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Clerodane diterpenoids from *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin

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Three clerodane diterpenoids, 6-acetyl teucrin F (**1**), teucrin E acetate (**2**) and 3α-acetoxy-teucvin (**3**) were isolated by the phytochemical investigation of the acetone extract from the aerial parts of *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin, (Lamiaceae). The structure and stereochemistry of the compounds were established by IR spectrum, HR-ESIMS and different ^{1}H NMR and 2D-NMR techniques. Compounds **1** and **2** are isolated for the first time from a natural source while compound **3** is a new natural product.

Keywords: *Teucrium scordium* subsp. *scordioides*, Lamiaceae, *neo*-clerodane diterpenes, *nor*-clerodane diterpenes.

INTRODUCTION

The genus *Teucrium* (Lamiaceae) covers about 360 species spread in different climatic zones. The plants are honey-bearing with medical use. Paws and infusion of the plants have been applied for centuries in open wound healing, treatment of gastrointestinal pains, diabetes, inflammations, rheumatism and other disorders. They are also used as diuretic, antipyretic, tonic, diaphoretic, analgesic [1]. Furanoid clerodane diterpenoids are the main biological active constituents of the plants from *Teucrium* genus [2-5].

The species *T. scordium* L. covers two subspecies, *scordium* and *scordioides* (Shreb.) Maire et Petitmengin. From 1985 to 1988 Papanov and Malakov isolated five new furanoid *neo*-clerodane diterpenoids from *T. scordium* subsp. *scordium*: teuscordinon (**4**) [6], 6-ketoteuscordin (**5**) and 6*α*-hydroxyteuscordin (**6**) [7], 6*β*-hydroxyteuscordin (**7**) [8], 2-keto-19-hydroxyteuscordin (**8**) [9], besides the previously known diterpenoids: 2*β*,6*β*-dihydroxyteuscordin (= teugin, **9**) [8, 10], teucrin E (**10**) [9, 11] and teucrin H$_4$ (**11**) [9, 12] (Fig. 1). In 1985 Jakupovic *et al.* [13] reinvestigated this species (collected at the Heidsee, Rosengarten-Raibach, near Schwäbisch-Hall, West Germany) and obtained 13 compounds; the known *neo*-clerodanes **4, 6, 7, 9**, **10**, dihydroteugin (**12**) [14], teucjaponin B acetate (**13**) [15], teucroxide (**14**) [16] and five new furano-clerodanes: 2,3-dehydroteucrin E (**15**), 2*β*,6*α*-dihydroxyteuscordin (**16**), 2*β*-hydroxyteuscordinone (**17**), 6,20-bisdeacetylteupyreinidin (**18**) and 6-deacetyl-teupyreinidin (**19**) (Fig. 1).

The experimental data about chemistry and pharmacology of *T. scordium* L. subsp. *scordioides* are scarce. The previous studies had shown the presence of polyphenols, *β*-sitosterol, tannins and bitter principles compounds [17], and the monoterpene menthofuran (11.9%) predominated in the essential oil of the plant [18]. In the literature there are no data about diterpenoid contents. Cytotoxicity and antimicrobial activity of cyclohexane, dichloromethane and methanol extracts of *T. scordium* subsp. *scordioides* have been studied [19]. Cyclohexane and dichloromethane extracts showed high cytotoxicity against MDA-MB-361 cells (IC_{50} = 130.33 ± 0.1 *μ*g/mL and I_{C50} = 189.89 ± 3.99 *μ*g/mL, respectively). Dichloromethane extract was more effective against MDA-MB-453 cell line with IC_{50} = 130.33 ± 0.1 *μ*g/mL. The methanol extract possessed no cytotoxicity against breast cancer cell lines, MDA-MB-361 and MDA-MB-453. The extracts of the plant had shown weak antibacterial activity on *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Bacillus subtilis* and didn't possess any activity against *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, and *Candida albicans*.

Continuing our ongoing research of *neo*-clerodane diterpene composition of *Teucrium* species [20] we investigated *T. scordium* L. subsp. *scordioides*. We report herein on the isolation and structure elucidation of three clerodane diterpenes, 6-acetyl teucrin F (**1**), teucrin E acetate (**2**) and 3α-acetoxy-teucvin (**3**) (Fig. 2).

