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

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Two new triterpenoids, isolated from the frond exudate of the fern *Notholaena rigida,* have been identified as 3β,12β,25-trihydroxy-(20 *R*-24 *R*)-epoxy-dammarane-12-acetate (**1**), and 24 *R*,25-diO-isopropyliden-9(19)-cyclolanostan-3β-ol (**5**), based on NMR spectroscopic studies.

Introduction

The farinose coating on the lower frond surface of the fern *Notholaena rigida* Dav. was shown previously to contain the flavones scutellarein-6,7,4′-trimethyl ether and scutellarein-6,7-dimethyl ether as well as minor amounts of apigenin, apigenin-4′-methyl ether, and apigenin-7,4′-dimethyl ether [1]. The exudate material also contains a considerable amount of terpenoids, one of which has been assumed earlier to be a triterpenoid with a cycloartenol skeleton [1]. The structure of this product as well as that of a second product isolated later have now been elucidated by detailed spectroscopic studies.

Materials and Methods

Dry fronds of *Notholaena rigida* were collected from plants growing along Highway 101 from Cd. Victoria close to Juamave, Ed. Tamaulipas, Mexico, in May 1983. Vouchers (G. Yatskievych & E. Wollenweber 83–109) are kept in the University of Arizona Herbarium in Tucson, Arizona (ARIZ), and in E.W.'s personal herbarium. Dry fern material was rinsed with acetone to dissolve the exudate. From 520 g of *N. rigida* 36 g of material were obtained after evaporation of the solvent. One portion of this material was subjected to column chromatography on silica (eluted with toluene and increasing amounts of methyl ethyl ketone and methanol), another portion was passed over Sephadex LH-20 (eluted with methanol) to separate terpenoids from flavonoids. In this way two of the terpenoids present were obtained as colourless crystals. TLC control of fractions was performed on silica and spots were visualized by spraying with Naturstoffreagenz A (for flavonoids) and $MnCl_2$ reagent (for terpenoids; *cf.* [2]). Mass spectra of the isolated products as well as of their trimethylsilyl derivatives were measured. The latter were prepared by treatment with BSTFA + 1% TMCS in the presence of an equal volume of pyridine at 50 °C for 2 h. Instruments used were Varian MAT 311 and VG 7070 E mass spectrometers. Samples were introduced *via* a solid probe, spectra were recorded at 70 eV. Elemental compositions of molecular ions and fragment peaks were determined at 10,000 resolution (10% valley). NMR spectra were recorded in $CDCl_3$ on a Bruker NMR spectrometer AC-300 at 300 MHz (for ^1H) and at 75.4 MHz (for ^{13}C). Chemical shifts are given against chloroform (δ 7.26 for ^1H and δ 77.0 for ^{13}C). sp^3 Hybridized carbons were distinguished using the DEPT technique. Melting points are uncorrected.

Compound **1.** ^1H NMR δ ppm (J, Hz): 0.75, 0.82, 0.92, 0.95, 0.97, 1.08, 1.16, 1.17 (3 H each, all s), 1.99 ($\underline{CH_3}CO-$, s), 3.18 (H-3, dd; 11.0, 4.2), 3.63 (H-24, dd; 7.5, 6.5) and 4.81 (H-12, ddd; 10.5, 10.5, 5.4). ^{13}C NMR: see Table I. EI-MS *m/z* (rel. int.): see Fig. 1.

