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TEUCRETOL, A *NEO*-CLERODANE DITERPENOID FROM *TEUCRIUM CRETICUM*

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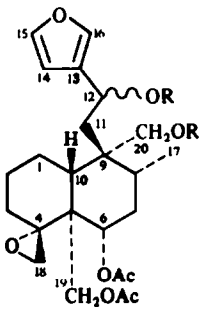
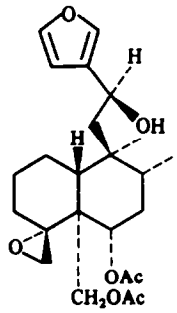
Key Word Index—*Teucrium creticum*; Labiatae; *neo*-clerodane derivatives; 6,19-diacetylteumassilin; 19-acetylgnaphalin; teucjaponin B; teucretol.

Abstract—From the aerial parts of *Teucrium creticum* a new *neo*-clerodane diterpenoid, teucretol, has been isolated, together with the previously known diterpenoids 6,19-diacetylteumassilin, 19-acetylgnaphalin and teucjaponin B. The structure of teucretol, 6,19-diacetoxy-4α,18;15,16-diepoxy-neo-cleroda-13(16),14-diene-12ξ, 20-diol, was established by chemical and spectroscopic means.

INTRODUCTION

In continuation of our studies on diterpenoid compounds from *Teucrium* species (family Labiatae) [1–4], we have now investigated *T. creticum* L. (synonym *T. rosmarinifolium* Lam.), an endemic species of the eastern Mediterranean basin. From the aerial parts of this plant we have isolated four neo-clerodane diterpenoids, three of which, 19-acetylgnaphalin [5–7], teucjaponin B [7, 8] and 6,19-diacetylteumassilin (3) [9], are already known. The other one is a new substance, teucretol, whose structure (1) was established by chemical and spectroscopic means.



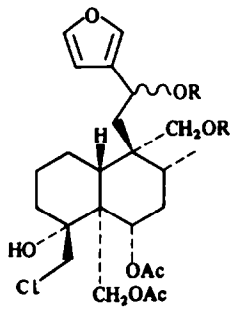


1 R = H
2 R = Ac
4 R = *p*-Nitrobenzoyl

3

RESULTS AND DISCUSSION

The new diterpenoid, teucretol (1), had a molecular formula $C_{24}H_{34}O_8$ and its IR spectrum showed hydroxyl (3460 cm^{-1}), furanic (3140, 1503, 875 cm^{-1}) and acetate (1730 *br*, 1260 cm^{-1}) absorptions. The ^1H NMR spectrum of compound 1 (Table 1) showed a number of similarities to that of 6,19-diacetylteumassilin (3), a *neo*-clerodane diterpenoid previously isolated from *T. massiliense* [9] and also found in the species now studied. Comparison between the ^1H NMR spectra of 1 (Table 1) and 3 [9] clearly established that compound 1 possessed a β-substituted furan ring, a 4α,18-oxirane group, two acetoxyl functions attached to the C-19 and C-6α positions and a C-12 hydroxyl group. In fact, the only difference between the ^1H NMR spectra of these compounds was the presence in teucretol (1) of a hydroxymethyl group attached to a quaternary carbon atom (δ3.60, 2H, *s*, Table 1) [6] instead of the C-20 methyl group of 6,19-diacetylteumassilin (3, δ0.70, 3H, *s*) [9]. These data suggested that teucretol (1) was the C-20 hydroxy derivative of 3. In agreement with this assumption, acetic anhydride-pyridine treatment of compound 1 gave a derivative (2) the IR spectrum of which was devoid of hydroxyl absorptions and whose ^1H NMR spectrum (Table 1) showed paramagnetically shifted signals of the



5 R = *p*-Nitrobenzoyl

C-12 and C-20 protons (Δδ + 1.08 and + 0.38, respectively).

