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Neo-clerodane diterpenoids from *Teucrium polium* subsp. *vincentinum* (rouy) D. Wood

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ABSTRACT

Four *neo*-clerodane diterpenoids, the new polivincins A – C and the known teulamifin B, were isolated by the phytochemical investigation of the acetone extract prepared from the aerial parts of *Teucrium polium* subsp. *vincentinum*, (Lamiaceae). The structure and stereochemistry of the compounds were established by IR, HRMS and different (Al-Khalil, 1995) NMR and 2D-NMR techniques.

1. Introduction

The genus *Teucrium* (Lamiaceae) has been thoroughly studied as a rich source of *neo*-clerodane diterpenoids with a great variety of biological activity. *T. polium*, a species belonging to the genus *Teucrium*, is a medicinal plant used in traditional folk medicine to treat many diseases such as abdominal pain, indigestion and diabetes (Al-Khalil, 1995; Said et al., 2002; Ljubuncic et al., 2006). The taxonomy studying of the section *Polium* of the genus determined 125 taxa and many variances (Navarro and El, 2000). Bruno and co-workers drew attention to the distribution of *neo*-clerodanes in the subspecies of the species *T. polium* (Bruno et al., 2003). They suggested that *T. capitatum* is taxonomically close to *T. polium* subsp. *polium* based on the presence of *capitatin* and *auropolin* in both species. The authors emphasized the fact that the diterpenoids, isolated by them from authentic *T. polium* subsp. *polium*, are distinctly different from those occurring in all the taxa indicated as *T. polium*. They underlined the necessity of a revision of these identifications. This question provokes our interest to determine diterpenoid content of the subspecies of *T. polium* growing in Bulgaria.

Five known *neo*-clerodane diterpenoids, teucrin P_1, teucrin H_3, montanin B, 19-deacetylteuscorodol, teucroxide and six new *neo*-clerodans, teupolins I – V and teulamifin B, were reported by Malakov et al. from Bulgarian plant *T. polium* designated as subsp. *polium* (Malakov et al., 1979, 1982; Malakov and Papanov, 1983; Malakov et al., 1988). Capitatin and auropolin were not detected. In Flora of Bulgaria (Jordanov et al., 1989) for species *polium* of genus *Teucrium* were described two subspecies, *capitatum* (L.) Arcangeli and *vincentinum* (Rouy) D. Wood. The authors noticed that subspecies polium of species polium was not spread in Bulgaria. Since, Malakov had gathered plant material from the area "Besaparski ridove" near Plovdiv, where *Teucrium polium* subsp. *capitatum* was spread, we decided to investigate the subsp. *vincentinum* (Rouy) D. Wood. The collected by us plant material was identified by Professor Rumen Mladenov from the Department of Botany and Education in biology, Plovdiv University, as *Teucrium polium* subsp. *vincentinum* (Rouy) D. Wood.

We report here on the structure elucidation of three new *neo*-clerodane diterpenoids, polivincins A–C (**1**-**3**) obtained from the acetone extract of the aerial parts of *Teucrium polium* subsp. *vincentinum*, besides the previously known teulamifin B (**4**) (Fig. 1).

2. Results and discussion

The bitter fraction obtained from the acetone extract of the plants was separated to three subfractions (**I-III**). For compound **1**, named polivicin A (received after TLC chromatography of **I**) was assigned the molecular formula $C_{24}H_{30}O_8$ based on the pseudo-molecular positive ion peak, in its HR-ESIMS, at *m/z* 469.1844 $[M + Na]^+$, (calcd. for $C_{24}H_{30}O_8Na$: 469.1838).

The IR spectrum displayed absorptions for furan ring (1508, 875 cm^{-1}), γ-lactone and ester carbonyl groups (1743 cm^{-1}, broad band). The ^{13}C NMR spectrum displayed the presence of twenty-four carbons, and a DEPT experiment identified three methyls, seven methylenes, seven methines (three for olefinic double bonds) and seven unprotonated carbon atoms, three of them are for carbonyl groups at δ_C 171.0 and 172.0 (acetates) and 177.6 (γ-lactone) (Table 1). The presence of furan ring in the structure of the molecule was corroborated by the signals at δ_C 125.0 (C-13), 108.1/δ_H 6.39 (dd, CH-14); 144.2/7.44 (t, CH-15) and 139.6/7.45 (m, CH-16) in the ^{13}C and ^{1}H NMR spectra.

