STRUCTURES OF TWO NEW DITERPENOIDS FROM Teucrium polium

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Two new acids of the neoclerodane series have been isolated from Teucrium polium L. (Lamiaceae); they have been called tepolin A and tepolin B and their structures have been established on the basis of spectral characteristics as 7,12,19-trihydroxy-6,18:15,16-diepoxycleroda-3,13(16),14-triene-9-carboxylic acid and 12,19-dihydroxy-6,18:15,16-diepoxycleroda-3,13(16)-14-triene-9-carboxylic acid, respectively.

Continuing an investigation [1, 2] of the chemical composition of golden germanda Teucrium polium L. (Lamiaceae) growing in Armenia, we have isolated from the ethyl-acetate-soluble part of a methanolic extract of the plant, two new diterpenoids which we have called tepolin A (I) and tepolin B (II), and have established their structures.

Both tepolin A (composition $C_{20}H_{26}O_7$) and tepolin B (composition $C_{20}H_{26}O_6$) contain in their structures a free carboxy group and a β-substituted furan ring. This was shown by their solubility in aqueous ammonia, their interaction with diazomethane, characteristic absorption bands in their IR spectra (3130, 1700, 1610, and 885 cm^{-1}), coloration by the Ehrlich reagent on thin-layer chromatography (TLC), the presence in the mass spectra of characteristic peaks of ions with m/z 81 and 95 [3], and, finally, their ^1H and ^{13}C NMR spectra (Tables 1 and 2). Both compounds contained hydroxy groups (ν_{max} 3429 cm^{-1}), and on acetylation they formed a triacetate (III) and a diacetate (IV), respectively.

A comparative analysis of the ^1H NMR spectra of tepolin A (I) and its triacetate (III) showed the presence in the structure of tepolin A (I) of $-CH_2-O-$, $>CH-O-$, $\diagdown C-CH_2-OH$, and two $HC(-OH)$ groups. The mass spectrum of tepolin A (I) contained the characteristic peaks of ions corresponding to the ejection from the molecular ion (M^+, m/z 378) of one and two molecules of water (m/z 360 and 342) and of a hydroxymethyl group (m/z 347) and also of water and hydroxymethyl and hydroxy groups (m/z 312) and of water, hydroxymethyl, and carboxy groups (m/z 284).

The ^{13}C NMR spectra of tepolin (I) and its triacetate (III) agreed with the PMR and mass spectra and contained the signals of 20 and 26 carbon atoms, respectively.

A comprehensive consideration of the spectral characteristics in the light of chemotaxonomic and biogenetic ideas on terpenoids of the Teucrium genus [4] permitted us to propose for tepolin A a structure of the neoclerodane type [5] corresponding to 7,12,19-trihydroxy-6,18:15,16-diepoxycleroda-3,13(16),14-triene-9-carboxylic acid (I).*

*The absolute configuration was not established; it is assumed that at the C(5) and C(8), C(9), and C(10) centers it coincides with that of other neoclerodane diterpenoids from Lamiaceae.

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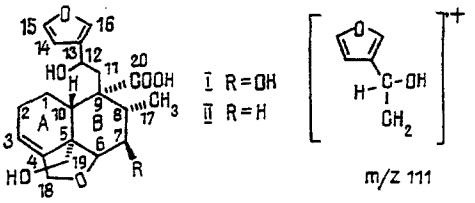

Arguments in favor of the correctness of the proposed structure (I) are provided by the following facts. By a series of experiments with $^1H \leftrightarrow H$ homonuclear double resonance we traced the spin–spin linkage of the H-1 and H-2, H-2 and H-3, and H-1 and H-10 protons. The value of one of the spin–spin coupling constants (SSCCs) of H-1 and H-10 (12.9 Hz) showed the axial orientation of H-10 in ring A. The preirradiation of H-10 in an experiment involving the nuclear Overhauser effect (NOE) caused an intensification of the H-8 signal and some intensification of the H-12 signal, which showed the diaxial positions of H-10 and H-8 in ring B and indicated the inclusion of H-12 within the equatorial substituent at C(9). The values of the SSCCs H-7 ↔ H-8 and H-6 ↔ H-7 (11.0 Hz and 3.1 Hz) showed the trans-diaxial arrangement of H-7 and and H-8 and the equatorial position of H-6, while the nature of the splitting of the H-8 and H-6 signals showed the absence of protons at C(9) and C(5).

The existence of an SSCC, even though a small one (less than 1 Hz) between the protons of the furan ring and the H-12 proton confirmed the position of the furan ring, again agreeing well with the presence in the mass spectrum of the peaks of an ion with m/z 111 and of ions connected with the ejection of 111 a.m.u. (m/z 267, 232, 201) on the cleavage of the C(9)–C(11) bond.

