New Triterpenes from the Frond Exudates of Some *Notholaena* Species

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Several triterpenes were isolated from the lipophilic frond exudates of three *Notholaena* species. In the terpenoid portion of *N. candida* we identified two novel cycloartane type triterpenes. *N. rigida* yielded protolyofoligenic acid, a cycloartane type product that has been reported once before from a higher plant. From *N. schaffneri,* a novel dammarane type compound was isolated. These products were identified by spectroscopic methods.

Introduction

During earlier studies on the occurrence of flavonoid aglycones in farinose fern frond exudates it has become obvious that in some species these lipophilic materials also contain terpenoids, sometimes in considerable amounts [1, 2]. For *Notholaena candida* (Mart. & Gal.) Hook. var. *copelandii* (C. C. Hall) Tryon we earlier reported the major triterpene constituent to be 6α-acetoxy-16β,22-dihydroxyhopan-24-oic acid [3]. Now two products with cycloartane skeleton have been identified from the same species. From the frond exudate of *Notholaena rigida* Dav. two triterpenes have been reported recently [4], one with a dammarane skeleton and the other with a cyclolanostane skeleton. One further product has now been identified from the same material. The frond exudate of *N. schaffneri* also yielded a triterpene. Herein we report the structure elucidation of these products by detailed spectroscopic studies.

Materials and Methods

Notholaena candida var. *candida* was collected in Costa Rica in 1979 by L. D. Gómez P. *N. rigida* was collected in May 1983 in Edo. Tamaulipas, México (for details see [4]). *N. schaffneri* (Fourn.) Underw. ex Davenp. var. *nealleyi* was collected on December 21, 1981 on north-facing limestone slopes above Cantera el Socorro, Edo. Durango, México. Voucher specimens (T. Reeves with L. Reeves & E. Wollenweber, 7516) were deposited at Morris, MN and at Darmstadt. The exudate was washed off the dry fern material with acetone and worked up as usual by column chromatography on Sephadex LH-20 and on silica. TLC control of fractions was performed on silica and terpenoid spots were visualized by spraying with $MnCl_2$ reagent [5]. Compound **1** was obtained from *N. schaffneri. N. candida* var. *candida* yielded compounds **2** and **3.** Compound **4** was isolated from *N. rigida.* – Crystalline products were analyzed by MS and NMR spectroscopy. Mass spectra were measured with Varian MAT 311 and VG 7070 E mass spectrometers at 70 eV. NMR spectra were recorded on a Bruker AC-300 NMR spectrometer at 300 MHz (for ^1H) and at 75.4 MHz (for ^{13}C) in either CD_3OD (compound **1**) or $CDCl_3$, respectively (compounds **2–4**). ^{13}C Multiplicities were assigned through DEPT experiments.

Compound **1.** ^1H NMR δ ppm (*J,* Hz): 0.27 (H-19, d, 4.0), 0.57 (H-19, d, 4.0), 0.87 (3H, s), 0.91 (3H, s), 0.92 (H_3-21, d, 5.0), 1.12 (H_3-26 and H_3-27, s), 1.15 (3H, s), 3.20 (H-24, bd, 7.0) and 3.37 (H-3, bs). ^{13}C NMR: see Table I. EI-MS *m/z* (rel. int.): 490 (M^+, 2), 272 (M^+-H_2O, 7), 454 (M^+-2H_2O, 9), 444 (3), 436 (M^+-3H_2O, 7), 426 (30), 409 (21), 299 (10), 281 (11), 248 (10), 59 (100), 43 (78) and 41 (65).

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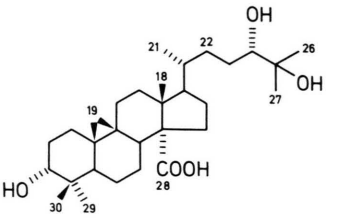
Compound **2.** ^{1}H NMR δ ppm (*J*, Hz): 0.30 (H-19, d, 4.0), 0.52 (H-19, d, 4.0), 0.84 (3H, d, 6.5), 0.84 (3H, s), 0.85 (3H, d, 6.5), 0.91 (3H, s), 0.93 (3H, s), 1.05 (3H, s), 2.08 (CH_3CO-, s) and 4.70 (H-3, bs). ^{13}C NMR: see Table I. EI-MS *m/z* (rel. int.): 500 (M^+, 3), 485 (M^+-CH_3, 2), 440 (M^+-AcOH, 34), 425 (M^+-AcOH$-CH_3$, 19), 397 (7), 318 (13), 175 (31), 95 (40), 55 (69), 43 (100) and 41 (75).

