

Article

Two New Norphenylpropanoid Glucosides and Hemipholin from the Flowers of *Ononis vaginalis*

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Received: May 12, 1997

Do extrato butanólico das flores de *Ononis vaginalis* Vahl. Symb. foram isolados dois novos glicosídeos norfenilpropanóides: 1 β -D-glicopiranosil-2-(4'-hidroxifenil) (*E*)-eteno (*trans*-vaginósídeo) e 1 β -D-glicopiranosil-2-(4'-hidroxifenil) (*Z*)-eteno (*cis*-vaginósídeo), e a flavanona C-glicosilada (+) 6-C- β -D-glicopiranosil (2*S*) naringenina (hemifolina). As estruturas destes compostos foram determinadas por métodos químicos e espectroscópicos.

The butanol extract of the flowers of *Ononis vaginalis* Vahl. Symb. afforded two new norphenylpropanoid glucosides, 1- β -D-glucopyranosyl-2-(4'-hydroxyphenyl) (*E*)-ethene (*trans*-vaginósídeo), 1- β -D-glucopyranosyl-2-(4'-hydroxyphenyl) (*Z*)-ethene (*cis*-vaginósídeo) as well as the known flavanone C-glucoside, (+)-6-C- β -D-glucopyranosyl (2*S*) naringenin (hemipholin). The structures of the isolated compounds were established utilizing chemical and spectroscopic methods.

Keywords: *Ononis vaginalis*, *fabaceae*, *norphenylpropanoid glucosides*, *flavanone C-glucoside*

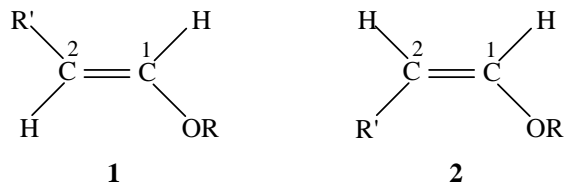
Introduction

The genus *Ononis* (Fabaceae) is represented in Egypt by eight species^{1,2}. Several plants belonging to the genus are known to be used in the treatment of jaundice, urinary tract inflammations and kidney stones³. Recently, a number of alkyl resorcinols were isolated from *Ononis* species⁴. This class of naturally occurring compounds are glycerol-3-phosphate dehydrogenase inhibitors⁵ and cleave DNA through selective strand scission⁶. Previous investigation of the non polar ether fractions of the flowers of *Ononis vaginalis* resulted in the isolation of seven flavonoids⁷. Among these, eupatilin is known to have a cytotoxic action against human carcinoma of nasopharynx⁸ and trifolin proved to have an insect growth regulator like action⁹. However, the more polar butanol fraction was not subjected to more detailed investigation.

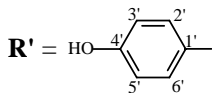
Results and Discussions

Compound **1** was isolated in the form of a yellowish gum. The UV λ_{\max} absorption at 263 nm indicated its aromatic nature. In the ¹H-NMR of **1** (Table 1), the two

doublets at 7.08 and 7.46 ppm ($J = 8.4$ Hz, 2H each) as well as their correlated carbons (base on a HETCOR experiment) at 117.7 and 130.4 ppm respectively, (see experimental) were diagnostic for a *p*-substituted benzene ring. Another two doublets at 6.39, 7.37 ppm ($J = 16$ Hz, 1H each) and their correlated carbons at 131.0 and 141.9 ppm were assigned for conjugated methines with their protons having the *trans*-orientation¹⁰. The chemical shifts of the carbons equivalent to C-1 in cinnamic acid and its derivatives is usually around 115 ppm¹¹, while the 141.9 ppm resonance in **1** indicates that C-1 is oxygenated. The quaternary carbon resonances at 136.1 and 158.5 ppm were in complete agreement with carbon and oxygen substitutions on the aromatic system. Both ¹H- and ¹³C-NMR showed signals diagnostic for β -D-glucopyranoside. FABMS showed an $[M^+ + H]$ at 299 m/z (100%) consistent with the molecular formula C₁₄H₁₈O₇ as indicated from ¹H, ¹³C-NMR and DEPT experiments. Positive reaction to FeCl₃, 12 nm UV bathochromic shift after the addition of NaOMe and the instability of the enol form (if the sugar part was attached to the phenolic OH) all support the position of glucose to be at C-1. Acid hydrolysis of **1** generated β -D-



