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Fern Constituents: Triterpenoids of *Polypodiodes amoena*

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From a Polypodiaceous fern, *Polypodiodes amoena* (type species of *Polypodiodes*) 29 triterpenoids including a new natural product, 20-hydroxylupane, were isolated and characterized. Comparison of the triterpenoids from this fern and other *Polypodiodes* species with those of ferns of the relating genera clearly indicated that the genus *Polypodiodes* is a genus chemotaxonomically distinguished from the genera *Polypodium* and *Goniophlebium*.

Keywords: Fern; *Polypodiodes amoena*; Polypodiaceae; 20-hydroxylupane; preoleanatetraene

The ferns, *Polypodiodes niponica* (Mett.) Ching (Polypodiaceae, Aone-kadura in Japanese), *P. formosana* (Bak.) Ching (Taiwan-aone-kadura) and *P. amamiana* (Tagawa) Saiki (Amami-aone-kadura),[1,2] are known to produce characteristic types of triterpenoids belonging to the oleanane and migrated oleanane groups as the main triterpenoid constituents.[3-7] To establish the chemotaxonomical identity of the genus *Polypodiodes*,[1] we investigated the triterpenoid constituents of the type species of the genus, *Polypdiodes amoena* (Wall.) Ching (=*Polypodium amoenum* Wall. ex Mett.; Arisan-denda). In the present paper is reported the isolation and characterization of the 28 known triterpenoids (**2–29**), and a new natural product, 20-hydroxylupane (**1**), and the chemotaxonomical discussion of the genus *Polypodiodes* on the bases of these compounds.

RESULTS AND DISCUSSION

An *n*-hexane extract of fresh rhizomes of *P. amoena* was fractionated into six fractions by silica gel column chromatography (CC). From the hydrocarbon, ester and acetate fractions, 27 known triterpenoids (**2–28**) were isolated which were identified by direct comparison (GC, ^{1}H-NMR) with authentic samples (Table 1), in addition to a mixture of cycloartanoid alcohols fatty acids esters as shown in Experimental. From the alcohol fraction, by repeated silica gel CC and prep. TLC, compound **1** and hydroxyhopane (**29**) were obtained.

Compound **1**, needles, mp 187–189° (acetone), $[\alpha]_D$ +82° (c=0.1, $CHCl_3$) was obtained from the benzene elutes (yield 0.0045%). The high resolution MS showed $[M^+]$ at *m/z* 428.4042 corresponding to the molecular formula $C_{30}H_{52}O$ (calcd. 428.4017). Its IR spectrum showed the presence of a hydroxyl group (3470, 1160 cm^{-1}) and EI–MS showed major fragment ions at *m/z* 428 (M^+), 410 (M^+–H_2O), 299, 272, 257, 231, 218, 205, 204 and 191, mostly of which were observed in the MS of lup-20(29)-ene (**2**).[8] The ^{1}H-NMR spectrum (Table 2) in $CDCl_3$ showed the signals for eight tertiary methyls, of which six (from H_3-23 to H_3-28) had chemical shifts similar to those of **2**. The remaining two signals were in somewhat down field indicating that the compound had one hydroxyisopropyl side chain in the molecule. Its ^{13}C–NMR spectrum, showed most of the carbons except for C-20, C-29 and C-30 gave signals closely similar to those of **2** (Table 3). The structure of **1** was established from the detailed analyses of its 2D NMR spectra, particularly HMBC and

