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A TRITERPENE ACID FROM *NEPETA HINDOSTANA*

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Key Word Index—*Nepeta hindostana*; Labiatae; triterpene acid; 2β,3α,23-trihydroxyurs-12-en-28-oic acid.

Abstract—A new triterpene acid has been isolated from alcoholic extract of *Nepeta hindostana* and its structure is elucidated as 2α,3β,23-trihydroxyurs-12-en-28-oic acid.

INTRODUCTION

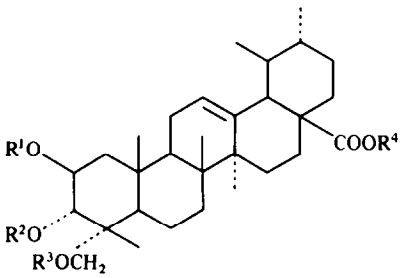
We have already reported the isolation and structure elucidation of the triterpenic alcohols nepeticin [1], nepetidin [2], lup-20 (29)-ene-1β,3β-diol [3] and nepehinol [4] as well as the glycoside of sitosterol and tetratriacontanol [5] from the hexane extract of *N. hindostana*. We have now isolated a new triterpene acid **1** from the alcoholic extract of the same plant.

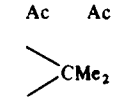
RESULTS AND DISCUSSION

Compound **1** was eluted in small quantity with benzene–EtOAc (85:15) from a column of silica gel and crystallized from methanol as colourless needles (20 mg), mp 270°. Its mass spectrum showed a molecular ion peak at *m/z* 488 corresponding to the formula $C_{30}H_{48}O_5$. On treatment with diazomethane, it furnished a monoester (**1a**), which on acetylation, gave a monoester triacetate. The vicinal position of the hydroxyl groups was confirmed through positive reaction with periodic acid. The IR spectrum of **1a** indicated the presence of hydroxyl (3400 cm^{-1}) and ester (1720 cm^{-1}) groups. The mass spectrum of **1** showed the characteristic retro-Diels–Alder fragmentation of ring C of Δ^{12}-amyrin derivatives [6] with a base peak at *m/z* 248 corresponding to fragment *a*, indicating the absence of hydroxyl groups in rings C, D and E. There was also a strong peak at *m/z* 203 due to the removal of the COOH group from fragment *a*.

The ^{1}H NMR spectrum of **1a** in $CDCl_3$ displayed signals due to six methyl groups at δ0.73, 0.85 (each *d*, *J* = 6 Hz), 0.94, 1.10 (each *s*, 3H), 0.98 (*s*, 6H), a single proton doublet centred at δ2.20 (*J* = 10 Hz) characteristic of H-18 of an ursane series [7], a singlet at δ3.60 due to a carbomethoxy group and an AB quartet centred at δ2.93 (2H) assigned to H-23. There were two doublets at 3.49 (*J* = 3 Hz) and 3.68 (*J* = 3 Hz) which can be assigned to H-2 and H-3, respectively, indicating the equatorial nature of these protons. A triplet at δ5.24 is due to the proton H-12. The ^{1}H NMR spectrum of **1b** showed three acetoxy methyl signals at δ1.95, 1.97 and 2.00 and an AB quartet centred at δ3.90 (*J* = 10 Hz) due to a methylene group bearing an acetoxy group. This chemical shift is within the range for an equatorial CH_2OAc [8]. The spectrum also showed the multiplet for two carbinylic protons together with an olefinic proton in the region δ5.18–5.30. These chemical shifts of the carbinylic protons are quite different from those reported for 2α,3β,23-trihydroxyurs-12-en-28-oic acid [9], 2α,3β,23-trihydroxyurs-12-en-28-oic acid [10] or 2β,3β,23-trihydroxyurs-12-en-28-oic acid [11]. These facts lead us to conclude that the three hydroxyl groups in compound **1** are at the 2β,3α and 23 positions. Until now only one triterpene alcohol having 2β,3α-hydroxyl groups has been isolated, namely 2β,3α-dihydroxyolean-12-en-28-oic acid [12] but unfortunately the NMR data of this compound are not reported. Compound **1a** forms only a monoacetonide (**1c**) which showed the signals of H-23, H-3 and H-2 carbinylic protons at δ3.32 (*br s*), 3.53 (*d*, *J* = 3 Hz) and 3.68 (*d*, *J* = 3 Hz), respectively. The former two signals of H-23 and H-3 are shifted downfield in **1c** while the signal of H-2 remained unshifted, which further confirmed that the acetonide formation was between H-3 and H-23. It also supports the proposed structure 2β,3α,23-trihydroxyurs-12-en-oic acid, because no acetonide formation is possible between the two axially oriented hydroxyl groups at C-2 and C-3 due to steric reasons.

This proposed structure is also confirmed by the ^{13}C NMR spectrum of **1a** (Table 1) which showed double



	R¹	R²	R³	R⁴
1	H	H	H	H
1a	H	H	H	Me
1b	Ac	Ac	Ac	Me
1c	H			H

bonded carbon signals at δ125.3 (C-12) and 138.5 (C-13). There were also signals of three carbon bearing oxygen functions at 78.58 (C-3), 71.35 (C-23), and 66.62 (C-2). Thus, all of the spectroscopic data cited above lead to the structure 2β,3α,23-trihydroxyurs-12-en-28-oic acid.