The existence of a five-membered oxide ring including the carbon atoms C(6) and C(18) followed, in particular, from the absence of a γ-effect of acetylation for the C(3) signal in the ^{13}C NMR spectrum of the triacetate (III). At the same time, the position of the CH_2OH group just at the C(5) atom agreed well with the value and sign of the γ-effect ($\Delta\delta$ = −6.38 ppm) for the C(4) atom in the ^{13}C NMR spectra on passing from the triol (I) to the triacetate (III). The same thing was indicated by the δ-effect for the C(3) and C(18) atoms, amounting to ~+2.2 ppm.

Finally, an unambiguous assignment of the signals of all the protonated carbonated carbon atoms in the ^{13}C NMR spectra of tepolin A (I) and its triacetate (III), causing no doubts, was made with the aid of ^1H–^{13}C two-dimensional correlation heteronuclear spectroscopy, COSY (Fig. 1).

A comparison of the ^1H NMR spectra of tepolin A (I) and its triacetate (III) and of tepolin B (II) and its diacetate (IV) enabled us to find the single structural difference

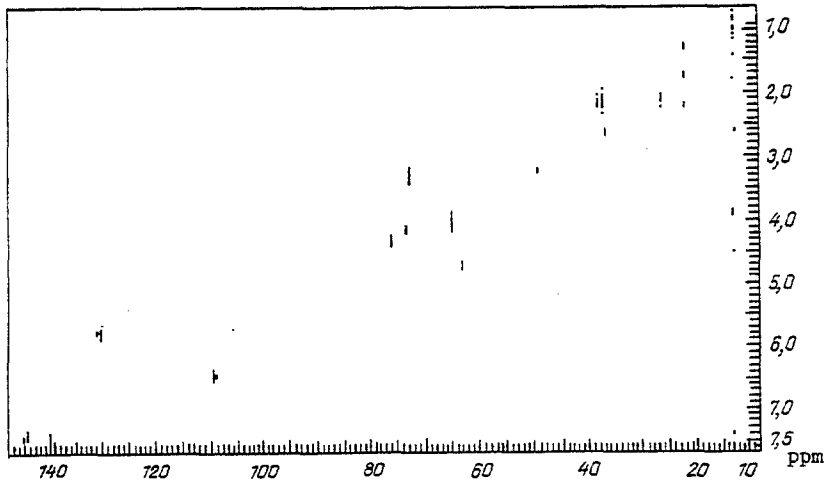

Fig. 1. ^{13}C–^1H two-dimensional heteronuclear COSY spectrum of tepolin A (I).

TABLE 1. Parameters of the PMR Spectra of Tepolins A (I) and B (II) (CD_3OD) and of Their Acetates (III) and (IV) ($CDCl_3$)

