Ent-Kaurenoic Acid: A Diterpene as Frond Exudate on Ferns of the Genus *Notholaena*

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The frond exudates of the two gymnogrammoid ferns, *Notholaena peninsularis* and *N. pallens*, consist mainly of a diterpene, accompanied by small amounts of flavonoid aglycones. The diterpenes from both sources are shown to be identical. Their structure has been established by spectroscopic methods as (−)-kaur-16-ene-19-oic acid. This result is confirmed by direct comparison with an authentic sample.

Introduction

Farinose exudates on fronds of gymnogrammoid ferns have been shown to consist of flavonoid aglycones in many species [1, 2]. Recently we found two labdane diterpenes as typical and major farina constituents in two chemical races of *Cheilanthes argentea* [3]. We have now been able to identify another diterpene as the major exudate constituent of two *Notholaena* species, in which it is excreted along with small amounts of flavonoids.

Notholaena peninsularis Maxon. & Weath. is a species that, according to Tryon's monography [4], occurs in Baja Californica, Mexico. The material studied here is from the first population of this species ever found in the Mexican inland. The upper surface of the fronds is "sparsely and minutely ceraceous-glandular, the lower surface densely white-ceraceous". Rhachis and stipe are also more or less ceraceous-glandular, which character discerns this species from the closely related *N. incana* Presl. [4].

Notholaena pallens Weath. is more widespread in Mexico than *N. peninsularis*. it has been confused previously with *N. palmeri* Baker, from which, according to Tryon, it is distinguished by its ceraceous stipe and the presence of scales on the ceraceous-glandular rhachis. The upper surface of the fronds is ceraceous-glandular and the lower densely white-ceraceous [4].

From both species we have now isolated a diterpene of the kaurane type, which is well known as a constituent of higher plants but has not been found previously as a farina constituent on a fern.

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Materials and Methods

Notholaena peninsularis was collected in 1979 near Malinalco, Edo. Mexico, by E. A. Ulrich. A voucher is kept at US (no. 2882322). *Notholaena pallens* was collected in 1981 a few miles SW of Matamoros, Edo. Durango, Mexico, by E. Wollenweber and T. Reeves. Vouchers are kept in the collectors' herbaria.

The air-dried fern fronds were rinsed with acetone to dissolve the farinose exudate. The concentrated material was dried onto silica and chromatographed over several columns of silica, eluted with toluene and increasing amounts of 2-butanone and methanol. The diterpene was recovered as the major constituent of several fractions in both cases. Total yield was more than 95% of the crude material.

Crystallized from methanol, the diterpene forms large colourless crystals or white needles, mp. 167–174° (from *N. peninsularis*) and 176–178° (from *N. pallens*). A mmp of 173–174° and direct comparison by TLC show that the two products are identical. On silica with solvent toluene/2-butanone 9 : 1 they form tailing spots at R_f 0.52–0.63 (depending very much on concentration), visible only after spraying with $SbCl_3$ and heating. $[\alpha]_D = -106°$ for both substances and the ^{1}H-NMR-spectra further prove their identity. Finally the MS also is the same for the products from both plant sources. *m/z* (rel. int.) 302 (73%, M^+), 287 (29), 259 (38), 243 (23), 241 (24), 213 (22). High resolution MS yields M^+ 302.2246; calc. for $C_{20}H_{30}O_2$: 302.2249. Methyl derivative (diazomethane) *m/z* 316 (100%, M^+), 301 (30), 273 (45), 257 (70), 241 (30), 213 (10). TMSi-derivative *m/z* 374 (100%, M^+), 359 (60), 331 (30), 257 670), 256 (50), 241 (40). ^{1}H-NMR ($CDCl_3$, 250 MHz; ppm/TMS): δ 0.94 and 1.24 (each s, 3H; 20-Me, 18-Me), 2.64 (m, 1H; 5-H), 4.74 and 4.79 (each br. sign., 1H; 17-H2). These signals show only

two methyl groups and two olefinic protons, but no protons at lower field. Together with the MS data this suggests a tetracyclic diterpene. This is confirmed by the ^{13}C-NMR-spectrum, which is in accordance with [5]. X-ray analysis indicates a kaurane skeleton. Details of these studies will be reported elsewhere.

In total these data establish the compound under investigation as (−)-kaur-16-ene-19-oic acid. Comparison with literature data confirms this result: m.p. (MeOH) 179−181 °, m.p. (MeOH/H_2O) 169−171 °, $[\alpha]_D = -110°$ ($c=3$, $CHCl_3$) [6]. Finally the correctness of this structure and configuration is confirmed by direct comparison with an authentic sample of this diterpene (co-TLC, ^{1}H-NMR; mmp with product from *N. pallens:* 168−170 °).

Discussion

(−)-Kaur-16-ene-19-oic acid was found probably for the first time in 1964 as leaf constituent of a *Bayeria* species [7]. Since then it has been reported from many plant sources as a minor constituent, for instance from roots and aerial parts of two *Helichrysum* species [8], from aerial parts of *Stevia setifera* [9], and from roots and stems of *Aristolochia triangularis* [10]. This is the first time, however, that this kaurenoid acid has been encountered as a constituent of farinose exudate on fern fronds, and only the second time that a diterpene in general has been found to form a fern farina. In this context it may be mentioned that on another fern, namely *Plagiogyra formosana* Nakai, the triterpene hydrocarbon 9(11)-fernene and its 21-epimer have recently been found to form the whitish layer on the lower surface of sterile pinnae. The same products cause the glaucous appearance of the under surface of the leaves of *Polypodium glaucinum* [11]. It is assumed that in the course of further studies in this field more di- and tri-terpenes, and maybe also sesquiterpenes, will be found as constituents of such fern exudates.

As in *Cheilanthes argentea* [3], flavonoids occur only in trace amounts in the farina of *Notholaena peninsularis* and *N. pallens.* Two of them are probably new natural products. Analysis is under way and the results will be reported elsewhere.

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