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Rare 2-Phenoxychromones from *Ononis serrata* Growing in Egypt

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Abstract – Two 2-phenoxychromones; a rare group of natural products, were isolated from the combined ethyl acetate and chloroform fractions of the ethanolic extract of *Ononis serrata* frossk (Leguminosae) growing in Egypt. Structures were elucidated by chemical, spectroscopic methods as well as single X-ray diffraction analyses. The cytotoxic and antimicrobial effects of the two compounds were examined. It is worth to mention that this is the first report on the isolation of 2-phenoxychromones from the family Leguminosae.
Key words – *Ononis serrata*, Leguminosae, 2-phenoxychromones, X-ray, cytotoxicity, antimicrobial.

Introduction

Chemical investigation of *Ononis* species growing in Egypt is one of the points of our interest. Previous study conducted in our laboratory resulted in the isolation of several plant phenolics belonging to different classes of secondary metabolities. (Amer *et al*, 1989; 2001; Abdel-Kader, 1997; 2001; Amer, 2001).

We previously reported on the isolation of seven flavonoids from the combined ethyl acetate and chloroform fractions of the ethanol extract of *O. serrata* (Amer *et al*, 2001). In this paper we report on the identification of two phenoxychromones from the same fraction. Antimicrobial and cytotoxic activities of the two isolates were evaluated.

Experimental

Plant materials – The whole plants of *Ononis serrata* Forssk collected near Rosetta, Egypt were described earlier (Amer *et al*, 2001).

Extraction and Isolation – The detailed fractionation procedures were described earlier (Amer *et al*, 2001). The ethanol extract of the whole dried powdered plants (1.5 kg) was fractionated with C_6H_{14}, $CHCl_3$ and EtOAc. A part (20 g) of the combined $CHCl_3$ and EtOAc extracts (32 g) was chromatographed over silica gel column (400 g). The early fraction (7-12) of the column eluted with 2% MeOH in CH_2Cl_2 (0.7 g) were rechromatographed over silica gel column (50 gm) eluted with 1% MeOH in CH_2Cl_2. 25 Fractions, 75 ml each were collected, screened by TLC and similar fractions were combined. Crystallization of fractions 5-9 from MeOH afforded 45 mg of 1 (R_f value = 0.62, $CHCl_3$/MeOH 9:1), while crystallization of fractions 12-18 from acetone-benzene afforded 400 mg of 2 (R_f value = 0.43, $CHCl_3$/MeOH 9:1).

Capillarisin [2-(*p*-hydroxyphenoxy)-6-methoxy-5,7-dihydroxy-chromone] 1 – $C_{16}H_{12}O_7$, creamy crystals, mp 225-226°C (MeOH). UV λ_{max}^{MeOH} nm: 232, 289, IR ν_{KBr}^{cm-1} : 3260, 1654, 1616, 1564, 1234, 1170. ^{1}H- and ^{13}C-NMR data (Table 1). EI-MS (rel. int., %) : 317 (M^++1, 50), 316 (M^+, 85), 301 ((M^+ - CH_3, 25), 298 (27), 273 (35), 110 (22), 93 (46), 69 (97), 65 (100), 63 (24), 53 (25).

6-Demethoxycapillarisin [2-(*p*-hydroxyphenoxy)-5,7-dihydroxy-chromone] 2 – $C_{15}H_{10}O_6$, colourless crystals, mp >280°C. UV. λ_{max}^{MeOH} nm: 230, 287, 310 (sh), IR ν_{KBr}^{cm-1} : 3500, 3150, 1649, 1618, 1569, 1500, 1431, 1369. ^{1}H- and ^{13}C-NMR data (Table 1). EIMS (rel. int., %): 287 (M^++1, 40), 286 (M^+, 100), 153 (82), 134 (59), 121 (30), 110 (56), 106 (43), 93 (22), 81 (34), 69 (94), 65 (75), 63 (27), 53 (35).

Antimicrobial and Cytotoxicity testing – The two compounds were subjected to antimicrobial testing against *Bacillus subtilis* (ATCC), *Micrococcus luteus* (ACTT10240), *Staphylococcus aureus* (ATCC6538), *Bordetella bronchiseptica*

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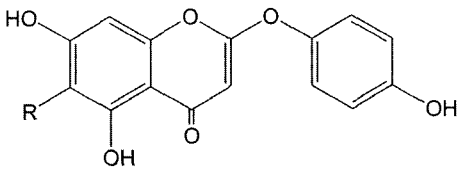
(ATCC4617), *Eschirichia coli* (ATCC8739), *Salmonella typhi* (ATCC3112), *Pseudomonus aeruginosa* (ATCC9027), and *Candida albicans* (ATCC8731). Cytotoxicity were tested utilizing Human Ovarian Cancer Cell (A 2780).

Single-Crystal X-ray Analysis of 6-demethoxycapillarisin 2 – Diffraction data of $C_{15}H_{10}O_6$, were collected from colourless crystals of dimensions 0.2×0.25×0.3 mm, using the automatic diffractometer Enraf Nonius FR 590. The crystal system is monoclinic with a = 5.2464 (2) A°, b = 29.2043(11) A°, c = 10.7650 (5) A°, v = 1632.01 (12) A°, P_{calc} = 1.259 gm/cm^3 for z = 4. The space group is $P_{2,2,2}$. Diffraction data : 3234 reflections were collected using MO radiation λ (Kα) = 0.71073 A°. Computing data collection: Kappa CCD.