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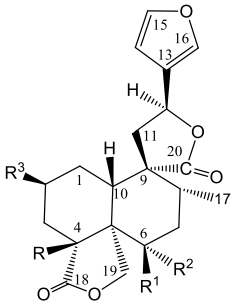
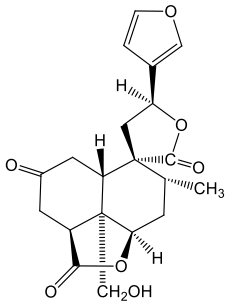
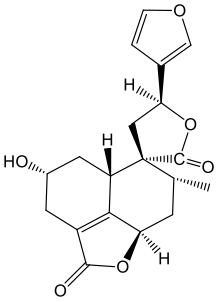


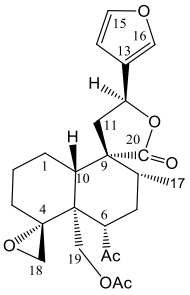


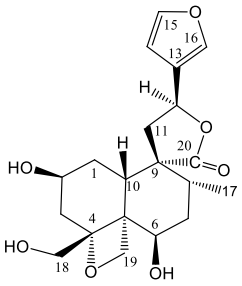


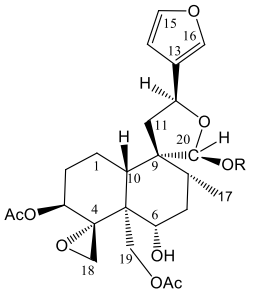
	R	R_1	R_2	R_3	Δ
teuscordinone (**4**)	H	= O		H	Δ^3
6-ketoteuscordin (**5**)	H	= O		H	-
6α-hydroxyteuscordin (**6**)	H	H	OH	H	Δ^3
6*β*-hydroxyteuscordin (**7**)	H	OH	H	H	Δ^3
teugin (**9**)	H	OH	H	OH	Δ^3
teucrin E (**10**)	H	H	OH	H	-
dihydroteugin (**12**)	H	OH	H	OH	-
2,3-dehydroteucrin E (**15**)	H	H	OH	H	Δ^2
2*β*,6*α*-dihydroxyteuscordin (**16**)	H	H	OH	OH	Δ^3
2*β*-hydroxyteuscordinone (**17**)	H	= O		OH	Δ^3

2-keto-19-hydroxyteuscordin (**8**)

teucrin H_4 (**11**)





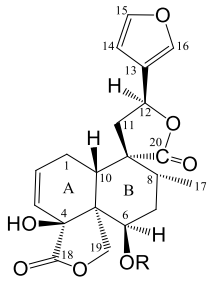


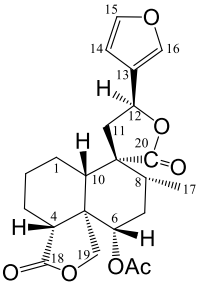
teucjaponin B acetate (**13**)

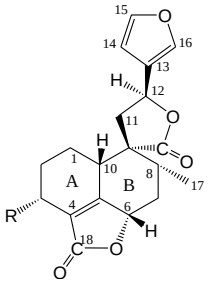
teucroxide (**14**)

	R
6,20-bisdeacetylteupyreinidin (**18**):	H
6-deacetylteupyreinidin (**19**):	Ac

Fig. 1. Structures of the previously isolated clerodane diterpenoids from *T. scordium* subsp. *scordium*.







	R
6-acetyl teucrin F (**1**):	Ac
teucrin F (**20**):	H

teucrin E acetate (**2**)

	R
3α-acetoxy-teucvin (**3**):	OAc
teucvin (**21**):	H

Fig. 2. Structures of the isolated and used in the discussion clerodane diterpenoids.

Although compounds **1** and **2** were previously semi-synthetically prepared by acetylation with acetic anhydride / pyridine of the corresponding hydroxyl derivatives isolated from *Teucrium hamaedris*, in the present study they were isolated for the first time from a plant source. For compound **1** synthesized before by acetylation of teucrin F (**20**), almost all ^{1}H and ^{13}C NMR signals had been

assigned [21]. We only interchanged reported values for C-2 and C-3. Compound **2**, synthesized before by acetylation of teucrin E (**10**), had been published with ^{1}H NMR data only [22]. In this study, fully assigned ^{13}C NMR data of compounds **1** and **2** are included in Table 1.

