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6α-ACETOXY-16β,22-DIHYDROXYHOPAN-24-OIC ACID, A TRITERPENE FROM THE FERN *NOTHOLAENA CANDIDA* VAR. *COPELANDII*

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Key Word Index—*Notholaena candida* var. *copelandii*; Pteridaceae; fern; frond exudate; novel triterpene; 6α-acetoxy-16β,22-dihydroxyhopan-24-oic acid.

Abstract—Farinose exudates on fronds of gymnogrammoid ferns generally consist of flavonoid aglycones. In *Notholaena candida* var. *copelandii* a new triterpene was found as a major component of the farina besides galangin 3-methylether and kaempferol 3-methylether. Extensive mass spectral and NMR studies revealed this triterpene to be a new natural product, 6α-acetoxy-16β,22-dihydroxyhopan-24-oic acid.

INTRODUCTION

Farinose frond exudates of gymnogrammoid ferns have been studied extensively in recent years [1, 2]. In most species analysed to date the exudate material was found to consist of mixtures of free flavonoid aglycones. Only a few cases have been reported where the farina also contains a certain amount of terpenoids. *ent*-8(17)-*E*-13-Labdadien-15-oic acid and its 3*R*-hydroxy derivative were isolated from *Cheilanthes argentea* [3] and (−)-kaur-16-ene-19-oic acid was found in *Notholaena pallens* and *N. peninsularis* [4]. 9(11)-Fernene and its 21-epimer, reported recently from *Polypodium glaucinum* and from *Plagiogyra formosana* [5] form a waxy layer rather than a farinose exudate on the fronds of these ferns. Some terpenoids isolated from a further *Notholaena* species still need to be analysed. We now report the structure of a novel natural triterpene acid, 6α-acetoxy-16β,22-dihydroxyhopan-24-oic acid (**1a**) isolated from the farina of *Notholaena candida* (Mart. & Gal.) Hook. var. *copelandii* (C. C. Hall) Tyron.

RESULTS AND DISCUSSION

Triterpene **1a**, $C_{32}H_{52}O_6$, mp 234–236°. Its IR spectrum showed bands at 3660 and 3420 cm^{-1} for hydroxyl groups, 3250–2700 br, 1710 and 1695 cm^{-1} for carboxyl and 1730 and 1250 cm^{-1} for acetyl groups. Its ^1H NMR spectrum (pyridine-d_5) revealed the presence of seven methyl groups, all singlets, between δ0.82 and 1.64 as well as a signal corresponding to the acetyl group (2.06). Only two protons appeared at low field, both having the same multiplicity (*ddd*, J = 11, 11 and 4 Hz) at 4.40 (CH–OH) and 6.07 (CH–OAc). The existence of two $J_{ax\text{-}ax}$ coupling constants (11 Hz) demonstrated that both substituents had the equatorial orientation. The remaining hydroxyl was a tertiary one.

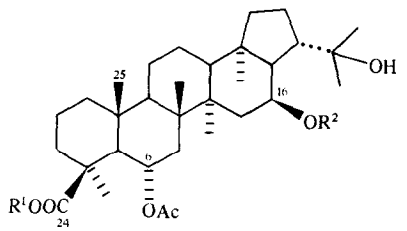
The methylated derivative (**1b**) showed a methyl signal at δ0.65 and the only methyl group so shielded should be the 10 β-Me if the COOMe group has a β-configuration [6]. On the other hand, the shift caused by this group on the 4 β-Me or 4 α-Me is very similar and not a proof of the configuration of the COOMe group. However, this substituent effect is very important for the 10 β-Me (−0.04 or −0.20 ppm). No methyls at this field have been observed in COOMe (C-23) or other hopane derivatives [7, 8].

Acetylation in the usual manner afforded the derivative **1b** which had two acetyl groups (singlets at δ2.02 and 2.09). Treatment of this compound with diazomethane yielded **1c**, showing a carbomethoxyl group (singlet at 3.55).

