Minor Diterpenoids from *Scutellaria polyodon*

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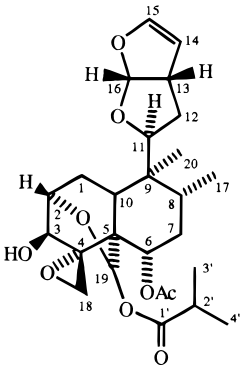
Four minor neoclerodane diterpene constituents were isolated from the aerial parts of *Scutellaria polyodon*. These compounds were characterized as the new scupolins J (**1**) and K (**2**) and the previously known scutalpin O (**3**) and scutalsin.

The genus *Scutellaria*, a good source of neoclerodane diterpenoids,[1] is known to be rich in derivatives showing potent antifeedant activity against pest insects.[2−6] Recently we reported the isolation of nine neoclerodane diterpenoids (scupolins A−I) from the acetone extract of the aerial parts of *Scutellaria polyodon* Juz. (Labiatae), and two similar known derivatives found in other *Scutellaria* species.[7] On extraction of a larger amount of this same plant material, we report herein the isolation of four minor diterpenoid constituents of *S. polyodon*, the new scupolins J (**1**) and K (**2**), and the already known scutalpin O (**3**) (from *S. alpina*)[8] and scutalsin (from *S. altissima*).[9]

Scupolin J (**1**), molecular formula $C_{26}H_{36}O_9$, showed in its IR spectrum absorptions for hydroxyl (3400 cm^{-1}) and ester (1730 and 1260 cm^{-1}) groups. Its ^1H NMR spectrum showed signals corresponding to a tetrahydrofurofuran moiety involving the C-11/C-16 carbons of a neoclerodane framework (H-11α, dd, δ 4.00; H-13β, m, δ 3.53; H-14, dd, δ 4.82; H-15, dd, δ 6.46; H-16β, d, δ 6.01), a 6α-acetoxyl proton (δ 4.68, dd), a 4α,18-oxirane (δ 2.88 and 3.08), and a 19,2α-hemiacetal function (δ 6.68, s) esterified with an isobutyryl group. In addition, scupolin J possessed a secondary hydroxyl group (δ_H 3.97, m, δ_C 71.0, d) that had to be placed in the 3β-axial position. Both ^1H and ^{13}C NMR spectra of scupolin J were similar to those of scupolin G,[7] with the only remarkable difference being the presence in **1** of a C-14,C-15 double bond instead of the saturated system occurring in scupolin G. Indeed, both diterpenes showed an almost identical pattern of values for the C-1/C-7 and C-10, C-18, C-19 carbons. Therefore, the structure of scupolin J is represented by **1**.

The ^1H NMR data of scupolin K (**2**), molecular formula $C_{26}H_{38}O_{10}$, were essentially the same as those present in the spectrum of scupolin J (**1**). The observed differences between the two spectra were in agreement with compound **2** being a 1:1 mixture of the C-15 epimers of the 14,15-dihydro-15-hydroxy derivative of scupolin J. In fact, protons H-11, H-15, and H-16 appeared as pairs of signals: H-11α, δ 3.99 and 4.54 (dd, 0.5 H each); H-15, δ 5.54 and 5.66 (br d, 0.5 H each), and H-16, δ 5.78 and 5.81 (d, 0.5 H each) instead of the corresponding unsplit signals occurring in **1**. The rest of each spectrum was identical in both compounds. The ^{13}C NMR spectrum of **2** was in complete agreement with the proposed structure and with the previously reported ^{13}C NMR data of other neoclerodane diterpenoids isolated from the genus *Scutellaria* containing the same C-11/C-16 moiety.[6,12,13] Indeed, it was possible to attribute the values to the protons and carbons of both epimers (Tables 1 and 2). Therefore, the structure of scupolin K is represented by **2**. Also, the ^{13}C NMR data of scupolin O (**3**), not previously reported, are presented in Table 2, indicating attributions for both (*R*)- and (*S*)-epimers at C-15.



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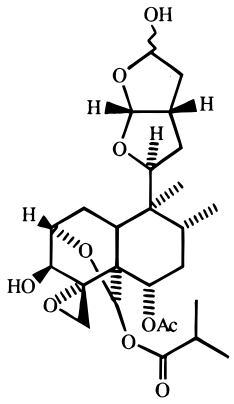

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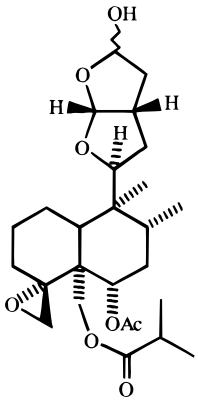

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Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. IR spectra were obtained on Perkin-Elmer 1310 spectrophotometer. ^1H NMR spectra were recorded in $CDCl_3$ solution using a Bruker AC 250 E apparatus at 250 MHz, and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.27). ^{13}C NMR

