A Chlorine-Containing *neo*-Clerodane Diterpene from *Teucrium pernyi*

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A novel chlorine-containing *neo*-clerodane diterpene, teupernin D, and two known compounds, teucvidin and teuflin, were isolated from the whole parts of *Teucrium pernyi*. The structure of teupernin D was characterized as (12*S*)-15,16-epoxy-8β-hydroxy-17-chloro-19-nor-10α-*neo*-cleroda-4,13,14-triene-18,6β:20,12-diolide on the basis of spectral evidence. The absolute configuration was established by the circular dichroism (CD) spectrum and confirmed by X-ray crystallographic analysis.

Keywords *Teucrium pernyi*; Labiatae; chlorine-containing diterpene; *neo*-clerodane; teupernin D

Introduction

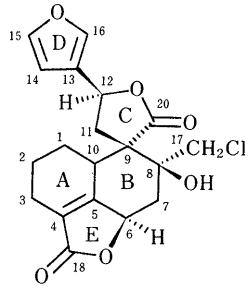
Teucrium species (Labiatae) are well-known for their abundance of *neo*-clerodane diterpenoids which sometimes show an insect antifeeding activity.[1] Previous chemical investigation on *T. pernyi* FRANCH. resulted in the isolation of three novel *neo*-clerodane diterpenes, teupernins A, B and C.[2] In continuous research on the plant, another novel chlorine-containing diterpene was isolated together with two known compounds, teucvidin[3] and teuflin.[4,5] In this paper the structure of the new diterpene is described.

Results and Discussion

Teupernin D (**1**) was isolated as colorless rectangles and its molecular formula $C_{19}H_{19}O_6Cl$ (required: 378.0870, found: 378.0863) was determined by high-resolution mass spectrometry (HRMS). A fragment at *m/z* 380 ($M^+ + 2$) in three times as strong as the molecular peak indicates the presence of chlorine in **1**. Infrared spectroscopy (IR) showed the absorption bands at 3300, 1760 and 1720 cm^{-1}, which correspond to a hydroxyl group, γ-lactone and α,β-unsaturated γ-lactone moieties, respectively. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, two α-furan protons (δ 7.47, 7.38) and one β-furan proton (δ 6.51) and an ABX system [δ 5.29 in triplet (t), 2.80 in double doublet (dd), 2.19 in dd] were observed, which indicated the presence of an β-substituted furan ring and a CH_2–CH–O moiety. Because of the similarity of ^{1}H- and carbon-13 (^{13}C) NMR spectral data of **1** to those of teucvidin, a nor-diterpene in *T. visidum* var. *miquelianum*,[3,6] the skeleton of **1** was predicted as a 19-nor-*neo*-clerodane. The only difference in the ^{1}H-NMR of **1** from teucvidin is that it was lacking the usual doublet at δ *ca.* 1.20 due to a secondary methyl group at C-8. In place of the doublet, two doublets at δ 4.59 and 3.91 were observed, which indicated that the methyl group was converted to a chloromethyl group connecting to a quaternary carbon. The presence of a hydroxyl group at C-8 was suggested by the evidence that no geminal proton could be found in the ^{1}H-NMR spectrum. Positions of the chlorine and the hydroxyl group were also supported by the chemical shifts of C-17 (δ 49.86 in triplet) and C-8 (δ 76.67 in singlet) in the ^{13}C-NMR spectrum which were shifted to a lower field than those of teucvidin.

The relative configuration of teupernin D was determined by nuclear Overhauser effect (NOE). When one of the chloromethyl protons at δ 3.91 was irradiated, NOE enhancements (8 and 10.3%) were observed in protons at C-6 (δ 4.81) and C-10α (δ 3.11), respectively, which revealed that the relationship between the chloromethyl group at C-8, and H-6 and H-10 is in *cis*-configuration. The other results of the NOE are shown in Fig. 1. The stereochemisty of C-12 could not be determined by the usual NOE experiment,[7,8] because the ring B is in a chair-type conformation. In this conformation, the space distances between H-14 and H-17, and between H-15 or H-16 and H-17 are over 5 Å in length either in C-12*S* or C-12*R* configuration. The configuration of C-12 was estimated in the following manner.