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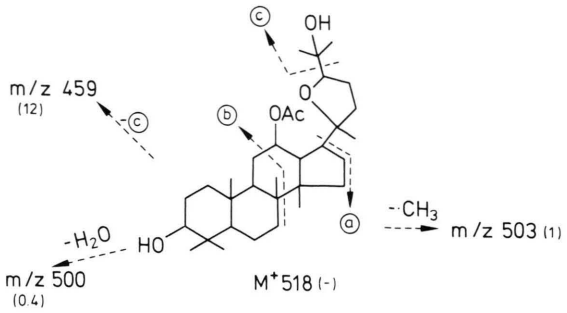
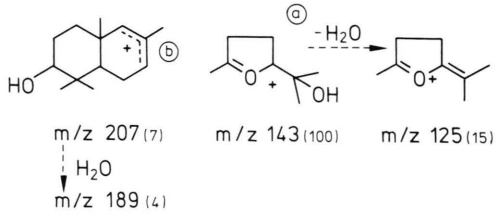
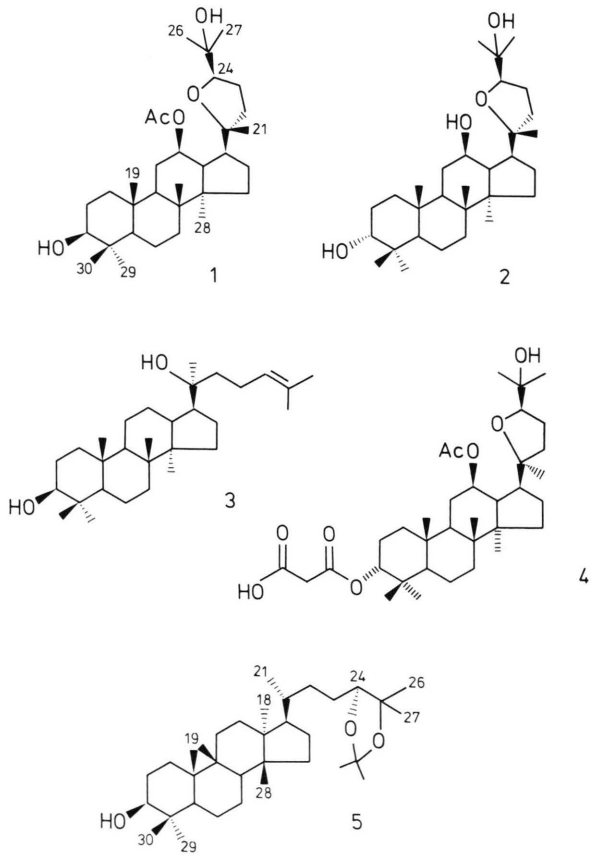
Compound **5.** ^{1}H NMR ($CDCl_3$) δ ppm (J, Hz): 0.33 (H-19, d; 4.4), 0.55 (H-19, d; 4.4), 0.80, 0.88, 0.96, 0.97, 1.10, 1.25, 1.33, 1.41 (3H each, all s), 0.90 (Me-21, d; 6.8), 3.28 (H-3, dd; 8.4, 4.5) and 3.64 (H-24, dd; 8.8, 4.0). ^{13}C NMR: see Table I. EI-MS *m/z* (rel. int.): 500 (M^+, 10), 485 (M^+-Me, 31), 482 (M^+-H_2O, 26), 467 (M^+-Me-H_2O, 7), 442 (9), 439 (6), 427 (8), 424 (7), 409 (21), 360 (16), 203 (17), 187 (12), 175 (23), 135 (25), 121 (30), 107 (37), 95 (45), 69 (43) and 43 (100).





