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A CHLORINE-CONTAINING AND TWO 17β-*NEO*-CLERODANE DITERPENOIDS FROM *TEUCRIUM POLIUM* SUBSP. *VINCENTINUM*

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Key **Word Index**—*Teucrium polium* **subsp.** *uincentinum;* Labiatae; neo-clerodane diterpenoids; chloro- and 17β-*neo*-clerodanes; teuvincentins A, B and C; X-ray diffraction analysis.

Abstract-From the aerial parts of *Teucrium polium* subsp. *vincentinum* three new neo-clerodane diterpenoids, teuvincentins A, B and C, have been isolated besides four already known diterpenes (19-acetylgnaphalin, eriocephalin, isoeriocephalin and 3-deacetyl-20-*epi*-teulanigin) and the flavones cirsiliol and apigenin. The structures of teuvincentin A [(12*S*,20*S*)-20-*O*-acetyl-19-acetoxy-18-chloro-15,16-epoxy-4α,7-dihydroxy-6-oxo-*neo*-clerodane-7,13(16),14-triene-20,12-hemiacetal], B [(12*S*,20*S*)-20-*O*-acetyl-4α,18;15,16-diepoxy-6α-hydroxy-17β-*neo*-clerodane-13(16),14-diene-7α,19;20,12-dihemiacetal] and C [(12*S*,20*S*)-20-*O*-acetyl-6α,19-diacetoxy-4α,18;15,16-diepoxy-7-oxo-17β-*neo*-clerodane-13(16),14-diene-20,12-hemiacetal] were established by chemical and spectroscopic means and, in the case of teuvincentins A and B, also confirmed by X-ray diffraction analyses of their acetyl derivatives.

INTRODUCTION

The naturally occurring neo-clerodane diterpenoids have attracted interest because of their biological activity as insect antifeedants and as antifungal, antitumour and antimicrobial agents. Although a large number of these compounds have been isolated from many plants in the last few years, the genus *Teucrium* (family Labiatae) is far and away the most abundant natural source of these substances [1].

In continuation of our studies on neo-clerodane diterpenoids from the *Teucrium* species [1, 2], we have investigated *T. polium* L. subsp. *uincentinum* (Rouy) D. Wood, a small shrub which grows in limited areas of Portugal. From the acetone extract of the aerial parts of this plant we have isolated the previously known neo-clerodane diterpenoids 19-acetylgnaphalin [1], eriocephalin (1) [1, 3], isoeriocephalin [1] and 3-deacetyl-20-*epi*-teulanigin [2], together with 5,3′,4′-trihydroxy-6,7-dimethoxyflavone (cirsiliol) [4] and 5,7,4′-trihydroxyflavone (apigenin) [4]. In addition, three new diterpenoids, teuvincentins A, B and C, have also been isolated from the same source and their structures (2, 3 and 4, respectively) established by chemical and spectroscopic means and, in the case of teuvincentins A and B, by X-ray diffraction analyses of their respective acetyl derivatives 6 and 10.

RESULTS AND DISCUSSION

The first of the new diterpenoids isolated from *T. polium* subsp. *vincentinum*, teuvincentin A (2), had a molecular formula $C_{24}H_{29}O_9Cl$ and its IR spectrum was consistent with the presence of hydroxyl groups (3480, 3460 cm^{-1}), a furan ring (3140, 3120, 1505, 875 cm^{-1}), acetoxyl groups (strong bands at 1745, 1240 cm^{-1}) and an a-diketone in its enolic form (diosphenol function, 1662 and 1628 cm-') [5]. The presence of a diosphenol chromophore in compound 2 was rigorously confirmed by its UV absorption at 287nm (log ε 4.14) [5]. The ^1H NMR spectrum of teuvincentin A (2, Table 1) was almost identical with that of 7,8-dehydroeriocephalin (5), a neo-clerodane diterpenoid previously isolated from *T. lanigerum* [5]. In fact, the only difference between the ^1H NMR spectra of compounds 2 (Table 1) and 5 [5] was the presence in the former of a 4α-hydroxy-18-chloro grouping (C-18 protons as an AB system, δ4.33 and 4.04, $J = 11.6$ Hz, see Table 1) [6] instead of the 4α,18-oxirane ring of the latter (C-18 protons at 62.41 *d*, $J_{gem} = 4.8$ Hz, and 2.65 *dd*, $J_{gem} = 4.8$ Hz, $J_{18,3\alpha} = 2.8$ Hz) [5].

Treatment of teuvincentin A (2) with acetic anhydride-pyridine at room temperature gave a monoacetyl derivative (6, $C_{26}H_{31}O_{10}Cl$), the IR spectrum of which showed hydroxyl (3450 cm^{-1}) and enol-acetate (1770 cm^{-1}) absorptions. The hypsochromic shift in the UV absorption of compound 6 (λ_{max} 250.5 nm, log ε 4.09) as compared with that of teuvincentin A (2, see above) further confirmed the acetylation of the C-7 enolic hydroxyl group.

Moreover, comparison between the ^{13}C NMR spectra of teuvincentin A and 7,8-dehydroeriocephalin (2 and 5, respectively, Table 2) clearly established that these compounds were different in their C-4 and C-18 substituents, i.e. an oxirane ring in 5 and a chlorohydrin grouping in teuvincentin A (2). Effectively, the C-9, C-l 1 to C-17, C-19 and C-20 carbon atom resonances were almost identical in both compounds (Table 2), whereas the observed differences [δ(2)–δ(6)] in the chemical shift of C-l

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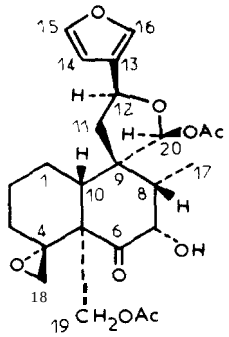

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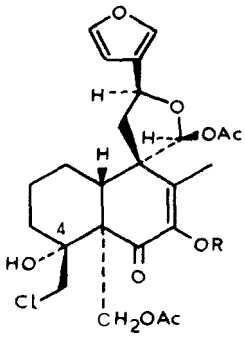

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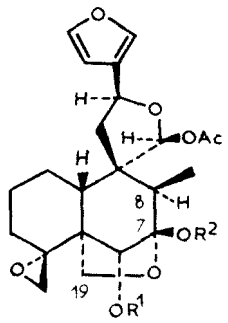
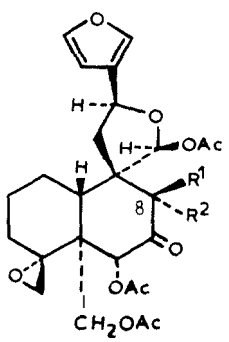
6 R = Ac



