Fern Constituents: Triterpenoids Isolated from the Leaves of *Cheiropleuria bicuspis*

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Eight triterpenoid hydrocarbons were characterized from the fresh leaves of *Cheiropleuria bicuspis*. They consist of hop-22(29)-ene, neohop-13(18)-ene, fern-7-ene, (18*R*)-dammara-13(17), 21-diene, eupha-7,21-diene, 13α(H)-malabaricatriene, γ-polypodatetraene and squalene, all of which have different carbon skeletons. A new triterpenoid alcohol was isolated and established as 1α,11α,30-trihydroxyhopane. We also detected friedelin, hydroxyhopane, dryocrassol, lupeol, fernenol, 1α,11α,22-trihydroxyhopane, a mixture of sterols and a mixture of fatty acid esters of cycloartanoids, methylsterols and sterols. These findings suggest that *Cheiropleuria bicuspis* is related to *Microsorium* (Polypodiaceae), but are nevertheless consistent with the independency of Cheiropleuriaceae.

Keywords *Cheiropleuria bicuspis*; Cheiropleuriaceae; fern constituent; triterpenoid hydrocarbon; hydroxyhopane; dryocrassol; 1α,11α,22-trihydroxyhopane; 1α,11α,30-trihydroxyhopane

Cheiropleuria bicuspis (Blume) Presl (Cheiropleuriaceae; sujihitotsuba in Japanese) is distributed in southern Japan, Taiwan, China, the Philippines, Java, Sumatra, Thailand and New Guinea. This single species forms a genus. In the course of chemotaxonomic studies on Polypodiaceous ferns, we have been interested in the constituents of the above fern, because some taxonomists have classified it into Polypodiaceae[1] and others into Cheiropleuriaceae.[2] Some oxygenated hopane group triterpenoids were isolated by Tanaka *et al.* from the methanol extract of this fern collected in Taiwan.[3] This paper concerns some components from the *n*-hexane extract of the fresh leaves collected in Okinawa Island. The chemotaxonomy of *Cheiropleuria bicuspis* is discussed.

Results and Discussion

Extraction of the fresh materials and chromatographic separation gave four fractions containing triterpenoid hydrocarbons (Table I). Investigation by gas chromatography (GC) and GC-mass spectroscopy (GC-MS) of each fraction showed the presence of hop-22(29)-ene (**1**),[4] neohop-13(18)-ene (**2**),[5] fern-7-ene (**3**),[6] (18*R*)-dammara-13(17),21-diene (**4**),[7] eupha-7,21-diene (**5**),[7] 13α(H)-malabaricatriene (**6**),[8] γ-polypodatetraene (**7**)[9] and squalene (**8**).[10] Compounds **1**, **2** and **3** belong to the hopane and migrated hopane group (pentacyclic); **4**, the dammarane group; **5**, the euphane group (tetracyclic); **6**, the malabaricane group (tricyclic); **7**, the polypodane group (bicyclic); **8**, the acylic triterpenoid group.

From the succeeding fractions, we detected friedelin (**9**),[11] hydroxyhopane (**10**),[12] dryocrassol (**11**),[13] lupeol (**12**),[14] fernenol (**13**),[15] compounds **14** and **15** as pentacyclic triterpenoids, together with a mixture of sterols (campesterol, stigmasterol and sitosterol) and a mixture of fatty acid (palmitic, linoleic and linolenic acids) esters of the following compounds: cycloartanoids (cycloartenol,[16] cyclolaudenol[17] and cycloeucalenol[18]), methylsterols (obtusifoliol[19] and citrostadienol[20]) and sterols (campesterol and sitosterol).

