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SCUTORIENTALIN D, A NEO-CLERODANE DITERPENOID FROM *SCUTELLARIA ORIENTALIS* SUBSP. *PINNATIFIDA*

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Key Word Index—*Scutellaria orientalis* subsp. *pinnatifida*; Labiatae; neo-clerodane diterpenoid; scutorientalin D.

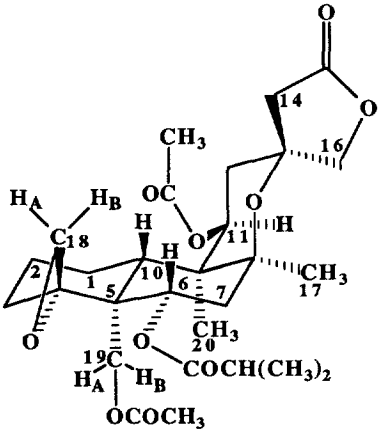
Abstract—A new neo-clerodane diterpenoid, scutorientalin D, was isolated from the acetone extract of the aerial parts of *Scutellaria orientalis* subsp. *pinnatifida*. The structure and configuration of this compound was established by spectroscopic and molecular mechanics methods as (13*S*)-11β, 19-diacetoxy-4α,18;8β,13-diepoxy-6α-isobutyroxy-neo-clerodan-15,16-olide. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

A number of biologically active neo-clerodane diterpenoids have been isolated during the last decade from *Scutellaria* species and their structures established by NMR spectroscopy and other methods [1–11]. In continuation of our search for new neo-clerodane diterpenoids in *Scutellaria* plants [12–15], we have now investigated *S. orientalis* subsp. *pinnatifida* growing near the town of Sliven in eastern Bulgaria. The acetone extracts of the aerial parts of this plant yielded a new neo-clerodane diterpenoid, scutorientalin D (**1**), whose structure, configuration and conformation were elucidated by means of spectroscopic methods (mainly 1D and 2D NMR) and molecular mechanics calculations.

RESULTS AND DISCUSSION

Compound **1** had the molecular formula $C_{28}H_{40}O_{10}$ as determined by mass spectrometry and ^{13}C NMR analysis. The ^1H NMR and ^{13}C NMR data (Table 1), as well as the proton connectivity pattern deduced from the H–H COSY and the selective decoupling experiments, suggested the structure **1** for this new diterpenoid. Its IR spectrum revealed the existence of a γ-lactone (1795 cm^{-1}) and ester group(s) (1729 *br*, 1254, 1231 cm^{-1}). The NMR data indicated the presence of two acetoxy groups (δ_H 2.08 *s* and 2.11 *s*, 3H each; δ_C 170.2 *s*, 171.0 *s* and 21.2 *q*, 2C) and an isobutyric ester function (δ_H 1.12 *d* and 1.16 *d*, 3H each; 2.40 *sept*; δ_C 175.6 *s*, 34.1 *d* and 18.7 *q*, 2C), together with characteristic signals of a neo-clerodane diterpene (δ_H 0.94 *s*, Me-20; 16.1 *q*, C-20) having a 4α, 18-oxirane [two C-18 protons at δ 3.01 *dd* (J_{gem} = 4.0 Hz, $J_{18B,3a}$ = 2.3 Hz) and 2.26 *d*; δ_C 64.8 *s*, C-4 and 48.4 *t*, C-18], in addition to an 8,13-ether bridge (δ_H 1.25 *s*, Me-17; δ_C 81.7 *s*, C-8; 77.7 *s*, C-13 and 24.1 *q*, C-17) and a 13-spiro-15,16-γ-lactone moiety (C-14 methylene protons as an AB-system at δ_H 2.55 *d* and 2.94 *d*, J_{gem} = 17.2 Hz; C-16 protons at δ_H 4.19 *d* and 4.38 *d*, J_{gem} = 8.9 Hz; δ_C 43.7 *t*, C-14; 172.7 *s*, C-15 and 77.1 *t*, C-16) like other neo-clerodane derivatives previously isolated from *Scutellaria* plants [1, 2, 9, 12–15]. Moreover, the C-12 methylene protons and the C-11 proton appeared as an ABX-system at δ_H 1.82 *br t* and 2.03 *dd* (J_{gem} = 13.5 Hz), and 5.34 *dd* (J_{vic} = 4.2 and 13.5 Hz), respectively; δ_C 34.8 *t*, C-12 and 72.5 *d*, C-11, indicating that there is a β-(equatorially) located acyloxy substituent at the C-11 position [1, 9, 13]. Selective INEPT experiments [16, 17] identified this substituent as one of the acetoxy groups, since irradiation of H-11 at δ 5.34 resulted in selective enhance-



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ments of C-10 ($^3J_{CH}$ = 10 Hz, *anti*-orientation), C-8 ($^3J_{CH}$ = 2 Hz, *gauche*-orientation), and the acetoxy C=O at δ 171.0 ($^3J_{CH}$ = 8 Hz), the latter indicating an almost *anti*-orientation of the carbonyl carbon with respect to H-11. The position of the acetoxy group at C-11 was independently proven also by selective decoupling of H-11 (δ 5.34) in the proton-coupled ^{13}C NMR spectrum [7], which affected only the carbonyl signal at δ 171.0.