3 $R^1 = R^2 = H$

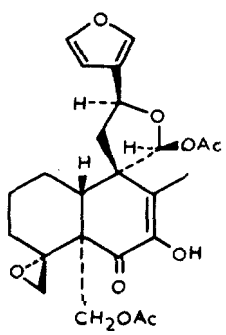
10 $R^1 = R^2 = Ac$

11 $R^1 = Ac$, $R^2 = H$



4 $R^1 = Me$, $R^2 = H$

12 $R^1 = H$, $R^2 = Me$



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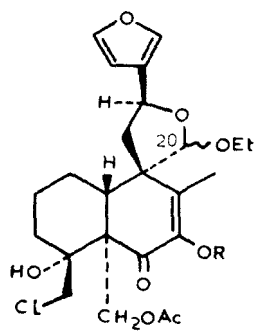
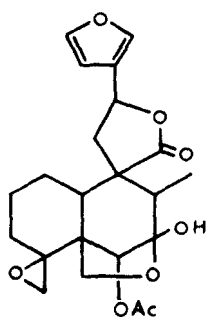


7 R = H

8 R = Ac



9

($\Delta\delta$ –0.68), C-2 (– 2.43), C-3 (–2.06), C-4 (+ 15.47), C-5 (+ 5.09), C-10 (– 3.22) and C-18 (– 1.11) were in complete agreement [6] with the structural variation at their C-4 and C-18 positions. Furthermore, the chemical shift difference in the C-6 ($\Delta\delta$ + 6.96), C-7 (+ 0.70) and C-8 (+ 4.04) carbons of compounds 2 and 5 (Table 2) were undoubtedly attributed to electronic interactions between the chlorine atom attached to C-18 and the C-6 ketone, since the β-carbon of the enone chromophore of teuvincentin A (2, C-8) appeared more paramagnetically shifted than the a-carbon (C-7) with respect to those of compound 5 (see above).

All the above data pointed towards a structure such as 2 for teuvincentin A, in which the 12*S*-and 20*S*-stereochemistry and the neo-clerodane absolute configuration were established as follows.

Table 1. ^{1}H NMR data of compounds **2**, **6**, **7** and **8** (CDCl$_3$, TMS as an int. standard)*

	2†	6	7	8
H-10β	2.59 *dd*	‡	‡	‡
H_A-11	2.43 *dd*	‡	2.09 *dd*	‡
H_B-11	2.94 dd	2.77 *dd*	2.66 *dd*	2.68 *dd*
H-12	5.47 dd	5.30 *t*	5.06 *t*	5.11 *t*
H-14	6.73 dd	6.39 dd	6.48 dd	6.45 dd
H-15	7.73 *t*	7.43 *t*	7.43 ‡	7.43 ‡
H-16	7.82 *m*	7.38 m	7.43 ‡	7.43 ‡
Me-17	1.89 *s*	1.92 *s*	1.98 *s*	2.01 *s*
H_A-18	4.04 *br d*	3.58 *br d*	3.63 dd	3.62 dd
H_B-18	4.33 *d*	3.98 *d*	3.92 *d*	3.97 *d*
H_A-19	4.87 *d*	4.45 *d*	4.43 d	4.47 *d*
H_B-19	5.54 *d*	5.06 *d*	5.05 *d*	5.05 *d*
H-20	6.83 *s*	6.41 *s*	5.06 *s*	5.13 *s*
OAc	2.44 *s*	2.27 *s*	2.09 *s*	2.27 *s*
	1.99 *s*	2.04 *s*		2.03 *s*
	—	1.97 *s*		
OH-4α§	5.90 *br s*	4.19 *br s*	4.13 *br s*	4.25 *br s*
OH-75	10.77 *s*		6.20 *s*	
OCH_2Me	—		3.76 *dq*	3.76 *dq*
			3.29 *dq*	3.29 *dq*
OCH_2Me	—	—	1.11 *t*	1.11 *t*
J (Hz)				
10β, 1a	13.0	‡	‡	‡
10β, 1β	3.0	‡	‡	‡
11A, 11B	14.3	13.5	14.0	13.9
11A, 12	8.7	7.6	8.2	8.0
11B, 12	7.2	7.6	8.1	8.0
14, 15	1.7	1.7	1.8	1.8
14, 16	0.8	0.9	0.9	0.9
15, 16	1.7	1.7	1.8	1.8
18A, 18B	11.6	11.9	11.5	11.9
19A, 19B	11.7	12.1	11.5	11.7
18A, 3a	<0.3	<0.3	1.3	1.1
OEt ^2J	—		9.7	9.1
^3J	—	—	7.0	7.1

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

†In pyridine-d$_5$ solution.

‡Overlapped signal.

§Disappeared after addition of D_2O.

Eriocephalin **(1)**, a neo-clerodane diterpenoid possessing a 12*S*,20*S*-configuration and whose structure has been established from an X-ray diffraction analysis [3], was transformed into 7,8-dehydroeriocephalin (5) by oxidation with chromium trioxide-pyridine [5]. Treatment of compound **5** with hydrochloric acid in methylene chloride solution yielded a substance identical in all respects (mp, $[\alpha]_D$, IR, UV, ^{1}H and ^{13}C NMR, MS, mmp, TLC) with natural teuvincentin A, thus establishing the structure depicted in 2 for this new diterpenoid.

It is of interest to note that reaction of 7,8-dehydroeriocephalin (5) with hydrochloric acid in chloroform solution gave a single product, to which the structure of 20-O-ethyl acetal derivative (7) was assigned on the basis of its ^{1}H and ^{13}C NMR spectroscopic and analytical data and those of the corresponding 7-acetyl derivative 8 (see Tables 1 and 2, and Experimental). Obviously, the formation of the acetal7 is due to the presence of ethanol as a stabilizer of the solvent.

