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Neo-clerodane diterpenoids from *Scutellaria velenovskyi* Rech. fil.

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A new *neo*-clerodane diterpenoid, (11S,13R,16S,19S)-3β,6α-diacetoxy-19-*trans*-tigloyloxy-2α,19;4α,18;11,16;15,16-tetraepoxyneoclerodane (scutevelin A), was isolated from the acetone extract of the aerial parts of *Scutellaria velenovskyi* Rech. Fil., in addition to the previously known 14,15-dihydrojodrellin T. The structure of the new compound was established by ^{1}D and ^{2}D NMR and other spectroscopic techniques and by comparison of its spectral data to those of the closely relative 14,15-dihydrojodrellin T.

Keywords: *Scutellaria velenovskyi*, Labiatae, *neo*-clerodane diterpenoids

INTRODUCTION

Neo-clerodane diterpenoids exhibit interesting biological properties – especially their action as insect antifeedant and antifungal agents [1-3]. *Scutellaria* (Labiatae) species was assessed to contain a great diversity of such compounds with different substructures. In Bulgaria, the investigation of this genus started in 1991 with the study of *Scutellaria alpina* [4]. In continuation of our ongoing efforts in searching for biologically active clerodanes we studied *Scutellaria velenovskyi*. Herein, we report the isolation and structural identification of two *neo*-clerodane diterpenoids, a new compound scutevelin A (1) and the already known 14,15-dihydrojodrellin T (2).

EXPERIMENTAL

Plant material

The plant material of *Scutellaria velenovskyi* Rech. fil. was collected in June 2016 in the region of Mezek near Svilengrad, Bulgaria and voucher specimens (N. 11927) were deposited in the Herbarium of the Agriculture University of Plovdiv, Bulgaria.

Extraction and isolation of the compounds

Dried and finely powdered aerial parts of *Scutellaria velenovskyi* (160 g) were extracted with acetone (2 × 2 L) at room temperature for 1 week. After filtration, the solvent was evaporated to dryness under reduced pressure yielding a gum (8.2 g), which was dissolved in 50 % aq. acetone (v/v, 100 mL). The solution was cooled to 4 ºC for 24 h and filtered. The filtrate was extracted with $CHCl_3$ (4 × 50 mL). The organic extract was dried with Na_2SO_4 and evaporated under vacuum (giving 1.4 g of a bitter residue). This residue was chromatographed over a Si gel column (Merck N. 7734, deactivated with 10 % H_2O, w/w, 30 g) with a light petroleum / ethyl acetate solvent gradient (from 10:0 to 3:7) as eluent. Eluate fractions (100 mL each) containing scutevelin A (compound **1**) based on TLC results were collected (8 flasks) and evaporated to obtain 24 mg of crude scutevelin A. After recrystallization from acetone 17 mg of pure substance was obtained. Analogously, 11 mg of compound **2** were obtained from 5 flasks.

Scutevelin A (1)

Colorless prisms from acetone, m.p. 190-192 °C. TLC: Rf 0.71 (EtOAc). IR bands, cm^{-1} (KBr): 2963, 2922, 2851, 1740, 1715, 1653, 1636, 1378, 1261, 1093, 1025, 907, 879, 801, 733, 669, 618, 599. ^{1}H and ^{13}C NMR: see Table 1. EIMS (70 eV, direct inlet) m/z (rel. int. in %): 489 [M – OAc]+ (33), 449 [M – OTig]+ (3), 389 [M – OAc, – OTig]+ (4), 316 [M – OAc, – OTig, – CH]+ (17), 224 (3), 171 (6).HREIMS m/z 571,2542 $[M+Na]^+$ (calcd for $C_{29}H_{40}O_{10}Na$: 571,2519).

RESULTS AND DISCUSSION

Two compounds with very close R_f values on TLC were obtained after chromatography of the acetone extract of the aerial parts of *Scutellaria velenovskyi*. The IR spectra of the compounds are very close. Both indicate presence of acetyl groups characterized by a band at 1740 cm^{-1} and tigloyl ester identified with the strong absorption for carbonyl function at 1715 cm^{-1} in combination with the intensive band for conjugated double bond at 1653 cm^{-1}. Both substances have molecular formula $C_{29}H_{40}O_{10}$ which corresponds to the observed in the HREIMS spectra $[M+Na]^+$ peaks at m/z 571.2542 for compound **1** and 571.2515 for **2**, respectively. In accordance with the observations in the IR spectra, the mass spectra display fragment ions at m/z 489, 449 and 389 corresponding to the loss of an acetoxyl, tigloyloxy unit or both functions,