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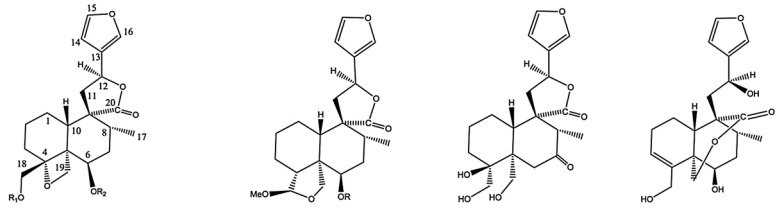

	R_1	R_2		R		
polivincin A (**1**):	Ac	Ac	polivincin B (**2**):	Ac	polivincin C (**3**)	teulamifin B (**4**)
montanin D (**5**):	H	H	teupolin XII (**6**):	H		

Fig. 1. Structures of the isolated and used in the discussion *neo*-clerodane diterpenoiods.

Table 1
Teuvincin A–C[a] NMR data.

position		1			2			3	
	δ 13C, nH	δ ^{1}H	m, J (in Hz)	δ ^{13}C, nH	δ 1H	m, J (in Hz)	δ 13C, nH	δ 1H	m, J (in Hz)
1α	16.4, CH_2	1.71	ov m[c]	23.1, CH_2	1.38	ov m[c]	21.4, CH_2	2.13	ov m[c]
1β		2.19	ov m[c]		1.88	ov m[c]		1.80	m
2α	29.0, CH_2	1.30	m	25.0, CH_2	1.89	ov m[c]	17.1, CH_2	2.14	ov m[c]
2β		1.68	ov m[c]		1.39	ov m[c]		1.72	m
3α	29.7, CH_2	1.81	m	26.3, CH_2	1.29	ov m[c]	30.1, CH_2	1.59	dd, 11.8, 7.3
3β		2.18	ov m[c]		1.79	ov m[c]		1.24	m
4	86.2, C			47.1, CH	2.18	dd, 12.6; 3.2	88.2, C		
5	46.7, C			51.2, C			47.6, C		
6α	73.0, CH	5.68	dd, 3.7; 2.1	73.2, CH	5.36	t, 2.8	53.7, CH_2	2.63	s
7α	21.3, CH_2	2.12	m	30.7 CH_2	2.24	ddd, 15.3; 13.1; 2.3	211,0, C		
7β		1.85	dt, 15.0; 3.8		1.87	dt, 15.4; 3.8			
8β	32.9, CH	1.78	m	33.2, CH	1.78	ov m[c]	32.1, CH	2.07	q, 4.5
9	51.9, C			48.0, C			69.6, C		
10β	38.8, CH	2.16	ov m[c]	44.5, CH	2.15	dd, 12.0, 6.0	31.7, CH	2.16	dd, 5.3; 2.3
H-11α	41.5, CH_2	2.47	dd, 14.0, 8.4	42.5, CH_2	2.45	dd, 14.0; 8.4	41.7, CH_2	2.47	dd, 13.1, 8.4
H-11β		2.33	dd, 14.0; 9.0		2.35	dd,15.7; 7.4		2.32	dd, 12.5; 6.3
12α	72.2, CH	5.40	t, 8.6	71.7, CH	5.38	dd, 10.7; 6.8	72.2, CH	5.38	t, 8.7
13	125.0, C			125.3, C			125.0, C		
14	108.1, CH	6.39	dd, 1.8; 0.8	108.1, CH	6.38	br s	108.1, CH	6.39	br s
15	144.2, CH	7.44	t, 1.7	144.1, CH	7.43	br d, 1.7	144.1, CH	7.43	dd, 3.5; 1.9
16	139.6, CH	7.45	m	139.5, CH	7.44	br s	139.6, CH	7.45	br s
Me-17	16.5, CH_3	0.93	d, 6.8	16.5, CH_3	0.95	d, 6.7	16.6, CH_3	0.95	d, 6.5
H-18b	66.9, CH_2	4.06[b]	d, 12.1	108.7, CH	4.46[b]	br s	66.6, CH_2	3.37	d, 11.5
H-18a		4.10	d, 12.1		4.14	d, 10.6		4.14	d, 11.6
H-19b	71.7, CH_2	4.77	d, 8.0	70.2, CH_2	3.96	d, 10.7	71.8, CH_2	4.04	d, 8.0
H-19a		4.16	d, 8.0					4.74	d, 8.0
20 (C = O)	177.6, C			177.0, C			177.9, C		
6^1 (C = O)	171.0, C			170.0, C					
6^2 (Me)	21.4, CH_3	2.06	s	21.4, CH_3	2.08	s			
18^1 (C = O)	170.0, C								
18^2 (Me)	20.9, CH_3	2.10	s						
MeO				54,4, CH_3	3.25	s			

[a]$CDCl_3$, ^{1}H 600.13 MHz, δref 7.26; ^{13}C 150.9 MHz, δref 77.0 ppm; [b] endo hydrogen with respect to ring B; [c] data from HSQC; ov overlapped signal.

