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3α,24-DIHYDROXY-URS-12-EN-28-OIC ACID FROM *VERBENA OFFICINALIS**

MUNDKINAJEDDU DEEPAK and SUKHDEV S. HANDA†

Regional Research Laboratory (CSIR), Canal Road, Jammu Tawi, 180001, India

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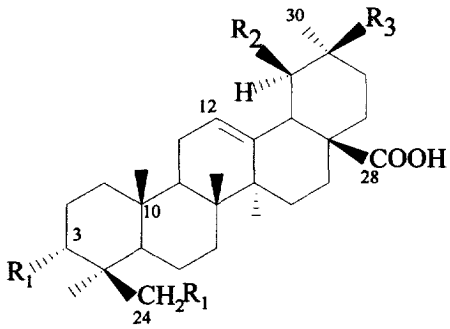
Key Word Index—*Verbena officinalis*; Verbenaceae; triterpenoids.

Abstract—A new triterpenoid, 3α,24-dihydroxy-urs-12-en-28-oic acid, has been isolated from the bioactive chloroform extract of aerial parts of *Verbena officinalis* along with 3α,24-dihydroxy-olean-12-en-28-oic acid and ursolic acid.

INTRODUCTION

Verbena officinalis Linn., a popular herb in folk medicine for the treatment of rheumatism and bronchitis and for use as a diuretic, is widely grown in all temperate regions of the earth [1]. The iridoids verbenalin [2] and hastatoside [3] and the phenyl propanoids verbascoside and eukovoside [4] have been isolated from this plant. A preliminary investigation of the chloroform extract of aerial parts exhibited anti-inflammatory activity (56% at 500 mg kg^{-1} bodyweight as compared to 79% at 50 mg kg^{-1} of Ibuprofen) in the carrageenan paw oedema model. Chemical investigation of the extract led to the isolation of a new triterpenoid, 3α,24-dihydroxy-urs-12-en-28-oic acid (**1**), its isomer 3α,24-dihydroxy-olean-12-en-28-oic acid (**3**) and large amounts of ursolic acid.



	R_1	R_2	R_3
1	OH	CH_3	H
2	OAc	CH_3	H
3	OH	H	CH_3
4	OAc	H	CH_3

RESULTS AND DISCUSSION

The EI mass spectrum of **1** contained retro Diels Alder (RDA) fragmentation peaks at *m/z* 248, 203, 189, 175 and 133, characteristic of Δ^{12} pentacyclic triterpenoids [5]. The molecular weight (472 by FAB-MS) corresponded to the molecular formula $C_{30}H_{48}O_4$. ^{1}H NMR signals at δ 5.25 (*br s*, H-12) and 2.18 (*d*, *J* = 11.0 Hz, H-18) and ^{13}C NMR signals at δ 125.9 (C-12), and 138.6 (C-13) indicated [6, 7] that the compound belonged to the urs-12-en series. From its IR absorptions at 1693 cm^{-1} (carbonyl), ^{13}C signal at 180.6 (COOH) and RDA fragments at *m/z* 248 (fragment "a") and 203 ("a"—COOH), it was concluded that the C, D and E rings were identical to those of ursolic acid and that the additional substitutions were either in ring A or B. The formation of the diacetate (**2**) of **1** suggested that these substitutions corresponded to two hydroxyl groups. The mass spectrum of **2** also contained RDA fragments of **1**, thus validating the above inferences.

Indications of an uncommon α (axial) hydroxyl group at the biogenetically probable C-3 position were provided by the ^{1}H NMR signal of **1** at δ 3.84 (*br s*) with a corresponding acetylation shift (1.1 ppm) in **2** to δ 4.99 (*br s*), an upfield ^{13}C signal at δ 70.3, and a shielded C-1 resonance at δ 33.5 due to γ-gauche interactions with C-3 OH [8, 9]. The longer time taken for

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† Author to whom correspondence should be addressed.

the completion of acetylation of **1** was also in favour of a sterically hindered hydroxyl group. An AB system (δ 3.47, 3.81, J = 11.0 Hz each *d*) in the ^{1}H NMR of **1** which was shifted to downfield in **2**, was attributed to an axial hydroxymethylene group attached to an asymmetric centre. Support for its assignment as C-24 CH_2OH was provided by a strong 1,3 diaxial interaction between 3α (*ax*) OH and 4β (*ax*) CH_2OH which gave rise to large upfield shift of C-3 (δ 70.3). Moreover, a shielded C-24 resonance (δ 65.3) ruled out the possibility of an equatorial hydroxymethylene group at C-4 which would have been comparatively deshielded by about 3 ppm [9]. Further evidence for the C-4 stereochemistry was obtained by comparison of the average chemical shift value for the acetoxy methylene protons of **2** [1/2 ($\delta A+\delta B$) = 4.11] with that of **4**, the latter's value being identical to the literature [10].

The spectral data of compounds **3** and **4** were comparable with those of **1** and **2** except for the characteristic differences between the oleanane and ursane skeletons. Carbon resonances of **4** at δ 41.0 (C-18), 122.5 (C-12) and 144.1 (C-13), the ^{1}H signal at δ 2.84 (*dd*, $J_{AX}+J_{BX}$ = 18.0 Hz, H-18) and the presence of six tertiary methyl groups gave clear evidence for an oleanane skeleton in **3** and **4**. The molecular formula of **3** was identical to **1** and all the spectral data were in accordance with the literature [10].

