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CONSTITUENTS OF *PRUNUS ARMENIACA*

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Phytochemical screening of the non-alcoholic extract of *Prunus armeniaca* has revealed the presence of a triterpenoid belonging to Ursane / Oleanane series and a steroid alongwith its glucoside for the first time from this source. Structures were confirmed by spectroscopic methods, using IR, ^{1}H-NMR,^{13}C-NMR and Mass spectra.

Keywords: *Prunus armeniaca,* Fruits, Steroidal glycosides and a triterpenoid.

Introduction

Prunus armeniaca is a member of family *Rosaceae* and *Genus Prunus*. It is commonly known as Apricot (English) and Zardalu in urdu. Apricot is normally found in areas of higher altitudes. The fruit is very popular, besides being a table fruit apricot is also employed in making jams and nector. In Pakistan, it is cultivated in the inner valleys of Baluchistan and Kashmir from the plains to 12,000m (Baquar 1989). *Prunus* species are reported to have antipyretic and leucodermatic activity in the treatment of leprosy. Apricot kernel oil closely resembles almond oil and employed as an adulterant or substitute for it. It is also used in medicine for earache and in variety of ailments (Chopra 1956, Gupta 1969). The kernel is used as an expectorant and a remedy for dry throat, laryngitis, lung diseases and abscesses. It is regarded as bechic, depurative, sedative for the respiratory centre, tonic and antispasmodic, a remedy for severe colds and bronchial asthma. In Indo China a special preparation of the fruit is chewed but not swallowed to protect the bronchial tubes from cold during winter (Lily 1980).

Triterpenoids/steroids are the compounds of wide occurance and structural diversity, which have always attracted attention, and their pharmacological activities. Keeping in view the biological / pharmacological importance, present studies were undertaken on this plant to carry out the isolation and structural studies of such compounds. Plant aqueous ethanolic extract also showed antibacterial activity, which is under process.

Materials and Methods

Plant material (2kg) fruits were purchased from local market, Karachi and verified by Botany Department, University of Karachi. The fruits were percolated with methanol at room temperature for 15 days (3x5 extractions), methanolic extract was concentrated and the residue obtained upon concentration was treated with n-hexane whereupon a white gummy solid separates out leaving behind n-hexane extract (**1**). Methanol soluble filtrate was further extracted with ethyl acetate and butanol saturated with H_2O. Ethyl acetate extract was evaporated and labelled as extract (**2**).

The compound isolated from non-alcoholic (hexane) fraction of the fruit was identified as α-amyrin acetate. Two other compounds were obtained from ethyl acetate fraction (non-alcoholic) and were identified as Stigmasterol and Stigmasterol glucoside.

Results and Discussion

The separation of compounds was achieved by column chromatography followed by preparative TLC and fractional crystallization. The compound generally belonged to Oleanane/ Ursane series (Fourneau and Hocquemiller 1996) and sterol derivatives (Jamshed and Fazal-ur-Rehman 1991) reported first time from this source.

Compound 1: It was eluted with pet ether-benzene 70:30, a white waxy solid gave Leibermann's burchard test positive. On re-crystallization with chloroform-methanol gave a white crystalline compound melting at 219-222°C, showing molecular ion peak, M^+ at m/z 468 ($C_{32}H_{52}O_2$) and a base peak m/e at 218.The base peak at 218 indicated the presence of α/β amyrin type of compound and arise due to retro Diels-Alder fragmentation. The fragment at m / z 203 and 189 arises from 218 fragment due to the loss of (M-CH_3) and (-CH_2CH_3). Its IR spectrum showed absorption at 2910, 2870 cm^{-1}due to CH stretching and 1735 (C=O), 1245 cm^{-1} (C-O), a sharp singlet at 1735 and 1245 cm^{-1} due to C-O single bond confirming

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Table 1
^{13}C-NMR ($CDC1_3$,75.43 MHz) Data of Compound (**1**)

