Diterpenes of *Cheilanthes argentea*, a Fern from Asia

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Cheilanthes argentea, *ent*-8(17),*E*-13-Labdadien-15-oic acid, 3 *R*-Hydroxy-*ent*-8(17),*E*-13-Labdadien-15-oic acid, Diterpenes, Ferns

The major components of the white exudate or farina from the abaxial side of fronds of the fern *Cheilanthes argentea* are the diterpenoid acids *ent*-8(17),*E*-13-labdadien-15-oic acid and its 3 *R*-hydroxy derivative. This is the first report of the first of these compounds as a natural product. Both are highly crystalline. Plants from the Asian mainland and Japan contain mostly the first compound whereas plants from Taiwan contain mostly the 3 *R*-hydroxy derivative. Thus, there are at least two distinct chemical races of the species *Cheilanthes argentea*.

Introduction

The Asiatic fern, *Cheilanthes argentea* (Gmel.) Kunze, is widely distributed in Eastern Asia and occurs in Sibiria from the Altai Mountains to Kamtschatka [1, 2] south into Japan [3] and China, in Taiwan [4], and India to the Malayan peninsula. Specimens of *Cheilanthes argentea* from Taiwan have more robust habit and less dissected laminae than those of Japan (Serizawa, private communication). As the fern is highly variable, however, most pteridologists have not considered these differences adequate to warrant the recognition of formal taxa. This fern characteristically grows on calcareous substrates or stone walls with moderate to full exposure to the sun and good drainage. As is true for many cheilanthoid ferns, the fronds coil during periods of drought and the abaxial side of the lamina is exposed to view. This lower frond surface is covered with "white ceraceous powder" [2] which varies in thickness and possibly in color (variations in color may in some cases be due to confusion of this fern with a related species *Cheilanthes chrysophylla* Hooker). This exudate consists of two major fractions; the lesser of these (about 10%) has been recently shown to consist of a series of novel flavanones [5]. We recently began study of the major portion of the exudate which was composed of diterpenes and appeared to vary within the geographic range of the species.

Materials and Methods

Material of *Cheilanthes argentea* was obtained from plants cultivated in the greenhouse of the Institut für Botanik at the TH in Darmstadt and as dried specimens from Dr. S. Serizawa, Aichi, Japan (Nos. 26084 and 27798), Dr. W.-C. Shieh, Taichung, Taiwan, and Dr. J.-H. Lin, Teipei-Hsien, Taiwan, and from a collection from the Peking Botanical Gardens (No. 475-64) at Kew. Vouchers of collections are maintained at Darmstadt (E.W.).

Air-dried fronds were rinsed with acetone and toluene to dissolve the exudate (4–5% yield), and the combined materials dissolved in boiling benzene. A slightly yellow diterpenoid compound which crystallized upon cooling was removed by filtration [5]. Material from Taiwanese plants (**I**) was recrystallized two times from benzene and that from Japanese plants (**II**) two times from ethanol. Purity of the compounds was determined by TLC on silica gel (toluene/2-butanone, 9:1, solvent A).

These compounds appeared as gray spots (TLC on silica gel in solvent A) after spraying with $SbCl_3$ (R_f 0.45 and 0.08 respectively). They may also be detected on polyamide TLC plates (toluene/petrol$_{100-140}$/2-butanone/methanol, 6:3:1:0.5, R_f **I** 0.80 and **II** 0.41) when viewed with UV_{254}.

Optical rotations were measured on a Perkin Elmer Polarimeter (Model 241) in $CHCl_3$. Mass spectra were recorded on a Varian MAT 112-S mass spectrometer (direct inlet system), ^1H NMR spectra on a Varian XL-200 and ^{13}C-NMR spectra on a Varian XL-100 spectrometer.

Results

The farina of *Cheilanthes argentea* plants collected in Taiwan yielded diterpenoid **I** as the major component whereas those from Japan, the Asian mainland, and in our greenhouses yielded primarily diterpenoid **II**.

