Unduloside, a New Iridoid Glycoside from *Verbascum undulatum*

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A new iridoid glycoside, unduloside (**1**), was isolated from the aerial parts of *Verbascum undulatum*. Its structure was elucidated on the basis of chemical and spectral data as 6-*O*-[(2″-*O*-*trans*-feruloyl)-α-L-rhamnopyranosyl]aucubin.

Verbascum is a large genus of the family Scrophulariaceae, comprising more than 300 species. Of these, *Verbascum undulatum* Lam., which is variable in color, density of the tomentum, and extent of lobing of the leaves, grows at low altitudes in the southern part of the Balkan peninsula.[1] In the present study on the aerial parts of *V. undulatum*, a new iridoid glycoside, unduloside (**1**), was isolated together with the three known iridoids, nigroside III (**2**),[2] nigroside II (**3**),[3] and sinuatol (**4**) (Chart 1).[3,4] In addition to the iridoids, a phenylpropanoid glycoside, verbascoside,[5−7] was also isolated. All these compounds were identified by means of spectral data (UV, ^{1}H NMR, ^{13}C NMR, 2D NMR, and DCIMS or ESMS) and chemical correlations (acetylation and alkaline hydrolysis).

Compound **1** was obtained as an amorphous powder with the molecular formula $C_{31}H_{40}O_{16}$ [ESMS *m*/*z* [M + Na]$^+$ 691]. Its UV spectrum suggested the presence of an aromatic acyl moiety. The ^{1}H- (Table 1) and ^{13}C-NMR spectral data were assigned by interpretation of COSY 45, DEPT 135, HETCOR, and COLOC experiments. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of **4** indicated that **1** is a monoacyl derivative of sinuatol (6-*O*-α-L-rhamnopyranosylaucubin) (**4**).[3] From two typical transolefinic proton signals in an AM system (δ 6.53 and 7.76, J_{AM} = 16 Hz), three aromatic protons coupled in an AMX system (δ 7.33, d, J = 2 Hz; 7.20, dd, J = 9, 2 Hz; 6.92, d, J = 9 Hz), and one aromatic methoxy group (δ 3.94 ppm) in the ^{1}H-NMR spectrum, the acyl moiety was suggested to be either *trans*-feruloyl or *trans*-isoferuloyl acid, with the chemical shifts of aromatic carbon signals corresponding more with those for ferulate rather than for isoferulate.[8,9] Further information on the structure of the acyl group was obtained from the results of long-range ^{1}H−^{1}H correlations in the COSY-LR NMR spectrum (mixing time 300 ms).[10] The appearance of a four-bond correlation between the methoxy protons at 3.94 ppm and the C-2‴ aromatic proton at 7.33 ppm suggested the structure of the acyl group as ferulic acid. The site of esterification was determined to be the 2″-position of the rhamnopyranosyl moiety, inasmuch as in the ^{13}C-NMR spectrum (Table 2, cf. **1** and **4**) significant acylation-induced shifts were observed for C-1″, C-2″, and C-3″ ($\Delta\delta$ −2.69, +1.87, −1.74 ppm, respectively) and by the fact that the ^{1}H-NMR signal of H-2″ was shifted downfield (δ 5.13 ppm) in comparison with **4**.[3] Acetylation of compound **1** afforded the octaacetate **5**, which showed seven aliphatic signals and one aromatic acetyl signal in the ^{1}H-NMR spectrum (Table 1). The observed mol wt (*m*/*z* [M + NH_4]$^+$ 1022) in the DCIMS indicated an increase of 336 mass units from **1**, which also accounted for eight acetyl groups. Finally, mild alkaline hydrolysis in MeOH afforded 6-*O*-(α-L-rhamnopyranosyl)aucubin or sinuatol (**4**). In conclusion, the structure of the novel compound **1** was determined to be 6-*O*-[(2″-*O*-*trans*-feruloyl)-α-L-rhamnopyranosyl]aucubin, for which we propose the trivial name unduloside. It is interesting to point out that a previous phytochemical study of *V. undulatum* gave evidence for the presence of iridoids (derivatives of harpagoside) in the roots but not in the aerial parts.[11]

