Natural Product Sciences
10(6) : 321-324 (2004)

Two Isoflavonoid Glucoside Derivatives from *Ononis serrata* Growing in Egypt

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Abstract – The *n*-butanol soluble fraction of the extract obtained from the whole plants of *Ononis serrata* afforded the pterocarpan derivative medicarpin-3-*O*-glucoside and the isoflavone glucoside rothindin. Structures were elucidated by chemical methods, detailed spectral analyses as well as comparison with the literature data.
Keywords – *Ononis serrata*, Leguminosae, medicarpin-3-*O*-glucoside, rothindin, chemotaxonomical significance

Introduction

Family Fabaceae (Leguminosae) is a rich source of flavonoid derivatives. Our investigation of *Ononis* species growing in the Mediterranean costal strip around Alexandria, Egypt resulted in the isolation of several flavonoid derivatives and other novel plant phenolic compounds (Amer *et al.*, 1989; 2001; 2004; Abdel-Kader, 1997; 2001; Amer, 2001).

Recent investigation of two *Ononis* species by other groups resulted in the isolation of new triterpenoid saponin and two flavonoid glycosides (Shaker *et al.*, 2004). More than 20 flavonoid aglycones were identified in the exudates of three *Ononis* species (Wollenweber, 2003). Antimicrobial testing of herbal plants used in the traditional medicine of Jordan proved that *O. spinosa* possess a moderate antifungal activity comparable to miconazole nitrate (Mahasneh *et al.*, 1999).

Previous investigation of the polar fractions of *O. serrata* resulted in the isolation of 9-*O*-methyl spinonin and 4-*O*-methyl myoinositol (ononitol) (Amer, 2001). In the present study two known flavonoid glucosides were identified from the *n*-butanol soluble fraction of *O. serrata* through chemical and spectral methods.

Experimental

General – Melting points were determined using a Kofler's hot stage instrument and are uncorrected. UV spectra were determined using a UV-1201 Shimadzu spectrometer and Pye-Unicam SP6-400 Unit. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a JEOL 500 NMR instrument at 500 and 125 MHz for ^{1}H and ^{13}C respectively. Proton and carbon chemical shifts are reported in parts per million (ppm) relative to residual deuteriated solvent peaks. DCI-MS were obtained on a 5989-Biff instrument at GlaxoSmithkline, King of Prussia, USA.

Plant Material – *Ononis serrata* Forssk (Fabaceae) was described earlier (Amer *et al.*, 2001) and was recollected in March 2002.

Extraction and Isolation – The powdered Plants of Ononis serrata Forssk (1.8 kg) were exhaustively extracted with 90% EtOH by cold maceration. The ethanolic extract was dried, re-dissolved in 500 mL of 80% EtOH and extracted with hexane (3×300 mL). The EtOH layer was diluted with water to 60% EtOH and subjected to successive extraction with $CHCl_3$ (3×400 mL), EtOAc (3×300 mL), and *n*-butanol (3×250 mL).

20 g of the *n*-butanol soluble fraction (30 g) was fractionated on RP_{18} silica gel (300 g) by VLC technique using buchner funnel (15 cm *i.d.*, 500 mL). Elution started with 100% H_2O, followed by addition of MeOH in 10% increments.

Fractions eluted with 60% MeOH in H_2O (0.9 g) were re-fractionated over silica gel column (40 g, 1.5 cm *i.d.*, 50 cm *l*) eluting with 5% MeOH in $CHCl_3$. Fractions 7-10 (200 mg) were subjected to PTLC on silica gel plates using $CHCl_3$/ MeOH (8.5: 1.5) as developing system. Two zones could be detected under UV light, scrapped off and eluted with $CHCl_3$/ MeOH mixture (1: 1). The zone with an R_f value = 0.59 afforded 31 mg of **1**, while that with an R_f value = 0.38 afforded 19 mg of 2.

Medicarpin-3-O-glucoside (1) – $C_{22}H_{24}O_9$, white crystals, mp 269-270°C (MeOH). UV λ_{max}^{MeOH} nm: 290.

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$[\alpha]_D^{25}$: −131° (*c* 1.0, MeOH). ^{1}H-and ^{13}C-NMR data (Table 1). DCI-MS (rel. int., %): 446 (M^++1+Na, 20), 433 (M^++1, 25), 432 (M^+, 100), 380 (18), 313 (76), 271 ($C_{16}H_{14}O_4$+1, 100), 270 ($C_{16}H_{14}O_4$, 8).

Enzymatic hydrolysis of (1) – A solution of **1** (10 mg) in 1 mL acetate buffer pH 5.0 was treated with 20 mg of β-glucosidase from almond (Sigma Chemical Co.) and the reaction mixture was stirred at 40°C for 24 hr. The reaction mixture was purified on RP_{18} silica gel (10 g) by VLC technique using buchner funnel (2 cm *i.d.*, 15 mL) and H_2O/ MeOH mixtures as eluents.