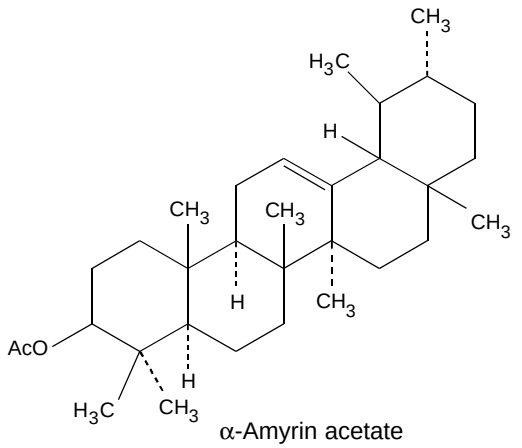
S No.	Multiplicity (DEPT)	^{13}C-NMR (δ)	S No	Multiplicity (DEPT)	^{13}C-NMR (δ)
1	CH_2	38.4	17	C	33.8
2	CH_2	23.6	18	CH	59.0
3	CH	80.7	19	CH	39.7
4	C	37.6	20	CH	39.7
5	CH	55.3	21	CH_2	31.3
6	CH_2	18.3	22	CH_2	41.5
7	CH_2	32.8	23	CH_3	28.1
8	C	40.1	24	CH_3	16.8
9	CH	47.6	25	CH_3	15.7
10	C	36.8	26	CH_3	16.8
11	CH_2	17.5	27	CH_3	23.2
12	CH	124.0	28	CH_3	28.1
13	C	139.0	29	CH_3	23.2
14	C	42.0	30	CH_3	21.4
15	CH_2	28.7		$COCH_3$-3	170.4
16	CH_2	26.7		$COCH_3$-3	21.2

Table 2
^{13}C-NMR ($CDC1_3$, 75.43 MHz) Data of Compound (**2**)

S No.	Multiplicity (DEPT)	^{13}C-NMR (δ)	S No.	Multiplicity (DEPT)	^{13}C-NMR (δ)
1	CH_2	37.2	16	CH_2	26.1
2	CH_2	28.2	17	CH	56.0
3	CH	71.8	18	CH_3	12.1
4	CH_2	40.3	19	CH_3	19.4
5	C	140.8	20	CH	36.1
6	CH	121.7	21	CH_3	19.0
7	CH_2	31.7	22	CH	138.3
8	CH	31.9	23	CH	129.3
9	CH	51.2	24	CH	50.2
10	C	36.5	25	CH	29.2
11	CH_2	21.1	26	CH_3	21.2
12	CH_2	39.7	27	CH_3	21.0
13	C	42.2	28	CH_2	23.1
14	CH	56.9	29	CH_3	12.2
15	CH_2	24.4	–	--	--

the ester grouping. Peak at m/e 408 (M^+-60) also indicated the presence of CH_3COO^- group. Presence of a sharp singlet at δ 2.01 ppm (3H,s -$COCH_3$) in (^{1}H- NMR) also provide the evidence. The NMR spectrum also showed eight methyl singlets from δ 0.78-1.16 and a distorted triplet at δ 5.02 due to proton at C-12 because of olefinic double bond and a broad singlet at δ 4.72 accounted for C-3 β-hydrogen, bearing OA_c group. The two other signals appeared at δ 170.4 and δ 21.2 in ^{13}C- NMR indicated the presence of carbonyl carbon and methyl carbon of acetate group (Table 1). On the basis of spectral studies and data that is available in literature the compound was identified as α-amyrin acetate. (Ahmed 2001)

Compound 2: It was obtained from ethyl acetate extract by repeated column chromatography using silica gel. The compound was eluted by chloroform-methanol with increasing percentage of methanol (10%, 2.5%, 50%, & 7.5%). The fractions obtained from 5% methanol were all similar to each other, showing a very prominent single spot on TLC when developed with spraying reagents. For further purification, the compound was re-crystallized with methanol-chloroform, and repeated re-crystallization gave white pure solid UV active compound (m.p=168°C), with intense coloured spot on TLC when sprayed with ceric sulphate and Leibermann's burchard reagents.



