FERN CONSTITUENTS: FOUR NEW ONOCERADIENES ISOLATED FROM *Lemmaphyllum microphyllum* PRESL

Kazuo MASUDA, Kenji SHIOJIMA and Hiroyuki AGETA*

Showa College of Pharmaceutical Sciences, 5-1-8 Tsurumaki, Setagaya-ku, Tokyo 154, JAPAN

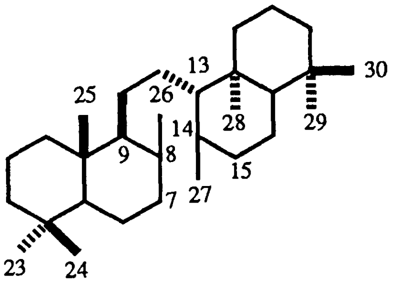
From the fresh whole plants of *Lemmaphyllum microphyllum*, family Polypodiaceae, we isolated, along with α-onoceradiene (**1**) and β-onoceradiene (**2**), four new onoceroid hydrocarbons, onocera-7,14-diene (**3**), onocera-7,13-diene (**4**), onocera-7,14(27)-diene (**5**) and onocera-8,14(27)-diene (**6**). Thier structures were established by spectral and chemical methods.

KEYWORDS triterpenoid; onoceradiene; onocera-7,14-diene; onocera-7,13-diene; onocera-7,14(27)-diene; onocera-8,14(27)-diene; onoceroid; fern constituent; *Lemmaphyllum microphyllum*; Polypodiaceae

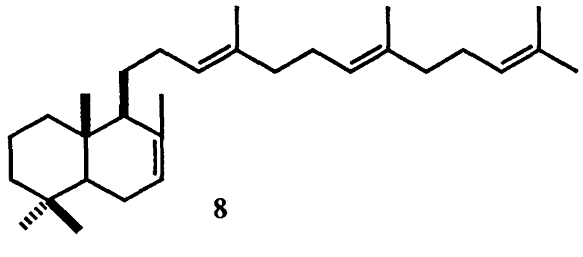
We have reported various kinds of triterpenoid hydrocarbons having a variety of carbon skeletons belonging to the hopane, dammarane, baccharane,1) lupane and oleanane series, and to the onocerane, serratane2) and polypodane3) groups from *Lemmaphyllum microphyllum* PRESL (Mame-dzuta, Polypodiaceae) and *L. microphyllum* var. *obovatum* (HARR.) C. CHR. (Ryukyu-mame-dzuta).4) As a result of further investigation of a n-hexane extract from the fresh whole plants (21.4 kg) of *Lemmaphyllum microphyllum* collected in Shizuoka Prefecture in June, four new onoceroid5) hydrocarbons were isolated along with α-onoceradiene (onocera-8(26),14(27)-diene, **1**), the main hydrocarbon of this fern,2) and β-onoceradiene (**2**). This communication deals with the structures of these compounds.

The extract was chromatograghed on Si gel with n-hexane to give the first mono-ene fractions and then more polar two hydrocarbon fractions. The latter fractions showed several peaks having rather fast retention times on GC.6) After the crystal of **1** was removed, mostly by recrystallization from acetone, the oily fractions were chromatograghed repeatedly on $AgNO_3$-Si gel to afford five crystalline (**1**, **2**, **3**, **5** and **6**) and one oily compound (**4**).

Compound **2**,7) mp 160-162°, $[\alpha]_D$ +122.2° ($CHCl_3$, c=1.0), Rt_R 1.60, was obtained in an estimated yield of 0.00001% of the dried materials. **2** had the molecular formula $C_{30}H_{50}$, as shown by high resolution MS spectrum



1 8(26),14(27)-diene
2 8,13-diene
3 7,14-diene
4 7,13-diene
5 7,14(27)-diene
6 8,14(27)-diene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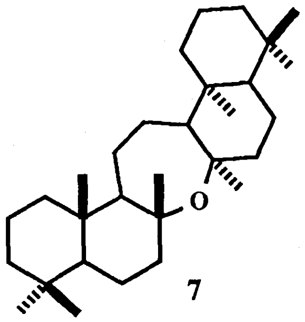

7

Table I. ^{1}H-Chemical Shifts (δ) in $CDCl_3$ Solution (on JEOL GX 270 at 270 MHz)

