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NEO-CLERODANE DITERPENOIDS FROM *TEUCRIUM PYRENAICUM*

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Key Word Index—*Teucrium pyrenaicum*; Labiatae; diterpenoids; new neo-clerodane derivatives; teupyrenone; teupyreinin; teupyreinidin; ^{13}C NMR data.

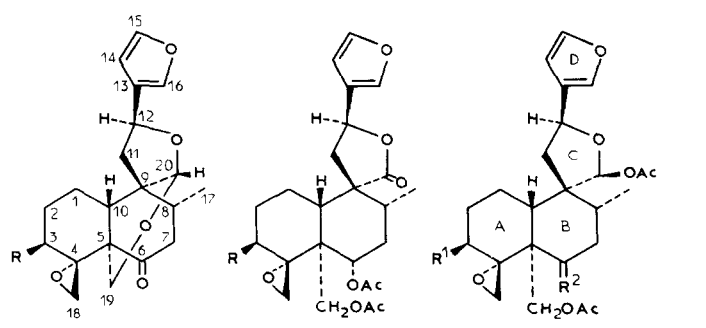
Abstract—From the aerial part of *Teucrium pyrenaicum* three new neo-clerodane diterpenoids have been isolated. Their structures, 3β-acetoxy-4α,18:12*S*,20*S*:15,16:19,20*S*-tetraepoxy-neo-cleroda-13(16),14-dien-6-one (teupyrenone), 3β,6α,19-triacetoxy-4α,18:15,16-diepoxy-neo-cleroda-13(16),14-dien-20,12*S*-olide (teupyreinin), and 3β,6α,19,20*S*-tetra-acetoxy-4α,18:12*S*,20*S*:15,16-triepoxy-neo-cleroda-13(16), 14-diene (teupyreinidin), were established mainly by spectroscopic means.

INTRODUCTION

In continuation of our studies on diterpenic compounds from *Teucrium* species [1–4], we have now investigated *T. pyrenaicum* L., from which three new neo-clerodane diterpenoids, teupyrenone (**1**), teupyreinin (**3**) and teupyreinidin (**5**), have been isolated. The structures of these new diterpenoids were established on the basis of spectroscopic evidence and by comparison with closely related compounds.

RESULTS AND DISCUSSION

The first of the new diterpenoids (teupyrenone, **1**), $C_{22}H_{26}O_7$, had an IR spectrum which showed furanic (3150, 3140, 1505, 880 cm^{-1}), acetate (1738, 1245 cm^{-1}) and ketone (1710 cm^{-1}) absorptions and no hydroxyl bands. The ^1H NMR spectrum showed signals for a secondary methyl group (δ 1.05, *d*, *J* = 7 Hz), a β-substituted furan ring (two α-furan protons at δ 7.45 and one β-furan proton at δ 6.42), an α,α-disubstituted oxirane ring (two protons forming an AB system at δ2.98 and 2.54, *J* = 4.5 Hz) and an acetalic carbon atom without vicinal protons (one-proton singlet at δ5.15). The closure of this acetal group was revealed by a one-proton double doublet at δ 5.11 (J_1 = 6.75, J_2 = 9 Hz) and by an AB system due to a methylene group attached to a fully substituted carbon atom (δ_A = 3.80, δ_B = 4.38, J_{AB} = 11.25 Hz). All these signals are also found in the ^1H NMR spectrum of a diterpenoid (**2**) isolated from *T. gnaphalodes*, whose structure, probably identical with teucrin P_1 [5, 6], was firmly established by X-ray analysis [1]. In addition, the ^1H NMR spectrum of **1** showed a signal of an acetoxyl group (a three-protons singlet at δ2.00), which must be equatorially attached to the C-3 carbon atom, because its geminal proton appeared as a double doublet ($J_{aa'}$ = 8.5, $J_{ae'}$ = 5.25 Hz) at δ5.00. An identical behaviour for a C-3 equatorial acetoxyl group has been previously found in the closely related diterpenoid 3-epicaryoptin [7, 8].