When a triplet (δ 5.29) assignable to H-12 was irradiated, an NOE enhancement (7.2%) was observed in H-1β at δ 1.55, which indicated that H-12 and H-1β were on the same plane defined by the γ-lactone of C-12 and C-20. As the circular dichroism (CD) curve of **1** was identical with



teupernin D (**1**)

Chart 1

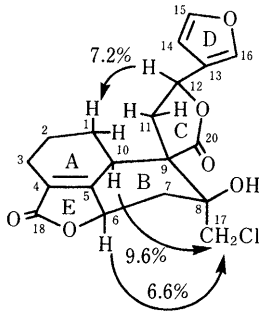


Fig. 1

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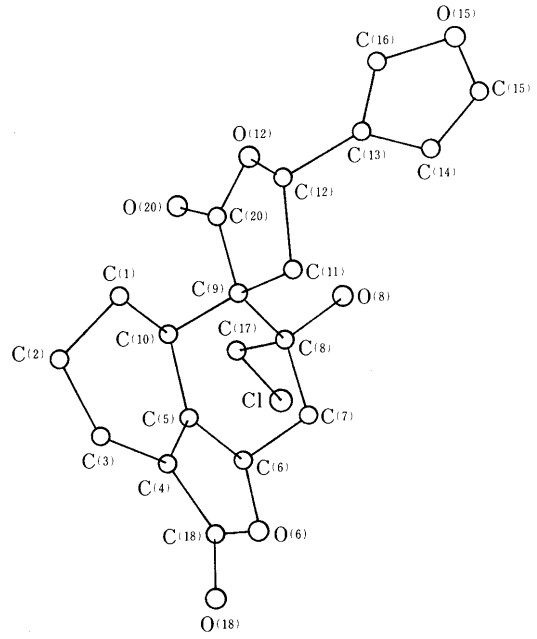

Fig. 2. Computer Generated Structural Drawing of **1**

TABLE I. Atomic Coordinates ($\times 10^4$) and Isotropic Thermal Parameter ($Å \times 10^3$) of Teupernin D (**1**)

	X	*Y*	*Z*	$U^{a)}$
C(1)	−2253 (9)	8082 (8)	−177 (5)	43 (3)
C(2)	−2085 (9)	8578 (8)	1007 (5)	45 (3)
C(3)	−305 (9)	8628 (8)	1452 (6)	46 (3)
C(4)	175 (9)	7262 (8)	1148 (5)	40 (2)
C(5)	−673 (8)	6276 (7)	414 (5)	36 (2)
C(6)	82 (9)	4996 (8)	318 (5)	40 (2)
C(7)	476 (9)	4695 (8)	−830 (5)	39 (2)
C(8)	−1137 (9)	4588 (8)	−1538 (5)	39 (2)
C(9)	−1589 (8)	6114 (7)	−1483 (5)	34 (2)
C(10)	−2050 (9)	6495 (8)	−325 (5)	39 (2)
C(11)	−105 (9)	7355 (7)	−2028 (5)	39 (2)
C(12)	−970 (9)	8123 (8)	−2866 (5)	42 (3)
C(13)	−101 (8)	8378 (8)	−3937 (5)	40 (2)
C(14)	678 (10)	7410 (9)	−4509 (6)	52 (3)
C(15)	1285 (11)	8140 (10)	−5447 (6)	61 (4)
C(16)	102 (9)	9600 (8)	−4550 (6)	50 (3)
C(17)	−2701 (10)	3272 (8)	−1153 (6)	49 (3)
C(18)	1605 (9)	6694 (8)	1526 (5)	45 (3)
C(20)	−3165 (10)	6064 (8)	−2225 (5)	42 (3)
O(6)	1068 (6)	5423 (5)	995 (4)	44 (2)
O(8)	−800 (6)	4323 (5)	−2629 (3)	43 (2)
O(12)	−2736 (6)	7097 (6)	−3009 (4)	51 (2)
O(15)	943 (7)	9504 (7)	−5464 (4)	60 (2)
O(18)	2738 (7)	7247 (6)	2193 (4)	54 (2)
O(20)	−4605 (7)	5266 (6)	−2128 (4)	54 (2)
Cl	−2169 (3)	1540 (2)	−1134 (2)	62 (1)

a) Equivalent isotropic *U* defined as one third of the trace of the orthogonalized Uij tensor.

