Two New Triterpenes from the Frond Exudate of the Fern *Notholaena rigida*

Fco. Javier Arriaga,[a], Angel Rumbero[b] and Eckhard Wollenweber[c]

[a] Tabacalera S.A., Centro I + D, c/Embajadores 51, E-28012 Madrid, Spain
[b] Departamento de Química Orgánica, Universidad Autónoma de Madrid, Cantoblanco, E-28049 Madrid, Spain
[c] Institut für Botanik der Technischen Hochschule, Schnittspahnstraße 3, D-64287 Darmstadt, Bundesrepublik Deutschland

Z. Naturforsch. **51c,** 423–425 (1996); received February 8/ March 11, 1996

Notholaena rigida, Pteridaceae, Farinose Frond Exudate, Novel Triterpenes, Dammarane Derivatives

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 24, 25-diO-isopropylidene-3β, 12β, 20(*S*)-trihydroxy-dammarane.

Introduction

In our first paper on the chemical composition of the farinose coating on the lower frond surface of the Mexican fern *Notholaena rigida* Dav. (Scheele *et al.,* 1987) we dealt with the flavone aglycones present in this exudate material, in which terpenoids are predominant. Later we reported some triterpenes from the same source, including two epoxydammarane derivatives (Arriaga-Giner *et al.,* 1991; Arriaga-Giner *et al.,* 1992) and an isopropylidene-cyclolanostanol (Arriaga-Giner *et al.,* 1991). From remaining fractions we have now isolated two further triterpenes. By detailed spectroscopic studies these were identified as being epimeric isopropylidene-dammarane derivatives

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). One of those fractions has now been 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 ^1H) and 75.4 MHz (for ^{13}C) in $CDCl_3$. Multiplicities were assigned through DEPT experiments. Mps are uncorrected.

Two pure products with the following properties were obtained.

Compound **1** forms colourless crystals, mp 215–216°. EI-MS *m/z* (rel. int.): 534 (M^+, -), 501 (M^+-Me-H_2O, 2), 483 (M^+-Me-$2H_2O$, 1) 465 (M^+-Me-$3H_2O$, 1) 458 (1), 440 (3), 422 (2), 407 (2), 369 (3),

^{13}C NMR Data for *Notholaena* triterpenoids **1** and **2**. a,b,c: Signals are interchangeable within the same column.

Carbon (Multiplicity)	**1**	**2**
1 t	39.0	39.1
2 t	27.5	27.4
3 d	79.0	78.9
4 s	39.0	39.0
5 d	55.9	55.9
6 t	18.4	18.4
7 t	34.9	34.8
8 s	39.9	39.8
9 d	50.0	50.2
10 s	37.2	37.1
11 t	30.7 a	31.5 b
12 d	70.4	71.1
13 d	48.2	47.7
14 s	51.5	51.7
15 t	31.0 a	31.0 b
16 t	26.4	26.4
17 d	53.0	54.0
18 q	15.7 b	15.8 a
19 q	16.2 b	16.2 a
20 s	73.6	73.7
21 q	26.9 c	26.7 c
22 t	33.0	32.2
23 t	23.7	23.6
24 d	84.6	84.2
25 s	81.0	80.6
26 q	22.8	23.1
27 q	25.7	26.2
28 q	28.1	28.1
29 q	15.4	15.5
30 q	16.9	16.9
Subst. s	106.9	106.6
q	28.5	28.7
q	27.0 c	27.1 c

Reprint requests to E. Wollenweber.
Telefax # 06151/166878.

0939–5075/96/0500–0423 $ 06.00

341 (5), 207 (17), 189 (20), 161 (15), 147 (26), 143 (47), 129 (89), 121 (30), 107 (32), 95 (30), 81 (40), 71 (49) and 59 (100).^{1}H NMR δ ppm (*J*, Hz): 0.78, 0.88, 0.90, 0.98, 0.98, 1.12, 1.22, 1.25, 1.34, 1.43 (3H each, all s), 3.22 (H-3, dd, 11.0, 5.2), 3.58 (H-12, ddd, 10.3, 10.3, 5.3) and 3.73 (H-24, dd, 9.5, 1.6). ^{13}C NMR: see Table I.

Compound **2** also forms colourless crystals, mp 224–226°. EI-MS *m/z* (rel. int.): 534 (M^+, -), 501 (M^+-Me-H_2O, 2), 483 (M^+-Me-2H_2O, 1) 465 (M^+-Me-3H_2O, 1) 458 (1), 440 (3), 422 (2), 407 (2), 369 (3), 341 (4), 207 (16), 189 (21), 161 (15), 147 (26), 143 (35), 129 (91), 121 (30), 107 (32), 95 (30), 81 (41), 71 (47) and 59 (100). ^{1}H NMR δ ppm (*J*, Hz): 0.78, 0.88, 0.89, 0.98, 1.00, 1.11, 1.18, 1.26, 1.35, 1.38 (3H each, all s), 3.20 (H-3, dd, 11.0, 5.1), 3.61 (H-12, ddd, 9.9, 9.9, 5.1) and 3.70 (H-24, brd, 8.5). ^{13}C NMR: see Table I.

Results and Discussion

The structures of the two tritepenes isolated were elucidated by detailed NMR spectroscopic studies.

The ^{1}H NMR spectrum of compound **1** exhibits ten methyl signals (all singlets) between 0.78 and 1.43 ppm. Three sets of one-proton resonances were observed at 3.22, 3.58 and 3.73 ppm which correspond to methine hydrogens adjacent to an oxygen substituent. The one-proton resonance at 3.22 ppm was assigned to H-3α on the basis of its splitting pattern and followed from the MS data which suggested a 3-hydroxy-dammarane skeleton. The assignment of the multiplet at 3.58 ppm to H-12α was made from the two axial-axial and one-axial-equatorial couplings and assuming the same substitution pattern found in related compounds previously isolated from this species (Arriaga-Giner *et al.*, 1991; 1992) and subsequent comparison from ^{13}C NMR data. The remaining one-proton signal at 3.73 ppm is assigned to a methine linked to an ether in the side-chain.

