Three Further Dammarane Type Triterpenes from the Frond Exudate of the Fern *Notholaena rigida*

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From the farinose frond exudate of the fern *Notholaena rigida* two new triterpenes have been identified. They were found to be the C-24 epimers of 3β-acetoxy-24, 25-diO-isopropylidene-12β, 20(S),24,25-tetrahydroxy-dammarane. A third triterpene is identical with the known compound pyxinol, (20S,24R)-20,24-epoxy-dammarane-3ß,12ß,25-triol.

Introduction

The lipophilic farinose coating on the lower frond surface of the Mexican fern *Notholaena rigida* Dav. is composed of terpenoids and flavonoids. In a first paper we dealt with the identification of flavone aglycones (Scheele *et al.,* 1987). Later we reported several triterpenes, including two epoxydammarane derivatives (Arriaga-Giner *et al.,* 1991; Arriaga-Giner *et al.,* 1992), an isopropylidene-cyclolanostanol (Arriaga-Giner *et al.,* 1991), and two epimeric isopropylidene-dammarane derivatives (Arriaga *et al.,* 1996). From remaining fractions we have now isolated three further triterpenes which were identified by detailed spectroscopic studies.

Material and Methods

Dry fronds of *Notholaena rigida* were collected near Cd. Victoria in Edo. Tamaulipas, México in May, 1983. Collection data, isolation and analysis procedures have been reported previously (Arriaga-Giner *et al.,* 1991). As in the preceding paper (Arriaga *et al.,* 1996), one of those fractions was subjected to "flash" chromatography on Si-gel using CH_2Cl_2-MeOH 15:1 as eluent. Mass spectra were measured on a VG Autospec at 70 eV *via* solid probe. NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz (for ^{1}H) and 75.4 MHz (for ^{13}C) in $CDCl_3$. Multiplicities were assigned through DEPT experiments. Mps are uncorrected.

Three pure products were obtained, which all appeared as colourless oils. They exhibit the following spectral properties:

Compound **1A** (R_f 0.64): EI-MS *m/z* (rel. int.): 576 (M^+, -), 561 (M^+-Me, 0.3), 543 (M^+-Me-H_2O, 2), 525 (M^+-Me-2H_2O, 1) 500 (1), 482 (6), 415 (5), 413 (5), 341 (5), 249 (5), 201 (10), 191 (10), 189 (22), 161 (11), 147 (21), 143 (44), 129 (100), 121 (23), 107 (23), 95 (23), 81 (30), 71 (39) and 59 (85). ^{1}H NMR δ ppm (J, Hz): 0.85, 0.85, 0,85, 0.90, 0.98, 1.11, 1.22, 1.24, 1.33, 1.43, 2.04 (3H each, all s), 3.58 (H-12, ddd, 10.1, 10.1, 4.9), 3.72 (H-24, brd, 8.6) and 4.49 (H-3, dd, 11.8, 7.4). ^{13}C NMR: see Table I.

Compound **1B** (R_f 0.60): EI-MS *m/z* (rel. int.): 576 (M^+, -), 561 (M^+-Me, 0.5), 543 (M^+-Me-H_2O, 2), 525 (M^+-Me-2H_2O, 0.5) 500 (1), 482 (5), 415 (5), 413 (5), 341 (6), 249 (5), 201 (12), 191 (14), 189 (22), 161 (13), 147 (22), 143 (64), 129 (100), 121 (28), 107 (28), 95 (29), 81 (36), 71 (46) and 59 (91). ^{1}H NMR δ ppm (J, Hz): 0.85, 0.85, 0,85, 0.91, 1.00, 1.11, 1.19, 1.24, 1.34, 1.42, 2.04 (3H each, all s), 3.63 (H-12, m), 3.68 (H-24, m) and 4.45 (H-3, dd, 11.8, 7.4). ^{13}C NMR: see Table I.

Compound **2** (R_f 0.52): EI-MS *m/z* (rel. int.): 476 (M^+, -), 461 (M^+-Me, 2), 443 (M^+-Me-H_2O, 3), 425 (M^+-Me-2H_2O, 2), 400 (31), 381 (12), 357 (6), 207 (22), 191 (16), 143 (100), 125 (30), 107 (27), 85 (46) and 59 (28). ^{1}H NMR δ ppm (*J*, Hz): 0.76, 0.84, 0,89, 0.96, 0.97, 1.09, 1.26, 1.27 (3H each, all s), 3.18 (H-3, dd, 10.5, 5.2), 3.50 (H-12, ddd, 10.4, 10.4, 4.5) and 3.84 (H-24, dd, 8.3, 6.5). ^{13}C NMR: see Table I.

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Results and Discussion

The ^1H NMR spectrum of compound **1A** exhibits eleven methyl signals (all singlets) between 0.85 and 2.04 ppm. Three sets of one-proton resonances were observed at 3.58, 3.72 and 4.49 ppm which correspond to methine hydrogens adjacent to an oxygen substituent. The one-proton resonance at 4.49 ppm was assigned to H-3α on the basis of its splitting pattern and followed from the MS data (*m/z* 249/189) and methyl singlet at 2.04 ppm which suggested a 3β-acetoxy-dammarane skeleton.

The ^{13}C NMR spectrum of compound **1A** displays 35 carbon atom signals (see Table I), showing five deshielded carbons between 70.2–84.4 ppm, a carbon signal at 106.8 ppm that suggested the existence of an acetonide, and a carbonyl at 170.9 ppm.

Table I. ^{13}C NMR Data for the *Notholaena* triterpenoids **1A** and **1B,** and **2** (and its derivative **2a**).
a,b,c,d: Signals are interchangeable within the same column.

