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Isolation and Structure Elucidation of a New Triterpenoid from *Prunus cerasoides* D. Don

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Abstract: One new metabolite, prunol (**1**), belonging to the pentacyclic triterpenoid skeleton was purified from the dichloromethane soluble fraction of the crude ethanolic extract of *Prunus cerasoides* D. Don. The structure elucidation was accomplished on the basis of one dimensional ^{1}H and ^{13}C NMR and two dimensional HMQC, HMBC, and COSY experiments. The molecular mass was determined by HRFAB-MS, while the major fragments were observed in the EI-MS. The comparative analysis of the NMR spectral data with the known analogues and the NOESY experiments were helpful in assigning the stereo centers in the molecule.

Keywords: Pentacyclic triterpenoids; *Prunus cerasoides*; ethanolic extract; NMR spectral data.

1. Introduction

Prunus is a genus of trees and shrubs, including the plums, cherries, peaches, apricots, and almonds. It is traditionally placed within the rose family, rosaceae [1]. The plant *Prunus cerasoides* D. Don is found in East Asia and commonly known as the Wild Himalayan Cherry. Its range extends in the Himalayas from Himachal Pradesh in India to southwest China and Burma. It grows in the forest from 1200 to 2400 meters above sea level [2–3]. The fruits and the leaves give a dark green dye. Seeds can be used in the manufacture of necklaces and rosaries. The wood is hard, strong, durable and aromatic, and branches are used as walking sticks. The branches and stems of the plant are also used for the treatment of gravel, kidney stones, asthma, thirst, leucoderma, liprosy, vomiting, and as antipyretic and refrigerant [4–5]. Because of their considerable value as both food and ornamental plants, many *Prunus* species have been introduced to the various parts of the World. All the members of the genus contain amygdalin and prunasin, which break down in water to form hydrocyanic acid (cyanide or prussic acid). In small amounts this exceedingly poisonous compound has been shown to stimulate respiration, and improve digestion. In excess, however, it can cause respiratory failure and even death [1]. Previously, some flavonoids and steroidal derivatives have been reported from *P. cerasoides* [2–3]. In the present paper, we report the isolation and structure elucidation of a new derivative of pentacyclic triterpenoids, named prunol (**1**).

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2. Materials and Methods

2.1. General

Optical rotations were measured on a JASCO DIP 360 polarimeter. IR spectrum was recorded on a Bruker VECTOR 22 spectrophotometer. EI-MS and HRFAB-MS were recorded on mass spectrometers JEOL JMS HX 110. ^{1}H and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers operating at 600 MHz (150 MHz for ^{13}C). The chemical shift values were reported in ppm (δ) units and the coupling constants (J) were given in Hz. For TLC, pre coated aluminium sheets (silica gel 60F-254, E. Merck) were used. UV at 254 and 366 nm were used for the visualization of the TLC plates. The TLC plates were then sprayed with the cerric sulphate reagent for the visualization of the UV inactive compounds. 5-10 % MeOH:DCM was used as solvent system.

2.2. Plant Material

The plant *Prunus cerasoides* D. Don (Rosaceae), was collected from Swat (Pakistan) in the year 2005, and was identified by Mr. Mehboob ur Rahman, Assistnat Professor, Department of Botany, Govt Jehanzeb Post Graduate Ccollege, Saidu Sharif, Swat, K.P.K., Pakistan. A voucher specimen (CP-14) has been deposited at the herbarium of the Department.

2.3. Extraction and Isolation

The air dried and powdered aerial parts (*P. cerasoides* 1.6 kg) were extracted exhaustively with 80% ethanol at room temperature. The filtrate was evaporated in vacuum to yield 150 g of the residue. The residue was partitioned in different solvents on the basis of increasing polarity to get *n*-hexane (12 g), dichloromethane (21 g), ethyl acetate (40 g), and *n*-butanol (25 g) extracts.

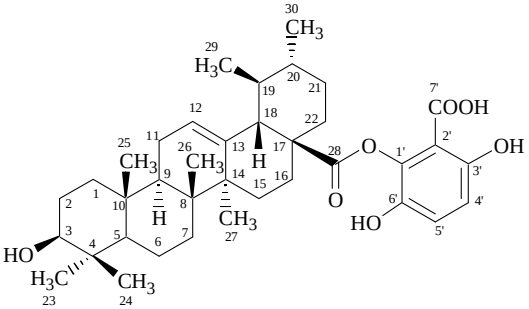

Figure 1. Structure of compound **1**

The dichloromethane (DCM) soluble fraction was loaded on silica gel column and eluted with gradually increasing polarity solvents; sixteen sub-fractions (SF 1 ton SF 16) were obtained. Sub-fractions collected at 5-7 % MeOH/DCM (SF 11 and SF 12) were again subjected to repeated column chromatography over silica gel by using 5 % MeOH/DCM, which afforded new compound **1** (Figure 1) along with some other semi-pure fractions.

