Verbaspinoside, a New Iridoid Glycoside from *Verbascum spinosum*[1]

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A new iridoid glycoside, verbaspinoside (**1**), was isolated from the aerial parts of *Verbascum spinosum*. Its structure was elucidated on the basis of chemical and spectral data as 6-*O*-[(2″-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosyl]-catalpol. Additionally, three known iridoids (aucubin, catalpol, and ajugol) and three phenylpropanoid glycosides [acteoside, angoroside A (**2**), and angoroside C (**3**)] were isolated and identified.

In a continuation of phytochemical studies on plants from Crete, the aerial parts of the endemic species *Verbascum spinosum* Lin. (Scrophulariaceae) were investigated. This plant differs from the other species belonging to the genus *Verbascum*, which are all herbs, in that it is a freely branched shrub up to 50 cm tall, with the branches ending in a spine.[2]

In the present study on *V. spinosum*, a new iridoid glycoside, verbaspinoside (**1**), was isolated, together with the three known iridoids, catalpol,[3,4] aucubin,[5,6] and ajugol.[7] In addition to the iridoids, three phenylpropanoid glycosides, acteoside,[8−10] angoroside A (**2**),[11] and angoroside C (**3**),[12,13] were also isolated. All these compounds were identified by means of spectral data (UV, ^{1}H NMR, ^{13}C NMR, 2D NMR, and ESMS) and chemical correlations. On the basis of these spectral data, some previous ^{1}H NMR and ^{13}C NMR assignments of angorosides A (**2**) and C (**3**) have been revised.

Compound **1** was obtained as an amorphous powder with the molecular formula $C_{30}H_{38}O_{15}$ (ESMS *m/z* $[M + Na]^+$ 661). Its UV spectrum suggested the presence of an aromatic acyl moiety and an iridoid enol ether system. The ^{1}H NMR spectrum (Table 1) showed the signals of five aromatic protons (δ 7.40−7.65) and two trans olefinic protons arising from a *trans*-cinnamoyl moiety (δ 6.61 and 7.75, AB system, J_{AB} = 16.2 Hz). Moreover, it showed all the typical protons of a catalpol diglycoside, with two characteristic anomeric protons appearing at δ 5.05 (d, J = 1.7 Hz) and 4.78 ppm (d, J = 7.9 Hz), indicating α-rhamnopyranose and β-glucopyranose to be sugar moieties. The ^{13}C NMR spectrum (Table 1) suggested also the presence of a cinnamoyl group, an α-rhamnopyranosyl unit, and a catalpol moiety. The location of the α-rhamnopyranosyl group was determined to be at the C-6 position in the catalpol unit, from the HMBC spectrum. The site of esterification by the *trans*-cinnamoyl group was determined to be the C-2″ position of the rhamnopyranosyl moiety, because the ^{1}H NMR signal of H-2″ was shifted downfield (δ 5.18) in comparison with 6-*O*-(α-L-rhamnopyranosyl)-catalpol.[14] The site of esterification was confirmed as the C-2″ position of the rhamnose unit from the HMBC spectrum, where a long-range coupling was observed between the signal at δ_C 168.0 (carbonyl of *trans*-cinnamoyl group) and the signal at δ_H 5.18 (H-2″). Acetylation of compound **1** afforded the heptaacetate (**4**), which showed seven aliphatic acetyl signals in the ^{1}H NMR spectrum.

Table 1. ^{1}H and ^{13}C NMR Data and COSY and HMBC Correlation of **1** in MeOD