Carbon (multiplicity)	**1A**	**1B**	**2**	**2a**
1 t	38.5	38.6	38.9	38.6
2 t	23.6a	23.6a	27.4	23.9
3 d	80.8	80.7	78.8	80.6
4 s	37.8	37.7	38.9	38.0
5 d	55.9	55.9	55.9	56.1
6 t	18.1	18.1	18.2	18.3
7 t	34.7	34.6	34.8	34.9
8 s	39.8	39.7	39.7	39.9
9 d	49.8	49.9	50.5	50.6
10 s	37.0	37.0	37.1	37.1
11 t	30.5b	30.8b	31.1a	32.2
12 d	70.2	71.0	70.9	71.0
13 d	48.1	47.6	47.9	48.3
14 s	51.3	51.5	52.0	52.1
15 t	30.9b	31.3b	31.3a	31.6
16 t	26.2	26.3	24.9	25.4
17 d	52.9	53.9	49.3	49.4
18 q	16.1c	16.1c	15.2b	16.6a
19 q	16.4c	16.4c	15.3b	16.4a
20 s	73.5	73.7	86.4	86.6
21 q	26.7d	26.9d	26.1	26.9b
22 t	32.9	32.0	32.6	32.7
23 t	23.5a	23.4a	28.5	28.6
24 d	84.4	84.1	85.4	85.5
25 s	80.8	80.4	70.0	70.3
26 q	22.6	22.9	27.5c	27.1b
27 q	25.5	26.1	27.9c	27.6b
28 q	27.9	27.9	27.9c	28.0b
29 q	15.6	15.7	16.3b	15.4a
30 q	16.7	16.8	18.1	18.3
Subst. s	106.8	106.5		
q	28.3	28.6		
q	26.9 d	27.0 d		
$CH_3-\underline{C=O}$ s	170.9	171.0		
$\underline{CH_3}-C=O$ q	21.2	21.2		

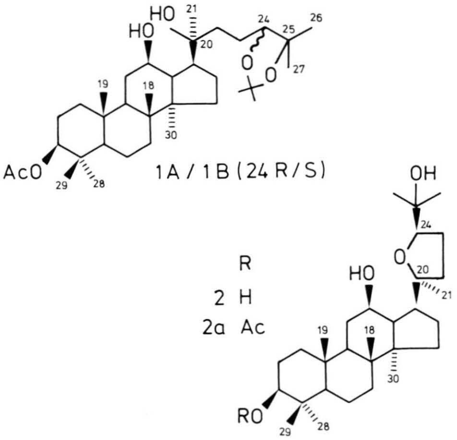

Fig. 1. Structural formulae for the *Notholaena* triterpenoids **1A** and **1B,** the 24*R* and 24*S* -epimers of 3β-acetoxy-12β, 20(*S*)-dihydroxy-24,25-di-O-isopropylidene-dammarane, and triterpenoid **2**, 3β,12β,25-trihydroxy-20 (*S*), 24(*R*)-epoxy-dammarane (and its derivative **2a**).

The ^1H NMR spectrum of compound **1B** shows substantially the same signals as compound **1A,** but two one-proton multiplets at 3.63 and 3.68 ppm are partially overlapped. No significant differences in methyl signals are observed.

Comparison of the ^{13}C NMR spectra of compounds **1A** and **1B** (see Table I) showed no differences with previously isolated triterpene-pentol epimers (Arriaga *et al.,* 1996) other than affecting the neighbourhood of the acetate (carbons 2–4). The chemical shifts for the signals attributed to C-17, C-21 and C-22 suggested the same stereochemistry at C-20 (Asakawa *et al.,* 1977). Thus, both compounds seem to be epimers at C-24.

The mass spectrometric fragmentation pattern of both compounds is also identical. The molecular ion corresponding the the formula of $C_{35}H_{60}O_6$ is not observed because losses of water and methyl are highly favoured. The structure of these new natural products, **1A** and **1B**, is fully in accordance with the structure of 3β-acetoxy-12β,20(*S*)-dihydroxy-24,25-di-O-isopropylidene-dammarane, but assignment of the two epimers at C-24 was not possible.

Compound **2** shows a ^1H NMR spectrum that differs in the methyl number and lack of the acetate. A comparison with related triterpenes isolated from this plant lead us to assume that the side chain is different, the acetonide being absent. Reviewing the literature we conclude that the spectroscopic data are fully in agreement with the structure of pyxinol, 3β,12β,25-trihydroxy-20(*S*), 24(*R*)- epoxy-dammarane, previously isolated from the lichen *Pyxine endochrysina* (Yosioka *et al.,* 1972). Its ^{13}C NMR spectrum, reported here for the first time, is closely related (see Table I) to that of its 3-acetyl derivative, **2a**, previously used in the structural elucidation of *Panax* saponins (Tanaka and Yahara, 1978). Again, the chemical shifts for C-21 and C-22 at ca. 26 and 33 ppm agree for 20*S* stereochemistry (21 and 39 ppm, respectively, for 20*R*) (Francisco *et al.,* 1984).

The pair of epimers **1A/1B** are the 3-O-acetyl derivatives of the parent compounds recently reported from the same plant (Arriaga *et al.,* 1996). Acetonides as natural products are rather unusual, but we have found five derivatives from the dammarane and cycloartane skeletons in this species (Arriaga *et al.,* 1996; Arriaga-Giner *et al.,* 1991). Pyxinol, compound **2**, is reported for the second time only as a natural product. As other dammarane-triterpenoids isolated from this plant, it has the side chain cyclized as tetrahydrofurane ring but the stereochemistry of C-20 differs from the other reported components. An isomer with reverse configuration at C-3 and C-20 has also been isolated as a natural product (Denisenko *et al.,* 1983).

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