The New Bishomoflavone Ononin and Its Glucoside from *Ononis vaginalis*

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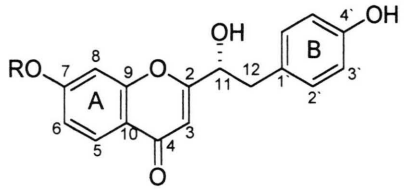
The new bishomoflavone ononin and its glucoside have been isolated from *Ononis vaginalis*. The structures were determined primarily by NMR spectroscopy. The assignment of NMR signals was performed by means of $^1H-^1H$ COSY, HMQC and HMBC experiments.

Introduction

The genus *Ononis* (Fabaceae) is represented in Egypt by eight species (Abdel-Kader, 1997). Several plants belonging to the genus are known to be used in the treatment of jaundice, urinary tract inflammations and kidney stones (Boulos, 1983). The flavonoids apigenin, chrysin, astragalin, trifolin, luteolin-3′,4′-dimethyl ether, cirsimatin and eupatilin have been isolated from *Ononis vaginalis* Vahl.. Eupatilin exhibits cytotoxic activity against human carcinoma of nasopharynx (Amer *et al.*, 1989) This prompted us to search for further flavonoids of *O. vaginalis*. In this report we desribe the isolation and structure elucidation of the new bishomoflavone ononin (**1**) and its glucoside (**2**).



1 R=H

2 R=β-D-Glucopyranosyl

Results and Discussion

The butanol fraction of the whole plants of *O. vaginalis* was obtained as described in the experimental section and chromatographed by column chromatography on Sephadex LH-20, on silica gel and centrifugally accelerated, radial TLC.

The LSI mass spectrum of **1** exhibited $[M-1]^-$ ion at *m/z* 297. The $[M-1]^-$ ion together with ^1H and ^{13}C NMR data allowed us to propose the molecular formula $C_{17}H_{14}O_5$.

In the ^1H NMR spectrum the three protons H-5, H-6 and H-8 of the ring A represented an ABX spin system. The signal of H-5 showed a coupling constant ^3J = 7.8 Hz which confirmed the ortho coupling to H-6. The signal of H-6 exhibited besides the ortho- also the meta coupling to H-8 with ^4J = 2.3 Hz. The protons at C-11 and C-12 formed an AMX spin system with the coupling constants $^3J_{AX}$ = 3.5 Hz, $^3J_{MX}$ = 5.9 Hz and $^2J_{AM}$ = 14.5 Hz. The HMBC cross signals of H-3/C-11 (^3J) and H-11/C-2 (^2J) indicated the linkage of the pyran ring with C-11 of the side chain. The HMBC signals H-11/C-1′ (^3J) and H-12/C-1′ (^2J) proved the linkage of the aromatic B ring with C-12 of the side chain. The two signals at δ 6.89 and 6.65 characterized the AA′BB′ spin system of the aromatic protons H-2′,H-6′/H-3′,H-5′. The strong downfield shift of the ^{13}C NMR signal at C-4′ δ 157.4 indicated the presence of the 4′-hydroxy group.

The $[M-1]^-$ ion at *m/z* 459 was observed in the LSI mass spectrum of **2**. The fragment ion at *m/z* 297 $[M-1-162]^-$ was a proof for the elimination of a hexose moiety.

The comparison of the ^{13}C NMR data (Agrawal, 1989) of ononin glucoside (**2**) and ononin (**1**)

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showed a good agreement in the flavone region. Six ^{13}C NMR signals of the hexose of **2** indicated the glycosylation of **1**. The coupling constants of the anomeric proton signal H-1″ $^3J_{1'',2''} = 7.3$ Hz (δ 5.01) and the signals H-2″, H-3″ and H-4″ $^3J_{2'',3''} \approx {}^3J_{3'',4''} \approx {}^3J_{4'',5''} \approx 9$ Hz (δ 3.42–3.45) proved the axial position of the protons H-1″–H-5″ and thus the presence of a β-glucopyranose. The D-form for glucose was determined as described in the experimental section. The HMBC cross peak H-1″/C-7 (δ 5.01/δ 158.2) showed the linkage of glucose in position 7 of the aglucone.

