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TWO TRITERPENOIDS FROM *GENTIANA NEUROTHECA*

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Key Word Index—*Gentiana neurotheca*; Gentianaceae; triterpenoids; 1α,2α,3β,30-tetrahydroxyurs-12-ene, 3β,24,30-trihydroxyurs-12-ene.

Abstract—Two new pentacyclic triterpenoids were isolated from the leaves of *Gentiana neurotheca* and characterized as 1α,2α,3β,30-tetrahydroxyurs-12-ene and 3β,24,30-trihydroxyurs-12-ene, respectively, based on their spectroscopic properties. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Gentian neurotheca grows in the mountain regions of the Cross River State, Nigeria. Both its leaves and flowers are widely used in traditional medical practices for the treatment of diverse ailments including arthritis and infectious hepatitis. The medicinal importance of the plant has given the impetus to this work. Investigation of the leaves of this plant has yielded two novel triterpenoids, 1α,2α,3β,30-tetrahydroxyurs-12-ene (**1**) and 3β,24,30-trihydroxyurs-12-ene (**2**).

RESULTS AND DISCUSSION

Compound **1** gave an orange colour on treatment with Liebermann Burchard reagent. The HREI mass spectrum showed a $[M]^+$ peak at *m/z* 474.7240 [Calcd for $C_{30}H_{50}O_4$: 474.7246] and fragment ion peaks at *m/z* 240 (A/B ring, $[C_{14}H_{24}O_3]^+$), 204 $[240\text{-}2H_2O]^+$, 234 (D/E ring, $[C_{16}H_{26}O]^+$), 218 $[234\text{-}CH_3]^+$, and 134 $[218\text{-}C_5H_{10}O]^+$ consistent with the ursene fragmentation pattern [1] and indicating that **1** possesses four hydroxy groups on an ursene skeleton. The retro Diels–Alder fission results indicated that three hydroxy groups were in the AB-portion of the ursene skeleton. The fragmentation of **1** is as shown in Fig. 1.

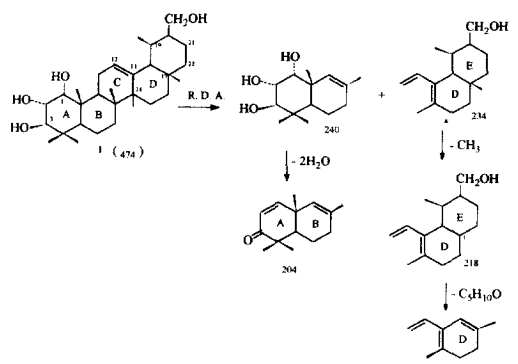

Fig. 1. Fragment ion peaks (*m/z*) in the mass spectrum of **1**. (R.D.A.: retro Diels–Alder fragmentation).

The ^{13}C NMR spectrum of **1** showed the presence of signals due to four oxygenated carbons at δ 68.2 (*t*), 71.7 (*d*), 74.7 (*d*) and 81.5 (*d*) together with signals for the carbons of a trisubstituted double bond at δ 123.9 (*d*) and 143.8 (*s*). The primary alcohol may be at C-29 or C-30 as the methylene protons gave a double doublet showing a coupling with an adjacent hydrogen atom. The position of the fourth hydroxy group is fixed at C-30 by comparison with the methyl shift values of the ^1H and ^{13}C NMR spectra signals of some reported ursene derivatives [2–6]. The ^1H NMR spectrum of **1** showed signals due to three methine protons at δ 4.71 (*br s*), 4.32 (*br, d, J* = 9.4 Hz) and 4.23 (*d, J* = 9.5 Hz) adjacent to hydroxy groups. The $^1H\text{-}^1H$ COSY of **1** indicated that the proton at δ 4.32 is not only coupled with the protons at δ 4.71 but also with the proton at δ 4.23. Thus, the three hydroxy groups are in ring A. The *J* values of the three proton signals indicated the presence of axial/equatorial and axial/axial coupling patterns, respectively.

$^{13}C\text{-}^1H$ COSY of **1** disclosed that the three signals at δ 4.71, 4.32 and 4.23 could be assigned to H-1β, H-2β, and H-3α, respectively, so the configuration of the three hydroxy groups would be 1α, 2α and 3β on ring A of an ursene skeleton. The structure of **1** is, therefore, 1α,2α,3β-30-tetrahydroxyurs-12-ene.

