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NOTE

Antioxidant flavonoids from *Alhagi maurorum*

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A new flavonoid, isorhamnetin-3-*O*-[-α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranoside (**1**), along with two known flavonoids 3′-*O*-methylorobol (**2**) and quercetin 3-*O*-β-D-glucopyranoside (**3**), was isolated from *Alhagi maurorum*. Their structures were established with the help of mass spectrometry, 1D and 2D NMR spectroscopy, and in comparison with the literature data. Compounds **1** and **2** exhibited moderate antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay.

Keywords: *Alhagi maurorum*; flavonoid glycosides; antioxidant; 2,2-diphenyl-1-picrylhydrazyl radical scavenging

1. Introduction

Alhagi maurorum is an undershrub, which is 60–100 cm tall, glabrous, or pubescent. It is distributed in Pakistan, Kashmir, Iran, Afghanistan, Russia, Turkey, Iraq, Syria, Palestine, Cyprus, and Africa [1]. It is widely used in folk medicine as an anti-asthmatic, aphrodisiac, antipyretic, appetizer, anti-rheumatic, digestible, tonic, diuretic, demulcent, expectorant, laxative, cholagogue, and refrigerant. It is also used for the treatment of bronchitis, skin diseases, and piles [2]. The flowers are used for the treatment of piles, migraine, and warts. Essential oil from the leaves of this plant is used for the treatment of rheumatism [3]. Water extract of the roots is used to enlarge the ureter and to remove the kidney stones, whereas the methanolic extract is known as an anti-diarrheal agent [4]. A literature survey revealed that alkaloids, flavonoids, and fatty acids are the major active constituents of this genus [5,6]. The pharmacological importance of this plant motivated us to explore this species for its chemical constituents, which resulted in the isolation of a new flavonoid glycoside (**1**), along with 3′-*O*-methylorobol (**2**) and quercetin 3-*O*-β-D-glucopyranoside (**3**) (Figure 1).

2. Results and discussion

The methanolic extract of air-dried whole plant material (8 kg) of *A. maurorum* was evaporated *in vacuo*, suspended in H_2O, and successively partitioned with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. As a result of a series of column chromatographic techniques of a chloroform-soluble fraction, compounds **1**–**3** were isolated as described in Section 3.

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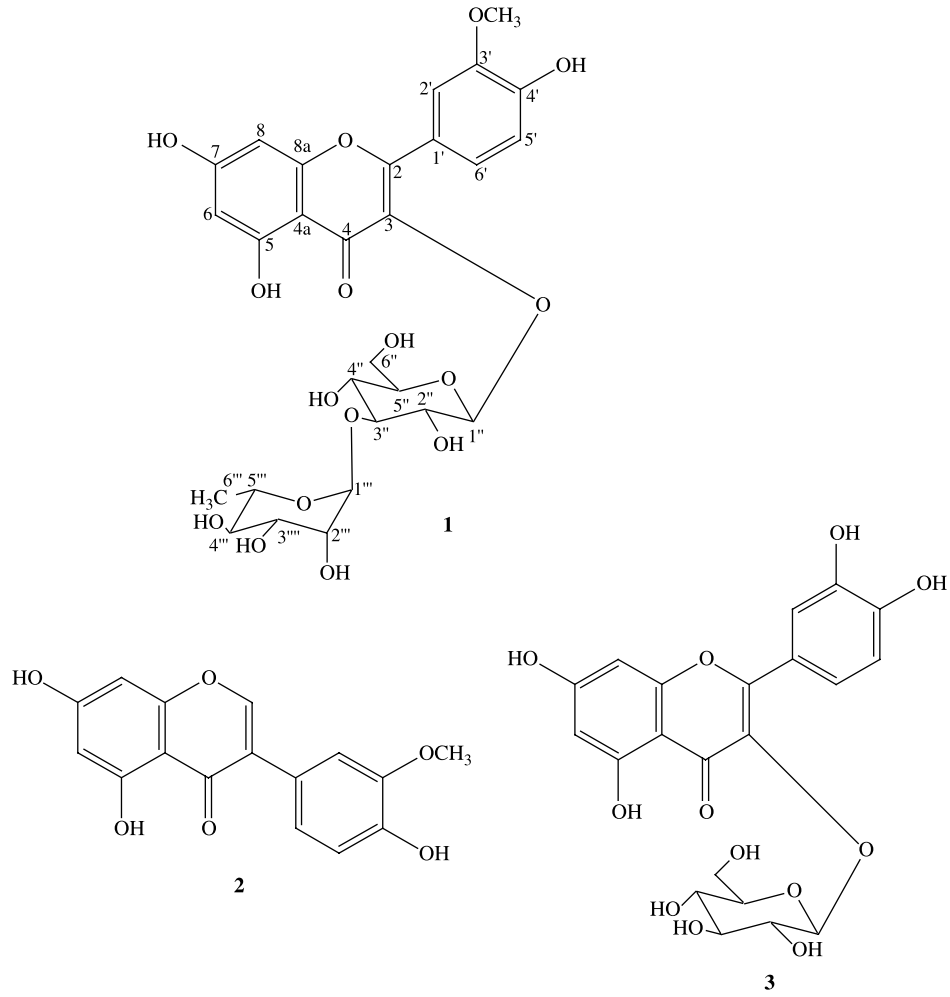