Table 1 Known Triterpenoids from *P. amoena*

	yield (mg)	ref.
lup-20(29)-ene (**2**)	60	8
squalene (**3**)	5	9
13βH-malabaricatriene (**4**)	2	10
podioda-7,17,21-triene (**5**)	trace	6
podioda-8,17,21-triene (**6**)	trace	6
preoleanatetraene (**7**)	47	7
eupha-7,21-diene (**8**)	7	11
tillucalla-7,21-diene (**9**)	6	11
shiona-3,21-diene (**10**)	4	11
dammara-18(28),21-diene (**11**)	3	11
hop-22(29)-ene (**12**)	9	12
neohop-13(18)-ene (**13**)	100	13
neohop-12-ene (**14**)	10	13
fern-7-ene (**15**)	9	13
fern-9(11)-ene (**16**)	52	13
filic-3-ene (**17**)	5	13
olean-18-ene (**18**)	5	14
taraxer-14-ene (**19**)	5	14
multuflor-7-ene (**20**)	8	14
friedel-3-ene (**21**)	27	14
friedel-4(23)-ene (**22**)	4	8
cycloartenyl acetate (**23**)	8	15
cyclolaudenyl acetate (**24**)	24	5
cyclomargenyl acetate (**25**)	6	5
cycloeucalenyl acetate (**26**)	14	5
cyclobalanyl acetate (**27**)	3	16
lupenyl acetate (**28**)	2	17
hydroxyhopane (**29**)	trace	8

Table 2 ^{1}H-NMR data (δppm) for **1, 2** and **30**

^{1}H	**1**	**2**	**30**
23	0.846	0.838	0.840
24	0.794	0.792	0.789
25	0.838	0.838	0.820
26	1.061	1.034	1.014
27	0.966	0.954	0.971
28	0.810	0.792	0.773
29	1.124	4.562bs 4.686bs	2.153
30	1.225	1.682	

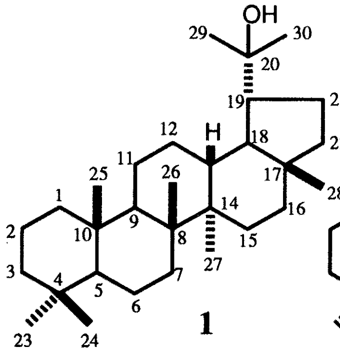
Table 3 ^{13}C-NMR data (δppm) for **1, 2** and **30**

^{13}C	**1**	**2**	**30**
1	40.27	40.30	40.23
2	18.68	18.71	18.67
3	42.11	42.13	42.06
4	33.26	33.26	33.26
5	56.25	56.37	56.31
6	18.65	18.65	18.61
7	34.52	34.25	34.15
8	41.57	41.05	40.94
9	50.34	50.51	50.33
10	37.38	37.38	37.47
11	21.28	20.81	20.78
12	29.22	25.23	27.26
13	37.41	38.02	36.19
14	43.56	42.86	42.70
15	27.54	27.41	27.26
16	35.60	35.63	35.00
17	44.71	43.04	43.08
18	48.31	48.33	49.79
19	49.93	48.01	52.67
20	73.54	151.05	213.00
21	28.78	29.87	27.66
22	40.27	40.03	39.88
23	33.37	33.38	33.37
24	21.58	21.57	21.57
25	16.14	16.10	16.10
26	16.22	16.06	15.97
27	14.85	14.57	14.49
28	19.23	109.27	–
29	24.81	18.01	17.99
30	31.48	19.32	29.10

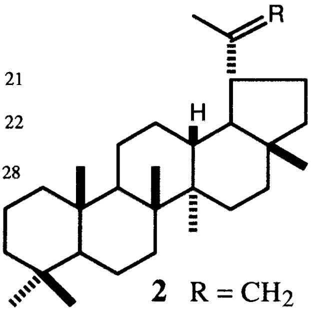
NOESY spectra. The relative stereochemistry at the ring juncture chiral centers was established on the bases of the NOE interactions observed in its NOESY spectrum (Fig. 1). Protons of C-13, 15 and 19 were detected by its HSQC spectrum {δ: 1.75 (H-13), δ 0.80 (H-15) and δ 1.80 (H-19)}. Finally the structure of **1** was confirmed as 20-hydroxylupane by its formation from 29-nor-20-oxolupane (**30**) *via* the Grignard reaction with CH_3MgI.