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respectively. The noticeable difference is that in the spectrum of **1** the strongest fragment ion is at m/z 449, while in **2** that is at m/z 389. The presence of the mentioned above substitutes in the molecules of **1** and **2** is supported by the observed in the ^{1}H and ^{13}C NMR spectra typical signals corresponding to a tigloyl and an acetyl ester. The downfield signal at δ_H 7.07/6.81 (lH, qq, J = 7.1, 1.1/7.3, 1.8 Hz, H-3'), signals for methyl groups at 1.80/1.82 (3H, br d, J = 7.6 Hz, H_3-4'), 1.88/1.81 (3H, br s, H_3-5') and carbon signals at δ_C 166.0/166.4 (C=O), 128.50/128.14 (C-2'), 139.02/137.77 (C-3') indicate the presence of a tiglate moiety (see Table 1). Signals for two acetate groups appeared at δ_H 2.05 / δ_C 169.42 and δ_H 1.78 / δ_C 169.95 for compound **1** and at δ_H 2.14 / δ_C 169.78 and δ_H 1.96 / δ_C 169.61 for **2**. In addition, signals for three geminal protons with ester groups are present at δ_H 4.64 (1H, dd, J = 11.1, 4.6 Hz), 5.38 (lH, t, J = 2.2 Hz) and 6.77 (1H, s) in the ^{1}H spectrum of **1** and at δ_H 4.64 (1H, dd, J = 11.1, 4.6 Hz), 5.38 (lH, t, J = 2.2 Hz) and 6.77 (1H, s) in the spectrum of **2**. Characteristic signals for two 4α,18-epoxy-neo-clerodane skeletons were easily distinguished (Tables 1 and 2) at δ_H 0.79 s / 0.80 s (Me-20), 0.787 d /0.791 d (Me-17).

^{1}H and ^{13}C NMR spectral data of 14,15-dihydrojodrellin T coincide in all respects with those of an authentic sample and with those reported in the literature [5, 6]. The 600 MHz ^{1}H NMR spectrum of **1** indicates all structural features common to **2** with the expected differences for the signals corresponding to the ring A and to the C-4/C-18 oxirane fragment. For instance, the signals from the two doublets, corresponding to the C-18 two hydrogens observed in **2** at δ_H 2.45, (lH, d, J = 4.6 Hz, H-18A) and 3.05 (lH, d, J = 4.7 Hz, H-18B), are replaced in **1** with δ_H 2.88 (lH, d, J = 4.3 Hz, H-18A) and 2.91 (lH, d, J = 4.3 Hz, H-18B). Such conciseness of the signals for 18A and 18B protons is a characteristic feature for compounds having electronegative substitute at third position. Another deviation of the signals in the ^{1}H NMR spectra of **1** and **2** is the shifted with 0.35 ppm downfield doublet of triplets characteristic of the C-2/C-19 etheral linkage (δ_H 4.07/4,42, H-2 dt). The signals from the hexahydrofurofuran substructure and the B ring were all well defined. The measured ^{1}H-broadband-decoupled ^{13}C NMR spectra of both compounds show 29 signals and the DEPT displayed 23 resonances for six methyls, six methylenes, ten methines (one of them olefinic) and seven quaternary carbons (one olefinic and three carbonyls). So we suggested that compound **1** was a positional isomer of **2** with *β*-ester group at carbon C-3 instead of C-1. This assumption is confirmed by comparison of their ^{13}C NMR spectra with that of scutecyprin, a compound with no substitutes in ring A, as it was described by us [7,8]. In the ^{13}C NMR spectrum of **1** the signal for the carbon atoms C-4 is downfield shifted with 2.89 ppm, compared with that of scutecyprin, while that for C-18 is high-field shifted with 5.65 ppm. In the ^{13}C NMR spectrum of **2** the value of these signals are not changed but the chemical shifts of carbon C-10 is downfield shifted by 7.42 ppm. The acceptance that C-3 is an oxygenated methine carbon atom is in agreement with the observed correlations:(1) in the HSQC spectrum the triplet at 5.38 ppm has a cross-peak with δ_C 71.69; (2) in the HMBC spectrum δ_H 5.38 has a cross-peak with δ_C 63.5 (C-4) and (3) in the ^{1}H-^{1}H COSY spectrum that signal has cross-peaks with signals of H-1α, H-2β and H-18B.