Figure 1. Structures of compounds **1**–**3**.

Compound **1** was isolated as a yellowish amorphous powder. The molecular formula $C_{28}H_{32}O_{16}$ was established through HR-FAB-MS [positive mode] showing a pseudo-molecular ion at *m/z* 625.1770 and common fragment ions in FAB-MS at *m/z* 479 and 317 corresponding to the loss of deoxyhexose $[(M+H)-146]^+$ and hexose $[(M+H)-(146+162)]^+$, respectively, from the molecular ion. The UV spectrum of **1** showed distinctive maxima at 254, 354, and 266 nm, similar to those of isorhamnetin-3-*O*-glycoside [7]. The IR spectrum of **1** revealed the presence of hydroxyl groups (3410 cm^{-1}), a methoxyl group (1590, 1055 cm^{-1}), and an aromatic system (1610–1535 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed distinctive resonances for one methoxy group at δ 3.93 and two anomeric protons at δ 5.20 (1H, d, $J = 7.5$ Hz) and 4.52 (1H, s). These data combined with ^{13}C NMR spectral data allowed the glycosidic units to be identified as β-glucopyranose and α-rhamnopyranose, respectively. Acid hydrolysis of **1** provided three products, which were separated by a solvent

Table 1. ^{1}H and ^{13}C NMR spectral data, HMBC, and COSY correlations of **1** (CD_3OD).

Position	$^{1}H^{a}$ multiplicity (J, Hz)	$^{13}C^{b}$ (DEPT)	HMBC^{c} (H → C)	COSY-45
2	–	158.8	–	–
3	–	135.4	–	–
4	–	179.2	–	–
4a	–	105.5	–	–
5	–	162.8	–	–
6	6.19 d (1.5)	100.1	4a, 5, 7, 8	–
7	–	166.4	–	–
8	6.39 d (1.5)	95.0	4a, 6, 7, 8a	–
8a	–	158.4	–	–
1′	–	122.9	–	–
2′	7.92 d (1.8)	114.5	2, 1′, 3′, 4′, 6′	–
3′	–	148.3	–	–
4′	–	150.8	–	–
5′	6.90 d (8.5)	116.1	1′, 3′, 4′, 6′	H-5′/H-6′
6′	7.61 dd (8.5, 1.8)	124.0	1′, 2′, 4′, 5′	H-5′/H-6′
1″	5.20 d (7.5)	104.4	3, 2″, 3″, 5″	H-1″/H-2″
2″	3.46 m	72.0	1″, 3″, 4″	H-2″/H-3″
3″	3.25 m	84.8	1‴, 2″, 4″, 5″	H-3″/H-4″
4″, 5″	3.35–3.50 m	72.5, 74.5	–	–
6″	3.85–3.65 m	60.7	–	–
1‴	4.52 s	102.4	3″	–
2‴	3.60 m	71.5	–	–
3‴	3.25 m	71.9	–	–
4‴	3.42 m	74.0	–	–
5‴	3.41 m	69.8	–	–
6‴	1.08 d (6.1)	17.8	–	–
OCH_3	3.93 s	56.8	–	–

