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NEW PENTACYCLIC TRITERPENOIDS FROM *PLUMERIA OBTUSA*

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Two new pentacyclic triterpenoids obtusic acid (1) and obtusilinic acid (2) have been isolated from fresh leaves of *Plumeria obtusa* and their structures elucidated through spectroscopic and chemical methods.

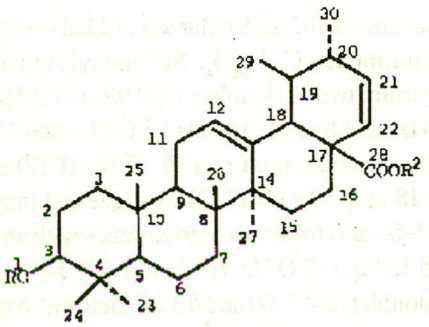
Key words: *Plumeria obtusa leaves*, Apocynaceae, Pentacyclic triterpenoids, Obtusic acid, Obtusilinic acid.

Introduction

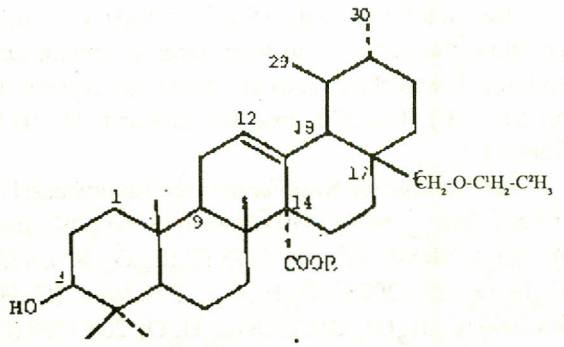
Different ornamental species of the genus Plumeria (Apocynaceae), a native of tropical America, are grown in the warmer regions of the world. In the indigenous system of medicine various parts of its species are reputed as purgative, emmenagogue and febrifuge [1,2]. Although other species of the genus had been investigated earlier for their chemical constituents [3-11], studies on *Plumeria obtusa* were undertaken only recently when four pentacyclic triterpenoids were isolated from its leaves [12]. Further investigation on the fresh leaves has resulted in the isolation and structure elucidation of two new pentacyclic triterpenoids obtusic acid (1) and obtusilinic acid (2). These bear potential pharmacological significance since various related pentacyclic triterpenes are reported in literature for a wide range of biological activity [13-21].

Experimental

IR (KBr) and UV (MeOH) spectra were recorded on JASCO-IRA-I and Pye-Unicam SP-800 spectrometers respectively; mass spectra were recorded on Finnigan MAT 112 and MAT 312 double focussing spectrometers connected to PDP 11/34 computer system. NMR spectra of **1** ($CDCl_3$) were recorded on a Bruker Aspect 3000 spectrometer, operating at 300 MHz for ^1H and 75 MHz for ^{13}C nuclei while the ^1H NMR spectrum of **2** ($CDCl_3$) was recorded on a Bruker AM 400 spectrometer operating at 400 MHz for ^1H unclei. The chemical shifts are reported in δ (ppm) and the coupling constants are in Hz. The ^{13}C NMR spectral assignments (Table 2) have been made partly through a comparison of the chemical shifts with the published data for similar compounds [23,27,28] and partly through the appearance of signals in GASPE spectrum. The plant was collected in Karachi and identified by Prof. S.I.Ali, Department of Botany, University of Karachi, and a voucher specimen (No.9317 KUH) has been deposited in the Herbarium.



1. $R^1=R^2=H$
1a. $R^1=H$, $R^2=Me$
1b. $R^1=Ac$, $R^2=Me$



2. R=H
2a R=Me

Extraction and isolation: The methanolic extract of fresh and uncrushed leaves (12 kg) collected from Karachi region in the month of April (1987) was concentrated under reduced pressure and shaken out with EtOAc and water. The former layer was treated with 4% aqueous solution of Na_2CO_3 to separate the acidic from the neutral fraction. The EtOAc layer containing the neutral fraction was washed with water, dried (anhydrous Na_2SO_4) and freed of the solvent. The residue was divided into petrol soluble and petrol insoluble portions and the petrol insoluble fraction was successively treated with petrol-EtOAc (7:3) and petrol EtOAc (1:1). The residue (3.50 g) obtained on removal of the solvent from the petrol-EtOAc (1:1) soluble fraction was subjected to flash column chromatography (silica gel, E.Merck 9385; $CHCL_3$,$CHC1_3$ -MeOH in order of increasing polarity). The fraction eluted with $CHCl_3$ -MeOH (9.50:0.5) through subsequent purification on thin layer silica gel cards (Riedel-deHaen, 37360; $CHCl_3$; MeOH 9:1) furnished pure **1** and **2** in

the order of decreasing polarity.