The above conclusion on the structure of teuvincentin A (2) was also supported by a single-crystal X-ray determination of its 7-acetyl derivative 6, which showed identical structural features and absolute configuration to those reported above (see Fig. 1). In addition, the crystal structure of compound 6 possesses the trans-fused rings A and B nearly coplanar (dihedral angle 13°), with ring A in a slightly distorted chair conformation (torsion angles from 52" to 59") and ring B in an envelope. conformation (torsion angles from 0° and 2" to 49") [7, 8], obviously due to the influence of the C-6, C-7, C-8 enone moiety. Ring C shows an envelope conformation, being the flap at C-20, and is nearly perpendicular to rings A and B, since the

Table 2. ^{13}C NMR chemical shifts ofcompounds 2, 5 and 7 (pyridine-d,, TMS as int. standard)

C	2	5	7
1	22.07 *t**	22.75 *t*	22.25 *t*
2	22.91 *t*	25.34 *t*	23.39 *t*
3	30.83 *t*	32.89 *t*	30.72 *t*
4	76.77 *s*	61.30 s	76.77 *s*
5	55.65 *s*†	50.56 *s*	55.52 *s*†
6	196.73 *s*	189.77 *s*	196.60 *s*
7	148.04 *s*	147.34 s	146.85 s
8	132.92 s	128.88 *s*	135.94 s
9	54.66 st	55.09 *s*	54.58 *s*†
10	47.77 *d*	50.99 *d*	47.40 *d*
11	44.61 *t*	43.17 *t*	46.01 *t*
12	74.95 *d*	75.14 *d*	72.10 *d*
13	128.80 *s*	128.88 *s*	128.31 *s*
14	109.56 *d*	109.52 *d*	109.79 *d*
15	*144.50 d*	144.38 *d*	144.25 *d*
16	140.22 *d*	140.04 *d*	140.18 *d*
17	17.84 *q*	17.28 *q*	17.67 *q*
18	49.97 *t*	51.08 *t*	50.02 *t*
19	63.33 *t*	64.00 *t*	64.59 *t*
20	99.42 *d*	99.91 *d*	106.12 *d*
OAc	169.78 s	169.87 s	169.92 *s*
	169.78 s	169.70 *s*	
	21.21 *q*	21.12 *q*	20.66 *q*
	20.63 *q*	20.58 *q*	
OEt			63.52 *t*
			15.02 *q*

*SFORD multiplicity.
†These assignments may be reversed.

dihedral angles between rings A and C, and B and C are 93 and 105" respectively. In compound 6 the thermal parameters U11 and U22 for the carbon and oxygen atoms of the ester groups are particularly large, specially for O-6, O-9, O-10, *C-23* and C-24, and the distances O-6-C-22 (1.16 Å), C-23-O-9 (1.31 Å) and C-23-C-24 (1.46 A) are short (for the atom numbering see Fig. 1). Moreover, the C-19 acetoxyl group *(O-9.* C-23, O-10 and

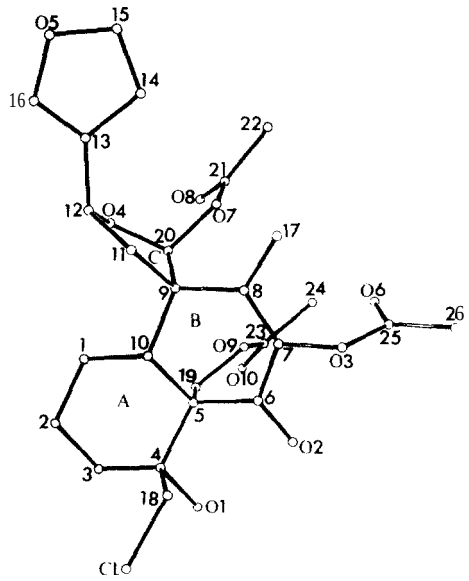


Fig. 1. X-ray molecular model of 7-acetylteuvincentin A (6).

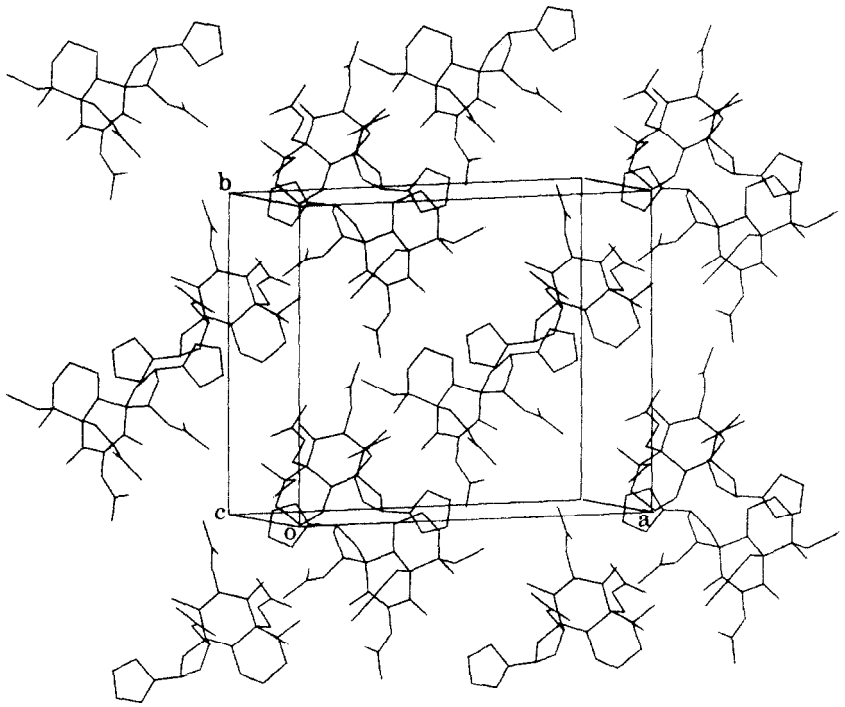


Fig. 2. Stereoscopic drawing (PLUTO [9]) of the molecular packing of 7-acetylteuvincentin A (6).

C-24) showed some difficulties to be interpreted in the difference synthesis, and we attribute this to the possibility that the C-19 acetate could produce some disorder. In the crystals the molecules of compound 6 are only held together by van der Waals forces and the shortest intermolecular distances are between O-8. . . .H-12 = 2.44 Å, O-4. . .C-11 = 3.10 Å, O-2. . .C-15 = 3.22 Å and O-1. . .C-22 = 3.25 Å (see Fig. 2).

