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CARAGISIDE D, A NEW ISOFLAVONE GLUCOSIDE FROM *Caragana conferta*

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*Caragiside D (**1**), a new isoflavone glucoside, has been isolated from the* n-*BuOH soluble subfraction of the MeOH soluble extract of the whole plant of* Caragana conferta *along with one known isoflavone glucoside, caragiside A (**2**), and two lignan glucosides, pinoresinol 4*-O-*glucoside (**3**) and syringaresinol 4*-O-*glucoside (**4**). The structures of these compounds were elucidated through spectroscopic techniques including MS and 2D NMR. The purity of compound **1** was confirmed by HPLC.*

Keywords: *Caragana conferta*, caragiside D, isoflavone glucoside.

The genus *Caragana* comprises over 80 species, of which 10 species have so far been identified in Pakistan [1]. Some of these species are used for the treatment of fever, hypertension, irregular menstruation, and fatigue [2, 3]. *Caragana conferta* Benth. ex Bake (Fabaceae) is a shrub that grows in Asia, Africa, and Southeast Europe. In Pakistan it is mainly found in the Gilgit and Kashmir Valleys [1]. The whole plant is used as folk medicine in China and Korea for the treatment of neuralgia, rheumatism, arthritis, and hypertension [4]. Literature survey revealed that many compounds have so far been reported from this species [5–10]. The ethnopharmacological and chemotaxanomic importance of this species prompted us to carry out further studies on *C. conferta*. Isolation studies on the *n*-BuOH-soluble subfraction resulted in one new isoflavone glucoside, named caragiside D (**1**), along with one known isoflavone glucoside named caragiside A (**2**) [5] and two lignan glucosides, pinoresinol 4-*O*-glucoside (**3**) [11] and syringaresinol 4-*O*-glucoside (**4**) [12]. The MeOH extract of the aerial parts of *C. conferta* was divided into subfractions soluble in *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. A part of the *n*-BuOH soluble subfraction was subjected to a series of column chromatographic techniques to afford compounds **1**–**4**. Their structures were established through spectroscopic techniques.

Caragiside D (**1**) was obtained as a yellow gummy solid, $[\alpha]_D^{25}$ +29° (*c* 0.20 in CH_3OH). It gave a violet coloration with $FeCl_3$ for a phenol. The negative mode electron spray ionization mass spectrum (ESI-MS) gave a quasi-molecular $[M - H]^-$ peak at *m/z* 461, while the HR-FAB-MS showed the $[M - H]^-$ peak at *m/z* 461.1014, corresponding to the molecular formula $C_{22}H_{22}O_{11}$. The negative mode FAB-MS further shows a peak at *m/z* 299 resulting from the loss of a hexose moiety. The ^1H NMR spectrum (Table 1) exhibited two singlets at δ 8.26 (1H, s, H-5) and 7.29 (1H, s, H-8), which were consistent with a 6,7-dioxygenated A ring of isoflavone. The protons of the 1,3,4-trisubstituted ring B were observed at 7.92 (1H, d, J = 1.8 Hz, H-2′), 7.23 (1H, d, J = 8.2 Hz, H-5′), and 7.51 (1H, dd, J = 8.2, 1.8 Hz, H-6′). The ^1H NMR spectrum gave the downfield singlet of an olefinic proton at δ 8.21. The signal of the methoxyl group appeared at 3.79 (3H, s). It also gave the signal for an anomeric proton at 4.89 (1H, d, J = 7.6 Hz, H-1′′), suggesting the *β*-configuration of the hexose unit. Further signals of the hexose moiety were observed between 3.47 and 3.97. Acid hydrolysis of **1** provided the sugar, which could be identified as D-glucose through co-TLC and the sign of its optical rotation. On the basis of these resonances, **1** was deduced to be an isoflavone glucoside.

