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TEUMARIN, A NEO-CLERODANE DITERPENOID FROM *TEUCRIUM MARUM*

GIUSEPPE SAVONA, FRANCO PIOZZI, ORIETTA SERVETTAZ*, FRANCISCO FERNÁNDEZ-GADEA† and BENJAMÍN RODRÍGUEZ†

Istituto di Chimica Organica dell'Università, Archirafi 20, 90123 Palermo, Italy; *Dipartimento di Biologia, Università di Milano, Italy; †Instituto de Química Orgánica, CSIC., Juan de la Cierva 3, Madrid-6, Spain

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Key Word Index—*Teucrium marum*; Labiatae; diterpenoid; neo-clerodane; teumarin.

Abstract—A new neo-clerodane diterpenoid, teumarin, was isolated from the aerial part of *Teucrium marum*. Its structure, 19-acetoxy-4α,18 : 15,16-diepoxy-2β,6β-dihydroxy-neo-cleroda-13(16),14-dien-20,12*S*-olide, was established by chemical and spectroscopic means and by comparison with closely related compounds.

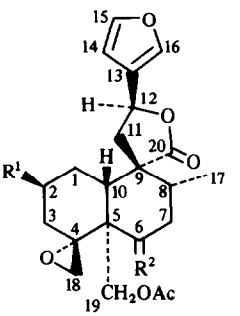
INTRODUCTION

In continuation of our studies on diterpenoids from *Teucrium* species [1–4], we now report the isolation and structure determination of a new compound from the aerial parts of *T. marum* L., a species collected in Sardinia, Italy. The structure of this new diterpenoid, teumarin, has been shown to be 19-acetoxy-4α,18 : 15,16-diepoxy-2β,6β-dihydroxy-neo-cleroda-13(16),14-dien-20, 12*S*-olide (**1**) by chemical and spectroscopic means and by comparison with closely related compounds.

RESULTS AND DISCUSSION

Teumarin (**1**) had a molecular formula $C_{22}H_{28}O_8$ and its IR spectrum showed hydroxyl (3460 cm^{-1}), furanic (3160, 3130, 1508, 880 cm^{-1}), γ-lactone (1760 cm^{-1}) and acetate (1737, 1260 cm^{-1}) absorptions. The presence of a furan ring was supported by UV absorption at λ_{max} 211 nm (log ε3.54). Acetic anhydride–pyridine treatment of teumarin (**1**) for 3 hr at room temperature gave a monoacetyl derivative (**2**, $C_{24}H_{30}O_9$), whereas the same treatment for 72 hr yielded a diacetyl derivative (**3**, $C_{26}H_{32}O_{10}$), the IR spectrum of which showed no hydroxyl absorption. Thus, teumarin (**1**) possesses two hydroxyl groups. The ^1H NMR spectra of compounds **1–3** (Table 1) showed signals of a β-substituted furan ring, a secondary methyl group, an α,α-disubstituted oxirane ring, a γ-lactone group and an acetylated hydroxymethylene grouping identical with those found in several neo-clerodane diterpenoids isolated from *Teucrium* species [5]. Consequently, the new diterpenoid possessed a structure of 19-acetoxy-4α,18 : 15,16-diepoxycleroda-13(16),14-dien-20,12-olide with two additional hydroxyl groups (**1**).

The two hydroxyl groups of teumarin (**1**) are secondary and axially oriented, because their geminal protons appeared at δ4.07 (*m*, $W_{1/2}$ = 7 Hz) and 4.33 (*t*, $J_1 = J_2$ = 3 Hz) in the ^1H NMR spectrum of **1** and were shifted to δ5.30 and 4.10, respectively, in the derivative **2**, and to δ5.30 and 5.05, respectively, in the peracetyl derivative **3**. The hydroxyl group which was more resistant to the acetylation reaction (geminal proton at δ4.33 in **1**, 4.10 in **2** and 5.05 in **3**) must be placed on the C-6β axial position, because when it was acetylated (compound **3**) the lower field signal of the C-18 oxiranic protons undergoes a strong diamagnetic shift (Δδ −0.84). An identical behaviour has been previously found between teucjaponin A (**4**) and its acetate (**5**) [6].



