Fern Constituents: Triterpenoids Isolated from the Leaves of *Cheiropleura bicusps*

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Eight triterpenoid hydrocarbons were characterized from the fresh leaves of *Cheiropleura bicusps*. They consist of hop-22(29)-ene, neohop-13(18)-ene, fern-7-ene, (18R)-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-trihydroxyxopane. We also detected friedelin, hydroxyxopane,
dryocassol, lupeol, fernenol, 1α,11α,22-trihydroxyxopane, a mixture of sterols and a mixture of fatty acid esters of
cycloartenoids, methylsterols and sterols. These findings suggest that *Cheiropleura bicusps* is related to *Microsorum* (Polypodiaeae), but are nevertheless consistent with the independence of Cheiropleuriaeae.

**Keywords** Cheiropleura bicusps; Cheiropleuriaeae; fern constituent; triterpenoid hydrocarbon; hydroxyxopane; dryocassol; 1α,11α,22-trihydroxyxopane; 1α,11α,30-trihydroxyxopane

*Cheiropleura bicusps* (BLUME) PRESL. (Cheiropleuriaeae; sujihitosubai 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 Polypodiaeaeous ferns, we have been interested in the constituents of the above fern, because some taxonomists have classified it into Polypodiaeae[1] and others into Cheiropleuriaeae.[2] Some oxygenated xopane 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 *Cheiropleura bicusps* 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] (18R)-dammara-13(17),21-diene (4),[7] eupha-7,21-diene (5),[7] 13α(H)-
malabaricatriene (6),[8] γ-polypodatetraene (7) and squalene (8).[7] Compounds 1, 2 and 3 belong to the xopane and migrated xopane group (pentacyclic); 4, the dammarane group; 5, the eupha group (tetracyclic); 6, the malabaricane group (tricyclic); 7, the polypodane group (biciclyclic); 8, the acyclic triterpenoid group.

From the succeeding fractions, we detected friedelin (9),[11] hydroxyxopane (10),[12] dryocassol (11),[13] lupeol (12),[14] fernenol (13),[15] compounds 14 and 15 as pentacyclic triterpenoids, together with a mixture of sterols (campesterol, stigmastanol and sitostanol) and a mixture of fatty acid (palmitic, linoleic and linolenic acids) esters of the following compounds: cycloartenoids (cycloartenol,[16] cycloaludene-
ol[17] and cycloaludenal,[18]) methylsterols (obutinosfiofiliol,[19] and citrostadienol[20]) and sterols (campesterol and sitosterol).

Compound 14, C₃₀H₄₅O₅, mp 285–286 °C, showed strong hydroxyl bands in the infrared (IR) spectrum. The
¹H-nuclear magnetic resonance (¹H-NMR) spectrum (270 MHz in CDCl₃) indicated the presence of eight tertiary methyl groups and two hydroxy-bearing methane protons (Table II). This compound was identical with an authentic sample of 1α,11α,22-trihydroxyxopane[20] by mixture melting point determination and IR, MS, and ¹H- and ¹³C-NMR comparisons.

**TABLE I. GC-MS Data for Triterpenoid Hydrocarbons**

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>Rₘ</th>
<th>MS fragments</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>61.5</td>
<td>2.60</td>
<td>410 (M⁺), 395, 367, 191, 189</td>
</tr>
<tr>
<td>2</td>
<td>8.9</td>
<td>1.91</td>
<td>410 (M⁺), 395, 367, 218, 205, 203, 191, 175</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>2.27</td>
<td>410 (M⁺), 395, 331, 257, 243, 231</td>
</tr>
<tr>
<td>4</td>
<td>7.9</td>
<td>1.20</td>
<td>410 (M⁺), 395, 367, 341, 227, 299, 297, 218, 205, 191, 149, 69</td>
</tr>
<tr>
<td>5</td>
<td>7.3</td>
<td>1.62</td>
<td>410 (M⁺), 395, 297, 271, 257, 241, 231, 203, 191, 189, 69</td>
</tr>
<tr>
<td>6</td>
<td>4.2</td>
<td>1.46</td>
<td>410 (M⁺), 395, 341, 231, 218, 204, 191, 137, 69</td>
</tr>
<tr>
<td>7</td>
<td>1.1</td>
<td>1.24</td>
<td>410 (M⁺), 395, 341, 273, 218, 217, 204, 191, 189, 137, 69</td>
</tr>
<tr>
<td>8</td>
<td>0.8</td>
<td>0.91</td>
<td>410 (M⁺), 395, 341, 286, 273, 205, 203, 137, 123, 69</td>
</tr>
</tbody>
</table>

The Rₘ and MS pattern of each compound were compared with those of the corresponding authentic sample. Rₘa, 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.

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A new compound (15), C_{10}H_{12}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 1H-NMR spectrum indicated the presence of six tertiary methyl groups, one secondary methyl group, two protons of a hydroxymethyl group and two hydroxy-bearing methane protons. The five methyl signals (H-23—H-27) and signals of two hydroxy-bearing methane 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 dryocassol (11), but not those of nerifiol (16).15,22 (Table II).