position	δ ^{1}H (m, J in Hz)	δ ^{13}C	COSY	HMBC
1	5.09 (d, 9.9)	95.1	H-9	H-1′/H-3/H-5/H-9
3	6.38 (dd, 6.0, 1.7)	142.3	H-4	H-1/H-5
4	5.08 (dd, 6.0, 4.6)	103.4	H-3/H-5	H-6/H-5/H-3
5	2.44 (m)	37.2	H-9/H-6/H-4	H-1/H-3/H-7/H-9/H-6
6	4.04 (d, 8.3)	84.3	H-5	H-1″/H-5/H-9
7	3.66 (s)	59.4		H-10a/H-9/H-6
8		66.5		H-1/H-10a/H-10b/H-9
9	2.58 (dd, 9.9, 7.9)	43.2	H-1/H-5	H-4/H-6/H-7/H-1/H-5/H-10b
10a	4.16 (d, 13.3)	61.5	H-10b	H-7
10b	3.81 (d, 13.3)		H-10a	
1′	4.78 (d, 7.9)	99.7	H-2′	H-1
2′	3.27 (dd, 9.1, 7.9)	74.8	H-1′/H-3′	H-3′
3′	3.41 (t, 9.1)	77.6	H-2′	H-2′
4′	3.25 (t, 9.1)	71.8		H-5′/H-3′
5′	3.32[a]	78.6	H-6′a/H-6′b	H-4′/H-1′
6′a	3.62 (dd, 12.0, 6.6)	62.9	H-6′b/H-5′	H-4′/H-5′
6′b	3.89 (dd, 12.0, 2.0)		H-6′a/H-5′	
1″	5.05 (d, 1.7)	97.6	H-2‴	H-6
2″	5.18 (dd, 3.3, 1.7)	74.4	H-1‴/H-3‴	H-1″/H-3″
3″	3.95 (dd, 9.9, 3.3)	70.4	H-2‴/H-4‴	
4″	3.50 (t, 9.9)	74.2	H-3‴/H-5‴	H-2″/H-6″/H-3″
5″	3.77 (m)	70.3	H-4‴/H-6‴	
6″	1.32 (d, 6.2)	18.0	H-5″	H-4″/H-5″
1‴		135.7		H-3‴/H-5‴/H-8‴
2‴	7.61−7.65[b]	129.3	H-3‴	H-4‴/H-7‴
3‴	7.40−7.44[b]	130.0	H-2‴	
4‴	7.40−7.44[b]	131.6		H-2‴/H-6‴
5‴	7.40−7.44[b]	130.0	H-6‴	
6‴	7.61−7.65[b]	129.3	H-5‴	H-7‴/H-4‴
7‴	7.75 (d, 16.2)	146.9	H-8‴	H-2‴/H-6‴
8‴	6.61 (d, 16.2)	118.6	H-7‴	H-7‴
9‴		168.0		H-2″/H-8‴/H-7‴

[a] Overlapping with CD_3OD. [b] Signal patterns unclear due to overlapping.

The observed molecular weight (*m/z* $[M + Na^+]$ 955) in the ESMS indicated an increase of 294 mass units compared with **1**, which accounted for seven acetyl groups. Mild alkaline hydrolysis of **1** afforded the known compound 6-*O*-(α-L-rhamnopyranosyl)-catalpol.[14] Accordingly, the structure of the new compound **1** was determined to be 6-*O*-[(2″-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosyl)-catalpol, for which we proposed the trivial name verbaspinoside.

To date, several acylated 6-*O*-(α-L-rhamnopyranosyl)-catalpol derivatives have been reported from plants belonging to the family Scrophulariaceae, mainly from various *Scrophularia* species[15] and from *Verbascum sinuatum*,[16] *V. saccatum*,[17] *V. laxum*,[18] *V. thapsus*,[19] and *V. pulverulentum*.[20] It is interesting to point out that angorosides A (**2**)

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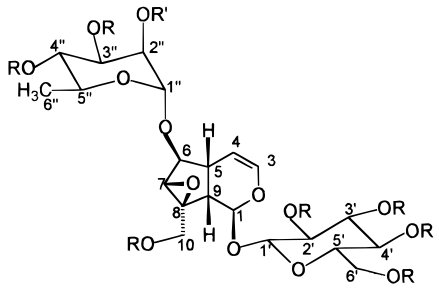
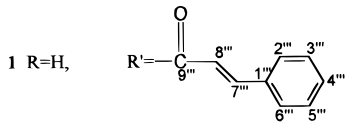
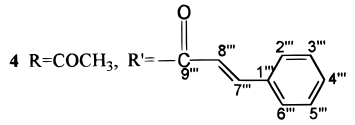
and C (**3**) have been previously reported only from plants belonging to the genus *Scrophularia*.

