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A New Antioxidant Triterpenoid from the Stem Wood of *Sorbus lanata*

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Abstract: In search for antioxidant constituents, a new triterpenoid (**1**) along with three known compounds (**2-4**) were isolated from *Sorbus lanata*. LCMS, HR-ESIMS, and advanced NMR (1D & 2D) data were used for the determination of structures and spectral assignments. The isolated compounds were also tested for their antioxidant activities in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay.

Keywords: *Sorbus lanata*; pentacyclic triterpenoid; DPPH radical scavenging assay.

1. Introduction

Sorbus lanata (D. Don.) Schauer, also known as hairy rowan, is a small tree with white-woolly flower clusters, found in the Himalayas (Afghanistan to Nepal). This plant belongs to genus *Sorbus* having wide variety of ethno-medical properties such as anti-inflammatory [1] antidiarrheal and diuretic [2-6] vasorprotective and vasorelaxant [7] and also used as source of vitamin C [8] and antioxidants[3, 9, 10].

In continuation to our previous investigations[11, 12], we are now going to report the isolation and structure elucidation of a new triterpenoid (**1**) along with three known secondary metabolites including 3*β*,23-dihydroxy-lup-20(29)ene-28-oic acid-23-caffeate (**2**)[13], and 3*β*,23-dihydroxy-lup-20(29)ene-28-oic acid-3*β*-caffeate (**3**)[14], and lyoniside (**4**)[15] from the stem wood of *S. lanata* (Figure 1). These constituents showed significant antioxidant activities in the DPPH radical scavenging assay using ascorbic acid as a standard antioxidant.

2. Materials and Methods

2.1. General

UV spectra were recorded on Spectra Max 190 in a 96-well plate reader using methanol as solvent. IR spectra were taken on a Perkin-Elmer FT-IR instrument for compounds mounted directly

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on the diamond cell. Optical rotations were determined on an ADP 220 polarimeter at 589 nm. ^{1}H-NMR spectra were executed in deuterated solvents such as $CDCl_3$, CD_3OD and CD_3COCD_3 with TMS as internal standard at 300 MHz, 400 MHz and 500 MHz, AVANCE-400, AVANCE-500, Varian-400 MR, Varian-S500a and Varian-S500b magnetic resonance spectrometers. ^{13}C-NMR spectra were obtained in the same solvents at 75 MHz, 100 MHz and 125 MHz using the same instruments. The 2D spectra (COSY, HSQC, H2BC, HMBC and NOSEY) were taken at 400 MHz and 500 MHz using the above magnetic resonance spectrometers. Low-resolution Electron Impact Ionization Mass Spectra were recorded on Finnigan MAT 311 with MASSPEC data system. HR-ESIMS were carried out on an APEX IV Fourier-transform Ion Cyclotron resonance instrument (Bruker Daltonics, Coventry, UK) using an Apollo ESI source. The nebulizer and drying gas was nitrogen at 180^{o}C. Samples were infused from solution (50% MeOH/DCM) by syringe pump at 100 microlitres per hour. Accurate masses to within 5ppm were obtained using a broad range external calibration using a mixture of PEG polymer grades. Silica gel (220-440 mesh) was used for column chromatography and Merck keisel gel 60 F254 pre-coated silica gel glass plates were used for analytical and preparative TLC. TLCs were visualized by UV light (λ = 254 nm, 366 nm) or by heating after spraying with 15% aqueous sulfuric acid saturated with ceric sulfate. Reverse phase HPLC was conducted using a Waters mass-directed autopurification system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex LUNA column (5µ, C_{18}, 100 Å, 10 x 250 mm) equipped with a Phenomenex Security Guard precolumn (Luna C_5 300 Å) eluting at 4 mL/min. Solvent A, HPLC grade H_2O + 0.05% formic acid; Solvent B, HPLC grade CH_3CN + 0.045% formic acid or HPLC grade CH_3OH + 0.045% formic acid. The post-column flow was split (100:1) and the minority flow was made up with MeOH + 0.045% formic acid to 1 mL/min for simultaneous analysis by diode array (Waters 2998), evaporative light scattering (Waters 2424) and ESI mass spectrometry in positive and negative modes (Waters Quatro Micro).