Teuvincentin A (2) and tafricanins A and B [6] are the only neo-clerodanes containing chlorine which have been

Table 3. ^{1}H NMR data of compounds **3**, **4**, **10** and 11 ($CDCl_3$, TMS as an int. standard)*

	3†	4	10	11
H-2β	1.43 ddt	‡	1.46 ddt	‡
H-3a	1.68 *br ct*	‡	1.74 tdd	‡
H-3β	1.10 dt	‡	1.11 *dt*	‡
H-6β	4.06 br *s*	5.32 *s*	5.31 *s*	5.13 *br s*
H-8a	3.25 q	3.22 q	3.81 *q*	2.90 *q*
H-10β	1.99 *br dd*	*2.38* dd	1.99 dd	‡
H_A-11	2.14 dd	2.18 *dd*	*2.09* dd	2.16 *dd*
Ha-11	2.43 *dd*	*2.35* dd	2.23 dd	*2.26* dd
H-12	5.30 *t*	5.21 *t*	5.13 *t*	5.13 t
H-14	6.69 *dd*	*6.34* dd	6.40 *dd*	6.37 dd
H-15	*7.66 t*	7.39 t	7.37 *t*	7.38 *t*
H-16	7.74 *m*	7.32 *m*	7.33 *m*	7.32 *m*
Me-17	1.28 d	1.36 *d*	1.09 *d*	1.12 *d*
H_A-18§	2.72 *d*	2.48 *d*	2.43 *d*	2.38 *d*
Ha-1811	3.35 dd	3.10 dd	2.79 dd	2.94 dd
H_A-19	4.38 *d*‖	4.31 d	4.43 *s*	4.27 *d*‖
Ha-19	4.48 *br d*§	5.01 *d*		4.21 *br d*§
H-20	6.94 *s*	6.46 *s*	6.54 *s*	6.44 *s*
OAc	1.94 *s*	2.07 *s*	2.20 *s*	2.16 *s*
		2.01 *s*	2.11 *s*	2.07 *s*
		1.83 *s*	1.98 *s*	
OH-67	5.19 *br s*	—	—	
OH-77	4.99 *s*	—	—	3.65 *s*
***J* (Hz)**				
1α,2β	12.6	‡	11.6	‡
1β,2β	3.2	‡	3.1	‡
2α,2β	12.6	‡	13.2	‡
2α,3α	2.3	‡	3.9	‡
2α,3β	3.2	‡	3.1	‡
2β,3α	12.6	‡	13.2	‡
2β,3β	3.2		3.1	‡
3α,3β	12.6	‡	13.2	‡
3α,18B	2.0	2.6	2.2	2.0
6β,19B	<0.3	0	0	<0.3
8α,17	7.3	7.6	7.3	7.8
10β,1α	12.0	13.0	11.5	‡
10β,1β	4.3	3.0	4.1	‡
11A,11B	13.7	13.7	13.5	13.3
11A,12	8.1	7.8	8.2	8.3
11B,12	8.4	8.4	8.2	8.3
14,15	1.7	1.7	1.8	1.6
14,16	0.7	0.6	0.6	0.7
15,16	1.7	1.7	1.8	1.6
18A,18B	3.9	3.4	4.4	4.4
19A,19B	8.3	12.1	0	8.4
19B,10β	10.3	0	0	‡

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

†In pyridine-d_5 solution.

‡Overlapped signal.

§*Exo* hydrogen with respect to ring B.

‖*Endo* hydrogen with respect to ring B.

¶Disappeared after additon of D_2O.

isolated from *Teucrium* species and all of them are chlorohydrins. In our opinion, teuvincentin A is not an artefact, because it was present in the initial acetone extract of the plant (TLC analysis) and its chemical precursor, 7,8-dehydroeriocephalin (5) [5], was recovered unchanged when it was treated with the same solvents and in the same way as the plant material and the extract. In connection with the presence of the chlorohydrin 2 in *T. polium* subsp. *vincentinum*, it is of interest to note that this species grows and was collected at the coastline of Cape São Vincente (Portugal), where there is a high concentration of chloride salts.

Another of the new diterpenoids isolated from the acetone extract of *T. polium* subsp. *vincentinum* was teuvincentin B (3, $C_{22}H_{28}O_8$), the ^1H and ^{13}C NMR spectra of which (Tables 3 and 4) showed signals attributable to a secondary methyl group, a /I-substituted furan ring, a 4α,18-oxirane ring and a 20-*O*-acetyl-20,12-hemiacetal grouping, identical with those found in other neo-clerodane diterpenoids isolated from *Teucrium* species [I, 2, 53. In addition, teuvincentin B possessed a secondary hydroxyl group placed between fully substituted carbon atoms (ν_{OH} 3420 cm- ^{1}; geminal proton at 64.06 *br s;* hydroxylic carbon at 673.62 d) and a hemiacetal group (ν_{OH} 3520 cm- ^{1}; hemiacetal carbon at δ 108.35 s) which must involve the C-7 and C-19 carbons, since the AB system of the C-19 protons showed a geminal J value of 8.3 Hz (see Table 3), typical of tetrahydrofuran derivatives [10]. Moreover, the chemical shifts of the C-19 methylene protons of teuvincentin B (an AB system centred at 64.43) and their geminal coupling constant (8.3 Hz) were identical with those observed for isopicropolin (64.4, J_{gem} = 8 Hz), a clerodan-20,12-olide derivative (9) which possesses a C-7-C-19 hemiacetal group and whose stereochemistry and absolute configuration have not been established [IO].

Table 4. ^{13}C NMR chemical shifts of compounds 3 and 4 (TMS as int. standard)

C	3*	4 t	C	3*	4†
1	24.20 *t*‡	22.33 *t*	14	109.61 d	108.49 *d*
2	24.90 *t*	24.81 *t*	15	144.20 *d*	143.75 *d*
3	32.76 *t*	31.44 *t*	16	140.29 *d*	139.55 *d*
4	63.27 *s*	64.55 *s*	17	15.85 *q*	17.48 *q*
5	51.93 *s*	49.02 *s*	18	52.86 *t*	51.42 *t*
6	73.62 *d*§	74.02 *d*	19	66.68 *t*	60.59 *t*
7	108.35 *s*	204.23 *s*	20	99.58 *d*	97.82 *d*
8	41.57 *d*	48.62 *d*	OAc	169.49 *s*	169.95 *s*
9	55.83 *s*	56.22 *s*		21.27 *q*	169.62 *s*
10	43.81 *d*	45.90 *d*			169.49 *s*
11	44.34 *t*	43.92 *t*			21.21 *q*
12	73.18 *d*§	71.07 *d*			20.47 *q*
13	129.71 *s*	128.10 *s*			20.07 *q*

*In pyridine-d, solution.
†In deuteriochloroform solution.
‡SFORD multiplicity.
§These assignments may be interchanged.