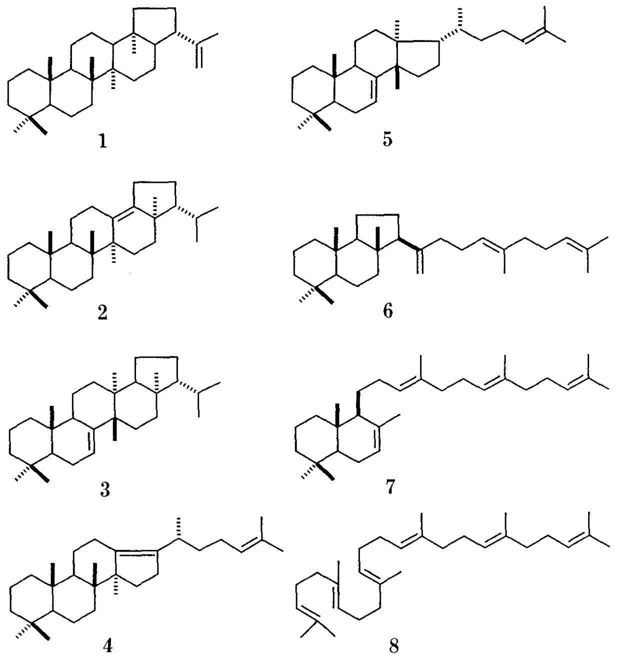


Chart 1

Compound **14**, $C_{30}H_{52}O_3$, mp 285—286 °C, showed strong hydroxyl bands in the infrared (IR) spectrum. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum (270 MHz in $CDCl_3$) indicated the presence of eight tertiary methyl groups and two hydroxy-bearing methine protons (Table II). This compound was identical with an authentic sample of 1α,11α,22-trihydroxyhopane[3] by mixture melting point determination and IR, MS, and ^{1}H- and ^{13}C-NMR comparisons.

Table I. GC-MS Data for Triterpenoid Hydrocarbons

	%[a]	Rt_R	MS fragments
1	61.5	2.60	410 (M⁺), 395, 367, 191, 189
2	8.9	1.91	410 (M⁺), 395, 367, 218, 205, 203, 191, 175
3	0.2	2.27	410 (M⁺), 395, 367, 257, 243, 231
4	7.9	1.20	410 (M⁺), 395, 367, 341, 327, 299, 297, 218, 205, 191, 149, 69
5	7.3	1.62	410 (M⁺), 395, 297, 271, 257, 241, 231, 203, 191, 189, 69
6	4.2	1.46	410 (M⁺), 395, 341, 231, 218, 204, 191, 137, 69
7	1.1	1.24	410 (M⁺), 395, 341, 273, 218, 217, 204, 191, 189, 137, 69
8	0.8	0.91	410 (M⁺), 395, 341, 286, 273, 205, 203, 137, 123, 69

The Rt_R and MS pattern of each compound were compared with those of the corresponding authentic sample. Rt_R: relative retention time. *a*) Each value is the percentage of all the triterpenoid hydrocarbon fractions, calculated from the weight of each of the four fractions and the peak area (%) of each compound in GC.

Table II. ^{1}H-NMR Spectral Data (270 MHz, $CDCl_3$, δ Value)

	H-23	H-24	H-25	H-26	H-27	H-28	H-29	H-30	H-1β	H-11β
10	0.848	0.796	0.818	0.960	0.960	0.767	1.181	1.208		
11	0.850	0.798	0.826	0.966	0.966	0.737	1.047 (d, 6.6)	3.386 (dd, 10.6, 6.6) 3.623 (dd, 10.6, 3.3)		
14	0.902	0.831	0.924	1.023[a]	1.001[a]	0.797	1.184	1.213	4.038 (br s)	4.062 (ddd, 10.1, 10.1, 6.5)
15	0.904	0.832	0.923	1.022[a]	1.002[a]	0.757	1.053 (d, 6.4)	3.398 (dd, 10.5, 6.5) 3.633 (dd, 10.5, 3.1)	4.038 (br s)	4.064 (ddd, 10.4, 10.4, 6.4)
16	0.846	0.791	0.814	0.951	0.951	0.712	3.347 (dd, 10.5, 7.5) 3.745 (dd, 10.5, 2.5)	0.938 (d, 6.6)		

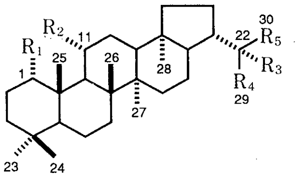
Multiplicity and coupling constants (J in Hz) are shown in parentheses. *a*) Assignments may be interchanged.