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Table 1. ^{1}H NMR Spectral Data of Compounds **1** and **2**

proton(s)	**1**	**2** (15*R*)	**2** (15*S*)
H-2β	4.36 (m)	4.36 (m)	4.36 (m)
H-3α	3.97 (m)	3.99 (m)	3.99 (m)
H-6β	4.68 (dd)	4.68 (dd)	4.68 (dd)
H-11α	4.00 (dd)	3.99 (dd)	4.54 (dd)
H-13β	3.53 (m)	3.10 (m)	2.85 (m)
H-14	4.82 (dd)	n.o.	n.o.
H-15	6.46 (dd)		
H-15α		5.54 (br d)	
H-15β			5.66 (br d)
H-16β	6.01 (d)	5.78 (d)	5.81 (d)
Me-17	0.89 (3H, d)	0.90 (3H, d)	0.92 (3H, d)
H_A-18	2.88 (d)	2.89 (d)	2.89 (d)
H_B-18	3.08 (d)	3.08 (d)	3.10 (d)
H-19α	6.68 (s)	6.68 (s)	6.68 (s)
Me-20	1.20 (3H, s)	1.17 (3H, s)	1.19 (3H, s)
OAc	1.94 (3H, s)	1.95 (3H, s)	1.95 (3H, s)
H-2′	2.57 (sept)	2.57 (sept)	2.57 (sept)
Me-3′	1.25 (3H, d)	1.25 (3H, d)	1.25 (3H, d)
Me-4′	1.24 (3H, d)	1.22 (3H, d)	1.22 (3H, d)
$J_{H,H}$ (Hz)	**1**	**2** (15*R*)	**2** (15*S*)
6β,7α	11.4	11.4	11.4
6β,7β	4.7	4.7	4.7
8β,17	6.1	6.2	6.2
11α,12α	11.8	11.4	10.7
11α,12β	5.0	4.8	6.2
13β,14	2.5		
13β,15	2.4		
13β,16β	6.2	5.4	5.4
14,15	2.5		
14α,15α		5.3	
14β,15β			3.0
18A,18B	4.4	4.4	4.4
2′,3′(4′)	6.9	6.9	6.9

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**3**

carbon	**1**	**2** (15*R*)	**2** (15*S*)	**3** (15*R*)	**3** (15*S*)
1	22.7 t	22.6 t	22.6 t	22.1 t	22.1 t
2	70.2 d	70.1 d	70.2 d	24.9 t	24.9 t
3	71.0 d	71.0 d	71.0 d	32.7 t	32.7 t
4	65.7 s	65.7 s	65.8 s	65.0 s	65.0 s
5	42.4 s	42.3 s	42.3 s	45.4 s	45.4 s
6	68.3 d	68.4 d	68.3 d	71.9 d	72.0 d
7	33.3 t	33.4 t	33.4 t	33.3 t	33.3 t
8	35.9 d	35.6 d	35.8 d	36.0 d	36.1 d
9	40.8 s	41.2 s	41.1 s	40.1 s	40.0 s
10	41.3 d	41.0 d	40.9 d	48.5 d	48.5 d
11	85.8 d	84.6 d	84.9 d	83.6 d	83.6 d
12	32.2 t	32.8 t	33.3 t	32.0 t	32.3 t
13	45.7 d	39.9 d	40.7 d	39.9 d	41.3 d
14	102.0 d	38.8 t	39.7 t	38.7 t	39.8 t
15	146.7 d	98.7 d	98.4 d	98.6 d	98.3 d
16	108.1 d	107.9 d	110.0 d	107.3 d	109.3 d
17	16.4 q	16.5 q	16.5 q	16.4 q	16.3 q
18	44.0 t	44.0 t	44.0 t	48.3 t	48.3 t
19	91.1 d	91.1 d	91.1 d	61.9 t	61.9 t
20	14.5 q	14.4 q	14.4 q	13.9 q	13.9 q
1′	175.3 s	175.3 s	175.3 s	177.0 s	177.0 s
2′	34.2 d	34.2 d	34.2 d	34.2 d	34.2 d
3′	18.3 q	18.3 q	18.3 q	18.8 q	18.8 q
4′	19.0 q	18.9 q	18.9 q	19.0 q	19.0 q
OAc	169.9 s	169.9 s	169.9 s	170.2 s	170.2 s
	21.3 q	21.3 q	21.3 q	21.3 q	21.3 q

spectra were recorded in $CDCl_3$ solution on the same apparatus at 62.7 MHz, and chemical shifts are reported with respect to the solvent signals (δ_{CDCl_3} 77.00). ^{13}C NMR assignments were determined by DEPT spectra. MS were recorded on a Finnigan TSQ70 instrument (70 eV, direct inlet). Elemental analysis was performed with a Perkin-Elmer 240 apparatus. Merck Si gel no. 7734 (70−230 mesh), deactivated with 15% H_2O w/v, was used for column chromatography. Radial chromatography was performed on a Harrison Chromatotron 7924 T apparatus using Merck Si gel no. 7749 60 PF_{254} as plate adsorbent.

