A New Triterpene Acid from the Fern *Notholaena grayi*

F. J. Arriaga-Giner
Centro I. + D., Tabacalera S.A., Planta Piloto,
c/Embajadores 51, E-28012 Madrid, Spain

Marta Bruix
Instituto de Estructura de la Materia, CSIC,
c/Serrano 119, E-28006 Madrid, Spain

E. Wollenweber
Institut für Botanik der Technischen Hochschule,
Schnittspahnstraße 3, D-W-6100 Darmstadt,
Bundesrepublik Deutschland

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In addition to several previously reported flavonoid aglycones, the white farinose material accumulating on the lower leaf surface of *Notholaena grayi* contains as major constituent a triterpene. This product has now been identified by detailed NMR spectroscopic studies as the novel (24*R*)-3α,24,25-trihydroxylanost-9(11)-en-28-oic acid.

Introduction

Previous studies on several specimens of *N. grayi* Davenp. have revealed, in the white frond exudate, flavonoid aglycones which form a constant pattern [1]. It was indicated later, in a tabulated survey of 23 specimens of *N. grayi,* that a further unknown flavonoid occurs in every species, in small or in trace amount [2]. When bulk material, collected in Arizona, was processed for the isolation of this flavonoid, a terpenoid constituent was also obtained in crystalline form. The structure elucidation of this latter product is reported here.

Materials and Methods

N. grayi was collected on December 30, 1979 on Mount Lemmon in the Catalina Mountains, Pima County, AZ at an elevation of about 3900 feet. Vouchers (G. Yatskievych 79-840) are kept at ARIZ and in E. W.'s herbarium in Darmstadt. The exudate material was recovered by rinsing the dry fern fronds with acetone. After evaporation of the solvent, the residue was separated by column chromatography on Sephadex LH-20, eluted with methanol, and on silica, eluted with toluene and increasing quantities of methylethyl ketone and methanol. From one of the terpenoid fractions a colourless product precipitated from concentrated acetone solution, m.p. 156–158 °C. After drying in a vacuum desiccator overnight the m.p. was 198 °C. The amorphous product **1** is readily soluble in ethanol, but hardly soluble in toluene.

^{1}H NMR spectra were obtained in $CDCl_3$ at 600 MHz on a Bruker AMX-600 spectrometer. Standard 1D, COSY, TOCSY, NOESY and ROESY experiments were recorded. NOE effects were measured using NOESY and ROESY experiments in order to take into account spin diffusion effects. After testing, 400 ms for NOESY and 200 ms for ROESY were found to be the optimal mixing time for this compound. ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer using $(CD_3)_2CO/CDCl_3$ as solvent. Multiplicities in ^{13}C NMR were assigned through DEPT experiments.

^{1}H NMR: see Table I. ^{13}C NMR δ ppm: 175.01 (s, C-28), 146.15 (s, C-9), 115.18 (d, C-11), 82.45 (d, C-24), 78.85 (s, C-25), 74.11 (d, C-3), 60.48 (s, C-14), 50.64 (d, C-17), 44.48 (d, C-5), 44.21 (s, C-13), 39.28 (d, C-8), 38.23 (s, C-4), 37.05 (t, C-12), 36.75 (s, C-10), 34.56 (d, C-20), 43.00, 29.51, 29.24, 28.04, 27.96, 25.01 (all t, not assigned), 27.25 (q, C-27), 24.95 (q, C-26), 24.84 (t, C-7), 21.69, 21.29, 20.39 (all q, not assigned), 20.09 (t, C-6), 17.03 (q, C-21) and 16.20 (q, C-18), EI-MS *m/z* (rel. int.): 490 (M^+, 0.3), 472 ($M^+ - H_2O$, 1), 454 ($M^+ - 2\,H_2O$, 3), 444 (3), 436 ($M^+ - 3\,H_2O$, 1), 426 (8), 409 (4), 393 (5), 327 (4), 299 (5), 175 (7), 91 (14), 69 (18), 59 (64), 43 (100) and 41 (50).