Assignments of the olefinic protons to the corresponding carbon atoms were in agreement with the data from HSQC spectrum and observed HMBC correlations from H-14 to C-15 and C-16, from H-15 to C-13 and from H-16 to C-14 and C-15. In the HMBC spectrum were displayed additional correlations from the downshifted at δ_H 5.40 proton H-12 (connected with carbon at δ_C 722) to carbon C-13 and from the resonated at δ_H 2.33 proton H-11β, which showed cross peak in the HSQC spectrum with carbon at δ_C 41.5, to carbons 9 (δ_C 51.9), 10 (δ_C 38.8) and 12 (δ_C 72.2). The proton H-11α (δ_H 2.48) showed cross peaks with the signal at δ_C 177.7 (carbonyl C-20) and C-9. In the COSY experiment, the olefin proton at δ 7.44 bounded to the carbon at δ 144.2 (C-15), correlated with the methine proton at δ 6.39, which correlated with the carbon at δ 108.1 (C-14) in the HSQC experiment. Another methine proton, resonated at δ_H 5.40 and connected with the oxygenated carbon C-12 (δ_C 72.2), correlated to the two doublet of doublets at δ 2.47 (H-11α) and 2.33 (H-11β). We placed the furan moiety at 12β position in accordance with the displayed in the NOESY spectrum cross peaks of Me-17 (δ_H 0.94) with H-14 (δ_H 6.39) and H-16 (δ_H 7.45), as well as of H-1α (δ_H 2.19) with H-12α (δ_H 5.40).

There were ^{1}H-^{1}H COSY cross peaks of H-6 (δ_H 5.68 dd) with both H_2-7 (δ_H 2.12 and 1.85) and an HSQC correlation to δ_C 73.0 (i.e. H-6 is geminal to acetate). The methylene protons H_2-18 showed HMBC cross peaks to C-3 (δ_C 29.7), C-4 (δ_C 86.2), C-5 (δ_C 46.7) and carbonyl carbon atom C-18^1 (δ_C 170.0).

In both acetate there were HMBC correlations δ_H 2.06 / δ_C 171.0 and δ_H 2.10 / δ_C 170.0. The positions of the two acetoxy groups were determined to be at C-6 and C-18 from HMBC correlations from H-7α to 6^1 (C = O), from H-18a to 18^1 (C = O) and H-18b to 18^1 (C = O).

We placed the C-6 acetoxy group at β position and H-6 in α position in agreement with the small value of 3.7 Hz and 2.1 Hz for the coupling constants in the ^{1}H NMR spectrum of the δ_H 5.68 dd, due to the equatorial methine proton H-6. This was supported by the observed interaction in the NOESY experiment of H-6 with the downshifted doublet at δ_H 4.16, which was assigned for one methylene proton (H-19b) of oxygenated C-19 (δ_C 71.7). The protons H-19a and H-19b HMBC heterocorelated to C-4, C-5, C-6 and C-10 (δ_C 38.8) and showed

additional NOESY interaction, in turn, with H-2*α* and with H-7*α*.

The last oxygenated quaternary carbon atom at δ_C 86.2 (C-4) was involved in an oxetane ring in the molecule supported by mentioned heterocorrelations to carbon C-4 in the HMBC spectrum from H-18a, H-18b and H-19b.