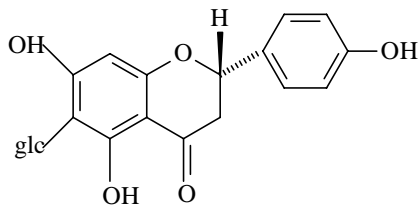
$R = \beta\text{-D-glucopyranosyl}$



glucose (identified by TLC comparison with authentic material) and an aglycone identified as (4-hydroxyphenyl)-acetaldehyde through comparing its $^1\text{H-NMR}$ data with those in the literature¹². As in cyanogenic glycosides; the glycosidic linkage is the main stabilizing factor for the aglycone. Consequently, hydrolysis of **1** resulted in the formation of the stable (4-hydroxy-phenyl)-acetaldehyde (keto form) rather than the unstable enol form.

Compound **2** showed essentially similar spectral characteristics to those of **1** (Table 1 and experimental). The major significant differences are in the $J_{1,2}$ value (16 Hz in **1** and 12 Hz in **2**) as well as H-1 and H-2 chemical shift (6.39, 7.37 ppm in **1** and 5.94, 6.33 ppm in **2**) indicating that **2** is the *cis*-isomer of **1**¹⁰.

Compound **3** was identified as hemipholin (6-C- $\beta\text{-D-glucopyranosyl}$ (2*S*) naringenin) through comparison of its data (see experimental) with those in the literature^{13,14} as well as its positive reaction with Gibb's reagent eliminating the possibility of the sugar to be attached to C-8¹⁵. The assignment of the unreported $^{13}\text{C-NMR}$ data for **3** were



performed based on HETCOR experiments as well as comparison with the data of 6,8-di-C- $\beta\text{-D-glucopyranosyl}$ (2*S*) naringenin¹⁶.

Experimental

General procedure

The melting point was determined using a Griffin melting point apparatus and was uncorrected. UV spectra were obtained in MeOH on a Pye Unicam Sp8-100 spectrometer. CD spectrum was recorded using a Jasco J-720 spectropolarimeter. NMR spectra were measured with a Bruker Ac-300 spectrometer at 300 and 75 MHz for ^1H - and $^{13}\text{C-NMR}$ respectively. Proton and carbon chemical shifts are reported in parts per million (ppm) relative to residual undeuterated solvent. The carbon chemical shifts for **1** in D_2O are reported relative to C-6 of glucose at 61.5 ppm. FAB and Electrospray mass spectra were recorded using a Quattro-SQ mass spectrometer. $\beta\text{-D-Glucose}$ was obtained from Sigma Chemical Co.

Plant material

Plant materials of *Ononis vaginalis* Vahl. Symb. (voucher MSA2) described earlier⁷ were collected on April 5, 1995 from El Agamy, Alexandria, Egypt.

Extraction and isolation

1.5 kg of fresh flowers of *Ononis vaginalis* were extracted with 95% ethanol (10 L). The concentrated ethanolic extract (150 mL) was diluted with 300 mL water and successively extracted with light petrol (3 x 500 mL), ether (3 x 500 mL), ethyl acetate (3 x 300 mL) and butanol (2 x 300 mL).

The dried butanol fraction (7 g) was fractionated by VLC over silica gel (400 g) using fritted funnel (10 x 8.5 cm) and eluted with EtOAc/ MeOH mixtures. Fractions 10-12 (0.5 g), eluted with 10% MeOH in EtOAc, were refractionated over a flash column (2 cm in diameter, 25 g silica gel) eluted with 10% MeOH in CHCl_3 . Fractions 4-7

Table 1. $^1\text{H-NMR}$ spectral data (300 MHz) for compounds 1 and 2 in CD_3OD .

No.	1	2
1	7.37 (d, $J = 16$ Hz)	6.33 (d, $J = 12$ Hz)
2	6.39 (d, $J = 16$ Hz)	5.94 (d, $J = 12$ Hz)
2', 6'	7.46 (d, $J = 8.4$ Hz)	7.53 (d, $J = 8.8$ Hz)
3', 5'	7.08 (d, $J = 8.4$ Hz)	7.01 (d, $J = 8.8$ Hz)
1''	4.93 (d, $J = 7.1$ Hz)	4.88*
2''-5''	3.41- 3.53 (m)	3.40-3.46 (m)
6''	3.70 (dd, $J = 4.8, 13.6$ Hz) 3.89 (dd, $J = 1.7, 13.6$ Hz)	3.69 (dd, $J = 4.6, 11.9$ Hz) 3.86 (dd, $J = 1.8, 11.9$ Hz)

* Signal partially obscured by water signal.