Results and Discussion

The mass spectrum of compound **1** shows a very small molecular ion at *m/z* 490, corresponding to the molecular fomula $C_{30}H_{50}O_5$. Three consecutive losses of water, giving rise to peaks at *m/z* 472, 454 and 436, suggest the presence of three hydroxyl groups. The ^{1}H NMR spectrum shows two oxygen-linked methines (two one proton multiplets at 3.43 and 3.62 ppm), thus indicating that one hy-

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Reprint requests to E. Wollenweber.

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droxyl is attached to a quaternary carbon. The remaining low field multiplet at 5.59 ppm is assigned to an olefinic proton of a trisubstituted double bond in a cyclohexane ring. Seven methyls are observed between 0.82−1.23 ppm, one at 0.92 as a doublet (6.6 Hz), those at 0.82 and 1.07 ppm as broad singlets. These facts suggest a lanostane skeleton, where three methyl groups are located in the side chain (C-21, C-26 and C-27) and the tertiary hydroxyl group at the end as $(CH_3)_2C(OH)$. ^{13}C NMR DEPT spectra confirm the presence of three oxygen-linked carbons, two of them being methines (82.45 and 74.11 ppm), on a quaternary carbon (78.85 ppm). Signals at 146.15 and 115.18 ppm for the olefinic carbons and the more deshielded signal at 175.01 ppm also agree with the proposed structure of a trihydroxylanostenoic acid.

Complete structural elucidation and assignment of ^1H NMR signals of **1** have been accomplished through 2D-^1H NMR experiments (COSY, TOCSY, NOESY and ROESY).

The olefinic proton (5.59 ppm) displays two COSY cross peak correlations with protons at 2.46 and 2.14 ppm, which are in turn mutually coupled. Furthermore, the proton at 2.46 ppm has a long range W-coupling with a methyl group at 0.82 ppm. This fact suggests that the double bond has to be located at C-9(11) because only this structure has an allylic proton (H-12α, 2.46 ppm) that could be W-coupled to a methyl group (Me-18, 0.82 ppm).

The two oxygen-linked methines present different spin systems. The one at 3.43 ppm is coupled to methylene protons at 1.71 and 2.00 ppm which also show cross correlations with two methylene protons at 1.50 and 1.90 ppm. One of these (1.90 ppm) has a long range W-coupling with the methyl group at 1.07 ppm. These data allow us to assign without doubt these spin systems to those corresponding to the protons at C-3 (3.43 ppm), C-2 (1.71, 2.00 ppm), C-1 (1.50, 21.90 ppm) and Me-19 (1.07 ppm) in the A-ring. The observed multiplicity for H-3 (br. t, 2.7 Hz) agrees with an equatorial orientation for such a proton. The spin system of the other oxygen-linked methine at 3.62 ppm is composed of protons with chemical shifts at 1.63, 1.23, 1.44, 1.46 and 0.92 ppm (Me-21) which are assigned to the side chain protons.

Finally, the remaining spin systems consisting of six (1.38, 1.49, 1.60, 1.85, 1.24 and 2.37 ppm) and five (1.26, 2.35, 1.33, 2.18 and 1.35 ppm) resonances are attributed to protons at position 5 to 8 and 15 to 17, respectively, of the lanostane nucleus on the basis of TOCSY cross correlations between H-11 and H-8β (2.37 ppm) and between Me-21 and H-17 (1.35 ppm).

The NOE data support the assignments, provide stereochemical information for all centres in the cyclic structure, and permit the correct identification of Me-29, Me-30 and Me-26/27. The axial orientation of H-1α and H-7α is confirmed, among others, through unambiguous NOEs with fixed H-5α, whereas axial disposition of H-2β and H-6β is also corroborated by NOEs with Me-19/Me-30 and H-8β, respectively. Furthermore, stereospecific assignment of protons at positions 12β and 15β is provided through observed NOEs with Me-18.