The presence of another acetoxy group at C-19 was evident from the long-range cross-peaks observed in the H–H COSY spectrum between the signals of the two C-18 protons (δ 3.01 and 2.26) and the CH_3CO protons at δ 2.11. Consequently, the isobutyroxy group was located at C-6, which was proven also by the NOE experiments. The latter permitted also the establishment of the relative stereochemistry of all the asymmetric centres in compound **1**. Thus, irradiation of the Me-20 protons at δ 0.94 led to positive NOE enhancements of the signals of H-1α (δ 1.57), H-7α (δ 1.82), H-11α (δ 5.34), H-16α (δ 4.38), H-19B (δ 4.71) and Me-17 (δ 1.25). Positive NOE enhancements were observed also upon irradiation of: Me-17 at δ 1.25 (for H-11α, H-16α, H-16β, Me-20 and H-2′ at δ 2.40); H-6β at δ 5.08 (for H-7β, H-10β and H-18B); H-11α at δ 5.34 (for H-12α, H-16α, Me-17 and Me-20). In addition to these results, 2D NOESY spectra revealed also the existence of the following cross-peaks: 19B/7α, 19A/3α and 19A/3β. Thus, the results from the NOE(SY) experiments permitted also the assignment of the methylene protons at C-16, C-18 (H-18B being the *endo*-H with respect to ring B) [13], as well as the conformation of the C-19 hydrogens (H-19B spatially closer to Me-20). The assignment of the methylene protons at C-14 became possible from the long-range COSY cross-peak observed for H-14α/H-16α, attributed to W-orientation. Other such cross-peaks observed were H-12β/H-16β and H-6β/H-19A, in accordance with the stereochemical assignment.

In agreement with all the above data, scutorientalin D (**1**) possessed the same stereochemistry as scutalpin D, the latter containing a tigloxy- instead of the isobutyroxy-substituent at the C-6 position [13]. On the other hand, scutorientalin D(**1**) has the same structural formula as the acetylation product of scutorientalin B [15], except for the different stereochemistry at C-11. This is clearly reflected in the ^1H NMR data: H-11α in **1** is axial, with very different vicinal coupling constants to both C-12 protons (13.0 and 4.2 Hz, Table 1), whereas in the case of acetyl-scutorientalin B (H-11β equatorial) both couplings are equal (2.6 Hz [13]).

The absolute stereochemistry of scutorientalin D (**1**) was not ascertained by direct methods but, on biogenetic grounds it could be assumed that it possesses a neo-clerodane absolute configuration [18], like other clerodanes isolated from *Scutellaria* plants and whose structures have been established by X-ray methods [7, 12].

The conformation of scutorientalin D (**1**) deduced from the NMR data was additionally ascertained by molecular mechanics calculations. They are performed using the standard force field MM2 of Allinger [19]. The computer-generated minimum-energy conformations were used as a basis for calculation of the vicinal coupling constants from the dihedral angles using the equaiton of Haasnoot *et al.* [20]. The calculated conformations also provided the interproton distances which were compared to the NOE results.

The results from the calculations showed that all three six-membered rings A, B and C in scutorientalin D (**1**) prefer chair-like conformations, whereas the five-membered lactone ring is close to an envelope, with C-13 out of plane. The H–C–C–H dihedral angles in rings A, B and C are within 60°±5° and 180°±10°, leading to calculated vicinal couplings in satisfactory agreement with the experimental ones (Table 1). For example, some of the calculated vicinal couplings are: H-11α/H-12β = 11.0 Hz (dihedral angle 171°); H-11α/H-12α = 4.6 Hz (56°); H-6β/H-7α = 11.5 Hz (179°); H-6β/H-7β = 3.7 Hz (62°); H-1α/H-10β = 12.3 Hz (177°); H-1β/H-10β = 2.6 Hz (64°). It might be expected that these conformational conclusions should be valid also for structurally similar neo-clerodane diterpenoids, such as scuterivulactones [1], scutalpins D [13], G [9] and others. A molecular mechanics study of the diterpenoid forskolin and some analogous compounds containing a similar three-six-membered ring system but with possibilities for intramolecular hydrogen bonding led to the conclusion that ring B favours a distorted-chair conformation, whereas rings A and C may interconvert between distorted chair and disorted twist-boat conformations of similar energy [21].