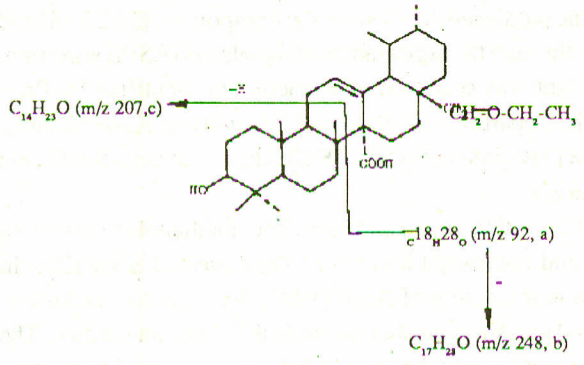
Spectral data of obtusic acid (1): Amorphous; UV and IR: (see results and discussion). HRMS: m/z 454.3506 ($C_{30}H_{46}O_3$, M^+), 246.2307 ($C_{16}H_{22}O_2$), 207.1769 ($C_{14}H_{23}O$) and 201.1551 ($C_{15}H_{21}$). NMR: (Tables 1 and 2).

Methylation of 1: To a solution of **1** in ether was added an ethereal solution of CH_2N_2 and kept at room temperature overnight. On usual work up the reaction mixture afforded 1a. EIMS: m/z 468 (M^+), 260, 207, 201 and 189. ^1H NMR: (Table 1).

Acetylation of 1a: To a solution of **1a** (6mg) in pyridine (1ml) was added acetic anhydride (1ml) and the reaction mixture allowed to stand at room temperature overnight. On usual work up 1b was obtained as an amorphous powder. EIMS: m/z 510 (M^+), 452, 392, 260, 249, 201 and 189. ^1H NMR: (Table1).

Spectral data of obtusilinic acid (2): Amorphous; UV:λ_{max} 210 nm; IRυ_{max} 3600-2650 (COOH), 3450 (OH) and 1700 cm^{-1} (CO); HRMS: m/z 500.3773 ($C_{32}H_{52}O_4$, M^+), 453.3366 ($C_{30}H_{45}O_3$),292.2073 ($C_{18}H_{28}O_3$), 291.1998 ($C_{18}H_{27}O_3$), 248.2499 ($C_{17}H_{28}O$), 207.1728 ($C_{14}H_{23}O$), 203.1599 ($C_{15}H_{23}$) and 189.1607($C_{14}H_{21}$); ^1H NMR: (Table 1).

Methylation of 2. A solution of **2** in ether was mixed with an ethereal solution of CH_2N_2 and kept at room temperature overnight. On usual work up 2b was obtained as a colourless gummy residue. EIMS: m/z 514 (M^+), 455, 396, 306, 248, 207 and 189; ^1H NMR: (Table 1).



Structure **2**

Results and Discussion

The composition of **1** was shown to be $C_{30}H_{46}O_3$ through HRMS. The UV spectrum showed absorption at 209.6 nm while the IR spectrum displayed bands at 3600-2600 (OH, COOH), 1710 (acid carbonyl) and 1598 cm^{-1} (C=C). It formed a monomethyl derivative 1a on reaction with CH_2N_2, which afforded the monoacetylmonomethyl derivative 1b on treatment with Ac_2O/pyridine showing the presence of a carboxyl function and a hydroxyl group in the molecule justifying all the three oxygen atoms. The ^1H NMR spectrum of **1** showed resonances for seven methyl signals, five as singlets and two as doublets which along with the molecular formula decided its triterpenoidal nature. A one-proton triplet at δ 5.25 (J=3.8 Hz, H-12), ^{13}C NMR chemical shifts [22-24] at δ 128.9 (C-12) and δ 138.0 (C-13), the characteristic retro-Diels-Alder fragmentation around ring C (m/z 246, 207, 201 and 189) and appearance of H-18 [22] as a doublet at δ 2.20 (J=11.2 Hz) established a Δ^{12}–α–amyrin skeleton of **1** with the carboxylic group at C-17. The relative intensities of the fragments at m/z 246 and 201 and the downfield signal of 14-Me [25] also favoured the carboxylic function at C-17 and the double bond at C-12. The hydroxyl group was placed at C-3 on biogenetic grounds and supported by retro-Diels-Alder fragments at m/z 207 and 189 and ^{13}C NMR shifts of rings A and B carbons which are comparable with those reported for ursolic acid and other 3β-hydroxytriterpenes [23]. The coupling constants of H-3 (δ 3.2 dd, J=10.3 and 5.1 Hz) further exhibited the β–orientation of the hydroxyl group. These features left one unsaturation to be accounted for in the molecule the nature of which was indicated as disubstituted through ^1H NMR spectrum (δ 5.95 dd, J=10.0 and 1.5 Hz, H-22; δ 5.52 dd, J =10.0 and 3.2 Hz, H-21) and ^{13}C NMR (δ 125.9, 133.4, C-21, C-22). The retro-Diels-Alder fragments discussed above showed that this double bond could be accomodated either in ring D or E. The multiplicities of the olefinic protons finally led to place the double bond at C-21 which was also supported by the interactions of H-21 with H-20 and H-20 with H-30 in the COSY-45 spectrum. These facts led to establish the structure of obtusic acid as 3β–hydroxyurs-12, 21-dien-28-oic acid (**1**). **1** is the first instance of isolation of an ursolic acid derivative with a double bond at C-21.

