Kaurane-Type Diterpenes from Fern Frond Exudates

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The viscid exudate found on fronds of the fern, *Cheilanthes kaulfussii,* consists of terpenoids in which flavonoid aglycones are dissolved. One of the terpenes is shown to be *ent*-kaur-16-en-19-oic acid. The farinose frond exudate of *Notholaena pallens* contains this acid as the major constituent and its 3*R*-hydroxy derivative, together with flavonoid aglycones as minor components. In *N. incana,* two of the diterpenes that form the farinose exudate along with flavonoid aglycones have been identified as 16*R*-*ent*-kauran-17-oic acid and 16*R*,17-dihydroxy-3-oxo-*ent*-kaurane (abbeokutone).

Introduction

"Farinose" or "ceraceous" frond exudates are produced by ferns of the genera *Cheilanthes, Notholaena, Pityrogramma* and some others belonging to the family Pteridaceae *sensu* Tryon [1]. These exudates have mostly been found to represent mixtures of flavonoid aglycones [2, 3], often forming characteristic profiles that can be evaluated taxonomically [4]. To our knowledge terpenoids have so far been encountered as fern exudate constituents in only few cases [5–7]. We now wish to report the identification of three further diterpenes from some additional fern species.

Materials and Methods

Cheilanthes kaulfussii Kunze was collected in May, 1983 (G. Yatskievych and E. Wollenweber), ca. 3 miles NW of Malinalco, Edo. Mexico, Mexico, where it is common in a roadside area on volcanic outcropping. A voucher specimen (G. Yatskievych 83-135) is kept in the Herbarium of Indiana University (IND). Plants of this as well as of the following species are also cultivated in a greenhouse at the Botanischer Garten der TH Darmstadt. *Notholaena pallens* Weath was collected in December, 1981 (T. Reeves, L. Reeves, and E. Wollenweber), some 19 miles south of Hidalgo del Parral, Edo. Durango, Mexico. Voucher specimens (T. Reeves 7508) are kept at the Biological Center of Boston University (BSN) and in the collectors' personal herbaria. *Notholaena incana* originates from two sites. One is rocky grassland ca. 5 roadmiles NW of Patagonia, Santa Cruz Co., Arizona, on Forest Road 143, off State Highway 82 (coll. E. Wollenweber and G. Yatskievych, December, 1981). The other is a roadside area of volcanic outcrop 3 miles NW of Malinalco, Edo. Mexico, Mexico, where the fern is common in the rocks (coll. G. Yatskievych and E. Wollenweber, May, 1983). Voucher specimens are kept at The University of Arizona Herbarium in Tucson (ARIZ), at IND, and in E. W.'s personal herbarium.

Fronds were carefully clipped in the field and air-dried in brown paper bags, amounts collected varying between 46 *(C. kaulfussii)* and 230 g *(N. incana)*. The frond exudate was recovered by rinsing the dry plant material with acetone. Yields of exudate material per frond dry weight were as follows: *Cheilanthes kaulfussii* 10.6%, *N. pallens* 6.5%, *N. incana* 4.5%. The concentrated solutions were dried onto silica and chromatographed over columns of silica, eluted with toluene and increasing amounts of methylethyl ketone and methanol. From relevant fractions the diterpenes were obtained as crystalline products. From *Cheilanthes kaulfussii,* a small amount of diterpene **1** was isolated, the properties of which agreed with those of the major diterpene reported previously from *Notholaena pallens* [6]. The remaining fractions of the latter species now yielded a second kaurenoid diterpene **2a**. From the frond exudate of *Notholaena incana* we have so far isolated two diter-

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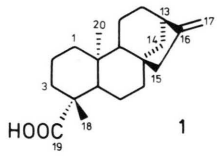
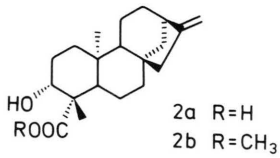
penoids, namely the major product **3**, and one minor product, **4**.

