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Neo-clerodane diterpenoids from *Teucrium polium*

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Abstract

Two new neo-clerodane type diterpenoids, teulolin A (15,16-epoxy-6β,7β,18,19-tetrahydroxy-neo-cleroda-3(4),13(16),14-trien-20,12(*S*)-olide, **1**) and teulolin B (15,16-epoxy-3α, 6β,7β,18,19-tetrahydroxy-neo-cleroda-4(18),13(16),14- trien-20,12(*S*)-olide, **2**) were isolated from the aerial parts of *Teucrium polium*. The structures of **1**–**2** were proposed on the basis of extensive NMR experiments and molecular modeling studies.

Keywords: *Teucrium polium*; Lamiaceae; Diterpenoids; Neo-clerodane derivative; Teulolins A, B

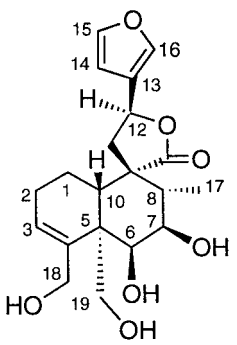
1. Introduction

The genus *Teucrium* (Lamiaceae) is represented by 27 species in the flora of Turkey (Davis, 1982). As part of our phytochemical investigations in this genus (Çalis, Wright, & Sticher, 1996), we have studied *Teucrium polium* L. which is used in the treatment of diabetis, hepatitis, hemorroid and stomach pain in Turkish folk medicine (Baytop, 1984). Our previous work on the aerial parts of this plant (Bedir, & Çalis, 1997) led to the isolation of two known phenylethanoid glycosides, verbascoside and poliumoside. Further examination of the polar fractions of the same source yielded two new minor neo-clerodane diterpenes, teulolins A (**1**) and B (**2**). The current report concentrates on the isolation and the detailed structure elucidation of compounds **1**–**2**.

2. Results and discussion

The water soluble portion of the methanolic extract was partitioned with $CHCl_3$. The diterpenoids contained in the water fraction were purified using a combination of polyamide CC, silica gel CC and RP-MPLC.

Teulolin A (**1**) was isolated as an amorphous powder. The IR spectrum of **1** showed absorptions of furan ring (3160, 1603, 1505, 876 cm^{-1}), hydroxyl (3367 cm^{-1}, br), olefin (1660 cm^{-1}) and lactone (1747 cm^{-1}) functionalities. **1** gave a molecular ion peak in its EI-mass spectrum at *m*/*z* 378, in agreement with the molecular formula $C_{20}H_{26}O_7$. These data together with the information from the ^{13}C NMR spectrum that displays 20 signals due to one carbonyl three endocyclic double bonds, one secondary methyl, five methylenes two of which are oxygen-bearing, two methine, one



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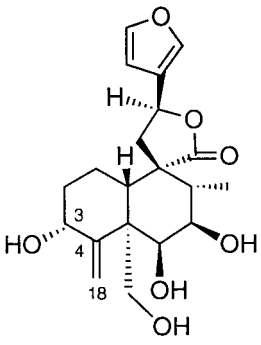