Compound **3.** ^{1}H NMR δ ppm (*J*, Hz): 0.52 (H-19, d, 4.0), 0.81 (H-19, d, 4.0), 0.84 (3H, d, 6.5), 0.85 (3H, d, 6.5), 0.90 (3H, s), 1.04 (3H, s), 1.08 (3H, s), 1.09 (3H, s) and 2.69 (H, ddd, m, 13.7, 13.7, 6.3). ^{13}C NMR: see Table I. EI-MS *m/z* (rel. int.): 456 (M^+, 25), 441 (M^+-CH_3, 25), 425 (7), 423 (8), 412 (11), 410 (12), 395 (16), 318 (40), 313 (22), 303 (12), 297 (13), 237 (18), 175 (55), 147 (48), 135 (38), 133 (47), 121 (56), 119 (45), 107 (78), 105 (43), 95 (100), 93 (52)m, 81 (54), 69 (47), 55 (52)m, 43 (68) and 41 (34).

Compound **4.** ^{1}H NMR δ ppm (*J*, Hz): 0.85, 0.85, 0.87, 0.93, 0.99, 1.10, 1.18, 1.19 (3H each, all s), 2.01 (CH_3CO-, s), 2.04 (CH_3CO-, s), 3.65 (H-24, dd, 7.5, 6.5), 4.48 (H-3, dd, 10.8, 5.0) and 4.83 (H-12, ddd, 10.5, 10.5, 5.4). ^{13}C NMR: see Table I. EI-MS *m/z* (rel. int.): 560 (M^+, not observed), 542 (M^+-H_2O, 2), 501 ($M^+-C_3H_7O$, 11), 441 (M^+-AcOH$-C_3H_7O$, 31), 381 (M^+-2AcOH$-C_3H_7O$, 20), 249 (6), 191 (37), 189 (22), 143 (91), 125 (49), 85 (66), 59 (82), 43 (100) and 41 (51).



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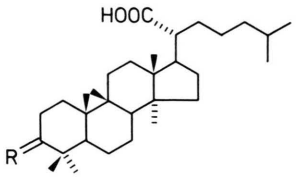

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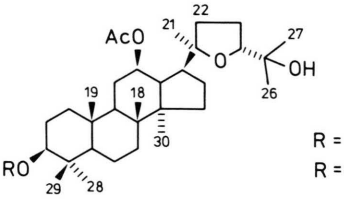


R = Ac 4

R = H 4a

Results and Discussion

Compound **1** is a triterpene belonging to the cycloartane group, based on the two one-proton doublets (J = 4 Hz) observed between 0.3–0.6 ppm. Its ^{1}H NMR spectrum also shows two methines geminal to oxygen-linked substituents (multiplets between 3.2–3.4 ppm) and six methyls, one of them as a doublet which must be located in the side chain. Its MS shows a small molecular ion at *m/z* 490, thus providing a molecular formula of $C_{30}H_{50}O_5$, and three consecutive losses of water, suggesting the existence of an additional hydroxyl attached to a quarternary carbon. The ^{1}H-decoupled ^{13}C NMR spectrum (see Table I) is in accordance with such assumptions, showing three oxygen-linked carbons (peaks between 70–80 ppm), one of them quarternary, while the lost methyl appeared as a carboxyl group at 177.81 ppm.

At this point, we can assume that the methyl doublet must be located in the side chain (C-21) and the tertiary hydroxyl in a 2-hydroxy isopropyl group. The multiplicity of the proton at 3.37 ppm suggests that it has the β-stereochemistry while no location is definite for the remaining hydroxyl and the carboxyl group.