EXPERIMENTAL

Structural data

^{1}H NMR spectra were recorded on Bruker Avance II+ spectrometers, operating at 600.01 MHz. ^{13}C NMR spectra were recorded at 150.89 MHz spectrometer. TMS was used as an internal standard and $CDCl_3$ as solvent (δ_C 76.00, δ_H 7.260). Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. The IR spectra were registered in KBr pellet on a Bruker Tensor 27 spectrometer from 4000 cm^{-1} to 400 cm^{-1} at resolution 4 cm^{-1} with 70 scans. The mass spectra were measured on Hewlett Packard 6890 GC System Plus/5973 MSD. The melting points were determined by a STUART SMP-10 digital melting point instrument. Specific optical rotation values were measured on Jasco P-2000 polarimeter (Tokyo, Japan) at D line of sodium lamp at 20 ºC by using 0.5 dm quartz cell. The $[\alpha]_D$ are given in deg.cm^3.g^{-1}.dm^{-1}, concentration in g.cm^{-3}.

Plant material

The aerial parts of *Teucrium scordium* L. subsp. *scordioides* (Shreb.) Maire et Petitmengin were collected in Jun 2019 around village Staro Orehovo near Varna, Bulgaria, and voucher specimens (n. 7212) were deposited in the Herbarium of the Higher Institute of Agriculture at Plovdiv, Bulgaria.

Extraction and isolation

Dried and finely powdered material (1.100 kg) were extracted with Me_2CO (3 × 5 L) at room temperature for a week. After filtration, the solvent was evaporated to dryness under reduced pressure and low temperature (<40 °C) yielding a gum (20.0 g), which was dissolved in aq. Me_2CO (40 % H_2O, v/v, 200 mL). This solution was cooled to 4 °C for 24 h and filtered. The filtrate was extracted with $CHCl_3$ (3 × 150 mL) and the organic layer was dried (Na_2SO_4) and evaporated in vacuum to afford a residue (5.8 g, bitter fraction). This residue was subjected to CC (70 g silica gel Merck n. 7734, deactivated with 10% H_2O, w/w). Pure petroleum ether (12 L), followed by a gradient of CH_2Cl_2 – CH_3OH mixtures (10:0 to 9.7:0.3) were used as eluting solvents. Eluting with CH_2Cl_2 – CH_3OH mixtures 9.8:0.23 resulted in the isolation of three diterpenoids, 25 mg of 6-acetyl teucrin F (**1**), 18 mg of teucrin E acetate (**2**) and 25 mg of 3α-acetoxy-teucvin (**3**).

*6-Acetyl teucrin F (**1**)*

Colorless powder. MP: 207-209 C° (from MeOH), $[\alpha]_D$ + 14 ± 1 (c = 0.77, MeOH), TLC: R_f 0.58 (EtOAc). IR ν_{max} (KBr): 3437, 2963, 2934, 1761, 1505, 1450, 1373, 1239, 1178, 1043, 1023, 875, 754, 603 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 1. Positive ESIMS (70 eV, direct inlet) m/z (rel. int. in %): 439 $[M+Na]^+$ (92.4), 423 (22.3), 399 (9.1), 357 (14.6). HRESIMS m/z 439.1367 $[M+Na]^+$, (calcd. for $C_{22}H_{24}O_8Na$: 439.1369).

*Teucrin E acetate (**2**)*

Colorless powder. MP: 201-203 C° (from MeOH), TLC: R_f 0.52 (EtOAc). IR ν_{max} (KBr): 3147, 2965, 2933, 1764, 1506, 1473, 1373, 1240, 1180, 1025, 875, 754, 727, 667, 603 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 1. Positive ESIMS (70 eV, direct inlet) m/z (rel. int. in %): 403 $[M+H]^+$ (95.4), 343 (55.6), 297 (8.5). HRESIMS m/z 403.1757 $[M+H]^+$, (calcd. for $C_{22}H_{26}O_7H$ 403.1753).