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TABLE 1. ^{1}H NMR and ^{13}C NMR Data of Caragiside D (**1**) (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2	8.21 (s)	152.6	4′	–	148.0
3	–	124.0	5′	7.23 (d, J = 8.2)	113.6
4	–	175.3	6′	7.51 (dd, J = 8.2, 1.8)	121.8
5	8.26 (s)	109.8	1″	4.89 (d, J = 7.6)	102.4
6	–	146.3	2″	3.53 (m)	75.6
7	–	154.4	3″	3.49 (m)	77.4
8	7.29 (s)	104.0	4″	3.47 (m)	70.8
9	–	152.4	5″	4.56 (m)	76.9
10	–	118.2	6″	3.97 (dd, J = 2.5, 11.2)	62.1
1′	–	126.3		3.73 (dd, J = 4.9, 11.2)	
2′	7.92 (d, J = 1.8)	119.1	3′-OMe	3.79 (s)	56.6
3′	–	149.7			

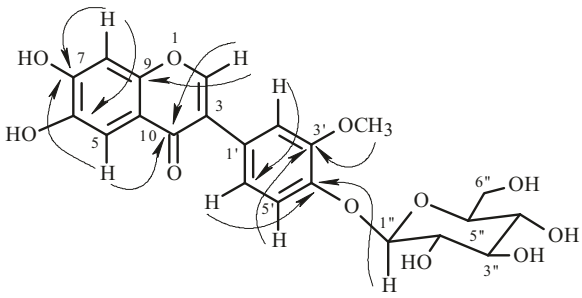

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Fig. 1. Important HMBC correlations of caragiside D (**1**).

The ^{13}C NMR spectrum of **1** (Table 1) showed 22 signals comprising one methyl, one methylene, eleven methines, and nine quaternary carbons. The signals at δ 152.6, 124.0 175.3, 152.4, and 118.2 were typical of C-2, C-3, C-4, C-9, and C-10 of an isoflavone moiety. Apart from further peaks of the aromatic carbons, the spectrum showed the signal of one anomeric carbon at δ 102.4, oxymethine carbons ranging between δ 70.8–77.4, and an oxymethylene carbon at δ 62.1. The point of attachment of the sugar moiety was shown to be C-4′ through HMBC correlations, in which the anomeric proton (δ 4.89) showed a ^{3}J correlation to C-4′ (δ 148.0) (Fig. 1). The position of the methoxy group on ring A was confirmed by HMBC experiment, in which the OCH_3 proton (δ 3.79) showed a ^{3}J correlation to C-3′ (δ 149.7). All these data were in complete agreement with the assigned structure of caragiside D (**1**) as 6,7-dihydroxy-3′-methoxy-4′-*O*-(*β*-D-glucopyranosyl) isoflavone.

EXPERIMENTAL

General Experimental Procedures. Optical activity was measured on a JASCO P-2000 Series polarimeter (JASCO Corporation, 2967-5, Tokyo, Japan). The IR spectra were recorded on a Shimadzu 460 spectrometer. The ^{1}H, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker AMX-500 spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts are in ppm (δ) relative to tetramethylsilane, and coupling constants (J) are reported in Hertz. ESI-MS were recorded on an Agilent Triple Quadrupole 6410 QQQ LC/MS mass spectrometer with ESI ion source (gas temperature 350°C, nebulizer pressure 60 psi and gas flow rate 12 L/min), operating in the negative and positive scan modes of ionization through the direct infusion method using CH_3OH–H_2O (1:1 v/v) at a flow rate of 0.4 mL/min. FAB and HR-FAB-MS (negative ion mode, matrix: glycerol) were performed on a JEOL JMS-HX110 mass spectrometer. Column chromatography was carried out on Sephadex LH-20 (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on precoated TLC plates (aluminum sheets, RP-18 F_{254}, Merck, Germany); detection was done at 254 nm and by spraying with ceric sulfate reagent. UFLC analysis was performed on a Prominence Shimadzu LC Solution (Kyoto, Japan); the system was equipped with a CBM-20A communications bus module, two LC-10AD pumps, a DGU-20A3 vacuum degasser, a CTO-10A(C) column oven, and an SPD 10A(V) diode array detector. A Shim-pack VP-ODS (150 mm × 4.6 mm, 5.0 μm, Shimadzu) analytical column and a preparative Shim-pack VP-ODS (20 × 250 mm, 5 μm, Shimadzu) column were used and kept at 40°C. The