Table 1. ^{1}H-NMR Spectral Data of Compounds **1, 5**, and **6**[a]

proton(s)	**1**[b]	**5**[c]	**6**[c]
H-1	4.99 (d, 7)	5.00−5.30[d]	5.00−5.25[d]
H-3	6.44 (dd, 6, 1.5)	6.27 (dd, 6, 1)	6.24 (dd, 6, 1)
H-4	5.23 (dd, 6, 3.5)	4.95 (dd, 6, 3)	4.90 (dd, 6, 3)
H-5	2.90 (m)	2.86 (m)	2.88 (m)
H-6	4.53 (m)	4.42 (m)	4.40 (m)
H-7	5.95 (br s)	5.83 (br s)	5.94 (br s)
H-9	2.90 (m)	3.12 (m)	3.10 (m)
H-10 a	4.44 (d, 13)	4.78 (br s)	4.78 (br s)
H-10 b	4.22 (d, 13)	4.75 (br s)	4.78 (br s)
H-1′	4.74 (d, 8)	4.90 (d, 8)	4.90 (d, 8)
H-2′	3.92−3.08[d]	5.00−5.30[d]	5.00−5.25[d]
H-3′	3.92−3.08[d]	5.00−5.30[d]	5.00−5.25[d]
H-4′	3.92−3.08[d]	5.00−5.30[d]	5.00−5.25[d]
H-5′	3.92−3.08[d]	3.73 (m)	3.70 (m)
H-6a′	3.92−3.08[d]	4.31 (dd, 12, 4)	4.29 (dd, 12, 4)
H-6′b	4.11 (dd, 12, 1.5)	4.19 (dd,12, 1.5)	4.17 (dd, 12, 1.5)
H-1″	4.95 (d, 1.5)	5.00−5.30[d]	5.00−5.25[d]
H-2″	5.13 (dd, 3.5, 1.5)	5.39 (dd, 3.5,1.5)	5.37 (dd, 3.5,1.5)
H-3″	3.92−3.08[d]	5.00−5.30[d]	5.00−5.25[d]
H-4″	3.92−3.08[d]	5.00−5.30[d]	5.00−5.25[d]
H-5″	3.92−3.08[d]	3.97 (m)	3.96 (m)
H-6″	1.38 (d, 6)	1.15 (d, 6)	1.25 (d, 6)
H-2‴	7.33 (d, 2)	7.25 (d, 2)	7.62 (d, 9)
H-3‴			7.13 (d, 9)
H-5″	6.92 (d, 9)	7.21 (d, 9)	7.13 (d, 9)
H-6‴	7.20 (dd, 9, 2)	7.11 (dd, 9, 2)	7.62 (d, 9)
H-7‴	7.76 (d, 16)	7.79 (d, 16)	7.75 (d, 16)
H-8‴	6.53 (d, 16)	6.58 (d, 16)	6.56 (d, 16)
−OMe	3.94 (s)	3.95 (s)	
MeCO		2.34[e]	2.35[f]

[a] Multiplicities and coupling constants (in Hz) are in parentheses. [b] Recorded in CD_3OD. [c] Recorded in $CDCl_3$. [d] Signal patterns unclear due to overlapping. [e] Compound **5** exhibited seven additional aliphatic acetyl signals at δ 2.15−1.99. [f] Compound **6** exhibited seven additional aliphatic acetyl signals at δ 2.10−1.95.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were determined in spectroscopic grade MeOH on a Shimadzu-160A spectrophotometer. NMR spectra were obtained with a Bruker AC 200

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Chart 1

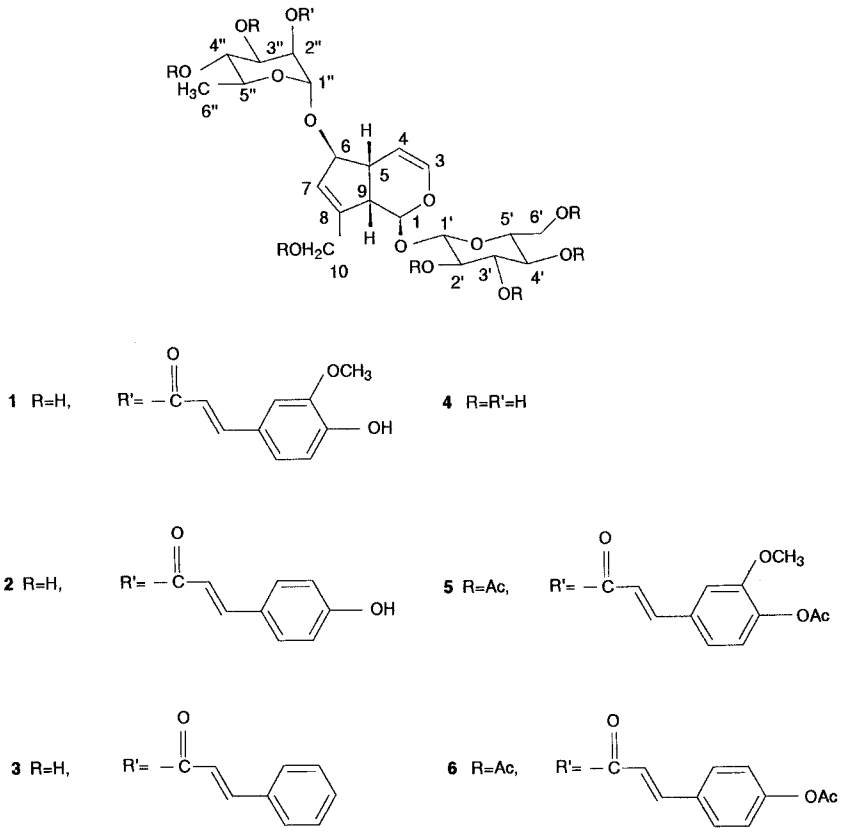

Table 2. ^{13}C-NMR Data for Compounds **1** and **4**[a] (CD_3OD)