*3α-Acetoxy-teucvin (**3**)*

Colorless powder. MP: 223-224 C° (from MeOH), $[\alpha]_D$ + 201 ± 1 (c = 0.9, MeOH), TLC: Rf 0.49 (EtOAc). IR ν_{max} (KBr): 3146, 2932, 1761, 1506, 1450, 1373, 1239, 1178, 1043, 1022, 974, 875, 753, 603 cm^{-1}. ^{1}H and ^{13}C NMR: see Table 1. Positive ESIMS (70 eV, direct inlet) m/z (rel. int. in %): 409 $[M+Na]^+$ (96.0), 349 (4.2), 327 (14.3), 309 (5.7). HRESIMS m/z 409.1270 $[M+Na]^+$ (calcd. for $C_{21}H_{22}O_7Na$: 409.1263).

RESULTS AND DISCUSSION

Based on the pseudo-molecular positive ion peak at m/z 439.1367 $[M+Na]^+$ in HR-ESIMS of compound **1** (see supplementary data section) was established molecular formula $C_{22}H_{24}O_8$ (calcd. for $C_{22}H_{24}O_8Na$: 439.1369) indicating 11 degrees of unsaturation. In its IR spectrum were observed absorptions consistent with the presence of a furan ring (3147, 1505 and 875 cm^{-1}), lactone and acetate functions (1761 broad, 1178 and 1239 cm^{-1}) and hydroxyl group (3437 cm^{-1}).

The ^{13}C NMR spectrum displayed the presence of twenty-two carbons, and a DEPT experiment identified two methyl (one of them from acetyloxy group), four methylene (one of them oxygenated, CH_2-19), nine methine (including three aromatic and two olefinic, five methines are oxidized) and seven quaternary carbon atoms (including two olefinic, one furan, one oxygenated and three for carbonyl groups – one from acetyloxy group and two from γ-lactones) (see Table 1).

Table 1. 6-Acetyl teucrin F (**1**), teucrin E acetate (**2**) and 3α-acetoxy-teucvin (**3**) NMR data [a]

Position	**1**			**2**			**3**		
	δ ^{13}C, nH	δ ^{1}H	m, *J* (in Hz)	δ ^{13}C, nH	δ ^{1}H	m, *J* (in Hz)	δ ^{13}C, nH	δ 1H	m, *J* (in Hz)
1*α*	25.19, CH_2	2.085	m	25.03, CH_2	1.57	m[c]	19.93, CH_2	2.13	ov m[c]
1β		2.589	m[c]		2.06	ov m[c]		1.68	ov m[c]
2*α*	129.85, CH	6.097	ddd, 10.0; 5.2; 2.1	22.5, CH_2	2.03	ov m[c]	27.92, CH_2	2.18	ov m[c]
2*β*					1.51	m[c]		1.69	ov m[c]
3*α*	125.56, CH	5.547	m	23.1, CH_2	1.45	m[c]	60.39, CH	5.601	br s
3*β*					1.90	m[c]		-	-
4	75.85, C	-		46.01, CH	2.165	br s	124.66, C	-	
5	47.84, C	-		45.98, C	-		167.46, C	-	
6*α*	68.04, CH	5.411	dd, 4.0; 2.0	78.33, CH	-		78.04, CH	4.815	dd, 5.5, 3.7
6*β*		-			4.757	dd, 12.1; 3.8			
7*α*	31.84, CH_2	2.343	ddd, 14.8, 12.7, 2.0	31.82 CH_2	1.81	ov[c]	34.76, CH_2	2.24	m[c]
7*β*		1.694	dt, 14.8; 4.0		2.21	ov[c]		2.37	m[c]
8*β*	32.88, CH	2.179-2.095	m	37.95, CH	1.77	ov[c]	35.74, CH	1.938	ddq, 12.6; 3.3; 6.8
9	51.57, C	-		50.89, C	-		53.59, C	-	
10*β*	37.23, CH	2.653-2.596	m	47.43, CH	1.79	ov[c]	42.00, CH	2.70	m[c]
11B	42.57, CH_2	2.574	dd, 14.3; 8.8	41.72, CH_2	2.365	dd, 14.5; 8.9	40.71, CH_2	2.584	br d, 8.5
11A		2.474	dd, 14.3; 8.8		2.445	dd, 14.5; 8.9		2.584	br d, 8.5
12*α*	72.12, CH	5.427	ov	71.88, CH	5.376	t, 8.9	71.76, CH	5.462	t, 8.5
13	124.59, C	-		124.69, C	-		124.08, C	-	
14	107.98, CH	6.406	dd, 1.8; 0.8	107.90, CH	6.376	dd, 1.8; 0.8	107.88, CH	6.392	br s
15	144.33, CH	7.459	t, 1.8	144.30, CH	7.451	br d, 1.8	144.33, CH	7.456	t, 1.6
16	139.62, CH	7.477	dt, 1.8; 0.8	139.62, CH	7.440	m	139.55, CH	7.465	br s
Me-17	16.44, CH_3	1.004	d, 6.8	16.40, CH_3	1.026	d, 6.6	16.88, CH_3	1.096	d, 6.8
18	176.04, C	-		176.54	-		170.72	-	
19B[b]	68.99, CH_2	4.536	d, 11.3	68.39, CH_2	4.788	d, 11.3	-	-	-
19A		4.118	d, 11.3		4.307	d, 11.3	-	-	-
20 (C=O)	177.51, C	-		178.49, C	-		174.92, C	-	
6^1 (C=O)	170.15, C	-		170.84, C	-		-		
6^2 (Me)	21.48, CH_3	2.041	s	20.97, CH_3	2.038	s	-		
3^1 (C=O)							170.50, C	-	
3^2 (Me)							21.17, CH_3	2.060	s