1 R = OAc
2 R = H
3 R = OAc
4 R = H
5 R^1 = OAc, R^2 = 
6 R^1 = H, R^2 = O

On the other hand, comparison between the ^{13}C NMR spectra of compounds **1** and **2** (Table 1) clearly revealed the presence of a C-6 keto function in both substances, an identical stereochemistry for their C-4, C-5, C-8–C-10, C-12 and C-20 assymetric centres, and also provided conclusive proof of the presence in teupyrenone (**1**) of an equatorial C-3 acetoxyl group. Effectively, the C-7–C-9, and C-11–C-20 carbon resonances were identical in both compounds, whereas their chemical shift differences in the C-1–C--6, C-10, C-18 and C-19 carbon atoms can only be explained by the introduction in teupyrenone (**1**) of an equatorial acetoxyl group on C-3. In particular, the small γ-effects shown by the C-1 and C-5 carbon atoms in compound **1** ($\Delta\delta$ −1.8 and −0.7, respectively), compared with the larger value shown by its C-18 carbon atom ($\Delta\delta$ −3.5), clearly confirmed this point [9].

Finally, the absolute configuration depicted in formula **1** for teupyrenone was inferred from its CD curve, which showed a positive Cotton effect ($\Delta\epsilon_{315}$ + 0.41), as compound **2** ($\Delta\epsilon_{303}$ + 0.93) [1].

Another of the new diterpenoids, teupyreinin (**3**), had a molecular formula $C_{26}H_{32}O_{10}$ and possessed a γ-lactone group (IR ν_{CO} 1765 cm^{-1}), three acetoxyl groups (strong IR absorption at 1730 and 1250 cm^{-1}; singlets at δ 2.10 (3H) and 1.98 (6H) in its ^1H NMR spectrum) and a β-substituted furan ring, an α, α-disubstituted oxirane ring and a secondary methyl group identical with those found in teupyrenone (**1**). The ^1H NMR spectrum of teupyreinin (**3**, Table 2) suggested a structure closely related to compound **4**, a substance previously obtained from 19-acetylgnaphalin [1, 10]. In fact, the only difference was the presence, in the ^1H NMR spectrum of compound **3**, of an additional secondary acetoxyl group, whose geminal proton appeared partially overlapped (δ5.30) with the signals of the C-12 lactonic proton and one of the C-19 protons.

Comparison of the ^{13}C NMR spectra of teupyreinin (**3**) and compound **4** (Table 1) firmly established structure **3** for the new diterpenoid, since the C-5–C-9 and C-11–C-20 carbon resonances were identical in both compounds, and the observed differences in the C-1–C-4, C-10 and C-18 carbon atoms (Table 1) were due to the presence in teupyreinin (**3**) of an equatorial acetoxyl group on C-3 [compare the γ-*trans* effect on C-1 ($\Delta\delta$ −1.5) and C-5 ($\Delta\delta$ +0.3) with the γ-*gauche* effect on C-18 ($\Delta\delta$ −6.1)]. Thus, teupyreinin (**3**) differs from compound **4** [1] only in an additional 3β-acetoxyl group.

The last diterpenoid teupyreinidin (**5**), had a molecular formula $C_{28}H_{36}O_{11}$. Its IR spectrum showed furanic (3150, 3140, 1505, 880 cm^{-1}) and strong acetoxyl absorptions (1735, 1245 cm^{-1}) and no hydroxyl bands. The ^1H NMR spectrum (Table 2) was almost identical with that of teupyreinin (**3**), except

Table 1. ^{13}C NMR data for compounds **1–6** (25.2 MHz, $CDCl_3$, TMS as int. standard)