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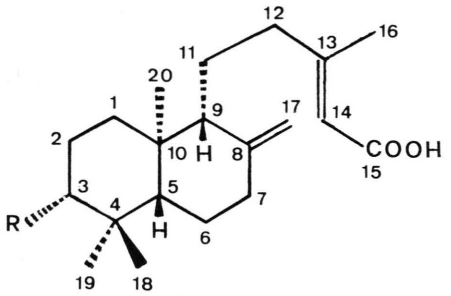

I R = H

II R = OH

Compound **I**: colorless needles, m.p. 93°, $C_{20}H_{32}O_2$ (M^{+} 304), $[\alpha]_D = -46.5°$ ($c = 1.15$). ^{1}H-NMR: 0.69, 0.82 and 0.88 (each s, 10-Me, 4-Me$_2$), 2.09 (*d*, $^4J = 1$ Hz, 13-Me, *E*-configuration), 4.52 and 4.88 (each br. *s*, $w_{1/2} = 4$ Hz, 17-H$_2$), 5.71 ("*d*", $^4J = 1$ Hz, 14-H). These data, together with the ^{13}C-NMR spectrum (Table I) establish **I** as *ent*-8(17),*E*-13-labdadien-15-oic acid (*ent*-anti-copalic acid or *ent*-9-epicopalic acid).

Compound **II**: colorless needles, m.p. 159–160°, $C_{20}H_{32}O_3$ (M^{+} 320), $[\alpha]_D = -42.6°$ ($c = 1.00$). ^{1}H-NMR: 0.69, 0.77 and 1.00 (each s, 10-Me, 4-Me$_2$), 2.16 (*d*, $^4J = 1$ Hz, 13-Me, *E*-configuration), 3.30 (*dd*, $^3J = 11$ and 4 Hz, 3-H), 4.51 and 4.87 (each br. *s*, $w_{1/2} = 4$ Hz, 17-H$_2$), 5.66 br. *s*, $w_{1/2} = 5$ Hz, 14-H). ^{13}C-NMR (Table I). Thus **II** is 3*R*-hydroxy-*ent*-8(17),*E*-13-labdadien-15-oic acid.

Table I. ^{13}C-chemical shifts of compounds **I** and **II** in $CDCl_3$, δ-Scale, TMS = 0.0 ppm.

Carbon	Shift [ppm]	
	Compound **I**	Compound **II**
1	40.1	37.1
2	19.4	27.9
3	42.2	78.9
4	33.6	39.4*
5	55.3	54.6
6	24.5	24.0
7	38.3	38.1
8	148.4	147.7
9	56.2	55.9
10	39.7	39.1*
11	21.5	21.9
12	39.1	39.9
13	164.1	163.7
14	115.0	115.1
15	172.5	171.8
16	19.2	19.2
17	106.5	106.9
18	33.6	28.3
19	21.7	15.4
20	14.5	14.5

Signals marked with * may be interchanged.

In addition to detailed study of the ^{1}H- and ^{13}C-NMR spectra and comparison of physical data [6–8], the structures of the bicyclic diterpenes **I** and **II** were confirmed by the characteristic mass spectral fragmentation pattern [9]. Absolute configurations were established by comparison of chiroptic data with known compounds [6–8].

Discussion

A number of species of ferns, especially those of the genera *Cheilanthes, Notholaena,* and *Pityrogramma,* are characterized by the "waxy induments" or "ceraceous powders" formed on the abaxial side of the frond. This material is more appropriately called simply an exudate or farina as it does not contain more than small amounts of waxes and these are probably inadvertently isolated from accompanying epidermal tissues. The major component of most of these exudates is flavonoid aglycones with varying degrees of methylation [10], although in some instances other compounds have been found. The exudates of *Notholaena dealbata* and *N. limitanea,* for example, contain dihydrostilbene derivatives [11]. This is the first report of the occurrence of diterpenes in the trichome exudates of ferns. These unusual compounds occur as major components in the exudate and a single compound predominates in each of the chemical races. In contrast to many other diterpenoid compounds these crystallized readily from crude isolates.

This is the first reported isolation of **I** as a naturally occurring compound, although compounds **I** and **II** (as their methyl esters) and a series of diterpenes with labdane and *ent*-labdane skeletons (equatorial side chain) have been isolated previously from resins and oils of members of the Fabaceae (Caesalpinoideae). Among these are *Oxystigma* [12, 13] and *Trachylobium* species [14] from Africa and *Hymenaea* [15] and *Copaifera* species [6, 16] from South America. Copaiba oil from *Copaifera* species is an item of commercial importance in South America. This diterpenoid acid is the enantiomer of the compound previously reported as "copaiferic acid" [16] and "*anti-copalic* acid" [7]. *Anti*-copalic acid was shown to be 8(17),*E*-13-labdadien-15-oic acid by partial synthesis from manool [7]. The crystalline compound has m.p. 88–90°, $[\alpha]_D = +47°$ ($CHCl_3$), and its methyl ester is an oil, $[\alpha]_D = +47°$ [7]. The structure of this compound has been unambiguously established [7, 16]. The methyl ester of this acid has $[\alpha]_D =$ ca $+46°$ (see *e.g.* [17]), whereas the enantiomeric methyl ester with $[\alpha]_D =$ ca $-46°$ belongs to the *ent-anti*-opalic series [12, 14].