[a] $CDCl_3$, ^{1}H 600.01 MHz, δ_{ref} 7.26; ^{13}C 150.9 MHz, δ_{ref} 77.0 ppm, TMS as an internal standard; [b] endo hydrogen with respect to ring B; [c] data from HSQC; ov overlapped signal.

The presence of furan ring in the molecule was confirmed by the signals at δ_C 124.59 (C-13), 107.98/δ_H 6.406 (dd, CH-14), 144.33/7.459 (t, CH-15) and 139.62/7.477 (dt, CH-16) in the ^{13}C and ^{1}H NMR spectra. Assignments of the furan protons to the corresponding carbon atoms were in agreement with the data from the HSQC spectrum and the observed HMBC correlations. The presence of two *γ*-lactone rings, formed between C-20 and C-12, as well as C-18 and C-19, was supported by the specific signals in the ^{1}H and ^{13}C NMR spectra. For carbonyl groups the signals were at δ_C 177.51 (C-20) and 176.04 (C-18), for oxygenated methine group – at δ_C 72.21/δ_H 5.427 t (CH-12) and for oxygenated methylene group – at δ_C 68.99/δ_H 4.536 d and 4.227 d (CH_2-19). These conclusions are in agreement with the correlations observed in the HMBC and COSY spectra.

Attachment of the acetyloxy group at C-6 was established by the HMBC correlations from H-6*α*

(δ_H 5.411, dd) to 6^1 (δ_C 170.15, C=O) and from 6^2 (δ_H 2.041, 3H) to C-6 (δ_C 68.04). The location of the acetyl ester in *β*-position and H-6 in *α*-position was in agreement with the small value of 4.2 Hz and 2.0 Hz for the coupling constants of the δ_H 5.411 dd due to the equatorial methine proton H-6. This conclusion was supported by the observed interaction in the NOESY experiment of H-6 with the downshifted doublet at δ_H 4.536, which was assigned for one of the methylene protons of oxygenated C-19 (δ_C 68.99).

Both olefin carbons, resonated at δ_C 129.85 and 125.56, are methine atoms. This fact determines the double bond to be formed between C-1 – C-2 or between C-2 – C-3. On the ground of the multiplicity and the coupling constants value (Table 1) of the signal for H-3 (dq, J = 10.0, 1.3) we concluded that in compound **1** Δ^2 olefinic bond was present. In case of presence of Δ^1 double bond the signal for H-1, equivalent to H-3 in compound with Δ^2 olefinic bond, would be with a more complicated multiplet structure due to the presence of an additional adjacent proton (H-10).

