Two New Acylated Iridoid Glycosides from *Verbascum undulatum*

Prokopios Magiatis, Eleni Melliou, Eugenia Tsitsa, Catherine Charvala, and Sofia Mitaku*

Laboratory of Pharmacognosy, University of Athens, Panepistimiopolis, Zografou, GR-15771 Athens, Greece. Fax (301) 7274594. E-mail: mitakou@pharm.uoa.gr

* Author for correspondence and reprint requests

Z. Naturforsch. **55c,** 667–669 (2000); received March 7/April 4, 2000

Verbascum undulatum, Scrophulariaceae, Iridoid Glycoside

On further phytochemical investigation of the aerial parts of *Verbascum undulatum*, two new acylated iridoid glycosides, 6-*O*-[3-*O*-(*trans*-3,4-dimethoxycinnamoyl)-α-L-rhamnopyranosyl] aucubin (**1**) and 6-*O*-[3-*O*-(*trans*-*p*-methoxycinnamoyl)-α-L-rhamnopyranosyl]aucubin (**2**) were isolated. These structures were determined by spectral methods, mainly by 1D and 2D NMR spectroscopy.

Introduction

The genus *Verbascum*, belonging to the family Scrophulariaceae, comprises more than 300 species. *Verbascum undulatum* Lam., is a biennial plant widely spread in the Balkan peninsula (Tutin, 1972). In previous papers (Magiatis *et al.*, 1998 and Skaltsounis *et al.*, 1996) we described the isolation of five iridoid glycosides, derivatives of 6-*O*-α-L-rhamnopyranosyl aucubin, and four phenylethanoid glycosides, from the aerial parts of *V. undulatum*. On further investigation of the aerial parts of the plant, two new acylated iridoid glycosides, 6-*O*-[3-*O*-(*trans*-3,4-dimethoxycinnamoyl)-α-L-rhamnopyranosyl]aucubin (**1**) and (6-*O*-[3-*O*-(*trans*-*p*-methoxycinnamoyl)-α-L-rhamnopyranosyl]aucubin (**2**) were isolated. This paper describes the isolation and the structure determination of compounds **1** and **2**. The isolated compounds were identified by means of spectral data (NMR, MS).

Results and Discussion

Compound **1**, $[\alpha]_D$ −80.5° (0.1 g/100 ml, MeOH), was obtained as an amorphous powder with molecular formula $C_{32}H_{42}O_{16}$, [ESMS *m/z* $[M+Na]^+$ 705]. The ^1H and ^{13}C-NMR spectral data were assigned by interpretation of COSY, DEPT 135, HMQC and HMBC experiments. Comparison of the ^1H and ^{13}C-NMR spectra of **1** with those of 6-*O*-(α-L-rhamnopyranosyl)aucubin or sinuatol (**3**) (Vesper *et al.*, 1994) indicated that **1** is a monoacyl derivative of **3**. Indeed, mild alkaline hydrolysis in MeOH afforded sinuatol (**3**). From two typical *trans* olefinic proton signals in an AM system (δ 6.48 and 7.71, J_{AM} = 16 Hz), three aromatic protons coupled in an AMX system (δ = 7.23, d, J = 2 Hz; 7.19, dd, J = 8.5, 2 Hz; 6.98, d, J = 8.5 Hz), and two aromatic methoxy groups (δ = 3.87, 3.86 ppm) in the ^1H-NMR spectrum, the acyl moiety was suggested to be the *trans*-3,4-dimethoxycinnamoyl group. The site of esterification was determined to be the 3″-position of the rhamnopyranosyl moiety, by the fact that the ^1H-NMR signal of H-3″ was shifted downfield (δ = 5.07 ppm) in comparison with **3**. Finally the site of esterification was confirmed to be the 3″-position by the HMBC spectrum (delay for evolution of long range coupling D_6 = 60 msec), where it was clear that the proton at δ = 5.07 ppm (H-3″) had a long range coupling with the carbon at δ = 169.1 ppm (carbonyl carbon of *trans*-3,4-dimethoxycinnamoyl moiety). In conclusion, the structure of the new compound **1** was determined to be 6-*O*-[3-*O*-(*trans*-3,4-dimethoxycinnamoyl)-α-L-rhamnopyranosyl] aucubin, for which we propose the trivial name unduloside II.

