

TERPENOIDS AND STEROIDS OF THE AERIAL PARTS OF *MIRABILIS JALAPA* LINN.

B.S. SIDDIQUI, Q.ADIL, S. BEGUM AND S. SIDDIQUI

H.E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

(Received November 14, 1993)

Three compounds including a diterpene, *trans*-phytol, a pentacyclic triterpenoid, methyl 3-oxo-urs-12-en-28-oate and a steroid, β -sitosterol acetate have been isolated for the first time from the fresh aerial parts of *Mirabilis jalapa* Linn. along with oleanolic acid, ursolic acid, β -sitosterol, brassicasterol and stigmasterol. Their structures have been established with the help of spectral data. This is the first report of 2D NMR studies (Hetero-COSY, COSY-45, NOESY, J-resolved) of *trans*-phytol.

Key words: *Mirabilis jalapa*, Terpenoids, Steroids.

Introduction

Mirabilis jalapa Linn. (Nyctagineae), commonly known as Gul'A'bbas (flower of A'bbas) is distributed throughout West Indies. Five varieties of this plant, with red, white, yellow, red and white, and red and yellow flowers, were introduced from the West Indies in 1596 [1]. It is a native of South America, widely cultivated in many tropical areas. The plant has been used in the indigenous system of medicine. Roots of the plant are said to be purgative [2], whereas leaves have been used to cure and lessen inflammation [2], proteinous substance has been reported to possess antitumor [3] and plant virus inhibitor activity [4]. Studies undertaken on *Mirabilis jalapa* by various groups of workers in view of its medicinal significance, have led to the isolation of soluble starch [5], pigments of centrospermae [6], sterols and fatty acids [7], sugars [8], amino acids [9] and triterpenes [10]. The present communication reports the isolation and characterization of eight compounds 1-8 from the aerial parts of the plant, including one diterpene *trans*-phytol 1, three pentacyclic triterpenes, methyl 3-oxo-urs-12-en-28-oate 2, oleanolic acid 3, ursolic acid 4, and four steroids β -sitosterol 5, β -sitosterol acetate 6, brassicasterol 7 and stigmasterol 8. Of these 1, 2 and 6 are hitherto unreported from this source, whereas 3-5, 7 and 8 have previously been reported [11,12]. The structure of 1 has been determined on the basis of detailed ^1H - and ^{13}C -NMR studies including 2D experiments (COSY-45, NOESY, J-resolved and hetero-COSY) which led to the complete assignments of the protons and carbons.

Experimental

Ms: Finnigan MAT 112 and 312 double focusing mass spectrometers connected to PDP 11/34 computer system, NMR spectra (CDCl_3): 400 MHz for ^1H and 75 MHz for ^{13}C nuclei. The chemical shifts are reported in δ (ppm) and coupling constants are in Hz. In the ^{13}C -NMR various carbons

have been identified through BB, DEPT and hetero-COSY spectra and comparison with the chemical shifts of the carbons reported earlier [19]; silica gel PF254 and silica gel 9385 have been used for tlc and flash column chromatography [20] (Model Eyela) respectively. The plant was identified by Prof. S.I. Ali (Department of Botany, University of Karachi) and a voucher specimen (No.23796 KUH) has been deposited in the herbarium of the Botany Department, University of Karachi.

Fresh aerial parts of *Mirabilis jalapa* (20 kg) were collected from the Karachi region in the month of April, 1991. Aerial parts were repeatedly percolated with MeOH at room temperature. The syrupy concentrate obtained on removal of the solvent from the methanolic extract under reduced pressure was extracted with EtOAc and water. The EtOAc layer was extracted with 4% aq. solution of Na_2CO_3 to separate the acidic from the neutral fraction. The EtOAc layer containing the neutral fraction was washed with H_2O and dried (Na_2SO_4). The residue obtained on removal of the solvent was divided into hexane soluble and hexane insoluble portions. The hexane insoluble fraction (4 g) was subjected to flash column chromatography (hexane, hexane-EtOAc in order of increasing polarity).

