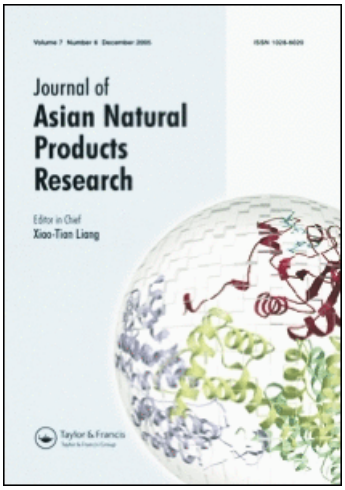

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Loasifolin, a new flavonoid from *Eremostachys loasifolia*

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NOTE

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Loasifolin (**1**), a new highly oxygenated flavonoid, has been isolated from *Eremostachys loasifolia* and its structure is assigned on the basis of spectroscopic data including 1D and 2D NMR techniques. 6,7-Dihydroxycoumarin (**2**) and luteolin 4′-*O*-β-D-glucopyranoside (**3**) have also been isolated for the first time from the genus *Eremostachys*.

Keywords: *Eremostachys loasifolia*; Labiatae; loasifolin; 1D/2D NMR

1. Introduction

The genus *Eremostachys* belongs to the family Labiatae and comprises more than 80 species which grow mostly in Central Asia [1]. *Eremostachys loasifolia* Benth. is one of the important species of the genus *Eremostachys*, which is widely distributed in Pakistan, particularly in Balochistan, and the northern province of Pakistan [1]. No phytochemical or pharmacological studies have so far been carried out on this species. The ethnopharmacological and chemotaxonomic importance of the genus *Eremostachys* prompted us to carry out phytochemical studies on this plant. We now report the isolation and structural elucidation of a new highly oxygenated flavonoid named loasifolin (**1**) along with 6,7-dihydroxycoumarin (**2**) [2] and luteolin 4′-*O*-β-D-glucopyranoside (**3**) [3], which are reported for the first time from the genus *Eremostachys*.

2. Results and discussion

Loasifolin (**1**) was obtained as an amorphous yellow solid and gave positive Mg–HCl (reddish) and $FeCl_3$ (violet) color tests. The UV spectrum exhibited absorption maxima at 267 and 340 nm, which are typical of a flavonoid moiety [4]. On addition of $AlCl_3$/HCl, it showed a bathochromic shift of 32 nm, suggesting the presence of a chelated hydroxyl group at C-5 of a flavonoid [5]. The IR spectrum showed absorption of a hydroxyl group (3400–3300 cm^{-1}), a conjugated carbonyl (1660 cm^{-1}), a conjugated double bond (1620 cm^{-1}), an aromatic moiety (1600–1400 cm^{-1}), and a carbon–ether bond (1345 cm^{-1}). The molecular formula was deduced as $C_{17}H_{14}O_7$ by HR-EI-MS showing an $[M]^+$ peak at *m/z* 330.0739. It was confirmed by broadband and distortionless enhancement by polarization transfer ^{13}C NMR spectra, which showed

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17 well-resolved signals comprising 2 methyl, 5 methine and 10 quaternary carbons. The signals at δ 160.0, 110.0, 175.1, 156.7, and 103.9 were typical of C-2, C-3, C-4, C-9, and C-10 of a flavonoidal moiety [6]. The signals of five oxygenated aromatic carbons were observed at δ 165.7, 160.7, 147.7, 146.4, and 145.6 besides the two methoxyl groups at δ 56.1 and 55.9, respectively. The ^{1}H NMR spectrum revealed the singlet resonances of the chelated hydroxyl group at δ 11.69 and of H-3 at δ 6.78. The signals in the aromatic region were assigned as two isolated AB systems, δ 6.47 and 6.36 (1H each, d, $J = 1.9$ Hz) as well as δ 7.76 and 7.03 (1H each, d, $J = 8.4$ Hz). The mass fragments resulting from a Retro-Diels-Alder cleavage in EI-MS at *m/z* 166 and 164 showed the presence of one hydroxyl and one methoxyl group in ring A and two hydroxyl groups and one methoxyl group in ring B. Their positions were ascertained through HMBC correlation studies (Table 1). The presence of the hydroxyl group at C-5 was confirmed through its ^2J correlation with C-5 (δ 160.7) and ^3J correlations with C-6 (δ 97.9) and C-10 (δ 103.9). The signal at δ 6.36 could be assigned to H-6 as it showed ^2J correlation with C-5 (δ 160.7) and C-7 (δ 165.7) as well as ^3J correlations with C-8 (δ 92.2) and C-10 (δ 103.9). The other *meta*-coupled doublet at δ 6.47 was assigned to H-8 based on its ^2J correlations with C-7 (δ 165.7) and C-9 (δ 150.7), and ^3J correlations with C-6 (δ 97.9) and C-10 (δ 103.9). One of the methoxyl groups could be located at C-7 as its protons at δ 3.87 showed ^3J correlation with C-7 (δ 165.7) as well as NOESY cross-peaks with both H-8 and H-6. The remaining methoxyl group was placed in ring B at C-6′ (δ 147.7), which in turn showed ^2J and ^3J correlations with H-5′ and H-4′. This was further confirmed by NOESY cross-peaks of methoxyl protons at δ 3.98 with H-5′. The assignments of *ortho*-coupled protons at C-5′ and C-4′ could further be authenticated through HMBC correlations given in Table 1, allowing us to assign the remaining hydroxyl groups to C-2′ and C-3′, respectively. The ^{1}H–^{1}H COSY and HMQC spectra were in complete agreement with the assigned structure of loasifolin (**1**)