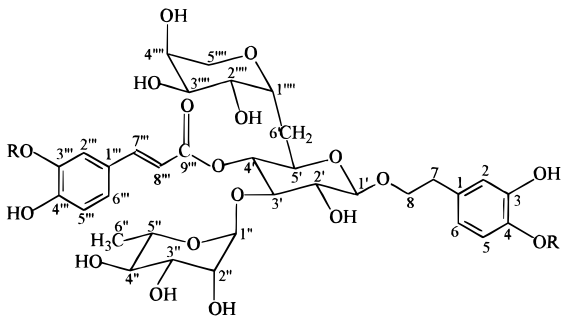




1 R=H,



4 R=COCH$_3$,



2 R=H

3 R=CH$_3$

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin−Elmer 341 polarimeter. UV spectra were determined in spectroscopic grade MeOH on a Shimadzu-160A spectrophotometer. NMR spectra were obtained with a Bruker AC200 spectrometer and a Bruker DRX400 spectrometer. Chemical shifts are given in δ values with TMS as internal standard. The 2D experiments (COSY, COSY LR, TOCSY, HMQC, TOCSY−HMQC, and HMBC) were performed using standard Bruker microprograms. ESMS was recorded with a Nermag R 10−10C spectrometer. Column chromatography was conducted using Si gel [Merck, 0.04−0.06 mm (flash) and 0.015−0.04 mm], with an applied pressure of 300 mbar. MPLC was performed with a Büchi model 688 apparatus on columns containing Si gel RP-18 (Merck, 0.02−0.04 mm).

Plant Material. Aerial parts was collected in June 1996, in mountain Lefka Ori, Crete (Greece). A voucher specimen (no. LK01) 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. spinosum* (1 kg) were first defatted with CH_2Cl_2 and then extracted with MeOH (2.5 L × 3). The MeOH-soluble extract was evaporated under reduced pressure to give a residue (100 g), a portion of which (15 g) was subjected to vacuum−liquid chromatography on Si gel (0.015−0.04 mm). Elution with a CH_2Cl_2−MeOH gradient yielded 9 fractions. Fraction 5 (1.7 g) was submitted to MPLC on RP-18. Elution with H_2O gave catalpol (20 mg), aucubin (44 mg), and ajugol (40 mg), and elution with H_2O−MeOH (70:30) gave acteoside (8 mg), verbaspinoside (**1**, 428 mg), and angoroside C (**3**, 55 mg). Angoroside A (**2**, 195 mg) was isolated by the same procedure from fraction 7 (865 mg) with H_2O−MeOH 70:30.