	Methyl and methylene signals								Olefinic protons attached to C[ ]
	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30	
1	0.868	0.788	0.634	4.544 4.808	4.544 4.808	0.634	0.788	0.868	
2	0.882	0.836	0.964	1.644	1.644	0.964	0.836	0.882	
3	0.858	0.876	0.738	1.700	1.700	0.738	0.876	0.858	[7,14] 5.380bs ($w_{1/2h}$=10)
4	0.854	0.872	0.728	1.778	1.596	0.936	0.834	0.886	[7] 5.390bs ($w_{1/2h}$=10)
5	0.860	0.872	0.706	1.696	4.534 4.820	0.670	0.802	0.872	[7] 5.368bs ($w_{1/2h}$=10)
6	0.884	0.832	0.926	1.590	4.656 4.838	0.658	0.794	0.870	
8	0.850	0.870	0.740	1.684	1.604	1.604	1.604	1.684	[13,17,21] 5.10-5.12 (J=6.3) [7] 5.376bs ($w_{1/2h}$=10)

Table II. ^{13}C-Chemical Shifts (δ) in $CDCl_3$ Solution (on JEOL GX 270 at 68MHz)

	Carbon number									
	1	2	3	4	5	6	7	8	9	10
1	39.0	19.5	42.3	33.6	55.7	24.5	38.5	149.2	58.0	39.6
2	37.7	19.3	41.9	33.5	51.9	19.3	34.1	126.0	141.7	39.1
3	39.4	18.9	42.4	33.0	56.3	23.9	122.2	135.6	50.3	36.7
4	39.5	19.0	42.4	33.0	56.9	23.9	122.1	135.6	50.3	37.0
5	39.2	19.0	42.4	33.0	55.6	23.9	121.9	135.8	50.3	36.7
6	37.2	19.2	41.9	33.6	52.0	19.2	33.7	125.4	141.6	39.0
8	39.3	18.9	42.4	33.0	54.4	23.9	122.1	135.6	50.3	36.8
	11	12	13	14	15	16	17	18	19	20
1	22.6	22.6	58.0	149.2	38.5	24.5	55.7	39.6	39.0	19.5
2	28.8	28.8	141.7	126.0	34.1	19.3	51.9	39.1	37.3	19.3
3	30.0	30.0	50.3	135.6	122.2	23.9	56.3	36.7	39.4	18.9
4	27.9	31.5	141.7	125.8	33.8	19.2	51.9	38.8	37.6	19.2
5	26.0	25.3	57.4	149.0	38.4	24.5	55.7	39.6	39.2	19.5
6	28.0	24.3	59.1	149.2	38.6	24.6	55.7	39.9	39.1	19.5
8	27.3	30.3	124.9	134.9	39.8	26.9	124.3	134.9	39.8	26.8
	21	22	23	24	25	26	27	28	29	30
1	42.3	33.6	33.6	21.7	14.5	106.2	106.2	14.5	21.7	33.6
2	41.9	33.5	33.5	21.8	20.9	20.6	20.6	20.9	21.8	33.5
3	42.4	33.0	33.2	21.9	13.6	22.6	22.6	13.6	21.9	33.2
4	41.8	33.4	33.2	21.9	13.6	22.9	20.2	20.3	21.7	33.4
5	42.2	33.6	33.2	22.0	13.6	22.5	106.3	14.7	21.8	33.6
6	42.3	33.6	33.4	21.8	20.3	20.0	106.0	14.6	21.7	33.4
8	124.5	131.1	33.2	21.9	13.6	22.2	16.2	16.0	17.7	25.7

(M^+ m/z 410.3908). The ^{1}H-NMR spectrum (Table I) of **2** showed only four tertiary methyl and no olefinic proton signals. Although **2** has thirty carbons in the molecule, the ^{13}C-NMR spectrum (Table II) of **2** showed fifteen carbon signals including two olefinic singlet carbon signals. This indicated that **2** has a tetracyclic ring system and a C_2 symmetry axis, which, like **1**, is located between C-11 and C-12. Finally the β-onoceradiene structures of **2** was determined by identifying it with the parent hydrocarbon derived from onoceranoxide (7), by treating it with BF_3-etherate in ether.[1]

Compound **3**, mp 155-156°, $[\alpha]_D$ +3.1° ($CHCl_3$, c=0.4), Rt_R 1.66, $C_{30}H_{50}$ (M^+ 410.3895) was obtained in 0.0002% yield. The ^{1}H-NMR spectrum of **3** (Table I) indicated the presence of a Δ^7–double bond in the molecule, since there were olefinic proton (δ 5.380bs, $w_{1/2h}$ 10Hz) and olefinic methyl proton (δ 1.664bs) signals. Also the EI low resolution MS spectrum showed major typical fragments: m/z (int.) 286 (15), 271 (17), and 204 (100), which are the same as fragments observed in taraxer-14-ene.[8] The symmetrical structure of **3** was proved by overlapped signals of the left and right counterparts on the ^{1}H- and ^{13}C-NMR spectra. And all signals of the ^{1}H- and ^{13}C-NMR spectra of **3** were very similar to those of the counterpart of γ-polypodatetraene(**8**) obtained from Aspidiaceous ferns.[3] Therefore compound **3** is considered to be onocera-7,14-diene.