Compound **2**, $[\alpha]_D$ −82.5° (0.1 g/100 ml, MeOH), was obtained as an amorphous powder with molecular formula $C_{31}H_{40}O_{15}$, [ESMS *m/z* $[M+Na]^+$ 675]. Comparison of the ^1H and ^{13}C-NMR spectra of **2** with those of **1** and **3** indicated that **2** is also a monoacyl derivative of **3** esterified at 3″ position. The presence of two *trans* olefinic protons (δ 6.44 and 7.71, J = 16 Hz), two pairs of ortho coupled aromatic protons (δ = 7.55 and 6.95, J = 8.7 Hz) and of one aromatic methoxy group (δ = 3.81 ppm) in the ^1H-NMR spectrum, showed clearly that the acyl moiety was the *trans*-*p*-methoxycinnamoyl group.

The site of esterification was determined to be the 3″-position of the rhamnopyranosyl moiety by the same way as in compound **1**. In conclusion, the structure of the new compound **2** was determined to be 6-*O*-[3-*O*-(*p*-methoxycinnamoyl)-α-L-rham-

0939–5075/2000/0700–0667 $ 06.00

nopyranosyl] aucubin, for which we propose the trivial name unduloside III.

It is interesting to point out that the *p*-methoxycinnamoyl group has been found in the structure of another sinuatol derivative (Agababyan *et al.*, 1987), whereas the *trans*-3,4-dimethoxycinnamoyl group is very rare in nature and very few examples of iridoids bearing this acyl group have been reported (Miyase *et al.*, 1991).

Experimental

The plant material was collected as described previously (Skaltsounis *et al.*, 1996). A voucher specimen (PROK008) is deposited in the herbarium of the Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens.

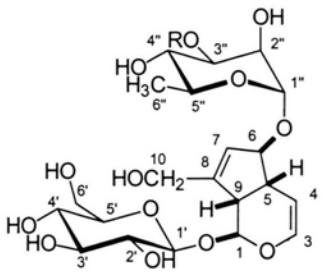
Isolation

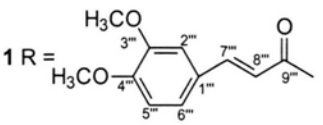
Dried, pulverized aerial parts of *V. undulatum* (1 kg) were first defatted with CH_2Cl_2 and then extracted with MeOH (2 l×4). The MeOH-soluble extract was evaporated under reduced pressure to give a residue (35 g), which was fractionated with reversed phase MPLC (H_2O, MeOH gradient) to afford a mixture of compounds **1** and **2**. The two new compounds were separated and purified with reversed phase preparative TLC (H_2O–MeOH 50:50 v/v).

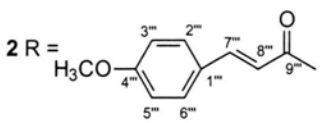
Spectroscopic data

Compound **1** (4 mg, 0.011%): ^{1}H NMR (CD_3OD, 400 MHz, δ ppm, *J* in Hz): 7.71 (1H, d, *J* = 16 Hz, H-7‴), 7.23 (1H, d, *J* = 1.7 Hz, H-2‴), 7.19 (1H, dd, *J* = 8.5, 2 Hz, H-6‴), 6.98 (1H, d, *J* = 8.5 Hz, H-5‴), 6.48 (1H, d, *J* = 16 Hz, H-8‴), 6.35 (1H, dd, *J* = 5.9, 1.8 Hz, H-3), 5.89 (1H, s, br H-7), 5.17 (1H, dd, *J* = 5.9, 3.9 Hz, H-4), 5.07 (1H, dd, *J* = 9.5, 3.5 Hz, H-3″), 4.95 (1H, d, *J* = 7 Hz, H-1), 4.85 (1H, d, *J* = 1.6 Hz, H-1″), 4.69 (1H, d, *J* = 7.9 Hz, H-1′), 4.50 (1H, m, H-6), 4.38 (1H, d, *J* = 15.2, H-10a), 4.19 (1H, d, *J* = 15.2, H-10b), 4.01 (1H, dd, *J* = 3.5, 1.6, H-2″), 3.87 (3H, s, OCH_3), 3.87 (1H, m, H-6a′), 3.87 (1H, dq, *J* = 9.5, 5.8, H-5″), 3.86 (3H, s, OCH_3), 3.70 (1H, t, *J* = 9.5, H-4″), 3.68 (1H, m, H-6b′), 3.41 (1H, t, *J* = 9, H-3′), 3.31 (1H, t, *J* = 9, H-4′), 3.30 (1H, m, H-5′), 3.26 (1H, dd, *J* = 9, 7.9, H-2′), 2.91 (1H, t, *J* = 7, H-9), 2.86 (1H, m, H-5), 1.33 (3H, d, *J* = 5.8, H-6′). ^{13}C NMR (CD_3OD, 50 MHz, δ ppm): 169.1 (C-9‴), 152.5 (C-4‴), 150.5 (C-3‴), 149.6 (C-8), 147.1 (C-7‴), 141.9 (C-3), 128.9 (C-1‴), 127.1 (C-7), 123.9 (C-6‴), 115.5 (C-8‴), 111.4 (C-2‴), 111.2 (C-5‴), 105.5 (C-4), 101.1 (C-1″), 99.9 (C-1′), 98.0 (C-1), 89.0 (C-6), 78.3 (C-5′), 77.9 (C-3′), 74.9 (C-3″, 2′), 71.6 (C-4″, 5″), 70.4 (C-4′, 2″), 62.7 (C-6′), 61.5 (C-10), 56.5 (2×OCH_3), 48.0 (C-9), 44.3 (C-5), 18.0 (C-6″).