Results

Compound **1** forms colourless crystals, m.p. 177–179 °C. The mass spectrum lacks a molecular ion. The fragment of highest mass-to-charge ratio occurs at *m/z* 503 ($C_{31}H_{51}O_5$) and corresponds to the loss of a methyl group from the molecular ion. This indicates an elemental composition of $C_{32}H_{54}O_5$ for the intact molecule (nominal molecular mass 518) which is in accordance with the number of carbon atoms detected by ^{13}C NMR spectroscopy (see below). The silylated compound shows a peak at *m/z* 647 as the ion of highest mass-to-charge ratio, and this corresponds to the presence of two hydroxyl groups. The base peak in the mass spectrum of compound **1** (*m/z* 143; shifted to *m/z* 215 in the TMS derivative), arises from side-chain cleavage of the tetracyclic triterpene with charge retention in the side-chain fragment. The base peak in the mass spectrum of the TMS ether is at *m/z* 131 and is attributed to a silylated 2-hydroxyisopropyl group as, *e.g.*, in hopan-22-ol TMS ether [3]. The conjugate fragments (loss of 2-hydroxyisopropyl group) occur at *m/z* 459 ($C_{29}H_{47}O_4$; underivatized triterpene) and *m/z* 531 (TMS ether), respectively. Loss of 60 mu from the M^+-15 ions in both compounds indicates the presence of an acetate group (*m/z* 443, $C_{29}H_{47}O_3$, and *m/z* 587, respectively). Fragments at *m/z* 400 and 472 (TMS derivative), respectively, involve the loss of both the 2-hydroxyisopropyl and the acetate group. The main fragments in the mass spectrum of compound **1** are consistent with those described earlier for a series of dammaranes [4–6] including small but significant fragments at *m/z* 207 and *m/z* 189 for 3-hydroxy dammaranes.



The ^{1}H NMR spectrum of compound **1** exhibits nine methyl signals (all singlets) indicating a triterpenoid structure. One of them is deshielded (1.99 ppm) and attributed to an acetoxy group. Three sets of one-proton resonances were observed at 3.18, 3.63 and 4.81 ppm which correspond to methine hydrogens adjacent to an oxygen substituent. Double resonance experiments demonstrated that they are not coupled to each other.

Following on from the MS data, which suggested a 3-hydroxy-dammarane skeleton, the one-proton resonance at 3.18 was assigned to H-3α on the basis of its splitting pattern which clearly indicated one axial-axial coupling ($J = 11.0$ Hz) and one axial-equatorial coupling ($J = 4.2$ Hz). The location of the acetate group to C-12β then followed from the splitting pattern of the one-proton resonance at 4.81 ppm as previously reported for a 12β-acetoxy-dammarane derivative [6]. The remaining observable one-proton resonance (3.63 ppm) was then assigned as H-24 of a (20,24)-epoxy-dammarane [6].

The ^{13}C NMR spectrum of **1** displays 32 carbon atom signals (Table I) whose chemical shifts are consistent with a 12β-acetoxy-3β,25-dihydroxy-(20,24)-epoxy-dammarane structure by comparison with related compounds (see structures **2, 3** and **4** in Table I) described in the literature [6–10]. Epimers at C-20 can be distinguished by examina-

Table I. ^{13}C NMR chemical shifts for compounds **1** and **5**, and literature data for aid in determining the assignment of **1**.

Carbon	Compd. **1**	Compd. **2** [8]	Compd. **3** [8]	Compd. **4** [6]	Compd. **5**
1	38.8 (t)		39.0		31.9 (t)
2	27.2 (t)		27.4		30.3 (t)
3	78.6 (d)		78.9		78.8 (d)
4	38.8 (s)		39.0		40.5 (s)
5	55.7 (d)		55.9		47.1 (d)
6	18.2 (t)		18.3		21.1 (t)
7	34.4 (t)		35.3		28.2 (t)
8	39.5 (s)		40.4	39.8	48.0 (d)
9	50.5 (d)		50.7	50.4	20.0 (s)
10	37.1 (s)		37.1		26.0 (s)
11	28.3 (t)	30.5		28.3	26.0 (t)[a]
12	75.5 (d)	70.7		75.6	35.5 (t)
13	46.2 (d)	49.0		46.3	45.3 (s)
14	52.1 (s)	51.8		52.3	48.8 (s)
15	31.1 (t)	31.4			32.9 (t)
16	26.0 (t)	26.9			26.3 (t)[a]
17	49.7 (d)	49.9			52.2 (d)
18	15.5 (q)[b]	16.1	16.2	15.9	18.2 (q)[c]
19	16.0 (q)[b]	15.6	16.5	15.6	29.9 (t)
20	85.7 (s)	86.4			36.3 (d)
21	22.2 (q)	21.3			18.0 (q)[c]
22	38.7 (t)	39.1			33.1 (t)
23	26.7 (t)	26.0			26.3 (t)[a]
24	83.3 (d)	86.5			83.8 (d)
25	70.9 (s)	70.3			80.2 (s)
26	24.1 (q)	24.7			22.9 (q)
27	27.5 (q)[c]	27.8			26.4 (q)[b]
28	27.9 (q)[c]		28.0		19.3 (q)
29	15.3 (q)[b]		15.4		25.4 (q)
30	17.5 (q)				14.0 (q)
Subst.	170.6 (s)				106.3 (s)
	21.8 (q)				28.6 (q)
					26.9 (q)[b]