^{1}H NMR data of **1** are very close to those reported by Malakov et al. for product received after acetylation of montanin D (**5**), received from *T. montanum* (Malakov et al., 1978). Quoted data were: δ_H 2.10 and 2.05 (each 3H, s)/vs 2.10 and 2.06 in **1**; 4.05 (H_2-18)/vs 4.06 and 4.10; 5.62 (1H, t, J = 4.0 Hz)/vs 5.68 (1H, dd, J = 3.7; 2.1 Hz) and an AB type quartet at δ_H 5.08 and 4.35 (1H each, J = 7.5 Hz, H_2-19)/vs 4.77 (1 H d, J = 8.0 Hz, H-19a) and 4.16 (1 H d, J = 8.0 Hz, H-19b). The differences in the data were probably due to the resolution ability of the used spectrometers for measurement of ^{1}H NMR spectra, operating at 600.130 MHz for **1** and quoted 80, 100 and 220 MHz by Malakov for hemi-synthetic product. Also, it is not clear the kind of solvent used by Malakov. He reported using solvents $CDCl_3$ and C_5D_5N jointly for all measurements without particularization of the conditions for individual compounds. The written above proton data were the only reported by Malakov, as ^{13}C NMR spectrum missed.

The HR-ESIMS of compound **2**, obtained from subfraction **II**, with the trivial name polivincin B, showed a pseudo-molecular positive ion peak at *m/z* 441.1861 $[M + Na]^+$, which indicated the molecular formula of $C_{23}H_{30}O_7$, (calcd. for $C_{23}H_{30}O_7Na$: 441.1889), while in its IR spectrum were observed absorptions consistent with the presence of a furan ring (3089, 1506, 1083 and 875 cm^{-1}), a γ-lactone (1762 and 1181 cm^{-1}) and acetate (1737 and 1246 cm^{-1}) groups.

The ^{13}C NMR spectrum displayed the presence of twenty-three carbons, and a DEPT experiment identified three methyls, six methylenes, nine methines (three from furan double bonds) and five quaternary carbon atoms, two of which are for carbonyl groups at δ_C 177.0 (γ-lactone) and 170.0 (acetate) (Table 1). The conjectural furan moiety in the molecule was confirmed by the signals, in the ^{1}H NMR spectrum of **2**, for aromatic protons at δ_H 7.44 (br s, H-16), 7.43 (br d, H-15) and 6.38 (br s, H-14). Observed triplet at δ_H 5.33 / δ_C 71.7, assigned for the H-12 proton, was characteristic signal for *neo*-clerodane diterpenoids possessing furan ring and γ-lactone (atom numbers C-11 – C-16 and C-20). The γ-lactone included carbon atoms C-9, C-11, C-12 and C-20 (δ_C 177.0). This connectivity was supported by the 2D NMR data. Olefin methines protons H-14, H-15, H-16 and the H-12 sp (Ljubuncic et al., 2006) methine proton were attached to corresponding carbons resonated at δ_C 108.1, 144.1, 139.5 and 71.7 on the ground of HSQC results. The abundance of ^{1}H –^{13}C HMBC correlations from H-14 to C-13, C-15, C-16, from H-15 to C-13, C-16, from H-16 to C-13, C-14, C-15, from H-11α to C-9, C-10, C-12, C-13, C-20 and from H-11*β* to C-8, C-9, C-10, C-12, C-13 confirmed the above connectivity and assignments including the position of quaternary carbons C-13 and C-20. ^{1}H – ^{1}H interactions in the COSY experiment, between the protons H-14/H-15, H-14/H-16, H-15/H-16 and between the both methylene protons H_2-11 with H-12, were observed. The S configuration to the C-12 chiral center was assigned based on the NOESY observed between the doublet for Me-17 (3H, δ_H 0.95) group and the H-14 (δ_H 6.38) and H-16 (δ_H 7.44) from the furan ring.