^{1}H and ^{13}C NMR data of **1** are identical in all respects with those reported by Rodriguez *et al.* [21] for 6-acetyl teucrin F obtained by treatment of teucrin F (**20**) with acetic anhydride/pyridine. Authors have assigned the signals at δ_C 125.56 of C-2 and 129.85 of C-3. Based on the observed HSQC correlations between the signals δ_C 129.85/δ_H 6.097 and δ_C 125.56/δ_H 5.547 we exchanged the signals of these methine carbons: δ_C 129.85 of C-2 and δ_C 125.56 of C-3.

The relative configuration of **1** was determined by the observed NOESY correlations H-1*β*/H-12*α*, H-11A/H-12*α*, H-11B/H-14 and H-11B/H-16. The NOESY correlations Me-17/H-11B, Me-17/H-14 and Me-17/H-16 indicated that the furan ring was in the *β*-configuration. Moreover, the correlations of H-6 with H-7*α*, H-19A, H-1*α* /H-19A and H-7*α* / H-19B, showed their co-facial relationship and were assigned in *α*-position. On the ground of all shown above data for **1** a structure of 6-acetyl teucrin F was assigned as depicted in Figure 2.

The HR-ESIMS of compound **2**, with the trivial name teucrin E acetate, showed a pseudo-molecular positive ion peak at m/z 403.1757 $[M+H]^+$, which indicated the molecular formula of $C_{22}H_{26}O_7$, (calcd. for $C_{22}H_{26}O_7H$: 403.1753). Compared to compound **1**, the molecular formula of teucrin E acetate contains two additional hydrogen atoms, and one oxygen atom less, corresponding to 10 degrees of unsaturation

In the IR spectrum of **2** were observed absorption bands consistent with the presence of a furan ring (3147, 1506, 1113 and 875 cm^{-1}), lactone and acetate groups (1764 broad, 1180 and 1240 cm^{-1}).

The ^{13}C NMR spectrum displayed the presence of twenty-two carbons, and a DEPT experiment identified two methyl, six methylene, eight methine (three from furan double bonds, four methines are oxygenated) and six quaternary (two of which were aliphatic, one was for aromatic carbon and the rest three for carbonyl groups at δ_C 176.54 and 178.49 for *γ*-lactones and 170.84 for acetate) carbon atoms (Table 1).

The ^{1}H and ^{13}C NMR spectral data of **2** revealed very similar structure to that of compound **1**. The only differences in the NMR spectra of **2** were the lack of the characteristic signals in the NMR spectrum of **1** for two olefin methine carbons C-2 and C-3 at δ_C 129.85, CH/δ_H 6.097 ddd and δ_C 125.56, CH/δ_H 5.547 dq, and for quaternary oxygenated carbon atom C-4 at δ_C 75.85, C-4). Instead, characteristic signals for C-4/H*β* methine group appeared at δ_C 46.01, CH /δ_H 2.165 br s.

The acetyloxy substituent was localized at C-6 again, according to the observed HMBC correlations from H-6 to C-6^1 (CO) and from the methyl protons H_3-6^2 to C-6, and the COSY correlations of H-6 to H-4, H-7*α*, H-7*β* and H-8. This time the acetyloxy group was determined to be *α*-oriented. This conclusion was based on the large value of the $J_{6,7}$ constant for the signal of the axial H-6*β* (δ_H 4.757 dd, J = 12.1, 3.8) and from the observed NOESY interaction between H-6*β*/H-4*β*, H-6*β*/8*β* and H-6*β*/10*β*.

The furan ring was placed in *β*-position according to the observed in the NOESY spectrum correlations of H-12*α* with H-1*β* and H-11*α*, of H-11β with H-14 and H-16 and of Me-17 with H-11*β*, H-14 and H-16. The interaction of H-6 with H-4, H-8 and H-10 demonstrated their co-facial relationship and *β*-orientation. On the other hand, the interactions of H-7*α* with H-19A, H_3-6^2 and H_3-17, and of H-19B with H-2*α* revealed their *α*-positions.

^{1}H NMR data of **2** were the same as those of the derivative obtained by acetylation of teucrin E (**10**) [22]. Therefore, the structure of compound **2** was elucidated as teucrin E acetate, as depicted in Figure 2.

