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11-EPISCUTECOLUMNIN C, A NEO-CLERODANE DITERPENOID FROM *SCUTELLARIA COLUMNAE*

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Key Word Index—*Scutellaria columnae var. columnae*; Labiatae; neo-clerodane diterpenes; 11-episcutecolumnin C.

Abstract—A new *neo*-clerodane diterpenoid, 11-episcutecolumnin C, has been isolated from the acetone extract of the aerial parts of *Scutellaria columnae var. columnae*. In addition to the previously known diterpenes, 11-episcutecyprin, scutegalin D, scutecolumnin C, scutecyprol B, scutaltisin and the iridoid glycoside globularin(scutellarioside **1**). The structure and configuration of 11-episcutecolumnin C was established by chemical spectroscopic and molecular mechanics methods as: (11R,13R,16S,19S),6α-acetoxy-4α,18; 11,16; 15,16-triepoxy neo-cleroda,19,2α hemiacetal. © 1998 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

In a previous communication, we reported on the isolation of two *neo*-clerodane diterpenoids, 11-episcutecyprin [1] and scutegalin D [2] from the aerial parts of *Scutellaria columnae var. columnae*, in our search for new *neo*-clerodane diterpenoids [3–8], we have re-investigated the acetone extract of *S. columnae var. columnae*. We report here on the isolation and structure elucidation of a new *neo*-clerodane derivative, 11-episcutecolumnin C (**1**), besides the already known 11-episcutecyprin, scutegalin D, scutecolumnin C (**2**) [9], scutaltisin [6], scutecyprol B [10] and an iridoid glycoside globularin (scutellarioside 1) [11].

RESULTS AND DISCUSSION

An inseparable, 3:7 mixture of scutecolumnin C(**2**) [9] and its C-11epimer (11-epicutecolumnin C) (**1**) was isolated from *S. columnae var. columnae*. This mixture showed only one spot on TLC with several solvents and we could not separate it into its constituents by crystallization.

Combustion analysis and mass spectrometry established that both constituents of the mixture possessed the same molecular formula ($C_{22}H_{32}O_7$). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of the mixture showed a series of signals corresponding to the minor constituent which were identical with those reported for scutecolumnin C (**2**) [9], whereas the remaining signals of the major constituent were only compatible with a structure such as **1**.

In order to obtain a pure sample of **1**, we carried out selective acid hydrolysis of 11-episcutecyprin (**3**). This removed the tigloyloxy group placed at the C-19 hemiacetal carbon yielding a compound with ^1H and ^{13}C NMR spectra identical to those of the major constituent 11-episcutecolumnin C (**1**). The differences between the ^1H NMR (Table 1) spectra of **1** and scutecolumnin C (**2**) were in the chemical shifts corresponding to CH_3-17($\Delta\delta + 0.12$ ppm), H-16α ($\Delta\delta - 0.10$ ppm) and H-11α($\Delta\delta - 0.42$ ppm), and the signals of Hα-15 (δ 3.96 *ddd*) and Hβ-15(δ 3.80 *ddd*), of 2H-15 in **2** (δ 3.87 m). Moreover, the ^{13}C NMR data (Table 2) showed that the C-7, C-11, C-15 and C-20 resonances in compound **1** were up-field shifted, with respect to those of scutecolumnin C (**2**), whereas the remaining signals appeared at almost identical field in both compounds (Table 1). The small differences in their chemical shifts were in complete agreement with those reported for scutecyprin [12] and its C-11 epimer[1].

The relative stereochemistry of **1** was established by NOE experiments. Irradiation at δ 3.63(H-11α) produced NOE enhancements (Table 3) of H-1α, H-16α and H-13α. Positive NOEs were observed upon irradiation of H-16α(δ 5.54) for H-11α and H-13α. These experiments showed that the protons at C-11, C-13 and C-16 were α-orientated. Moreover, on irradiating H-19α(δ 5.74), NOE effects were observed for H-7α, Me-20 and Me-17 thus establishing a *cis*-1,3 diaxial relationship between these groups and consequently, a *trans*-junction of the A and B rings of **1** [4].