The compound **1** is considered to be a primary product from a lupanyl cation to lupane triterpenoids on the biogenetic pathway like **29** in hopane skeleton. Although **1** is the first example of 20-hydroxylupanes from natural resources, the isolation of three triterpenoids, **1**, **2**, and **28**, belonging to the lupane group, might be a characteristic feature of *A. amoena*, because they were not found in the ferns studied of *Polypodiodes*, *Goniophrebium*, and *Polypodium* before.

Of the triterpenoids in Table 1, tricyclic compounds, **4**, **5** and **6**, oleanene and migrated oeleanene compounds, **18**, **19**, **20**, **21** and **22**



1



2 R = CH_2
30 R = O

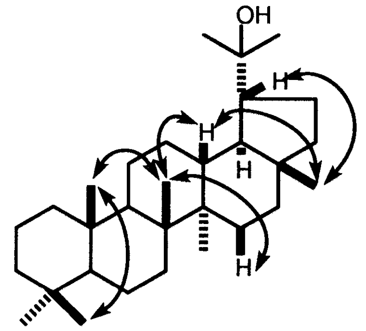
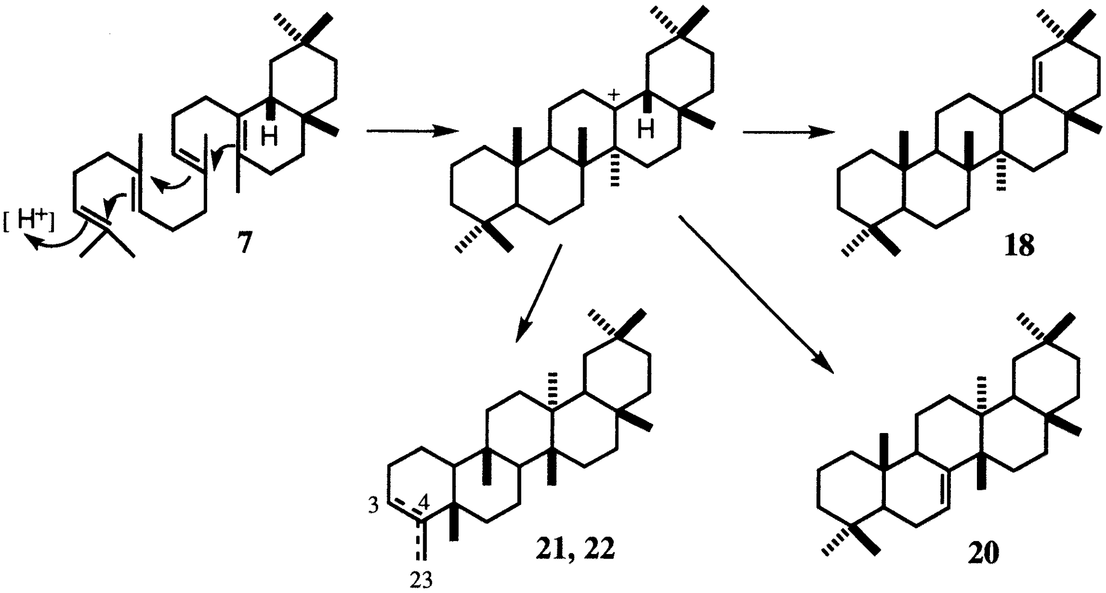


Fig.1. NOEs obtained from NOESY of **1**



7

18

21, 22

20

Fig. 2. Biogenetic hypothesis of oleanene and migrated oleanenes

were commonly obtained from the ferns of the genus *Polypodiodes*, but the compounds **18–22** were hardly found in the *Polypodium* and *Goniophlebium* ferns. From all the *Polypodiodes* ferns examined, a considerable amount of preoleanatetraene (**7**) was isolated. The presence of **7** is interesting because it suggests a new alternative biogenetic route of oleanene and migrated oleanene compounds from **7** (Fig. 2). Consequently, it was clearly indicated that *Polypodiodes amoena* is very close to *P. niponica*,[3,5,6] *P. formosana*[3,5,7] and *P. amamiana*[4] chemotaxonomically.