(90 mg) were subjected to PTLC using EtOAc/ MeOH/ H₂O (30:5:4) as developing system to afford 50 mg of **3** after crystallization from MeOH.

Fractions 14 and 15 (0.3 g), eluted with 15% MeOH in EtOAc, were refractionated over a flash column (2 cm in diameter, 20 g silica gel) eluted with 12% MeOH in CHCl₃. Fractions 6-9 (81 mg) were rechromatographed using PTLC silica gel plates and EtOAc- MeOH- H₂O (30:5:4) as developing system (double development). Two bands were visualized under UV light, scraped off and eluted from silica with EtOAc- MeOH (1:1). After evaporation of the solvents, the first band (*R_f* value = 0.55) afforded 19 mg of **1** while the second band (*R_f* value = 0.47) afforded 5 mg of **2**.

trans-Vaginoside [*1-β-D-glucopyranosyl-2-(4'-hydroxyphenyl) (E) - ethene*] (**1**)

Yellowish gum. [α]_D²⁶ + 24° (*c* 0.71, MeOH). UV (MeOH) λ_{\max} nm: 263, 204, (NaOMe) 275, 212. FABMS *m/z* (rel. int.): 299 (100, M⁺+H), 279 (9, M⁺-H), 291 (7), 289 (12). ¹H-NMR: Table 1. ¹³C-NMR (D₂O): 61.5 (C-6"), 70.4 (C-4"), 73.9 (C-2"), 76.5 (C-5"), 77.1 (C-3"), 100.8 (C-1"), 117.7 (C-3', C-5'), 130.4 (C-2', C-6'), 131.0 (C-2), 136.1 (C-1'), 141.9 (C-1), 158.5 (C-4').

cis-Vaginoside [*1-β-D-glucopyranosyl-2-(4'-hydroxyphenyl) (Z) - ethene*] (**2**)

Yellowish gum. [α]_D²⁶ + 18° (*c* 0.52, MeOH). UV (MeOH) λ_{\max} nm: 264, 206, (NaOMe) 277, 215. FABMS *m/z* (rel. int.): 299 (100, M⁺+H), 279 (6, M⁺-H), 291 (3), 289 (5). ¹H-NMR: Table 1.

Acid hydrolysis of **1** and **2**

Compound **1** (9 mg) and compound **2** (3 mg) were dissolved in 0.5 mL MeOH and 1 mL of 0.1 N HCl was added to each solution. After heating at reflux for 3 h. the solutions were extracted with CHCl₃ (3 x 5 mL). The residue left after the evaporation of solvents proved to be identical. The aqueous layer afforded 3 and 0.9 mg of β-D-glucose, while the organic layer afforded 4 mg and 1 mg of (4-hydroxy-phenyl)-acetaldehyde in case of **1** and **2** respectively.

(4-hydroxy-phenyl)-acetaldehyde

Yellowish oil. ¹H-NMR (CDCl₃): 3.61 (2H, d, *J* = 2.3 Hz, H-2), 6.77 (2H, d, *J* = 8.9 Hz, H-3', 5'), 7.11 (2H, d, *J* = 8.9 Hz, H-2', 6'), 9.73 (1H, t, *J* = 2.3 Hz, H-1).

Hemipholin [(+) -6-C-β-D-glucopyranosyl (2*S*) naringenin] (**3**)

Yellow crystals *m.p.* 208- 209 °C. UV (MeOH) λ_{\max} nm: 329(sh), 288, 223, (NaOMe) 327, 243, (AlCl₃) 379, 309, 224, (AlCl₃/HCl) 379, 310, 224, (NaOAc) 327, 241. CD (MeOH): [θ]₂₁₁ 0, [θ]₂₂₃ +16926, [θ]₂₅₂ 0, [θ]₂₆₂ -3906, [θ]₂₉₂ -11284, [θ]₃₀₃ 0, [θ]₃₁₉ +14765, [θ]₃₃₁ 0, [θ]₃₄₃ -12152, [θ]₃₆₅ 0, .

