HOMOISOFLAVANONES FROM *Lespedeza juncea*

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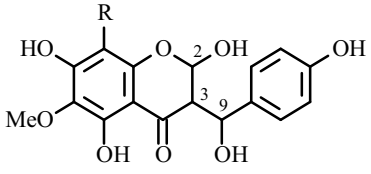
*Two unusual homoisoflavanone compounds named lesjunceol (**1**) and lesjuncerol (**2**) were isolated from the aerial parts of the methanolic extract of* Lespedeza juncea *apart from the other known constituents myrcetin and β-sitosterol. All the compounds were characterized on the basis of chemical derivatization and spectroscopic methods.*

Key words: *Lespedeza juncea*, lesjunceol and lesjuncerol.

Lespedeza juncea of the family Fabaceae growing wildly in Kashmir has been used in traditional medicine [1]. The genus *Lespedeza* of this family is reported to be a rich source of flavonoids, flavanones, isoflavones, prenylated isoflavones, ethyl caffeate, protocatechuic acid, betulinic acid, *β*-sitosterol, catechins, daidzein, isoliquiritigenin, and alkaloids [2–7]. The plant species belonging to the genus has well-known antioxidant activity and is an artificial leaf opening substance [8–11]. *Lespedeza juncea* is a small creeping woody herb growing at altitudes from 1200–2400 m. To the best of our knowledge this plant species has never been investigated for its phytochemical characterization. Keeping in view the biological importance of *Lespedeza* species, we have undertaken a research program to investigate the molecular characterization of *Lespedeza juncea*. The methanolic extract of the aerial parts of the plant led to the isolation and characterization of two new homoisoflavanones lesjunceol (**1**) and lesjuncerol (**2**).



1, 2

1: R = H; **2:** R = CH_3

Column chromatography of the methanolic extract of aerial part of *Lespedeza juncea* resulted in the isolation of the following compounds.

Lesjunceol (1). Analysis from mass spectrum showed *m/z* at 371 [M+Na], corresponding to the molecular formula $C_{17}H_{16}O_8Na$. The UV spectrum showed λ_{max} at 242.3 and 302.2, suggestive of the flavonoid structure. Bathochromic shift with NaOAc (25 nm) and $AlCl_3$ (15 nm) indicated the -OH groups at C-5 and C-7 or C-4′. The IR spectrum showed characteristic absorption bands for an a,b-unsaturated system at 1678 cm^{-1}, bridged ethers at 1310 cm^{-1}, aromatic stretching at 2954 cm^{-1}, and a broad hydroxyl stretching at 3450 cm^{-1}. The ^1H NMR showed the presence of two aromatic moieties. There are four proton doublets at δ 7.41 (J = 8.5), 7.34 (J = 8.5), 7.32 (J = 8.6), and 7.38 (J = 8.6), and a one-proton singlet at δ 6.10. The presence of the four doublets indicated that C-4′ is substituted in aromatic ring B, and the singlet at 6.10 was typical of methine proton of polysubstituted ring A. The aliphatic proton doublets at δ 4.14 (J = 7.7), 3.25 (J = 7.7), 4.3 and 5.11 (J = 7.8) were indicative of the presence of -CH-CH-CH- in the structure. The singlet at δ 3.82 was assigned to the protons of $-OCH_3$ attached to the aromatic ring. Based on the spectroscopic data, a homoisoflavanone skeleton with penta-substituted ring A and a single substituted ring B was proposed. ^{13}C NMR (DEPT) experiments indicated the presence of seventeen carbon atoms including eight quaternary carbons, eight methines, and one methyl as methoxyl (Table 1).

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TABLE 1. ^{13}C NMR (500 MHz, CD_3OD, δ, ppm) of Lesjunceol (**1**) and Lesjuncerol (**2**)

C atom	**1**	**2**	C atom	**1**	**2**
2	67.1	68.2	9	72.2	75.1
3	51.2	52.2	1′	139.2	139.1
4	198.2	191.8	2′	129.5	130.1
4a	102.2	103.2	3′	128.4	127.7
5	154.2	154.5	4′	155.2	154.5
6	128.1	128.2	5′	127.5	129.1
7	158.3	157.3	6′	135.5	134.2
8	94.5	98.5	$O\underline{C}H_3$-6	60.1	59.4
8a	157.5	150.2	$\underline{C}H_3$-8		15.2

The number of hydroxyls was confirmed by preparing the acetylated derivative of compound **1** in Ac_2O/pyridine. The formation of pentaacetate confirmed the presence of five hydroxyl groups. The positions of ring A substituents were confirmed from H–H COSY (not shown). Hydroxyl groups were assigned to C-5, C-7, and C-4′, and methoxyl group to C-6; the last position was assigned on the basis of the correlation observed between the -OCH_3 singlet at δ 3.82 and C-6 (δ 128.1), C-7 (δ 158.3), C-8a (δ 157.5), and C-4a (δ 102.2). In addition, the long-range correlation observed between H-2 doublet and C-8a was crucial to assign C-2 at δ 67.1.