Fraction eluted with hexane-EtOAc (9.9:0.1) furnished *trans*-phytol 1 (27 mg) as a pure constituent, while hexane-EtOAc (9.5:0.5) eluate furnished pure β -sitosterol 5 (15 mg) and β -sitosterol acetate 6 (9 mg) as a more polar eluant, whereas a fraction eluted with hexane-EtOAc (8:2), furnished brassicasterol 7 (9 mg), stigmasterol 8 (7 mg), methyl 3-oxo-urs-12-en-28-oate 2 (12 mg), oleanolic acid 3 (6 mg) and ursolic acid 4 (11 mg) in order of polarity.

Trans-phytol 1. Oily (27 mg), UV λ_{max} (MeOH) 201 nm; IR ν_{max} (CHCl_3) 3400, 2900-2850, 1620, 1375 and 1190 cm^{-1} , ^1H - and ^{13}C -NMR (Table-1); HRMS m/z (rel.int.%) 278.2968 ($\text{C}_{20}\text{H}_{38}$, $\text{M}^+ - \text{H}_2\text{O}$), (35) 207.1800 ($\text{C}_{14}\text{H}_{23}\text{O}$), (10), 125.1293

(C₉H₁₇)(100), 85.1026 (C₆H₁₃)(41), 71.0494 (C₄H₇O)(13).

Methyl 3-oxo-urs-12-en-28-oate 2.-Colourless plates from EtOH (12 mg), mp 193-194°; UV λ_{max} (MeOH) 202 nm; IR ν_{max} (CHCl₃) 2950-2850, 1740-1700 br., 1410, 1090 cm⁻¹; ¹H-NMR δ (ppm) 5.23 (1H, t, *J* 3.63 Hz, H-12), 3.57 (3H, s, O-Me), 2.48 (1H, ddd, -*J*15.94, 10.87, 7.28 Hz, H-2 β), 2.33 (1H, ddd, *J* 15.94, 6.99, 3.78 Hz, H-2 α), 2.21 (1H, d, *J* 11.29 Hz, H-18), Methyl doublets, 0.89 (*J* 6.19 Hz), 0.82 (*J* 6.46 Hz), Methyl singlets 1.05, 1.03, 1.00, 0.99 and 0.76; HRMS *m/z* (rel.int.%) 468.3608 (C₃₁H₄₈O₃, M⁺) (17), 409.3469 (C₂₉H₄₅O₂, (20), 262.1861 (C₁₇H₂₆O₂, r.D.A. fragment) (100), 205.1611 (C₁₄H₂₁O) (52), 187.1475 (C₁₄H₁₉)(74).

Results and Discussion

Compound 1 did not show the molecular ion peak in EI, FD and HRMS, however, the FABMS (+ve) spectrum showed M⁺ + 1 peak at *m/z* 297. Hence the molecular formula C₂₀H₄₀O was derived through exact measurement of various mass fragment ions in the EI spectrum and ¹³C-NMR spectroscopy

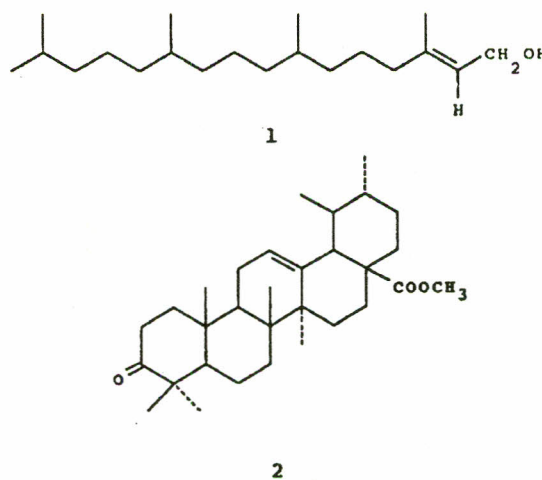
TABLE 1. ¹H-¹³C-HETERO-COSY OF 1.

