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TEUGNAPHALODIN, A NEO-CLERODANE DITERPENOID FROM *TEUCRIUM GNAPHALODES*

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Key Word Index– *Teucrium gnaphalodes*; Labiatae; diterpenoid; neo-clerodane derivative; teugnaphalodin.

Abstract—A new neo-clerodane diterpenoid, teugnaphalodin, has been isolated from the aerial part of *Teucrium gnaphalodes*. Its structure, 6β,18; 15,16-diepoxy-4α,6α,12*S*-trihydroxy-neo-clerodane-13(16),14-dien-20,19-olide, has been established by chemical and spectroscopic means.

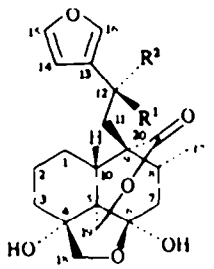
INTRODUCTION

In previous communications [1, 2] we reported the isolation and structure elucidation of four neo-clerodane diterpenoids from the acetone extract of the aerial part of *Teucrium gnaphalodes*. We have re-examined this plant and isolated a fifth diterpenoid, teugnaphalodin (**1**), in addition to gnaphalin, 19-acetylgnaphalin, gnaphalidin and teucrin P_1 [2, 3] previously found in this source.

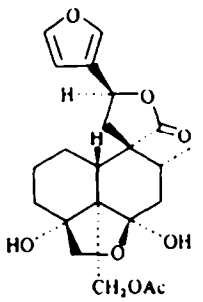
RESULTS AND DISCUSSION

Teugnaphalodin (**1**), $C_{20}H_{26}O_7$, had an IR spectrum which was consistent with the presence of hydroxyl groups (3480, 3400 cm^{-1}), a furan ring (3150, 3130, 1510, 875 cm^{-1}) and, probably, a δ-lactone function (1700 cm^{-1}). The ^1H NMR spectrum of the new diterpenoid (**1**, Table 1) showed signals attributable to a secondary methyl group and a β-substituted furan ring, identical with those found in the other neo-clerodane diterpenoids isolated from *T. gnaphalodes* [1–3]. In addition, the ^1H NMR spectrum of teugnaphalodin (**1**) showed three one-proton signals (δ8.43 *s*, 6.83 *d*, J = 2.5 Hz, and 6.66 *s*) which disappeared after addition of D_2O and were assigned to two tertiary hydroxyl groups and to a secondary one. There were two AB systems at δ4.27 and 4.41 (J = 9.6 Hz) and at 4.92 and 5.17 (J = 12.1 Hz) of two methylene groupings bearing oxygen atoms and attached to fully substituted carbon atoms, and finally, an ABX system (δ_A 2.50, δ_B 2.96, δ_X 5.28; J_{AB} = 15.5 Hz, J_{AX} = 10.4 Hz, J_{BX} = 2.5 Hz) the X part of which appeared as a doublet of triplets before addition of D_2O, thus establishing that this proton was coupled (J = 2.5 Hz) with the hydroxylic proton at δ6.83 and suggesting that teugnaphalodin (**1**) possessed a free secondary alcohol function at the C-12 position of a clerodane hydrocarbon skeleton such as teumassilin, a diterpenoid previously isolated from *Teucrium massiliense* [4].

All the above data pointed towards a structure such as **1** for the new diterpenoid and this was confirmed as follows. Treatment of compound **1** with acetic anhydride–pyridine at room temperature yielded a monoacetyl derivative (**2**, $C_{22}H_{28}O_8$), the IR spectrum of which showed hydroxyl absorptions at 3500 and 3420 cm^{-1} and its ^1H NMR spectrum (Table 1) showed paramagnetically shifted ($\Delta\delta$ = + 1.08) the signal of the X part of the ABX system. Chromium trioxide–pyridine oxidation of teugnaphalodin (**1**) gave compound **3** ($C_{20}H_{24}O_7$), in which the keto group arising from the oxidation of the secondary alcohol



	R^1	R^2
1	OH	H
2	OAc	H
3	O	



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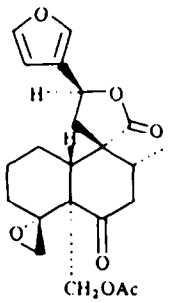

