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A NEW ISOFLAVONE FROM *Astragalus peregrinus*

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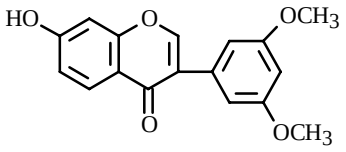
In addition to diadzen, genisten, luteolin, apigenin, and apigenin-7-O-neohesperidoside, the methanol extract of the aerial parts of Astragalus peregrinus *yielded a new isoflavone identified as 7-hydroxy-3′,5′-dimethoxyisoflavone.*

Key words: *Astragalus peregrinus*, Fabaceae, new isoflavone, anticancer activity.

Plants of *Astragalus* genus are interesting not only for the variety of chemical compounds in them but also for their biological activity [1]. In continuation of our study on the genus *Astragalus* that started with the investigation of the flavonoid content of *A. spinosus* [2], the present communication deals with the isolation and structure elucidation of six flavonoids including one new isoflavone from *A. peregrinus*. The anticancer activity is also evaluated.

The MeOH extract of *A. peregrinus* was fractionated on a polyamide column. Purification was achieved by a combination of PPC and silica gel TLC and Sephadex LH-20. Compound **1** was isolated. The isolated known compounds were identified by comparison of their spectral data with those previously reported [3, 4, 5].



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Compound **1** was isolated as a yellowish white powder. It shows chromatographic properties similar to those reported for isoflavone aglycones [6]. The UV spectral data of **1** with diagnostic shift reagents indicated an isoflavone with the 7-hydroxyl group and absence of the *ortho*-dihydroxyl pattern at the B-ring [7].

EI-MS showed a molecular ion peak at *m/z* 298 (50%) in accordance with isoflavone bearing one hydroxyl and two methoxyls group. A fragment at *m/z* 283 (100%) is due to the loss of the $-CH_3$ group. The most important fragments were observed at *m/z* 136 (10%), 160 (11%) and 163 (11%) for A_1^+, B_1^+ and B_2^+, confirming attachment of the hydroxyl group to the A-ring and the two methoxyl groups to the B-ring [8, 9].

The ^{1}H-NMR spectrum of **1** showed the characteristic H-2 signal of isoflavone as a singlet at δ 8.20 [10]. The two aromatic protons H-5 and H-6 appeared at δ 7.9 (d, J = 9 Hz) and 6.80 (dd, J = 9, 2 Hz). The signal at δ 6.65 (d, J = 2 Hz) was assigned to H-8. The B-ring protons appeared as two signals integrated to three protons at δ 7.15 (d, J = 2 Hz, H-4′) and δ 7.0 (d, J = 2Hz, H-2′, 6′). The chemical shifts of these three protons seem to be shifted upfield in comparison with those reported for the B-ring hydroxyl groups of isoflavone [11]. This is due to the electron donating ring substituents. The two methoxyl groups appeared as sharp singlet at δ 3.90. From the above results compound **1** is identified as 7-hydroxy-3′, 5′-dimethoxyisoflavone.

Tested against U251 human cells, responsible for brain tumor, compound **1** showed cytotoxicity with IC_{50} of 9.5 mg/ml.

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EXPERIMENTAL

Plant Material. *A. peregrinus* aerial parts were collected in March 2000 from Borg El-Arab, 60 km from Alexandria, and authenticated by Dr. S.A. Kawashty. A voucher specimen was deposited in the Herbarium at NRC (CAIRC).

Extraction and Isolation. Dried plant (1 kg) was extracted with 80% MeOH. The concentrated extract was subjected to a polyamide column eluted with H_2O-EtOH mixture with increasing amount of EtOH. PPC using H_2O, 15% AcOH, BAW (BuOH-AcOH-H_2O, 4:1:5, upper phase) afforded pure samples of diadzen, genisten, luteolin, apigenin, and apigenin-7-O-neohesperidoside. A combination of TLC ($CHCl_3$-EtOAc-acetone, 5:1:4) and Sephadex LH-20 afforded **1**.

7-Hydroxy-3′,5′-dimethoxyisoflavone (1). UV-spectra (MeOH, λ_{max}, nm): 247, 260sh, 285; +NaOMe: 257, 332; +$AlCl_3$: 248, 264sh, 287; $AlCl_3$+HCl : 250, 262sh, 287, 380; +NaOAc : 257, 303sh, 337; NaOAc+H_3BO_3: 251, 260sh, 284. ^{1}H NMR (270 MHz, DMSO-d_6, δ, J/Hz,): 8.20 (1H, s, H-2), 7.90 (1H, d, J = 9, H-5), 6.80 (1H, dd, J = 9, 2, H-6), 6.65 (1H, d, J = 2, H-8), 7.15 (1H, d, J = 2, H-4′), 7.00 (2H, d, J = 2, H-2′, 6′), 3.90 (6H, s, 6H, 2′OCH_3).

Bioassay. Measurement of the cytotoxicity of compound **1** was carried out according to the reported procedure [12]. The relation between the surviving fraction and drug concentrations is plotted to get the survival curve of the cell line after the specified compound.

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