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

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 1z,11z,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 cycloartenol esters, suggests that Cheiridopsis bicuspis is closely related to Polyopodialaceae ferns, such as Microsorum, from a chemotaxonomic point of view. On the other hand, the isolation of some hopane group triterpenoids having hydroxy groups at 1z and 11z is very characteristic (the only example from the plant kingdom) and might suggest the independency of Cheiridopiliaeaceae.

**Experimental**

Melting points were measured with a Yanagimoto microapparatus and are uncorrected. The 1H- and 13C-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 N₂. Cholesterol 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 CHCl₃-MeOH as the solvent system. High-performance liquid chromatography (HPLC) was carried out on a Sension Pak ODS 5µ column (8 mm x 25 cm) with CH₃CN-CHCl₃ (9:1) using Waters 600 (Multisolvent Delivery System) and Waters 410 (RI) equipment.

**Plant Materials** The fresh leaves (2.7 kg) of *Cheiropleura bicuspis* were collected in December, Toyo, Kusumia, 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-Et₂O (9:1), and 14 and 15 with benzene-Et₂O (1:1). The pure compounds described below were purified by further repeated chromatography (AgNO₃-silica gel and/or silica gel) and/or HPLC and recrystallization.

**Triterpenoid Hydrocarbons** Each of the triterpenoid hydrocarbon fractions (fractions 1 (9.2 mg), fraction II (50.5 mg), fraction III (7.1 mg) and fraction IV (7.6 mg)) was subjected to GC and GC-MS. The results are shown in Table 1.

**Hop-22(29)-ene** (1) Colorless needles (8 mg) from EtOAc, mp 210–211°C. Identical (IR, GC, and MS) to an authentic sample.

**Furostane** (10) Colorless needles (5 mg) from EtOAc, mp 253–255°C. Identical (IR and MS) with an authentic sample.

**Droserosol** (11) Colorless needles (1 mg) from Et₂O-acetone, mp 257°C. Identical (IR, GC, and MS) with an authentic sample.

**Lupéol (12) and Fermenol (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.

**Triterpenoids** (14) Colorless needles (5 mg) from pet. ether (CH₃CN), mp 285–286°C. IR (νmax cm⁻¹): 3400, 1700, 1005, and 998 cm⁻¹. m/z (rel. int.): 442 (M⁺, H₂O, 2), 424 (8), 406 (100), 207 (3), 189 (73), 149 (29).

**Saponin** (15) Colorless needles (4 mg) from pyridine-CH₃CN, mp 296–297°C. [α]D₂⁰ value: 49.7° (c = 0.1), pyridine. IR (νmax cm⁻¹): 3400, 1070, 1045, 1035, 1010, 750, 720, 425, 409 (12), 343 (100), 234 (85), 221 (61), 207 (92), 203 (32), 189 (22), 149 (84). HRMS m/z: 442.3813 (M⁺-H₂O, Calcd for C₂₄H₃₆O₂: 442.3798).

**Mixture of Steroids** Colorless powder (11 mg). GC and GC-MS: Rf₉₈ 2.24 (27% of total peaks) [m/z 400 (M⁺), 385, 382, 367, 315, 209, 235, 239, 213 (campesterol), 2.41 (18%) [412 (M⁺)], 397, 394, 379, 351, 300, 271, 255, 231, 213 (stigmastanol), 2.73 (55%) [414 (M⁺)], 399, 396, 381, 329, 303, 273, 255, 231, 213 (stigmasterol).

**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 fractions. The neutral portion was repeatedly chromatographed on silica gel to give three fractions. Fraction A (42 mg), GC and GC-MS: Rf₉₈ 3.15 (38% of total peaks) [m/z 426 (M⁺), 411, 408, 393, 353, 315, 297, 286 (cycloartenol), 3.58 (62%) [440 (M⁺), 425, 422, 407, 379, 353, 315, 300, 297] (cycloartenol). Fraction B (15 mg), GC and GC-MS: Rf₉₈ 2.56 (66% of total peaks) [m/z 426 (M⁺), 411, 393, 327, 259, 245, 227 (obusistofilosil, 2.86 (28%) [426 (M⁺), 411, 408, 393, 300, 283 (cycloartenol), 3.67 (16%) [426 (M⁺), 411, 328, 285, 269, 267, 245, 227 (cycloartenol). This fraction was further subjected to HPLC to give obusistofilosil (colorless needles from MeOH), mp 165–167°C and cycloartenol (crude), each of which was confirmed by H-NMR. Fraction C (60 mg), GC and GC-MS: Rg₉₈ 2.24 (51% of total peaks) [m/z 400 (M⁺) (cycloartenol), 2.74 (49%) [414 (M⁺)] (stigmasterol). The acid portion (95 mg) was methylated with CH₃ONa and the products were characterized by GC (oven temp: 200°C and GC-MS: t₉ 6.00 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 Rg₉₈ and t₉ and MS pattern of all the compounds described above were compared with those of corresponding authentic samples to confirm identifications.

**Acknowledgement** We are grateful to Professor N. Tanaka, Science University of Tokyo for sending samples and Mr. Y. Takase of this college for measurements of NMR and MS.