α-Amyrin acetate

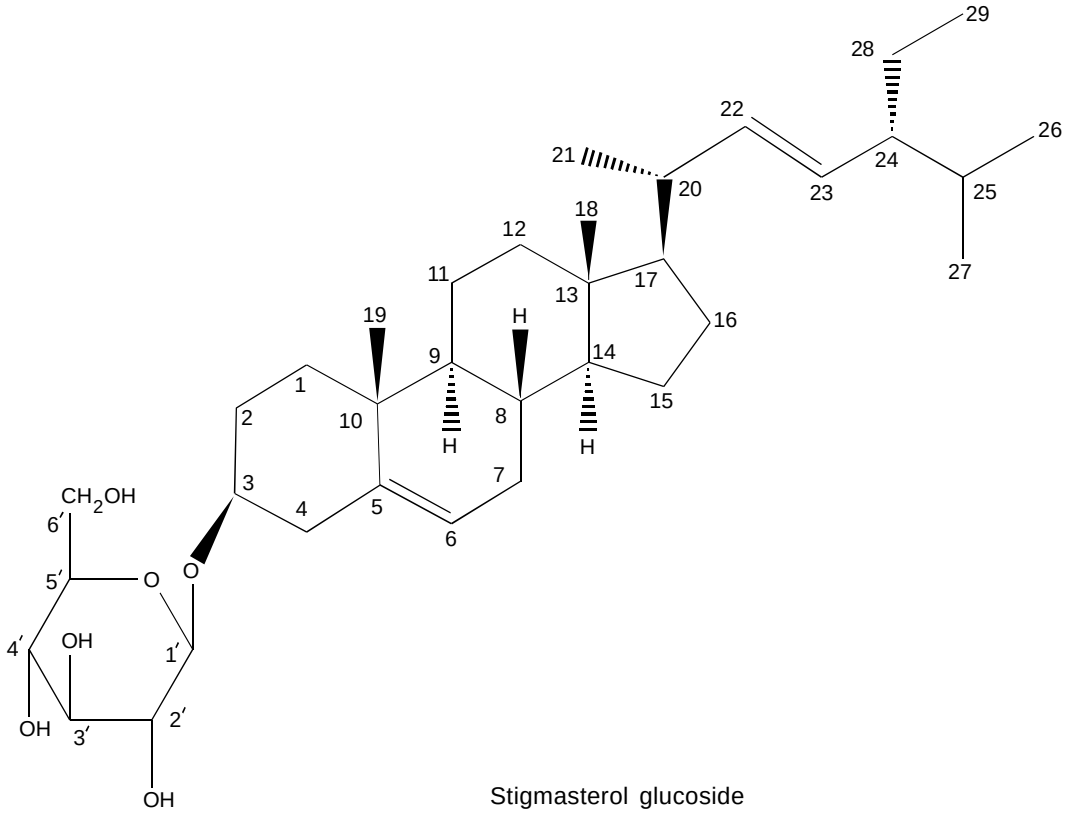
EI mass showed the molecular ion peak M^+at m/z 412.3809 ($C_{29}H_{48}O$) and base peak at 203, other peaks in mass spectra appeared at 369, 301, 273 (M-139), 220 and 164. IR absorption exhibited υ_{max} at 3412 cm^{-1} (OH-br), 2900 (CH-str), 1680 due to C=C bond, and 1041 (C-O-C). The ^{13}C-NMR showed 29 carbon signals indicating six methyl, nine methylene, eleven methine and three quaternary carbons (Table2). The six methyls appeared in the ^{1}H-NMR spectrum at δ 0.69 (s,H-18), 0.97 (s, H-19),1.00 (d, *J* = 6.4 Hz, H-21), 0.81(d, *J* = 6.1 Hz, H-26), 0.76(d, *J* = 6.0 Hz, H-27) and 0.78 (t, *J* = 7.5Hz, H-29). The olefinic signals, each of one proton resonated at δ 4.97 (dd, *J* = 15.4, 8.5 Hz, H-23), 5.11 (dd, *J* = 15.5, 8.5 Hz, H-22) 5.31 (br.s, H-6) and their associated carbons resonated at δ129.3(C-23), 138.3 (C-22) and 121.8 (C-6), respectively, which indicated the two double bonds in the molecule. The methine carbon resonated in the ^{13}C-NMR spectrum at δ 71.8 (C-3) and in the ^{1}H-NMR spectrum at δ 3.46 (m, H-3) revealed that hydroxyl group was attached to C-3.