TABLE II. H-Atom Coordinates ($\times 10^4$) and Isotropic Thermal Parameter ($Å^2 \times 10^3$) of Teupernin D (**1**)

	X	*Y*	*Z*	*U*
H(1a)	−3378	8092	−433	50
H(1b)	−1377	8778	−592	50
H(2a)	−2956	7868	1416	50
H(2b)	−2265	9557	1066	50
H(3a)	−359	8699	2226	50
H(3b)	538	9509	1190	50
H(6)	−720	4060	526	50
H(7a)	1422	5509	−1079	50
H(7b)	791	3769	−877	50
H(10)	−3146	5793	−157	50
H(11a)	503	8073	−1503	50
H(11b)	695	6905	−2378	50
H(12)	−972	9079	−2599	50
H(14)	828	6428	−4391	50
H(15)	1872	7635	−5930	50
H(16)	−361	10367	−4254	50
H(17a)	−3019	3486	−435	50
H(17b)	−3662	3193	−1632	50

TABLE III. Bond Length (Å) of Teupernin D (**1**)

C(1)–C(2)	1.531 (9)	C(1)–C(3)	1.533 (11)
C(2)–C(3)	1.521 (11)	C(3)–C(4)	1.470 (11)
C(4)–C(5)	1.333 (9)	C(4)–C(18)	1.467 (11)
C(5)–C(6)	1.478 (11)	C(5)–C(10)	1.493 (10)
C(6)–C(7)	1.499 (9)	C(6)–O(6)	1.447 (8)
C(7)–C(8)	1.546 (10)	C(8)–O(8)	1.412 (8)
C(9)–C(10)	1.546 (9)	C(11)–C(12)	1.530 (10)
C(12)–C(13)	1.487 (9)	C(13)–C(14)	1.418 (12)
C(13)–C(16)	1.335 (10)	C(14)–C(15)	1.363 (11)
C(15)–O(15)	1.365 (12)	C(16)–O(15)	1.335 (9)
C(17)–Cl	1.770 (9)	C(20)–O(12)	1.348 (8)
C(20)–O(20)	1.192 (8)	C(18)–O(6)	1.348 (9)
C(18)–O(18)	1.229 (8)	C(1′)–C(2′)	1.535 (11)

TABLE IV. Bond Angles (°) of Teupernin D (**1**)

C(2)–C(1)–C(10)	112.2 (6)	C(1)–C(2)–C(3)	111.1 (6)
C(2)–C(3)–C(4)	110.5 (6)	C(3)–C(4)–C(5)	123.9 (7)
C(3)–C(4)–C(18)	130.2 (6)	C(5)–C(4)–C(18)	105.9 (7)
C(4)–C(5)–C(6)	111.0 (6)	C(4)–C(5)–C(10)	125.6 (7)
C(6)–C(5)–C(10)	123.0 (6)	C(5)–C(6)–C(7)	112.4 (6)
C(5)–C(6)–O(6)	103.8 (5)	C(7)–C(6)–O(6)	113.2 (6)
C(1)–C(10)–C(5)	109.7 (6)	C(1)–C(10)–C(9)	115.2 (6)
C(5)–C(10)–C(9)	107.7 (6)	C(11)–C(12)–C(13)	115.1 (5)
C(12)–C(13)–C(14)	128.1 (7)	C(12)–C(13)–C(16)	125.7 (7)
C(14)–C(13)–C(16)	106.2 (6)	C(13)–C(14)–C(15)	105.8 (8)
C(14)–C(15)–O(15)	109.6 (8)	C(13)–C(16)–O(16)	111.8 (7)
O(12)–C(20)–O(20)	121.9 (7)	C(4)–C(18)–O(6)	110.4 (6)
C(4)–C(18)–O(18)	128.9 (7)	O(6)–C(18)–O(18)	120.7 (7)
C(6)–O(6)–C(18)	108.3 (6)	C(15)–O(15)–C(16)	106.5 (6)

that of teucvidin,[3] H-10 and H-6 were predicted in α-orientation. Therefore, the structure of teupernin D was characterized as (12*S*)-15,16-epoxy-8β-hydroxy-17-chloro-19-nor-10α-*neo*-cleroda-3,13,14-triene-18,6β:20,12-diolide.