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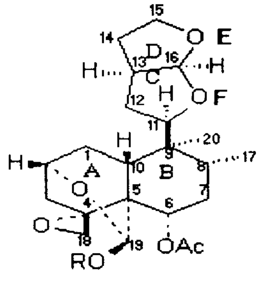
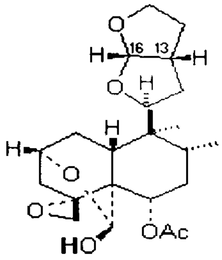


1 R=H



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3 R=

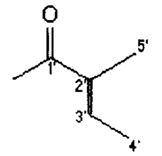

Table 1. ^{1}H NMR spectral data of compounds **1** and **2** (250 MHz, $CDCl_3$, TMS as int. standard)†

H	**1**	**2***	Δppm	*J*(Hz)	**1**	**2***
1α	~2.11″	—		2β,3α	2.0	2.7
1β	~1.51″	—		2β,3β	2.4	2.5
2β	4.21 *m*‡	4.17 *m*	+0.04	3α,1α	2.7	2.7
3α(eq)	2.60 *dt*	2.58 *dt*	+0.02	3α,3β	14.3	14.4
3β	1.72 *dd*	1.76 *dd*	−0.04	6β,7α	11.6	11.6
6β	4.70 *dd*	4.68 *dd*	+0.01	6β,7β	5.2	4.5
7α	~1.65″	—		11α,12A	11.7	10.9
7β	~1.42″	—		11α,12B	4.7	5.8
8β	~2.01″	—		13α,14A =		—
				13α,12A	5.4	
10β	~1.89″	—		13α,14B =		—
11α	3.63 *dd*	4.05	−0.42	13α,12B	8.7	—
12A	~1.61″	—		15α,15β	8.1	—
				15α,14B	5.4	
				15β,14A	7.9	
12B	~1.84″	—		15β,14B	4.9	—
13α	2.80 *ddd* 13β	2.84 *m*	−0.04	16α,13α	5.5	—
14A	~1.52″	—		16β,13β	—	5.0
14B	~1.78″	—		17,8β	6.6	6.1
15α	3.96 *ddd*	3.87 *m*	−0.09	18A,18B	4.1	4.1
15β	3.80 *ddd*	3.87 *m*	+0.07	19α,OH	1.7	—
16α	5.54α 16β	5.64 *d*	−0.10			
Me-17	1.03 *d*	0.91	+0.12			
18A	2.42 *d*	2.43 *d*	−0.01			
18B	2.96 *d*	2.95 *d*	−0.03			
19α	5.74 *d*	5.72 *s*	+0.03			
Me-20	1.04 *s*	1.08 *s*	−0.04			
OAc	2.05 *s*	2.05 *s*	0			
OH	3.52 *d*					

* Taken from ref. [9]

† Spectral parameters were obtained by first-order approximation. All assignments were confirmed by double resonance experiments and were in agreement with the ^{1}H-^{13}C COSY spectra.

‡ $W_{1/2}$ = 8.1 Hz.

″ Overlapped signal.

Table 2. ^{13}C NMR spectral data of compounds 1 and 2* (62.9 MH$_z$, CDCl$_3$ TMS as int standard)†

C	1	2	$\Delta\delta$c
1	28.7 *t*	28.8 *t*	−0.1
2	66.5 *d*	66.8 *d*	−0.3
3	36.5 *t*	36.7 *t*	−0.2
4	60.6 *s*	60.8 *s*	−0.2
5	42.4 *s*#	42.5 *s*	−0.1
6	69.7 *d*	69.9 *d*	−0.1
7	31.1 *t*	32.7 *t*	−1.6
8	34.5 *d*	35.0 *d*	−0.5
9	41.4 *s*#	41.4 *s*	0.0
10	41.2 *d*	40.4 *d*	+0.8
11	83.4 *d*	85.9 *d*	−2.5
12	32.2 *t*	32.9 *t*	−0.7
13	42.5 *d*	41.8 *d*	+0.7
14	33.4 *t*	33.5 *t*	−0.1
15	66.1 *t*	68.3 *t*	−2.2
16	107.7 *d*	108.3 *d*	−0.6
17	17.3 *q*	16.8 *q*	+0.5
18	49.6 *t*	49.7 *t*	−0.1
19	93.0 *d*	93.1 *d*	−0.1
20	12.2 *q*	13.9 *q*	−1.7
OAc	169.1 *s*	169.3 *s*	−0.2
	21.3 *q*	21.4 *q*	−0.1

* Taken from ref. [9].