Table 3
^{13}C-NMR ($CDC1_3$+ CD_3OD, 75.43 MHz) Data of Compound (**3**)

S No.	Multiplicity (DEPT)	^{13}C-NMR (δ)	S No.	Multiplicity (DEPT)	^{13}C-NMR (δ)
1	CH_2	39.2	19	CH_3	19.3
2	CH_2	29.3	20	CH	36.7
3	CH	79.6	21	CH_3	19.1
4	CH_2	40.0	22	CH	138.8
5	C	141.1	23	CH	129.9
6	CH	122.4	24	CH	46.6
7	CH_2	32.4	25	CH	29.8
8	CH	32.5	26	CH_3	20.0
9	CH	51.9	27	CH_3	19.6
10	C	37.3	28	CH_2	23.6
11	CH_2	21.6	29	CH_3	12.4
12	CH_2	40.3	1′	CH	101.7
13	C	42.8	2′	CH	71.0
14	CH	57.5	3′	CH	76.6
15	CH_2	25.8	4′	CH	74.3
16	CH_2	26.8	5′	CH	77.2
17	CH	56.7	6′	CH_2	62.5
18	CH_2	12.2	–	--	--

On the basis of above spectral data, TLC and comparison with authentic sample the compound was identified as stigmasterol. (Funes 1978)

Compound 3: It was isolated from ethyl acetate soluble part of methanolic extract. The molecular mass of (**3**) was confirmed as 574 ($C_{35}H_{58}O_6$) with the help of peak observed in the negative FAB mass spectrum at m/z 573 [M-H]. Other fragments in mass appeared at m/z 432 (M-162 glucose), 369 (M-43 $CH(CH_3)_2$, 273 (M-139), loss of side chain. Other fragments at 203/205 the base peak of steroidal skeleton. Its IR showed intense absorption band at 2853, 2921 cm^{-1} (CH stretch), a strong doublet at 1696 and 1649 (two C = C) bonds and also strong bands at 1461, 1025 cm^{-1}. The ^{13}C-NMR spectrum showed the presence of 35 signals, which were resolved as six methyl, ten methylene, sixteen methine and three quaternary carbons. Six-anomeric carbon in ^{13}C-NMR spectra showed resonance absorption between δ 60 and 71 (Table 3), a signal at β 3.18 due to proton (C-3) and a methine carbon at 101.7 indicated the presence of sugar moiety in the molecule. The compound was subjected to hydrolysis. The sugar confirmed was β-D glucose through magnitude of coupling constant of anomeric carbon at δ 4.35(J = 7.7 Hz) and co-TLC of hydrolyzed product with the authentic sugar sample. The values were found very similar to that previously reported in the literature (Zlatanov 1998). The sapogenine was found to be



Stigmasterol glucoside

stigmasterol.

The ^{1}H-NMR spectrum showed three olefinic signals at δ 4.92 (dd, *J* = 15.2, 8.2 Hz, H-23) 5.09 (dd, *J* = 15.2, 8.4 Hz, H-22) and 5.31 (br.s, H-6). The six methyl confirmed by the ^{13}C-NMR spectrum appeared in the ^{1}H-NMR spectrum at δ 0.69 (s, H-18), 1.00 (s, H-19), 0.88 (d, *J* = 6.2 Hz, H-21) 0.82 (d, *J* = 6.3 Hz, H-26), 0.76 (d, *J* = 6.4Hz, H-27) and 0.77 (t, *J* = 7.0 Hz, H-29). Compound **3** was identified as 3-O-β-D-glucopyranosyl-stigmasterol and was confirmed by matching the spectral data with that of reported in literature.

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