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# FERN CONSTITUENTS: THREE FERNENOIC ACIDS AND ONE ADIANENOIC ACID ISOLATED FROM RHIZOMES OF *MICROSORIUM BRACHYLEPIS* AND *M. NORMALE*

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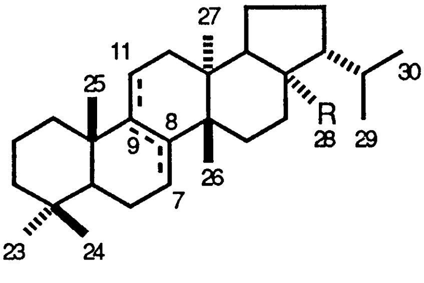
From the fresh rhizomes of the ferns, *Microsorium brachylepis* and *M. normale* (Polypodiaceae) we isolated new triterpenoid acids, fern-7-, fern-8- and fern-9(11)-en-28-oic acids, and adian-5-en-28-oic acid as their methyl esters. Their structures were determined by spectral analysis and chemical correlations.

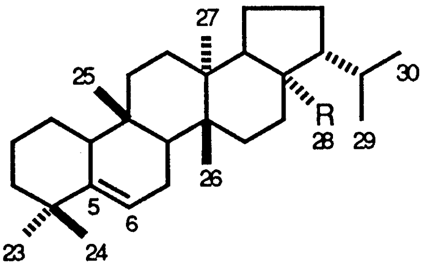
**KEYWORDS** fern constituent; *Microsorium brachylepis*; *Microsorium normale*; Polypodiaceae; pentacyclic triterpenoid; migrated hopane; fern-7-en-28-oic acid; fern-8-en-28-oic acid; fern-9(11)-en-28-oic acid; adian-5-en-28-oic acid

Davallic acid has been reported from a Formosan fern, *Davallia divaricata* BLUME and its structure has been established as fern-9(11)-en-24-oic acid.[1, 2] Since then retigeric acid A (2α,3β-dihydroxyfern-9(11)-en-23-oic acid) and retigeric acid B (2α,3β-dihydroxyfern-9(11)-ena-23,25-dioic acid) have also been reported from foliose lichens, *Lobaria retigera* (BORY) TREV., *L. kazawaensis* (ASAH.) YOSHIM. and *L. saccharinensis* ASAH.[3, 4] Thus only a few examples of fernenoic acids are known in non-flowering plants. In the course of the chemotaxonomic studies on Polypodiaceous ferns, we investigated the triterpenoid components of the fresh rhizomes of *Microsorium brachylepis* (BAKER) NAKAIKE (Nukaboshi-kuriharan in Japanese), collected at Mt. Katsu-u in Okinawa in June and *M. normale* CHING (O-kuriharan in Japanese), collected at Wulai in Taiwan in August. We isolated three fernen- and one adianen-28-oic acids (**1** - **4**) from the former, and **1**, **3** and **4** from the latter as their methyl esters (**1a** - **4a**). From both species we detected by GC-MS analysis many kinds of triterpenoid hydrocarbons having various carbon skeletons. In addition, onoceranoxide[5] and some cycloartanoid derivatives (fatty acid esters, acetates, ketones and alcohols) were isolated and identified from the former species. This communication deals with the isolation and characterization of the compounds **1a** - **4a**.

The n-hexane extract of the fresh rhizomes (564 g) of *M. brachylepis* was separated into 9 fractions by Si gel chromatography with a n-hexane-benzene-ether solvent system. On TLC the fraction 6 (280 mg) eluted with n-hexane-benzene (1:1) showed an acute lilac spot (a typical triterpenoid acid spot) together with some other spots, after spraying with conc-$H_2SO_4$ followed by heating. The above fraction was further purified with Si gel chromatography to give white crystals which showed a single spot on TLC. However, the $^1$H-NMR spectrum of these crystals in $CDCl_3$ showed numerous signals at the tertiary methyl proton region. After these were methylated with diazomethane, the GC[6] revealed four peaks, indicating at least four components, at $Rt_R$ 3.10 (**1a**, 12%, in total peaks),