	R^1	R^2
1	OH	βOH, αH
2	OAc	βOH, αH
3	OAc	βOAc, αH
4	H	βOH, αH
5	H	βOAc, αH
6	H	O
7	OAc	O

As the signal of the C-6α equatorial proton appeared as a triplet in the ^1H NMR spectra of compounds **1–3** (Table 1), it was clear that the other hydroxyl group of the new diterpenoid must be placed on C-1α, C-2β or C-3α axial positions. Its location at the C-2β position was suggested by the fact that in the ^1H NMR spectrum of compound **2** (Table 1) a one-proton signal appeared at δ1.48 (*ddd*) which was assigned to the C-1α axial proton ($J_{1\alpha,1\beta} = J_{1\alpha,10\beta}$ = 15 Hz, $J_{1\alpha,2\alpha}$ = 3.5 Hz) because on irradiation at δ5.30 (H-2α) it was transformed into a

Table 1. ^{1}H NMR data of compounds **1**, **2**, **3** and **7** (90 MHz, $CDCl_3$, TMS as int. standard)*

	1	**2**	**3**	**7**
H-1α	†	1.48 *ddd* $J_{1\alpha,1\beta} = J_{1\alpha,10\beta} = 15$ Hz $J_{1\alpha,2\alpha} = 3.5$ Hz	†	†
H-2α	4.07 *m* $W_{1/2} = 7$ Hz	~ 5.30 *m*‡	~ 5.30 *m*‡	5.30 *m* $W_{1/2} = 8$ Hz
H-6α	4.33 *t* $J_{6\alpha,7\alpha} = J_{6\alpha,7\beta} = 3$ Hz	4.10 *t* $J_{6\alpha,7\alpha} = J_{6\alpha,7\beta} = 3.6$ Hz	5.05 *t* $J_{6\alpha,7\alpha} = J_{6\alpha,7\beta} = 3.5$ Hz	
H-7α	†	†	†	3.55 *t* $J_{7\alpha,7\beta} = J_{7\alpha,8\beta} = 14.7$ Hz
H-7β	†	1.55 *dd* $J_{7\beta,7\alpha} = 14$ Hz, $J_{7\beta,6\alpha} = 3.6$ Hz, $J_{7\beta,8\beta} = 0$ Hz	†	†
2H-11	†	2.40 *d* $J_{11A,12} = J_{11B,12} = 8.7$ Hz	2.41 *d* $J_{11A,12} = J_{11B,12} = 8.6$ Hz	†
H-12	5.36 *t* $J_{12,11A} = J_{12,11B} = 8.5$ Hz	5.33 *t* $J_{12,11A} = J_{12,11B} = 8.7$ Hz	5.36 *t* $J_{12,11A} = J_{12,11B} = 8.6$ Hz	5.45 *t* $J_{12,11A} = J_{12,11B} = 8.6$ Hz
H-14	6.37 *m* $W_{1/2} = 4$ Hz	6.39 *m* $W_{1/2} = 4$ Hz	6.40 *m* $W_{1/2} = 4$ Hz	6.43 *m* $W_{1/2} = 4$ Hz
H-15	7.42 *m*‡ $W_{1/2} = 4.5$ Hz	7.40 *t* $J_{15,16} = J_{15,14} = 1.2$ Hz	7.44 *m*‡ $W_{1/2} = 4.5$ Hz	7.49 *m*‡ $W_{1/2} = 4.5$ Hz
H-16	7.42 *m*‡	7.43 *m*	7.44 *m*‡	7.49 *m*‡
Me-17	0.97 *d* $J_{17,8} = 6$ Hz	1.00 *d* $J_{17,8} = 6.5$ Hz	1.00 *d* $J_{17,8} = 6.5$ Hz	1.11 *d* $J_{17,8} = 6.5$ Hz
H_A-18§	3.80 *dd* $J_{18A,18B} = 5.4$ Hz $J_{18A,3\sigma} = 1.2$ Hz	3.81 *dd* $J_{18A,18B} = 5.4$ Hz $J_{18A,3\alpha} = 1.5$ Hz	2.96 *dd* $J_{18A,18B} = 4.8$ Hz $J_{18A,3\alpha} = 1.2$ Hz	3.67 *dd* $J_{18A,18B} = 5.7$ Hz $J_{18A,3\alpha} = 1.8$ Hz
H_B-18‖	2.43 *d* $J_{18A,18B} = 5.4$ Hz	2.31 *d* $J_{18A,18B} = 5.4$ Hz	2.33 *d* $J_{18A,18B} = 4.8$ Hz	2.30 *d* $J_{18A,18B} = 5.7$ Hz
2H-19	4.83 *s*	4.83 *s*	4.93 and 4.82 AB system, $J_{AB} = 12$ Hz	5.47 and 5.02 AB system, $J_{AB} = 12.5$ Hz
OCOMe	2.03 *s*	2.06 *s*, 2.06 *s*	2.08 *s*, 2.08 *s*, 2.08 *s*	2.09 *s*, 2.06 *s*