For 3*α*-acetoxy-teucvin (**3**) the molecular formula $C_{21}H_{22}O_7$ was established by the pseudo-molecular positive ion peak in its HRESIMS at m/z 409.1270 $[M+Na]^+$ (calcd. for $C_{21}H_{22}O_7Na$: 409.1263) indicating 11 degrees of unsaturation. The odd number of C-atoms suggested a structure with a 19-*nor*-clerodane skeleton or a presence of a methoxy group in the molecule.

In the IR spectrum of **3** absorption bands consistent with the presence of a furan ring (3146, 1506, 1069 and 875 cm^{-1}), lactone and acetate groups (1761 broad, 1178 and 1240 cm^{-1}) were observed.

The ^{13}C NMR spectrum displayed the presence of twenty-one carbons, and a DEPT experiment identified two methyl, four methylene, eight methine (three of them aromatic, oxygenated carbons were five) and seven quaternary (including three carbonyl, at δ_C 174.92 from γ-lactone, 170.72 from α,β-unsaturated γ-lactone, 170.50 from acetate; three olefinic at δ_C 124.08, 124.66, 167.46 and one aliphatic at δ_C 53.59) carbon atoms.

No methoxy group signals were observed in the ^1H and ^{13}C NMR spectra. Characteristic signals for the methylene protons H_2-19 were missing, too. Acetyloxy group signals (δ_C 170.50, C=O; δ_C 21.17/δ_H 2.060 s, CH_3) and a signal at δ_H 5.601 br s for its geminal proton were present in the spectra. The aforementioned data define **3** as a diterpenoid with 19-*nor*-furoclerodane dilactone skeleton with an acetyloxy substituent. Spectral data for the C-11 – C-16 substructure were very close to that for diterpenes **1** and **2**. The formation of C-20 – C-12 γ-lactone and β-oriented furan ring at C-12 in **3** was confirmed by the analysis of the 2D experiments, analogous to that described by the structural elucidation of **1.** Thus, the correlations from methylene protons H_2-11 to C-12, C-13, C-20, from H-12 to C-13, C-14, C-16, from H-14 to C-13, C-15, C-16, from H-15 to C-16 and from H-16 to C-13, C-14, C-15 are presented in the HMBC spectrum. The β-orientation of the furan ring was confirmed by the NOESY interaction of H_3-17 with H-14 and H-16.

The signals at δ_C 124.66 and 167.46, for quaternary olefin carbon atoms, were assigned to C-4 and C-5. They were part of an α,β-unsaturated γ-lactone constructed between C-18 and C-6. This conclusion was supported by the characteristic signal at δ_H 4.815 (dd) for an axial methine proton H-6 geminal to an oxygen atom and by the HMBC correlations from H-7β to C-6 and from methyl protons H_3-17 to C-6. The other possible position of the double bond between C-5 and C-10 was excluded due to the observed COSY correlations of H-10 with H-1β and H-1α and NOESY correlations of H-10 with H-1β, H-2β, H-3β and H-11A.

The linkage of the fragment C-18–O– to the C-6 with alpha bond was established by the observed NOESY correlation of the axial H-6β with H-7β, H-8β and H-10β indicating that all these protons were co-facial and β-oriented.

The acetyloxy group was attached to C-3 on the ground of the H^1-H^1 COSY correlation of the geminal to the acetyloxy group proton H-3β (δ_H 5.601) with H-6β (δ_H 4.815) and with H-10β (δ_H 2.685). This conclusion was supported by the HMBC correlation from H-1α (δ_H 2.13) to C-3 (δ_C 60.39) and from H-2α (δ_H 2.18) to the methyl carbon of the acetoxy group C-3^2 (δ_C 21.17). The *a*-orientation of the acetate group was determined by the small value of the *J* constants of the signal (br s) for the pseudo-equatorial H-3 by the coupling with the adjacent protons H-2α and H-2β. This assumption was confirmed by the NOESY interaction of H-3 with H-2α and with H-2β indicating that H-3 was in pseudo-equatorial position and in this case beta-oriented.

Therefore, the new compound **3** was assigned as 3α-acetoxy derivative of teucvin (**21**) isolated by Fujita and Uchida from *Teucrium viscidurn* var. *miquelianum* [23].