Compound **4**, oil, $[\alpha]_D$ +87.8° ($CHCl_3$, c=0.7), Rt_R 1.63, $C_{30}H_{50}$ (M^+ 410.3910) was obtained in a 0.0005 % yield. Since there were eight tertiary methyl signals and one olefinic proton signal in the ^{1}H-NMR spectrum (Table I) and thirty carbon signals in the ^{13}C-NMR spectrum (Table II), the compound **4** is asymmetrical. Both the ^{1}H- and ^{13}C-signals of the left counterpart of **4** are very similar to those of **3** and the right counterpart of **4** is also similar to **2**, so compound **4** appears to be onocera-7,13-diene.

Compound **5**, mp 83-85°C, $[\alpha]_D$ +15.6° ($CHCl_3$, c=0.5), Rt_R 1.45, $C_{30}H_{50}$ (M^+ 410.3924) and compound **6**, mp 174-175°C, $[\alpha]_D$ +94.8° ($CHCl_3$, c=0.6), Rt_R 1.42, $C_{30}H_{50}$ (M^+ 410.3889) were obtained in yields of 0.00085 and 0.001% respectively. The ^{1}H-NMR spectrum of **5** showed signals of a Δ^7–double bond proton (δ 5.368 bs $w_{1/2h}$ 10 Hz) and exomethylene protons (δ 4.534bs and 4.820bs). But the ^{1}H-NMR spectrum of **6** showed signals of exomethylene protons (δ 4.656bs and 4.838bs) only. In contrast with **1**, **2** and **3**, there were thirty carbons signals in their ^{13}C-NMR (Table II) because of thier assymetrical structures. As the ^{13}C-NMR signals of the right counterparts of **5** and **6** match each other and are similar to those of **1**, and also the left counterpart of **5** and **6** were similar to those of **3** and **2** respectively, compounds **5** and **6** appear to be onocera-7,14(27)-diene and onocera-8,14(27)-diene respectively. To comfirm the structure, **5** was compared with the parent hydrocarbon derived from onocera-7,14(27)-diene-3,21-dione, a component of *Lansium domesticum*,[9] by Wolff-Kishner-Barton reduction. The physico-chemical data of **5** were identical with those of the derived hydrocarbon in all respects.

Finally the chemical relationships between these onoceradienes have been established by treating each compound with 20% BF_3-etherate in ether at 25°C for 24 h. **2** was obtained from **1**, **3**, **5** and **6**, all of which were identified by GC-MS and ^{1}H-NMR, and **4** was obtained from **5** and **6**.

It is interesting to note that in addition to α-onoceradiene, five possible biosynthetic isomers of onoceradienes have been found in *Lemmaphyllum microphyllum* as natural products. The percentages of every compounds in total onoceradienes (140 mg) were: **1** (41.6%); **2** (0.3%); **3** (4.3%); **4** (10.7%); **5** (19.4%) and **6** (23.7%).

REFERENCES AND NOTES

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3) K. Shiojima, Y. Arai, K. Masuda, T. Kamada, and H. Ageta, Tetrahedron Lett., **51**, 5733 (1983).
4) Our observations in chemical constituents of this fern indicate that this fern must be a species independent from *Lemmaphyllum microphyllum*.
5) We have proposed the term "onoceroid" for triterpenoids biosynthesized from squalene by both-end cyclization.[2]
6) GC were run on a Hitachi 163 with Chromosorb G HP coated with SE-30 (1.4%) at 260°C in a flow of nitrogen (20 ml/min). Cholestane was used as an internal reference; its retention time was set at 3.0 min.
7) $[\alpha]_D$ and ^{13}C-NMR of **2** were taken with a synthetic sample because of an insufficient experimental yield.
8) H. Ageta and Y. Arai, Phytochemistry, **22**, 1801, (1983).
9) K. Habaguchi, M. Watanabe, Y. Nakadaira, K. Nakanishi, A. K. Kiang, and F. Y. Lin, Tetrahedron Lett., **1968**, 3731. We are grateful to Professor Y. Hayashi (Osaka City University) and Professor M. Nishizawa (Tokushima Bunri University) for the gift of onocera-7,14(27)-diene-3,21-dione.

(Received November 18, 1988)