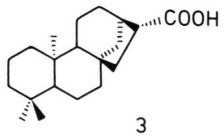
TLC was performed on silica with the solvents toluene/methylethyl ketone 9:1 and toluene/dioxane/HOAc 18:5:1. Spots were visualized by spraying with $MnCl_2$ reagent (3 g $MnCl_2$ dissolved in 150 ml H_2O, 750 ml MeOH and 30 ml conc. H_2SO_4 added), followed by heating to 120 °C. Optical rotations were measured on a Perkin-Elmer Polarimeter Mod. 241 in $CHCl_3$, CD spectra on a JASCO-J-500A Dichrograph in MeOH and NMR spectra on Varian XL-200 (200 MHz/^{1}H) and FT-80A (80 MHz/^{1}H; 20 MHz/^{13}C) in $CDCl_3$ (δ in ppm, TMS = 0). Mass spectra were recorded on Varian MAT 311 and 112S.

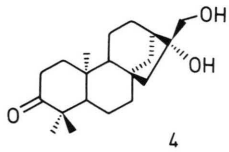
Results

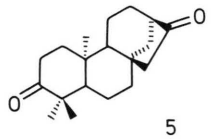
ent-Kaur-16-en-19-oic acid (**1**, (−)-kaur-16-en-19-oic acid)* m.p. 177−178 °C (EtOH), was identified on the basis of its physical and spectral properties and by direct comparison with an authentic sample [6].











Compound **2a**, colourless prisms (benzene), m.p. 221−223 °C, $C_{20}H_{30}O_3$ (M^+ 318), $[\alpha]_D$ = −88.1° (c = 1.4), is a hydroxy derivative of **1**. The ^{1}H NMR signals at δ 0.98 (s, Me-10), 1.46 (s, Me-4), 2.70 (br, s, $w_{1/2}$ = 10 Hz, H-13), 3.14 (dd, $^3J_{aa}$ = 11, $^3J_{ea}$ = 5.5 Hz, H-3), 4.74 and 4.80 (each br, s, $w_{1/2}$ = 6 Hz, H_2-17) together with the ^{13}C NMR data (Table I) are in accordance with a kaur-16-ene skeleton and suggest an equatorial hydroxy group at C-3. Methylation of **2a** with diazomethane in ether yielded the ester **2b**, colourless needles (pentane), m.p. 151−153 °C, $C_{21}H_{32}O_3$ (M^+ 332), $[\alpha]_D$ = −98.3° (c = 0.53), ^{1}H NMR at δ 3.67 (s, MeO-19).

Ester **2b** was compared directly with an authentic sample [8] and was shown to be identical in every respect, including the CD curve (213 nm/Δε = −1.36, 217sh/Δε = −1.07), thus establishing the genuine compound **2a** as 3*R*-hydroxy-*ent*-kaur-16-en-19-oic acid (3-hydroxy-*ent*-kaur-16-en-19-oic acid = *ent*-3β-hydroxykaur-16-en-19-oic acid = (−)-3α-hydroxykaur-16-en-19-oic acid)*.

The major compound **3**, colourless needles (benzene/petrol), m.p. 216−217 °C, $C_{20}H_{32}O_2$ (M^+ 304), $[\alpha]_D$ = −66.1° (c = 0.35) is a saturated diterpenoid acid. ^{1}H NMR signals at δ 0.81, 0.85 and 1.00 (each s, Me_2-4, Me-10), the absence of signals above 2.7 ppm and the ^{13}C NMR data (183.8, s, CO-16; see Table I) confirm the kauranoid constitution **3**. Final proof of the structure was accomplished by direct comparison with an authentic sample [9]. The superimposable CD curve (212 nm/Δε = −1.37) unambiguously confirmed the same absolute configuration

Table I. ^{13}C NMR.

