A New Iridoid from *Scrophularia auriculata* ssp. *pseudoauriculata*

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Received February 26, 1998

A new iridoid glycoside, scrovalentinoside (**1**), was isolated from the MeOH extract of the aerial parts of *Scrophularia auriculata* L. ssp. *pseudoauriculata*. The structure of the new compound **1** was elucidated as 6-*O*-(2″,3″-di-*O*-acetyl-4″-*O*-*p*-methoxy-cinnamoyl)-α-L-rhamnopyranosyl catalpol by spectroscopic methods. The known iridoid glycoside, scropolioside A; two saponins, verbascosaponin A and verbascosaponin; and the phenylethanoid glycoside, verbascoside, were also isolated.

The genus *Scrophularia* (Scrophulariaceae) is represented by 30 species in the European flora.[1] It is well known for its variety of iridoids, which are of value for taxonomic evaluation of this genus.[2,3] Other phytochemical studies of Asian species[4−6] have revealed the presence of phenolics[3] and saponins, most of them oleanane triterpene glycosides.

Some *Scrophularia* species, such as *S. ningpoensis*, have been used traditionally in Chinese folk medicine, for treatment of inflammation.[2] As a part of our study of Iberian plants we had isolated from *Scrophularia auriculata* L. ssp. *pseudoauriculata* (Senn.) Bolòs et Vigo (*S. valentina* Rouy),[7] a species used in inflammatory dermal diseases,[8] two new antiedematous catalpol rhamnosides.[9] We report here on the isolation and structure elucidation of further constituents of this plant.

Air-dried and powdered aerial parts of *S. auriculata* L. ssp. *pseudoauriculata* were macerated with MeOH, and the resulting extract was tested against tetradecanoylphorbol acetate (TPA)-induced ear edema showing a 65% inhibition. Liquid−liquid partition of this extract with Cl_2CH_2 and further gel filtration on Sephadex and RP-8 Lobar chromatography yielded a new iridoid glycoside, scrovalentinoside (**1**), together with a known iridoid glycoside, scropolioside A;[10] two known saponins, verbascosaponin A and verbascosaponin;[11] and the phenylethanoid glycoside, verbascoside.[9] Verbascosaponin A is an artifact formed during the extraction process because it was not present in the extract when the fresh aerial parts of this species were immediately macerated with EtOH at room temperature.

Scrovalentinoside (**1**) presented a quasimolecular ion in the FABMS at *m/z* 775 $[M + Na - H]^+$, suggesting the molecular formula $C_{35}H_{44}O_{18}$. Another peak at *m/z* 391 indicated the presence of a diacetyl-methoxycinnamoyl-deoxyhexose residue. The ^1H NMR spectrum of **1** displayed a signal pattern similar to that of scropolioside D, previously identified in *S. ilwensis*.[3] It showed one methoxy group (δ 3.64), two aromatic signals corresponding to two equivalent protons each (δ 6.84 and 7.40, d, $J = 8.8$ Hz), and two olefinic protons (δ 6.36 and 7.75, d, $J = 16$ Hz) arising from a *p*-methoxy-*trans*-cinnamoyl moiety. Additionally, two acetyl groups (δ 1.84 and 2.02) were observed as well as a doublet at δ 1.27 (the methyl of a deoxyhexose) and two signals for anomeric protons at δ 5.10 (d, $J = 1.7$ Hz) and 4.89 (d, $J = 7.9$ Hz), indicating the presence of α-L-rhamnose and β-D-glucose residues, according to data reported by Çalis et al.[3] This was confirmed from analysis of the ^{13}C NMR spectrum, which exhibited 35 carbon signals, nine corresponding to the aglycon, 12 for two sugar moieties, nine for a *trans*-cinnamoyl residue, one methoxy group (δ 55.8), and two acetyl groups (δ 171.6 and 20.8, δ 171.6 and 20.8, respectively). Full assignments of the ^1H and ^{13}C NMR signals were accomplished using HMBC, HMQC, $^1H-^1H$ COSY, and NOESY experiments (Table 1).

The HMQC sequence (H−C direct correlation) established the connectivities between C-2″ (δ 71.4) and H-2″ (δ 5.45), C-3″ (δ 70.6) and H-3″ (δ 5.57), and C-4″ (δ 72.1) and H-4″ (δ 5.38). In the HMBC, long-range connectivities ^3J were observed between *C*OCH$_3$ (δ 171.6) and H-2″ (δ 5.45), *C*OCH$_3$ (δ 171.6) and H-3″ (δ 5.57), and *C*O (δ 167.9) of the *trans*-cinnamoyl group

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S0163-3864(98)00067-6 CCC: $15.00
Published on Web 07/31/1998

Table 1. ^{13}C and ^{1}H NMR Spectral Data of Scrovalentinoside (**1**) ($CD_3OD-C_6D_6$ 9:1; 400 MHz)