Notes: [a]^{1}H NMR spectrum was recorded at 500 MHz.
[b]^{13}C NMR spectrum was recorded at 125 MHz.
[c]HMBC spectrum was recorded at 400 MHz.

extraction. The ethyl acetate layer contains 3′-methylquercetin. The glycone could be separated from the aqueous layer, purified using preparative thin layer chromatography by a solvent system (EtOAc–MeOH–H_2O–AcOH; 4:2:2:2), and identified as D-glucose and L-rhamnose through their optical rotation signs and comparison of the retention time of their trimethylsilyl (TMS) with the standards in gas chromatography. The inter-glycosidic linkages and the site of attachment between the glycoside and the aglycone were determined from long-range HMBC correlations, in which the anomeric proton of β-Glu (δ 5.20) showed ^{3}J correlation with C-3 (δ 135.4) of the aglycone, confirming the site of attachment of glucose at C-3 and Rha H-1‴ (δ 4.52) with Glu C-3″ (δ 84.8) and characterizing the inter-glycosidic linkage between these sugars as (1‴ → 3″). The 'D' and 'L' configurations of β-Glu and Rha were assumed by comparing the coupling constant and ^{13}C NMR spectral data of several reported flavonoid glycosides [8,9]. The aromatic region of the ^{1}H NMR spectrum showed two doublets at δ 7.92 (1H, d, $J = 1.8$ Hz), 6.90 (1H, d, $J = 8.5$ Hz) and a double doublet at δ 7.61 (1H, dd, $J = 8.5$, 1.8 Hz), supporting the presence of the 1,3,4-trisubstituted benzene ring. The downfield shift of H-2′ (δ 7.92) confirmed the position of the methoxy group at C-3′ [8], which was further supported by HMBC ^{3}J correlation

of —OMe (δ 3.93) with C-3 (δ 148.3). The aromatic region also showed the signals of two *meta*-coupled protons at δ 6.39 (1H, d, $J = 1.5$ Hz) and 6.19 (1H, d, $J = 1.5$ Hz). The above-mentioned aromatic data supported the presence of the isorhamnetin nucleus in the molecule [8]. The ^{13}C NMR spectrum of **1** (Table 1) disclosed a total of 28 carbon signals; 2 methyl, 1 methylene, 15 methine, and 10 quaternary carbon atoms. The downfield signals at δ 179.2, 166.4, 158.4, 150.8, 148.3 indicated the presence of a flavonoid nucleus with a quercetin-like arrangement [8]. The signals for the anomeric carbons were resonated at δ 104.4 and 102.4, respectively. The downfield signals of C-3″ at δ 84.8 indicated the attachment of both sugars at C-3″ position, confirming a rungiose moiety in the molecule. On the basis of these evidences, the structure of **1** was determined to be isorhamnetin 3-*O*-rungioside. Although the glycone unit rungiose is not common in natural products, many natural flavonoid glycosides have been reported in the literature to have rungiose as a glycone part [10,11]. Compound **1** is a new natural product.

The structures of the known compounds **2** [12] and **3** [13] were determined by the comparison of spectroscopic data and similar TLC profiles with the authentic samples.

Compounds **1** and **2** were evaluated for their antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Both compounds exhibited low activity, with IC_{50} values of 357.7 and 121.3 μg/ml, respectively (Table 2).

Table 2. Determination of DPPH scavenging activity of **1** and **2**.

S. no.	Sample code	IC_{50} (μg/ml)[a]
1	**1**	357.7 ± 0.79
2	**2**	121.3 ± 0.63
3	Quercetin[b]	0.89 ± 0.07

Notes: [a]All experiments were done in triplicate (mean ± SEM).
[b]Standard.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded on a Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). FAB-MS and HR-FAB-MS spectra were recorded on a Jeol JMS-HX 110 spectrometer with a data system. The ^{1}H NMR spectra were recorded on Bruker AMX-400 and 500 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) are in Hz. The ^{13}C NMR spectra were recorded at 125 MHz on the same instrument. Column chromatography was carried out using silica gel of 70–230 and 230–400 mesh. Aluminum sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck; purchased from a local authorized dealer of E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm), followed by ceric sulfate as the spraying reagent.