C-Atom	δ [ppm] (20 MHz, $CDCl_3$, TMS = 0)			
	1	**2a**	**3**	**4**
1	40.5	39.2	38.2	39.1
2	18.9	28.0	18.7	33.8
3	37.6	77.9	42.1	218.1
4	43.6	48.6	33.3	47.0
5	54.9	54.9	56.1*	54.1
6	21.6	21.6	21.6	21.5
7	41.1	41.0	41.0$^+$	40.7
8	44.0	43.8	45.2	44.3
9	56.9	56.3	56.2*	55.2
10	39.5	39.2	39.3	38.3
11	18.2	18.4	18.7	18.6
12	32.9	32.9	31.3	25.9
13	43.7	43.6	45.5	45.1
14	39.5	39.5	40.5$^+$	36.7
15	48.8	48.6	44.8	52.6
16	155.6	155.2	41.5	81.6
17	102.8	103.1	183.8	66.0
18	28.8	23.8	33.7	27.1
19	184.7	182.8	21.6	20.7
20	15.4	15.5	17.5	17.6

*/$^+$ Assigned signals may be interchanged.

* Since there exist discrepancies in the current literature concerning the nomenclature of such diterpenoids, correct alternative names are cited here.

for the isolated and the reference compound, establishing **3** as 16*R*-*ent*-kauran-17-oic acid = (*ent*-16β-kauran-17-oic acid = (−)-16α-kauran-17-oic acid)*.

Compound **4**, colourless needles (EtOH), m.p. 187–188 °C, $C_{20}H_{32}O_3$ (M^+ 320), $[\alpha]_D$ = −71.9° (c = 0.85), IR 3570, 3440, 1700 cm^{-1} is a kauranoid dihydroxy ketone: ^1H NMR signals at δ 1.03 and 1.08 (6H) (each s, Me_2-4, Me-10), 2.07 (br, s, $w_{1/2}$ = 11 Hz, H-13), 2.47 (dd, ^3J = 6.1, 8.6 Hz, H-2), 3.68 and 3.79 (AB, ^2J = 11 Hz, 16*R* configuration [10]). ^{13}C NMR signals at 52.6 (t, C-15, 16*R* configuration [10]), 66.0 (t, C-17), 81.6 (s, C-16), 218.1 (s, C-3), see also Table I.

The low field position of the carbonyl-C could account for either position at C-3 or C-14 [11], whereas the IR absorption at 1700 cm^{-1} suggested an unstrained 6-ring ketone (C-3). On treatment with HIO_4/MeOH, **4** yielded the 3,16-dicarbonyl compound **5**, $C_{19}H_{28}O_2$ (M^+ 288), IR 1745, 1708 cm^{-1} which clearly differed from the corresponding 14,16-diketone (IR 1765, 1730 cm^{-1}) [12].

The correctness of structure **4** was confirmed by direct comparison with an authentic sample of this diterpene ("abbeokutone" [13]): all the physical and spectral data were identical in every respect (CD 290 nm/Δε = −1.02), establishing also the absolute configuration as depicted. Thus, compound **4** is 16*R*,17-dihydroxy-3-oxo-*ent*-kaurane (16α,17-dihydroxy-3-oxo-*ent*-kaurane = *ent*-16β,17-dihydroxy-3-oxokaurane = (−)-16α,17-dihydroxy-3-oxokaurane)*.

Discussion

Among the diterpenes of ferns, the *ent*-kauranoids constitute the largest group [14]. The compounds **2a**, **3**, and **4** are reported here for the first time as constituents of ferns, and the occurrence of 3-oxygenated kauranoids is new, whereas this oxidation pattern seems to be common in the corresponding labdane- and pimarane derivatives [14].

The diterpenes **3** and **4** are quite rare natural products: to our knowledge, the acid **3** has been identified only once before as a natural product (isolated and characterized as its methyl ester): as a minor component in *Baccharis minutiflora* (Asteraceae) [15], whereas it had been prepared earlier by two different partial synthetic routes [9, 16]. "Abbeokutone" (**4**) was first isolated and characterized from the bark of *Didymosalpinx abbeokutae* (Rubiaceae) [13]. Recently it was shown (characterized as its 17-O-acetate) to be a minor constituent of *Hymenopappus newberryi* (Asteraceae) [17].