A compound called "copalic acid" was first isolated as an oil ($[\alpha]_D = -6.9°$ ($CHCl_3$)) from "Brazil Copal" and the structure tentatively assigned as *ent*-8(17),*E*-13-9-epilabdadien-15-oic acid (axial side chain) [15]. Later the pure, crystalline compound was isolated from Brazilian Copaiba oil and the proposed structure confirmed [6]. Copalic acid has m.p. 106–107°, $[\alpha]_D = -4.4°$ ($CHCl_3$), and its methyl ester is an oil, $[\alpha]_D = -11.2°$ [6]. Compounds of this series are diastereomers (C(9)-epimers) of the *anti*-copalic and *ent-anti*-copalic series and only these are to be called "copalates", a term which should not be used to refer to the former ones (see *e.g.* [12]).

Compound **II** is the 3 *R*-hydroxy derivative of **I** which has been previously isolated from Brazilian Copaiba oil [6]. The configuration at C (3) was not definitively established. **II** is the enantiomer of the saponification product from 3 *S*-acetoxy-8(17),*E*-13-labdadien-15-oic acid which occurs naturally in the leaves of *Metasequoia glyptostroboides* [8].

In an effort to ascertain the distribution of compounds **I** and **II** over the geographical range of the species, we examined specimens from Japan (14 samples), from mainland Asia (14 samples) and from Taiwan (7 samples). In all 14 specimens from Japan, compound **II** was the major component. These exudates also contained several rarely encountered flavonoids [5] and often 7-O-methyl kaempferol and 7,4′-di-O-methyl kaempferol [18] and other minor components. Comparison of chromatographic and physical data suggests that one of these minor components is eupalitin (7-O-methyl-6-methoxy kaempferol) (Wollenweber, unpublished data). The 14 samples from the Asian mainland all produce compound **II**, but differ somewhat in flavonoid composition. In contrast, plants of Taiwan produce compound **I** and differ in flavonoid composition. As the Taiwanese plants differ markedly in the deterpenoid content of their exudate (compound **I** is restricted to Taiwan), these chemical races may be recognized in the laboratory but doubtfully in the field. Plants growing in Taiwan represent a geographically isolated group of populations of *Cheilanthes argentea*.

[1] M. M. Il'in, Flora of the USSR, **Vol. I**, p. 74 (p. 58, English translation). Izdatelstvo Akademii Nauk SSR, Leningrad 1934.

[2] K. H. Hooker, Synopsis Filicum, 2nd Ed., p. 142, London 1974.

[3] J. Ohwi, Flora of Japan (English Ed.) p. 41, Smithsonian Institution Washington, D.C. 1965.

[4] Editorial Committee, Flora of Taiwan, **Vol. I**, p. 282, Taipei 1975.

[5] E. Wollenweber, V. H. Dietz, D. Schillo, and G. Schilling, Z. Naturforsch. **35c**, 685 (1980).

[6] J. R. Mahajan and G. A. L. Ferreira, An. Acad. Brasil. Cienc. **13**, 611 (1971).

[7] D. Do Khac Manh, M. Fétizon, and M. Kone, Tetrahedron **31**, 1903 (1975).

[8] S. Braun and H. Breitenbach, Tetrahedron **33**, 145 (1977).

[9] C. R. Enzell and R. Ryhage, Arkiv Kemi **23**, 367 (1967).

[10] E. Wollenweber, Am. Fern J. **68**, 13 (1978).

[11] E. Wollenweber and J. Favre-Bonvin, Phytochemistry **18**, 1243 (1979).

[12] W. Sandermann, K. Bruns, and W. Reichheim, Tetrahedron Letters **1967**, 2685.

[13] C. W. L. Bevan, D. E. U. Ekong, and J. I. Okogun, J. Chem. Soc. (C), **1968**, 1067.

[14] G. Hugel, A. C. Oehlschlager, and G. Ourisson, Tetrahedron **22**, Suppl. 8, Part I, 203 (1968).

[15] T. Nakano and C. Djerassi, J. Org. Chem. **26**, 167 (1961).

[16] F. Delle Monache, E. Corio, I. Leoncio d'Albuquerque, and G. B. Marini-Bettolo, Ann. Chim. (Roma) **1969**, 539.

[17] D. F. Zinkel, J. K. Toda, and J. W. Rowe, Phytochemistry **10**, 1161 (1971).

[18] V. H. Dietz, Doctoral Thesis, Technische Hochschule Darmstadt (1980).