** (2 mg), mp 155—160 °C, $[\alpha]_D$ +37.1° (*c* = 0.1, $CHCl_3$) from peak **1** (first eluate), and **2** (3 mg), mp 254—256 °C, $[\alpha]_D^{23}$ +51.0° (*c* = 0.1, $CHCl_3$), from peak 3 (third eluate) and **3** (2 mg), mp 174—178 °C, $[\alpha]_D^{23}$ +63.1° (*c* = 0.1, $CHCl_3$) from peak 2 (second elute).

Cycloartanoid Esters (13) and Sitosterol Esters (14) Fraction 3 showed the same spot on TLC as the ester fraction of *C. spinulosa*,[1] and on by SiO_2 chromatography it afforded **13** (oily, more polar, 320 mg), ^{1}H-NMR δ: 0.843, 0.854, 0.879, 0.891, 0.903, 1.026 (3H, d, *J* = 6.7), 1.032d, 0.341 (1H, d, *J* = 4.2), 0.575 (1H, d, *J* = 4.2), 2.037, 2.296, 2.802, 5.363 and **14** (waxy, less polar, 25 mg). ^{1}H-NMR δ: 0.679 (3H, H-18), 1.020 (3H, H-19), 0.928 (3H, d, *J* = 6.4, H-21), 0.813 (3H, d, *J* = 6.9, H-26), 0.834 (3H, d, *J* = 6.9, H-27), 0.843 (3H, t, *J* = 7.0, H-29), 5.348 (1H, d, *J* = 4.2, H-5), 1.25 (–CH_2). **13** was hydrolyzed with 5% KOH in ethanol, and treated in the usual manner to give the alcohol fraction, cycloartenol (Rt_R 3.08) *m/z*: 426 (M^+), 411, 408, 393, 297, 286; 31-norcyclolaudenol (Rt_R 3.16) *m/z*: 426 (M^+), 411, 408, 393, 297, 286; cyclolaudenol (Rt_R 3.58) *m/z*: 440 (M^+), 425, 422, 407, 300, 297, and the acid fraction, linoleic acid (M^+ *m/z*: 280) and linolenic acid (M^+ *m/z*: 278). **14** was also hydrolyzed to give sitosterol (M^+ *m/z*: 414, 399, 396, 381) containing campesterol (M^+ *m/z*: 400, trace) and campestanol (M^+ *m/z*: 402, trace), and palmitic acid (M^+ *m/z*: 256).

Adiantone (8), Glaucanone (9), Fern-9(11)-en-12-one (10) and α-Tocopherol (15) Fraction 4 was chromatographed through Al_2O_3 followed by HPLC to give **15**, **8**, **9**, and **10** in order of elution. **15** (yellow oil, 7 mg), ^{1}H-NMR δ: 0.839 (3H, d, *J* = 6.7), 0.847 (3H, d, *J* = 6.4), 0.865 (3H, d, *J* = 6.7), 1.226 (3H, s), 2.111 (6H, s), 2.159 (3H, s), 4.789. **8** (2 mg), mp 227—229 °C. IR ν_{max}^{KBr} cm^{-1}: 1000 (C=O). ^{1}H-NMR δ: 0.845 (3H, H-23), 0.791 (3H, H-24), 0.812 (3H, H-25), 0.962 (3H, H-26), 0.933 (3H, H-27), 0.581 (3H, H-28), 2.119 (3H, H-29). **9** (4 mg), 244—245 °C. ^{1}H-NMR δ: 0.839 (3H, H-23), 0.781 (3H, H-24), 0.804 (3H, H-25), 0.839 (3H, H-26), 1.026 (3H, H-27). **10** (10 mg), mp 220—222 °C. IR ν_{max}^{KBr}: 1672 (C=O). ^{1}H-NMR δ: 0.883 (3H, H-23), 0.917 (3H, H-24), 1.106 (3H, H-25), 0.825 (3H, H-26), 1.024 (3H, H-27), 0.784 (3H, H-28), 0.899 (3H, d, *J* = 6.7, H-29), 0.843 (3H, d, *J* = 6.7, H-30), 5.645 (1H, d, *J* = 3.0, H-11).