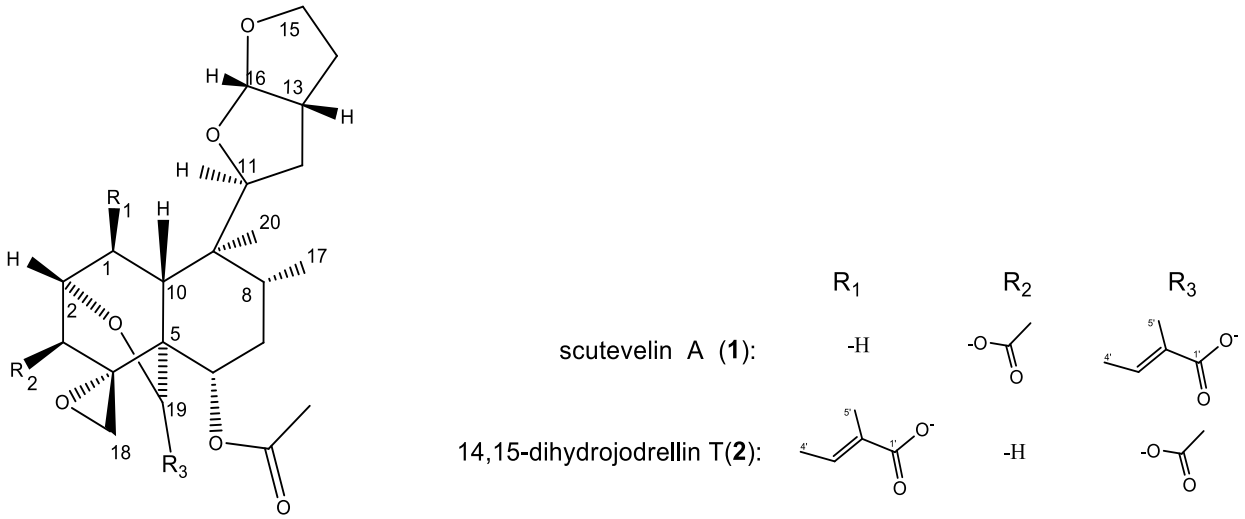

Figure 1. Structures of the *neo*-clerodanes.

Finally, the attachment of the tigloyloxy and the two acetoxyl groups to the *neo*-clerodane skeleton in **1** needed to be rigorously established. The carbonyl resonance at δ_C 169.95 and downfield doublet of doublets at δ_H 4.64 (J=11.1, 4.6), assigned for an oxygenated methine proton, are very common structural features for clerodane diterpenoids with acetate at C-6α position [5-7] as 14,15-dihydrojodrelin T (see Table 2). This conclusion was also supported by the HMBC correlations from H-6 to C-4 (δ_C 63.49), C-5 (δ_C 42.51), C-7 (δ_C 33.40), C-19 (δ_C 90.70), C=O (δ_C 169.95) and ^{1}H-^{1}H COSY correlations of H-6 with H-7*α* and H-7*β*. The tiglate group was assigned to be attached to C-19 based on the HMBC correlation of H-19/C-1'. The NOESY correlations of H-11 with H-3, H_3-17, H_3-20 and of H-19 with H_3-20 indicated that these protons were cofacial and *α*-oriented. Respectively, the NOESY correlations of H-6 with H-8 and H-10 reveal its *β*-position.

Table 1. ^{1}H and ^{13}C NMR spectral data[a] and ^{1}H-^{1}H COSY and HMBC correlations for scutevelin A (**1**).