	compound **1**	
atom no.	^{13}C	^{1}H
1	95.2 (CH)	5.14 (1H, d, J = 9.6 Hz)
3	142.4 (CH)	6.33 (1H, dd, J = 6.0, 1.7 Hz)
4	103.2 (CH)	5.07 (1H, dd, J = 6.0, 4.6 Hz)
5	37.1 (CH)	2.55 (1H, dddd, J = 8.1, 7.3, 4.6, 1.7 Hz)
6	85.1 (CH)	4.03 (1H, dd, J = 8.1, 0.8 Hz)
7	59.5 (CH)	3.61 (1H, s)
8	66.5 (C)	
9	43.3 (CH)	2.68 (1H, dd, J = 9.6, 7.3 Hz)
10	61.6 (CH_2)	4.24 (1Ha, d, J = 13.2 Hz)
		3.90 (1Hb, d, J = 13.2 Hz)
1′	99.8 (CH)	4.89 (1H, d, J = 7.9 Hz)
2′	74.8 (CH)	3.43 (1H, m)
3′	77.7 (CH)	3.55 (1H, t, J = 8.7 Hz)
4′	71.7 (CH)	3.43 (1H, m)
5′	78.5 (CH)	3.40 (1H, m)
6′	62.9 (CH_2)	3.75 (1Ha, dd, J = 12.9, 1.7 Hz)
		3.99 (1Hb, dd, J = 12.9, 5.8 Hz)
1″	97.8 (CH)	5.10 (1H, d, J = 1.7 Hz)
2″	71.4 (CH)	5.45 (1H, dd, J = 3.4, 1.7 Hz)
3″	70.6 (CH)	5.57 (1H, dd, J = 10.1, 3.4 Hz)
4″	72.1 (CH)	5.38 (1H, t, J = 10.1 Hz)
5″	68.4 (CH)	4.17 (1H, dq, J = 10.1, 6.3 Hz)
6″	18.0 (CH_3)	1.27 (3H, d, J = 6.3 Hz)
1‴	127.9 (C)	
2‴	131.2 (CH)	7.40 (1H, d, J = 8.8 Hz)
3‴	115.5 (CH)	6.84 (1H, d, J = 8.8 Hz)
4‴	163.3 (C)	
5‴	115.5 (CH)	6.84 (1H, d, J = 8.8 Hz)
6‴	131.2 (CH)	7.40 (1H, d, J = 8.8 Hz)
OCH_3	55.8 (CH_3)	3.64 (3H, s)
CH-α	115.1 (CH)	6.36 (1H, d, J = 16.0 Hz)
CH-*β*	147.4 (CH)	7.75 (1H, d, J = 16.0 Hz)
CO	167.9	
$OCOCH_3$	171.6 (C)	
	171.6 (C)	
$OCOCH_3$	20.8 (CH_3)	2.02 (3H, s)
	20.8 (CH_3)	1.84 (3H, s)

and H-4″ (*δ* 72.1), indicating clearly the locations of the two acetoxy and one *trans*-cinnamoyl moieties. Consequently, the structure of **1** was elucidated to be 6-*O*-(2″,3″-di-*O*-acetyl-4″-*O*-*p*-methoxy-cinnamoyl)-α-L-rhamnopyranosyl catalpol, a new natural compound for which the name scrovalentinoside is now proposed. Verbascosaponin and verbascosaponin A, previously identified in *Verbascum phlomoides*,[11] are also described for the first time in the genus *Scrophularia*.

Since the simplest rhamnopyranosylcatalpol was first isolated from *S. nodosa*,[12] many similar substances, mainly di- or tri-acyl derivatives,[3,10,13] have been reported in the genus. By way of contrast, mono-acyl-rhamnopyranosyl catalpols are found in *Verbascum*[10] and *Premna*[14,15] (Scrophulariaceae).

Experimental Section

General Experimental Procedures. NMR spectra were run on a 400 MHz (*δ*, ppm) (Bruker AMX) instrument in CD_3OD/C_6D_6 (9:1). FABMS were carried out in a VG Auto Spec (Fisons). IR spectra were recorded as KBr disks in a Perkin−Elmer 843 spectrophotometer. Optical rotations were measured with a Perkin−Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV-2101PC spectrophotometer. Analytical TLC was carried out on Merck Si gel F_{254} aluminum sheets and Merck RP-8 plates visualized with 1% sulfuric acid−anisaldehyde.

Plant Material. Aerial parts of *S. auriculata* L. ssp. *pseudoauriculata* were collected during the flowering season (May 1996) in Polinyà de Xúquer (Valencia, Spain). A voucher specimen no. VF06744 has been deposited in the herbarium of the Faculty of Pharmacy (Valencia, Spain).

Extraction and Isolation. Air-dried and powdered aerial parts of *S. auriculata* (1 kg) were extracted with MeOH at room temperature. The MeOH extract (69 g) was evaporated under reduced pressure, dissolved in H_2O, and fractionated with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2 extract (2 g) was subjected to gel filtration over Sephadex LH-20 and eluted with MeOH. Further purification of the saponin-rich fractions 3−5 (480 mg), by a Lobar LiChroprep RP-8 (Merck) column with MeOH−H_2O (7:3), yielded verbascosaponin A (50 mg)[11] and verbascosaponin[11] (104 mg). The following two fractions (6 and 7, 900 mg) contained a mixture of iridoids that were separated by a Lobar RP-8 column with MeOH−H_2O (6.5:3.5) and yielded compound **1** (166 mg) and scropolioside A (158 mg)[10] Fraction 8 yielded verbascoside (38 mg).[9] The known compounds were identified by comparison of $[\alpha]_D$ and spectral data with those reported in the literature.

Scrovalentinoside (1): amorphous powder, $[\alpha]_D$ −136° (CH_3OH; *c* 0.1); UV λ_{max} (MeOH) 203, 228, 313 nm; IR (KBr) ν_{max} 3400 (O−H), 1715 (C═O), 1640 (C═C) cm^{-1}; FABMS *m/z* $[M + Na - H]^+$ 775, $[M + H]^+$ 753, [diacetyl-*p*-methoxycinnamoyl-rhamnosyl]$^+$ 391, [cinnamoyl]$^+$ 131.

Acknowledgment. This work was supported by the Secretaría de Estado de Universidades e Investigación of the Spanish Government (grant PM95-0150).

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NP980067O