† Multiplicities were determined by DEPT pulse sequences.

These assignments may be interchanged.

On the other hand irradiation at δ 4.70(H-6β) caused a positive NOE enhancement in the signals of H-8β, H-10β and H$_B$-18 and a negative NOE effect on H$_A$-18 [3]. Thus establishing that these protons are β-orientated.

From the above data, it was evident that 11-episcutecolumnin C (**1**) possessed the structure depicted in **1** and can be assigned as (11R,13R,16S,19S)6α-acetoxy-4α,18;11,16;15,16-triepoxy neo-cleroda,19, 2α-hemiacetal. Final proof that 11-episcutecolumnin C (**1**) has the structure and stereochemistry depicted in formula **1** was obtained by selective hydrolysis of 11-episcutecyprin (**3**) [1] (see Experimental).

The absolute stereochemistry of 11-episcutecolumnin C (**1**) was not ascertained by direct methods but, on biogenetic grounds, it was assumed that it possessed a *neo*-clerodane absolute configuration [13] like other diterpenes isolated from *Scutellaria* species and whose structures have been established by X-ray methods [3, 14].

The conformation of 11-episcutecolumnin C (**1**) deduced from the NMR data was additionaly ascertained by molecular mechanics calculations. These were performed using the standard force field MMX computer program PC model 2.0 of Allinger [15].

The computer generated minimum-energy conformations were used as a basis for calculations of the vicinal coupling constant from the dihedral angles using the equation of Haasnoot *et al.* [16].

The MM calculated conformations also provided the interproton distances. The MM results showed (Table 3) that ring A is in the boat conformation and

Table 3. Selected MM calculated distances between the hydrogen atoms and dihedral angles of compound **1**, compared with the ^{1}H NMR data

Dihedral angles Type	Value(in deg)	$J_{H,H}$ (Hz)	Distances Type	Value (in Å)	NOE (%)
Hα-C$_1$-C$_2$-Hβ	−60.29		6H*-10H	2.51	8.8
Hα-C$_1$-C$_{10}$-Hβ	147.57		6H*-18HB	2.22	13.2
Hβ-C$_1$-C$_{10}$-Hβ	24.36		6H*-8H	3.40	6.9
Hβ-C$_2$-C$_3$-Hβ	57.66	2.4	8H-15Hβ	4.68	
Hβ-C$_2$-C$_3$-Hα	58.88		10H-12Hβ	3.34	
Hβ-C$_{10}$-C$_5$-C$_{19}$	153.11		11H*-1Hα	3.93	4.7
Hβ-C$_6$-C$_7$-Hα	163.32	11.6	11H*-19H	5.52	2.4
Hβ-C$_6$-C$_7$-Hβ	50.66	5.2	11H*-13H	2.60	1.7
Hα-C$_{11}$-C$_9$-C$_{20}$	62.03		11H*-16H	3.25	5.2
H-C$_{11}$-C$_{12}$-Hα	47.55	4.7	13H-16H*	2.43	8.5
H-C$_{11}$-C$_{12}$-Hβ	172.95	11.7	19H*-20H$_{1,2,3}$	2.21,3.36,3.93	8.4
H-C$_{13}$-C$_{16}$-H	29.18	5.5	19H*-17H$_{1,2,3}$	2.21,3.36,3.93	1.5
Hα-C$_{15}$-C$_{14}$-Hα	−21.28	4.9	19H*-7Hα	2.42	8.7
Hβ-C$_{15}$-C$_{14}$-Hβ	−18.84	<1	OE-17H$_{1,2,3}$	5.29,5.66,6.33	
Hβ-C$_{15}$-C$_{14}$-Hα	101.58	7.9	OF-17H$_{1,2,3}$	3.91,4.79,4.81	
H-C$_{19}$-O-H	72.34				
C$_{14}$-C$_{13}$-C$_{16}$-OF	144.94				
C$_{12}$-C$_{13}$-C$_{16}$-OE	−94.41				

* Irradiated protons.