Furthermore, structure 1 for teucretol was also supported by the ^{13}C NMR spectra of the natural compound and its acetyl derivative 2 (Table 2). The chemical shift of the C-2–C-7, C-12–C-16, C-18 and C-19 carbon atoms of

Table 1. ^{1}H NMR data of compounds **1**, **2**, **4** and **5** ($CDCl_3$; TMS as internal standard)*

	1	2	4†	5†
H-6β	4.75 *br dd*	4.71 *br dd*	4.74 *br dd*	5.10 *br dd*
H_A-11	‡	‡	2.30 *dd*	2.33 *dd*
H_B-11	‡	‡	2.58 *dd*	2.63 *dd*
H-12	4.86 *dd*‡	5.94 *dd*	6.34 *dd*	6.43 *dd*
H-14	6.41 *t*	6.38 *dd*	6.46 *dd*	6.45 *dd*
H-15	7.39 *m*‡	7.39 *t*	7.42 *t*	7.42 *t*
H-16	7.39 *m*‡	7.43 *m*	7.53 *m*	7.53 *m*
Me-17	0.92 *d*	0.85 *d*	1.02 *d*	1.05 *d*
H_A-18§	2.22 *d*	2.23 *d*	1.99 *d*	3.69 *d*
H_B-18‖	3.01 *dd*	3.02 *dd*	2.93 *dd*	3.91 *d*
H_A-19	4.43 *br d*	4.37 *br d*	4.40 *br d*	4.69 *br d*
H_B-19	4.89 *d*	4.89 *d*	4.83 *d*	4.88 *d*
H_A-20	3.60 *s*	3.95 *d*	4.38 *d*	4.39 *d*
H_B-20		4.01 *d*	4.47 *d*	4.64 *d*
OAc	2.11 *s*	2.13 *s*	2.11 *s*	2.11 *s*
	1.95 *s*	2.12 *s*	1.96 *s*	2.01 *s*
	—	2.03 *s*	—	—
		1.96 *s*		—
J (Hz)				
6β,7α	9.9	10.5	11.0	10.8
6β,7β	4.4	4.8	5.5	4.6
11A,11B	‡	‡	16.0	16.4
11A,12	‡	3.5	3.2	2.8
11B,12	‡	7.8	7.9	8.0
14,15	1.4	1.8	1.8	1.8
14,16	1.4	0.9	0.9	0.9
15,16	‡	1.8	1.8	1.8
17,8β	5.9	6.1	6.6	6.4
18A,18B	3.8	3.8	3.9	11.3
18B,3α	1.8	2.2	1.7	0
19A,19B	12.1	12.2	12.1	13.4
19A,6β	<0.4	<0.4	<0.4	<0.4
20A,20B	0	12.2	12.0	12.1

*All these assignments have been confirmed by double resonance experiments.

†*p*-Disubstituted aromatic protons: **4**, 8.38–8.14 (8H, $J_o = 9.0$ Hz, $J_m = 2.0$ Hz); **5**, 8.36–8.12 (8H, $J_o = 9.0$ Hz, $J_m = 2.1$ Hz).

‡Overlapped signal.

§*Exo* hydrogen with respect to ring B.

‖*Endo* hydrogen with respect to ring B.

teucretol were identical with those of 6,19-diacetylteumassilin (**3**) [9], whereas the observed differences in the δ values of the C-1, C-8–C-11, C-17 and C-20 carbons (see Table 2 and ref. [9]) were only compatible with the existence in teucretol of a primary hydroxyl group at the C-20 position [9, 10]. On the other hand, comparison between the ^{13}C NMR spectra of compounds **1** and **2** (Table 2) clearly established that teucretol (**1**) possessed hydroxyl substituents at the C-12 and C-20 positions and acetoxyl groups attached to its C-6 and C-19 carbon atoms, since acetylation of **1** caused paramagnetic shifts in C-12 and C-20 ($\Delta\delta + 1.1$ and $+ 1.7$, respectively) and diamagnetic shifts of the C-8, C-9, C-10, C-11 and C-13 carbons ($\Delta\delta - 1.0, -1.4, -1.6, -3.7$ and -4.5, respectively), whereas the rest of the carbons appeared at identical or almost identical field in both compounds (Table 2).