Verbaspinoside (1): white amorphous powder; $[\alpha]^{20}_D$ −110.9° (*c* 0.72, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.10), 222 (4.05), 280 (4.16) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 1; 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 (CH_2Cl_2−Me_2CO 97:3) gave the heptaacetate **4** (92%); $[\alpha]^{20}_D$ −57.4° (*c* 0.62, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ϵ) 229 (3.70), 282 (4.33) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (1H, d, *J* = 16.2 Hz, H-7‴), 7.58−7.53 (2H, m, H-2‴, H-6‴), 7.41−7.37 (3H, m, H-3‴, H-4‴, H-5‴), 6.53 (1H, d, *J* = 16.2 Hz, H-8‴), 6.30 (1H, dd, *J* = 6.0, 1.7 Hz, H-3), 5.41 (1H, dd, *J* = 3.3, 1.7 Hz, H-2″), 5.36 (1H, dd, *J* = 3.3, 9.9 Hz, H-3″), 5.20 (1H, t, *J* = 9.5 Hz, H-3), 5.17−5.10 (2H, m, H-4″, H-4′), 5.06 (1H, dd, *J* = 6.0, 4.6 Hz, H-4), 5.00 (1H, d, *J* = 1.7 Hz, H-1″), 4.97−4.91 (2H, m, H-1′, H-2′), 4.80 (1H, d, *J* = 13.1 Hz, H-10a), 4.74 (1H, d, *J* = 9.9 Hz, H-1), 4.31 (1H, dd, *J* = 12.4, 2.2 Hz, H-6′a), 4.13 (1H, dd, *J* = 12.4, 3.3 Hz, H-6′b), 3.96 (1H, m, H-5″), 3.95 (1H, d, *J* = 13.1 Hz, H-10b), 3.92 (1H, d, *J* = 8.3 Hz, H-6), 3.66 (1H, m, H-5′), 3.56 (1H, s, H-7), 2.61 (1H, dd, *J* = 9.9, 7.9 Hz, H-9), 2.49 (1H, m, H-5), 1.22 (3H, d, *J* = 6.2 Hz, H-6″); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 166.0 (C-9‴), 146.3 (C-7‴), 141.1 (C-3), 134,0 (C-1‴), 130.7 (C-4‴), 128.9 (C-3‴/C-5‴), 128.3 (C-2‴/C-6‴), 117.0 (C-8‴), 102.4 (C-4), 96.6 (C-1″), 96.5 (C-1′), 94.2 (C-1), 83.4 (C-6), 72.5 (C-3′), 72.2 (C-5′), 71.1 (C-4″), 70.5 (C-2′), 69.9 (C-2″), 68.8 (C-3″), 68.2 (C-4′), 66.8 (C-5″), 62.3 (C-8), 62.1 (C-10), 61.0 (C-5′), 58.0 (C-7), 41.6 (C-9), 35.4 (C-5), 17.4 (C-6″); ESMS *m*/*z* [M + Na]$^+$ 955.

Alkaline Hydrolysis of 1. A solution of **1** (20 mg) in 5% methanolic KOH (3 mL) was kept at room temperature for 2 h. The mixture was neutralized with 2% HCl and filtered. The filtrate was evaporated to dryness in vacuo, and the residue, 6-*O*-(α-L-rhamnopyranosyl)-catalpol (81%), was identified by ^{1}H NMR and ^{13}C NMR data; $[\alpha]^{20}_D$ −123.1° (*c* 0.54, MeOH).

Angoroside A (2): ^{1}H NMR (MeOD, 400 MHz) δ 7.62 (1H, d, *J* = 16.1 Hz, H-7‴), 7.08 (1H, d, *J* = 1.4 Hz, H-2‴), 6.98 (1H, dd, *J* = 8.3, 1.4 Hz, H-6‴), 6.81 (1H, d, *J* = 8.3 Hz, H-5‴), 6.74 (1H, d, *J* = 2.0 Hz, H-2), 6.70 (1H, d, *J* = 8.3 Hz, H-5), 6.59 (1H, dd, *J* = 8.3, 2.0 Hz, H-6), 6.30 (1H, d, *J* = 16.1 Hz, H-8‴), 5.20 (1H, d, *J* = 1.5 Hz, H-1″), 5.00 (1H, t, *J* = 9.8 Hz, H-4′), 4.39 (1H, d, *J* = 7.8 Hz, H-1′), 4.24 (1H, d, *J* = 6.8 Hz, H-1⁗), 4.04 (1H, m, H-8b), 3.95 (1H, br, H-2″), 3.88 (1H, dd, *J* = 11.2, 1.4 Hz, H-6b′), 3.85−3.76 (4H, m, H-3′, H-5′, H-4⁗, H-5b⁗), 3.74 (1H, m, H-8a), 3.62−3.55 (4H, m, H-6a′, H-3″, H-5″, H-2⁗), 3.50 (1H, dd, *J* = 9.5, 3.3 Hz, H-3⁗), 3.46 (1H, br, H-5a⁗), 3.41 (1H, dd, *J* = 8.8, 7.8 Hz, H-2′), 3.30 (1H, t, *J* = 9.8 Hz, H-4″), 2.80 (2H, t, *J* = 7.3 Hz, H-7), 1.11 (3H, d, *J* = 6.3 Hz, H-6″); ^{13}C NMR (MeOD, 50 MHz) δ 168.3 (C-9‴), 149.8 (C-4‴), 148.3 (C-7‴), 146.7 (C-3‴), 146.0 (C-3), 144.6 (C-4), 131.5 (C-1), 127.6 (C-1‴), 123.3 (C-6‴), 121.3 (C-6), 117.1 (C-5), 116.5 (C-2), 116.3 (C-5‴), 115.2 (C-2‴), 114.6 (C-8‴), 104.9 (C-1⁗), 104.0 (C-1′), 103.0 (C-1″), 81.6 (C-3′), 76.0 (C-2′), 74.8 (C-5′), 74.0 (C-3⁗), 73.7 (C-4″), 72.4 (C-2″, C-3″), 72.3 (C-8), 72.0 (C-2⁗), 70.4 (C-4′, C-5″), 69.4 (C-4⁗), 68.9 (C-6′), 66.8 (C-5⁗), 36.5 (C-7), 18.4 (C-6″).