EXPERIMENTAL

Mps: uncorr. Plant materials were collected near the town of Sliven (eastern Bulgaria) in July 1994 and voucher specimens were deposited in the Herbarium of the Higher Institute of Agriculture at Plovdiv, Bulgaria.

Extraction and isolation of scutorientalin D (**1**). Dried and powdered aerial parts of *Scutellaria orientalis* subsp. *pinnatifida* (220 g) were extracted with Me_2CO (3 × 3 l) at room temp. for 4 days. The combined extracts were evapd under vacuum almost to dryness (6.0 g), MeOH (500 ml) was added and then was extracted with petrol (5 × 150 ml). The MeOH phase was concd (3.0 g) and chromatographed (CC, silica gel Merck No 7734 deactivated with 15% H_2O, 130 g; column dimensions 700 × 28 mm). Elution with petrol–EtOAc (9:1, 400 ml) gave crude **1** (24 mg) which was recrystallized from petrol–EtOAc (4:1, 2.5 ml) to yield pure scutorientalin D (19 mg), mp 221–223°C; $[\alpha]_D^{20}$ −17.5° ($CHCl_3$; *c* 0.285). IR ν_{max}^{KBr} cm^{-1}: 1795 (spiro γ-lactone), 1729 (C=O ester), 2953, 1654, 1598, 1563, 1495, 1458, 1362, 1254, 1231, 1214, 1200, 1163, 1024, 914, 853. FAB$^+$ MS *m/z* 537 $[M+H]^+$; EI-MS (70 eV, direct inlet) *m/z* (rel. int.): 506 $[M-CH_2O]^+$ (1.5), 465 (2.5), 393 (3), 375 (4), 329 (3), 315 (3), 51 (35), 43 (100). ^1H NMR and ^{13}C NMR: see Table 1.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (62.9 MHz) parameters of compound **1** (in $CDCl_3$, TMS as int. standard, chem. shifts in ppm, J in Hz)*

H (C)	δ_H	J_{HH}	δ_C†
1α	1.57 *qd*	1α, 10β ~ 1α, 2β ~ 1α, 1β ~ 13	21.7 *t*
1β	~2.27 *m*‡	1β, 10β 3.2	
2α	1.96 *m*	1α, 2α ~ 3.5	25.1 *t*
2β	1.47 *qt*	2β, 2α ~ 2β, 3α ~ 13.0	
3α	~2.1 *m*‡	2β, 1β ~ 2β, 3β ~ 4.2	32.4 *t*
3β	1.06 *m*	3α, 3β 13.2	
4			64.8 *s*
5			45.2 *s*
6β	5.08 *dd*	6β, 7α 11.5	67.6 *d*
7α	1.82 *dd*	6β, 7β 5.0	38.4 *t*
7β	1.68 *dd*	7α, 7β 14.0	
8			81.7 *s*
9			42.2 *s*
10β	~2.30 *m*‡		42.8 *d*
11α	5.34 *dd*	11α, 12β 13.0	72.5 *d*
12α	2.03 *dd*	11α, 12α 4.2	34.8 *t*
12β	1.82 *br t*	12α, 12β 13.5	
13			77.7 *s*
14α	2.55 *d*	14α, 14β 17.2	43.7 *t*
14β	2.94 *d*		
15			172.7 *s*
16α	4.38 *d*	16α, 16β 8.9	77.1 *t*
16β	4.19 *d*		
17	1.25 *s*		24.1 *q*
18A	2.26 *d*	18A, 18B 4.0	48.4 *t*
18B	3.01 *dd*	18B, 3α 2.3	
19A	4.48 *d*	19A, 19B 12.2	61.7 *t*
19B	4.71 *d*		
20	0.94 *s*		16.1 *q*
1′			175.6 *s*
2′	2.40 *sept*		34.1 *d*
3′,4′	1.12 *d*, 1.16 *d*	2′, 3′; 2′, 4′ 7.0	18.7 *q*(2C)
11-Ac	2.08 *s*		21.2 *q*, 170.2 *s*
19-Ac	2.11 *s*		21.2 *q*, 171.0 *s*

*The ^{1}H NMR parameters were obtained by first-order approximation. All assignments were confirmed by selective decoupling, ^{1}H–^{1}H and ^{1}H–^{13}C 2D correlation experiments.

†Multiplicities from DEPT-135 experiments.

‡Overlapped signals.

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