Treatment of teuvincentin B (3) with acetic anhydride-pyridine for 72 hr at room temperature gave the peracetyl derivative 10 ($C_{26}H_{32}O_{10}$, no hydroxyl absorption in its IR spectrum) besides major quantities of the acetate **11** ($C_{24}H_{30}O_9$, ν_{OH} at 3450 cm^{-1}), thus confirming the presence of a hydroxyl and a hemiacetal group in the molecule of this new diterpenoid.

The relative configuration of all the asymmetric centres of teuvincentin B was established by NOE experiments on the natural diterpenoid (3) and its acetyl derivatives 10 and **11.** Effectively, the data collected in Table 5 rigorously established that the C-l 7 methyl group, the furan ring and the C-20–*O*-acetyl group are on the same side of the plane defined by the C-20-C-12 hemiacetal ring, since irradiation at δ1.28 in 3 and at δ1.09 in 10 (Me-17 protons) caused NOE enhancements in the signals of the H-14 and H-16 furanic protons, whereas no effect was observed in the signals of the H- 12 and H-20 protons [11, 12]. Moreover, the C-17 methyl group is *cis*-oriented with respect to the H-6 and H-IO protons (NOE enhancements in these signals of 12.1– 11.3% and 2.9–2.8%, respectively), the H-6 and H-18 *endo*-protons also possess a *cis*-relationship in the substituted decalin ring (see Table 5, irradiation at δ 5.13 in compound 11) and, finally, the H-8 proton and the C-19 methylene grouping are also *cis*-oriented, since a slight NOE enhancement (0.3%) was observed in the signal of the H-19 *endo*-proton when the proton at C-8 was irradiated (see Table 5, compound 10).

From all the above data, it was evident that teuvincentin B possessed the structure and relative stereochemistry depicted in 3, in which the C-17 methyl group had a biogenetically unusual 8β-configuration, not previously found in any of the nen-clerodane diterpenoids until now isolated from plants belonging to the *Teucrium* genus [1–3, 5, 6, 10–12]. In order to elucidate this point

Table 5. NOE experiments on compounds 3.4, **10** and **11**

	Irradiation 6 (proton)	Observed NOE enhancement (%)											
		H-6β	H-8α	H-10β	H-12	H-14	H-16	Me-17	H_A-18*	H_B-18†	H_A-19†	H_B-19*	H-20
3	1.28 (Me-17)	12.1	13.6	2.8	0	1.1	0.8	—	0	0	0	0	0
4	1.36 (Me-17)	7.8	5.6	0.8	0	0	0.3	—	0	2.1	0	0	0
	5.32 (H-68)	—	0	5.6	0	0	0	2.1	0	13.5	0	0	0
	6.46 (H-20)	0	0	0	3.1	0	0	0	0	0	2.8	0	
10	1.09 (Me-17)	11.3	11.8	2.9	0	1.3	1.0	—	0	0	0	0	0
	3.81 (H-8α)	0	—	0	0	0	0	2.0	0	0	0.3	0	0
11	5.13 (H-6/3)		0	4.7	0	0	0	2.2	0	12.3	0	0	0

* *Exo* hydrogen with respect to ring B.
† *Endo* hydrogen with respect to ring B.

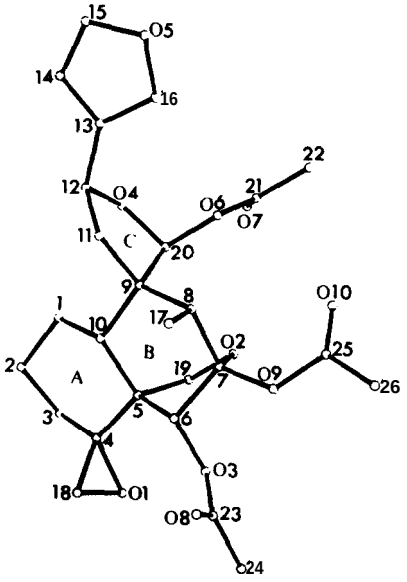


Fig. 3. X-ray molecular model of 6,7-diacetylteuvincentin B (**10**).

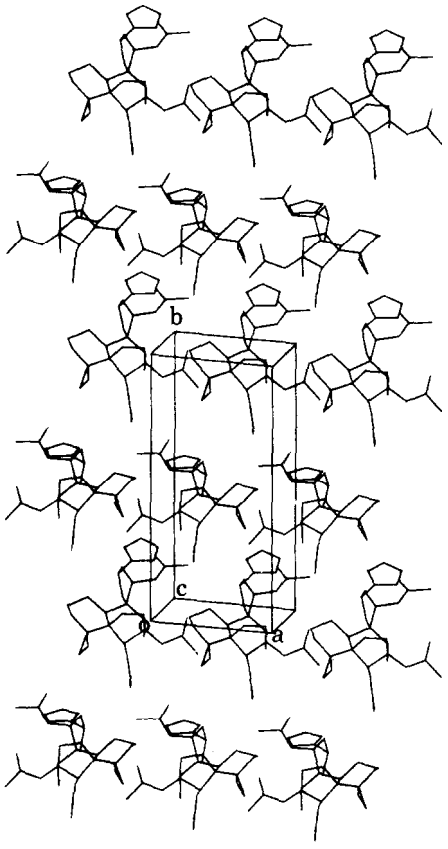


Fig. 4. Stereoscopic drawing (PLUTO [9]) of the molecular packing of 6,7-diacetylteuvincentin B (**10**).

conclusively and establish the absolute configuration of teuvincentin B, a single-crystal X-ray determination of its derivative 10 was undertaken. The X-ray molecular model of compound 10 is shown in Fig. 3, confirming all the above deductions on the structure of teuvincentin B (3) and establishing a neo-clerodane absolute configuration for this new diterpenoid. Furthermore, the crystal structure of 6,7-diacetylteuvincentin B (10, Fig. 3) shows rings A and B nearly coplanar (dihedral angle 6°), both in a slightly distorted chair conformation as shown by the range of the torsion angles (from 46" to 61" and from 42" to 79°, respectively) [7,8], whereas ring C possesses an envelope conformation, being the flap at C-20, and it is nearly perpendicular to rings A and B (dihedral angles 104" and 96°, respectively). In the crystals the molecules of compound 10 are only held together by van der Waals forces, being the shortest intermolecular distances between O-7.. ..C-15 = 3.21 Å and O-7.. ..C-18 = 3.34 Å (see Fig. 4).