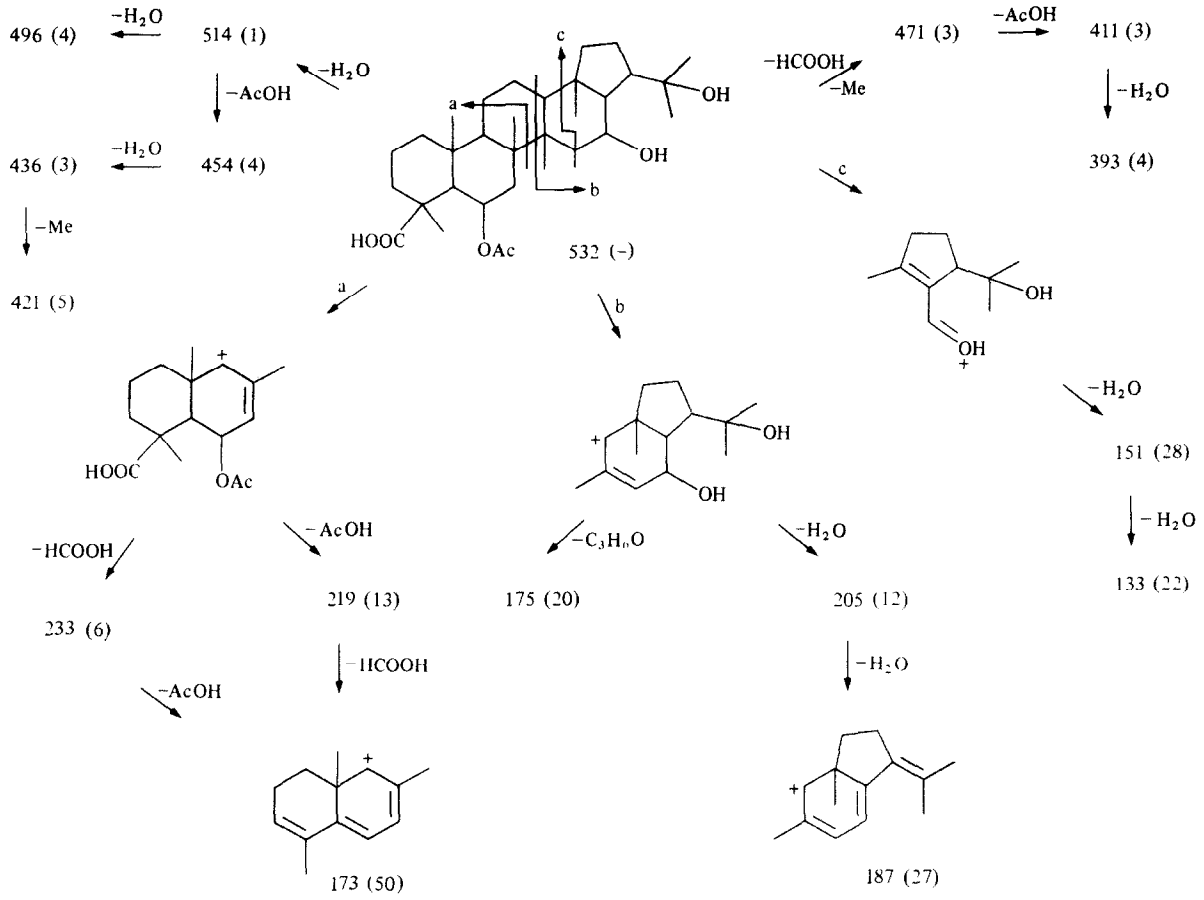
The mass spectrum of **1a** showed fragments typical of 22-hydroxyhopanes [9, 10] and suggested that the other two oxygen functions were attached to C-6 and C-16 of ring B and D, respectively (Scheme 1). The position of the carboxyl group cannot be deduced from the mass spectral data.

The final proof for the structure and stereochemistry of the triterpene **1a** came from the ^{13}C NMR spectrum of the natural product and its acetyl derivative **1b**. The determination of the multiplicity of the signals of the ^{13}C NMR spectra has been made through the DEPT subspectra [11].

Assignment of the signals was achieved by comparison with the reported data for 6α,16β-diacetoxyhopan-22-ol and 6α-acetoxyhopan-16β,22-diol which were previously



	R^1	R^2
1a	H	H
1b	H	Ac
1c	Me	Ac



Scheme 1. Mass spectral fragmentation pattern for compound **1a** (relative intensities in parentheses).

isolated from the lichen genus *Physcia* [12]. The ^{13}C NMR chemical shifts of the signals of **1a** and **1b** are summarized in Table 1. Especially noteworthy are the singlets at δ73.2, demonstrating the presence of the hydroxyisopropyl side chain. It was concluded that the carboxyl group was located in ring A at C-24, in accord with the existence of two methyl signals which appeared at δ30 (C-23 and C-30) [13, 14].

The evidence described is fully in accordance with the proposed structure of 6α-acetoxy-16β,22-dihydroxyhopan-24-oic acid for **1a**. This is the first time that this triterpene acid has been found in nature.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were obtained on a Bruker WP200SY at 200 and 50.13 MHz, respectively. Mass spectra were determined at 70 eV in a GC-MS Hewlett–Packard 5985. Mps are uncorr.

Plant material. Notholaena candida var. *copelandii* was collected by T. Reeves, L. Reeves and E. Wollenweber on 23 Dec. 1981 at the NW end of the Cerro de la Silla near Monterrey, N.L., Mexico. At this locality it grows on lower slopes of very rugged limestone and in protected sites under outcrop cliffs. The fern fronds were carefully clipped in the field and air-dried in a paper-sack. Vouchers (T. Reeves 7537D) are kept in the personal herbaria of T.R. at Morris, Minnesota and of E. W. at Darmstadt, W. Germany.