Table III. ^{13}C-NMR Spectral Data (68 MHz, δ Value)

	10	**14** (*d*-pyridine)	**15**	**11**	**15** ($CDCl_3$)	**16**
C-1	40.5	73.1	73.1	40.5	73.4	40.4
C-2	19.0	25.8	25.8	18.7	25.5	18.7
C-3	42.3	36.7	36.4	42.2	36.3	42.2
C-4	33.4	33.8	33.8	33.2	33.6	33.2
C-5	56.4	48.4	47.8	56.3	47.7	56.2
C-6	19.0	19.1	19.1	18.8	18.8	18.7
C-7	33.6	35.6	35.6	33.4	35.0	33.4
C-8	42.2	43.3	43.2	41.9	43.0	41.9
C-9	50.7	47.4	47.4	50.6	47.0	50.5
C-10	37.6	43.3	43.4	37.5	43.3	37.5
C-11	21.3	68.6	68.6	21.1	69.9	21.0
C-12	24.5	33.8	33.9	24.1	33.5	24.0
C-13	50.2	49.2	49.2	49.5	48.8	49.3
C-14	42.2	42.4	42.2	41.9	42.1	41.8
C-15	34.8	34.7	33.8	33.8	33.8	33.6
C-16	22.4	22.4	23.0	22.6	22.7	22.0
C-17	54.7	54.7	54.6	54.4	54.5	54.2
C-18	44.4	44.2	44.4	44.5	44.3	44.5
C-19	41.8	41.6	41.8	41.8	41.6	41.6
C-20	27.0	27.0	27.7	27.2	27.3	26.3
C-21	51.6	51.5	43.7	42.8	42.8	41.0
C-22	72.5	72.4	40.7	39.6	39.7	38.7
C-23	33.6	33.7	33.7	33.4	33.4	33.4
C-24	21.8	21.7	21.7	21.6	21.5	21.6
C-25	16.1	18.1	18.0	15.7	17.8	15.8
C-26	17.0	17.4	17.6	16.6	17.2	16.7
C-27	17.3	17.5	16.9	16.5	16.8	16.2
C-28	16.5	16.2	15.7	15.9	15.6	15.9
C-29	29.9	29.9	19.1	18.1	18.2	68.1
C-30	31.5	31.5	66.9	67.7	67.8	21.6

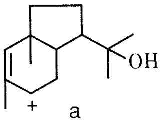
A new compound (**15**), $C_{30}H_{52}O_3$, mp 296—297 °C, which has a lower *Rf* value than **14** on thin-layer chromatography (TLC), also showed strong IR bands due to hydroxyl groups. The ^{1}H-NMR spectrum indicated the presence of six tertiary methyl groups, one secondary methyl group, two protons of a hydroxymethyl group and two hydroxy-bearing methine protons. The five methyl signals (H-23—H-27) and signals of two hydroxy-bearing methine protons (H-1β and H-11β) agree very closely with those of **14**.[21] One secondary methyl signal and two proton signals of a hydroxymethyl group were observed and they closely resemble those of dryocrassol (**11**), but not those of neriifoliol (**16**)[13,22] (Table II).

The ^{13}C-chemical shifts of rings A, B and C of **15** are almost the same as those of **14**,[23] and the ^{13}C-chemical shifts of rings D and E of **15**, resemble those of **11**, but not those of **16** (Table III).