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carbinyl and two secondary carbinol signals, as well as two quaternary carbon atoms indicated **1** to be a tetracyclic compound. Taking into account the results from our comprehensive 1-D and 2-D NMR studies and previous knowledged derived from metabolites isolated from the genus *Teucrium* (Çalis et al., 1996), it is evident that **1** possesses a highly oxidized neo-clerodane nucleus. The ^{1}H NMR data established the following fragments: The signals resonating at δ_H 6.26 (br. s, H-14), 7.29 (br. s, H-15) and 7.32 (br. s, H-16) were readily assigned to a terminal furan ring (Çalis et al., 1996). Another spin-system is comprised of two sets of well-resolved triplets at δ_H 2.34 (J=8.3 Hz, H_2-11) and 5.29 (J=8.3 Hz, H-12) and the shifts of the attached carbons (δ_C 45.0 t and 72.4 d) and of the two additional quaternary carbons (δ_C 53.3 s and 178.4 s) were consistent with the presence of a five-membered spirolactone moiety (Çalis et al., 1996). These two fragments were easily connected to each other on the basis of heteronuclear ^2J and ^3J ^{1}H, ^{13}C long-range correlations from an HMBC experiment as shown in Table 2. To complete the assignment the chemical shifts of the decalin ring-protons and its substitution pattern had to be determined for which task the ^{1}H–^{1}H DQF-COSY spectrum proved to be most useful. Detailed examination of this spectrum indicated the presence of four spin systems. The first spin system included the signals of a methine, two methylene and an olefinic proton. Thus, H_2-1 (δ 1.65 m) coupled with the partially overlapped signals of H-10 (δ 2.05 m) and H_2-2 (δ 2.15 m) which in turn is vicinally coupled with the vinylic proton at δ 5.67 (br. s, H-3). The latter, together with the crucial ^{1}H–^{13}C long-range correlations observed in the HMBC spectrum of **1** Table 2 clearly indicated the presence of the double bond across C-3/C-4. The second ^{1}H–^{1}H spin system could be traced from the secondary methyl protons (δ 0.96 d, J=6.7 Hz, H_3-17) to H-8 (δ 1.82, m, J=6.7, 10.7 Hz) and from there to an oxymethine proton at δ 3.84 (H-7, dd, J=1.8, 10.7 Hz). The latter proton showed a vicinal coupling to another carbinol proton at δ 3.47 (m) which was assigned as H-6, thus completing the spin system and also placing both secondary hydroxy functions at C-7 and C-6. The remaining two separated spin systems were affixed to the primary hydroxy-bearing C atoms, C-18 and C-19, on the basis of 2-D DQF-COSY and HMQC spectral data (Table 1). Observation of the cross peaks in the HMBC spectrum from C-4 to both H_2-18 and H_2-19 as well as the additional long-range couplings between H_2-18/C-3, H_2-18/C-10, H_2-19/C-5 and H_2-19/C-10 ascertained this deduction. All these fragments (the furan and the spirolactone moieties), were unambiguously assembled into the basic framework of **1** from the results of the HMBC experiment (Table 2).

The relative stereochemistry of the chiral centers in **1** was resolved by a combination of 2-D ROESY data and an analysis of the coupling constants and was supported by data from a molecular modeling study. The cross peaks observed in the ROESY spectrum between H-8, H-10 and H_2-11 implied that these protons were on the same molecular face (β) while observation of the key ROESY couplings from H-6 to H_3-17 and H-7 to H_2-19 revealed them to be on the opposite side of the molecule (α). Examination of the 3-D computer-generated model of **1** (Fig. 1a) further supported that the these protons lie in close proximity to one another. All these geometric facts were also compatible with the *trans* junction of the A/B rings. Due to the overlap of H-6 signal with those of H_2-18 and the solvent (CD_3OD), the stereolocation of OH-6 could not be inferred from the ROESY data, but a small coupling constant (J=1.8 Hz) between H-6 and H-7 proved the α,α-equatorial position of these protons, and therefore, the C6,C7 *cis*-β,β-diol structure of **1**. This left the configuration of H-12 to be established. The 3-D model of **1** indicates that rings A and B lie in one plane whereas the third ring that forms the spirolactone is located perpendicular to that plane (Fig. 1a). The presence of a distinctive dipolar coupling from H-12 to H_2-1 clearly proves the *S* configuration for H-12 which is further supported by the absence of an ROE interaction between H-12 and H_3-17 (Bruno, Fazio, Piozzi, Rodriguez, & de la Torre, 1995). The interatomic dis-

Table 1
NMR data of **1**–**2** (δ in ppm, J in Hz) (300 and 75.5 MHz, $CDCl_3$–MeOD, 3:1). Signals were assigned on the basis of DEPT 135, DQF-COSY, HMQC and HMBC experiments

Atom	1		2	
	δ_H	δ_C	δ_H	δ_C
1	1.65 (m)	18.8 t	1.50[a]–1.95[a]	34.2 t
2	2.15[a]	25.3 t	1.80[a]	21.6 t
3	5.67 (br. s)	130.4 d	4.25 (d, 4.3)	79.3 d
4		142.0 s		153.7 s
5		48.5 s		50.4 s
6	4.00 (m)	70.0 d	3.79 (d, 1.8)	73.2 d
7	3.84 (dd, 1.8/10.7)	69.4 d	3.54 (dd, 1.8/8.8)	70.9 d
8	1.82 (m, 6.7/10.7)	38.4 d	2.01[a]	37.1 d
9		53.3 s		54.4 s
10	2.05[a]	44.4 d	2.21[a]	46.1 d
11	2.34 (t, 8.3)	45.0 t	2.35 m	44.5 t
12	5.29 (t, 8.3)	72.4 d	5.33 (t, 8.7)	72.6 d
13		124.9 s		124.7 s
14	6.26 (br. s)	107.7 d	6.29 (br. s)	107.8 d
15	7.29 (br. s)	143.9 d	7.32 (br. s)	144.0 d
16	7.32 (br. s)	139.4 d	7.35 (br. s)	139.5 d
17	0.96 (d, 6.7)	11.8 q	0.95 (d, 6.5)	11.7 q
18	4.00 m	63.8 t	5.42 (br. s), 4.84 (br. s)	103.8 t
19	3.60 (d, 11.6)	63.8 t	3.25 (d, 8.3)	71.4 t
	4.75 (d, 11.6)		4.75 (d, 8.3)	
20		178.4 s		172.2 s