Hydroxyhopane (11) Fraction 5 was recrystallized from acetone to

remove aliphatic compounds, and the soluble fraction was purified by HPLC to give **11** (15 mg), mp 243—245 °C. IR ν_{max}^{KBr} cm^{-1}: 3450, 1020 (OH). ^{1}H-NMR δ: 0.848 (3H, H-23), 0.790 (3H, H-24), 0.812 (3H, H-25), 0.954 (3H, H-26), 0.954 (3H, H-27), 0.712 (3H, H- 28), 1.179 (3H, H-29), 1.207 (3H, H-30).

Sitosterol Mixture (16) Fraction 7 was recrystallized repeatedly from methanol to give **16** (500 mg), mp 138—141 °C. The GC pattern of **16** indicated it was a mixture of sitosterol (M^+ 414, 88%), stigmasterol (M^+ 412, 6%) and campesterol (M^+ 400, 6%).

20α,22-Dihydroxyhopane (4) and Hopan-30-oic Acid (12) Fraction 8 was methylated with CH_2N_2 to separate **4** and **12** from aliphatic compounds. The crude reaction product was chromatographed through dry SiO_2 gel {solvent: hexane (9): EtOAc (1)} to give an acid methyl ester and a diol fraction. Each fraction was purified by HPLC to give the methyl ester (**12a**, 4 mg) of **12**, mp 234—237 °C. IR ν_{KBr}^{KBr} cm^{-1}: 1725 (CO), $[\alpha]_D^{23}$ +76.7° (c=0.2, $CHCl_3$). EI-MS m/z: 456 (M^+, 6), 441 (M^+-CH_3, 5), 369 ($M^+-C_3H_7$, 8), 235 (100), 191 (71). ^{1}H-NMR δ: 0.846 (3H, H-23), 0.791 (3H, H-24), 0.813 (3H, H-25), 0.951 (3H, H-26), 0.951 (3H, H-27), 0.752 (3H, H-28), 1.178 (3H, d, J=6.7, H-29), 3.643 (3H, $COOCH_3$) and **4** (6 mg), mp 222—224 °C, $[\alpha]_D^{23}$ +3.5° (c=0.2, C_5H_5N).

Saturated Fatty Acids Fraction 9 was purified by using dry SiO_2 chromatography (solvent: hexane (9): EtOAc (1)) to give waxy fatty acids (100 mg). ^{1}H-NMR δ: 0.867 (3H, t, J=6.8), 1.255 (CH_2). MS m/z: 284 (M^+, stearic acid, main), m/z: 256 (M^+, palmitic acid, trace) and m/z: 270 (M^+, heptadecanoic acid, trace).

Synthesis of Methyl Hopan-30-oate (12a′) A mixture of AcOH (9 ml) and H_2O (1 ml) containing CrO_3 (1.0 g) was dropped into a solution of dryocrassol (50 mg) in AcOH (5 ml) and benzene (10 ml), and the mixture was refluxed for 6 h. The reaction product was treated with CH_2N_2 to give the methyl ester, which was chromatographed on dry SiO_2 followed by HPLC to give 6 mg of **12a′**, mp 233—235 °C. ^{1}H-NMR δ: 0.846 (3H, H-23), 0.791 (3H, H-24), 0.813 (3H, H-25), 0.952 (3H, H-26), 0.952 (3H, H-27), 0.750 (3H, H-28), 1.177 (3H, d, J=6.7, H-29), 3.643 (3H, $COOCH_3$).

Acknowledgements The authors are grateful to Mr. Yôichi Takase and Mr. Hideki Suzuki of the central analytical center of this college for ^{1}H-NMR, ^{13}C-NMR and MS measurements.

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