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

†Could not be identified.

‡Overlapped signal.

§*Endo* hydrogen with respect to ring B.

‖*Exo* hydrogen with respect to ring B.

double doublet ($J_{1\alpha,1\beta} = J_{1\alpha,10\beta} = 15$ Hz). The width at half height of the C-2α equatorial proton (7–8 Hz) was also in agreement with this conclusion, since the sum of $J_{2\alpha,1\alpha} + J_{2\alpha,1\beta} + J_{2\alpha,3\alpha} + J_{2\alpha,3\beta}$ in a ring A with a chair conformation is 9.6 Hz [7].

All the above conclusions were confirmed by the ^{13}C NMR spectra of compounds **1**–**3** (Table 2), which also provided conclusive proofs of an A/B ring *trans* junction and a 12*S* configuration for teumarin (**1**). Effectively, the chemical shifts of the C-6 to C-9, C-11 to C-17, C-19 and C-20 carbon atoms of teumarin (**1**) were identical with those found for the same carbon atoms of teucjaponin A (**4**, Table 2), a diterpenoid isolated from *T. japonicum* [6] and recently found by us in *T. massiliense* [unpublished results]. Since teucjaponin A (**4**) has been correlated with 19-acetylgnaphalin (**6**) [6; unpublished results] and the structure of **6** has been firmly established by X-ray diffraction analysis [8], it was evident that teumarin (**1**) possessed a *trans* clerodane structure and a 12*S* configuration. On the other hand, comparison of the ^{13}C NMR spectra of compounds **1** and **4** (Table 2) clearly confirmed the presence in teumarin (**1**) of a C-2β axial hydroxyl group: α-effect on C-2 ($\Delta\delta$ +41.3), β-effects on C-1 ($\Delta\delta$ +7.6) and C-3 ($\Delta\delta$ +7.2), γ-gauche effects on C-10 ($\Delta\delta$ −6.7) and C-4 ($\Delta\delta$ −3.2), and δ-effects on C-5 and C-18 ($\Delta\delta$ −0.5 and +1.2, respectively).

Finally, the neo-clerodane [9] absolute configuration of teumarin (**1**) was inferred from the CD curve of its 6-ketoderivative **7**, which showed a negative Cotton effect ($\Delta\varepsilon_{297}$ −0.74), as 19-acetylgnaphalin (**6**, $\Delta\varepsilon_{298.5}$ −0.48) [8, 10].