Trans junction in the decalin core of polivincin B was determined by the characteristic signals in the ^{1}H NMR spectrum at δ_H 2.15 for methine proton H-10 (1 H dd, J = 12.0; 6.0), the doublet at δ_H 0.95 for Me-17 (3 H d, J = 6.7) and the NOESY correlations of methylene proton H-19a with protons 1*α*, 2*α*, 3*α* and of H-19b with 6*α* and 7*α*. Signals in high-frequency region for geminal protons to oxygen atoms were displayed in the ^{1}H and ^{13}C spectra for methine at δ_H 5.36 (1H t, J = 2.8, H-6*α*)/δ_C 73.2 (CH) and methine at δ_H 4.46 (1H br s, H-18α)/δ_C 108.7 (CH), which heterocorrelated to C-3 (δ_C 26.3), C-4 (δ_C 47.1), C-19 (δ_C 70.2) and 18[1] (δ_C 54.4). The resonances of methylene protons, geminal to oxygen too, at δ_H 4.14 (1 H d, J = 106 H-19a) and 3.96 (1 H d, J = 107 H-19b) showed HMBC cross peaks to C-4, C-6 (δ_C 73.2) and C-18 (δ_C 108.7). The signal for methyl protons at δ_H 3.25 (3H s, MeO-18[1]), bound to the carbon at δ_C 54.4 (CH_3), correlated in HMBC spectrum to δ_C 108.7 (C-18). For the discussed above protons ^{1}H – ^{1}H COSY correlations between H-19a/H-19b, H-6α/H-7*α* (δ_H 2.24), H-6α/H-7*β* (δ_H 1.87) were evident. All above data, together with the characteristic signal for the methine proton at δ_H 2.18 (dd, J = 12.6; 3.2, H-4) disclosed the presence in the structure of an acetal moity formed by the C-18 carbon with the H-19 hydroxymethyl and a methanol molecule. Methoxy group was attached at C-18 in endo position with respect to the ring B of the decalin ring on the ground on the cross peak in the NOESY experiment between H-18a/H-3α and MeO-18[1]/H-6α. The NOESY interaction of the proton H-10 with H-1, H-2, H-4, H-8 and H-11 protons indicated, that they all were cofacial and *β* oriented.

Acetoxy group, resonated at *δ* 2.08 (3H s)/170.0 (C) and 21.4 (CH_3), was located at 6*β* position in accordance with small value of coupling constants of the germinal equatorial 6α proton, and also the NOESY interactions between H-6α/7α, H-6α/Me-18 (Al-Khalil, 1995) and H-6α/19a.

Further, the protons in the upfield region of the spectrum were a multiplet for methine at δ_H 1.78, which was bound to carbon at δ_C 33.2 (C-8), correlated in the ^{1}H – ^{1}H COSY spectrum with H-7*α*, H-7*β* and Me-17; there were seven overlapped protons detected in the 1.38–1.79 ppm range.

Finally, the spectral data of polivincin B were very close to those of teupolin XII (**6**) isolated by Fiorentino et al. in 2003 (Fiorentino et al., 2011). Observed differences were the downshifted signal in ^{1}H NMR spectrum for the proton H-6α, from δ_H 4.23 in **6** to δ_H 5.36 in the spectrum of **2**, besides the additional signals in ^{1}H and ^{13}C NMR spectra of **2** for acetoxy group at δ_H 2.08/δ_C 170.0 (CO) and 21.4 (CH_3).

For the new compound polivincin C (**3**), isolated from the subfraction **III,** was established molecular formula $C_{20}H_{26}O_7$ by the negative $[M-H]-$ molecular ion peak, in its HRESIMS, at *m/z* 377.1599, (calcd for $C_{20}H_{25}O_7$: 377.1600). The IR spectrum displayed absorptions for hydroxyl (3439 cm^{-1}), furan (1506, 875 cm^{-1}), γ-lactone and carbonyl (1759 cm^{-1}, broad band) functionalities. The ^{13}C NMR spectrum displayed the presence of twenty carbons, and a DEPT experiment identified one methyl, seven methylenes, six methines and six unprotonated carbon atoms, as two of them are for carbonyl groups at δ_C 211.0 (ketone) and 177.9 (γ-lactone). The signals at δ_C 125.0 (C-13), δ_C 108,2/δ_H 6.39 br s (H-14), δ_C 144.1/δ_H 7.43 dd (H-15) and δ_C 139.6/δ_H 7.45 br s (CH-16) in the ^{1}H and ^{13}C NMR spectra indicated the presence of furan ring in the structure of the molecule (Table 1). Attachments of the furan protons to the corresponding carbons were in agreement with HSQC spectrum and observed HMBC correlations from H-14 to C-16, from H-15 to C-16 and from H-16 to C-13 and C-14. In the HMBC spectrum were displayed additional correlations to carbon C-13 from the downshifted at δ_H 5.38 proton H-12 and from the resonated at δ_H 2.32 proton H-11*β*, which showed cross peak in the HSQC spectrum, with carbon at δ_C 41.7. Two protons, H-11*β* (δ_H 2.32) and H-8 (δ_H 2.07) showed cross peak with the signal at δ_C 177.9 (carbonyl C-20). In the COSY experiment, the furan proton at δ_H 7.43, which was bound to the carbon at δ_C 144.1 (C-15), correlated with the methine proton at δ_H 6.39, which correlated with the carbon at δ_C 108.2 (C-14) in the HSQC experiment. Another methine proton, resonated at δ_H 5.38 and connected with the oxygenated carbon C-12 (δ_C 72.2), correlated to the two doublet of doublets at *δ* 2.47 (H-11*α*) and 2.32 (H-11*β*). We placed the furan moiety at 12*β* position in accordance with the displayed in the NOESY spectrum cross peaks of Me-17 (δ_H 0.94) with H-14 and H-16, as well as between the protons H-11*α*/H-12*α*, H-11*α*/H-1*α* (δ_H 2.13) and H-12*α*/H-1*α*. The last proton (H-1*α*) showed NOESY interaction with signals at δ_H 4.04 (H-19a) and 4.74 (H-19b), which indicated its *α*-orientation.