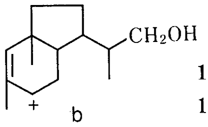


	R_1	R_2	R_3	R_4	R_5
10	H	H	OH	CH_3	CH_3
11	H	H	H	CH_3	CH_2OH
14	OH	OH	OH	CH_3	CH_3
15	OH	OH	H	CH_3	CH_2OH
16	H	H	H	CH_2OH	CH_3

Chart 2



	a	a—H_2O
	m/z 207	m/z 189
10	(9, rel. int.)	(87)
14	(3)	(73)



	b	b—H_2O
	m/z 207	m/z 189
11	(100)	(10)
15	(92)	(22)

Chart 3

In the MS (Chart 3), there is some difference between the characteristic fragment a of **14** and b of **15**: the intensity of fragment a is remarkably lower than that of its dehydrated peak (a—H_2O). This indicates that fragment a is more liable to dehydration than fragment b. A similar situation was also observed in the MS of **10** and **11**.

On the basis of all the above evidence, **15** was established as 1α,11α,30-trihydroxyhopane, in which the absolute configuration at C-22 is *S*.[13]

The presence of many triterpenoid hydrocarbons with various kinds of carbon skeletons, lupeol and fernenol (these are the first examples from fern plants), and also a large amount of cycloartanoid esters, suggests that *Cheiropleuria bicuspis* is closely related to Polypodiaceous ferns, such as *Microsorium*, from a chemotaxonomic point of view. On the other hand, the isolation of some hopane group triterpenoids having hydroxyl groups at 1α and 11α is very characteristic (the only example from the plant kingdom) and might suggest the independency of Cheiropleuriaceae.

Experimental

Melting points were measured with a Yanagimoto microapparatus and are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM GX-270 spectrometer using tetramethylsilane as an internal standard. Electron impact mass spectra (EI-MS) and high-resolution mass spectra

(HRMS) were measured at 30 eV (direct inlet) with a JEOL JMS D300 spectrometer and the relative intensities of peaks were reported with reference to the most intense peak higher than m/z 100. GC was run on a Hitachi 163 apparatus using a glass column containing Chromosorb G HP coated with SE-30 (1.4%) at 260 °C in a flow of N_2. Cholestane was used as an internal reference, whose retention time was set at 3.0 min. GC-MS was run on a JGC 20K-JMS D300 system using the same absorbant as described above in a flow of He. TLC was carried on precoated Silica gel 60 plates (Merck) with *n*-hexane–EtOAc or $CHCl_3$–MeOH as the solvent system. High-performance liquid chromatography (HPLC) was carried out on a Senshu Pak ODS 5μ column ($8\,mm \times 25\,cm$) with CH_3CN–$CHCl_3$ (9 : 1) using Waters 600 (Multisolvent Delivery System) and Waters 410 (RI) equipmemt.

Plant Materials The fresh leaves (2.7 kg) of *Cheiropleuria bicuspis* were collected in December at Benoki, Kunigami, Okinawa Prefecture, Japan. A voucher specimen has been deposited in the Herbarium of Showa College of Pharmaceutical Sciences, Tokyo.

Extraction and Separation The fresh plant materials were extracted with *n*-hexane to give the extract (14.4 g) and water (1690 ml). The hexane extract was chromatographed on silica gel to elute paraffins and triterpenoid hydrocarbons with *n*-hexane, **9** and a mixture of fatty acid esters with *n*-hexane–benzene (1 : 1), **10**, **11**, **12** and **13** with benzene, a mixture of sterols with benzene–Et_2O (9 : 1), and **14** and **15** with benzen–Et_2O (1 : 1). The pure compounds described below were purified by further repeated chromatography ($AgNO_3$-silica gel and/or silica gel) and/or HPLC and recrystallization.

Triterpenoid Hydrocarbons Each of the triterpenoid hydrocarbon fractions {fr. I (9.2 mg), fr. II (50.5 mg), fr. III (7.1 mg) and fr. IV (7.6 mg)} was subjected to GC and GC-MS. The results are shown in Table I.

Hop-22(29)-ene (1) Colorless needles (8 mg) (from EtOAc), mp 210—211 °C. Identical (IR, GC and MS) with an authentic sample.[4)]

Friedelin (9) Colorless needles (3 mg) (from $CHCl_3$–MeOH), mp 268—270 °C. Identical (IR, GC, MS, and ^1H-NMR) with an authentic sample.

