FERN CONSTITUENTS: TWO NEW MALABARICATRIENES ISOLATED FROM *Lemmaphyllum microphyllum* var. *obovatum*

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From the fresh whole plants of *Lemmaphyllum microphyllum* var. *obovatum* (Polypodiaceae), we isolated two new oily tricyclic triterpenoid hydrocarbons, 13βH-malabaricatriene (**1**) and 13αH-malabaricatriene (**2**), having the malabaricane skeleton. Their structures were established by physicochemical methods. This is the first example of the isolation of tricyclic triterpenoid hydrocarbons from a natural source.

KEYWORDS fern constituent; *Lemmaphyllum microphyllum* var. *obovatum*; Polypodiaceae; malabaricane; tricyclic triterpenoid; oily hydrocarbon; 13βH-malabaricatriene; 13αH-malabaricatriene

We have reported the new oily tetracyclic[1] and bicyclic[2] triterpenoid hydrocarbons from *Lemmaphyllum microphyllum* PRESL (Mamedzuta) and *L. microphyllum* var. *obovatum* (HARR) C. CHR., (Ryukyu-mamedzuta),[3] family Polypodiaceae. These triterpenoids were rather unstable when exposed to the air. During the course of the studies,[1, 2] we found by GC-MS analysis that the latter species contained new type of triterpenoid hydrocarbons (M^+ m/z 410, $C_{30}H_{50}$). We now describe the isolation and structure of the two new oily triterpenoid hydrocarbons with the tricyclic malabaricane skeleton.

The fresh whole plants (14.1 kg) of *L. microphyllum* var. *obovatum*, collected at Naka-gusuku in Okinawa Prefecture in February, were extracted with n-hexane, and the extract was chromatographed on Si gel with n-hexane. The n-hexane eluant was rapidly chromatographed on 20%-$AgNO_3$-Si gel with a gradient of n-hexane to benzene. The fractions eluted with benzene were further purified with 20%-$AgNO_3$-Si gel chromatography and sandaracopimaradiene (**3**)[4] was removed by HPLC to afford first 13βH-malabaricatriene (**1**), 15 mg (estimated yield, 0.0006% of the dried plant materials), oil, Rt_R 1.16, $[\alpha]_D$ +16.3° ($CHCl_3$, c=0.6), $C_{30}H_{50}$ (M^+ m/z 410.3905), and next 13αH-malabaricatriene (**2**), 2 mg (0.00008%), oil, Rt_R 1.46, $[\alpha]_D$ -23.3° ($CHCl_3$, c=0.1), $C_{30}H_{50}$ (M^+ m/z 410.3938).

The IR absorption bands of **1**, ν_{max}^{film} cm^{-1}: 3192, 1640 and 889, indicated the presence of exomethylene in the molecule. The 100 MHz ^{1}H-NMR of **1** (in $CDCl_3$) also showed the exomethylene proton signals at δ 4.604bs and 4.876bs, and two trisubstituted olefinic protons signal at δ 5.100bt (2H, J=2Hz). There were also four tertiary methyl proton signals at δ 0.858 (C-23), 0.824 (C-24), 0.824 (C-25) and 0.980 (C-26), and three olefinic methyl proton signals at δ 1.598 (C-28), 1.598 (C-29) and 1.686 (C-30). The EI low resolution mass spectrum of **1** (Chart 2) showed the base peak, losing a C_{13} side chain at m/z 231 ($C_{17}H_{27}$). The above data indicated that **1** consists of a tricyclic ring, two with six and one with five members, and a $C_{13}H_{21}$ side chain having one exomethylene and two trisubstituted double bonds. Comparison of the ^{13}C-NMR (TABLE I) of **1** with those of colysanoxide (**4**) obtained from *Colysis elliptica* Ching [5] (Polypodiaceae) and squalene (**5**),[6] showed that the carbon signals of the ring A of **1** were similar to those of **4** and part of the side chain (from C-17 to C-22 and C-28 to C-30) was very similar to those of **5**. Therefore **1** appears to be a malabaricane hydrocarbon except for the confirmation of an absolute configuration of the tricyclic part. To confirm the structure, **1** was treated with 30% BF_3-etherate in ether at 20°C for 3 h to give isomerized product which showed no exomethylene absorption band in its IR spectrum. The product, without purification, was subjected to ozonolysis followed by treatment with zinc powder and acetic acid to give a methyl ketone (**6**), oil, $[\alpha]_D$ +51.8° ($CHCl_3$, c=0.4), $C_{19}H_{32}O$ (M^+ m/z 276.2456), IR ν_{max}^{film} cm^{-1}: 1706. In the ^{1}H-NMR (in $CDCl_3$) of **6**, there were four tertiary methyl signals, at δ 0.836 (C-23), 0.812 (C-24), 0.812 (C-25) and 1.050 (C-26), and a methylketone at δ 2.106. The ORD and CD of **6** showed a positive Cotton