Compound **2** (5 mg, 0.014%): ^{1}H NMR (CD_3OD, 400 MHz, δ ppm, *J* in Hz): 7.71 (1H, d, *J* = 16 Hz, H-7‴), 7.55 (2H, d, *J* = 8.7 Hz, H-2‴,6‴), 6.95 (2H, d, *J* = 8.7 Hz, H-3‴,5‴), 6.44 (1H, d, *J* = 16 Hz, H-8‴), 6.35 (1H, dd, *J* = 5.9, 1.8 Hz, H-3), 5.89 (1H, s, br H-7), 5.17 (1H, dd, *J* = 5.9, 3.9 Hz, H-4), 5.07 (1H, dd, *J* = 9.5, 3.5 Hz, H-3″), 4.93 (1H, d, *J* = 7 Hz, H-1), 4.85 (1H, d, *J* = 1.6 Hz, H-1″), 4.68 (1H, d, *J* = 7.9 Hz, H-1′), 4.48 (1H, m, H-6), 4.38 (1H, d, *J* = 15.2, H-10a), 4.18 (1H, d, *J* = 15.2, H-10b), 3.99 (1H, dd, *J* = 3.5, 1.6, H-2″), 3.87 (1H, m, H-6a′), 3.87 (1H, dq, *J* = 9.5, 5.8, H-5″), 3.81 (3H, s, OCH_3), 3.70 (1H, t, *J* = 9.5, H-4″), 3.68 (1H, m, H-6b′), 3.41 (1H, t, *J* = 9, H-3′), 3.31 (1H, t, *J* = 9, H-4′), 3.30 (1H, m, H-5′), 3.26 (1H, dd, *J* = 9, 7.9, H-2′), 2.90 (1H, t, *J* = 7, H-9), 2.83 (1H, m, H-5), 1.29 (3H, d, *J* = 5.8, H-6′). ^{13}C NMR (CD_3OD, 50 MHz, δ ppm): 169.6 (C-9‴), 162.8 (C-4‴), 149.6 (C-8), 146.3 (C-7‴), 141.9 (C-3), 130.0 (C-2‴,6‴), 128.5 (C-1‴), 127.1 (C-7), 115.4 (C-3‴,5‴,8‴), 105.5 (C-4), 101.1 (C-1″), 99.9 (C-1′), 98.0 (C-1), 89.0 (C-6), 78.3 (C-5′), 77.9 (C-3′), 74.9 (C-3″.2′), 71.6 (C-4″, 5″), 70.4 (C-4′, 2″), 62.7 (C-6′), 61.5 (C-10), 56.5 (OCH_3), 48.0 (C-9), 44.3 (C-5), 18.0 (C-6″).







3 R = H

Agababyan E. Yu., Arutyunyan L. S. and Mnatsakanyan V. A. (1987), Iridoids glycosides of *Verbascum laxum.* Chem. Nat. Compd. (Engl. Transl.) **23**, 75–79.

Magiatis P., Mitaku S., Tsitsa E., Skaltsounis A. L. and Harvala C. (1998), Verbascoside derivatives and iridoid glycosides from *Verbascum undulatum.* Nat. Prod. Lett. **12**, 111–115.

Miyase T., Akahori C., Kohsaka H. and Ueno A. (1991), Acylated iridoid glycosides from *Buddleja japonica* Hemsl. Chem. Pharm. Bull. **39**, 2944–2951.

Tutin T. G. (1972), Flora Europaea. University Press, Cambridge, UK, vol. **3**, p.461.

Skaltsounis A. L., Tsitsa-Tzardis E., Demetzos C. and Harvala C. (1996), Unduloside, a new iridoid glycoside from *Verbascum undulatum.* J. Nat. Prod. **59**, 673–675.

Vesper T. and Seifert K. (1994), Iridoids from *Verbascum nigrum.* Liebigs Ann. Chem. 751–753.

Satz und Druck: AZ Druck und Datentechnik GmbH, Kempten