It may be noted that despite the large number of literature reports [11, 12] of triterpenoids with hydroxylations on 2,3,23, or 2,3,24 carbons with all combinations of stereochemistries, the occurrence of compounds with only 3α,24 dihydroxy substitutions in the ursane and oleanane series seems to be rare.

EXPERIMENTAL

General

Mps: uncorr; IR: KBr; ^{1}H- and ^{13}C-NMR: TMS as int. reference; TLC: Silica gel followed by 10% H_2SO_4 in MeOH and heating at 120° for 5–10 min; CC: silica gel (100–200 mesh), monitored by TLC; EIMS: direct inlet system.

Plant material

The aerial parts of *Verbena officinalis* Linn. were collected from Chakrata (U.P.), India, in November 1995. The identity of the plant was confirmed by Dr T. S. Sareen, Professor of Taxonomy, Department of Botany, Panjab University, and a voucher specimen has been deposited at the Herbarium of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

Extraction and isolation

Ground aerial parts (1.3 kg) were extracted with petrol by cold maceration and percolation for 72 h. The concentrated petrol extract gave sitosterol upon CC. The Marc was air dried and further extracted with $CHCl_3$ by the same method for 5 days. It was concentrated to about 200 ml, the insoluble material present was filtered and the filtrate (A) was set aside. The insoluble solid was washed with Et_2O (3 × 15 ml) and Me_2CO (2 × 10 ml) to give a light green solid (500 mg). The solid was subjected to CC and eluted with $CHCl_3$–EtOAc (3:1) to yield ursolic acid and compound **1**. The filtrate (A) upon concentration and CC ($CHCl_3$–MeOH, 97:3) gave a mixture of ursolic acid and compound **3** which were separated by CC ($CHCl_3$–EtOAc, 3:1). Compounds **1** and **3** were purified by washings with Et_2O and crystallizations from MeOH.

Compound **1**. Mp 242–244°; $[\alpha]_D^{25}$ +44° (MeOH; *c* 0.255); IR$_{max}$ cm^{-1}: 3416, 2931, 1693, 1455, 1396, 1031; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.18 (1H, *d*, J = 11.0 Hz, H-18), 3.47, 3.81 (2H, AB-*q*, J = 11.0 Hz each *d*, H-24), 3.84 (1H, *br s*, H-3), 5.25 (1H, *br s*, H-12); FAB-MS (positive) *m/z*: 495 [M+Na]$^+$; EIMS (70 eV) *m/z*: 248 (100), 203 (75), 189 (18), 175 (31), 161 (11), 145 (14), 133 (34); ^{13}C NMR (50 MHz, CD_3OD): δ 33.5 (C-1), 17.5 (C-29), 21.0 (C-30), 53.4 (C-18), 65.3 (C-24), 70.3 (C-3), 125.9 (C-12), 138.6 (C-13), 180.6 (C-28).

Acetylation of **1**. Compound **1** was acetylated with Ac_2O and pyridine for 24 h at room temp. and worked up in the usual manner to yield **2** as amorphous solid.

Compound **2**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.08, 2.13 (each 3 H, *s*, MeCO), 2.23 (1H, *d*, J = 11.0 Hz, H-18), 3.89, 4.34 (2H, AB-*q*, J = 11.0 Hz each *d*, H-24), 4.99 (1H, *br s*, H-3), 5.30 (1H, *br s*, H-12) FAB-MS (positive) *m/z*: 579 [M+Na]$^+$; ^{13}C NMR: Table 1.

Table 1. ^{13}C NMR (75 MHz, $CDCl_3$) data of compound **2***

C	**2**	C	**2**
1	33.7	21	30.7
2	22.6	22	36.7
3	73.5	23	22.1
4	42.4	24	66.8
5	50.8	25	15.8
6	18.3	26	17.0
7	33.5	27	23.8
8	40.0	28	182.3
9	47.5	29	17.1
10	37.1	30	21.2
11	23.4	OAc	171.0
12	125.8		171.9
13	138.3		21.0
14	42.6		21.4
15	28.1		
16	24.1		
17	48.3		
18	52.7		
19	39.2		
20	39.0		

* Assignments based on DEPT experiments.

Compound **3**. Mp 274–276° $[\alpha]_D^{25} = +31°$ (MeOH; *c* 0.170); IR and EIMS identical to lit.; FAB-MS (positive) *m/z*: 495 $[M+Na]^+$; ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.6 (C-30), 33.1 (C-29), 33.6 (C-1), 41.6 (C-18), 64.4 (C-24), 68.8 (C-3), 121.9 (C-12), 144.1 (C-13), 179.1 (C-28).

Compound **4** (*diacetate of* **3**). ^1H NMR (300 MHz, $CDCl_3$): δ 2.05, 2.08 (each 3H, *s*, MeCO), 2.84 (1H, *dd*, $J_{AX}+J_{BX}$ = 18.0 Hz, H-18), 3.85, 4.29 (1H, AB-*q*, *J* = 11.0 Hz each *d*, H-24), 4.96 (1H, *t* like, H-3), 5.29 (1H, *t* like, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1, 21.5 (CO<u>C</u>H3), 23.6 (C-30), 33.1 (C-29), 41.0 (C-18), 66.8 (C-24), 73.5 (C-3), 122.5 (C-12), 144.1 (C-13), 170.8, 171.4 (<u>C</u>OCH3), 182.1 (C-28).

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