The ^{13}C NMR spectrum of compound **1** displays 33 carbon atom signals (see Table I). The multiplicity of the five deshielded carbons between 70.4–84.6 ppm confirmed the existence of three hydroxy groups (one tertiary) and the two remaining C-O bonds joined to the uncommon fully substituted sp^3 carbon signal at 106.9 ppm suggested the existence of an acetonide. Comparison of the chemical shifts with those obtained for the 20R and 20S epimers of protopanaxadiol (compounds **1** and **8** in Asakawa *et al.*, 1977)), related dammarane-type sapogenins isolated from Ginseng, is consistent with the proposed structure at the four rings A-D. Thus, differences are observed in the side-chain where the acetonide is located. The assignment of the C-20 configuration was made by examination of the chemical shifts of the adjacent carbons because C-21 and C-17 are shielded in the (20*R*) epimer relative to that of the (20*S*) epimer whilst for C-22 the opposite phenomenon is observed. The chemical shifts observed for C-21 and C-17 in compd. **1** at 26.9 and 53.0 ppm, and 33.0 ppm for C-22 clearly indicate, therefore, the (20*S*) configuration (Asakawa *et al.*, 1977). However, the configuration at C-24 could not be established by this method.

The ^{1}H NMR spectrum of compound **2** shows substantially the same signals as **1,** but the two one-proton multiplets at lower field are partially overlapped and the minor coupling of the signal corresponding to H-24 is not observed here. No significant differences in methyl signals are observed either. Comparison of the ^{13}C NMR spectrum of compound **2** with that of **1** (see Table) showed no differences in the chemical shifts for the signals attributed to C-17, C-21 and C-22 suggesting the same stereochemistry at C-20. Thus, both compounds seem to be epimers at C-24.

As expected, the mass spectrometric fragmentation patterns of both compounds are identical and no differences due to the C-24 configuration are observed. The molecular ion corresponding to the formula of $C_{33}H_{58}O_5$ is not observed because losses of water and methyl are highly favoured; however, three consecutive losses of water can be observed in two different series of ions as can be expected from the hydroxy groups. Moreover, significant fragments for 3-hydroxy-dammaranes (Kitagawa *et al.*, 1983) at *m/z* 207 and *m/z* 189 are observed while the second strongest peak at *m/z* 129 arises from the side chain.

The structures of these new natural products, **1** and **2**, are fully in accordance with 24,25-diO-isopropylidene-dammarane-3ß,12β,20(*S*),24,25-pentol, but assignment of the two epimers at C-24 was not possible (Fig. 1).

Both compounds are closely related to a triterpene isolated from *N. rigida* previously (com-

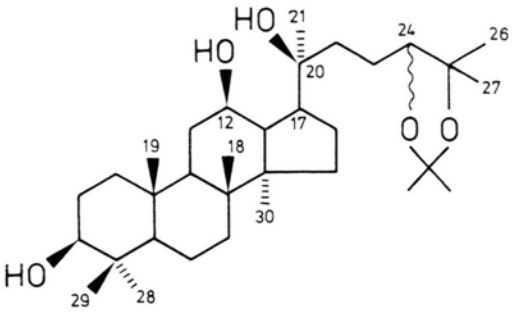

Fig. 1. Structural formula for compounds **1** and **2**, the 24*R* and 24*S* -epimers of 24,25-diO-isopropylidene-dammarane-3β,12β, 20(S),24,25-pentol.

pound **4** in: Arriaga-Giner *et al.*, 1992). From this product they can be derived by deacylation, heterocycle opening and subsequent formation of the acetonide. Acetonides as natural products are rather unusual. Since during both the extraction and isolation processes acetone was used, doubts arose as to the genuineness of these products. Once more we therefore checked leaf washes (from plants cultivated in a greenhouse at Darmstadt), freshly prepared with toluene, with chloroform, and with ethyl acetate, as we had done before in the case of a cycloartane acetonide previously isolated from this species (compound **5** in: Arriaga-Giner *et al.*, 1991). Immediate TLC analysis revealed unambiguously that the newly reported compounds **1** and **2** are, indeed, genuine natural triterpene acetonides.

Arriaga-Giner F. J., Rullkötter, J., Peakman T.M. and Wollenweber E (1991), New triterpenes from the frond exudate of the fern *Notholaena rigida*. Z. Naturforsch. **46c**, 507–512 .

Arriaga-Giner F. J., Rumbero A. and Wollenweber E. (1992), New triterpenes from the frond exudates of some *Notholaena* species Z. Naturforsch. **47c**, 508–511.

Asakawa J., R. Kasai R, K. Yamasaki K. and Tanaka O. (1977),. ^{13}C NMR study of ginseng sapogenins and their related dammarane type triterpenes. Tetrahedron **33**, 1935–1939.

Kitagawa I., Wang H.K., Tagaki A., Fuchida M., Miura I. and Yoshikawa M. (1983), Saponin and sapogenol. XXXIV. Chemical constituents of *Astragali radix*, the root of *Astragalus membranaceus* Bunge (1). Cycloastragenol, the 9,19-cyclolanostane-type aglycone of astragalosides, and the artifact aglycone astragenol. Chem . Pharm. Bull. **31**, 689–697.

Scheele C., Wollenweber E. and Arriaga-Giner F.J. (1987), New flavonoids from cheilanthoid ferns. J. Nat. Prod. **50**, 181–187.