Lesjuncerol (2). The mass spectrum showed *m/z* 385 [M+Na], corresponding to the molecular formula $C_{18}H_{18}O_8Na$. UV showed λ_{max} at 243 and 308 characteristic of homoisoflavanones. The bathochromic shift with NaOAc (23 nm) and $AlCl_3$ (10 nm) indicated the OH groups at C-5 and C-7 or C-4′. The IR showed characteristic absorption bands at 1692 cm^{-1} for an a, b-unsaturated system, aromatic stretching at 2952 cm^{-1}, and a broad -OH stretching at 3410 cm^{-1}. The ^1H NMR spectrum showed the presence of two aromatic rings. The four proton doublets δ 7.31 (J = 8.5), 7.21 (J = 8.5), 7.25 (J = 8.6), and 7.32 (J = 8.6) were indicative of the protons attached to the aromatic ring. The two singlets at δ 3.78 and δ 2.45 indicated one of the methoxyl attached to the aromatic ring and the other, that of the allylic proton attached to the aromatic ring. The aliphatic protons at δ 4.10 (1H, d, J = 7.8, H-2), 3.25 (1H, dd, J = 7.8, 4.4, H-3), and at δ 5.25 (1H, d, J = 7.7, H-9) were again indicative of the presence of -CH-CH-CH- in the molecule. Based upon this, a homoisoflavanone structure with all the positions of the aromatic ring A and one of the positions of ring B substituted is proposed.

^{13}C NMR (DEPT) indicated the presence of eighteen carbons, including nine quaternary carbons, seven methines, and two methyls, one as methoxyl and one as methyl (Table 1). Formation of pentaacetate from **2** confirmed the presence of five hydroxyls. The substituted positions on the aromatic ring were again confirmed by H-H COSY. Hydroxyl groups were again assigned at position C-5, C-7, and C-4′, and methoxyl at C-6. We observed a correlation of the -OCH_3 singlet at δ 3.78 and C-6 (δ 128.2), in addition to the correlation of -CH_3 at C-8 and also with C-6, C-7 (δ 157.2), C-8a (δ 150.2), and C-4a (δ 103.2). Apart from this, the long-range correlation observed between the H-2 doublet and C-8a was important for assigning C-2 at δ 68.3.

EXPERIMENTAL

Melting points are uncorrected and were determined on a BUCHI melting point apparatus. UV spectra were recorded in methanol in nm on a Specord S 100. IR were recorded on a Bruker Vector 22 spectrometer as KBr pellets with absorption given in cm^{-1}. ^1H NMR and ^{13}C NMR were run on a 500 MHz Bruker Avance 300 instrument using TMS as internal standard. Mass spectra were recorded on an Autospec M instrument at an ion source temperature of 200°C, an electron energy of 70 eV, and a mass resolution of approximately 500. Column chromatography was run using silica gel (60–120 mesh), TLC was run on a silica gel G and fluorescent aluminium TLC using solvents $CHCl_3$:MeOH. Spots were visualized on TLC under UV light, ferric chloride, cerric ammonium sulfate, and exposure to iodine vapor in an iodine chamber.

Plant Material. The aerial parts of the plant were collected in July 2005 from Shopian (Kashmir), India. A voucher specimen was deposited in the herbarium of the institute.

Extraction and Isolation. Air dried and coarsely powdered (aerial part) plant material (1.8 kg) was extracted exhaustively with hexane for 28 hrs. The defatted plant material was dried and extracted with methanol for 48 hrs. The methanol

extract was concentrated under reduced pressure to give a crude extract of 92 g. The dried methanolic extract (60 g) was dissolved in the minimum amount of methanol and adsorbed on silica gel to form a slurry. The air-dried slurry was subjected to silica gel column chromatography. The column was eluted with different percentages of petroleum ether, chloroform, ethylacetate, and finally with methanol. The following compounds were isolated.

Lesjunceol (1). Elution of an the column with $CHCl_3$–MeOH (80:20; v/v) afforded amorphous light yellow powder of **1** (63 mg), mp 240.2°C; UV (MeOH, λ_{max}, nm): 242, 281, 302; IR (KBr, ν, cm^{-1}) 3450 (OH), 2954 (Ar), 1678 (C=O), 1310, 702, 668; ^{1}H NMR (500MHz, CD_3OD, δ, ppm, J/Hz): 4.14 (1H, d, J = 7.7, H-2), 3.34 (1H, dd, J = 7.7, 4.3, H-3), 6.10 (1H, s, H-8), 5.11 (1H, J = 7.8, H-9), 7.41 (1H, J = 8.5, H-2′), 7.34 (1H, J = 8.5, H-3′), 7.32 (1H, d, J = 8.6, H-5′), 7.38 (1H, d, J = 8.6, H-6′), 3.82 (3H, s, OCH_3).

Lesjuncerol (2). Elution of the column with EtOAc–MeOH (75:25; v/v) afforded a dull white amorphous powder of **2** (52 mg), mp 190.4°C; UV (MeOH, λ_{max}, nm): 243, 280, 308; IR (KBr, ν, cm^{-1}): 3410 (OH), 2956 (Ar), 1692 (C=O), 1303, 735, 670; ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 4.10 (1H, d, J = 7.6, H-2), 3.25 (1H, dd, J = 7.6, 4.2, H-3), 5.25 (1H, J = 7.8, H-9), 7.31 (1H, J = 8.5, H-2′), 7.21 (1H, J = 8.5, H-3′), 7.25 (1H, J = 8.6, H-5′), 7.32 (1H, J = 8.6, H-6′), 3.78 (3H, s, OCH_3), 2.45 (3H, s, CH_3).

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