[a, b, c]: Signals are interchangeable within the same column. – For compounds **2, 3** and **4** only the relevant chemical shifts have been included. In the case of compound **4** the assignments have been made by interpretation of the data reported previously [6].

tion of the chemical shifts of C-21 and C-22 [7, 8]. Thus, the chemical shift of C-21 is shielded in the (20 *R*) isomer relative to that of the (20 *S*) isomer whilst for C-22 the opposite phenomenon is observed. Comparison with related 12β,25-dihydroxy-(20,24)-epoxy-dammarane derivatives [8, 9] indicates that for (20 *R*) stereochemistry chemical shifts for C-21 and C-22 at *ca.* 21 and 39 ppm and for (20 *S*) stereochemistry at *ca.* 27 and 33 ppm are observed. The chemical shifts for C-21 and C-22 in **1** at 22.2 and 38.7 ppm clearly indicate, therefore, the (20 *R*) configuration.

Epimers at C-24 are also revealed by ^{13}C NMR spectroscopy [8, 9]. Again by comparison with related 12β,25-dihydroxy-(20,24)-epoxy-dammarane derivatives [8, 9] it is apparent that the chemical shifts of C-26 and C-27 are resolved from each other by 1–2 ppm when C-21 and the 2-hydroxyisopropyl group at C-24 are arranged in a *trans* spatial relationship to each other and by 3–4 ppm when they are *cis*. On this basis the difference of 3.4 ppm in the chemical shifts for C-26 and C-27 in **1** indicates a *cis* relationship and hence the (24 *R*) stereochemistry. Compound **1** is assigned, therefore, as 12β-acetoxy-3β,25-dihydroxy-(20 *R*,24 *R*)-epoxy-dammarane.

Compound **5** forms colourless crystals, m.p. 189 °C. Its ^1H NMR spectrum revealed the existence of nine methyl groups, one being secondary (doublet), between 0.91 and 1.42 ppm. The presence of two very shielded one-proton doublets ($J = 4.4$ Hz) at 0.33 and 0.55 ppm suggested the existence of a cyclopropane ring in the triterpene skeleton. Moreover, two methine groups bearing oxygen atoms were observed as double doublets at 3.28 and 3.65 ppm, respectively, remaining unaffected by double irradiation experiments.

The ^{13}C NMR spectrum provided significant information (see Table I), with carbon atom resonances in complete agreement with a 9(19)-cyclolanostane skeleton possessing two secondary and a tertiary hydroxyl group, no double bonds nor carbonyl groups, but an uncommon fully substituted sp^3 carbon with a signal at 106.31 ppm. Such a resonance as well as the nine methyls, in addition to the carbon atom forming the cyclopropane ring in a molecular formula of $C_{33}H_{56}O_3$ (M^+ 500), suggested the presence of an acetonide.