Product isolation. Dry fronds (130 g) were rinsed with Me_2CO to dissolve the white farinose exudate from their lower surfaces. After evaporation of the solvent *ca* 4.7 g of crude exudate material remained. Fractional crystallization from Me_2CO yielded 1.2 g of galangin 3-methylether with kaempferol 3-methylether and finally, *ca* 20 mg of the terpenoid alone. The latter portion was crystallized once more from C_6H_6–Me_2CO to give pure **1a**.

6α-Acetoxy-16β,22-dihydroxyhopan-24-oic acid (**1a**). Colourless crystals, mp 234–236°. IR ν_{max}^{KBr} cm^{-1}: see Results and Discussion. MS *m/z* (rel. int.): see Scheme 1. ^{1}H NMR (pyridine-d_5): δ0.82 (3H, *s*), 1.06 (3H, *s*), 1.10 (3H, *s*), 1.22 (3H, *s*), 1.32 (3H, *s*), 1.44 (3H, *s*), 1.64 (3H, *s*), 2.06 (3H, *s*, Ac), 4.40 (1H, *ddd*, *J* = 11, 11, 4 Hz, H-16α) and 6.07 (1H, *ddd*, *J* = 11, 11, 4 Hz, H-6β). ^{13}C NMR (pyridine-d_5): see Table 1.

6α,16β-Diacetoxy-22-hydroxyhopan-24-oic acid (**1b**). To a soln of **1a** (50 mg) in pyridine (1 ml) was added Ac_2O (1 ml) and the mixture kept at room temp. overnight. Usual work and recrystallization from MeOH gave colourless needles of **1b** (50 mg), mp 154–156°. IR ν_{max}^{KBr} cm^{-1}: 3600, 3300–2700, 1730, 1710, 1695 and 1250. ^{1}H NMR ($CDCl_3$): δ0.82 (3H, *s*), 0.84 (3H, *s*), 1.09 (3H, *s*), 1.12 (3H, *s*), 1.13 (3H, *s*), 1.15 (3H, *s*), 1.40 (3H, *s*), 2.02 (3H, *s*, Ac), 2.09 (3H, *s*, Ac), 5.19 (1H, *ddd*, *J* = 11, 11, 4 Hz) and 5.49 (1H, *ddd*, *J* = 11, 11, 4 Hz). ^{13}C NMR ($CDCl_3$): see Table 1. MS *m/z* (rel. int.): 574 $[M]^+$ (–), 514 $[M - AcOH]^+$ (3), 496 $[M - AcOH - H_2O]^+$ (8), 454 $[M - 2AcOH]^+$ (12), 436 $[M - 2AcOH - H_2O]^+$ (8) 421 (8) 396 (11) 221 (51) 187 (59) 173 (100) 147 (58) 109 (61) 59 (80) and 43 (64).

Table 1. ^{13}C NMR chemical shifts for compounds **1a** and **1b**

Carbon	**1a** (pyridine-d_5)	**1b** ($CDCl_3$)
1	40.0	39.2
2	19.6	18.8
3	41.1*	40.5*
4	44.6†	44.1†
5	59.2	58.6
6	72.8	72.4
7	40.9*	40.4*
8	43.0	42.5
9	49.2	48.7
10	40.4	40.0
11	21.7	21.3
12	24.0	23.5
13	49.2	48.7
14	44.4†	44.0†
15	44.5	41.1*
16	66.6	73.0
17	61.7	57.0
18	45.8	45.5
19	42.0	41.6
20	27.9	27.6
21	51.9	51.6
22	73.2	73.2
23	32.7	32.2
24	179.4	181.1
25	17.5	17.0
26	18.4	17.8‡
27	15.4	14.9
28	18.0	17.6‡
29	27.5	27.5
30	31.3	30.3
MeCOO	169.5	169.9–170.1
MeCOO	21.9	21.8–21.5

*†‡Assignments of values with the same superscript may be interchanged.

6α,16β-Diacetoxy-22-hydroxyhopan-24-oic acid methyl ester **1c**). Methylation of **1b** (10 mg) with CH_2N_2 in Et_2O and ecrystallization from MeOH yielded **1c** (10 mg) as colourless crystals, mp 130–132°. IR ν_{max}^{KBr} cm^{-1}: 3600, 1745, 1730 and 1250. ^1H NMR ($CDCl_3$): δ0.65 (3H, *s*), 0.77 (3H, *s*), 1.02 (3H, *s*), 1.05 (3H, *s*), 1.06 (3H, *s*), 1.08 (3H, *s*), 1.26 (3H, *s*), 1.96 (3H, *s*, Ac), 2.03 (3H, *s*, Ac), 3.55 (3H, *s*, COOMe), 5.12 (1H, *ddd*, J = 11, 11, 4 Hz) and 5.39 (1H, *ddd*, J = 11, 11, 4 Hz).

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