| | | |
|---|---|---|
| **1** | $\Delta^7$ | R=COOH |
| **1a** | $\Delta^7$ | R=COOCH$_3$ |
| **1b** | $\Delta^7$ | R=H |
| **2** | $\Delta^8$ | R=COOH |
| **2a** | $\Delta^8$ | R=COOCH$_3$ |
| **2b** | $\Delta^8$ | R=H |
| **3** | $\Delta^{9(11)}$ | R=COOH |
| **3a** | $\Delta^{9(11)}$ | R=COOCH$_3$ |
| **3b** | $\Delta^{9(11)}$ | R=H |



| | |
|---|---|
| **4** | R=COOH |
| **4a** | R=COOCH$_3$ |
| **4b** | R=H |

2.50 (**2a**, 4%), 2.70 (**3a**, 24%) and 2.87 (**4a**, 60%). This methyl ester mixture was subjected to 20%-$AgNO_3$-Si gel chromatography repeatedly to afford four crystalline compounds: methyl fern-7-en-28-oate (**1a**), 2 mg (0.001%, estimated yield of the dried rhizomes), mp 162-163°C, $[\alpha]_D$ +1.1° ($CHCl_3$, c=0.1), $C_{31}H_{50}O_2$ ($M^+$ m/z 454.3763); methyl fern-8-en-28-oate (**2a**) 1 mg (0.0006%), mp 188-189°C, $[\alpha]_D$ +43.9° ($CHCl_3$, c=0.2), $C_{31}H_{50}O_2$ ($M^+$ m/z 454.3780); methyl fern-9(11)-en-28-oate (**3a**), 17 mg (0.011%), mp 155-157°C, $[\alpha]_D$ +9.3° ($CHCl_3$, c=0.4), $C_{31}H_{50}O_2$ ($M^+$ m/z 454.3794); and methyl adian-5-en-28-oate (**4a**), 60 mg (0.038%), mp 168-169°C, $[\alpha]_D$ +71.6° ($CHCl_3$, c=1.0), $C_{31}H_{50}O_2$ ($M^+$ m/z 454.3854). In the same way described above, three crystalline compounds: **1a**, 28 mg (0.010%), **3a**, 154 mg (0.056 %) and **4a**, 13 mg( 0.005%) were isolated from the fresh rhizomes (1176 g) of *M. normale.*

The EI low resolution mass spectra of **1a** - **3a** gave major fragments at m/z 454 ($M^+$), 439 ($M^+$-$CH_3$), 379 ($M^+$-$CH_3$-$HCOOCH_3$), 257 (a), 243 (b) and 231 (c). The last three[7] (Chart 1) indicate that they have the fernane skeleton with different double bond positions. Since the fragments a - c are the same ones as observed in their parent hydrocarbons (**1b** - **3b**), the methoxycarbonyl group of **1a** - **3a** can be located in ring E. This is further supported by the $^1$H-NMR study described below. There were five tertiary methyl, two secondary methyl and one methoxycarbonyl groups in **1a** - **3a** (TABLE I). Each olefinic proton of **1a** and **3a** showed the same splitting pattern and a similar chemical shift as that of the corresponding compounds (**1b** and **3b**), while **2a** showed no olefinic proton signal. In addition, the C-27 methyl protons of **1a** - **3a** appeared at a remarkably higher field (Δ 0.157, 0.132 and 0.156 ppm for **1a**, **2a** and **3a** respectively) than those of **1b** - **3b**. This indicates a 1,3 diaxial disposition of the C-27 methyl group and methoxycarbonyl group; therefore a methoxycarbonyl group is located at C-17α.