Accordingly with the biogenetic pathway of the *neo*-clerodane diterpenoids, the C-17 methyl group must be in the 8α-configuration (C-5α–H-10β, H-10β–C-9α, C-9α–H-8β all *trans*-backbone arrangement), and the unexpected 8β-configuration of this methyl group in teuvincentin B (3) must be explained biogenetically by considering that the C-7 carbonyl function causes an epimerization at the C-8 asymmetric centre prior to the formation of the C-7-C-19 hemiacetal ring.

The last of the new diterpenoids isolated from *T. polium* subsp. *vincentinum*, teuvincentin C, was an amorphous substance very difficult to purify and was characterized as its acetyl derivative 4. Compound 4 had a molecular formula $C_{26}H_{32}O_{10}$ and its spectroscopic data (Tables 3 and 4, and Experimental) suggested a structure closely related to the acetyl derivative of isoeriocephalin (**12**), a neo-clerodane diterpenoid previously found in *T. lanigerum* and whose structure is well known [13]. In fact, the ^{1}H NMR spectra of compounds 4 (Table 3) and **12** [13] were identical except in the chemical shift of their H-8 proton (at 63.22 *q*, $J = 7.6$ Hz in 4 and at 62.67 *q*, $J = 7.0$ Hz in compound 12). Moreover, the ^{13}C NMR spectra of these compounds (4, Table 4; 12, ref. [13]) showed almost identical chemical shift for their C-1–C-5, C-9, C-12–C-16 and C-18–C-20 carbon atoms, whereas the observed differences [δ(**4**)–δ(**12**)] in the resonances of the C-6 ($\Delta\delta$ –2.48), C-7 (+3.43), C-8 (−4.18), C-10 (−4.70), C-11 (− 1.38) and C-17 (+ 6.78) carbons clearly established that teuvincentin C was the C-8 epimer of compound 12. The behaviour of the new diterpenoid (4) under NOE experiments (see Table 5) further confirmed this point.

As in the case of teuvincentin B (3, see above), the unusual 8β-configuration of the C-17 methyl group of teuvincentin C (4) must be attributed to an epimerization caused by the presence of a ketone at C-7.

The absolute stereochemistry of teuvincentin C was not ascertained. However, it is reasonable to assume that it possesses a neo-clerodane configuration the same as teuvincentins A and B, and the other previously known diterpenoids co-occurring in the same species. Moreover, all the diterpenoids until now isolated from plants belonging to the *Teucrium* genus have a neo-clerodane absolute configuration [1–3, 5, 6, 10–13].

From a biogenetic point of view, it is important to note that teuvincentins B and C are the first 17β-*neo*-clerodane derivatives isolated from *Teucrium* species. However, both compounds possess a carbonyl group at the C-7

position [teuvincentin C (4); in its hemiacetalic form in teuvincentin B (3)] to which this biogenetically unusual B/I-configuration of the C-17 methyl group must be attributed.

EXPERIMENTAL

Mps: uncorr. Plant materials were collected in May 1986 at Cape São Vicente (Portugal) and voucher specimens were deposited in the Herbarium of the 'Instituto Superior de Agronomia', Lisbon (Portugal).

Extraction and isolation. Dried and powdered ***Teucrium polium*** subsp. *vincentinum* aerial parts (3 kg) were extracted 3 x with Me_2CO (10 I) at room temp. for 3 days. The extract (126 g) was chromatographed on a silica gel column (Merck, No. 7734, deactivated with 15% H_2O, 1 kg) eluted with n-hexane and *n*-hexane–EtOAc mixtures giving six fractions which, in turn, were rechromatographed (silica gel, the same eluent mixtures) yielding the following compounds in order of increasing chromatographic polarity: teuvincentin A (2, 70 mg), apigenin (371 mg) [4], teuvincentin B (3, 40 mg), eriocephalin (1, 6.9 g) [1, 3], 19-acetylgnaphalin (64 mg) [1], cirsiliol (1.7 g) [4], isoeriocephalin (179 mg) [1, 133, teuvincentin C (4, 74 mg) and 3-deacetyl-20-*epi*-teulanigin (1 1 mg) [2].

The previously known compounds (eriocephalin, 19-acetylgnaphalin, isoeriocephalin, 3-deacetyl-20-*epi*-teulanigin, apigenin and cirsiliol) were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, UV, ^1H NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

Teucincentin A (2). Mp 200–201°, decomp. (EtOAc–*n*-hexane); $[\alpha]_D^{20}$ −92.7° (CHCl,; c 0.192); IR ν_{max}^{KBr} cm-': 3480. 3460 (OH), 3140, 3120, 1505, 875 (furan), 1745, 1240 (OAc), 1662, 1628 (diosphenol), 3030, 2970, 2880, 1460, 1450, 1435, 1380, 1160, 1095, 1060, 1045, 970. 940, 765, 730, 715, 640; UV λ_{max}^{MeOH} nm (logs): 287 (4.14); ^1H NMR (300 MHz, pyridine-d,): see Table 1; ^{13}C NMR (75.4 MHz, pyridine-d,): see Table 2; EIMS (direct inlet) ***m/z*** (rel. int.): 498 $[M]^+$ (0.07), 496 [M]' (0.23), 461 (1.5), 437 (7), 408 (17), 377 (19), 317 (39), 229 (14), 121 (11), 119 (11), 105 (12), 95 (14), 94 (18), 91 (13), 81 (20), 77 (15), 43 (100). (Found: C, 57.83; H, 5.87; Cl. 7.46. $C_{24}H_{29}O_9Cl$ requires: C, 58.00; H, 5.88; Cl, 7.14%.)