As compounds **1** and **2** were thick oils, we were interested to prepare a crystalline derivative in order to determine the absolute configuration and the C-12 stereochemistry of teucretol (**1**) by an X-ray diffraction analysis. Treatment of teucretol (**1**) with *p*-nitrobenzoyl chloride–pyridine yielded two compounds: the di-*p*-nitrobenzoate **4** and the chlorohydrin **5**. This last compound was transformed into the former by treatment with Amberlite IR-400 resin [11]. Unfortunately, compound **4** was an amorphous powder which did not crystallize, the derivative **5** was a crystalline substance, but its crystals from several solvents were not suitable for X-ray analysis. Thus, the stereochemistry at the C-12 centre and the absolute configuration of teucretol were not ascertained. However, the decalin moiety of compound **1** is believed to belong to the *neo*-clerodane series like 19-acetylgnaphalin [5–7], teucjaponin B [7, 8] and 6,19-diacetylteumassilin

Table 2. ^{13}C NMR chemical shifts of compounds **1** and **2** ($CDCl_3$, TMS as internal standard)

C	1	2	C	1	2
1	22.7 *t**	22.2 *t*	15	143.3 *d*	143.7 *d*
2	25.2 *t*	25.2 *t*	16	138.1 *d*	139.8 *d*
3	32.9 *t*	32.5 *t*	17	16.8 *q*	16.2 *q*
4	65.3 *s*	65.4 *s*	18	48.6 *t*	48.7 *t*
5	45.3 *s*	45.1 *s*	19	62.2 *t*	61.9 *t*
6	72.4 *d*	71.8 *d*	20	63.5 *t*	65.2 *t*
7	33.2 *t*	33.2 *t*	OAc	171.1 *s*	170.8 *s*
8	35.6 *d*	34.6 *d*		170.0 *s*	170.7 *s*
9	43.2 *s*	41.8 *s*		21.2 *q*	169.9 *s*
10	49.4 *d*	47.8 *d*		21.1 *q*	169.8 *s*
11	40.4 *t*	36.7 *t*		—	21.5 *q*
12	63.2 *d*	64.3 *d*		—	21.2 *q*
13	130.5 *s*	126.0 *s*		—	21.1 *q*
14	108.2 *d*	108.4 *d*		—	21.0 *q*

*SFORD multiplicity.

(**3**) [9], co-occurring in the same species. Moreover, all the diterpenoids previously isolated from *Teucrium* species [12], and whose structures have been rigorously established, belong to the *neo*-clerodane series. These biogenetic reasons are not adequate for suggesting a configuration at C-12 in teucretol, since there are some *neo*-clerodanes isolated from *Teucrium* which possess a 12*R*-absolute stereochemistry, although a 12*S*-configuration is the more common feature [12].

From a biogenetic point of view it is important to note that teucretol (**1**) is the first C-20 hydroxylated *neo*-clerodane found in *Teucrium* species. All the diterpenoids until now isolated from plants of this genus possessed the C-20 carbon as methyl, aldehyde, hemiacetal or acetal group, or forming the carboxyl moiety of a lactone function [12].

EXPERIMENTAL

Mps: uncorr. For general details on methods, see refs [1–4, 6, 7, 9, 10]. Plant materials were collected in April 1986 near Lytrodonda (Cyprus) and voucher specimens were deposited in the Herbarium of the Dipartimento di Biologia of the University of Milan, Italy.

Extraction and isolation of the diterpenoids. Dried and finely powdered *T. creticum* L. aerial parts (400 g) were extracted with Me_2CO (4 l) at room temp. for a week. The extract (23 g) was chromatographed on a silica gel column (Merck, No. 7734, deactivated with 15% H_2O, 400 g) eluted with *n*-hexane and *n*-hexane–EtOAc mixtures. Elution with *n*-hexane–EtOAc (1:1) gave 6,19-diacetylteumassilin (**3**, 200 mg) [9] and elution with EtOAc–*n*-hexane (2:1) successively gave teucretol (**1**, 980 mg), 19-acetylgnaphalin (80 mg) [5–7] and teucjaponin B (600 mg) [7, 8].

The previously known diterpenoids, 6,19-diacetylteumassilin (**3**) [9], 19-acetylgnaphalin [5–7] and teucjaponin B [7, 8], were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^1H NMR, ^{13}C NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

Teucretol (**1**). A syrup; $[\alpha]_D^{27} - 8.8°$ ($CHCl_3$; *c* 0.341); IR ν_{max}^{NaCl} cm^{-1}: 3460, 3140, 2940, 2880, 1730 (*br*), 1503, 1370, 1260, 1030, 875; ^1H NMR (200 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 2; EIMS (70 eV, direct inlet) *m/z* (rel. int.): 450 $[M]^+$ (0.3), 432 (0.2), 389 (0.4), 355 (1.2), 317 (4), 203 (12), 187 (10), 175 (12), 173 (14), 159 (17), 145 (14), 107 (15), 105 (19), 97 (39), 95 (39), 91 (21), 81 (25), 43 (100). $C_{24}H_{34}O_8$: M_r 450. (Found: C, 63.81; H, 7.57. $C_{24}H_{34}O_8$ requires: C, 63.98; H, 7.61%).