[a] Signal pattern is unclear due to overlapping.

Table 2
^{1}H–^{13}C long range (HMBC) correlations observed for compounds **1** and **2**

H	**1** (C, δ in ppm)	**2** (C, δ in ppm)
1	25.3 (C-2), 48.5 (C-5), 44.4 (C-10)	21.6 (C-2), 50.4 (C-5), 46.1 (C-10)
2	18.8 (C-1), 142.0 (C-4)	79.3 (C-3), 153.7 (C-4), 50.4 (C-5), 46.1 (C-10)
3	48.5 (C-5), 63.8 (C-18)	21.6 (C-2), 50.4 (C-5), 71.4 (C-19)
6	142.0 (C-4), 48.5 (C-5), 69.4 (C-7), 38.4 (C-8)	50.4 (C-5), 70.9 (C-7), 37.1 (C-8), 46.1 (C-10)
7	48.5 (C-5), 38.4 (C-8)	73.2 (C-6)
8	69.4 (C-7), 53.3 (C-9), 11.8 (C-17), 178.4 (C-20)	70.9 (C-7), 54.4 (C-9), 11.7 (C-17), 172.2 (C-20)
10	48.5 (C-5), 53.3 (C-9), 45.0 (C-11), 63.8 (C-18/C-19), 178.4 (C-20)	21.6 (C-2), 50.4 (C-5), 54.4 (C-9), 71.4 (C-19), 172.2 (C-20)
11	38.4 (C-8), 44.4 (C-10), 72.4 (C-12), 124.9 (C-13)	37.1 (C-8), 54.4 (C-9), 46.1 (C-10), 72.6 (C-12), 124.7 (C-13)
12	45.0 (C-11), 124.9 (C-13), 107.7 (C-14), 139.4 (C-16)	44.5 (C-11), 124.7 (C-13), 107.8 (C-14), 139.5 (C-16)
14	124.9 (C-13), 143.9 (C-15), 139.4 (C-16)	124.7 (C-13), 144.0 (C-15), 139.5 (C-16)
15	124.9 (C-13), 107.7 (C-14), 139.4 (C-16)	124.7 (C-13), 107.8 (C-14), 139.5 (C-16)
16	124.9 (C-13), 107.7 (C-14), 143.9 (C-15)	124.7 (C-13), 107.8 (C-14), 144.0 (C-15)
17	69.4 (C-7), 38.4 (C-8), 53.3 (C-9)	70.9 (C-7), 37.1 (C-8), 54.4 (C-9)
18	130.4 (C-3), 142.0 (C-4), 44.4 (C-10)	79.3 (C-3), 153.7 (C-4), 50.4 (C-5), 71.4 (C-19)
19	142.0 (C-4), 70.0 (C-6), 44.4 (C-10)	79.3 (C-3), 153.7 (C-4), 50.4 (C-5), 73.2 (C-6), 46.1 (C-10)

tance of 3.9 and 4.8 Å between H-12 and H_2-1 for 12(*R*) conformer as measured from the 3-D model is too large to give rise to an observable ROE of that magnitude. In contrast, in the 12(*S*) conformer, H-12 and H_2-1 are separated by ROE-observable distances (2.3 and 3.4 Å, Fig. 1a). On the basis of these facts, the structure of **1** was established as 15,16-epoxy-6β,7β,18,19-tetrahydroxy-neo-cleroda-3(4),13(16),14 trien-20,12(*S*)-olide.