Peak matching of the molecular ion of **2** led to determine its molecular formula as $C_{32}H_{52}O_4$. Six methyl signals in the ^1H NMR spectrum, two as doublets (δ 0.99, J=7.3 Hz and δ 0.94, J = 6.9 Hz) and four as singlets (δ 0.92, 0.88,0.78 and 0.77), an olefinic proton multiplet (δ 5.44, H-12) and the doublet of H-18 at δ 2.18 (J=11.OHz) suggested that **2** also belongs to Δ^{12}–α–amyrin series of triterpenes with an ethoxy substituent (δ 3.29q, J=7.O Hz, H-1' and 1.14t, J=7.O Hz, H-2'). Two AB doublets at δ 3.60 and δ 3.25 each with a geminal coupling constant of 11.8Hz (H-28a and H-28b) further showed that the ethoxy group is located at one of the methyl carbons. Retro-Diels-Alder fragmentation around ring C resulted in fragments at m/z 292.2076 (fragment a, $C_{18}H_{28}O_3$ and 207.0750 (fragment c, $C_{14}H_{23}O$). The relative intensity of fragment a (23%) and that of the ion at m/z 248.2140 ($C_{17}H_{28}O$; fragment b; 31%) resulting through the loss of CO_2 from fragment 'a' suggested that the carboxyl group (3600-2650, 1700 cm^{-1}; methyl derivative **2a**; δ OCH_3:3.36) is at C-14, since functional groups at the allylic position are more labile

TABLE 1. ^{1}HNMR DATA OF TRITERPENOIDS (δ/Hz)

Proton	1	1a	1b	2	2a
3α	3.20dd(10.3,5.1)	3.21dd(10.3,5.2)	4.41dd(9.2,6.9)	3.21dd(9.2,4.0)	3.22dd(9.6,3.8)
12	5.25t(3.8)	5.23t(3.3)	5.23t(3.3)	5.44m	5.45m
18	2.20d(11.2)	2.20d(11.0)	2.23d(11.0)	2.18d(11.0)	2.20d(11.1
20	1.95m	1.97m	1.95m	-	-
21	5.52dd(10.0,3.2)	5.52dd(10.0,3.3)	5.53dd(9.9,3.3)	-	-
22	5.95dd(10.0,1.5)	5.93dd(10.0,1.4)	5.93dd(9.9,1.2)	-	-
C-methyls	0.98s	0.98s	0.97s	0.92s	0.91s
	0.96s	0.93s	0.91s	0.88s	0.89s
	0.79s	0.75s	0.74s	0.78s	0.77s
	0.77s	0.72s	0.74s	0.77s	0.76s
	1.05s	1.07s	1.10s	-	-
28a	–	-	-	3.60d(11.8)	3.59d(11.6)
28b	–	-	-	3.25d(11.8)	3.25d(11.6)
29	0.85d(6.3)	0.85d(6.3)	0.87d(5.8)	*0.99d(7.3)	0.97d(7.0)
30	1.14d(6.6)	1.16d(6.3)	1.11d(6.2)	*0.94d(6.9)	0.94d(7.0)
1'	-	-	-	3.29q(7.0)	3.31q(6.9)
2'	-	-	-	1.14t(7.0)	1.15t(6.9)
OCH_3	-	3.35s	3.32s	-	3.36s
$OCOCH_3$	-	-	2.07s	-	-

*Values in a vertical column may be interchanged.

TABLE 2. ^{13}C NMR CHEMICAL SHIFTS FOR OBTUSIC ACID (1)

Carbon	δ value	Carbon	δvalue
1	38.3	16	23.3
2.	27.2	17	47.6
3.	78.9	18	52.7
4.	38.9	19	39.0
5.	55.2	20	38.1
6.	18.3	21	125.9*
7.	33.0	22	133.4*
8.	38.6	23	28.1
9.	47.5	24	15.6
10.	37.0	25	16.1
11.	22.8	26	17.1
12.	128.9	27	23.5
13.	138.0	28	177.5
14.	41.7	29	18.9
15	27.7	30	21.1

*Values may be interchanged

to undergo a rapid loss and thus decrease the intensity of retro-Diels-Alder fragment (i.e. fragment a) [24]. The carboxyl group at C-14 was also favoured by the downfield chemical shift [26] of H-12 at δ 5.44 as against ca. 5.2 in compounds lacking a carboxyl group at this position.

Further loss of the ethoxy ion from fragment b results in an ion at m/z 203.1799 ($C_{15}H_{23}$). The ions noted above showed that the ethoxy group is located at C-28 and the hydroxyl function (υ_{max} 3450 cm^{-1}) somewhere in ring A or B. The latter was placed at C-3 on biogenetic grounds and supported by the chemical shift and coupling constants of H-3 (δ 3.21 dd, J=9.2 and 4.OHz) which are comparable with those reported for this proton in similar compounds [27]. Its coupling constants exhibited β–disposition of the hydroxyl group. In light of these observations obtusilinic acid has been assigned the structure as 28-ethoxy, 3β–hydroxyurs-12-en-27-oicacid (2).

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