12,20-*Diacetylteucretol* (**2**). Treatment of **1** (112 mg) with Ac_2O–pyridine 48 hr at room temp. gave **2** (130 mg): a syrup; $[\alpha]_D^{25} - 20.0°$ ($CHCl_3$; c 0.330); IR ν_{max}^{NaCl} cm^{-1}: 3140, 3110, 2960, 2880, 1740 (br), 1505, 1370, 1240 (br), 1090, 1025, 877; ^1H NMR (200 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 2; EIMS (70 eV, direct inlet) *m/z* (rel. int.): 534 $[M]^+$ (0.1), 474 (4.5), 295 (2.5), 201 (8), 187 (8), 173 (7), 111 (10), 107 (7), 95 (11), 94 (15), 81 (10), 43 (100). $C_{28}H_{38}O_{10}$: M_r 534.

Preparation of the derivatives **4** *and* **5**. An excess of *p*-nitrobenzoyl chloride was added to a solution of **1** (95 mg) in pyridine (5 ml) and the solution was heated for 13 hr. Work-up in the usual manner yielded a mixture of compounds **4** and **5** (148 mg), which was subjected to PLC over silica gel plates eluted with *n*-hexane–EtOAc (3:2) yielding **5** (65 mg, less polar compound) and **4** (60 mg, most polar constituent).

Compound **4**. An amorphous powder which melted at 90–100°; $[\alpha]_D^{20} + 6.5°$ ($CHCl_3$; *c* 0.215); IR ν_{max}^{KBr} cm^{-1}: 3140, 3110, 3080, 3050, 2930, 2880, 1730 (br), 1610, 1530, 1505, 1350, 1270 (*br*), 1100, 1015, 875, 785, 720; ^1H NMR (200 MHz, $CDCl_3$): see Table 1; EIMS (70 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ absent, 581 $[M - p$-nitrobenzoic acid$]^+$ (20), 466 (11), 401 (5), 322 (5), 281 (12), 187 (16), 167 (78), 150 (57), 146 (53), 121 (53), 104 (31), 81 (31), 65 (69), 43 (100). (Found: C, 60.79; H, 5.48; N, 3.70. $C_{38}H_{40}N_2O_{14}$ requires: C, 60.96; H, 5.39; N, 3.74%.)

Compound **5**. Mp 152–155° (EtOAc–*n*-hexane, or MeOH); $[\alpha]_D^{20} + 13.6°$ ($CHCl_3$; *c* 0.044); IR ν_{max}^{KBr} cm^{-1}: 3525, 3160, 3110, 3080, 3050, 2960, 2880, 1740, 1725, 1610, 1530, 1505, 1350, 1270, 1250, 1120, 1100, 877, 785, 720; ^1H NMR (200 MHz, $CDCl_3$): see Table 1; EIMS (70 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ absent, 581 $[M - HCl - p$-nitrobenzoic acid$]^+$ (17), 466 (8), 401 (4), 313 (4), 281 (10), 187 (13), 167 (21), 150 (50), 146 (56), 104 (29), 95 (16), 94 (16), 91 (24), 81 (27), 43 (100). (Found: C, 58.23; H, 5.21; N, 3.67; Cl, 4.49. $C_{38}H_{41}N_2ClO_{14}$ requires: C, 58.12; H, 5.26; N, 3.57; Cl, 4.52%.)

Treatment of **5** *with Amberlite IR-400 resin.* The compound (**5**, 15 mg) in dry dimethylformamide (3 ml) was stirred for 40 hr with dry IR-400 resin in the anionic form (200 mg). The resin was filtered off, water was added to the filtrate and the product was recovered in EtOAc. The residue was chromatographed on silica gel with EtOAc–*n*-hexane (3:2) as eluent yielding a substance (8 mg) identical (IR, ^1H NMR, MS, TLC) in all respects with compound **4** described above.

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