*2.4. Prunol (**1**)*

White amorphous solid: $[\alpha]_D^{30}$ -56 (*c* 0.05, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 276 nm (ε = 3.44), 215 nm (ε = 2.67) nm; IR (KBr): ν_{max} 3415, 2922, 1705, 1635, 1450, 1395 cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD): see Table 1; ^{13}C NMR (CD_3OD, 150 MHz)**:** see Table 1; FAB-MS: *m/z* 609 $[M + H]^+$; HRFAB-MS: *m/z* 609.3251 $[M + H]^+$, calcd. 609.3247

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was isolated as white amorphous powder. The UV spectrum showed the absorption maxima at 276 nm and 215 nm which indicated the presence of aromatic ring in conjugation with a carbonyl group [6]. The IR spectrum showed absorption bands for hydroxyl group (3423 cm^{-1}), benzene ring and aromatic methine (1600 and 1458 cm^{-1} and 2926 cm^{-1}), and carboxyl group (1690 and 1185 cm^{-1}). The molecular formula of **1** was established as $C_{37}H_{52}O_7$ on the basis of high resolution (+)-FAB-MS (*m/z* 609.3251 $[M + H]^+$, calcd. 609.3247). The twelve degree of unsaturation in the molecule was attributed to benzene ring (3 + 1), C(12/13) unsaturated pentacyclic skeleton (5 + 1), and two carbonyl groups (2). The other prominent fragment ions in the EI-MS (Figure 2) at *m/z* 456, 438, 262, 248, 207, 203, and 133 coupled with ^{1}H NMR spectral data (Table 1) indicated compound **1** to be a substituted phenyl ester of a pentacyclic triterpenoid [7].