Position	δ ^{13}C, nH	δ ^{1}H	m, *J* (in Hz)	^{1}H-^{1}H COSY	HMBC
1	23.1, CH_2	2.23 (*α*) 1.79 (*β*)[d]	dt[f], 14.9; - ov m	1*β* , 2*β*, 3*α*[b], 10*β* 1*α*, 10*β*	2[ew], 10[ew]
2	67.7, CH	4.07 (*β*)	ov m	1*α*, 3*α*,	3[b], 4, 19[b]
3	71.7, CH	5.38 (*α*)	t[e], 2.2	1*α*[b] , 2*β*, 18B	4[b]
4	63.5, C				
5	42.5, C				
6	67.9, CH	4.64	dd, 11.1; 4.6	7*α*, 7*β*	4[b], 5[b], 7, 19[b], 1'[b]
7	33.4[g], CH_2	1.65 (*α*)[d] 1.39 (*β*)[d]	ov m m	6 6, 17[b]	
8	35.0, CH	1.66[d]	ov m	17	
9	41.4, C				
10	40.8, CH	2.05 (*β*)[d]	ov m	1*α*,1*β*	
11	85.8, CH	4.05	ov m	12α, 12*β*	10, 7 (12)[b], 20[b]
12	33.5[g], CH_2	1.66 (*α*)[d] 1.97 (*β*)[d]	ov m m	11, 12*β* 11, 12*α*, 13*β*	
13	41.8, CH	2.86 (*β)*	m	12*β*, 14*β*, 16*β*	15[ew]
14	32.6, CH_2	1.69 (*α*)[d] 2.15 (*β*)[d]	ov m m	13*β*, 14*β*, 15 14*α*, 15	 13[b], 15[b]
15	68.3, CH_2	3.87	m	14*α*, 14*β*	13[b], 16
16	108.2, CH	5.64 (*β*)	d, 5.0	13	11, 14[b], 13, 15
17	16.7, CH_3	0.89	d, 5.8	7*β*[b], 8	
18	44.6, CH_2	2.91 (B[c]) 2.88 (A)	d, 4.3 d, 4.3	18A 18B	4, 5[ew], 3[ew], 4, 5[ew]
19	90.7, CH	6.77	s	-	1′, 2, 4,5[b]
20	14.0, CH_3	1.14	s		
1′ (C=O)	166.0, C				
2′	128.5, C				
3′	139.0, CH	7.07	qq, 7.1;1.1	4′, 5′[b]	1′[ew], 4′[ew], 5′[ew]
4′	14.7, CH_3	1.80	br d, 7.6	3′	2′, 3′, 1′[b]
5′	11.9, CH_3	1.88	br s	4′[b]	2′, 3′, 1′
3^1 (C=O)	169.4, C				
3^2 (Me)	20.8[f], CH_3	2.05	ov s		C=O
6^1 (C=O)	170.0, C				
6^2 (Me)	20.9[f], CH_3	1.78	s		C=O

[a] $CDCl_3$, ^{1}H 600.13 MHz, δ_{ref} 7.26; ^{13}C 150.9 MHz, δ_{ref} 77.0 ppm; ov = overlapped; br = broad [b] weak; [ew] extremely weak; [c] endo hydrogen; [d] data from HSQC; [e] apparent multiplicity; [f,g] signals with the same letters may be interchangeable.

Table 2. ^{1}H and ^{13}C NMR spectral data for 14,15-dihydrojodrelin T (**2**).

Position	δ ^{13}C, nH	δ ^{1}H	m, J (in Hz)
1	66.8, CH	5.51	m
2	69.6, CH	4.42	dt, 5.0; 2.6
3	31.1, CH_2	2.47 1.86	br d, 15.3 dd, 15.0;3.0
4	59.7, C		
5	43.0, C		
6	67.7, CH	4.65	dd, 12.0; 4.8
7	32.6, CH_2	1.64-1.72 (α) 1.38-1.43 (β)	ov m m
8	35.3, CH	1.56-1.60	ov m
9	40.7, C		
10	48.2, CH	2.03	d, 2.6
11	86.0, CH	4.06	dd, 11.3; 5.4
12	32.9, CH_2	1.64-1.72 (α) 1.96 (β)	ov m ov m
13	41.7, CH	2.63-2.68	m
14	33.2, CH_2	1.64-1.72 (α) 2.06-2.12 (β)	m ov m
15	68.3, CH_2	3.77-3.80	m
16	108.1, CH	5.35	d, 5.0
17	16.1, CH_3	0.89	d, 6.6
18	50.3, CH_2	3.05 (B^c) 2.45 (A)	d, 4.7 d, 4.6
19	90.4, CH	6.68	s
20	15.8, CH_3	1.24	s
1′ (C=O)	166.4, C		
2′	128.1, C		
3′	137.8, CH	6.81	qq, 7.3;1.8
4′	14.6, CH_3	1.82	ov br d
5′	12.0, CH_3	1.81	ov br s
3^1 (C=O)	169.8, C		
3^2 (Me)	21.8, CH_3	2.14	s
6^1 (C=O)	169.6, C		
6^2 (Me)	21.2, CH_3	1.96	s

[a] $CDCl_3$, ^{1}H 600.13 MHz, δ_{ref} 7.26; ^{13}C 150.9 MHz, δ_{ref} 77.0 ppm; ov = overlapped; br = broad [b] weak; [ew] extremely weak; [c] endo hydrogen; [d] data from HSQC; [e] apparent multiplicity; [f,g] signals with the same letters may be interchangeable.

CONCLUSION

The structure of a newly isolated *neo*-clerodane diterpenoid scutevelin A was unambiguously assigned by its spectral data that are very close to those of theknown 14,15-dihydrojodrellin T.

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