Plant Collection. *S. polyodon* Juz. was cultivated at Tuscolano on Garda Lake (Experimental Field of the Botanic Garden of the University of Milano) from seeds provided by the Jardin Botanique de Lausanne, Switzerland. Plant materials were collected in July 1998, and voucher specimens (no. 7677) have been deposited in the Herbarium of the Dipartimento di Biologia, University of Milano, Italy.

Extraction and Isolation. The dried and finely powdered aerial parts of *S. polyodon* (3.4 kg) were extracted with Me_2CO (3 × 10 L) at room temperature for 1 week. After filtration, the solvent was evaporated at low temperature (35 °C), yielding a gum (110 g), which was chromatographed over Si gel dry column with a solvent gradient from 100% petroleum ether (bp 50−70 °C) to 100% EtOAc and finally with EtOAc−MeOH (19:1, 9:1). The fraction that eluted with petroleum ether−EtOAc (1:1) (1.2 g) was subjected to radial chromatography, with $CHCl_3$−MeOH (23:2) as eluent, and then to column chromatography, with petroleum ether−EtOAc (3:2) as eluent, to afford, in order of increasing chromatographic polarity, scutecolumnin A[10] (5 mg), jodrellin B[11] (80 mg), scupolin H[7] (70 mg), scupolin I[7] (100 mg), and scutalpin O[8] (**3**, 40 mg). The fraction that eluted with petroleum ether−EtOAc (3:7) (2.5 g) was rechromatographed in turn by radial chromatography (CH_2Cl_2−MeOH 19:1) and column chromatography (petroleum ether−EtOAc 4:6) to afford the following compounds, in order of increasing polarity: scupolin C[7] (60 mg), scupolin A[7] (800 mg), scupolin F[7] (30 mg), scupolin J (**1**, 10 mg), scupolin G[7] (100 mg), and scupolin E[7] (30 mg). The fraction that eluted with petroleum ether−EtOAc (1:9) (1.7 g) was rechromatographed in turn by radial chromatography (CH_2Cl_2−MeOH 9:1) and column chromatography (EtOAc), respectively, to give, in order of increasing polarity, scupolin D[7] (500 mg), scupolin B[7] (20 mg), scupolin K (**2**, 20 mg), and scutalsin[9] (30 mg). The chromatographic fractions containing diterpenoids were decolored by filtration through a pad of a mixture (1:1) of activated charcoal and Celite, eluting with EtOAc. The previously known compounds were identified by their $[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR, and mass spectra and by comparison (TLC) with authentic samples.

Scupolin J (1): mp 167−170 °C (EtOAc−petroleum ether); $[\alpha]^{20}_D$ +4.4° (*c* 0.25, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3400 (OH), 3050 (oxirane), 2930, 1730, 1260 (ester groups), 1620 (vinyl ether), 1420, 1155, 1080, 960, 900, 740 cm^{-1}; ^{1}H NMR (250 MHz), see Table 1; ^{13}C NMR (62.7 MHz), see Table 2; EIMS *m*/*z* $[M]^+$ absent, 449 (9) $[M - COCH_3]^+$, 405 (17) $[M - OCOCH(CH_3)_2]^+$, 294 (7) $[M - COCH(CH_3)_2 - C_6H_7O_2]^+$, 234 (24) $[M - OCOCH(CH_3)_2 - C_6H_7O_2 - HOAc]^+$, 188 (22), 157 (27), 111 (100), 85 (47), 57 (38), 43 (24); *anal.* C 63.26%, H 7.29%, calcd for $C_{26}H_{36}O_9$ C 63.40% H 7.37%.

Scupolin K (2): amorphous solid; IR (CH_2Cl_2) ν_{max} 3450 (OH), 3050 (oxirane), 2990, 1730, 1265 (ester groups), 1420, 1155, 1080, 965, 900, 745 cm^{-1}; ^{1}H NMR (250 MHz), see Table 1; ^{13}C NMR (62.7 MHz), see Table 2; EIMS *m*/*z* $[M]^+$ absent, 423 (16) $[M - OCOCH(CH_3)_2]^+$, 294 (7) $[M - OCOCH(CH_3)_2 - C_6H_9O_3]^+$, 234 (20) $[M - OCOCH(CH_3)_2 - C_6H_9O_3 - HOAc]^+$, 188 (30), 159 (16), 129 (17), 111 (99), 83 (70), 55 (64), 43 (100); *anal.* C 61.01%, H 7.41%, calcd for $C_{26}H_{38}O_{10}$ C 61.16%, H 7.50%.

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