Angoroside C (3): ^{1}H NMR (MeOD, 400 MHz) δ 7.68 (1H, d, *J* = 15.9 Hz, H-7‴), 7.23 (1H, d, *J* = 1.8 Hz, H-2‴), 7.11 (1H, dd, *J* = 8.5, 1.8 Hz, H-6‴), 6.85 (1H, d, *J* = 7.9 Hz, H-5), 6.83 (1H, d, *J* = 8.5 Hz, H-5‴), 6.77 (1H, d, *J* = 2.2 Hz, H-2), 6.72 (1H, dd, *J* = 7.9, 2.2 Hz, H-6), 6.39 (1H, d, *J* = 15.9 Hz, H-8‴), 5.21 (1H, d, *J* = 1.7 Hz, H-1″), 5.00 (1H, t, *J* = 9.8 Hz, H-4′), 4.41 (1H, d, *J* = 7.9 Hz, H-1′), 4.26 (1H, d, *J* = 6.7 Hz, H-1⁗), 4.08 (1H, m, H-8b), 3.94 (1H, dd, *J* = 3.3, 1.8 Hz, H-2″), 3.91 (3H, s, MeO-3‴), 3.87 (1H, dd, *J* = 11.5, 1.7 Hz, H-6b′), 3.84 (3H, s, MeO-4), 3.84−3.74 (5H, m, H-8a, H-3′, H-5′, H-4⁗, H-5b⁗), 3.63−3.56 (4H, m, H-6a′, H-3″, H-5″, H-2⁗), 3.51 (1H, dd, *J* = 9.1, 3.3 Hz, H-3⁗), 3.47 (1H, br, H-5a⁗), 3.42 (1H, dd, *J* = 9.0, 7.9 Hz, H-2′), 3.31 (1H, t, *J* = 9.8 Hz, H-4″), 2.85 (2H, t, *J* = 7.3 Hz, H-7), 1.12 (3H, d, *J* = 6.1 Hz, H-6″); ^{13}C

NMR (MeOD, 50 MHz) δ 168.3 (C-9‴), 150.2 (C-4‴), 149.4 (C-3‴), 148.2 (C-7‴), 147.7 (C-3), 147.5 (C-4), 133.0 (C-1), 127.5 (C-1‴), 124.5 (C-6‴), 121.2 (C-6), 117.1 (C-2), 116.5 (C-5‴), 114.9 (C-8‴), 112.8 (C-5), 111.7 (C-2‴), 105.1 (C-1‴), 104.5 (C-1′), 103.0 (C-1″), 81.5 (C-3′), 76.2 (C-2′), 75.0 (C-5′), 74.1 (C-3‴), 73.7 (C-4″), 72.4 (C-2″), 72.2 (C-3″, C-2‴), 72.0 (C-8), 70.5 (C-4′, C-5″), 69.5 (C-4‴), 69.0 (C-6′), 66.8 (C-5‴), 56.42 (MeO-3‴, MeO-4), 36.6 (C-7), 18.4 (C-6″).

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References and Notes

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