The rest four signals of oxygenated carbons, two unprotonated and two methylene, were assigned in the decalin core for carbons C-4 (δ_C 88.2, C), C-7 (δ_C 211.0, CO), C-18 (δ_C 66.6, CH_2) and C-19 (δ_C 71.8, CH_2). Assignments were in consistence with 2D spectra. In HMBC spectrum were observed cross peaks from the doublets at δ_H 3.37 and

4.14, which are bounded with carbon resonated at δ_C 66.6 (C-18) as it was seen in HSQC experiment, in turn to δ_C 88.2 (C-4), 47.6 (C-5) and to 30.1 (C-3), C-4, C-5. The doublets at δ_H 4.04 and 4.74, which showed in HSQC direct bound with carbon resonated at δ_C 71.8 (C-19), had HMBC correlations, in turn to C-4, C-5 and C-9 (δ_H 69.6).

The signal for ketone functional group at δ_C 211.0, was unambiguously assigned for C-7 on the ground on observed HMBC correlations from the singlet for two protons at δ_H 2.63 (CH_2-6) to δ_C 211.0 (C-7), 301 (C-3), 69.6 (C-9) and from the doublet of doublets at δ_H 2.17 (H-10) to δ_C 53.7 (C-6) and to 211.0 (C-7). Also, the usual multiplet for H-8, in diterpenoids with unsubstituted carbon C-7, was reduced into quartet at δ_H 2.07. This simplified signal (H-8) displayed interaction, in the ^{1}H-^{1}H COSY spectrum, with the doublet for three protons at δ_H 0.94 (Me-17), and HMBC cross peak to the carbonyl atom at δ_C 177.9 (C-20). On the other hand, Me-17 protons heterocorelated to δ_C 32.1 (C-8) and 53.7 (C-6).

The interaction between protons H-18a and H-19a, observed in the NOESY experiments, confirmed the α-orientation of the H-18 hydroxymethylene group and the β-orientation of the tertiary hydroxyl group at C-4, respectively. Other NOESY interactions, H-3α (δ_H 1.58)/H-1α, H-3α/H-2α (δ_H 2.15), 10β/1β (δ_H 1.80) and 10β/2β (δH 1.72), were observed.

The last fraction **III** provided second compound identical in all respects (IR, HRESIMS, ^{1}H NMR, ^{13}C NMR and 2D NMR experiments) with teulamifin B (**4**), previously isolated by Malakov in 1988 (Malakov et al., 1988).

From the acetone extract of *Teucrium polium* subsp. *vincentinum* four furoclerodane lactone diterpenoids, the new polivincins A–C and previously known teulamifin B, were isolated and structural elucidated by extensive spectroscopic investigation. The absolute configuration of compounds **1**-**3** was not ascertained. However, on biogenetic grounds, it may be supposed that **1–3** belong to the trans neo-clerodane series like the other diterpenoids isolated from *Teucrium polium* species (Bruno et al., 2003; Malakov et al., 1978; Fiorentino et al., 2011). The presence of capitatin and auropolin were not detected in our plant material and the four isolated by us compounds were different from all diterpenoids reported previously by Malakov as constituents of the plant *Teucrium polium.* Our suggestion, that Malakov and coauthors had studied *Teucrium polium* subsp. *capitatum,* was corroborated.

3. Experimental

3.1. Structural data

^{1}H NMR spectra were recorded on Bruker Avance II + spectrometers, operating at 600.130 MHz. ^{13}C NMR spectra were recorded at 150.903 MHz spectrometer. TMS was used as internal standard and $CDCl_3$ as solvent. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. The IR spectra were registered in KBr pellet on a Vertex 70 spectrometer from 4000 cm^{-1} to 400 cm^{-1} at resolution 4 cm^{-1} with 25 scans. The mass spectra were measured on Hewlett Packard 6890 GC System Plus/5973 MSD. The melting points were determined by a SMP-10 digital melting point apparatus (Fig. 1).