CONCLUSION

The 6-acetyl teucrin F (**1**) and teucrin E acetate (**2**) isolated in this study are *neo*-clerodane diterpenoids belonging to a furoclerodane series with 10β-18,19;20,12-diolide functions. It can be noted that compounds from the same series have been previously found in *T. scordium* L. subsp. *scordium*. In contrast, diterpenoids with furoclerodane-10β-18,6α;20,12-diolide structure as that of the new 3α-acetoxy-teucvin (**3**) have not been detected in *T. scordium* L. *subsp. scordium* so far. The accumulation of furoclerodane compounds with 10β-18,19;20,12-diolide functions in *Teucrium scordium* L. subsp. *scordioides* and *T. scordium* L. subsp. *scordium* could be of chemotaxnomic interest.

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REFERENCES

1. A. Ulubelen, G. Topcu, U. Sonmez, *Studies in Nat. Prod. Chem.*, **23**, 591 (2000).
2. L. Rodriguez-Hahn, B. Esquivel, J. Cardenas, *Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products*, **II** (3), 121 (1994).
3. M. Bruno, F. Piozzi, S. Rosselli, *Nat. Prod. Rep*., **19**, 357 (2002).
4. F. Piozzi, M. Bruno, S. Rosselli, A. Maggio, *Heterocycles*, **65** (5), 1221 (2005).

5. A. Fiorentino, B. D'Abrosca, S. Pacifico, M. Scognamiglio, G. D'Angelo, M. Gallicchio, A. Chambery, P. Monaco, *Phytochem.*, **72**, 2037 (2011).
6. G. Y. Papanov, P. Y. Malakov, F. Bohlmann, *Phytochem.*, **20**, 170 (1981).
7. G. Y. Papanov, P. Y. Malakov, *Z. Naturforsch*., **36b**, 112 (1981).
8. G. Y. Papanov, P. Y. Malakov, *Z. Naturforsch*., **37b**, 519 (1982)
9. G. Y. Papanov, P. Y. Malakov *Phytochem.*, **24** (2), 297 (1985).
10. M. Bruno, G. Savona, C. Pascual, B. Rodriguez, *Phytochem*. **20**, 2259 (1981).
11. D. P. Popa, A. M. Reinbol'd, *Khim. Prirodn. Soedin*., **67, 27** (1972).
12. E. Gacs-Baitz, L. Radics, G. B. Oganessian, V. A. Mnatsakanian, *Phytochemistry*, **17**, 1967 (1978)
13. J. Jakupovic, R. N. Baruah, F. Bohlmann, W. Quack, *Planta Medica*, **51,** 341 (1985).
14. G. Savona, M. Z.Garcia-Aalvarez, B. Rodriguez, *Phytochem.*, **21**, 721 (1982).
15. J. Fayos, F. Fernandez-Gadea, C. Pascual, A. Perales, F. Piozzi, M. Rico, B. Rodriguez, G. Savona, *J. Org. Chem.* **49**, 1789 (1984).
16. M .C. Garcia-Alvarez, G. Lukacs, A. Neszmelyi, F. Piozzi, B. Rodriguez, G. Savona, *J. Org. Chem.* **48**, 5123 (1983).
17. R. J. Grubešić, D. Kremer, S. Vladimir-Knežević, J. V. Rodríguez, *Cent. Eur. J. Biol*., **7**, 542 (2012).
18. N. Radulovic, M. Dekic, M. Joksovic, R. Vukicevic, *Chem. & Biodiv*., 9, 106 (2012).
19. T. Kundakovic, M. Milenkovic, A. Topic, T. Stanojkovic, Z. Juranic, B. Lakušic, *African J. Microbiol. Res*., **5**, 2950 (2011).
20. P. I. Bozov, P. N. Penchev, *Phytochem. Lett.*, **31**, 237 (2019).
21. M. C. Rodriguez, J. Barluenga, G. Savona, F. Piozzi, O. Servettaz, B. Rodriguez, *Phytochem*., **23**, 1465 (1984).
22. A. M. Reinbol'd, D. P. Popa, *Chemistry of Natural Compounds*, **10**, 600 (1974).
23. E. Fujita, I. Uchida, *J. C. S. Perk&t,* **I**, 1547 (1974).