Compound **2** has the same molecular formula $C_{20}H_{26}O_7$ as **1** deduced from its EI-mass spectrum (*m*/*z* 378) and ^{13}C NMR data. Comparison of its IR, ^{1}H and ^{13}C NMR data with those of **1** showed that **2** has many spectral features in common with **1**. In fact, **2** differed from **1** only in the substitution pattern of ring A Table 1. The differences in their NMR spectra could be accounted for by the absence of the signals of both the C3=C4 double bond and the primary hydroxy group attached to H_2-18 in **2**. Instead, an exomethylene (δ_H 4.84 br. s, 5.42 br. s; δ_C 103.8 t, 153.7 s) and an additional secondary hydroxy function (δ_H 4.25 d, J=4.3 Hz; δ_C 79.3 d) were introduced in **2**. Prominent HMBC correlations (Table 2) between H_2-2/C-4, H_2-18/C-4, H_2-18/C-5 and H_2-18/C-19 allowed us to position the exocyclic double bond at C-4/C-18. From the additional ^{1}H, ^{13}C long-range couplings between H-2/C-3, H-3/C-2, H-3/C-5, H_2-18/C-3 and H_2-19/C-3 in conjuction with the results of ^{1}H,^{1}H DQF-COSY spec-

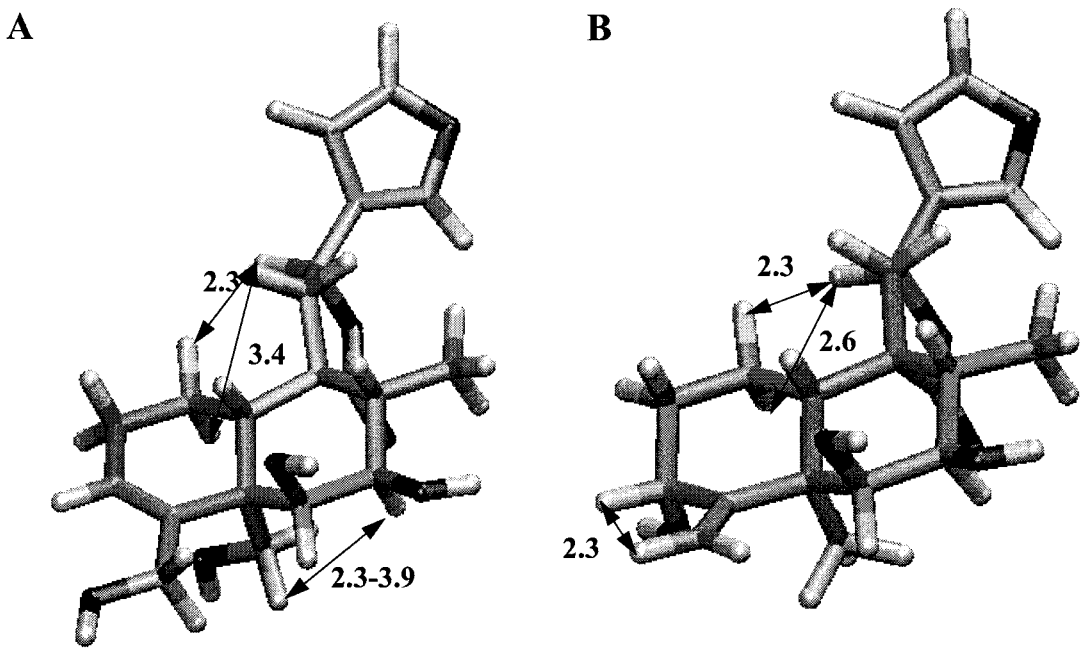

Fig. 1. Graphical representation of **1** (a) and **2** (b) derived from molecular modeling study.

trum, it was evident that the secondary hydroxyl was attached to C-3.

The stereochemical assignments within **2** were accomplished in the similar manner as described for **1**. From ROE data it was obvious that **2** has the same configurations at the chiral centers C-5, C-6, C-7, C-8, C-9, C-10 and C-12 as **1**, and only the stereochemistry at C-3 remained to be determined. The α-orientation of OH(C-3) function was concluded from the following observations: As indicated by molecular modeling calculations, the interproton distance between H-3 and H_2-18 (2.3 Å) in the 3-Hβ conformer is smaller than the corresponding distance in the 3-Hα conformer (3.4 Å). This is in accordance with the ROE data that display a strong dipolar coupling between these protons. Furthermore, the interproton distances between H-3 and other relevant protons (H-3/H-10, 4.1 Å, H-3/H-6 5.0 Å, H-3/H_2-19 3.8-5.2 Å, depending on the rotational state of the C-5/C-19 bond) justified the absence of ROE couplings. A value of 4.3 Hz for the coupling constant between H_2-2 and H-3 further strengths the β-position of H-3. Fig. 1b illustrates a 3-D representation of compound **2** highlighting the interatomic distances between pertinent protons. Thus, **2** is 15,16-epoxy-3α,6β,7β,19-tetrahydroxy-neo-clerода-4(18),13(16),14 trien-20,12(*S*)-olide. For this compound, we propose the trivial name of teulolin B.