EXPERIMENTAL

Mps are uncorr. For general details on methods see refs [1–4]. Assignments of ^{13}C NMR chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Plant materials were collected in July 1982, in East Sardinia, Italy, and

Table 2. ^{13}C NMR chemical shifts ($CDCl_3$, TMS as int. standard) of compounds **1–4**

C	1	2	3	4
1	30.9 *t**	28.3 *t*	28.5 *t*	23.3 *t*
2	66.0 *d*	69.6 *d*	69.7 *d*	24.7 *t*
3	40.4 *t*	37.3 *t*	37.0 *t*	33.2 *t*
4	59.1 *s*	58.7 *s*	58.3 *s*	62.3 *s*
5	45.3 *s*	45.3 *s*	44.4 *s*	45.8 *s*
6	65.4 *d*	65.5 *d*	69.0 *d*	65.6 *d*
7	35.3 *t*	35.4 *t*	31.0 *t*	35.4 *t*
8	32.4 *d*	32.4 *d*	33.3 *d*	32.4 *d*
9	51.8 *s*	51.6 *s*	51.4 *s*	52.1 *s*
10	39.6 *d*	40.6 *d*	41.9 *d*	46.3 *d*
11	44.8 *t*	44.9 *t*	44.7 *t*	45.3 *t*
12	72.2 *d*	71.8 *d*	71.8 *d*	71.9 *d*
13	125.3 *s*	125.2 *s*	125.1 *s*	125.5 *s*
14	108.2 *d*	108.2 *d*	108.1 *d*	108.2 *d*
15	144.2 *d*	144.2 *d*	144.3 *d*	144.2 *d*
16	139.8 *d*	139.7 *d*	139.8 *d*	139.7 *d*
17	16.4 *q*	16.4 *q*	16.2 *q*	16.4 *q*
18	54.3 *t*	53.7 *t*	51.9 *t*	53.1 *t*
19	63.7 *t*	63.3 *t*	62.4 *t*	63.6 *t*
20	178.1 *s*	177.2 *s*	176.7 *s*	177.5 *s*
OAc	171.5 *s*	171.0 *s*	170.5 *s*	171.1 *s*
	21.2 *q*	170.3 *s*	170.0 *s*	21.2 *q*
		21.3 *q*	169.2 *s*	
		21.1 *q*	21.5 *q*	
			21.2 *q*	
			21.0 *q*	

*SFORD multiplicity.

voucher specimens were deposited in the Herbarium of the 'Dipartimento di Biologia' of the University of Milan, Italy.

Isolation of the diterpenoid. Dried and finely powdered *T. marum* aerial parts (630 g) were extracted with Me_2CO (10 l.) as previously described for other *Teucrium* species [1–4]. The extract (57 g) was chromatographed on a silica gel column (Merck No. 7734, deactivated with 15% H_2O). Elution with EtOAc–*n*-hexane (5:2) yielded teumarin (**1**, 1.2 g) as the sole diterpenic constituent.

Teumarin (**1**). Amorphous solid which melts at 98–107°; $[\alpha]_D^{20}$ +34.1° ($CHCl_3$; *c* 0.135); IR ν_{max}^{KBr} cm^{-1}: 3460, 3160, 3130, 2980, 2890, 1760, 1737, 1508, 1445, 1395, 1370, 1260, 1240, 1190, 1160, 1050, 1030, 950, 880, 765; UV λ_{max}^{EtOH} nm (log ε): 211 (3.54); ^1H NMR (90 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.): 420 $[M]^+$ (0.3), 402 (0.5), 389 (1.5), 360 (6), 354 (1.3), 347 (20), 342 (5), 330 (13), 329 (18), 285 (4), 267 (5), 249 (5), 248 (5), 236 (11), 218 (7), 201 (7), 185 (7), 179 (15), 178 (11), 173 (9), 164 (8), 161 (14), 145 (12), 133 (15), 121 (11), 107 (11), 105 (18), 95 (45), 94 (21), 91 (20), 81 (33), 79 (15), 77 (15), 69 (14), 67 (13), 55 (15), 53 (12), 43 (100). (Found: C, 62.68; H, 6.59. $C_{22}H_{28}O_8$ requires: C, 62.84; H, 6.71%.)