EXPERIMENTAL

The melting points were determined on a Gallenkamp apparatus and are uncorr. ^{1}H NMR recorded at 200 MHz and ^{13}C NMR was recorded on Bruker-300 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. TLC was performed on silica gel plates using the following solvent systems: (a) C_6H_6–EtOAc (7:3); (b) $CHCl_3$–MeOH (17:3); (c) $CHCl_3$–MeOH (9:1). Spots were detected by spraying with ceric sulphate soln in 10% H_2SO_4 followed by heating.

Extraction and separation. The plant material was extracted × 3 each with hexane and with cold EtOH. The combined ethanolic extract was evaporated at red. pres. to afford a gummy residue. This residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated and evaporated under red. pres. to afford crude material, which was chromatographed on a silica gel column. Elution of the column was carried out with gradients of increasing polarity in the order of hexane, C_6H_6, EtOAc and MeOH.

2α,3β,23-Trihydroxyurs-12-en-28-oic acid (**1**). The fractions eluted with C_6H_6–EtOAc (17:3) afforded compound **1**, which on recrystallization with MeOH yielded colourless shining crystals of **1** (20 mg), mp 270°. MS *m/z* (rel. int.): 488 $[M]^+$ (0.5), 444 $[M-CO_2]^+$ (0.5), 425 $[M-COOH-H_2O]^+$ (0.2), 410 $[M-COOH-H_2O-Me]^+$ (0.2), 248 [RDA fragment *a*]$^+$ (100), 203 $[a-COOH]^+$ (52), 189 (10), 133 (50), 119 (24).

Methylation of compound **1**. Compound **1** (18 mg) was dissolved in MeOH and treated with CH_2N_2 at room temp. for 2 hr. After evaporation of the solvent from the reaction mixture, the methyl ester (**1a**) was obtained which was crystallized from MeOH as colourless crystals, mp 210–212°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 2920, 2860, 1720 (ester), 1000. ^{1}H NMR ($CDCl_3$): δ0.73 (*d*, *J* = 6 Hz, sec. Me), 0.80 (*d*, *J* = 6 Hz, sec. Me), 0.95 (*s*, Me), 0.99 (*s*, 6H, 2 × Me), 1.10 (*s*, Me), 2.20 (*d*, *J* = 10 Hz, H-18), 3.60 (*s*, COOMe), 3.49 (*d*, *J* = 3 Hz, H-3), 3.68 (*d*, *J* = 3 Hz, H-2), 2.93 (ABq, *J* = 10 Hz, 2 × H-23), 5.20 (*t*, H-12). MS *m/z* (rel. int.): 502 $[M]^+$ (1), 469 $[M-H_2O-Me]^+$ (0.05), 443 (1), 436 $[M-2H_2O-2Me]^+$ (0.02), 262 [RDA fragment *a*]$^+$ (100), 203 $[a-COOMe]^+$ (99), 189 (26), 173 (27), 133 (91), 95 (22).

Acetylation of compound **1a**. Compound **1a** (10 mg) was treated with Ac_2O, and 2–3 drops of pyridine was added. The reaction mixture was kept overnight at room temp. and ice was added. The mixture was extracted with Et_2O and the Et_2O layer washed with H_2O. After evaporation of the solvent the residue was crystallized with MeOH to furnish colourless rods of methyl ester triacetate (**1b**). mp 108–110°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2850, 1720. ^{1}H NMR ($CDCl_3$): δ0.75 (*s*, Me), 0.86 (*d*, *J* = 6 Hz, sec. Me), 0.90 (*s*, Me), 0.97 (*d*, *J* = 6 Hz, sec. Me), 0.98 (*s*, Me), 1.06 (*s*, Me), 1.95, 1.97, 2.00 (each *s*, 3H, 3 × OAc), 3.61 (*s*, COOMe), 3.90 (*dd*, *J* = 10 Hz, 2 × H-23), 5.18–5.30 (*m*, 3H, H-2, H-3 and H-12). MS *m/z* (rel. int.): 628 $[M]^+$ (6.8), 568 $[M-AcOH]^+$ (9.0), 508 $[M-2AcOH]^+$ (6.3), 448 $[M-3\times AcOH]^+$ (15.9), 407 (15.6), 389 (13.6), 262 [RDA fragment *a*]$^+$ (100), 203 $[a-COOMe]^+$ (100), 133 (100).

Acetonide of compound **1a**. Acid **1a** (5 mg) was treated with dry Me_2CO (10 ml) and dry $CuSO_4$ (20 mg) and stirred at room temp. After 10 min it was filtered and solvent was removed from the filtrate to furnish acetonide **1c**. It showed a single spot on TLC in solvent system (c), which was less polar than the original compound. MS *m/z* (rel. int.): 542 $[M]^+$ (11), 482 $[M-60]^+$ (5), 466 (7), 427 (6.5), 262 [RDA fragment *a*]$^+$ (100), 203 $[a-COOMe]^+$ (100), 189 (27), 133 (74). ^{1}H NMR ($CDCl_3$), 300 MHz): δ0.74 (*s*, Me), 0.86 (*s*, Me), 0.95 (*d*, *J* = 6.5 Hz, Me), 0.99 (*d*, *J* = 6 Hz, Me), 1.10 (*s*, Me), 1.11 (*s*, Me), 1.26 (*s*, 6H, 2 × Me), 2.24 (*d*, *J* = 10.94 Hz, H-18), 3.32 (*br s*, 2 × H-23), 3.53 (*d*, *J* = 3 Hz, H-3), 3.59 (*s*, COOMe), 3.68 (*d*, *J* = 3 Hz, H-2), 5.26 (*br s*, H-12).

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