Table 4. Selected MM calculated distances between the hydrogen atoms and dihedral angles of compound 3, compared with the ^{1}H NMR data

Dihedral angles		$J_{H,H}$	Distances		NOE*
Type	Value (in deg)	(Hz)	Type	Value (in Å)	(%)
Hα-C_1-C_2-Hβ	−48.56		6H*-10H	2.37	3.4
Hα-C_1-C_{10}-Hβ	124.13	11.0	6H*-18HB	2.17	11.3
Hβ-C_1-C_{10}-Hβ	5.93		6H*-8H	2.38	3.3
Hβ-C_2-C_3-Hβ	−55.42		10H-12Hβ	2.57	
Hβ-C_2-C_3-Hα	63.78	2.0	8H-15Hβ	3.18	
H-C_{10}-C_5-C_{19}	168.51		11H*-1Hα	2.22	7.3
H-C_6-C_7-Hα	−177.64	12.1	11H*-13H	2.85	2.8
H-C_6-C_7-Hβ	65.78	5.1	11H*-16H	2.86	4.5
H-C_{11}-C_9-C_{20}	55.29		11H*-17$H_{1,2,3}$	4.06,4.58,5.30	2.4
H-C_{11}-C_{12}-Hα	44.74	4.7	11H*-20$H_{1,2,3}$	2.54,2.92,3.75	3.7
H-C_{11}-C_{12}-Hβ	167.27	11.7	15Hβ-17H$^*_{1,2,3}$	2.86,3.84,4.61	0.6
H-C_{13}-C_{16}-H	5.02	5.5	19H*-20$H_{1,2,3}$	2.06,3.06,3.77	10.0
Hα-C_{15}-C_{14}-Hα	41.84	4.8	19H*-7Hα	2.28	8.7
Hβ-C_{15}-C_{14}-Hβ	43.78	<1	OE-17$H_{1,2,3}$	3.29,4.86,4.95	
Hβ-C_{15}-C_{14}-Hα	−79.90	8.2	OF-17$H_{1,2,3}$	2.39,3.45,3.98	
H-C_{19}-O-R†	−51.64				
C_{14}-C_{13}-C_{16}-OF	124.27				
C_{12}-C_{13}-C_{16}-OE	−116.48				
O-$C_{1'}$-$C_{2'}$	112.71				
$C_{1'}$-$C_{2'}$-$C_{3'}$	117.16				

* Irradiated protons.

† *R* = Tig.

that ring B prefers the chair conformation. The five membered ring C (from the hexahydrofuro-furan ring) is almost planar, whereas ring D is an envelope with an oxygen out of plane (the distance H_{16} -Hα_{15} is 2.69 Å whereas H_{16}-H_{11} is 3.25 Å). Moreover, the MM calculated dihedral angle for C_{14}-C_{13}-C_{16}-OF is 144.94° for C_{12}-C_{13}-C_{16}-OE is −94.41°, and the distance H_{16}-H_{13} is 2.43 Å. The experimental vicinal coupling between H_{16}-H_{13} is 5.5 Hz. These MM results showed that the oxygen atom in ring D is orientated to ring B, the OE-17$H_{1,2,3}$ distances are 5.29/5.66/6.63 Å and OF-17$H_{1,2,3}$ are 3.91/4.79/4.81 Å. On the other hand, the MM obtained dihedral angle C_{20}-C_9-C_{11}-H is 62.03° which showed that CH_3-20 and the hexahydrofurofuran moiety are anti-orientated. The MM obtained dihedral angle H-C_{19}-O-H is 72.34° and the distance between the proton of the hydrohyl group at C-$_{19}$ and the carbonyl oxygen atom from the acetoxyl group is 2.55 Å. This indicated a hydrogen bond, the presence of which was confirmed by the IR spectrum (~1708 cm^{-1} instead of ν 1736 cm^{-1} for >C=O). All of the data mentioned above were in a complete agreement with the results from the ^{1}H NMR investigation for compound **1** (Table 3).