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Table 1. ^{1}H NMR spectral data of compounds 1–4 (300 MHz, TMS as internal standard)*

	1†	2†	3‡	4‡
H-7α	§	§	1.61 *dd*	2.22 *dd*
H-7β	§	2.52 *dd*	2.24 *dd*	§
H-8β	§	2.37 *ddq*	2.54 *ddq*	§
H_A-11	2.50 *dd*	2.60 *dd*	3.04 *d*	2.31 *dd*
H_B-11	2.96 *dd*	3.04 *dd*	3.55 *d*	2.48 *dd*
H-12	5.28 *dt*	6.36 *dd*	-	5.40 *dd*
H-14	6.73 *dd*	6.69 *dd*	6.74 *dd*	6.40 *dd*
H-15	7.62 *t*	7.63 *t*	7.45 *t*	7.44 *t*
H-16	7.76 *m*	7.86 *m*	8.09 *m*	7.46 *m*
Me-17	1.04 *d*	0.95 *d*	0.93 *d*	1.07 *d*
H_A-18	4.27 *d*	4.27 *d*	4.04 *d*	4.04 *d*
H_B-18	4.41 *d*	4.34 *d*	4.29 *d*	4.13 *d*
H_A-19	4.92 *d*	4.89 *d*	4.59 *d*	5.00 *d*
H_B-19	5.17 *d*	5.14 *d*	4.74 *dd*	5.07 *d*
OH	8.43 *s*	8.68 *s*	4.11 *s*	4.61 *s*
	6.83 *d*	-	--	--
	6.66 *s*	6.57 *s*	3.92 *s*	4.16 *s*
OAc	—	2.11 *s*	-	2.12 *s*
J (Hz)				
7α, 7β	§	14.3	14.7	15.6
7α, 8β	§	12.6	13.4	13.8
7β, 8β	§	4.8	4.9	§
8β, 17	6.4	6.6	6.8	6.6
11A, 11B	15.5	16.0	18.8	14.0
11A, 12	10.4	10.5		7.3
11B, 12	2.5	2.8		9.9
14, 15	1.7	1.7	1.7	1.8
14, 16	1.0	1.0	0.9	0.8
15, 16	1.7	1.7	1.7	1.8
18A, 18B	9.6	9.9	10.2	10.0
19A, 19B	12.1	12.1	12.3	13.2
19B, 10β	0	0	1.3	0
12, 12(OH)	2.5	—	-	—

*Spectral parameters were obtained by first order approximation. All these assignments have been confirmed by double resonance experiments.

†In pyridine-d_5 solution.

‡In deuteriochloroform solution.

§Overlapped signal.

Table 2. ^{13}C NMR chemical shifts (in δ values from TMS) of compounds 1, 2 and 3

	1*	2*	3†
C-1	22.6 *t*‡	22.4 *t*	23.4 *t*
C-2	23.7 *t*	23.9 *t*	23.4 *t*
C-3	30.2 *t*	30.1 *t*	29.6 *t*
C-4	81.6 *s*	81.4 *s*	81.5 *s*
C-5	47.8 *s*	48.0 *s*	47.7 *s*
C-6	107.2 *s*	107.0 *s*	107.1 *s*
C-7	40.1 *t*	40.9 *t*	40.3 *t*§
C-8	35.5 *d*	35.8 *d*	36.6 *d*
C-9	49.3 *s*	49.1 *s*	48.3 *s*
C-10	43.0 *d*	42.9 *d*	42.4 *d*
C-11	37.5 *t*	34.8 *t*	40.5 *t*§
C-12	62.7 *d*	65.2 *d*	192.3 *s*
C-13	132.2 *s*	126.7 *s*	127.8 *s*
C-14	109.4 *d*	109.3 *d*	108.5 *d*
C-15	143.5 *d*	144.0 *d*	144.5 *d*
C-16	138.9 *d*	140.7 *d*	147.4 *d*
C-17	16.6 *q*	16.5 *q*	16.3 *q*
C-18	76.9 *t*	77.0 *t*	76.4 *t*
C-19	67.4 *t*	67.4 *t*	66.8 *t*
C-20	172.7 *s*	171.8 *s*	172.4 *s*
OAc		169.9 *s*	
	—	21.1 *q*	--

*In pyridine-d_5 solution.