Compound **2** also gave an orange colour on treatment with Liebermann Burchard reagent. The HREI mass spectrum showed a $[M]^+$ peak at *m/z* 458.7236 [Calcd for $C_{30}H_{50}O_3$: 458.7247] and fragment ion peaks at 224 (A/B ring, $[C_{14}H_{24}O_2]^+$), 190 [M-234-MeOH-H_2]$^+$, 175 $[190\text{-}0]^+$, 234 (D/E ring,

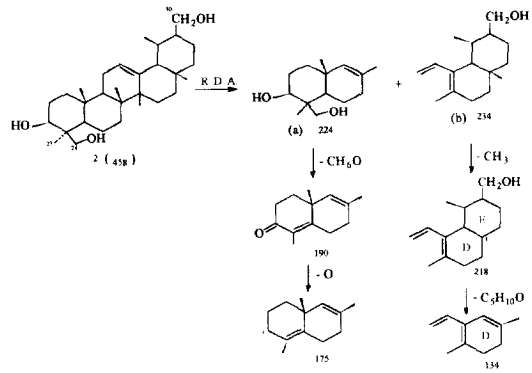

Fig. 2. Fragment ion peaks (m/z) in the mass spectrum of **2** (R.D.A.: retro Diels–Alder fragmentation).

$[C_{16}H_{26}O]^+$), 218 $[234\text{-}CH_3]^+$, 134 $[218\text{-}C_5H_{10}]^+$ consistent with ursene fragmentation pattern [1]. These fragments were derived from retro Diels–Alder fragmentation as shown in Fig. 2. The ^{13}C NMR spectrum of **2** showed the presence of three oxygenated carbons at δ 80.9, 65.2 and 68.4. The ^1H NMR spectrum of **2** showed the presence of two oxygenated methylene protons at δ 4.20 and 3.87 (1H each, *d*, J = 11.0 Hz), 4.20 and 3.93 (1H each, *dd*, J = 11 and 7.1 Hz; 11 and 3.6 Hz), signals due to six tertiary methyls and one secondary methyl in the region δ 0.81–1.30, a signal for one methine proton at δ 4.25 could be assigned to H-3α and so the configuration of the hydroxy group at C-3 should be β. There was a triplet at δ 5.25 (J = 3.5 Hz) due to H-12. Its IR spectrum showed bands at 1640 and 840 cm^{-1} (trisubstituted double bond). The olefinic carbons C-12 and C-13 in the ^{13}C NMR spectrum of **2** appeared at δ 125.1 and 138.5, respectively. The upfield value of C-13 suggested clearly that the compound is a derivative of ursene rather than an oleanane [7]. The ready loss of the methyl group at C-17 and $C_5H_{10}O$ from the retro Diels–Alder fragment '*b*' gave a fragment at m/z 134. One primary alcohol is shown to be on ring A of the molecule by the retro Diels–Alder fragmentation (Fig. 2). The other primary hydroxy group again may be at C-29 or C-30 as in **1** because each of the methylene protons of the alcohol gave a double doublet showing a coupling with an adjacent hydrogen atom. The position of the hydroxy group is fixed at C-30 by comparison with the methyl shift values of ^1H and ^{13}C NMR spectral signals of some reported ursene derivatives [2–6]. Furthermore, from the EI mass spectral data, it follows that the other primary hydroxy group is attached to ring A and the position is at C-23 or C-24. Usually, the chemical shift of the C-24 methyl group is at about δ 12.8 [8] if the hydroxy group is linked at C-23 and the chemical shift of the C-23 methyl group is about δ 23.5 if the hydroxy group is linked at C-24 [9]. The chemical shift of C-23 is at δ 28.1 in **2** and thus the other primary hydroxy group must be linked at C-24 of ring A. The structure of **2** is thus 3β,24,30-trihydroxyurs-12-ene.

EXPERIMENTAL

Mps: uncorr: IR spectra: $CHCl_3$, ^1H and ^{13}C NMR on Bruker AMX 400: TMS as int. Stand., MS: 70 ev, on Varian MAT 700 spectrometer., 2D NMR experiments (^1H-^1H and ^{13}C-^1H COSY) were carried out with standard pulse sequence.

Plant material. The plant material was collected from Cross River State, Nigeria and authenticated by Dr John of the Botany Division of the Department of Biological Sciences of the University of Calabar. A voucher specimen documenting its collection is deposited in the Herbarium of the University of Calabar, Calabar.