Teuvincentin B ***(3).*** M p 194–197° (EtOAc–*n*-hexane); $[\alpha]_D^{20}$ -27.1" (CHCI,; c 0.170); IR ν_{max}^{KBr} cm^{-1}: 3520, 3420 (OH), 3140, 3120, 1505, 875 (furan), 3050 (oxirane), 1740, 1240 (OAc), 2980, 2860, 1445, 1370, 1095, 1015, 940, 815, 750, 695, 625; 'HNMR (300 MHz, pyridine-d,): see Table 3; ^{13}C NMR (75.4 MHz, pyridine-d,): see Table 4; EIMS (direct inlet) m/z (rel. int.): 420 [M]' (0.6), 402 (2.3). 360 (3.3), 220 (6), 202 (6), 191 (7), 190 (7), 163 (30), 121 (20), 105 (21), 97 (13), 95 (48), 94 (99), 91 (36), 81 (57), 79 (31), 55 (37), 43 (100). (Found: C. 62.59; H, 6.86. $C_{22}H_{18}O_8$ requires: C, 62.84; H, 6.71%.)

Teuvincentin C (4). This diterpenoid was isolated from the chromatographic process as an impure and amorphous solid and was characterized as its acetyl derivative 4, obtained by treatment of the impure sample with Ac_2O–pyridine (24 hr, room temp.) and subsequent chromatographic purification (silica gel column; EtOAc–*n*-hexane; 1: 1): mp 220–225° (EtOAc); $[\alpha]_D^{22}$ -8.1" (CHCI,; *c* 0.246); IR ν_{max}^{KBr} cm^{-1}: 3155, 3130, 1505, 876 (furan), 3060 (oxirane), 1745, 1245 (OAc), 2980, 2875, 1455, 1385, 1105, 1075, 1010, 955, 820; ^1H NMR (300 MHz, $CDCl_3$): see Table 3; ^{13}C NMR (50.3 MHz, $CDCl_3$): see Table 4; EIMS (direct inlet) ***m/z*** (rel. int.): absence of $[M]^+$, 462 $[M-42]^+$ (0.5), 445 $[M-MeCOO]^+$ (1.6), 444 $[M-AcOH]^+$ (1), 402 (4), 385 (2), 163 (7), 153 (14), 111 (21), 95 (16), 94 (36), 93 (6), 91 (11), 81 (18), 55 (10), 43 (100). (Found: C, 61.79; H, 6.46. $C_{26}H_{32}O_{10}$ requires: C, 61.89; H, 6.39%.)

Table 6. Crystallographic and physical data of compounds 6 and 10

	6	10
Stoichiometry	$C_{26}H_{31}O_{10}Cl$	$C_{26}H_{32}O_{10}$
Space group	$P2_12_12_1$	$P2_1$
a (Å)	19.540 (2)	8.837 (1)
b (Å)	16.372 (1)	17.366 (1)
c (A)	8.358 (1)	8.660 (1)
β (°)		109.281 (3)
Z	4	2
D_c (g/cm^3)	1.339	1.336
μ (cm^{-1})	17.305	8.194

7-Acetylteuvincentin A (6). Ac_2O–pyridine treatment of 2 (20 mg) 48 hr at room temp. yielded 6 (20 mg, after crystallization from EtOAc–*n*-hexane): mp 192-194"; $[\alpha]_D^{21}$ − 118.3" ($CHCl_3$; c 0.180); IR ν_{max}^{KBr} cm-': 3450 (OH), 3150. 3120, 1502, 872 (furan), 1770 (enol acetate), 1740, 1240 (OAc), 1660, 1630 (enone), 2960, 2880, 1460, 1380, 1175, 1155, 1135, 1095, 1040, 1015, 965, 855, 810, 740, 730; UV λ_{max}^{MeOH} nm (log E): 250.5 (4.09); ^1H NMR (200 MHz, CDCI,): see Table 1; EIMS (direct inlet) *m/z* (rel. int.): 540 $[M]^+$ (0.01), 538 [M] + (0.04). 503 (0.4), 502 (0.9), 443 (2). 419 (2), 317 (9), 95 (6), 94 (17), 81 (1 1), 77 (7). 43 (100). $C_{26}H_{31}O_{10}Cl$: M_r 540 (^{37}Cl) and 538 (^{35}Cl).

Teuvincentin A (2) from eriocephalin (1). CrO_3–pyridine (900 mg in 18 ml) treatment of 1 (900 mg) for 18 hr at room temp. gave 7,8-dehydroeriocephalin (5,514 mg, after CC over silica gel, *n*-hexane–EtOAc, 4: 1), identical in all respects [mp. mmp, $[\alpha]_D$, IR, 'HNMR, ^{13}C NMR (50.3 MHz, pyridine-d,: see Table 2), MS] with the previously described compound [5].

A soln of **5** (351 mg) in CH_2Cl_2 (35 ml) at 0° was treated with aq. conc. HCI (3.1 ml) for 1 hr with stirring. Work-up in the usual manner gave a residue which was crystallized from EtOAc yielding a compound [mp 197-199" decomp.; $[\alpha]_D^{25}$ -94.3" (CHCI,; c 0.162)] identical in all respects (mmp, IR, UV, 'HNMR, ^{13}C NMR, MS, TLC) with natural teuvincentin A (2).

Compound 7 from 7,8-*dehydroeriocephalin* ***(5).*** Treatment of **5** (50 mg) with aq. conc. HCl (0.3 ml) in $CHCl_3$ soln (20 ml) as described above yielded the 20-*O*-ethyl acetal derivative 7 (48 mg). Mp 190-194" (EtOAc); $[\alpha]_D^{25}$ − 147.2" ($CHCl_3$–MeOH, 9:1; *c* 0.248); IR ν_{max}^{KBr} cm^{-1}: 3475 (OH), 3140. 3130, 3120, 1505, 875 (furan), 1730, 1240 (OAc), 1655, 1620 (diosphenol), 2965, 2870, 1445, 1390, 1295, 1190, 1100, 1050. 1040, 1030. 1015, 795, 740; UV λ_{max}^{MeOH} nm (logs): 289 (3.99); ^1H NMR (300 MHz, CDCI,): see Table 1; ^{13}C NMR (50.3 MHz, pyridine-d,): see Table 2; EIMS (direct inlet) ***m/z*** (rel. int.): absence of $[M]^+$, 410 (3), 408 (10), 335 (10), 319 (11), 317 (33), 299 (19), 121 (17), 119 (15), 95 (24), 94 (24), 91 (18), 81 (46), 77 (26), 55 (19), 43 (100). (Found: C, 59.43; H, 6.19; Cl. 7.59. $C_{24}H_{31}O_8Cl$ requires: C, 59.68; H, 6.47: Cl, 7.34%.)