Hydroxyhopane (10) Colorless needles (5 mg) (from EtOAc), mp 253—255 °C. Identical (IR and MS) with an authentic sample.[12)]

Dryocrassol (11) Colorless needles (1 mg) (from Et_2O–acetone), mp 243—245 °C. Identical (IR, GC and MS) with an authentic sample.[13)]

Lupeol (12) and Fernenol (13) A mixture (5 mg) was subjected to HPLC to give **12** (crude) and **13** (crude), each of which was identical (HPLC, GC and GC-MS) with an authentic sample.[14,15)] These identifications were also confirmed by ^1H-NMR.

1α,11α,22-Trihydroxyhopane (14) Colorless needles (5 mg) (from pyridine–CH_3CN), mp 285—286 °C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1070, 1005. MS m/z (rel. int.): 442 ($M^+ - H_2O$, 2), 424 (8), 406 (100), 207 (3), 189 (73), 149 (29).

1α,11α,30-Trihydroxyhopane (15) Colorless needles (4 mg) (from pyridine–CH_3CN), mp 296—297 °C, $[\alpha]_D^{23}$ +49.7° (c=0.1, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400, 1070, 1045, 1035, 1010, 750. MS m/z (rel. int.): 442 ($M^+ - H_2O$, 27), 424 (23), 409 (12), 343 (100), 234 (85), 221 (61), 207 (92), 203 (32), 189 (22), 149 (84). HRMS m/z: 442.3813 ($M^+ - H_2O$, Calcd for $C_{30}H_{50}O_2$: 442.3798).

A Mixture of Sterols Colorless powder (11 mg). GC and GC-MS: Rt_R 2.24 (27% of total peaks) {m/z 400 (M^+), 385, 382, 367, 315, 289, 273, 255, 231, 213} (campesterol), 2.41 (18%) {412 (M^+), 397, 394, 379, 351, 300, 271, 255, 231, 213} (stigmasterol), 2.73 (55%) {414 (M^+), 399, 396, 381, 329, 303, 273, 255, 231, 213} (sitosterol).

A Mixture of Fatty Acid Esters of Cycloartanoids, Methylsterols and Sterols Colorless gum (800 mg). Hydrolysis of the fatty acid esters (280 mg) with 5% KOH–EtOH under reflux for 2 h gave the neutral and acidic factions. The neutral portion was repeatedly chromatographed on silica gel to give three fractions. Fraction A (42 mg), GC and GC-MS: Rt_R3.15 (38% of total peaks) {m/z 426 (M^+), 411, 408, 393, 365, 339, 315, 297, 286} (cycloartenol), 3.58 (62%) {440 (M^+), 425, 422, 407, 379, 353, 315, 300, 297} (cyclolaudenol). Fraction B (15 mg), GC and GC-MS: Rt_R 2.56 (46% of total peaks) {m/z 426 (M^+), 411, 393, 327, 259, 245, 227} (obtusifoliol), 2.86 (28%) {426 (M^+), 411, 408, 393, 300, 283} (cycloeucalenol). 3.67 (16%) {426 (M^+), 411, 328, 285, 269, 267, 245, 227} (citrostadienol). This fraction was further subjected to HPLC to give obtusifoliol {colorless needles (from MeOH), mp 146.5—148 °C} and cycloeucalenol (crude), each of which was confirmed by ^1H-NMR. Fraction C (60 mg), GC and GC-MS: Rt_R 2.24 (51% of total peaks) (m/z 400, M^+) (campesterol), 2.74 (49%) (414, M^+) (sitosterol). The acidic portion (95 mg) was methylated with CH_2N_2 and the products were characterized by GC (oven temp: 200 °C) and GC-MS: t_R 6.06 min (12% of total peaks) (m/z 270, M^+) (methyl palmitate), 10.92 min (86%) (292, M^+; 294, M^+) (a mixture of methyl linoleate and methyl linolenate). The Rt_R or t_R and MS pattern of all the compounds described above were compared with those of corresponding authentic samples to confirm identifications.