†In deuteriochloroform solution.

‡SFORD multiplicity.

§These assignments may be reversed.

was conjugated (ν_{CO} 1680 cm^{-1}; λ_{max} 254 nm, log ε 3.48) with the furan ring. These facts clearly established [4] that the secondary hydroxyl group of the new diterpenoid (1) is at the C-12 position. Furthermore, comparison of the ^{1}H NMR spectra (Table 1) of compounds 2 and 4 (a synthetic product obtained by us from 19-acetylgnaphalin (5) by H_3PO_4 treatment [5]), showed that they are very similar and the difference in the chemical shift of the C-12 proton (δ6.36 in 2 and 5.40 in 4) must be due to the fact that compound 2 possesses a C-12 acetoxyl group and a C-20/C-19 δ-lactone instead of the C-19 acetate and the C-20/C-12 γ-lactone functions of compound 4.

In addition, the chemical shifts of the C-1 to C-10 and C-18 carbon atoms of compound 1 (Table 2) are almost identical with those reported [5] for the corresponding carbon atoms of teupolin V, a *trans*-neo-clerodane diterpenoid which possesses an hemiacetalic bridge between the C-6 β and C-18 positions and a tertiary α-hydroxyl group at C-4, whereas the C-11 to C-17, C-19 and C-20 signals of the new diterpenoid (1) appeared at the same position as those in a synthetic *trans*-neo-clerodane diterpenoid possessing a C-12 hydroxyl group and a C-20/C-19 δ-lactone function [6].

Finally, application of Horeau's method [7] to teugnaphalodin (1, see Experimental) established the absolute configuration of the C-12 hydroxyl group as *S*. The absolute configuration of the substituted *trans*-decalin moiety of teugnaphalodin was not ascertained. However, compound 1 is believed to belong to the neo-clerodane [8] series like gnaphalin, 19-acetylgnaphalin (5), gnaphalidin and teucrin P_1 [1–3], co-occurring in the same species. Moreover, all the diterpenoids until now isolated from *Teucrium* species, and whose structures have been rigorously established, belong to the neo-clerodane series.

On the basis of all the above data, structure 1 was established for teugnaphalodin.

EXPERIMENTAL

Mps are uncorr. For general details on methods see refs [1, 2]. Plant materials were collected in July 1984, near Arganda, Madrid, Spain, and voucher specimens were deposited in the Herbarium of the Royal Botanical Garden of Madrid.

Extraction and isolation of the diterpenoids. Dried and finely powdered *Teucrium gnaphalodes* L'Hér. aerial parts (6.5 kg) were extracted with Me_2CO (15 l.) at room temp. for a week. The extract (260 g) was chromatographed on a silica gel column (Merck No. 7734, deactivated with 15% H_2O, 2 kg), eluted with petrol (alkanes, fats and waxes), then petrol–EtOAc mixtures.

The petrol–EtOAc (2:1) successively eluted teucrin P_1 (300 mg) [2, 3] and gnaphalidin (600 mg) [1]; petrol EtOAc (1:1) eluted 19-acetylgnaphalin (5, 6 g) [1, 2] and gnaphalin (2.5 g) [1, 2], and petrol–EtOAc (1:3) eluted teugnaphalodin (1, 1.1 g).

The previously known diterpenoids were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^{1}H NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

Teugnaphalodin (1). Mp 159 162° (EtOAc *n*-hexane); $[\alpha]_D^{21}$ − 70.2° (MeOH; *c* 1.084); IR ν_{max}^{KBr} cm^{-1}: 3480, 3400 (br), 3150, 3130, 2950, 2890, 2870, 1700, 1510, 1450, 1390, 1250, 1200, 1175, 1155, 1070, 1060, 1020, 1000, 890, 875, 810, 745; ^{1}H NMR (300 MHz, pyridine-d_5): see Table 1; ^{13}C NMR (20.15 MHz, pyridine-d_5): see Table 2; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 378 [M]$^+$ (8), 360 (3), 348 (2), 342 (3), 330 (5), 317 (25), 312 (14), 185 (36), 145 (22), 134 (55), 105 (43), 95 (83), 91 (77), 77 (53), 69 (48), 53 (43), 41 (100). (Found: C, 63.45; H, 7.09. $C_{20}H_{26}O_7$ requires: C, 63.48; H, 6.93%.)