2.2. Plant Material

The stem wood along with leaves and fruits of *Sorbus lanata* were collected in August 2008 from Gilgit region in the north of Pakistan. The plant material was identified by Mr. Naveed Ahmad, Lecturer in Botany, Department of Botany, University of Peshawar, Peshawar, Pakistan. A voucher specimen (JR-57/2008) was deposited in the herbarium of the same department.

2.3. Extraction and Isolation

The dried and crushed wood of *Sorbus lanata* (12 kg) was subjected to cold extraction with methanol. The methanolic extract (750 g) was suspended in water and successively partitioned in hexane, ethyl acetate and butanol soluble fractions. The ethyl acetate fraction (180 g) was further defatted with hexane for several times. The defatted ethyl acetate fraction (150 g) was subjected to column chromatography (CC) on silica gel. The column was first eluted with hexane-ethyl acetate (100:0 → 0:100) and then with dichloromethane (DCM)-methanol (MeOH) (98:2 → 90:10) as solvent systems. The fraction obtained in DCM: MeOH (98:2) was subjected to column chromatography to afford compound **4** (39.6 mg). The fraction collected in hexane: EtOAc (20:80) was further subjected to CC on flash silica using hexane-EtOAc mixture, to give eight sub-fractions. The sub-fractions obtained in hexane: EtOAc (60:40) were combined on the basis of thin layer chromatography (TLC) profiles. This combined fraction was again subjected to CC on flash silica to obtain compound **1** (40.3 mg) in MeOH: DCM (2:98). The fraction obtained in hexane: EtOAc (40:60) was subjected to purification through reverse phase HPLC (High Performance Liquid Chromatography), using a WATERS mass-directed autopurification system, compound **2** (20.3 mg) and **3** (23.8 mg) were obtained using a solvent combination of acetonitrile-water (45:90).

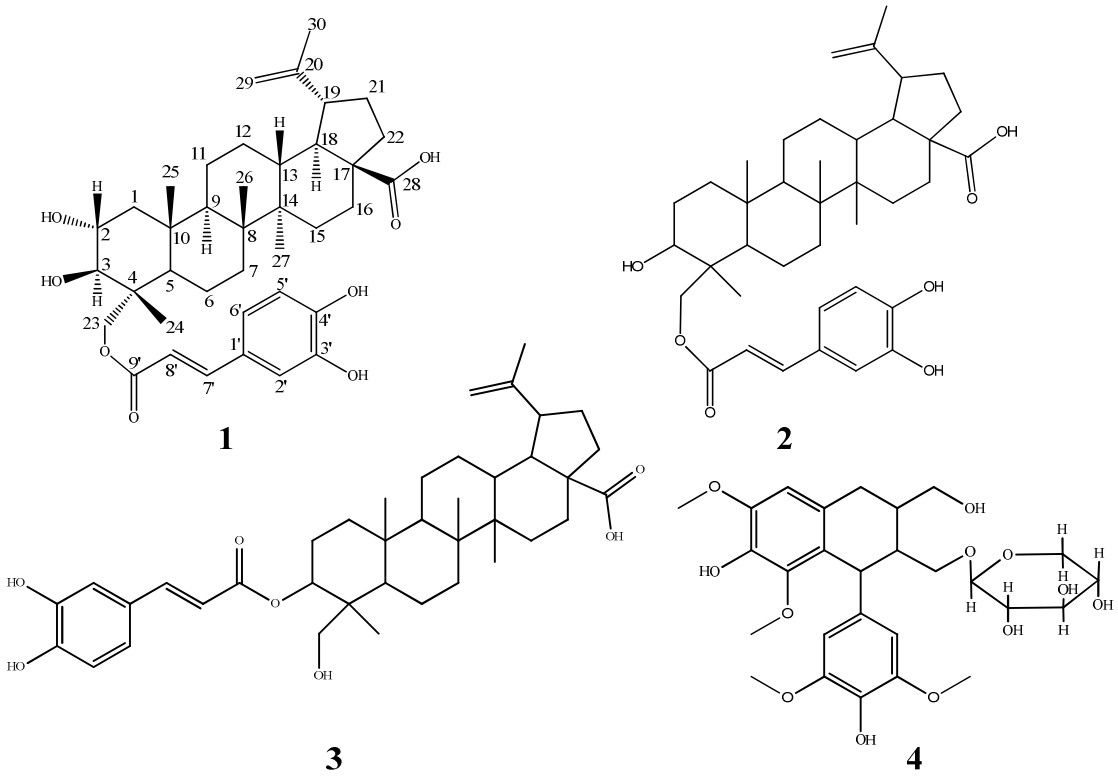

Figure 1. Structure of Compounds **1-4**

*2.4. Sorbanolic Acid (**1**)*:

White amorphous solid, $[\alpha]_D^{20}$ + 10 (*c* 0.001, CH_3COCH_3). IR υ_{max} cm^{-1}: 3358, 1686, 1602, 1516, 1447, 1378. ESIMS (-): *m/z* 649 $[M-H]^-$. HR-ESIMS (+): ($[M+Na]^+$ *m/z* 673.3712; calcd 673.3716). ^{1}H-NMR, ^{13}C-NMR and HMBC NMR data in Table 1.

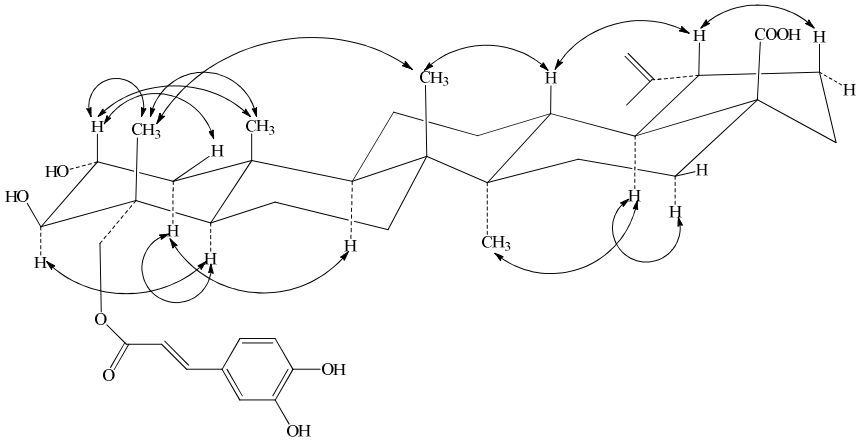

Figure 2. Selected NOESY correlations of sorbanolic acid (**1**)

Table 1. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz) and HMBC-NMR (500 MHz) correlations of Sorbanolic Acid (**1**) in CD_3COCD_3

H/C.	^{1}H NMR (δ) Coupling Constants J_{HH} (Hz)	^{13}C NMR (δ)	HMBC
1*α*	2.01, dd (*J* = 10.0, 5.0)	47.8	C-2, C-3, C-5, C-10, C-25
1*β*	0.88, m		C-2, C-3, C-5, C-9, C-10, C-25
2	3.70, m	68.9	C-3
3	3.32, m	77.5	C-1, C-2, C-4, C-23, C-24
4	-	43.1	-
5	1.31, m	48.8	C-3, C-4, C-7, C-23, C-24
6	1.46, m	18.9	C-7, C-8
7*α*	1.34, m	34.9	C-5, C-8, C-9, C-26
7*β*	1.45. m		C-8
8	-	41.6	-
9	1.46, m	51.6	C-1, C-7, C-10, C-25
10	-	38.9	-
11*α*	1.28, m	21.9	C-8, C-9, C-13
11*β*	1.47, m		C-10
12*α*	1.08, m	26.3	C-13, C-14
12*β*	1.74, m		C-9, C-14
13	2.35, m	39.0	C-12, C-14, C-18, C-27
14	-	43.2	-
15*α*	1.14, m	30.4	C-13, C-14, C-16, C-17, C-27
15*β*	1.52, m		C-14, C-27
16*α*	1.43, m	32.8	C-14, C-15, C-17, C-18
16*β*	2.22, m		C-15, C-28
17	-	56.8	-
18	1.62, t (*J* = 11.32, 11.32)	49.9	C-13, C-14, C-17, C-19, C-20, C-28
19	3.05, m	47.9	C-13, C-18, C-20, C-21, C-29, C-30
20	-	151.6	-
21*α*	1.38, m	31.4	C-17, C-20, C-22
21*β*	1.91, m		C-17, C-18, C-19
22*α*	1.47, m	37.5	C-17, C-28
22*β*	1.92, m		C-17, C-18, C-19, C-21, C-28
23*α*	4.00, d (*J* = 11.2)	66.1	C-3, C-4, C-5, C-24, C-9′
23*β*	4.09, d (*J* = 11.2)		C-3, C-4, C-5, C-24, C-9′
24	0.79, s	13.6	C-3, C-4, C-5, C-23
25	0.97, s	18.3	C-10
26	0.96, s	16.6	C-7, C-8, C-9, C-14
27	0.99, s	14.9	C-8, C-14, C-15
28	-	177.5	-
29*α*	4.59, dd (*J* = 2.4, 1.4)	110.0	C-18, C-20, C-21, C-30
29*β*	4.72, d (*J* = 2.4)		C-18, C-20, C-21, C-30
30	1.71, s	19.5	C-19, C-20, C-21, C-29
1′	-	127.6	-
2′	7.17, d (*J* = 2.2)	115.3	C-4′, C-6′, C-7′
3′	-	146.4	-
4′	-	148.8	-
5′	6.88, d (*J* = 8.2)	116.5	C-1′, C-2′, C-3′, C-4′, C-6′
6′	7.04, dd (*J* = 8.2, 2.2)	122.5	C-2′, C-4′, C-5′, C-7′
7′	7.57, d (*J* = 15.9)	145.6	C-1′, C-2′, C-6′, C-9′
8′	6.32, d (*J* = 15.9)	115.8	C-1′, C-9′
9′	-	167.3	-