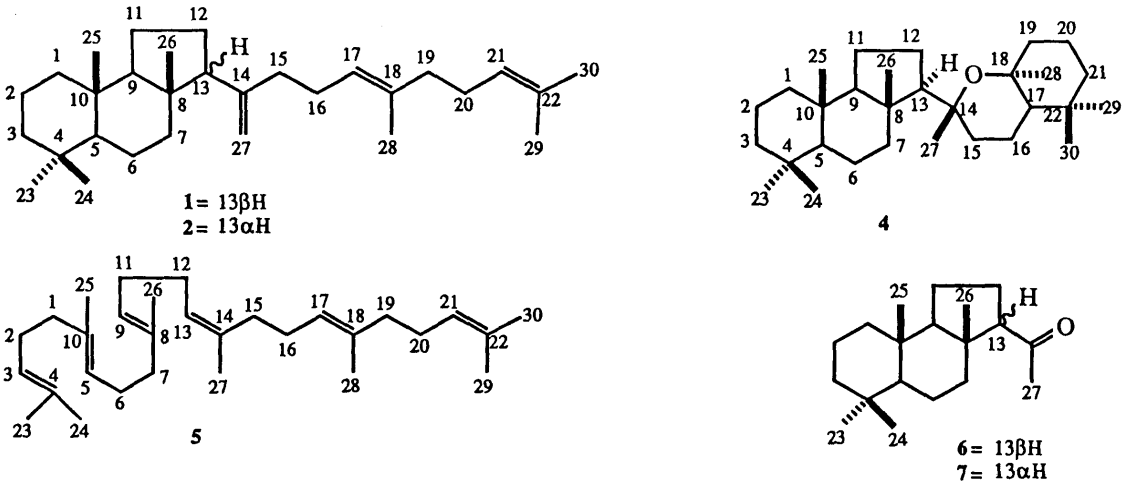

Chart 1

TABLE I. ^{13}C-Chemical Shifts (δ) in $CDCl_3$ Solution (on JEOL FX 100 at 25 MHz)

	Carbon numbers														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	40.5	18.5	42.5	33.0	57.0	19.3	39.3	45.5	55.6	36.8	20.7	24.9	56.6	154.5	36.7
4	40.5	18.5	42.7	33.0	57.6	19.3	43.3	44.5	63.6	37.3	19.6	23.2	62.7	74.0	31.5
5	39.9	26.8	131.1	124.6	131.1	26.9	39.9	134.7	124.4	134.9	28.4	28.4	124.4	134.7	39.9

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	27.8	124.3	135.0	39.7	26.9	124.4	131.2	33.5	21.4	15.6	26.8	108.7	16.0	17.7	25.7
4	15.5	47.9	75.8	43.3	20.5	42.2	33.9	33.5	21.3	15.6	16.0	28.5	24.8	20.5	32.0
5	26.9	124.4	134.9	39.9	26.8	124.6	131.1	25.7	17.6	16.0	16.0	16.0	16.0	17.6	25.7

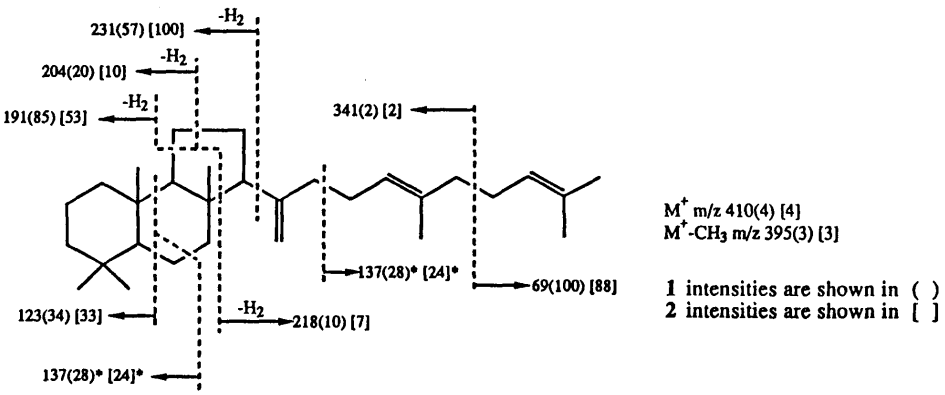

Chart 2. Mass Fragments of **1** and **2** on JEOL D-300 (30 eV)