Methyl resonances at 0.87 and 0.94 ppm have NOE connectivities with H-3β and H-6α protons as we can expect for Me-29 and Me-30, but only that at 0.87 ppm has additional NOEs with H-2β, H-6β and Me-19 which is consistent with an axial C-30 methyl group. The unassigned methyls at 26/27 positions (1.09 and 1.23 ppm) are confirmed by NOEs with the side chain proton H-24. Fig. 1 shows all NOE connectivities involving cyclic methyl protons. Particularly significant is an NOE

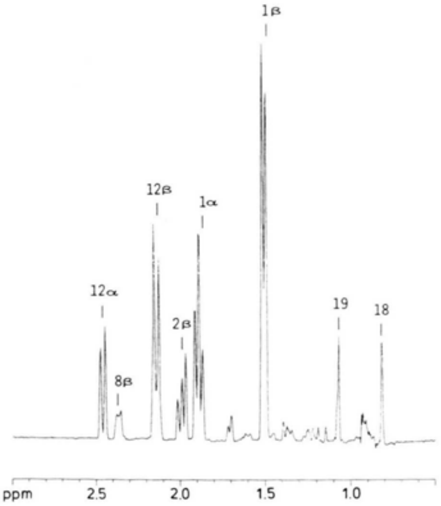


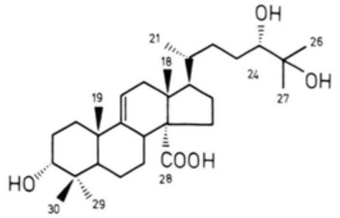
Fig. 1. Compound **1** and selected NOE connectivities involving methyl protons.

between Me-19 and Me-30 which is consistent with groups located on the same face of the molecule.

Proofs of the olefinic proton position in a relatively crowded area are NOE effects with H-12α, H-8β, H-12β H-2β, H-1α, H-1β, Me-19 and Me-18 (Fig. 2). NOE between H-11 and H-1β is more intense than NOE with H-1α; NOE with H-12β is also more intense than with H-12α, whereas with methyl groups both NOE intensities are nearly the same. All these facts agree with the location of the double bond in C-9(11) position of the lanostane ring.

The 24*R* stereochemistry is assumed to be the same as in protolyofoligenic acid because of the multiplicity of the H-24 (dd, 8.7 and 3.7 Hz) which is similar to that observed during correlation of such a 24*R* acid with cycloartenol while that corresponding to the 24*S* epimer should appear as triplet [3].

Thus, the structure for this compound is (24*R*)-3α,24,25-trihydroxylanost-9(11)-en-28-oic acid, which is found for the first time as a natural product. It is an isomer of the previously isolated, and unusual, protolyofoligenic acid [(24*R*)-3α,24,25-trihydroxy-9(19)-cyclolanostan-28-oic acid]. The latter terpenoid has so far only been found in *Lyonia ovalifolia* leaves [4] and, more recently, in the frond exudate of *Notholaena schaffneri* [5]. In this context it should be mentioned that *Notholaena rigida* yielded another very uncommon compound, namely the natural acetonide of the 3-epimer of such an acid [6].



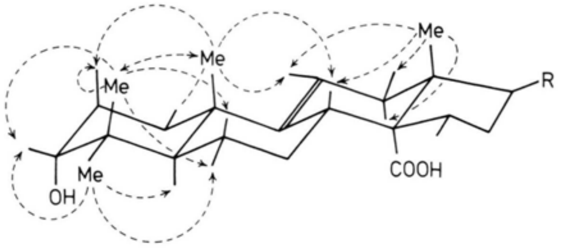


Fig. 2. Row cross section in the OESY spectrum of compound **1** corresponding to H-11 frequency. NOEs between this resonance and other protons are labelled.

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