Carbon No.	**1**	**2**[1]	**3**	**4**[1]	**5**	**6**[11]
C-1	21.8 *t**	23.6 *t*	21.4 *t*	22.9 *t*	22.1 *t*	23.3 *t*
C-2	29.5 *t*	23.6 *t*	30.7 *t*	24.9 *t*	31.9 *t*	24.9 *t*
C-3	69.1 *d*	30.9 *t*	66.7 *d*	32.6 *t*	66.9 *d*	33.0 *t*
C-4	57.5 *s*	58.3 *s*	65.2 *s*	64.6 *s*	65.0 *s*	61.1 *s*
C-5	50.8 *s*	51.5 *s*	45.7 *s*	45.4 *s*	45.9 *s*	54.3 *s*
C-6	207.6 *s*	209.5 *s*	71.1 *d*	71.5 *d*	70.7 *d*	205.6 *s*
C-7	45.5 *t*	45.4 *t*	32.7 *t*	32.1 *t*	34.9 *t*	45.0 *t*†
C-8	36.3 *d*	36.1 *d*	40.5 *d*	38.9 *d*	41.2 *d*	41.7 *d*
C-9	46.2 *s*	47.0 *s*	51.1 *s*	50.8 *s*	52.7 *s*	51.6 *s*
C-10	43.7 *d*	45.0 *d*	49.9 *d*	52.9 *d*	51.0 *d*	55.1 *d*
C-11	39.5 *t*	39.9 *t*	43.1 *t*	43.1 *t*	44.3 *t*	45.7 *t*†
C-12	70.6 *d*	70.7 *d*	71.5 *d*	71.8 *d*	72.5 *d*	71.1 *d*
C-13	126.9 *s*	126.8 *s*	125.3 *s*	125.0 *s*	124.2 *s*	125.7 *s*
C-14	108.3 *d*	108.4 *d*	107.8 *d*	107.0 *d*	108.5 *d*	108.4 *d*
C-15	143.5 *d*	143.5 *d*	144.1 *d*	144.0 *d*	143.2 *d*	143.4 *d*
C-16	138.9 *d*	138.9 *d*	139.0 *d*	139.4 *d*	139.3 *d*	139.4 *d*
C-17	16.7 *q*	16.8 *q*	16.7 *q*	16.4 *q*	17.1 *q*	18.0 *q*
C-18	47.4 *t*	50.9 *t*	42.1 *t*	48.2 *t*	43.2 *t*	49.2 *t*
C-19	61.7 *t*	62.8 *t*	61.4 *t*	61.5 *t*	61.0 *t*	61.8 *t*
C-20	100.3 *d*	100.4 *d*	175.4 *s*	175.7 *s*	98.5 *d*	97.8 *d*
MeCO–	169.3 *s*	—	170.4 *s*	170.1 *s*	170.9 *s*	170.4 *s*
			169.6 *s*	169.8 *s*	169.7 *s*	169.3 *s*
			169.0 *s*		169.5 *s*	
					169.2 *s*	
MeCO–	20.9 *q*	—	21.0 *q*	21.1 *q*	21.8 *q*	21.3 *q*
			21.0 *q*	21.1 *q*	21.0 *q*	20.7 *q*
			20.8 *q*		20.9 *q*	
					20.8 *q*	

*SFORD multiplicity.

†These assignments may be reversed.

Table 2. ^{1}H NMR data of compounds **1**, **3** and **5** (90 MHz, $CDCl_3$, TMS as int. standard)*