3.2 Plant material

The whole plant of *A. maurorum* (8 kg) was collected from Cholistan Desert (Bahawalpur) in May 2007 and identified by Dr Muhammad Arshad, Plant Taxonomist, Cholistan Institute for Desert Studies (CIDS), The Islamia University of Bahawalpur, where a voucher specimen (66/CIDS/07) is deposited.

3.3 Extraction and isolation

The shade-dried, ground whole plant of *A. maurorum* (8 kg) was extracted

with MeOH (3× 33 liters). The combined MeOH extract was concentrated, and the residue (400 g) was divided into *n*-hexane-, chloroform-, ethyl acetate-, *n*-butanol-, and water-soluble subfractions. The chloroform-soluble subfraction (95 g) was subjected to column chromatography over silica gel, eluting with *n*-hexane, *n*-hexane–chloroform, chloroform, chloroform–methanol, and methanol in increasing order of polarity. The fractions obtained from *n*-hexane–chloroform (3:7) showed a strong UV-absorbing spot with some impurities and were re-chromatographed over silica gel using *n*-hexane–chloroform (2.8:7.2) to obtain 3′-*O*-methylorobol (**2**). The fraction obtained from chloroform–methanol (9.1:0.9) was chromatographed over silica gel using chloroform–methanol (9:1) as the eluent to provide quercetin 3-*O*-β-D-glucopyranoside (**3**). The fraction obtained from chloroform–methanol (8.5:1.5) was chromatographed over silica gel and eluted with chloroform–methanol (8.4:1.6) to get compound **1**.

3.3.1 *Compound **1***

A yellowish amorphous powder (32 mg), $[\alpha]_D$ −25.75 ($c = 0.25$, MeOH); IR ν_{max} (KBr): 3410, 1610–1535, 1055 cm^{-1}; UV λ_{max} (MeOH): 254, 354, 266 nm; FAB-MS (positive-ion mode) *m/z*: 625 $[M+H]^+$, 479, 317; HR-FAB-MS (positive-ion mode) *m/z*: 625.1770 (calcd for $C_{28}H_{33}O_{16}$, 625.1768); For the ^1H and ^{13}C NMR data, see Table 1.

3.3.2 *Acid hydrolysis of compound **1***

A solution of **1** (8 mg) in MeOH (5 ml) containing 1N HCl (4 ml) was refluxed for 4 h, concentrated under reduced pressure, and diluted with H_2O (8 ml). It was extracted with EtOAc and the residue recovered from the organic phase was found to be 3′-methylquercetin. The aqueous phase was concentrated and purified by preparative thin layer chromatography using a solvent system (EtOAc–MeOH–H_2O–AcOH; 4:2:2:2) and was identified as D-glucose and L-rhamnose by the sign of its optical rotation ($[\alpha]_D^{20}$ −52) and ($[\alpha]_D^{20}$ +8.1), respectively. These sugars were also confirmed by comparison of the retention time of their TMS ether (D-glucose α-anomer 4.1 min, β-anomer 7.8 min, and L-rhamnose 8.6 min) with the standards.

3.4 *DPPH scavenging activity*

The DPPH, stable free radical, was used for the estimation of antioxidant activity [14]. Compounds were dissolved in DMSO, and 5 μl of each compound (final concentration of 1 mg/ml) was mixed with 200 μM DPPH to make the total volume up to 100 μl in a 96-well plate. The contents were mixed and incubated at 37°C for 30 min. The absorbance was measured at 517 nm. Quercetin was used as the control. The experiments were done in triplicate. The percentage of DPPH scavenging activity was calculated by the formula given below. The IC_{50} value was calculated by Ez-Fit™ v. 5.03 (Perrella Scientific, Amherst, NH, USA):

$$\text{DPPH scavenging activity} = 100 - [(\text{OD of the test sample}/\text{OD of the control}) \times 100].$$

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