C	¹³ C-chemicals shifts δ (ppm)	Correlated ¹ H-chemical shifts			
		δ (ppm)	No. of H-atom	Multiplicity	<i>J</i> (Hz)
1	59.48	4.14	2H	d	6.96
2	123.22	5.40	1H	m	—
3	140.50	—	—	—	—
4	39.91	1.98	2H	t	6.61
5	25.20	1.53	2H	quintet	6.61
6	36.7	1.28	1H	m	—
	"	1.24	1H	m	—
7	32.74	1.41	1H	m	—
8	37.48	1.14	2H	m	—
9	24.51	1.27	1H	m	—
	"	1.20	1H	m	—
10	37.55	1.13	2H	m	—
11	32.85	1.37	1H	m	—
12	37.35	1.16	2H	m	—
13	24.83	1.40	1H	m	—
	"	1.35	1H	m	—
14	39.50	2.10	2H	m	—
15	28.02	1.26	1H	m	—
16	22.73	0.88	3H	d	6.35
17	16.24	1.66	3H	br,s	—
18	19.78	0.83*	3H	d	6.56
19	19.78	0.84*	3H	d	6.10
20	22.64	0.87	3H	d	6.35

Assignments have been made with the help of DEPT, J-resolved, COSY-45, HMQC experiments. *Values may be interchanged.

(BB, DEPT and hetero-COSY). The ¹H-NMR showed four methyl signals as doublets at δ 0.88 (*J* 6.35 Hz), 0.83 (*J* 6.56 Hz), 0.84 (*J* 6.10 Hz) and 0.87 (*J* 6.35 Hz). One downfield methyl signal was observed at δ 1.66 as a broad singlet along with one olefinic proton at δ 5.40 (1H, m). A two-proton signal was observed at δ 4.14, *d* (*J* 6.96 Hz) indicating a CH₂OH group in the molecule (IR ν_{max} 3400 cm⁻¹) which was supported by the ¹³C-NMR shift (δ 59.48). In the light of above observations structure of 1 was decided to be 3,7,11,15-tetramethyl-2-hexadecen-1-ol. Compound 1 has earlier been reported from *Gracilaria andersoniana* [13], however, this is the first report of its isolation from this plant. A complete assignment of the protons based on 2D NMR experiments is also reported.

The molecular ion peak of compound 2 was observed at *m/z* 468, the exact measurement (468.3608) of which gave the molecular formula as C₃₁H₄₈O₃. The ¹H-NMR showed two doublets of double doublets at δ 2.48 (*J* 15.94, 10.87, 7.28 Hz) and 2.33 (*J* 15.94, 6.99, 3.78 Hz) for H-2 β and H-2 α respectively, indicating an adjacent keto group (IR ν_{max} 1690 cm⁻¹). One olefinic proton at δ 5.23 (*t*, *J* 3.63 Hz), H-18 doublet at δ 2.21 (*J* 11.29 Hz) and the O-Me signal at δ 3.57 along with r.D.A. fragment at *m/z* 262.1861 (C₁₇H₂₆O₂) were suggestive of a carboxymethyl substituent at C-17 of urs-12-en skeleton. Further, absence of H-3 signal of 3-hydroxy compounds and DBE showed that the hydroxyl group at C-3 has been oxidized to a keto function. Hence, 2 is methyl 3-oxo-urs-12-en-28-oate. It has been obtained earlier from *Dipterocarpaceae* [14], however, this is the first instance of its isolation from this source.



The characterization of the Known compounds 3-8 has been carried out through comparison of the data with those reported earlier for oleanolic acid [15], ursolic acid [15,16], β -sitosterol [17], β -sitosterol acetate [17], brassicasterol [18] and stigmasterol [17].

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