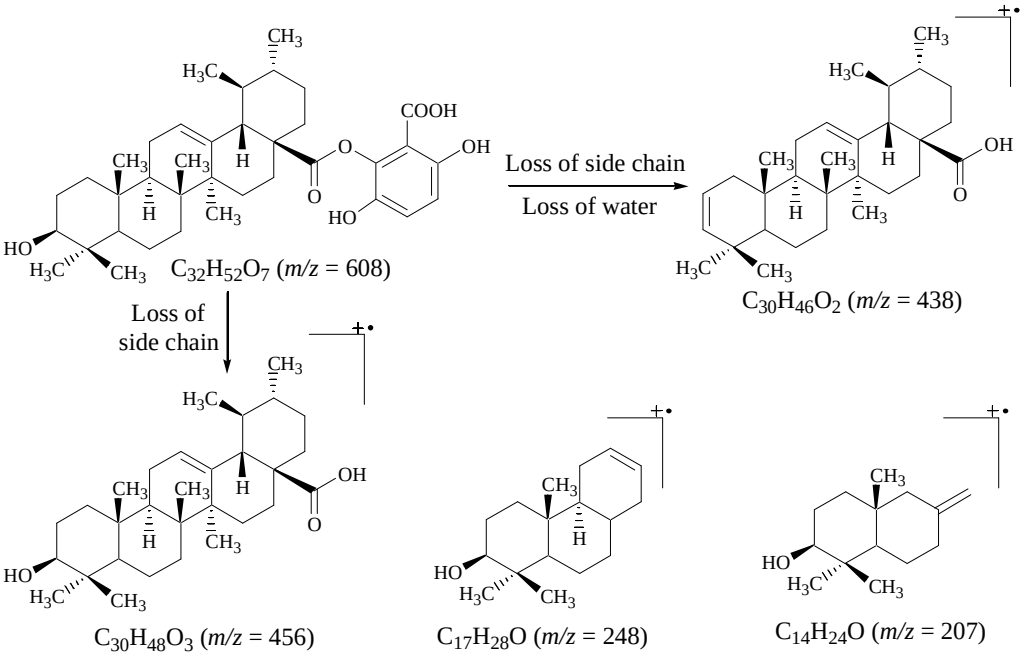

Figure 2. Major mass fragments in compound **1**

The ^{1}H NMR spectrum showed the presence of five tertiary methyl singlets and two secondary methyl doublets at δ 0.77, 0.85, 0.94, 0.96, 1.10, and at δ 0.87, 0.95 respectively. This indicated the ursolic acid skeleton of the molecule [8]. The proton geminal to the hydroxyl group H-C(3) was observed at δ 3.14 as a multiplet. The olefinic proton H-C(12) of the ring C appeared at δ 5.21. Two downfield doublets at δ 7.75 and 7.20 (each 1H, d, *J* = 8.1 Hz) indicated the presence of a substituted phenyl ring having two *ortho*-coupled protons. The tetra-substituted phenyl ring was further supported through ^{13}C NMR spectrum (Table 1). The substitution pattern of the phenyl ring at C(28) was thus inferred to have two *ortho*-coupled protons, two OH groups and a carboxyl group. This substituted phenyl moiety with two OH groups and a carboxyl group was further confirmed through mass spectrometry by the presence of fragment ion at *m/z* 171 in the EI-MS (Figure 2) corresponding to the loss of benzoic acid derivative side chain. The ^{13}C NMR spectrum (BB, and DEPT) of **1** displayed thirty seven signals, including seven methyl (C(23), C(24), C(25), C(26), C(27), C(29), and C(30)), nine methylene, nine methine and twelve quaternary carbons (Table 1). The two downfield quaternary carbons (C(28) and C(7′)) at δ 182.3 and 170.4 along with four quaternary carbons (C(1′) to C(3′) and C(6′)) at δ 145.5, 123.4, 150.1, and 143.8, respectively, and two methine carbons (C(5′) and C(4′)) at δ 128.7, and 127.0, respectively, in the aromatic region, confirmed that compound **1** is the substituted phenyl ester of ursolic acid. Furthermore, the olefinic moiety in ring-C was confirmed by the presence

of signals at δ 125.6, and 139.2 in ^{13}C NMR spectrum. The ^{1}H-^{13}C connectivities were determined through HMQC spectrum and the long-range ^{1}H-^{13}C HMBC correlations (Figure 3) were helpful for linking different sub-structures together for the final confirmation of structure **1**. The HMBC correlations from H-C(3) to C(1), C(2), C(4), C(23), and C(24), from H-C(9) to C(5), C(8), and C(10), from H-C(12) to C(11), C(13), C(14), and C(18), from H-C(18) to C(13), C(14), C(16), and C(28), and from H-C(22) to C(28), thus indicated the relative positions of these groups in the molecule. The substitution of the phenyl ring was confirmed by the HMBC interactions from H-C(4′) to C(3′), and C(6′); and from H-C(5′) to C(4′), and C(6′) along with the ^{1}H-^{1}H COSY correlations H-C(4′)↔H-C(5′) (Figure 3). The structure of compound **1** closely resembles to that of a reported compound, basilol [9], with the difference in substitution pattern of the phenyl ring and pentacyclic skeleton. The reported compound, basilol, is the 4′-formyl substituted phenyl ester of oleanolic acid, whereas the new compound **1** is 2-carboxy-3,6-dihydroxy substituted phenyl ester of ursolic acid. The relative stereochemistry of the asymmetric centers in the molecule was determined by the NOESY correlations (Figure 3) of H-C(3)↔H-C(23)↔H-C(9) and H-C(25)↔H-C(26)↔H-C(29) and also on the basis of biogenetic considerations (Figure 4).

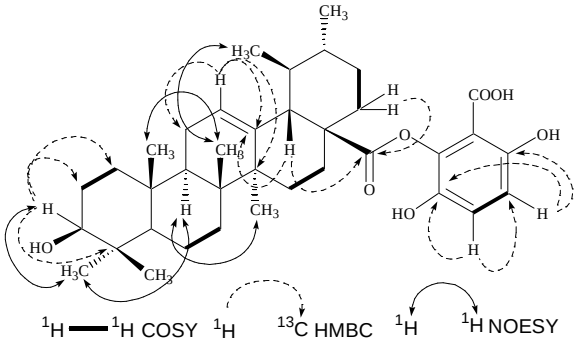

Figure 3. Important COSY, HMBC, and NOESY interactions in compound **1**

Thus on the basis of the above spectral studies the structure of compound **1** was established as 2′-carboxy-3′,6′-dihydroxyphenyl-3*β*-hydroxyurs-12-en-28-oate (prunol) as a new constituent from *P. cerasoides*.

*3.2 Biogenesis of compound **1***

Compound **1** possesses the ursane skeleton belonging to the pentacyclic triterpene system. The pentacyclic triterpene system arises from squalene [10], which is a basic precursor for the most of steroids and terpenoids.