mobile phase consisted of water containing 0.1% triflouroacetic acid (A) and CH_3OH (B). The flow rate was set at 0.4 mL/min, and the injection volume was 50 μL for the analytical column at 4.0 mL/min flow rate; the injection volume was 200 μL for the preparative column. The DAD detection was achieved in the range of 254 nm.

Plant Material. The whole plant of *Caragana conferta* Benth. ex Bake (7.0 kg) was collected from Gilgit (Pakistan) and identified by the senior scientist of the National Agriculture Research Center, Islamabad, Pakistan. A voucher specimen has been deposited in the Herbarium of the Department of Botany, University of Karachi (voucher No. 319).

Extraction and Isolation. The shade-dried plant material (7.0 kg) was extracted with methanol (25 × 3 L) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (350 g). It was suspended in water and successively extracted with *n*-hexane (120 g), chloroform (70 g), ethyl acetate (50 g), and *n*-butanol (60 g). A part of the *n*-BuOH soluble fraction (50 g) was dissolved in water and loaded on a Sephadex LH-20 column (flow rate 100 mL/h, 12 × 70 cm), and elution was successively carried out with water and mixtures of H_2O and MeOH in decreasing order of polarity, leading to two major subfractions I–II. Subfraction I (5.0 g) obtained from H_2O–MeOH (6:4) was a binary mixture, which was rechromatographed over Sephadex LH-20 (flow rate 40 mL/h, 8 × 70 cm) using H_2O–MeOH (1:1) as eluent to afford syringaresinol 4-*O*-glucoside (**4**, 10 mg) and pinoresinol 4-*O*-glucoside (**3**, 12 mg). Subfraction II (4.0 g) eluted with H_2O–MeOH (1:1) showed two major spots on TLC and was subjected to HPLC with flow rate 1.0 mL/min using H_2O–MeOH (4:6) to afford caragiside A (**2**, 15 mg) and caragiside D (**1**, 13 mg).

Caragiside D (1). Yellow gummy solid, $[\alpha]_D^{25}$ +29° (*c* 0.20, MeOH). IR (KBr, ν, cm^{-1}): 3445, 2950, 1660, 1610, 1590, 462, 1120. ^1H and ^{13}C NMR data, see Table 1. ESI-MS (–ve ion mode) *m/z* 461; FAB-MS (–ve ion mode) *m/z*: 461, 299; HR-FAB-MS (–ve ion mode) *m/z* 461.1014 $[M - H]^-$ (calcd for $C_{22}H_{21}O_{11}$).

Acid Hydrolysis of Caragiside D (1). Caragiside D (1 mg) was dissolved in 0.5 mL of a solution of 1 N HCl–methanol (1:1). The mixture was refluxed at 70°C for 85 min. The reaction mixture was concentrated *in vacuo*, water was added and the whole was extracted with ethyl acetate. The aqueous portion was filtered, the filtrate was evaporated, and D-glucose was identified from the sign of its optical rotation ($[\alpha]_D^{25}$ +52.0°) and co-TLC (ethyl acetate–methanol–water, 10:4:1, R_f 0.12) with an authentic sample of D-glucose (Merck).

HPLC Analysis for the Purity of Compound 1. Caragiside D (1.0 mg) was dissolved in 3 mL Millipore water (HPLC grade), and the solution was filtered through 0.45 μm Millipore filter. Compound **1** was detected at Rt 13.195 min using methanol–water (1:1) at 0.4 mL/min flow rate.

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