H	I	III	II	IV
H-1	1,31 m $J_{1,10}$ = 12,9	1,41 m $J_{1,10}$ = 13.1	1.31 m $J_{1.10}$ = 12,8	1,42 m $J_{1,10}$ = 13,0
H-1′	2,23 m $J_{1',10}$ = 2.7	2,25 m $J_{1',10}$ = 2,5	2,17 m $J_{1',10}$ = 2,6	2,26 m $J_{1',10}$ = 2,3
H-2	2,15 m	2,18 m	2,17 m	2,19 m
H-2′	2,2 m	2,34 m	2,24 m	2,36 m
H-3	5,78 br.t $J_{3,2}$ = $J_{3,2'}$ = 3,6	5,94 br.t $J_{3,2}$ = $J_{3,2'}$ = 3,6	5,79 br.t $J_{3,2}$ = $J_{3,2'}$ = 3,6	6,02 br.t $J_{3,2}$ = $J_{3,2'}$ = 3,6
H-5	4.14 d $J_{6,7}$ = 3,1	4,11 d $J_{6,7}$ = 3,1	4 19 t $J_{6,7}$ = $J_{6,7'}$ = 2.8	4,11 br.t $J_{6,7}$ = $J_{6,7'}$ = 3,0
H-7	3 37 dd $J_{7,8}$ = 11,0	4,85 dd $J_{7,8}$ = 11,5	1,55 ddd $J_{7,7'}$ = 14,9	1.68 ddd $J_{7,7'}$ = 14,9
H-7′	—	—	1,79 ddd $J_{7',8}$ = 4,3	1,86 ddd $J_{7',8}$ = 4,3
H-8	2,21 dq $J_{8,17}$ = 6,5	2,4 dq $J_{8,17}$ = 6 6	2,42 ddq $J_{7,8}$ = 12,6	2,38 ddq $J_{7,8}$ = 12.6
H-10	2, 8 dd	2,48 dd	2.67 dd	2,47 dd
H-11	2,16 dd $J_{11,11'}$ = 15,5	2,39 dd $J_{11,11'}$ = 16,0	2,16 dd $J_{11,11'}$ = 15.2	2,38 dd $J_{11,11'}$ = 16,0
H-11′	2,36 dd $J_{11',12}$ = 3,5	2,58 dd $J_{11',12}$ = 3,5	2,37 dd $J_{11',12}$ = 3.8	2,64 dd $J_{11',12}$ = 3,8
H12	4 74 dd $J_{12,11}$ = 8,5	5 92 dd $J_{12,11}$ = 8,9	4,77 dd $J_{12,11}$ = 8,2	6,00 dd $J_{12,11}$ = 9,3
H-14	6,43 br.d $J_{14,15}$ = 1 8	6 40 br.d $J_{14,15}$ = 1,8	6 44 br.d $J_{14,15}$ = 1,8	6,43 br.d $J_{14,15}$ = 1,8
H-15	7,39 m $J_{15,16} \leqslant 1$	7,35 m $J_{15,16} < 1$	7,39 m $J_{16,16} \leqslant 1$	7,37 m $J_{15,16} \leqslant 1$
H-16	7,42 br.s $J_{16,12} < 1$	7,43 br.s $J_{16,12} \leqslant 1$	7 43 br.s $J_{16,12} \leqslant 1$	7,46 br.s $J_{16,12} \leqslant 1$
H-17	0,81 d	0,80 d	0,72 d $J_{17,8}$ = 6,6	0,83 d $J_{17,8}$ = 6,6
2 -18	4,35 and 4 41 (AB) J_{AB} = 12,4	4,35 and 4,41(AB), J_{AB} = 12,4	4,39 and 4,41(AB), J_{AB} = 12,3	4,19 and 4,41(AB). J_{AB} = 12,4
2′-19	3.92 and 4.20(AB) J_{AB} = 12,2	4,46 and 4 63(AB) J_{AB} = 12,3	3,91 and 4,15(AB), J_{AB} = 12,0	4,50 . 4,63(AB), J_{AB} = 12,4
CH_3CO	—	1,99 s; 2,0 s; 2,0 s	—	2,02 s; 2,07 s

*Chemical shifts, (δ), ppm from TMS; SSCC, Hz.

TABLE 2. ^{13}C NMR Chemical Shifts of Tepolins A (I) and B (II) (CD_3OD) and of the Acetate (III) ($CDCl_3$) (δ, ppm from TMS)

Carbon atom	I	*	II	*	III	*
1	21,34	2	21,41	2	19,8	2
2	26,06	2	26,17	2	25,11	2
3	130,42	1	131,75	1	132,55	1
4	140,26[a]	0	140,7[a]	0	133,8[a]	0
5	50,68[b]	0	49,75[b]	0	49,24[b]	0
6	73,65	1	70,42	1	69,67	1
7	72,91	1	38,11	2	74,68	2
8	37,99	1	31,48	1	34,41	1
9	42,61[b]	0	41,40[b]	0	41,22[b]	0
10	36,64	1	37,34	1	35,16	1
11	37,22	2	37,24	2	32,95	2
12	63,11	1	63,14	1	64,49	1
13	131,9[a]	0	131,9[a]	0	125,43[a]	0
14	109,65	1	109,74	1	108,66	1
15	144,58	1	144,57	1	143,55	1
16	139,93	1	140,03	1	140,22	1
17	12,19	3	16,80	3	11,53	3
18	76,30	2	76,69	2	74,03	2
19	65,14	2	65,19	2	66,45	2
20	175,45	0	175,50	0	170,36	0
$\underline{C}H_3CO$	—		—		171,47; 170,64; 170,29	0
$CH_3\underline{C}O$	—		—		21,31; 21,09	3
					20,95	

*Number of attached protons according to the APT [8].
a,bThe assignment within a column may be reversed.

between tepolin A (I) and tepolin B (I), which consisted in the absence from tepolin B (II) of the hydroxy group at C(7), as was also shown by the ^{13}C NMR spectrum of tepolin B (II) (Table 2). Thus, tepolin B (II) is 12,19-dihydroxy-6,18:15,16-diepoxycleroda-3,13(16),14-diene-9-carboxylic acid.