The (*R*)-configuration in position 11 of **1** and **2** was determined as follows: Oxidative hydrolysis (methanolic NaOH, H_2O_2) of the compounds yielded 4-hydroxyphenyllactic acid (Joule and Smith, 1978), which was trimethylsilylated and identified by gas chromatography on a chiral C-DEX B column as trimethylsilyl-(*R*)-4-hydroxyphenyllactic acid. The trimethylsilyl derivatives of (*R*)- (R_t 15.52, R_i 1108.7) and (*S*)-4-hydroxyphenylactic acid (R_t 8.65, R_i 1035.1) were used as reference compounds.

The bishomoflavone 7-hydroxy-2-[2-(4-hydroxyphenyl)-ethyl]-4H-1-benzopyran-4-one with a similar structure as **1** was synthesized and tested for inhibition of aldose reductase, an enzyme involved in the appearance of diabetic complications (Costantino *et al.*, 1999).

Experimental

General

Negative ion MS: MAT 8500 (Finnigan), matrix glycerol. NMR: 500.13 MHz (^1H) and 125.76 MHz (^{13}C), reverse probehead, δ in ppm, solvent CD_3OD, CD_3OD signals were used as int. standard (^1H: 3.30, ^{13}C: 49.0), temp. 290 K, HMQC: phase-sensitive using TPPI, BIRD sequence, GARP decoupled, HMBC: using TPPI, delay to achieve long range couplings: 71 msec ($J_{C,H}$ = 14 Hz).

CC: silica gel (0.063–0.2 mm); TLC: silica gel (0.25 mm precoated plates 60 F254, Merck, 0.25 mm precoated plastic sheets SIL G/UV_{254} Macherey-Nagel), the spots were sprayed with 10% H_2SO_4 in MeOH, 'sugar reagent' (4% ethanolic aniline–4% ethanolic diphenylamine–H_3PO_4, 5:5:1) and phosphomolybdic acid reagent (Aldrich). Chromatotron was used for centrifugally accelerated, radial TLC. GLC (H_2; 15 min 80°, 80–280° with 3° min^{-1}) was carried out on a Fisons GC 8130 instrument using a fused silica capillary column coated with DB 1 phase (30 m × 0.32 mm, J&W). GLC (H_2; 50–100° with 0.5° min^{-1} , 100–200° with 1.0° min^{-1}, 15 min 200°) was measured on a Carlo Erba HRGC 5060 with a fused silica capillary column coated with C-DEX B phase (30 m × 0.32 mm, J&W)

Isolation

O. vaginalis was collected in 1996 in the Libyan Desert of Egypt and identified by Dr. M. Elgebaly from the National Research Centre (NRC) Cairo. A voucher specimen of the plant is deposited at the Herbarium of the NRC, Department of Chemotaxonomy. Dried powder of the whole plant of *O. vaginalis* (3 kg) was exhaustively extracted with 80% MeOH. After removal of the solvent by evaporation, the residue was successively partitioned between H_2O and *n*-BuOH. The butanolic fr. was evaporated under red. pres. at 50 °C to obtain a crude extract (18 g). CC on Sephadex LH-20 eluting with MeOH–H_2O 8:2 v/v followed by CC on silica gel eluting with $CHCl_3$–MeOH–H_2O 7:1:0 → 7:2:0.25 v/v gave five frs.: I (2.5 g), II (1.2 g), III (300 mg), IV (250 mg) and V (1.5 g). III was further purified by means of chromatotron eluting with $CHCl_3$–MeOH–H_2O 7:2:0.25 v/v to give pure **1** (4 mg) and **2** (3 mg).

Determination of the D-form of glucose

A sample (ca 250 μg) of **2** was hydrolysed with 0.5 ml 5% HCl for at least 3 h at 80 °C. After evaporation of the acid under red. pres., 0.5 ml (*R*)-2-BuOH was added, dried HCl gas was bubbled through the soln. for 30 s and the reaction mixture was heated for 3 h at 80 °C under N_2 in a sealed vessel. Trimethylsilylation was performed with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. GC (DB 1): (*R*)-2-butyl-L-Glc: R_t 81.92, R_i 2086; (*R*)-2-butyl-D-Glc: R_t 82.25, R_i 2088. Identification of the sugars were done by comparison of the R_i values and co-injection with the appropriate standard. R_i according to (van den Dool and Kratz, 1963). Consequently it was shown for **2** that glucose belongs to the D-series.

