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TRITERPENES, LICHEXANTHONE AND AN ACETYLENIC ACID FROM *MINQUARTIA GUIANENSIS*

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Key Word Index—*Minquartia guianensis*; Olacaceae; triterpenes; lichexanthone; polyacetylenic acid.

Abstract—Lichexanthone, 17-hydroxy,9,11,13,15-octadecatetraynoic acid and fatty acid esters (mixture) of erythrodiol (12-oleanene-3,28-diol, 3-β-form), and betulin (20-(29)-lupen-3,28-diol) were isolated. The structure of the new triterpene, 13,28-epoxy-3-acetoxy-11-oleanene, 3β,13β form, was determined by X-ray diffraction. The triterpenes occur mainly as C-3-*O*-palmitates.

INTRODUCTION

An infusion of the bark of *Minquartia guianensis* Aubl. is used by traditional healers against various intestinal infections [1], lung cancer and tuberculosis [2] in eastern Ecuador. The Quichuas and Waoranis use the bark as a fish poison [3, 4]. The wood is resistant to rot [5] indicating the presence of antimicrobial metabolites. This species was collected as part of our project on chemical investigations of Ecuadorian medicinal plants. A cytotoxic acetylene (**5**) was previously isolated from the bark [2].

RESULTS AND DISCUSSION

From the petrol fraction the two triterpenes erythrodiol (**1**) and betulin (**3**) were isolated as fatty acid esters (at C-3). Palmitic acid, myristic acid and stearic acid were the principal acids as shown by base catalysed esterification in methanol–dioxane and GC analysis of the methyl ester fraction. Minor amounts of oleic, linolic and arachidic esters were observed. The ester mixture **2** and **4** constituted 0.41 and 0.55%, respectively, of the dry weight of the bark. Apart from the palmitate of erythrodiol these esters have not been reported previously in the literature. A small amount of a new triterpene was also isolated as its fatty acid ester. Methanolysis gave the triterpene (**7**), mp 246–248°, M_r 440, corresponding to $C_{30}H_{48}O_2$. The ^{1}H NMR spectrum showed a characteristic AB spectrum at δ5.33 and 5.83, J=10.5 Hz for the olefinic protons and seven methyls as singlets in the δ0.74–1.05 region. There was one hydroxyl group (3-OH, C-3H, δ3.20, *dd*) and an ether linkage present (H_2C-28, δ3.68, *d*, 3.22, *d*) in the molecule. The triterpene itself did not give suitable crystals for a X-ray determination, but the acetate could successfully be used (Fig. 1, structure **8**). The triterpene (**7**) could be formed biosynthetically by oxidative cyclization of **1**. From the methanolic fraction the unusual acetylenic acid, minquartic acid (**5**) was isolated in exceptionally high quantities and it comprised approximately 2% of the dry weight. The petrol extract contained a small amount of yellow crystalline compound which was identified as lichexanthone (**6**). Xanthones are normally characteristic metabolites of lichens.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR: Varian Gemini 200 at 200 and 50 MHz, respectively. TLC: silica gel 60, $PF_{254+360}$, layer (0.2 × 20 × 20 cm) on glass plates. CC: Kieselgel 60 (0.063–0.200 mm) Merck.

Plant material. Minquartia guianensis was collected in 1990 at the biological station 'Jatun Sacha' in the Provincia del Napo, Ecuador. Voucher specimens are kept in Herbario Economico del Ecuador, Escuela Politecnica Nacional, Quito and in the Dept of Organic Chemistry, Chemical Institute, Aarhus University, Denmark.

Extraction. The bark was dried at 40° in the dark in a ventilated hood. The ground material (5.8 kg) was extracted 3 × with petrol (bp 40–60°) and then with MeOH for 4–6 days each time at room temp. Evapn of the solvents *in vacuo* gave 169 and 514 g of crude material, respectively. Part of the MeOH extract (315 g) was extracted 3 × with EtOAc with mechanical stirring. Decantation of the solvent and evapn *in vacuo* gave 180 g of a crude material. The semi-solid residue was extracted 3 × with H_2O for 2 hr to give 121 g of a crude fr. after evapn of the H_2O *in vacuo*. The insoluble residue (14 g)

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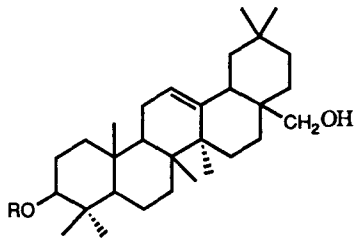
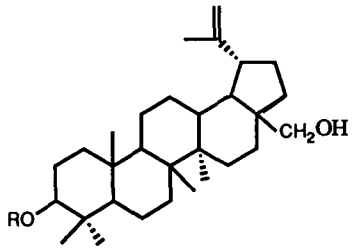