Reviewing the literature published for related cycloartanes having three hydroxyls and one carboxyl group, we found that the ^{13}C NMR data of **1** closely correlates with that reported for protolyofoligenic acid, an unusual triterpenoid which has only been previously isolated from *Lyonia ovalifolia* leaves [6]. Minor differences between spectroscopic data must be assumed due to different solvents used. A 24*R* stereochemistry is also assumed for **1** as in protolyofoligenic acid, due to the observed multiplicity of the H-24 proton (broad doublet, J = 7.0 Hz). This is the same phenomenon as that observed previously in the correlation of this acid with cycloartenol [7].

Compound **2,** $C_{32}H_{52}O_4$, is another cycloartane type triterpenoid (two one-proton doublets, J = 4 Hz, at 0.30 and 0.52 ppm) apparently related to **1**

Table I. ^{13}C NMR chemical shifts for compounds **1–4** ($CDCl_3$ solns. except 1 in CD_3OD; DEPT multiplicities).

Carbon	**1**	**2**	**3**	**4**	**4a**
1	31.78t°	29.76t*	33.29t	38.67t*	38.83t
2	30.56t°	28.14t	37.37t	23.39t	27.19t
3	76.70d	78.82d	216.44s	80.43d	78.72d
4	40.49s	38.69s	50.16s	37.69s	38.88s
5	47.50d	41.97d	48.18d	55.64d	55.72d
6	21.89t	20.74t	21.28t	17.96t	18.20t
7	28.07t+	27.14t+	27.16t+	34.27t	34.48t
8	48.10d	47.86d°	47.80d*	39.44s	39.56s
9	20.50s	19.77s	20.97s	50.35d	50.50d
10	28.95s	26.39s	26.09s	36.85s	37.06s
11	28.77t#	25.09t	25.09t	28.21t	28.35t
12	35.02t	34.88t	34.88t	75.28d	75.53d
13	41.56s	45.07s	45.16s	46.11d	46.25d
14	63.10s	48.65s	48.53s	51.96s	52.14s
15	34.41t	32.49t	32.51t	31.02t	31.18t
16	29.13t#	26.14t+	26.57t+	25.91t	26.05t
17	53.80d	47.85d°	47.46d*	49.52d	49.76d
18	18.78q*	17.74q	17.77q	15.94q*	16.02q
19	29.45t#	29.87t	29.89t	15.39q*	15.51q
20	36.69d	49.07d	49.04d	85.56s	85.73s
21	18.86q*	182.99s	182.16s	22.12q	22.24q
22	32.96t	29.67t*	29.41t	38.32t*	38.83t
23	28.62t+	25.41t	25.68t	26.66t	26.78t
24	79.00d	38.81t	38.81t	83.20d	83.31d
25	72.80s	27.75d	27.77d	70.86s	70.99s
26	25.72q	22.32q	22.33q	24.08q	24.15q
27	26.69q	22.75q	22.76q	27.32q°	27.54q
28	177.81s	19.33q	19.24q	27.83q°	27.97q
29	24.98q	25.37q	22.25q	16.36q	15.34q
30	21.95q	21.25q	20.73q	17.36q	17.53q
Subst.		170.80s		170.38s	170.61s
				170.72s	
		21.25q		21.70q	21.85q
				21.14q	

Values with the same superscript may be interchanged in the same column.

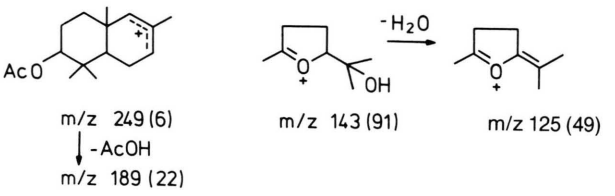
but showing clear differences in its ^1H NMR spectrum. Thus six methyls are also observed but two of them are now doublets, probably due to the isopropyl end of the side chain, and a singlet attributed to an acetoxy group is seen at 2.08 ppm. A low field multiplet is observed at 4.70 ppm whose multiplicity suggests a 3β-hydrogen geminal to the acetoxy group. The MS shows a small molecular ion at *m/z* 500, a prominent peak at *m/z* 440 (34%) arising from a loss of acetic acid, and a base peak at *m/z* 43. Its ^{13}C NMR spectrum (see Table I) confirms the above facts, showing only one oxygen-linked carbon (C-3, 78.82 ppm) and two carbonyls, one the expected acetyl (170.8 ppm) and the other a carboxyl group at 182.99 ppm corresponding to the lost methyl.