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References and Notes

1) E. B. Copeland, "Genera Filicum, "Ronald Press, New York, 1947, p. 178; J. Ohwi, "Flora of Japan, Pteridophyta," Shibundo, Tokyo, 1957. p. 143; T. Nakaike, "New Flora of Japan, Pteridophyta," Shibundo, Tokyo, 1982, p. 634.
2) T. Nakai, *Bot. Mag. Tokyo*, **42**, 213 (1928); R. C. Ching, *Sunyatsenia*, **5**, 254 (1940); H. Ito, "Filices Japonenses Illustratae," Koseikaku Tokyo, 1944, p. 448; M. Tagawa, "Coloured Illustrations of the Japanese Pteridophyta," Hoikusha, Tokyo, 1959, p. 154; T. Nakaike, "Enumeratio Pteridophytarum Japonicarum, Filicales," Univ. of Tokyo Press, Tokyo, 1975, p. 361.
3) N. Tanaka, H. Wada, M. Kojima, T. Murakami, Y. Saiki, C. M. Chen and Y. Iitaka, *Chem. Pharm. Bull.*, **35**, 4016 (1987).
4) H. Ageta, K. Iwata and K. Yonezawa, *Chem. Pharm. Bull.*, **11**, 408 (1963).
5) H. Ageta, K. Shiojima and Y. Arai, *Chem. Commum.*, **1968**, 1105.
6) H. Ageta, K. Iwata and S. Natori, *Tetrahedron Lett.*, **1964**, 3413. H. Ageta and Y. Arai, *Phytochemistry*, **22**, 1801 (1983).
7) *a*) Y. Arai, K. Masuda and H. Ageta, *Chem. Pharm. Bull.*, **30**, 4219 (1982); *b*) To facilitate comparison of tetracyclic triterpenoid with pentacyclics, we proposed a new numbering system for the side chain and the eight methyl groups: K. Masuda, K. Shiojima and H. Ageta, *ibid.*, **31**, 2530 (1983).
8) K. Masuda, K. Shiojima and H. Ageta, *Chem. Pharm. Bull.*, **37**, 1140 (1989).
9) K. Shiojima, Y. Arai, K. Masuda, T. Kamada and H. Ageta, *Tetrahedron Lett.*, **24**, 5733 (1983).
10) A pure sample of squalene was purchased from Tokyo Kasei Co., Ltd.
11) T. Takahashi, *Nippon Kagaku Zasshi*, **87**, 10 (1966).
12) H. Ageta, K. Iwata and Y. Otake, *Chem. Pharm. Bull.*, **11**, 407 (1963).
13) H. Ageta, K. Shiojima, Y. Arai, T. Kasama and K. Kajii, *Tetrahedron Lett.*, **1975**, 3297.
14) A sample derived from lupenyl acetate; Y. Arai, M. Wama and H. Ageta, *Yakugaku Zasshi*, **102**, 1089 (1982).
15) H. Ageta and T. Ageta, *Chem. Pharm. Bull.*, **32**, 369 (1984).
16) H. E. Audier, R. Beugelmans and B. C. Das, *Tetrahedron Lett.*, **1966**, 4341; Y. Arai, K. Shiojima and H. Ageta, *Chem. Pharm. Bull.*, **37**, 560 (1989).
17) H. Ageta and Y. Arai, *Phytochemistry*, **23**, 2875 (1984).
18) F. F. Knapp and H. J. Nicholas, *Phytochemistry*, **8**, 207 (1969).
19) T. Kato, S. Tanaka, M. Ueda and Y. Kawase, *Agric. Biol. Chem.*, **39**, 169 (1975).
20) W. Sukrow, W. Littman and B. Raduchel, *Chem. Ber.*, **110**, 1523 (1977).
21) We assigned the ^1H-NMR signals (in $CDCl_3$) of **14** on the basis of those of **10** and **11**.
22) G. N. Pandy and C. R. Mitra, *Tetrahedron Lett.*, **1967**, 1353.
23) We revised the assignment of some ^{13}C-chemical signals of **14** on the basis of measurements at 68 MHz.