3. Results and Discussion

3.1. Structure elucidation

Sorbanolic acid (**1**) was obtained as a white amorphous solid. Its molecular formula was determined to be $C_{39}H_{54}O_8$ by positive mode HR-ESIMS ($[M + Na]^+$ *m/z* 673.3712; calcd 673.3716). The UV spectrum exhibited absorption maxima at 218 ad 329 nm, suggesting the presence of an aromatic ring in the molecule. The IR spectrum showed absorptions bands for hydroxyl (3358 cm^{-1}), α, β- unsaturated carbonyl (1686 cm^{-1}), and aromatic (1602 and 1516 cm^{-1}) functionalities. Analysis of the NMR data (Table 1) suggested that **1** is based on a pentacyclic triterpene alphitolic acid [16] linked to a *trans* caffeoyl moiety. The presence of a *trans* caffeoyl moiety [17] was deduced from the olefinic protons at δ_H 6.32 (1H, d, J = 15.9 Hz, H-8′) and δ_H 7.57 (1H, d, J = 15.9 Hz, H-7′) and by a 1, 3, 4-trisubstituted benzene ring at δ_H 7.04 (1H, dd, J = 8.2, 2.1 Hz, H-6′), δ_H 6.88 (1H, d, J = 8.2 Hz, H-5′) and δ_H 7.17 (1H, d, J = 2.1 Hz, H-2′). The caffeoyl moiety was fixed at C-23 (δ_C 66.1) for HMBC correlations (Table 1) of the geminal protons at δ_H 4.00 (H_a-23) and 4.09 (H_b-23) to the carbonyl signal at δ_C 167.3, and to C-3, C-4 and C-5 (δ_C 77.5, 43.17, and 48.8, respectively). The olefinic protons H-7′ and H-8′ also showed HMBC correlations to the same carbonyl carbon. The relative *trans*-configuration of H-2 and H-3, was supported by coupling constant (J = 9.5 Hz) and 2D NOESY experiment (Figure 2) wherein NOEs were seen between H-2 (δ_H 3.70) and H-24 (δ_H 0.79)/H-25 (δ_H 0.97), between H-3 (δ_H 3.32) and H-5 (δ_H 1.31). H-2 and H-3 did not show NOESY cross peak, thereby confirming their staggered configuration. The stereochemical configurations of all other groups were also confirmed on the basis of 2D-NOESY NMR experiment. Thus, the structure of Sorbanolic acid (**1**) was assigned as 2α,3β,23-trihydroxy-lup-20(29)en-28-oic acid-23-caffeate.

3.2. Antioxidant activity

All the four compounds were tested for their antioxidant activity in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay using a modified established protocol [18, 19]. The IC_{50} values for compounds (**1-4**) were found to be 24.2, 9.2, 6.0, 21.2 μM, respectively. The assay was done in comparison to ascorbic acid (IC_{50} = 33.9 μM) which was taken as positive control.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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