effect ($[\Phi]_{312}$ +4530.6°, $[\Phi]_{265}$ -4426.4°, $[\Theta]_{293}$ +96699.0°). As a result of the comparison of all of the above data, **6** was identical in all respect with the 13βH-methyl ketone (**6'**) derived from malabaricol, a component of the oleoresin of *Ailanthus malabarica* DC.[7, 8, 9] In addition to the above comparison, **6** was also compared with the degradation product of **4**[5] of the 13αH-methyl ketone (**7**). The optical rotation, ORD and CD of **7** ($[\alpha]_D$ -123.5° ($CHCl_3$, c=0.5), ORD $[\Phi]_{312}$ -4890°, $[\Phi]_{269}$ +6940°, CD $[\Theta]_{294}$ -12600°) showed opposite signs as compared to **6**, and the ^{1}H-NMR of **6** was also different from **7**. Therefore the structure of **1** proved to be (17*E*, 21*E*)-13βH-malabarica-14(27),17,21-triene.[10] The minor compound **2** showed almost the same fragment pattern as **1** in the

EI low resolution mass spectrum except for the relative intensity of the peaks (Chart 2). The presence of an exomethylene group in **2** was confirmed by its IR spectrum (ν_{max}^{film} cm^{-1}: 3190, 1683 and 888) and ^{1}H-NMR (δ 4.738bs, 4.888bs, C-27). The other methyl protons were observed at δ 0.848 (C-23), 0.826 (C-24), 0.848 (C-25), 0.662 (C-26), 1.598 (C-28), 1.598 (C-29) and 1.680 (C-30) and trisubstituted olefinic protons occurred at δ 5.100bt (C-17H and C-21H). The signal of the C-26 methyl group appeared at a remarkably higher field, due to shielding by the exomethylene group. The optical rotation of **1** and 13βH-methyl ketone (**6**) were positive, and of **2** and 13αH-methyl ketone (**7**) were negative. On the basis of this and the biosynthetic indications, **2** was confirmed to be (17*E*, 21*E*)-13αH-malabarica-14(27),17,21-triene, a C-13 epimer of **1**. Both **1** and **2** were also detected in *L. microphyllum* by GC-MS analysis.

A few documented sources of the malabaricane triterpenoids are the flowering plants, *Ailanthus malabarica* [7, 8, 9] and *Pyrethrum santolinoides*, [11] and the sponge, *Jaspis stellifera*.[12, 13] This is the first example of the isolation of malabaricane hydrocarbons from a natural source. It is interesting that *Lemmaphyllum microphyllum* var. *obovatum* contains the linear (squalene), bicyclic,[2] tricyclic (this paper), tetracyclic,[1] and the pentacyclic triterpenoid hydrocarbons.[14]

REFERENCES AND NOTES

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3, mp 30-31°C, $[\alpha]_D$ -10.7°, IR ν_{max}^{KBr} cm^{-1}: 3080, 1634, 908, MS: M^+ m/z 272.2515 ($C_{20}H_{32}$), ^{1}H-NMR (δ): 0.796 (C-18), 0.848 (C-19), 0.876 (C-20), 1.040 (C-17), 4.872dd (J=10.3, 1.7Hz, C-16H), 4.902dd (J=18.7,1.7 Hz, C-16H), 5.206bs (C-14H), 5.792dd (J=10.3, 7.8Hz, C-15H), ^{13}C-NMR (δ): 39.5 (C-1), 19.1 (C-2), 42.2 (C-3), 33.3 (C-4), 54.9 (C-5), 22.7 (C-6), 36.1 (C-7), 137.3 (C-8), 50.7 (C-9), 39.4 (C-10), 18.8 (C-11), 34.7 (C-12), 37.4 (C-13), 128.5 (C-14), 149.1 (C-15), 109.9 (C-16), 26.0 (C-17), 33.8 (C-18), 22.9 (C-19), 15.0 (C-20). The isolation of **3** is the first example from fern plants.
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