The sugar was identified as D-glucose in the H_2O eluate by TLC comparison with reference sugars. The chromatogram was developed with $CHCl_3$/MeOH (6:4) and visualized by thymol/H_2SO_4 spray reagent.

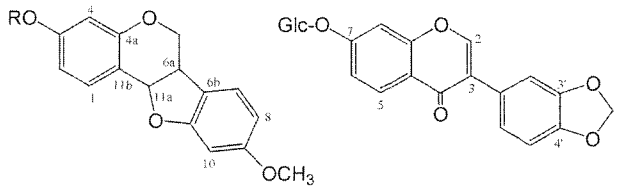
Medicarpin (1a) – The MeOH eluate was evaporated to give the aglycone **1a** (4 mg) identified as medicarpin by direct comparison with sample isolated from *O. vaginalis* (Abdel-Kader, 2001), ^{1}H- and ^{13}C-NMR data (Table 1) and EI-MS (rel. int., %): 270 (M^+, 100), 269 (M^+-1, 70), 255 (M^+-CH_3, 51), 161 (26), 148 (42), 135 (26), 69 (32).

Rothindin (pseudobaptigenin-7-O-β-D-glucoside) (2) – $C_{22}H_{20}O_{10}$, pale yellow crystals, mp 237- 238°C (MeOH). UV λ_{max}^{MeOH} nm: 262, 288 (sh). $[\alpha]_D^{25}$: −45° (*c* 0.7, MeOH). ^{1}H-NMR (CD_3OD) δ: 8.43 (s, H-2), 8.03 (d, *J*= 8.9, H-5), 7.22 (d, *J*= 2.3, H-8), 7.17 (d, *J*= 1.7, H-2'), 7.15 (dd, *J*= 8.9, 2.3, H-6), 7.06 (dd, *J*= 8.0, 1.7, H-6'), 6.96 (d, *J*= 8.0, H-5'), 6.03 (s, $\underline{C}H_2$), 5.09 (d, *J*= 7.4, H-1"), 3.68 (dd, *J*= 4.7, 13.8, H-6"), 3.16- 3.44 (m, H-2"-H-6"). ^{13}C-NMR (CD_3OD) δ: 153.9 (C-2), 125.5 (C-3), 174.6 (C-4), 126.9 (C-5), 115.7 (C-6), 161.5 (C-7), 103.4 (C-8), 157.0 (C-9), 118.4 (C-10), 123.4 (C-1'), 109.4 (C-2'), 147.0 (C-3'), 146.9 (C-4'), 108.2 (C-5'), 122.5 (C-6'), 101.1 (CH2), 99.9 (C-1"), 73.1 (C-2"), 76.5 (C-3"), 69.6 (C-4"), 77.2 (C-5"), 60.6 (C-6"). DCI-MS (rel. int., %): 468 (M^++1+Na, 11), 445 (M^++1, 16), 444 (M^+, 38), 283 ($C_{16}H_{10}O_5$+1, 57), 282 ($C_{16}H_{10}O_5$, 100), 207 (30), 178 (100).

Table 1. ^{1}H- and ^{13}C-NMR data of **1** and **1a** (δ values, *J* in parenthesis in Hz)[a].

Position	**1**[b]		**1a**[c]	
	^{1}H	^{13}C	^{1}H	^{13}C
1	7.38 d (8.7)	132.5	7.37 d (8.5)	132.4
2	6.71 dd (8.7, 2.3)	110.9	6.54 dd (8.5, 2.3)	110.1
3	-	161.1	-	161.3
4	6.42 d (2.3)	96.9	6.41 d (2.3)	97.2
4a	-	156.7	-	156.8
6	3.67 m 4.28 bd (6.2)	66.5	3.62 t (10.9) 4.23 dd (10.9, 4.9)	66.8
6a	3.67 m	39.8	3.52 m	39.7
6b	-	119.7	-	119.4
7	7.24 d (8.2)	125.7	7.12 d (8.8)	125.0
8	6.46 dd (8.2, 2.6)	106.6	6.45 dd (8.8, 2.5)	106.7
9	-	160.8	-	160.9
10	6.55 d (2.6)	104.5	6.43 d (2.5)	103.9
10a	-	159.0	-	157.4
11a	5.60 d (4.8)	78.3	5.49 d (6.5)	78.8
11b	-	114.7	-	112.7
1'	4.83 d (7.6)	100.7		
2'	3.12- 3.41 m	73.6		
3'	3.12- 3.41 m	76.9		
4'	3.12- 3.41 m	70.1		
5'	3.12- 3.41 m	77.5		
6'	3.41 m 3.67 m	61.1		
OCH_3	3.96 s	55.8	3.76 s	55.8

[a] Assignments made by combination of COSY, DEPT, HMQC data and comparison with the literature.
[b] Obtained in CD_3OD.
[c] Obtained in $CDCl_3$.



1 R= Glc
1a R= H

2

Discussion

The ^{1}H- and ^{13}C-NMR data (Table 1 and experimental) of both compounds **1** and **2**, as well as enzymatic hydrolysis of **1** all indicated the presence of only one β-D-glucose unit in each compound.