Compound **2** has been identified previously in several plant families (see *e.g.* [8, 18, 19]). However, it was mostly isolated and characterized only after derivatization as methyl ester **2b**, or as the corresponding 3-O-acetate, or after saponification of these derivatives, thus giving rise to considerable inconsistencies in the physical data. A reliable characterization was performed by partial synthesis [8, 20]. Thus, the unusually high m.p. 221–223 °C recorded by us may be due to the isolation, in this case, of the pure genuine compound directly from the plant source in reasonable amounts.

Some years ago we have shown [21] that the triterpene 9(11)-fernene and its 21-epimer occur as a thin epicuticular layer on rhachis and pinnae of the fern *Polypodium glaucinum* Mart. & Gal., causing their glaucous appearance. Fernene also forms a thin chalky covering on the lower leaf surface of *Plagiogyra formosana* Nakai [21]. The conspicuous "farinose" or "ceraceous" white and yellow coatings that are characteristic for many gymnogrammoid ferns mostly consist of a more or less complex mixture of flavonoid aglycones [2, 3]. For one species of *Notholaena,* however, it has been shown previously that another triterpene, namely 6-acetoxy-16,22-dihydroxyhopan-24-oic acid, contributes much to the "farina" [7], and a triterpene isolated from *N. grayi* is presently under study. Diterpenes have so far been encountered in only a few species as more or less important constituents of such frond exudates. Thus, in *Cheilanthes argentea* most of the "farina" consists of *ent*-8(17)-*E*-13-labdadien-15-oic acid or its 3*R*-hydroxy derivative, depending on the chemotype [5]. *ent*-Kaur-16-ene-19-oic acid (**1**) is the major constituent of the frond exudate in *Notholaena pallens* and in *N. peninsularis* [6].

The kaurenoic acid **1** has now also been found as a constituent of the exudate of *Cheilanthes kaulfussii.* This fern does not exhibit a "ceraceous indument", but all surfaces are covered with glandular hairs. The amount of exudate they produce is considerable, and it makes the fronds slightly sticky. As has been reported previously [22], several flavonoid aglycones, comprising the novel product pinobanksin-3-cinnamate, are dissolved in the terpenoid material. One of the terpenoid components has now been found to be identical with *ent*-kaur-16-ene-19-oic acid (**1**), the diterpene we found previously in *Notholaena pallens*

and *N. peninsularis*. This finding again underlines the close relationship between *Cheilanthes* and *Notholaena*, on account of which the delimination of these two genera still is a taxonomic problem.

From the frond exudate of *N. pallens*, the kaurenoic acid **1** had been isolated as the major constituent. A minor diterpene from this species has now been identified to be its 3*R*-hydroxy derivative **2a**. The quantitative relation of **1** and **2a** is the same in the 10 specimens of *N. pallida* that could be checked for their presence (*cf.* [4]; in Table 3 of this ref. comp. **1** = "DM-7" and **2a** = "DM-9"). Amongst the flavonoid aglycones excreted by this species, apigenin-7,4′-dimethyl ether and apigenin monomethyl ethers are prevailing [4].

Methyl derivatives of kaempferol and of apigenin form the flavonoid profile that is characteristic of *Notholaena incana* [23]. One of the major constituents of the farinose frond exudate, however, is diterpene **3**, while compound **4** is one of its minor components. Other terpenoids were detected on thin layer chromatograms but have not been isolated to date. Some specimens of the closely related *N. delicatula* exhibit compound **4**, too, plus a prominent non-polar diterpene. It would be of interest to check whether or not the flavonoid profiles reported previously for *N. incana*, *N. delicatula*, and the "intermediate" specimens [23] have their parallel in diterpene profiles. Unfortunately most of the many samples available, obtained from herbarium fragments, are too scanty to allow such a comparative study, most having been used up for the flavonoid work.

At present one is inclined to consider kaurane-type diterpenes to be typical terpenoids not only in ferns in general [14], but also in fern exudates. It should be kept in mind, however, that in our extensive phytochemical studies on "farinose" ferns emphasis was clearly laid on the analysis of flavonoid aglycones. Further studies on the relevant terpenoid fractions may still reveal surprising results.

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