Extraction and isolation. The leaves of *G. neurotheca* collected were dried in the shade. The powdered leaves (4.5 kg) were exhaustively extracted with $CHCl_3$ by the cold percolation method and the concd extract subjected to CC over silica gel G using C_6H_6–EtOAc (3:1) as eluent. Frns eluted were tested for homogeneity and similar pure frs were mixed together and evapd to provide two compounds. Compound **1** (50 mg) needles from $CHCl_3$–MeOH, $C_{30}H_{50}O_4$, mp 168–170°; IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2955, 2875, 1460, 1377, 1213, 1113, 1032, 986, 879, 840, 768, 677.

^1H NMR (300 MHz, $CDCl_3$): δ 0.81 (3H, *s*, H-28), 0.82 (3H, *d*, J = 6 Hz, H-29), 0.86 (3H, *s*, H-23), 0.87 (3H, *s*, H-24), 0.98 (3H, *s*, H-25), 1.05 (3H, *s*, H-26), 1.11 (3H, *s*, H-27), 5.14 (1H, *t*, J = 3.5 Hz, H-12), 4.71 (1H, *br*, *s*, H-1α), 4.32 (1H, *d*, J = 9.4 Hz, H-2α), 4.23 (1H, *d*, J = 9.5 Hz, H-3β), 1.99 (1H, H-5), 2.28 (1H, *q*, J = 6.3 Hz, H-9), 3.13 (2H, *m*, H-11), 2.74 (1H, *d*, J = 11.6 Hz, H-18).

^{13}C NMR: δ 74.1 (C-1), 71.7 (C-2), 81.5 (C-3), 44.9 (C-4), 49.4 (C-5), 19.1 (C-6), 34.0 (C-7), 40.6 (C-8), 49.6 (C-9), 43.8 (C-10), 28.0 (C-11), 123.9 (C-12), 143.8 (C-13), 42.2 (C-14), 28.4 (C-15), 23.9 (C-16), 33.7 (C-17), 42.0 (C-18), 46.5 (C-19), 31.0 (C-20), 34.3 (C-21), 33.3 (C-22), 23.9 (C-23), 15.7 (C-24), 13.5 (C-25), 17.8 (C-26), 23.5 (C-27), 28.5 (C-28), 17.1 (C-29), 68.2 (C-30). EIMS: m/z 474 $[M]^+$, $(C_{30}H_{50}H_4]$, 240 (A/B ring, $[C_{14}H_{24}O_3]^+$, 204 $[240\text{-}2H_2O]^+$, 234 (D/E ring, $[C_{16}H_{26}O]^+$), 218 $[234\text{-}CH_3]^+$ and 134 $[218\text{-}C_5H_{10}O]^+$.

Compound **2**. (30 mg), powder from MeOH, $C_{30}H_{50}O_3$, mp 198.5–200.5°; IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3418, 3018, 2950, 2875, 1460, 1375, 1210, 1113, 1028, 984, 878, 840, 768, 675.

^1H NMR: δ 0.81 (3H, *s*, H-28), 0.82 (3H, *d*, J = 6 Hz, H-29), 0.86 (3H, *s*, H-23), 4.20 (1H, *d*, J = 11 Hz, H_β-24), 3.87 (1H, *d*, J = 11 Hz, H_β-24), 1.30 (3H, *s*, H-25), 1.11 (3H, *s*, H-26), 1.23 (3H, *s*, H-27), 4.20 and 3.93 (1H each, *dd*, J = 11 and 7.1 Hz; 11 and 3.6 Hz, H-30), 5.55 (1H, *t*, J = 3.5 Hz, H-12), 2.71 (1H, *d*, J = 11.6 Hz, H-18) ^{13}C NMR (25 MHz, $CDCl_3$): δ 38.5 (C-7), 40.0 (C-8), 47.6 (C-9), 36.0 (C-10), 23.5 (C-11), 125.1 (C-12), 138.5 (C-13), 42.0 (C-14), 26.9 (C-15), 29.3 (C-16), 33.6 (C-17), 58.8 (C-18), 36.8 (C-19), 43.9 (C-20), 28.1 (C-21), 40.8 (C-22), 28.1 (C-23), 65.2 (C-24), 16.1 (C-25), 16.8 (C-26), 23.5 (C-27), 28.5 (C-

28), 17.1 (C-29), 68.4 (C-30). EIMS: *m/z* 458 ($C_{30}H_{50}O_3$) and fragment ion peaks at 224 (A/B ring, $[C_{14}H_{24}O_2]^+$), 190 [M-234-MeOH-H_2]$^+$, 175 [190-O]$^+$ 234 (D/E ring, $[C_{16}H_{26}O]^+$, 218 [234-CH_3]$^+$, 134 [218-$C_5H_{10}O]^+$.

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