3.2. Plant material

The stems of *Teucrium polium* subsp. *vincentinum* L. were collected in July 2018 from sand dunes of seaside resort Atleman in Kiten, Bulgaria, and voucher specimens (n. 7212) were deposited in the Herbarium of the Higher Institute of Agriculture at Plovdiv, Bulgaria.

3.3. Extraction and isolation

Dried and finely powdered aerial parts of *Teucrium polium* subsp. *vincentinum* (Rouy) D. Wood (0.850 kg) were extracted with Me_2CO (4 × 3 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 (15.7 g), which was dissolved in aq. Me_2CO (40% H_2O, v/v, 100 mL). This solution was cooled to 4 °C for 24 h and filtered. The filtrate was extracted with $CHCl_3$ (3 x 100 mL) and the organic layer was dried (Na_2SO_4) and evaporated in vacuum to afford a residue (2.4 g, bitter fraction). This residue was subjected to CC (55 g silica gel Merck n. 7734, deactivated with 10% H_2O, w/w). Pure petroleum ether (10 L), followed by a gradient of CH_2Cl_2 – CH_3OH mixtures (10:0 to 9.8:0.2) were used as eluting solvents. Initially, three diterpene fractions **I** - **III** were obtained. Preparative TLC of the fractions (n-hexane-EtOAc, 1:4 was used as eluent) afforded pure compounds as follows, 12.5 mg of polivincin A (**1**) from fraction **I**, 9.2 mg of polivincin B (**2**) from fraction **II**, 8.0 mg of polivincin C (**3**) and 11.3 mg of teulamifin B (**4**) from fraction **III.**

***Polivincin* A (1).** Colorless resin. TLC: R_f 0.75 (EtOAc). IR ν_{max} (KBr): 3447, 2929, 1743, 1637, 1508, 1459, 1383, 1246, 1163, 1046, 982, 875, 758, 667 cm^{-1}.

^{1}H and ^{13}C NMR: see Table 1.

Positive ESIMS (70 eV, direct inlet) m/z (rel. int. in %): 469 $[M + Na]^+$ (96.2), 425 (37.2), 381 (38.1), 353 (26.7), 304 (13.8). HRESIMS m/z 469.1844 $[M + Na]^+$, (calcd. for $C_{24}H_{30}O_8Na$: 469.1838).

***Polivincin* B (2).** Amorphous solid. MP: 97–99 C°, TLC: R_f 0.72 (EtOAc). IR ν_{max} (KBr): 2931, 1762, 1737, 1655, 1506, 1458, 1383, 1325, 1246, 1212, 1181, 1157, 1111, 1083, 1062, 1024, 988, 955, 929, 875, 853, 800, 779, 730, 708, 647, 602 cm^{-1}.

^{1}H and ^{13}C NMR: see Table 1.

Positive ESIMS (70 eV, direct inlet) m/z (rel. int. in %): 441 $[M + Na]^+$ (97.1), 393 (53.9), 358 (6.6), 304 (29.7), 127 (4.0). HRESIMS m/z 441.1861 $[M + Na]^+$, (calcd. for $C_{23}H_{30}O_7Na$: 441.1889).

***Polivincin* C (3).** Colorless oil. TLC: Rf 0.69 (EtOAc). IR ν_{max} (KBr): 3439, 2928, 1759, 1506, 1459, 1383, 1321, 1159, 1056, 1023, 930, 875, 797, 731, 669, 647, 601 cm^{-1}.

^{1}H and ^{13}C NMR: see Table 1

Negative ESIMS (70 eV, direct inlet) m/z (rel. int. in %): 377 $[M-H]-$ (95.1), 287 (13.4), 255 (9.1), 183 (6.7), 137 (3.2). HRESIMS m/z 377.1599 $[M-H]-$ (calcd for $C_{20}H_{25}O_7$: 377.1600).

3.4. Supplementary data

Tables of complete spectral data, IR spectra, HRESIMS spectra and the ^{1}H NMR, ^{13}C NMR and 2D NMR spectra (with enlarged detailed sections for multiplets and cross peaks) are included in a "Supplementary Data" section.

Declarations of interest

none.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.04.013.

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