Determination of the configuration in position 11 of **1** *and* **2**

A sample (0.5 mg) of the appropriate compound was oxidatively hydrolysed with 5 ml of methanolic NaOH/1 ml of H_2O_2 for 6 h at 50 °C. The reaction mixture was acidified with 1 N HCl and extracted three times with 1 ml of Et_2O. The combined organic extracts were evaporated and the residue trimethylsilylated with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. The trimethylsilylated 4-hydroxyphenyllactic acid was identified by GC on a chiral C-DEX B column. Trimethylsilyl-(*R*)-4-hydroxyphenyllactic acid (R_t 15.52, R_i 1108.7) and trimethylsilyl-(*S*)-4-hydroxyphenyllactic acid (R_t 8.65, R_i 1035.1) were used as reference compounds. It was shown that **1** and **2** possess (*R*)-configuration in position 11.

Spectroscopic data

Ononin (**1**): ($C_{17}H_{14}O_5$, M_r 298); $[\alpha]_D^{25}$ +46 (MeOH; c 0.08). LSI-MS negative ion mode *m/z* (rel. int.): 297 $[M-H]^-$ (30). ^1H NMR and ^{13}C NMR: Table I.

Ononin glucoside (**2**): ($C_{23}H_{24}O_{10}$, *M*r 460); $[\alpha]_D^{25}$ −27 (MeOH; c 0.06). LSI-MS negative ion mode *m/z* (rel. int.): 459 $[M-H]^-$ (41), 297 $[M-H-Glc]^-$ (25). ^1H NMR and ^{13}C NMR: Table I.

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Table I. ^1H and ^{13}C NMR spectral data for **1** and **2** in CD_3OD.

	1		**2**	
	^1H	^{13}C	^1H	^{13}C
2		168.7		168.9
3	6.16	112.6	6.11	113.6
4		177.3		176.6
5	7.34 (d, $J_{5,6}$ = 7.8)	132.1	7.35 (d, $J_{5,6}$ = 8.6)	132.6
6	6.42 (dd, $J_{5,6}$ = 7.8, $J_{6,8}$ = 2.3)	109.3	6.58 (dd, $J_{5,6}$ = 8.6, $J_{6,8}$ = 2.0)	111.4
7		160.0		158.2
8	6.42 (d, $J_{6,8}$ = 2.3)	104.0	6.79 (d, $J_{6,8}$ = 2.0)	103.6
9		163.6		163.4
10		110.9		112.7
11	5.85 (J_{AX} = 3.5, J_{MX} = 5.9)	86.0	6.10	86.3
12	3.28 (J_{AM} = 14.5, J_{AX} = 3.5)	39.3	3.19 (J_{AM} = 14.8, J_{AX} = 3.1)	39.3
	2.84 (J_{AM} = 14.5, J_{MX} = 5.9)		2.75 (J_{AM} = 14.8, J_{MX} = 6.4)	
1′		127.8		127.9
2′	6.89 (dd, $J_{2',3'}$ = 8.5, $J_{2',6'}$ = 2.8)	132.0	6.91 (dd, $J_{2',3'}$ = 8.4, $J_{2',6'}$ = 2.8)	131.9
3′	6.65 (dd, $J_{2',3'}$ = 8.5, $J_{3',5'}$ = 2.8)	115.9	6.75 (dd, $J_{2',3'}$ = 8.4, $J_{3',5'}$ = 2.8)	115.7
4′	–	157.4		157.1
5′	6.65 (dd, $J_{5',6'}$ = 8.5, $J_{3',5'}$ = 2.8)	115.9	6.75 (dd, $J_{5',6'}$ = 8.4, $J_{3',5'}$ = 2.8)	115.7
6′	6.89 (dd, $J_{5',6'}$ = 8.5, $J_{2',6'}$ = 2.8)	132.0	6.91 (dd, $J_{5',6'}$ = 8.4, $J_{2',6'}$ = 2.8)	131.9
1″			5.01 (d, $J_{1'',2''}$ = 7.3)	101.8
2″			3.42–3.45	74.6
3″			3.42–3.45	78.4
4″			3.42–3.45	71.2
5″			3.51	78.2
6″			3.95/3.75	62.5

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