carbon	**1**	**4**
1	98.01	98.03
3	142.00	141.76
4	105.45	105.48
5	44.30	44.36
6	89.23	88.85
7	127.04	127.07
8	149.35	149.22
9	48.40	[b]
10	61.48	61.42
1′	99.95	99.89
2′	74.87	74.85
3′	78.23	78.19
4′	71.50	71.50
5′	77.85	77.83
6′	62.62	62.59
1″	98.38	101.07
2″	74.34	72.47
3″	70.49	72.23
4″	74.32	73.94
5″	70.29	70.13
6″	18.14	18.00
1‴	129.27	
2‴	111.68	
3‴	149.35	
4‴	150.81	
5‴	116.34	
6‴	124.36	
7‴	147.35	
8‴	115.74	
9‴	166.59	
OCH_3	56.48	
OCO	168.69	

[a] Data taken from Seifert *et al.*[3] [b] Overlapping with CD_3OD.

spectrometer [^{1}H (200 MHz) and ^{13}C (50 MHz)], and TMS was the internal standard. Chemical shifts are reported in δ (ppm) values. DICMS (using NH_3 as reagent gas) and ESMS were recorded with a Nermag R 10-10C spectrometer. Column chromatography was performed on columns of Si gel [Merck 0.04−0.06 mm (flash) and 0.015−0.04 mm].

Plant Material. The plant material was collected in August 1993, from Attiki, Greece, and was identified by Dr. T. Constandinidis, Department of Botany, University of Patras, Greece. A voucher specimen (no. 275) is deposited in the herbarium of the Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens.

Extraction and 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), a portion of which (5 g) was subjected to vacuum−liquid chromatography on Si gel (0.015−0.04 mm). Elution with a CH_2Cl_2−MeOH gradient yielded 10 fractions. Fractions 3−4 were rechromatographed (EtOAc−MeOH gradient, Si gel 0.015−0.04 mm) to afford unduloside (**1**) (60 mg) and nigroside III (**2**) (10 mg). Fractions 7−8 were rechromatographed with a EtOAc−MeOH gradient (Si gel 0.015−0.04 mm) to afford nigroside II (**3**) (30 mg) and verbascoside (150 mg). Fraction 10 was further purified by flash chromatography (EtOAc−MeOH gradient) to yield sinuatol (**4**) (8 mg).

Unduloside (1): white amorphous powder; $[\alpha]_D$ −61.6° (*c* 0.63, MeOH); UV λ max (log ϵ) (MeOH) 300 (4.05), 312 (4.20) nm; ^{1}H-NMR data, see Table 1; ^{13}C-NMR data, see Table 2; ESMS *m*/*z* $[M + Na]^+$ 691, $[M + K]^+$ 707.

Nigroside III (2): white amorphous powder; [α]D −150° (*c* 0.5, MeOH); UV λ max (log ϵ) (MeOH) 299 (4.05) nm; ^{1}H- and ^{13}C-NMR data, comparable with literature values;[2] ESMS *m*/*z* [M + Na] 661.

Acetylation of 1. Treatment of **1** (10 mg) with Ac_2O (1 mL) and pyridine (1 mL) at room temperature overnight followed by flash column chromatography (hexane−EtOAc 70:30) gave the octaacetate **5** (90%): ^{1}H-NMR data, see Table 1; DCIMS *m/z* $[M + NH_4]^+$ 1022, 331.

Alkaline Hydrolysis of 1. A solution of **1** (10 mg) in MeONa (0.1 M, 2 mL) was kept at room temperature for 3 h. The mixture was neutralized with AcOH and filtered. The filtrate was evaporated to dryness *in vacuo*. Flash column chromatography (EtOAc−MeOH gradient) afforded sinuatol (**4**) (85%).

Acetylation of 2. Treatment of **2** (10 mg) with Ac_2O (1 mL) and pyridine (1mL) at room temperature overnight followed by flash column chromatography (hexane-EtOAc, 70:30) gave the octaacetate **6** (90%): ^{1}H-NMR data, see Table 1; DCIMS *m/z* $[M + NH_4]^+$ 992, 331.

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