Table 1. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectral data and HMBC correlations for **1** recorded in $CDCl_3$.

Position	δ_H	δ_C	HMBC (H → C)
2	–	160.0	–
3	6.78 (1H, s)	110.0	2, 4, 10, 1′
4	–	175.1	–
5	–	160.7	–
6	6.36 (1H, d, $J = 1.9$ Hz)	97.9	5, 7, 8, 10
7	–	165.7	–
8	6.47 (1H, d, $J = 1.9$ Hz)	92.2	6, 7, 9, 10
9	–	156.7	–
10	–	103.9	–
1′	–	110.0	–
2′	–	145.6	–
3′	–	146.4	–
4′	7.76 (1H, d, $J = 8.4$ Hz)	121.7	2′, 3′, 5′, 6′
5′	7.03 (1H, d, $J = 8.4$ Hz)	114.6	2′, 3′, 4′, 6′
6′	–	147.7	–
OMe-7	3.87 (3H, s)	55.9	7
OMe-6′	3.98 (3H, s)	56.1	6′
OH-5	11.69	–	5

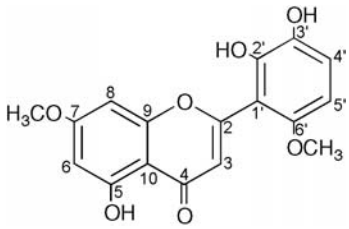

Figure 1. Structure of **1**.

as 5,2′,3′-trihydroxy-7,6′-dimethoxyflavone (Figure 1).

3. Experimental

3.1 General experimental procedures

The UV and IR spectra were recorded on Hitachi-UV-3200 and JASCO 302-A spectrometers, respectively. ^{1}H, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker AM-400 spectrometer. Chemical shifts (δ) are expressed in ppm relative to TMS as the internal standard and coupling constants (J) are given in Hz. The HR-EI-MS were measured on a JEOL JMS-HX-110 mass spectrometer. Silica gel (230–400 mesh; E. Merck, Darmstadt, Germany) was used for column chromatography. Silica gel plates (Si 60 F_{254}; E. Merck) were used for TLC.

3.2 Plant material

The whole plant material of *E. loasifolia* Benth. was collected from Miuan Ghundi near Lakpass, Quetta Valley, Balochistan, Pakistan, and identified by Prof. Dr Rasool Bakhsh Tareen, Plant Taxonomist, Department of Botany, University of Balochistan, where a voucher specimen (No. el. Rbt. 01. 2005) has been deposited in the herbarium.

3.3 Extraction and isolation

The air-dried whole plant material (20 kg) was extracted with EtOH (50 liters, 10 days each ×3) at room temperature. The combined ethanolic extract was evaporated to yield the residue (750 g), which was divided into *n*-hexane (81 g), chloroform (70 g), ethyl acetate (200 g), *n*-butanol (163.5 g), and water-soluble (63 g) subfractions. The chloroform-soluble fraction was subjected to column chromatography over silica gel eluting with *n*-hexane–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH in increasing order of polarity to obtain three major fractions A–C. Fraction B obtained from $CHCl_3$–MeOH (9.8:0.2) was further purified by column chromatography eluting with $CHCl_3$–MeOH (9.5:0.5) to afford loasifolin **1** (8 mg) and compound **2** (7 mg) from the top and tail fractions, respectively. Fraction C obtained from $CHCl_3$–MeOH (6:4) yielded compound **3** (5 mg).

*3.3.1 Loasifolin (**1**)*

Amorphous yellow solid. Mp 144°C. UV λ_{max} (MeOH) nm (log ε): 220 (3.61), 267 (5.16), 340 (4.20). IR (KBr) ν_{max} (cm^{-1}): 3400–3300, 1660, 1620, 1600–1400, 1345. For ^{1}H and ^{13}C NMR spectral data, see Table 1. EI-MS: *m/z* 330 (100), 315 (10), 312 (15), 299 (20), 166 (20), 164 (32), 139 (44), 135 (30), 124 (21), 108 (25), 94 (30), 78 (18). HR-EI-MS: *m/z* 330.0739 $[M]^{+}$ (calcd for $C_{17}H_{14}O_7$, 330.0724).

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