Discussion

Compounds **1** and **2** were isolated from the non-polar fractions of the combined $CHCl_3$/EtOAc extracts along with seven other flavones (Amer *et al*, 2001). These two compounds were different from other flavonoids in both TLC behaviours and spectral data. They develop an orange red colour with anisaldehyde/H_2SO_4 spray reagent. Both **1** and **2** give very faint yellow colour with ammonia and Marquis reagent, violet colour with Mandalin's reagent. Erdman's reagent develops orange red colour with **1** and yellow colour with **2**.

^{1}H- and ^{13}C-NMR data of **1** (Table 1) looks like a simple flavone with 5,6,7,4'-tetraoxygenation including one methoxyl group. The singlet at δ 6.40 assigned for H-8 in **1** was replaced by two meta-coupled doublets at δ 6.04, 6.13 (*J* = 2.1 Hz) in **2** assigned for H-8 and H-6 respectively. The disappearance of the methoxyl singlet from the ^{1}H-NMR indicated that **2** is the demethoxy derivative of **1**. However, UV, ^{1}H-, ^{13}C-NMR (Table 1) and MS data (experimental) did not match a regular flavone skeleton. The odd values were the ^{1}H-NMR singlets at δ 4.98 and 4.97 in **1** and **2** respectively. The chemical shifts of some characteristic carbons in flavones such as C-2, C-3, C-1', C-2' and C-6' could be changed in **1** and **2**. Comparing the MS of **1** (M^+ at 316 *m/z*) and **2** (M^+ at 286 *m/z*) with sorbifolin (M^+ at 300 *m/z*) and apigenin (M^+ at 270 *m/z*), the two simple flavones have same substitution pattern, revealed that **1** and **2** have an additional oxygen atom.



1: R= OCH_3
2: R= H

Compound **2** was crystallized from acetone/benzene and the crystals were subjected to single X-ray diffraction analysis (Fig. 1) which proved that the extra oxygen atom is located between C-2 and C-1'. Consequently **2** were identified as 6-demethoxy capillarisin previously isolated from three Compositae plants (Kijjoa *et al*, 1999; Tanko and Hashimoto, 1981; Komiy *et al*, 1976) and *Rosa rugosa* (Rosaceae) (Hashidoko, 1991). Compound **1** was identified as the more rare capillarisin only isolated from *Artemisia capillaris* (Komiya, 1975).

Table 1. ^{1}H- and ^{13}C-NMR data of compounds **1** and **2**.

Position	**1**[a]		**2**[a]	
	^{13}C	^{1}H	^{13}C	^{1}H
2	168.3		168.3	
3	87.0	4.98 (1H, s)	87.0	4.97 (1H, s)
4	183.8		184.0	
5	150.2		161.3*	
6	132.1		99.4	6.04 (1H, d, *J*= 2.1)
7	153.4		163.7*	
8	94.5	6.40 (1H, s)	94.1	6.13 (1H, d, *J*= 2.1)
9	156.5*		155.3**	
10	102.7		102.5	
1'	143.5		143.6	
2'	122.2	7.13 (2H, d, *J* = 9)	121.4	6.82 (2H, d, *J*= 8)
3'	116.9	6.84 (2H, d, *J* = 9)	116.4	6.67 (2H, d, *J*= 8)
4'	157.2*		155.6**	
5'	116.9	6.84 (2H, d, *J* = 9)	116.4	6.67 (2H, d, *J*= 8)
6'	122.2	7.13 (2H, d, *J* = 9)	121.4	6.82 (2H, d, *J*= 8)
OCH_3	60.4	3.70 (3H, s)		

[a]Spectra were measured in $CDCl_3$ in 400 MHz NMR Spectrometer.
*, ** Signals can be interchanged.

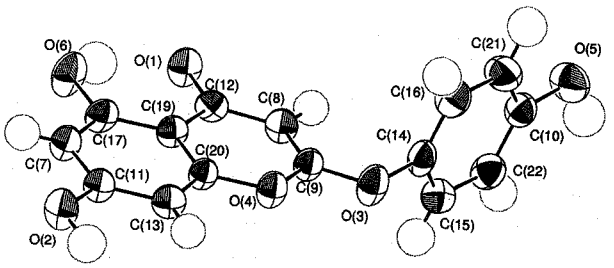


Fig. 1. X-ray structure of compound **2**.

^{13}C-NMR data of **1** was not previously reported. However, the results obtained from an HMQC experiments indicated that the ^{1}H-NMR singlets at δ 4.98 and 4.97 are correlated to carbons at 86.96 and 86.97 ppm in **1** and **2** respectively. These findings clearly prove that the assigments of C-3 and C-8 (Hashidoko, 1991; Kijjoa, 1999) must be revised. These new assignments of C-3 and C-8 fit with the calculated values by means of ACD Lab simulation program.

In the antimicrobial assay the two compounds were active against *Bacillus subtilis* with an MIC = 32 and 48 μg/ml respectively. Compound **1** was also active against *Bordetella bronchiseptica* with an MIC = 64 μg/ml. Compounds **1** and **2** showed weak cytotoxic effect against A 2780 (Human Ovarian Cancer Cell) with an IC_{50} = 23 and 21 μg/ml respectively.

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