1 R = H

2 R = fatty acids



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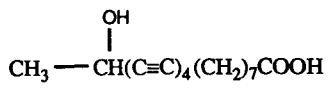
4 R = fatty acids



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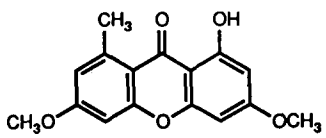

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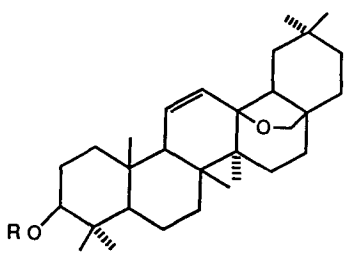

7 R = H

8 R = $COCH_3$

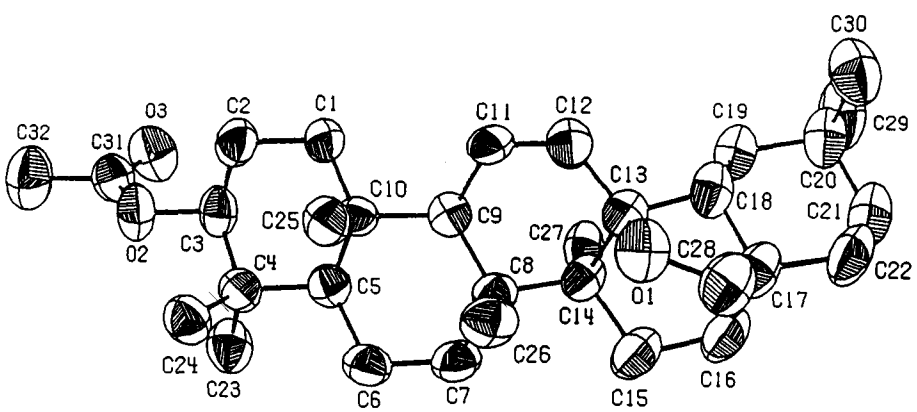

Fig. 1. Perspective view of **8** showing atomic labelling scheme.

was discarded. The H_2O extract consisted mainly of carbohydrates. It was not further investigated.

General procedure for the methanolysis of fatty acid esters of triterpenes (transesterification). The fatty acid esters of the triterpenes (100 mg) were treated in dry MeOH–dioxane (1:2, 10 ml) with sodium methoxide (10 mg) under N_2 for 20 hr. The soln was evapd to 1/3 of the original vol., $CHCl_3$ (5 ml) and H_2O were added and the aq. phase neutralized to pH 3. The organic phase was sepd, dried over Na_2SO_4 and evapd. The residue was sepd by TLC silica gel ($CHCl_3$–MeOH, 98.5:1.5) into a terpene fr. ($R_f \sim 0.5$) and a methyl ester fr. ($R_f \sim 0.9$). The methyl esters were analysed by GC (DB Wax, 30 M × 0.25 mm) with standard refs.

Isolation and purification. The petrol extract (15 g) was fractionated on silica gel with gradient elution by petrol–EtOAc. The first non-polar frs eluted with petrol contained straight chain hydrocarbons, squalene, fatty

acid esters, glycerides, polyprenols. A yellow band was eluted with petrol–EtOAc (98:2) to give yellow crystals (50 mg), mp 192–194°, (lit. [6] 187–190°) which was identified as lichexanthone (**6**) by comparison with spectral data [7, 8]. ^{13}C NMR ($CDCl_3$): δ23.7, 55.6, 56.0, 92.7, 97.2, 98.9, 104.6, 113.4, 115.9, 144.0, 157.5, 160.0, 164.3, 164.4, 166.4, 183.0. IR ν_{max}^{KBr} cm^{-1}: 1655 (s).

Petrol–EtOAc (92:8) eluted fatty acid esters of triterpene (C-3-OH, 2.2 g). Base catalysed methanolysis in dioxane–MeOH gave erythrodiol (**1**) mp 231–234°, from EtOH (lit. [9] 235–237°), identified by its ^{13}C NMR spectrum [10]. ^{1}H NMR ($CDCl_3$): δ3.18 (1H, *d*, J = 11 Hz), 3.2 (2H, *dd*), 3.51 (1H, *d*, J = 11 Hz), 5.17 (1H, *t*, J = 4 Hz). The principal acids were palmitic acid (70%), myristic acid (12%), stearic acid (9%) and oleic acid (3.5%). The stearate has previously been isolated from *Erythroxylon novogratense* [11]. The 28-monoacetate and 3,28-diacetate of **1** were prepared. The ^{1}H NMR spectral data of the 28-monoacetate, mp 183–185° agreed with the lit. data [12]. ^{13}C NMR ($CDCl_3$): 3.28-diacetate of **1**, δ15.7, 16.9, 18.4, 21.1, 21.5, 22.4, 23.7, 25.7, 26.1, 28.2, 29.9, 31.1, 31.6, 32.7, 33.4, 34.2, 36.0, 37.0, 37.9, 38.5, 40.0, 41.9, 42.8, 46.5, 47.7, 55.5, 71.1, 81.2, 123.3, 144.1, 171.6, 171.9.