Electrospray MS *m/z* (rel. int.): 457 (27, M⁺+Na), 435 (66, M⁺+H), 391 (28), 276 (16), 214 (50), 163 (34), 124 (100). ¹H-NMR (CD₃OD): 2.73 (dd, *J* = 3, 17 Hz, H-3), 3.12 (dd, *J* = 13, 17 Hz, H-3), 3.34-3.44 (*m*, H-3"-H-5"), 3.67 (dd, *J* = 3, 13 Hz, H-6"), 3.85 (dd, *J* = 5, 12 Hz, H-6"), 4.11 (*m*, H-2"), 5.34 (dd, *J* = 3, 13 Hz, H-2), 5.95 (*s*, H-8), 6.79 (d, *J* = 8.6 Hz, H-3', H-5'), 7.29 (d, *J* = 8.6 Hz, H-2', H-6'). ¹³C-NMR (CD₃OD): 43.9 (C-3), 62.9 (C-6"), 71.8 (C-4"), 72.6 (C-2"), 75.2 (C-1"), 80.2 (C-3"), 80.4 (C-2), 82.5 (C-5"), 96.4 (C-8), 103.2 (C-10), 106.0 (C-6), 116.3 (C-3', 5'), 129.1 (C-2', 6'), 130.9 (C-1'), 159.0 (C-4'), 163.2 (C-9), 164.2 (C-5), 167.4 (C-7), 198.0 (C-4).

Acknowledgments

This work was supported in part by the University of Alexandria. The author is very grateful to Dr. Tommaso A. Foderaro for carrying out the CD spectrum of compound **3**.

References

- Muschler, R. In *A Manual Flora of Egypt*; Friedlaender Shon, Karlstrasse, Berlin 1912, II, 476.
- Tackholm, V. In *Students Flora of Egypt*; Cairo University, 2 nd. Ed., 1974, p 229.
- Boulos, L. In *Medicinal Plants in North Africa*; Reference Publications, Inc. 1983, p 126.
- Barrero, A.F.; Cabrera, E.; Rodriguez, I.; Fernandez-Gallego, E.V. *Phytochemistry* **1994**, 39, 189.
- Tsuge, N; Mizokami, M.; Imai, S.; Shimazu, A.; Seto, H. *J. Antibiot.* **1992**, 45, 886.
- Scannell, R.T.; Barr, J.R.; Murty, V.S.; Reddy, K.S.; Hecht S.M. *J. Am. Chem. Soc.* **1988**, 110, 3650.
- Amer, M.E.; Abdel-Kader, M.S.; Mahmoud, Z.F.; Abdel-Salam, N.A.; Yang, S.S.; Mabry, T.J. *Rev. Latinoamer. Quim.* **1989**, 20, 152.
- Kupchan, S.M.; Sigel, C.W.; Hemingway, R.J.; Knox, J.R., Udayamurthy, M.S. *Tetrahedron* **1969**, 25, 1603.
- Abdel-Kader, M.S. *M. S. Thesis* 1989, Alexandria University.
- Silverstein, R.M.; Bassler, G.C.; Morrill, T.C. In *Spectrometric Identification of Organic Compounds*; John Wiley & Sons, Inc. Fifth Ed., 1991, p 221.
- Pouchert, C.J.; Behnke, J. *The Aldrich Library of ¹³C and ¹H FTNMR Spectra*; Aldrich Chemical Company, Inc., 1993, Ed. I.
- Padwa, A.; Wisnieff, T.J.; Walsh, E.J. *J. Org. Chem.* **1989**, 54, 299.
- Budzianowski, J.; Skrzypczakowa, L. *Phytochemistry* **1978**, 17, 2044.
- Lorente, F.T.; Ferreres, F.; Barberan, F.A.T. *Phytochemistry* **1982**, 21, 1461.
- Tahara, S.; Ingham, J.L.; Nakahara, S.; Mizutani, J.; Harborne, J.B. *Phytochemistry* **1984**, 23, 1889.
- Okamura, N.; Yagi, A.; Nishioka, I. *Chem. Pharm. Bull.* **1981**, 29, 3507.