EXPERIMENTAL

General Mps were measured on a Yanagimoto micro apparatus and were recorded uncorrected. Optical rotations were measured at 23 °C on a JASCO DIP-140 digital polarimeter. The ^1H- (500 MHz) and ^{13}C-NMR (125.65 MHz) spectra were recorded on a JEOL α-500 spectrometer and the chemical shifts were expressed on the δ (ppm) scale with TMS as int. standard. MS was measured on a JEOL JMS D-300 spectrometer. TLC was performed on precoated Kieselgel 60 plates (0.2 mm) by using *n*-hexane–EtOAc (9:1) as the developing phase and the detection was carried out by spraying conc. H_2SO_4 followed by heating. HPLC was performed by using a C_{18} reverse phase column with various solvent systems {viz. $CHCl_3$-CH_3OH (1:1) for triterpenoid hydrocarbons, CH_3CN-$CHCl_3$ (3:2) for fatty acid esters and CH_3CN-$CHCl_3$ (4:1) for triterpenoid alcohols}.

Plant materials *P. amoeana* was collected in Alisan, Taiwan. The voucher specimen has been deposited in the Herbarium of Shôwa Pharmaceutical University, Tokyo.

Extraction and Separation Fresh rhizomes (660 g) were extracted with hot *n*-hexane to give a crude extract (5.6 g). The extract was subjected to silica gel CC with *n*-hexane, *n*-hexane-benzene and benzene-ether mixtures of increasing polarity to give six main fractions, *viz.* triterpenoid hydrocarbons fraction (Fr. 1, 0.37 g), triterpenoid esters fraction (Fr. 2, 1.45 g), triterpenoid acetates fraction (Fr. 3, 0.55 g), triterpenoid alcohols fraction (Fr. 4, 0.21 g), sterols fraction (Fr. 5, 0.81 g) and tar fraction (Fr. 6, 1.20g). Fr. 1 has further subjected to various chromatographic separations, CC, prep. HPLC and prep. TLC to give pure com-pounds, **2**–**22**, as shown in Table 1. These compounds were identified by direct comparison (GC, ^{1}H-NMR) with authentic samples. The ester fraction (Fr. 2) was saponified, and the alcohols were identified to be a mixture of cycoartenol (28 %), cyclolaudenol (28 %), cyclomargenol (5 %) and sitosterol (24 %) by GC and HPLC. The fatty acids were identified as linolenic acid (81 %, M^+ m/z 292), oleic acid (5 %, M^+ m/z 296), palmitic acid (13 %, M^+ m/z 270) and stearic acid (1 %, M^+ m/z 298). The acetate fraction (Fr. 3) was chromatographed on silica gel, followed by HPLC to give **23**–**28**, which were identified by direct comparison (GC, MS, ^{1}H-NMR) with authentic samples. The alcohol fraction (Fr. 4) yielded 20-hydroxylupane (**1**, 3 mg) and **29** on purification by CC followed by prep. TLC.

Preparation of 1 from 2 A solution of **2** (500 mg) was treated with O_3 at –20 °C for 20 min, and then with zinc and acetic acid. Purification of the product by CC yielded 29-nor-20-oxolupane (**30**, 220 mg), mp 165–166 °C (acetone), IR: 1698 cm^{-1}. A solution of **30** (200 mg) in abs. ether (50 ml) was treated with CH_3MgI prepared from magnesium (1g) and iodomethane (10 ml) in abs. ether (30 ml) for 3 hr. The reaction mixture was then treated with methanol and 10 % HCl to give a solid which was purified by flush silica gel chromatography and prep. TLC. Recrystallization gave **1'** (5 mg) as colorless needles, identical (^{1}H-NMR) with **1** obtained from *P. amoena.*

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