Application of Horeau's method to teugnaphalodin (1). This was performed in the usual manner [7]. Compound 1 (66.0 mg, 0.175 mmol), (±)-α-phenylbutyric anhydride (244.8 mg, 0.79 mmol) in pyridine (2 ml) soln, 16 hr at room temp.: $\alpha_1 = -3.108$; $\alpha_2 = -2.334$; $\alpha_1 - 1.1\alpha_2 = -0.541$. Configuration (12*S*).

12-*Acetylteugnaphalodin* (2). Ac_2O–pyridine treatment of 1 (300 mg) for 24 hr at room temp. yielded compound 2 (275 mg, after crystallization from EtOAc–*n*-hexane): mp 228–230°; $[\alpha]_D^{21}$ − 82.5° ($CHCl_3$–MeOH, 2:1; *c* 0.640); IR ν_{max}^{KBr} cm^{-1}: 3500, 3420, 3160, 3020, 2955, 2900, 2880, 1750, 1710, 1510, 1445, 1380, 1240, 1173, 1165, 1070, 1050, 1025, 1010, 980, 890, 875, 820, 740; ^{1}H NMR (300 MHz, pyridine-d_5): see Table 1; ^{13}C NMR (20.15 MHz, pyridine-d_5): see Table 2; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 420 [M]$^+$ (0.4), 403 (2), 402 (1), 389 (3), 378 (83), 360 (90), 342 (12), 329 (19), 205 (19), 191 (19), 121 (26), 105 (38), 97 (57), 95 (64), 94 (60), 91 (57), 81 (47), 69 (47), 55 (45), 43 (100). (Found: C, 62.35; H, 6.81. $C_{22}H_{28}O_8$ requires: C, 62.84; H, 6.71%.)

Compound 3 from teugnaphalodin (1). CrO_3–pyridine treatment of 1 (300 mg) in the usual manner gave 3 (290 mg after chromatography on a silica gel column eluted with *n*-hexane–EtOAc, 2:1), an amorphous solid which melted at 90–96°; $[\alpha]_D^{21}$ − 25.1° ($CHCl_3$; *c* 0.675); IR ν_{max}^{KBr} cm^{-1}: 3430, 3130, 2940, 2870, 1715, 1680, 1560, 1510, 1415, 1350, 1160, 1000, 975, 875, 745; UV λ_{max}^{MeOH} nm (log ε): 254 (3.48); ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (20.15 MHz, $CDCl_3$): see Table 2; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 376 [M]$^+$ (33), 358 (10), 281 (12), 263 (13), 249 (38), 205 (20), 175 (18), 110 (20), 105 (13), 95 (100), 91 (20), 77 (15), 69 (16), 55 (20). (Found: C, 63.62; H, 6.29. $C_{20}H_{24}O_7$ requires: C, 63.82; H, 6.43%.)

Compound 4 from 19-acetylgnaphalin (5). 19-Acetylgnaphalin (200 mg) was added to a soln of 0.1 N H_3PO_4 (40 ml) and MeOH (40 ml) and the reaction mixture was stirred and refluxed at 105° for 15 hr. Work-up in the usual manner [5] yielded a mixture (TLC) of the starting material (5) and compound 4. After chromatographic separation (silica gel column eluted with $CHCl_3$–MeOH, 99:1), pure 4 (80 mg) was obtained: mp 96 98° (EtOAc *n*-hexane); $[\alpha]_D^{19}$ − 8.8° ($CHCl_3$; *c* 0.204); IR ν_{max}^{KBr} cm^{-1}: 3510, 3455, 3420, 3250, 3170, 3140, 2980, 2950, 2870, 1745, 1710, 1510, 1455, 1380, 1275, 1165, 1025, 985, 930, 880, 820, 725; ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 420 [M]$^+$ (0.2), 402 (12), 389 (16), 360 (16), 329 (48), 318 (48), 317 (56), 313 (20), 220 (19), 161 (14), 123 (50), 105 (15), 95 (60), 94 (32), 91 (26), 81 (36), 69 (20), 55 (20), 43 (100). (Found: C, 62.93; H, 6.66. $C_{22}H_{28}O_8$ requires: C, 62.84; H, 6.71%.)

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