EXPERIMENTAL

For analytical and preparative thin-layer chromatography (TLC) we used standard Silufol plates in the solvent systems: ethyl acetate—methanol—water (18:2:1) and (16:2:1) for compounds (I) and (II), respectively, and chloroform—ether (19:1) for compounds (III) and (IV). The spots were revealed on the chromatograms by spraying with the Erhlich reagent (a 2% solution of p-dimethylaminobenzaldehyde in ethanol), followed by treatment in a chamber with vapors from concentrated hydrochloric acid. For column chromatography we used neutral alumina (Brockmann activity grade IV) and type KSK silica gel (63-100 mesh).

Melting points were determined on a Boëtius instrument, and optical activities on a Polamat A instrument; IR spectra were taken on a UR-20 spectrometer in KBr, UV spectra on a Specord UV-VIS in methanol, and mass spectra on a MKh-1320 spectrometer.

PMR spectra were taken on a Bruker WM-250 instrument at room temperature. Difference NOE spectra were recorded by the method of Hall and Sanders [6], and difference homonuclear selective double resonance spectra were taken by a procedure modified from that described in [6] and providing for an increased selectivity of the experiment [7].

^{13}C NMR spectra were obtained on a Bruker WM-300 spectrometer with a working frequency of 75 MHz for carbon-13. In recording the ^{13}C–^{1}H two-dimensional heteronuclear correlation spectrum we used the standard XHCORRD procedure from the mathematical information of the Bruker firm as applied to an ASPECT-3000 computer. The relaxation delay was 1 s, and the delay D3 and D4, 3.2 and 1.2 ms, respectively (optimized for an SSCC $^{1}J_{C-H}$ ~ 150 Hz). The spectral window for the ^{13}C channel amounted to 130 ppm, and for the ^{1}H channel to 6 ppm. The dimensions of the matrix of 2 K × 512 W ensured a resolution of no worse than 5 Hz/point along the ^{13}C axis and 8 Hz/point along the ^{1}H axis. Before Fourier transformation, the spectra in the time domain were multiplied by a sinusoidal function with zero shift.

Isolation of Tepolins A (I) and B (II). The ethyl acetate fraction (35.0 g of dry residue) obtained from the methanolic extract of 2.0 kg of golden germanda [2], was chromatographed on a column of alumina (1 kg) in the ethyl acetate–methanol–water (90:7:3) system. This gave 3.9 g of a fraction colored by the Ehrlich reagent on TLC, which was chromatographed on a column of silica gel (100 g). Elution with chloroform–methanol (97:3) led to the isolation of 0.57 g of fraction "a", and by chloroform–methanol (20:1) to 0.53 g of fraction "b". The preparative TLC of fraction "a" and "b" gave, respectively, 154 mg of tepolin A (I) and 138 mg of tepolin B (II).

Tepolin A (I) – a white amorphous substance insoluble in water but soluble in 5% aqueous NH_4OH solution with R_f 0.43; $[\alpha]_D^{22}$ −14° (c 0.68; CH_3OH). UV spectrum (λ_{max}): 202 nm. Mass spectrum, m/z (%): 378 (M^+, 82), 360(19), 347(2), 342(7), 334(7), 330(11), 312(100), 285(11), 284(10), 267(17), 256(17), 232(16), 229(22), 214(33), 213(78), 203(37), 202(32), 201(25), 170(33), 111(60), 105(56), 97(44), 95(96); 81(55). For the ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

The triacetate (III) – white amorphous substance with R_f 0.13. For its ^1H and ^{13}C NMR, see Tables 1 and 2.

Tepolin B (II) – colorless acicular crystals insoluble in water but soluble in 5% aqueous NH_4OH solution, with mp 188-190°C (from chloroform), R_f 0.54, $[\alpha]_D^{20}$ −38° (c 1.0; CH_3OH). UV spectrum (λ_{max}): 202 nm.

Mass spectrum, m/z (%); 362 (M^+, 100), 344(10), 326(3), 314(5), 296(6), 251(3), 234(7), 220(8), 216(20), 203(9), 171(24), 111(50), 105(25), 97(27), 95(34), 81(20). For its ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

The diacetate (IV) – colorless amorphous substance with R_f 0.14; its PMR spectrum is given in Table 1.

Reaction of Tepolins A (I) and B (II) with Diazomethane. An excess of an ethereal solution of diazomethane was added to solutions of samples of (I) or (II) (5 mg in each case in 0.5 ml of CH_3OH), and the mixture was left for 3 h. After the solvent had been distilled off, in each case a single component was detected by the TLC method, with R_f 0.54 and 0.68, respectively.

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