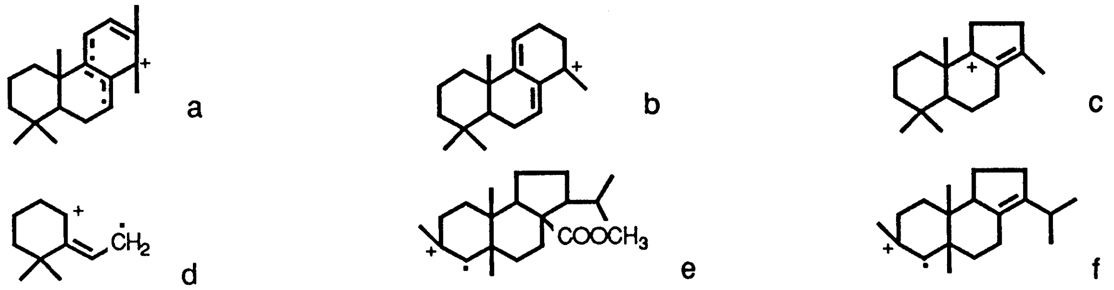


Chart 1

The forth compound **4a** showed one of characteristic fragments at m/z 136 (d) that were observed in adian-5-ene (**4b**)[8] and the other major fragments at m/z 439 ($M^+$-$CH_3$), 379 ($M^+$-$CH_3$-$HCOOCH_3$), 318 (e) and 258 (f) in its EI low resolution mass spectrum (Chart 2). The fragment e (base peak) in **4a** was 44 mass units ($CO_2$) more than a corresponding fragment (m/z 274) which was caused by a cleavage of ring B in **4b**, and the fragment f resulted in a loss of $HCOOCH_3$ from e. The $^1$H-NMR of this compound (TABLE I) indicated the presence of a $\Delta^5$-double bond in the molecule, since the olefinic proton signal showed a typical splitting pattern like that of **4b**. **4a** has a methoxycarbonyl group at C-17α by the same indication explained in **1a** - **3a**.

TABLE I. $^1$H-NMR Chemical Shifts (δ) in $CDCl_3$ on JEOL GX 270 at 270 MHz

| | Proton signals attached to | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | C-23 | C-24 | C-25 | C-26 | C-27 | C-28 | C-29 and C-30 | | Olefinic proton |
| **1 a** | 0.827 | 0.872 | 0.737 | 1.024 | 0.749 | 3.604* | 0.827d (6.1) | 0.872d (6.1) | 5.341ddd (3.1, 3.1, 3.7) |
| **1 b** | 0.844 | 0.878 | 0.742 | 0.996 | 0.906 | 0.742 | 0.830d (6.6) | 0.898d (6.6) | 5.354ddd (3.2, 3.2, 3.7) |
| **2 a** | 0.864 | 0.820 | 0.938 | 0.973 | 0.637 | 3.616* | 0.827d (6.1) | 0.897d (6.1) | |
| **2 b** | 0.875 | 0.828 | 0.946 | 0.946 | 0.769 | 0.769 | 0.826d (6.2) | 0.888d (6.2) | |
| **3 a** | 0.832 | 0.883 | 1.047 | 0.756 | 0.667 | 3.628* | 0.830d (6.0) | 0.899d (6.0) | 5.298ddd (2.2, 2.2, 5.3) |
| **3 b** | 0.858 | 0.892 | 1.054 | 0.736 | 0.824 | 0.760 | 0.830d (6.4) | 0.890d (6.4) | 5.286ddd (3.0, 3.4, 4.0) |
| **4 a** | 1.013 | 1.054 | 0.812 | 0.988 | 0.774 | 3.621* | 0.824d (6.1) | 0.884d (6.1) | 5.503ddd (2.9, 2.9, 5.9) |
| **4 b** | 1.002 | 1.058 | 0.835 | 0.924 | 1.002 | 0.781 | 0.823d (6.4) | 0.893d (6.4) | 5.513ddd (2.5, 2.5, 4.9) |

* Values indicate methyl proton signals of methoxycarbonyl groups. Coupling constants (Hz) are shown in parentheses.

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

| | Carbon numbers | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| **1a** | 38.7 | 19.2 | 42.5 | 33.3 | 48.1 | 24.7 | 116.9 | 144.9 | 51.6 | 35.6 | 16.3 | 31.8 | 36.5 | 41.6 | 29.8 |
| **1b** | 38.7 | 19.1 | 42.5 | 33.2 | 48.1 | 24.6 | 116.3 | 145.4 | 51.6 | 35.6 | 16.1 | 32.5 | 36.1 | 41.6 | 30.4 |
| **2a** | 37.2 | 19.3 | 41.8 | 33.4 | 51.2 | 19.3 | 26.8 | 134.7 | 133.4 | 37.8 | 22.2 | 28.4 | 37.0 | 41.0 | 29.1 |
| **2b** | 37.3 | 19.3 | 41.8 | 33.3 | 51.1 | 18.9 | 27.0 | 134.9 | 133.7 | 37.8 | 20.4 | 27.0 | 36.7 | 41.1 | 30.4 |
| **3a** | 41.5 | 19.6 | 42.5 | 33.7 | 44.9 | 18.1 | 19.6 | 40.2 | 151.6 | 37.7 | 115.5 | 35.5 | 37.1 | 38.1 | 29.7 |
| **3b** | 41.5 | 19.6 | 42.5 | 33.6 | 44.9 | 18.0 | 19.6 | 40.0 | 151.7 | 37.7 | 115.7 | 36.8 | 36.8 | 38.1 | 29.4 |
| **4a** | 23.8 | 21.8 | 40.8 | 34.9 | 145.4 | 117.4 | 34.6 | 51.5 | 35.7 | 44.3 | 26.0 | 28.2 | 38.8 | 39.3 | 29.9 |
| **4b** | 23.8 | 21.9 | 40.9 | 34.8 | 145.6 | 117.6 | 34.3 | 51.8 | 35.7 | 44.3 | 25.9 | 29.2 | 38.7 | 39.4 | 29.2 |

| | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1a** | 33.1 | 54.1 | 56.3 | 21.7 | 30.9 | 60.2 | 31.7 | 33.0 | 21.3 | 13.0 | 24.5 | 19.5 | 176.2 | 22.2 | 23.0 |
| **1b** | 36.4 | 42.9 | 54.2 | 20.1 | 28.3 | 59.7 | 30.7 | 32.9 | 21.2 | 12.9 | 24.1 | 21.1 | 14.1 | 22.1 | 23.0 |
| **2a** | 32.7 | 54.1 | 54.6 | 22.1 | 30.0 | 60.3 | 31.8 | 33.3 | 21.8 | 20.2 | 19.4 | 14.0 | 176.5 | 22.1 | 22.9 |
| **2b** | 36.0 | 43.0 | 52.8 | 19.4 | 28.4 | 59.8 | 30.8 | 33.3 | 21.8 | 20.3 | 22.1 | 15.8 | 14.6 | 22.1 | 23.0 |
| **3a** | 33.2 | 54.2 | 54.0 | 21.9 | 31.1 | 60.3 | 31.7 | 32.8 | 21.7 | 25.0 | 15.5 | 14.1 | 171.0 | 22.1 | 22.9 |
| **3b** | 36.3 | 43.0 | 52.1 | 20.2 | 28.3 | 59.7 | 30.8 | 32.8 | 21.7 | 25.1 | 15.9 | 15.4 | 14.0 | 22.2 | 23.0 |
| **4a** | 30.9 | 54.1 | 54.0 | 21.8 | 31.8 | 60.7 | 31.7 | 29.8 | 29.7 | 16.2 | 17.6 | 13.6 | 176.8 | 22.1 | 22.9 |
| **4b** | 35.5 | 42.9 | 51.9 | 20.0 | 28.4 | 60.1 | 30.8 | 30.0 | 29.8 | 16.1 | 17.9 | 15.1 | 15.8 | 22.0 | 22.9 |

Methyl signals of methoxycarbonyl groups are: 50.7 (**1a**), 50.6 (**2a**), 50.6 (**3a**) and 50.7 (**4a**).

The $^{13}$C-chemical shifts of the left counterparts (rings A - C) of **1a** - **4a** are almost the same as those of their parent hydrocarbons, **1b** - **4b**, respectively (TABLE II). And the signals of a five-member ring moiety including an isopropyl group in **1a** - **4a** are very similar to each other. The carbon signals of C-17 and C-18 shifted to a lower field and the signals of C-16 and C-27 shifted to a higher field in **1a** - **4a** than those of **1b** - **4b**. On the bases of the above evidence, **1a**, **2a**, **3a** and **4a** are proved to be methyl fern-7-en-28-oate, methyl fern-8-en-28-oate, methyl fern-9(11)-en-28-oate and methyl adian-5-en-28-oate respectively.

To confirm these structures, **1a**, **3a** and **4a** were treated with 1N-$H_2SO_4$-$CH_3COOH$-benzene at 20°C for 12 h under $N_2$ gas. **2a** was obtained from **1a**, **3a** and **4a**, and **3a** was also obtained from **4a**. It has been reported that fern-8-ene was obtained along with 8βH-fern-9(11)-ene from **4b**$^{9)}$ under the same conditions as described above. But in the case of **4a**, 8βH isomer was not obtained. The reaction product seems to be different depending on a substituent group effect.

These four compounds are the first examples of naturally occurring 28-oic acids of all the migrated hopane groups. The occurrence of them in the *Microsorium* group is of chemotaxonomic interest. Further chemical studies on some *Microsorium* ferns are still in progress.

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