MM calculation were also used for 11-episcutecyprin (**3**) and the results (Table 4) showed that the conformations of the ring C is almost planar, whereas ring D is an envelope with CH_2-15 out of plane and the distances H_8-$H_{15\beta}$ is 3.18 Å and OE-17$H_{1,2,3}$ is 3.29/4.86/4.95 Å. These MM-calculation results showed that CH_2-15 in ring D was probably orientated to the ring B. This was confirmed from NOE experiments (Table 4).

EXPERIMENTAL

General

Mps: uncorr. Plant materials were collected near the town of Kardjali (south Bulgaria) in July 1996 and voucher specimens (N 17506) are deposited in the Herbarium of the Department of the Botanica of the Higher Institute of Agriculture of Plovdiv, Bulgaria.

Extraction and isolation of the diterpenoids

Dried and powdered aerial parts of *S. columnae subsp. columnae* (1050 g) were extracted with Me_2CO (3 × 5 l) at room temp. for 7 days. The combined extracts were evapd. *in vacuo* to near dryness (30 g). The extract was subjected to CC on silica gel (Merck N 7734; deactivated with 15% H_2O, 400 g) with petrol, petrol–EtOAc mixts and $CHCl_3$–MeOH mixts as eluents. The fr. eluted with petrol–EtOAc (7:3) (3 g) was rechromatographed over silica gel (50 g); elution with petrol–EtOAc (4:1) gave a mixture of four compounds (320 mg) which was subjected to radial chromatography (silica gel disc., $CHCl_3$–MeOH, 24:1) to give the following compounds in order of increasing chromatographic polarity; 11-episcutecyprin (**3**, 30 mg), mixture of scutecolumnin C (**2**) and 11-episcutecolumnin C (**1**, 68 mg), scutegalin D (50 mg), scutecyprol B (28 mg) and scutaltisin (50 mg). The $CHCl_3$–MeOH (9:1) fraction (520 mg) was subjected to CC on silica gel (7 g) eluting with $CHCl_3$-MeOH (23:2) yielding an iridoid glucoside globularin (scutellarioside 1, 200 mg).

The previously known compounds, 11-episcutecyprin (**3**), scutecolumnin C (**2**), scutegalin D, scutecyprol B, scutaltisin and globularin (=scutellarioside 1) were indentified by their mp., $[\alpha]_D$, and ^1H NMR and mass spectra.

11-*Episcutecolumnin C* (**1**). Mp. 189–191°; $[\alpha]_D20°$ 0 ($CHCl_3$; c 0.376). IR ν_{max}^{KBr} cm^{-1}: 3462, 2961, 2945, 1708, 1430, 1374, 1306, 1280, 1246, 1107, 1022, 980, 940, 776; ^1H NMR: Table 1; ^{13}C NMR: Table 2; EIMS (70 eV, direct inlet) *m/z* (rel. int.): 408 $[M]^+$ (0.2), 349(0.4), 348(0.2), 218 (4), 172(5), 171(3), 113(100), 111(4), 69(74), 67(18), 55(80), 43(41). (Found: C 64.39; H 8.07; $C_{22}H_{32}O_7$ requires C 64.68; H 7.90%).

Selective hydrolysis of the tiglate ester of 3 *to give* 1

To a soln of **3** (33 mg) in Me_2CO (5 ml) two drops of $3MH_2SO_4$ were added. Then the reaction mixt was stired at room temp. for 30 min. Work-up (dilution with H_2O, extraction with $CHCl_3$) and evapn of the solvents yielded 21 mg of **1**, which was recrystallizated from EtOAc–n-hexane to give 17 mg of **1**.

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