To the best of our knowledge, teulolin B is the first neo-clerodane diterpene retaining an exocyclic double bond at C-4/C-18 isolated from *Teucrium* species.

3. Experimental

3.1. Plant material

The plant material was collected from Antalya, Gündoğmuş, Ümit village, Turkey in July 1993. Voucher specimens are deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

3.2. Extraction and isolation

Air-dried and powdered aerial parts of the plant material (280 g) were extracted with MeOH at room temperature. After removal of the solvent in vacuo, the residue (62.9 g) was dissolved in water and defatted with $CHCl_3$. The water fraction (22.2 g) was fractionated on polyamide column using a gradient mixtures of MeOH–H_2O to yield 12 fractions. Fraction 3 was divided into three portions (a–c) and subsequent work was conducted on frs 3a and 3b. Fr. 3a (900 mg) was subjected to RP MPLC (Sepralyte 40 μm) eluting with MeOH–H_2O mixtures (stepwise gradient, 30–45% MeOH) and then chromatographed on a silica gel column ($CHCl_3$–MeOH, 95:5) to afford *1* (7 mg). Repeated silica gel CC of fr. 3b (900 mg) employing $CHCl_3$–MeOH mixtures as eluent furnished *2* (8 mg).

3.3. Teulolin a (*1*)

Amorphous, colorless powder. $[\alpha]_{20}^{D}$ −34° (*c* 0.2, $CHCl_3$). IR (KBr; υ_{max}) 3367 (br.), 1747, 1660, 1603, 1505 and 876 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$–MeOD, 3:1) and ^{13}C NMR (75.5 MHz, $CDCl_3$–MeOD, 3:1) Table 1. EI-MS (70 eV) m/z 378 $[M]^+$ (1.5), 360 $[M\text{-}H_2O]^+$ (21), 342 $[M\text{-}2H_2O]^+$ (15), 295 (50), 269 (10), 251 (15), 189 (38), 166 (36), 149 (29), 133 (37), 118 (39), 105 (31), 95 (56), 86 (100), 85 (34), 81 (77), calculated for $C_{20}H_{26}O_7$.

3.4. Teulolin b (*2*)

Amorphous, colorless powder. $[\alpha]_{20}^{D}$ +14° (*c* 0.31, $CHCl_3$). IR (KBr; υ_{max}) 3419 (br.), 1756, 1668, 1505 and 875 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$–MeOD, 3:1) and ^{13}C NMR (75.5 MHz, $CDCl_3$–MeOD, 3:1) Table 1. EI-MS (70 eV) m/z 378 $[M]^+$ (1), 360 $[M\text{-}H_2O]^+$ (2), 342 $[M\text{-}2H_2O]^+$ (3), 316 (7), 297 (8), 271 (7), 247 (11), 219 (15), 193 (20), 133 (18), 111 (26), 95 (85), 81 (63), 57 (100), calculated for $C_{20}H_{26}O_7$.

3.5. Molecular modeling

Structures were built and displayed using the molecular modeling program SYBYL (version 6.3 Tripos Inc). Therein, minimization was performed using the force-field implemented within the MOPAC module, a program for semiempirical calculations, that uses AM1 as the Hamiltonian. Interproton distances mentioned above were extracted from the resulting conformers.

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References

Baytop, T. (1984). *Therapy with medicinal plants (past and present)*. Istanbul: Istanbul University Publications.

Bedir, E., & Çalis, I. (1997). *H. U. Ecz. Fak. Derg.*, *17*, 9.
Bruno, M., Fazio, C., Piozzi, F., Rodriguez, B., & de la Torre, M. C. (1995). *Phytochemistry*, *40*, 1481.
Çalis, I., Wright, A. D., & Sticher, O. (1996). *J. Nat. Prod.*, *59*, 457.
Davis, P. H. (1982). In *Flora of Turkey and the East Aegean Islands*, *Vol. 7*. Edinburgh: University Press.