7-Acetyl derivative of 7 ***(Compound 8).*** Ac_2O–pyridine treatment of 7 (20 mg) for 24 hr at room temp. yielded 8 (19 mg) as a colourless thick oil: $[\alpha]_D^{21}$ − 109.6" (CHCI,; c 0.437); IR ν_{max}^{NaCl} cm^{-1}: 3480 (OH), 3140, 3130, 1510, 880 (furan), 1770 (enol acetate), 1750, 1240 (OAc), 1663, 1638 (enone), 2980, 2880, 1450, 1380, 1220, 1105, 1055, 1040, 915, 800; UV λ_{max}^{MeOH} nm (log ε): 252 (4.0); ^1H NMR (300 MHz, CDCI,): see Table 1; EIMS (direct inlet) m/z (rel. int.): 526 $[M]^+$ (0.2), 524 [M] $^+$ (0.6), 489 (21), 45 1 (18), 450 (18), 419 (27), 317 (36), 299 (22), 281 (14), 254 (11), 95 (12), 94 (13), 91 (8), 81 (17), 43 (100). $C_{26}H_{33}O_9Cl$: ***M,*** 526 ("Cl) and 524 (^{35}Cl).

Acetic anhydride-pyridine treatment of teuvincentin B (3) to give compounds 10 and 11. Ac_2O–pyridine treatment of 3 (30 mg) for 72 hr at room temp. gave a mixture of two substances (TLC). CC (silica gel, *n*-hexane–EtOAc, 2: 1) yielded the 6,7-diacetyl derivative (10, 10 mg, less polar constituent) and the 6-monoacetyl derivative (11, 18 mg).

6,7-*Diacetylteuvincentin B* (10). Mp 220–223° (EtOAc–*n*-hexane); $[\alpha]_D^{20} + 38.5°$ ($CHCl_3$; c 0.171); IR ν_{max}^{KBr} cm-': 3170, 3150, 3130, 1500, 875 (furan), 3070, 3055 (oxirane), 1750, 1250 (OAc), 2970, 2875, 1450, 1380, 1370, 1225, 1220, 1120, 1105, 1100, 1025, 1000, 960, 805, 750; ^1H NMR (300 MHz, $CDCl_3$): see Table 3; EIMS (direct inlet) *m/z* (rel. int.): 504 [M]' (O.l), 462 (O.l), 445 (3), 444 (2), 402 (15), 280 (5), 163 (10), 121 (11), 95 (20), 94 (37), 91 (11), 81 (23), 43 (100). (Found: C, 62.03; H, 6.26. $C_{26}H_{32}O_{10}$ requires: C, 61.89; H, 6.39%.)

6-*Acetylteuvincentin B* (11). Mp 191-193" (EtOAc–*n*-hexane); $[\alpha]_D^{21}$ -6.2" (CHCl,; c 0.065); IR ν_{max}^{KBr} cm-': 3450 (OH), 3160, 3130, 1505, 875 (furan), 3060 (oxirane), 1740, 1725, 1265, 1240 (OAc), 2980, 2900, 2870, 1445, 1375, 1160, 1095, 1060, 1015, 1000, 945, 910, 880, 805, 745; ^1H NMR (300 MHz, $CDCl_3$): see Table 3; EIMS (direct inlet) m/z (rel. int.): absence of $[M]^+$, 403 $[M - MeCOO]^+$ (2.5), 402 $[M - AcOH]^+$ (5), 342 (3), 175 (5), 163 (14), 121 (9), 107 (9), 105 (13), 95 (23), 94 (58), 91 (18), 81 (28), 79 (IS), 55 (15), 43 (100). (Found: C, 62.21; H, 6.68. $C_{24}H_{30}O_9$ requires: C, 62.32; H, 6.54%.)

X-ray ***structure determination of 7-acetylteuvincentin A (6)*** and **6,7-*diacetylteuvincentin B*** (10). Colourless crystals of compounds 6 and 10 with approximate dimensions of 0.1 x 0.15 x 0.08 and $0.20 \times 0.25 \times 0.28$ mm respectively, were used for the crystal diffraction analysis. The cell dimensions given in Table 6 were determined from least-squares analysis of 17 high-angle reflections, range of 2θ from 20° to 48°, and 25 high-angle reflections, range of 28 from 20" to 63°, for compounds 6 and 10, respectively. The lattice parameters and the intensities were measured on a Philips PW 1100 four-circle diffractometer, with graphite-monochromated CuKα radiation ($\omega/2\theta$ scan mode). A total of 2295 (6) and 2347 (10) independent reflections with 2" $<65°$ were scaned, from these 1208 for 6 and 2212 for 10 with $I > 2\sigma$ (I) were considered as observed and used for the structure determination and refinement. Two reference reflections for each compound were periodically measured during the data collection process in order to check crystal alignment and/or decomposition; the intensities showed no variation and no crystal decay. The intensities were corrected for Lorentz and polarization effects. The structures were solved by direct methods [14] and by difference maps. Least-squares calculations refined the structures to give H atoms in several difference synthesis; they were included in the final refinement as fixed contributors with isotropic thermal factors, the non-hydrogen atoms were treated anisotropically. The weighting scheme was chosen to give no trends in $\langle w\Delta^2 \rangle$ over ranges of F_0 and $\sin\theta/\lambda$. The final R values are $R = 11.1$ and $R_w = 9.1$ for 6, and $R = 4.1$ and $R_w = 4.7$ for 10. A final difference synthesis shows the residual electron density being no greater than ± 0.64 eÅ^{-3} and ± 0.33 eÅ^{-3} for 6 and 10 respectively. Scattering factors were taken from the literature [15]. All the calculations were performed on a VAX 750/11 computer using the X-Ray System Crystallography package [16] and other local programs. In the determination of the absolute configuration the anomalous dispersion effects of Cl, 0 and C for 6, and 0 and C for 10 were used. On consideration of reflections with $F_0 > 10\sigma(F_0)$ there are 108 and 58 Bijvoet pairs with $F_c > 1.00$ and $F_c > 0.10$ for 6 and 10 respectively, showing an averaged Bijvoet difference (A') of 1.829 for the correct enantiomer vs 3.18 for the wrong one (A-) in the case of compound 6 and 0.293 for (A') vs 0.376 for the wrong one (A-) for compound 10 [17].

Lists of atomic coordinates as obtained from the final refinement, structure factors, anisotropic thermal parameters for non-hydrogen atoms and H atom parameters of compounds 6 and 10 are deposited as supplementary material at the Cambridge Crystallographic Data Centre.

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