	1	**3**	**5**
H-3α	5.00 *dd* $^3J_{3\alpha,2\alpha}$ = 5.25 Hz $^3J_{3\alpha,2\beta}$ = 8.50 Hz	5.30§	~5.2§
H-6β	—	4.87 *dd* $^3J_{6\beta,7\beta}$ = 4.5 Hz $^3J_{6\beta,7\alpha}$ = 11.0 Hz $^4J_{6\beta,19A}$ = 1.8 Hz	~4.8§
H-12	5.11 *dd* $^3J_{12,11A}$ = 6.75 Hz $^3J_{12,11B}$ = 9.0 Hz	5.42 *t* $^3J_{12,11A}$ = 8.5 Hz $^3J_{12,11B}$ = 8.5 Hz	~5.1§
H-14	6.42 *m* $W_{1/2}$ = 4 Hz	6.40 *m* $W_{1/2}$ = 4 Hz	6.40 *m* $W_{1/2}$ = 4.5 Hz
H-15	7.45 *m* $W_{1/2}$ = 4.5 Hz	7.45 *m* $W_{1/2}$ = 4.5 Hz	7.40 *m* $W_{1/2}$ = 4.5 Hz
H-16	7.45 *m*	7.45 *m*	7.40 *m*
Me-17	1.05 *d* $^3J_{17,8}$ = 7 Hz	1.11 *d* $^3J_{17,8}$ = 7 Hz	1.02 *d* $^3J_{17,8}$ = 7 Hz
H_A-18†	2.54 *d* $^2J_{18A,18B}$ = 4.5 Hz	2.59 *d* $^2J_{18A,18B}$ = 4.5 Hz	2.62 *d* $^2J_{18A,18B}$ = 4.5 Hz
H_B-18‡	2.98 *d*	2.80 *dd* $^4J_{18B,3\alpha}$ = 0.8 Hz	2.91 *dd* $^4J_{18B,3\alpha}$ = 1.5 Hz
H_A-19†	3.80 *d* $^2J_{19A,19B}$ = 11.25 Hz	4.52 *dd* $^2J_{19A,19B}$ = 13.0 Hz $^4J_{19A,6\beta}$ = 1.8 Hz	4.12 *dd* $^2J_{19A,19B}$ = 12.0 Hz $^4J_{19A,6\beta}$ = 1.5 Hz
H_B-19‡	4.38 *d*	5.18 *d*	4.85 *d*
H-20	5.15 *s*		6.15 *s*
OCOMe	2.00 *s*	2.10 *s*, 1.98 *s* 1.98 *s*	2.25 *s*, 2.10 *s* 1.99 *s*, 1.99 *s*

*Spectral parameters were obtained by first order approximation.
†*Exo* hydrogen respect ring B.
‡*Endo* hydrogen respect ring B.
§Overlapped signal.

for the presence of an additional acetyl group attached to a C-20 hemi-acetalic function (a one-proton singlet at δ 6.15 and a three-proton singlet at δ 2.25) instead of the C-20 lactone group found in compound **3**. This hemi-acetalic arrangement has been previously found in other clerodane diterpenoids gnaphalidin (**6**) [10] and eriocephalin [11] isolated from *Teucrium* species. The ^{13}C NMR data of compound **5** (Table 1) revealed that this compound had the same C and D ring system as gnaphalidin (**6**) and eriocephalin [11]. Thus, its C-20 configuration was *S*, since this was the one found in eriocephalin by X-ray analysis [11]. On the other hand, the C-17–C-19 and rings A and B carbon resonances of the new diterpenoid (**5**) were almost identical with those observed for the same carbon atoms of teupyreinin (**3**) (Table 1). The small differences showed for C-1 ($\Delta\delta$ +0.7), C-2 (+1.2), C-7 (+2.2), C-8 (+0.7), C-9 (+1.6) and C-10 (+1.1) carbon atoms of teupyreinidin (**5**) with respect to compound **3**, were due to the C-20 functional difference between these two compounds [11]. Thus, teupyreinidin possesses the structure depicted in formula **5**.

The absolute configuration of these two last diterpenoids was not ascertained; however, compounds **3** and **5** are believed to belong to the neo-clerodane [12] series like teupyrenone (**1**), co-occurring in the same species. Moreover, all the diterpenoids until now isolated from *Teucrium* species [2, 13–15], and whose structures have been rigorously established, belong to the neo-clerodane series.

From a biogenetic point of view, it is curious that all the hydroxyl groups of the diterpenoids isolated from *T. pyrenaicum* are naturally acetylated.

EXPERIMENTAL

Mps (Kofler apparatus) are uncorr. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Plant materials were collected in July 1981, near Rodiezmo (León, Spain), and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy (Madrid "Complutense" University).