2-Acetylteumarin (**2**). Ac_2O–pyridine treatment of **1** (200 mg) for 3 hr at room temp. yielded the derivative **2** (180 mg after crystallization): mp 183–185° (from EtOAc–*n*-hexane); $[\alpha]_D^{20}$ +42.9° ($CHCl_3$; *c* 0.451); IR ν_{max}^{KBr} cm^{-1}: 3525, 3160, 3140, 3130, 3115, 3090, 2980, 2950, 1750, 1730, 1718, 1508, 1475, 1440, 1395, 1370, 1330, 1250, 1230, 1190, 1180, 1155, 1095, 1040, 1025, 985, 965, 930, 880, 870, 830, 760, 730, 715; ^1H NMR (90 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.): 462 $[M]^+$ (1), 444 (0.5), 402 (4), 384 (1), 371 (4), 360 (1), 342 (7), 330 (6), 329 (7), 324 (4), 312 (13), 311 (15), 296 (6), 267 (6), 231 (5), 218 (12), 203 (7), 202 (7), 201 (6), 185 (9), 179 (21), 178 (12), 161 (15), 159 (14), 157 (10), 145 (11), 134 (16), 133 (17), 121 (10), 119 (16), 118 (11), 107 (11), 105 (17), 95 (37), 94 (21), 91 (20), 81 (29), 69 (13), 55 (10), 43 (100). (Found: C, 62.58; H, 6.67. $C_{24}H_{30}O_9$ requires: C, 62.32; H, 6.54%.)

2,6-Diacetylteumarin (**3**). Ac_2O–pyridine treatment of **1** (200 mg) for 72 hr at room temp. yielded the derivative **3** (200 mg): an amorphous solid which melts at 80–86°; $[\alpha]_D^{20}$ +12.7° ($CHCl_3$; *c* 0.722); IR ν_{max}^{KBr} cm^{-1}: 3140, 3120, 2970, 2940, 2880, 1765, 1740, 1505, 1460, 1445, 1375, 1240, 1195, 1180, 1160, 1025, 985, 925, 877, 810, 755, 700; ^1H NMR (90 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.): 504 $[M]^+$ (0.3), 444 (0.4), 431 (0.3), 416 (0.3), 402 (0.8), 384 (1), 371 (1.7), 354 (1.2), 342 (1.8), 329 (1.3), 324 (2.8), 313 (3), 312 (5), 311 (2.5), 299 (3), 296 (4.5), 294 (2.5), 267 (3), 251 (4), 231 (3), 230 (3), 218 (4), 202 (8), 179 (12), 157 (14), 143 (10), 134 (10), 133 (10), 118 (15), 105 (15), 96 (22), 95 (41), 94 (19), 91 (16), 81 (27), 69 (11), 55 (10), 43 (100). (Found: C, 61.74; H, 6.61. $C_{26}H_{32}O_{16}$ requires: C, 61.89; H, 6.39%.)

Compound **7**. CrO_3–pyridine treatment of 2-acetylteumarin (**2**, 70 mg) for 24 hr at room temp. yielded the ketone **7** (52 mg after crystallization): mp 130–132° (from Me_2CO–*n*-hexane); $[\alpha]_D^{22}$ +85.7° ($CHCl_3$; *c* 0.407); IR ν_{max}^{KBr} cm^{-1}: 3160, 3140, 3120, 3090, 2985, 2960, 2940, 2880, 1755, 1730, 1720, 1510, 1475, 1385, 1375, 1365, 1320, 1250, 1225, 1190, 1175, 1150, 1120, 1085, 1045, 1025, 980, 930, 880, 830, 750, 730, 720, 660, 635; CD nm ($\Delta\varepsilon$): 340 (0), 297 (−0.74), 245 (0) (EtOH, *c* 0.606); ^1H NMR (90 MHz, $CDCl_3$): see Table 1; EIMS (direct inlet) 75 eV *m/z* (rel. int.): 460 $[M]^+$ (0.2), 430 (3), 400 (3), 388 (13), 369 (25), 340 (4), 328 (38), 327 (39), 310 (19), 309 (27), 232 (15), 216 (13), 179 (42), 161 (23), 149 (30), 133 (27), 132 (23), 121 (18), 105 (14), 95 (52), 94 (30), 91 (32), 81 (40), 79 (20), 77 (19), 69 (34), 53 (14), 43 (100). (Found: C, 62.82; H, 6.18. $C_{24}H_{28}O_9$ requires: C, 62.60; H, 6.13%.)

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