The ^{1}H-NMR and COSY experiments of **1** showed two ABX systems (δ 6.42, 6.71, 7.38 and 6.46, 6.55, 7.24) (Table 1) indicating two tri-substituted aromatic systems. In addition, ^{1}H-, ^{13}C-NMR, COSY, DEPT and HMQC experiments indicated the presence of a CH_2-O (3.67, 4.28 and 66.5 ppm), CH (3.67, 39.8 ppm), CH-O (5.60, 78.3 ppm) system diagnostic for 3, 9- substituted pterocarpan skeleton (Chalmers *et al.*, 1977; Li *et al.*, 2002). The Chemical shifts of C-3, C-9 at 161.1 and 160.8, respectively, indicated oxygenation at the two positions. The glycosidic linkage must occupy one of these positions while the other is the site of attachment of the OCH_3 (3.96 and 55.8 ppm in ^{1}H- and ^{13}C-NMR respectively). Compound **1** was unambiguously identified

as medicarpin-3-*O*-glucoside after enzymatic hydrolysis and full characterization of the aglycone **1a** by spectral analyses (Table 1 and experimental) and direct comparison with sample isolated from *O. vaginalis* (Abdel-Kader, 2001). DCI-MS confirmed the identity by the M^+ at 432 *m/z* calculated for $C_{22}H_{24}O_9$. The spectral data of **1** are in complete agreement with those published for medicarpin-3-*O*-glucoside (Sakagami *et al.*, 1974; Al-Khalil *et al.*, 1995). Although medicarpin is widespread in the Fabaceae, medicarpin-3-*O*-glucoside was previously reported only from *Medicago sativa* (Sakagami *et al.*, 1974), *Ononis natrix* (Al-Khalil *et al.*, 1995) and *Glycyrrhiza pallidiflora* hairy root cultures (Li *et al.*, 2002).

The UV absorption at 262, 288 (sh) nm, the aromatic CH-O at δ 8.43, 153.9 in ^{1}H- and ^{13}C-NMR respectively were diagnostic for an isoflavone skeleton (Mabry *et al.*, 1974; Agrawal *et al.*, 1989). In the ^{1}H-NMR of **2** (experimental) the ABX system at δ 6.96 (1H, d, *J*= 8.0 Hz), 7.06 (1H, dd, *J*= 8.0, 1.7 Hz), 7.17 (1H, d, *J*= 1.7 Hz) as well as the two oxygenated quaternary carbons at 146.9 and 147.0 ppm were assigned for an ortho-substituted ring B of the isoflavone. The UV shift reagents were unable to produce any shifts with **2**, a sign for the absence of free hydroxyl groups on the aglycone skeleton. This fact, in addition to the presence of a methylene dioxy group at δ 6.03 and 101.1 in the ^{1}H- and ^{13}C-NMR respectively (experimental) indicated that this group must be attached to C-3' and C-4'. Another ABX system in the ^{1}H-NMR of **2** at δ 7.15 (1H, dd, *J*= 8.9, 2.3 Hz), 7.22 (1H, d, *J*= 2.3 Hz), 8.03 (1H, d, *J*= 8.9 Hz) was assigned for a mono-substituted ring A. The failure of **2** to produce any UV shifts with $AlCl_3$ as well as the ^{1}H-NMR doublet at δ 8.03 (8.9 Hz) correlated by an HMQC experiment to the carbon at 126.9 ppm are all characteristic for un-substituted C-5 (Mabry *et al.*, 1974; Agrawal *et al.*, 1989). In the ^{13}C-NMR, the signal at 161.5 ppm was consequently assigned to oxygenated C-7 which is the only site available for the glycosidic *O*-linkage. The proposed structure for **2** was further confirmed by the DCI-MS showing an M^+ at 444 *m/z* calculated for $C_{22}H_{20}O_{10}$. The data of **2** were identical with those reported for rothindin (pseudobaptigenin-7-*O*-β-D-glucoside) isolated for the first time from *Rothia indica* (Nair *et al.*, 1976). In addition, rothindin was also isolated from *R. trifoliata* (Rao and Rao, 1985), *Ononis spinosa* (Haznagy *et al.*, 1978), *Cladrastis platycarpa, C. shikokiana* (Ohashi and Imamura, 1978), *Trifolium pratense* (Fraishtat *et al.*, 1980), *Thermopsis alterniflora* (Yuldashev *et al.*, 1989) and *Caragana intermedia* (Jia *et al.*, 1991). These plants are all members of the Fabaceae and represent five tribes of the sub-family Papilionoideae. The rare occurrence of 5-deoxyisoflavones in nature may give indication that the presence of rothindin in such plants is chemotaxomonically significant.

Acknowledgements

This work was supported in part by the University of Alexandria. My thanks are due to the Analytical Services Unit at Virginia Tech for spectral analyses and Prof. A. Freyer for the DCI-MS.

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(Accepted November 30, 2004)