The ^{13}C NMR data of compound **5** are exactly the same as those reported for 3,24,25-cycloartanetriol, isolated from *Mangifera indica* [11], as well as those for the corresponding 24(25)-epoxide [12], except for values for C-24 and C-25, which should carry the acetonide. Reviewing the literature, we found that the four isomers (at C-3 and C-24) of such a 3,24,25-cycloartanetriol were synthesized earlier in order to correlate protolyofoligenic acid with cycloartenol [13, 14]. Among several products, the authors obtained, in fact, the corresponding four 24,25-di-O-isopropylidene derivatives. While carbon resonances were not reported, we can establish the C-24 configuration as *R*, based on ^1H NMR multiplicity of H-24, which appears as double doublet in the spectrum of compound **5** (reported as broad doublet and broad triplet for *R* and *S* configuration, respectively [13]).

The mass spectrometric fragmentation pattern of compound **5** is also in accordance with those of its afore-mentioned derivatives. B and C ring cleavages as well as losses of water, methyl and side chain dominate in the mass spectrum with no influence of C-3 or C-24 configuration [15]. Thus, based on the full spectroscopic evidence, compound is 24 *R*,25-diO-isopropylidene-9(19)-cyclolanostan-3 β-ol.

Discussion

A wide variety of triterpenoids, belonging to two major groups, is known to occur in ferns [16]. However, up to now such products have only rarely been reported to be accumulated externally, on the fern's surfaces. Fernenes form the "waxy" epicuticular layer on *Polypodium aureum* leaf and young rhizome [17] as well as the "chalky" coating on the lower frond surface of *Plagiogyra formosana* [17 and further *Plagiogyra* species, where hopanes have also been found [18]. A triterpene acid forms the major part of the conspicuous "farina" on the lower frond surface of *Notholaena candida* var. *copelandii,* which also contains several flavonoid aglycones [19]. With compounds **1** and **5** we have now found two further fern exudate triterpenoids.

To the best of our knowledge, compound **1** is a new natural product. Triterpenoids with a dammarane skeleton are fairly common in angiosperms [20, 21], but have been less frequently found in ferns [*e.g.* 22–24]. They may, however, be more widely distributed than is presently known:

geochemical evidence of the occurrence of dammaranes and dammarenes in many oceanic sediments suggests that this type of triterpenoids, like hopanoids and fernenes, is also biosynthesized by microorganisms [25, 26]. Various 3β,12β,25-trihydroxy-substituted (20,24)-epoxy-dammaranes like compound **1** have been reported before to occur, *e.g.*, in *Betula platyphylla* [27], *B. papyrifera* ssp. *humilis* [6], *B. spec.* [28], but in each case these compounds had a 20*S*,24*R* configuration. 3α-Hydroxydammaranes are also common in *Betula* species. Thus, the occurrence of 3β,12β,25-trihydroxy-(20*R*,24*R*)-epoxy-dammarane-12-acetate (**1**), particularly in a fern, is unique.

Several cycloartenols have been reported before as fern constituents [16], but none as externally deposited products. Compound **5** is unusual in being an acetonide. It was first assumed, therefore, to be an artifact, formed during exudate processing with acetone. It can be shown, however, that "leaf washs" prepared *e.g.* with ethylacetate, toluene, or chloroform also contain this product. We believe, therefore, that compound **5** is, in fact, a natural acetonide of a triterpenoid. The acetone ketal of marmin, a 7-geranyloxycoumarin derivative from the fruit of *Geijera parviflora* Lindl. (Rutaceae), is the only naturally occurring acetonide reported before, but also in this case the authors considered it an artifact of their acetone treatment [29]. The only likely precursor for the formation of such an artifact would be the corresponding epoxide. It was shown, however, in a careful study, that epoxides react with acetone to form acetonides only in the presence of anhydrous $CuSO_4$, whereas no reaction at all was observed in the absence of $CuSO_4$ [30].

The leaf exudate of *N. rigida* contains several further terpenoids that have not been isolated yet. They all show the same colour reaction with $MnCl_2$ as do compounds **1** and **5**, so they are probably very closely related structurally. TLC comparison further reveals that in *N. rigida* samples collected in different localities, the terpenoid profiles as well as the flavonoid profiles are more or less identical, varying only quantitatively.

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