For the structure confirmation of teupernin D, **1** was subjected to X-ray crystallographic analysis. The crystals for the X-ray analysis were recrystallized in a mixture of $CHCl_3$ and MeOH. The results well agreed with the structure proposed by the spectral analyses. A computer generated drawing and related data are shown in Fig. 2 and Tables I—IV.

The compound containing chlorine is sometimes regarded as an artifact; teupernin D is, however, detected in an acetone extract of the fresh plant on thin layer chromatography.

The structures of the other two compounds isolated in the present study were determined to be teucvidin[3] and teuflin[4,5] respectively, after comparison of IR, MS and NMR spectral data.

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Experimental

Plant Material *Teucrium pernyi* was collected at Lushan mountain in Jiangxi province, China. The voucher specimen was deposited in the Herbarium of the Lushan Botanical Garden, Chinese Academy of Sciences.

Extract and Isolation The whole plants (7.0 kg, naturally dried and pulverized) were extracted with EtOH (95%) by percolation for a week. After removing EtOH under reduced pressure, the syrup (1.0 kg) was extracted successively by petroleum ether (bp 60—69 °C), $CHCl_3$, and EtOAc. Chromatographic separation of the $CHCl_3$ extract gave minute diterpenes of **1** (20 mg), teucvidin (54 mg) and teuflin (47 mg) in addition to teupernins A, B and C.

Teupernin D (1) Colorless rectangle, mp 205—207 °C ($CHCl_3$–MeOH). Circular dichroism (CD) (MeOH, $c=0.00017$) nm ($\Delta\varepsilon$): 201 (+0.60), 223.5 (−9.18), 250 (+0.70). IR ν_{max} cm^{-1}: 3300 (OH), 2910, 1760 (C=O), 1720 (C=O), 1430, 1260, 1180, 1010, 860, 800. Electron impact-mass spectrum (EIMS) (direct inlet) *m/z* (rel. int.): 380 [M^++2] (2.5), 378 [M^+] (6.1), 360 (2.8), 342 (5.5), 332 (39.6), 311 (61.8), 255 (19.3), 186 (20.7), 95 (100), 94 (99.5), 91 (42.0). ^{1}H-NMR (400 MHz, $CD_3OD+CDCl_3$) δ: 2.19 (1H, dd, $J_{11b,12}=8.6$, $J_{gem}=13.8$ Hz, H-11B), 2.80 (1H, dd, $J_{11a,12}=8.6$, $J_{gem}=13.8$ Hz, H-11A), 3.11 (1H, br t, $W_{1/2}=16.0$ Hz, H-10α), 3.91 (1H, d, $J_{gem}=12.6$ Hz, H-17B), 4.59 (1H, d, $J_{gem}=12.6$ Hz, H-17A), 4.81 (1H, br t, $W_{1/2}=18.5$ Hz, H-6), 5.29 (1H, t, $J_{11,12}=8.4$ Hz, H-12), 6.51 (1H, s, H-14), 7.38 (1H, s, H-15), 7.47 (1H, s, H-16). ^{13}C-NMR (100 MHz, C_5D_5N) δ: 20.46 (t, C-1), 21.69 (t, C-3), 23.73 (t, C-2), 34.54 (t, C-7), 37.82 (t, C-11), 38.73 (d, C-10), 49.86 (t, C-17), 59.68 (s, C-8), 72.96 (d, C-12), 76.17 (d, C-6), 76.67 (s, C-8), 109.34 (d, C-14), 126.03 (s, C-13), 128.88 (s, C-4), 141.40 (d, C-16), 144.48 (d, C-15), 161.04 (s, C-5), 172.65 (s, C-18), 177.60 (s, C-20).

X-Ray Structure Determination of 1 $C_{19}H_{19}O_6Cl$, monoclinic in the space group $P2_1$, with $Z=2$, $a=8.031$ (3), $b=9.255$ (5), $c=12.385$ (4) Å and $V=884.19$ (69) Å^3. The calculated density is 1.34 g/cm^3. Independent Friedel pairs (2381) were measured on a R_{3m}/E four-circle diffractometer with graphite-monochromated CuK_α radiation in ω-scan mode. Friedel pairs (2327) were observed according to the criterion $I>3\sigma(I)$. The structure was resolved by direct methods (SHELXTL program), the E-map gave the rough position of 17 non-hydrogen atoms. After four cycles of Karle's addition, full-matrix least-squares and Fourier difference refinement, the coordinates of all non-hydrogen atoms (carbons, oxygens and chlorine) were established. The positions of hydrogens were determined by the geometric method.