The dammarenyl cation is the transient product of the folding of squalene oxide on to a cyclase enzyme in a chair-chair-chair-boat conformation [11]. This cation undergoes further carbocation promoted cyclizations to form tertiary lupenyl cation with pentacyclic ring system. Ring expansion in the lupenyl cation gives rise to the oleanyl system, which on further hydride migrations and loss of proton converted into *α*-amyrin (Figure 4). Compound **1** possesses the identical skeleton to that of the *α*-amyrin. It may have arises from esterification of ursolic acid (Figure 4), which is the oxidation product of *α*-amyrin at C(28) [11].

Table 1. ^{1}H and ^{13}C NMR data and key HMBC correlations for compounds **1**.

S. No (C)	^{13}C (δ)	^{1}H (δ)	Multiplicity	HMBC correlation
1	40.0	0.95, m & 1.63, m	CH_2	
2	29.3	1.95, m	CH_2	
3	79.7	3.14, m	CH	1, 2, 4, 23, 24
4	39.9		C	
5	56.8	0.75, br s	CH	
6	19.5	1.47, m, 1.62, m	CH_2	
7	34.4	1.29, m, 1.35, m	CH_2	
8	40.8		C	
9	49.2	1.54, m	CH	5, 8, 10, 11
10	38.1		C	
11	24.4	1.20, m	CH_2	
12	126.7	5.21, t, *J* = 3.6 Hz	CH	11, 13, 14, 18
13	139.8		C	
14	43.3		C	
15	27.9	1.59, m	CH_2	
16	25.4	1.92, m	CH_2	
17	47.1		C	
18	54.5	2.19, d, *J* = 11.4 Hz	CH	13, 14, 16, 28
19	40.4	1.63, m	CH	
20	40.5	1.55, m	CH	
21	31.9	1.48, m	CH_2	
22	38.2	1.62, m	CH_2	28
23	28.8	0.96, s	CH_3	
24	16.4	0.77, s	CH_3	
25	16.1	0.94, s	CH_3	
26	17.9	0.85, s	CH_3	
27	21.7	1.10, s	CH_3	
28	182.3		C	
29	17.7	0.87, d, *J* = 6.6 Hz	CH_3	
30	24.1	0.95, d, *J* = 6.6 Hz	CH_3	
1′	145.5		C	
2′	123.4		C	
3′	150.1		C	
4′	127.0	7.75, d, *J* = 8.1 Hz	CH	3′, 6′
5′	128.7	7.20, d, *J* = 8.1 Hz	CH	4′, 6′
6′	143.8		C	
7′	170.4		C	

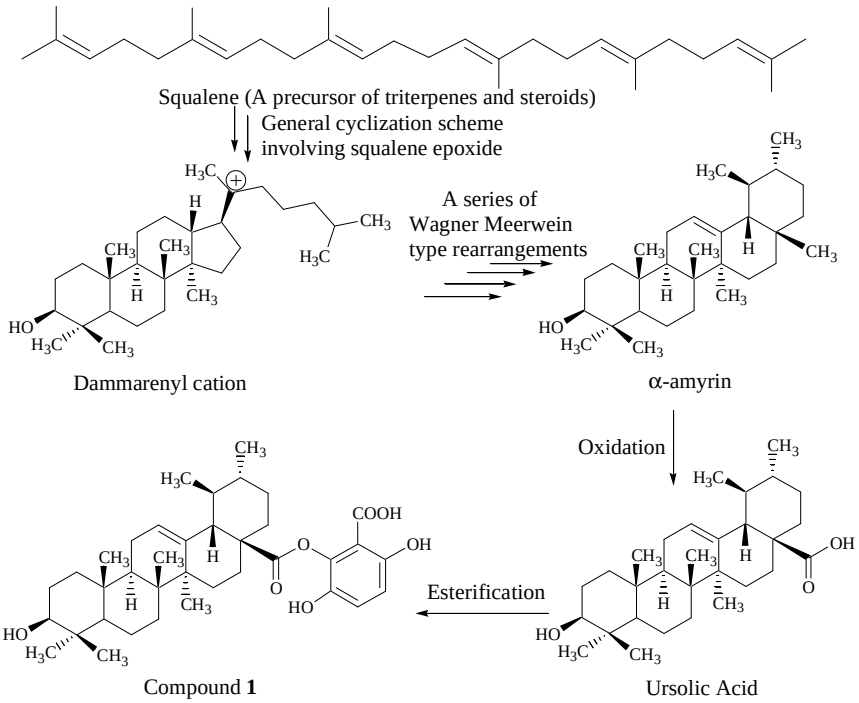

Figure 3. Biogenetic pathway for compound **1**

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