A 2nd triterpene esterified at C-3-OH with fatty acid **4** (2.9 g) was eluted with the same eluent. Hydrolysis gave betulin **3** identified by ^{1}H and ^{13}C NMR spectral data and transformation to the diacetate [13, 14]. The principal fatty acids were the palmitic, stearic and myristic acids, ~10:2:1.

Small amounts, *ca* 100 mg, of a 3rd triterpene esterified at C-3-OH were eluted with petrol–EtOAc (96:4). It gave, on base catalysed methanolysis, white crystals of **7** mp 246–248° from EtOH MS: 440 $[M]^+$, 410, 268, 157, 129, 85; $C_{30}H_{48}O_2$. ^{1}H NMR ($CDCl_3$): δ0.74–1.05 (7 Me, s), 1.1–2.1 (*m*), 3.20 (1H-3, *dd*), 3.22 (1H-28, *d*, J = 7.5 Hz), 3.68 (1H-28,*d*, J = 7.5 Hz), 5.33 (1H-11, *dd*, J = 10.5 and 2.8 Hz), 5.82 (1H-12, *d*, J = 10.5 Hz). ^{13}C NMR ($CDCl_3$): δ15.4, 18.1, 18.3, 19.8, 19.9, 24.0, 25.7, 26.1, 27.6, 28.3, 30.1, 31.3, 31.8, 32.2, 34.1, 35.3, 36.8, 37.6, 38.7, 39.4, 42.0, 42.0, 44.2, 51.5, 53.7, 55.3, 79.4, 85.3, 131.3, 132.6. Compound **7** gave an acetate, **8**, mp 172–173°, from EtOH MS: 482 $[M]^+$; $C_{32}H_{50}O_3$. ^{1}H NMR ($CDCl_3$): δ3.25 (1H-28, *d*, J = 7.5 Hz), 3.71 (1H-28, *d*, J = 7.5 Hz), 4.49 (1H-3, *t*, J = 9 Hz), 5.37 (1H-11, *dd*, J = 10.5 and 2.8 Hz), 5.83 (1H-12, *d*, J = 10.5 Hz).

X-Ray data for **8**. A crystal of dimension 0.56 × 0.35 × 0.20 mm was mounted on a HUBER diffractometer. Unit cell dimensions were determined from setting angles of 30 reflections measured at $\pm\theta$ and at low and high χ with graphite monochromated Mo K_α (λ = 0.71073 Å) radiation. Crystal data: $C_{32}H_{50}O_3$, M_r = 482.75, monoclinic space group $P2_1$, a = 7.6190(10), b = 16.5352(23), c = 11.2643(15) Å, β = 90.829(8)°; V = 1418.95(32)Å^3; Z = 2, D^c = 1.130 g cm^{-3}; μ(Mo) = 0.65 cm^{-1}; T = 294 K. Data collection: $2.0 \leq 2\theta \leq 50.0°$; $\omega - 2\theta$ scan technique, 2578 unique observed reflections, 1467 with $I > 3\sigma(I)$, were corrected for background, Lorentz and polarization effects, and absorption.

The structure was solved by direct methods using SHELX-86 on a DEC 3000 work station. The structure was refined by the least-squares minimization of $\Sigma w(|F_o| - |F_c|)^2$. Hydrogen atoms were located on a difference map, the methyl group of the acetate group was disordered and the hydrogen atoms for this group were included at calcd positions (C-H = 0.95 Å). The final R-values were $R(F) = 0.035$ and $wR(F) = 0.040$. A full list of coordinates, thermal parameters and bond distances and angles have been deposited at the Cambridge Crystallographic Data Center.

The acetylenic acid 5,17-*hydroxy*, 19,11,13,15-*octadecatetraynoic acid, minquartic acid.* Part of the EtOAc fr. (8.0 g), obtained from the MeOH extraction as described above, was extracted 3 × with $CHCl_3$ to give a product (5.7 g), the main component of which was **5**. Compound **5** (2.9 g) was obtained from 5 g of the $CHCl_3$ extract by CC on silica, $CHCl_3$–MeOH, 6:1. Mp 93–94° from $CHCl_3$, (lit. [2] 95°). The ^{1}H NMR spectrum of **5** agreed with the published data [2] for minquartic acid. The compound was unstable at room temp. and in light.

The crude petrol and MeOH extracts showed no significant activity against micro-organisms and insects. Xanthones were reported to show mutagenic activity [15].

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