Extraction and isolation of the diterpenoids. Dried and finely powdered. *T. pyrenaicum* L. aerial parts (1 kg) were extracted with Me_2CO (9 l.) at room temp. for 1 week. After filtration, the solvent was evaporated yielding a gum (60 g) which was subjected to dry CC over Si gel (800 g, Merck No. 7734, deactivated with 15% H_2O). Elution with *n*-hexane–EtOAc (3:2) yielded a mixture of compounds **1**, **3** and **5**

(1.1 g). This mixture was subjected to further chromatography over a Si gel (150 g) column, which was eluted with *n*-hexane–EtOAc (4:1) yielding, in order of elution, teupyreinidin (**5**, 104 mg), teupyrenone (**1**, 45 mg) and teupyreinin (**3**, 760 mg).

Teupyrenone (**1**). Mp 213–215° (from EtOAc–*n*-hexane); $[\alpha]_D^{18} - 46.5°$ ($CHCl_3$; *c* 0.80); IR ν_{max}^{KBr} cm^{-1}: 3150, 3140, 3080, 2980, 2960, 2940, 2910, 1738, 1710, 1505, 1460, 1390, 1370, 1245, 1220, 1160, 1070, 1030, 897, 880, 820, 740; UV λ_{max}^{EtOH} nm (ϵ): 211 (5500), 285 (80). CD $\Delta\epsilon_{301}0$, $\Delta\epsilon_{315} + 0.41$, $\Delta\epsilon_{337}0$ (EtOH; *c* 0.56); ^1H NMR (90 MHz, $CDCl_3$): see Results and Discussion and Table 2; ^{13}C NMR (25.2 MHz, $CDCl_3$): see Table 1; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 402 $[M]^+$ (11), 342 (6), 331 (3), 325 (5), 312 (8), 303 (3), 296 (4), 283 (28), 266 (9), 265 (8), 261 (7), 201 (13), 189 (13), 175 (12), 173 (12), 163 (15), 134 (24), 95 (45), 94 (100), 81 (43). (Found: C, 65.79; H, 6.63. $C_{22}H_{26}O_7$ requires: C, 65.66; H, 6.51%.)

Teupyreinin (**3**). Mp 112–114° (from MeOH); $[\alpha]_D^{18} - 9.4°$ ($CHCl_3$; *c* 1.00); IR ν_{max}^{KBr} cm^{-1}: 3150, 3140, 2980, 2960, 2890, 1765, 1730, 1505, 1450, 1390, 1370, 1250, 1185, 1140, 1080, 1030, 880, 755; UV λ_{max}^{EtOH} nm (ϵ): 212 (5800); ^1H NMR (90 MHz, $CDCl_3$): see Table 2; ^{13}C NMR (25.2 MHz, $CDCl_3$): see Table 1; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 504 $[M]^+$ (0.5), 461 (1), 444 (1), 415 (1), 401 (1), 372 (5), 354 (2), 342 (4), 329 (3), 324 (4), 312 (24), 157 (16), 145 (16), 105 (20), 96 (81), 95 (80), 91 (26), 81 (54), 79 (20), 55 (28), 43 (100). (Found: C, 61.55; H, 6.58. $C_{26}H_{32}O_{10}$ requires: C, 61.89; H, 6.39%.)

Teupyreinidin (**5**). An amorphous powder which melts at 102–108° $[\alpha]_D^{18} + 26.7°$ ($CHCl_3$; *c* 0.415); IR ν_{max}^{KBr} cm^{-1}: 3150, 3140, 2980, 2950, 2880, 1735, 1505, 1445, 1395, 1370, 1245, 1165, 1080, 1035, 950, 895, 880, 805, 735; UV λ_{max}^{EtOH} nm (ϵ): 211 (5000); ^1H NMR (90 MHz, $CDCl_3$): see Table 2; ^{13}C NMR (25.2 MHz, $CDCl_3$): see Table 1. EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 548 $[M]^+$ (0.3), 489 (4), 488 (3), 445 (48), 429 (8), 428 (4), 423 (2), 416 (6), 399 (2), 369 (2), 368 (2), 187 (24), 145 (36), 95 (90), 94 (96), 91 (50), 81 (88), 55 (60), 43 (100). (Found: C, 61.72; H, 6.68. $C_{28}H_{36}O_{11}$ requires: C, 61.31; H, 6.56%.)

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