A comparison of related carbons for **2** and **1,** as well as the absence of the methyl doublet (C-21) in the ^1H NMR spectrum of **2** led us to assume that the acid group is located at C-21. Thus, the compound must be assumed to be 3α-acetoxy-9,19-cyclolanostan-21-oic acid, which is described for the first time herein as a natural product.

Compound **3** shows very similar chromatographic behaviour to **2.** However, its ^1H NMR spectrum, which indicates a cycloartane skeleton with 6 methyls, two of them more shielded and appearing as doublets, shows some characteristic differences from the former. It has no low field shifted protons nor methyl singlets indicative of acetoxy groups and the two one-proton doublets corresponding to the cyclopropane ring are shifted

to 0.52 and 0.81 ppm, respectively, typical for cycloartane triterpenoids having a keto group at C-3 [8, 9]. In this way, the deshielded multiplet at 2.69 ppm is assigned to the H-2 β based on its splitting pattern and its position adjacent to the carbonyl. The MS shows a relatively intense molecular ion (M^+ 456, 25%) providing a molecular formula of $C_{30}H_{48}O_3$ whilst no losses of water are observed. Finally, the ^{13}C NMR spectrum (Table I) confirms the above-mentioned facts. No sp^3 oxygen-linked carbons are observed (no peaks between 51–100 ppm) while two carbonyls are present, one of them corresponding to the carboxyl group located at C-21 (182.163 ppm) and the other (216.44 ppm) to the expected keto group at C-3. The structure of **3** is assigned, therefore, as 3-keto-9,19-cyclolanostan-21-oic acid which is reported for the first time in nature.

Compound **4** differs from the three previous triterpenoids in many spectroscopic features. Its ^1H NMR spectrum shows eight shielded methyls, all singlets, between 0.85–1.2 ppm and two deshielded methyls (2.01 and 2.04 ppm) which are attributed to two acetoxy groups. Three one-proton multiplets at low field are observed, one of them at 3.65 ppm, attributable to a methine geminal to an ether linkage, and two more at 4.48 and 4.83 ppm, methines geminal to the acetoxy groups. The ^{13}C NMR DEPT subspectra (Table I) reveal five C–O carbons (peaks between 70–85 ppm) and two carbonyls corresponding to the expected acetates. Thus, the existence of a cyclic ether and a tertiary hydroxyl is assumed in accordance with the ^1H NMR multiplet at 3.65 ppm and the two shifted methyls at 1.18 and 1.19 ppm which must be those corresponding to a 2-hydroxyisopropyl group. The ^{13}C NMR spectrum is practically the same as that reported recently for compound **4a,** a dammarane triterpene isolated from the frond exudate of *Notholaena rigida* [4], except for minor differences for carbons in the A-ring, notably in the neighbourhood of C-3 where the additional acetate is located. The MS shows no molecular ion for the expected molecular formula of $C_{34}H_{56}O_6$, but a small peak at *m/z* 542 from a loss of water. However, three consecutive losses of 59, 60 and 60 a.m.u. reveal the loss of the hydroxyisopropyl group and two molecules of acetic acid from the corresponding acetates. Strong peaks at *m/z* 143 and *m/z* 125 as well as the characteristic A-ring fragmentation are in accordance with previously reported related dammarane triterpenoids [10] (see Scheme). Thus, the structure of this new natural product **4** is fully in accordance with (20 *R*, 24 *R*)-20,24-epoxydammarane-3 β,12 β,25-triol-3,12-diacetate. – It may be mentioned here that two closely related triterpenes were isolated recently from the frond exudate of *Notholaena greggii* [11].



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