With the *R*-factor, $R(+)=0.0469$ and $R(-)=0.0507$, the absolute configuration of teupernin D was determined to be $R(+)$ configuration. Ring A in a torsion chair, ring B in a chair and ring C in a chair type, and H-6 in α-, CH_2Cl at C-17 in α-, OH-8 in β-, H-10 in α-, and H-12 in α-orientation, respectively.

Teucvidin Colorless rectangles, mp 208—209 °C ($CHCl_3$). $[\alpha]_D$ −72.1° ($CHCl_3$, $c=0.14$). CD (MeOH, $c=0.00017$) nm ($\Delta\varepsilon$): 198 (+7.29), 228 (−14.87). IR ν_{max} cm^{-1}: 3500—3300, 3150, 3100, 2900, 1745, 1695, 1500, 1180, 1030, 800. EIMS (direct inlet) *m/z* (rel. int.): 328 [M^+] (3.3), 236 (7.6), 96 (16.0), 95 (100), 80 (7.8). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35 (3H, d, $J_{8,17}=7.0$ Hz, H-17), 1.91 (1H, dd, $J_{11b,12}=8.0$, $J_{gem}=14.0$ Hz, H-11*B*), 2.60 (1H, $J_{11a,12}=8.0$, $J_{gem}=14.0$ Hz, H-11A), 3.24 (1H, m, H-10α), 5.00 (1H, br q, $J_{6\alpha,7\alpha}=7.0$, $J_{6\alpha,7\beta}=10.0$ Hz, H-6), 5.35 (1H, t, $J=8.0$ Hz, H-12), 6.36 (1H, m, H-14), 7.34 (2H, m, H-15, 16). ^{13}C-NMR (25.1 MHz, $CDCl_3$) δ: 14.30 (q, C-17), 20.02 (t, C-1), 21.38 (t, C-3), 23.36 (t, C-2), 35.81 (d, C-10), 35.69 (t, C-7), 38.72 (d, C-8), 39.00 (t, C-11), 52.09 (s, C-9), 71.94 (d, C-12), 76.10 (d, C-10), 107.91 (d, C-14), 125.22 (s, C-13), 139.46 (d, C-16), 144.39 (d, C-15), 162.24 (s, C-5), 172.59 (s, C-18), 177.64 (s, C-20).

Teuflin Colorless crystals, mp 190—192 °C ($CHCl_3$). IR ν_{max} cm^{-1}: 3500—3400, 3150, 2940, 2850, 1750, 1700, 1500, 1180, 1160, 960, 870, 830. EIMS *m/z* (rel. int.): 328 [M^+] (87.7), 300 (5.5), 283 (39.8), 233 (100), 201 (44.1), 189 (21.8), 105 (16.3), 95 (78.6), 94 (21.4), 91 (22.3), 81 (30.6), 77 (20.8). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.22 (3H, d, $J=7.3$ Hz, Me-17), 2.06 (1H, dd, $J_{11b,12}=10.0$ Hz, $J_{gem}=13.7$ Hz, H-11B), 2.66 (1H, dd, $J_{11a,12}=7.3$ Hz, $J_{gem}=13.7$ Hz, H-11a), 2.72 (1H, br d, $W_{1/2}=16.0$ Hz, H-10β), 5.37 (1H, dd, $J_{11a,12}=7.3$ Hz, $J_{11b,12}=10.0$ Hz, H-12), 5.73 (1H, br d, $W_{1/2}=15.0$ Hz, H-6α), 6.39 (1H, m, H-14), 7.44 (1H, m, H-15), 7.48 (1H, s, H-16). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.93 (q, C-17), 19.04 (t, C-1), 23.68 (t, C-3), 24.04 (t, C-2), 32.17 (t, C-7), 36.24 (d, C-8), 43.21 (t, C-11), 43.50 (d, C-10), 51.36 (s, C-9), 71.90 (d, C-12), 76.97 (d, C-6), 108.21 (d, C-14), 124.23 (s, C-4), 124.64 (s, C-13), 140.20 (d, C-16), 144.65 (d, C-15), 166.49 (s, C-5), 173.95 (s, C-18), 176.23 (s, C-20).

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