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# 12-*EPI*-TEUSCORDONIN AND OTHER NEOCLERODANES FROM *TEUCRIUM BICOLOR*

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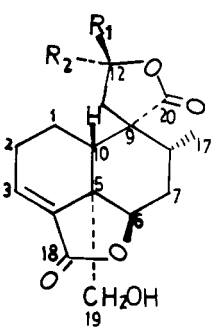
ABSTRACT.—A new neoclerodane, (12*R*)-*epi*-teuscordonin [**1**] has been isolated from *Teucrium bicolor*. The boat conformation of ring B and the configuration at C-12 in **1** were established by nOe experiments. The known diterpenoids montanin C, teucvin, 12-*epi*-teucvin, teupolin I, 12-*epi*-teupolin I, and (12*S*)-teucrin H-2 were also isolated from the extract. Montanin C [**2**] was found to be moderately toxic in the brine shrimp bioassay.

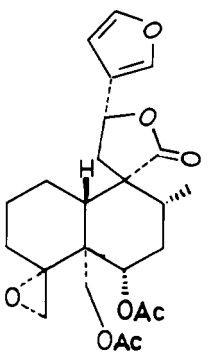
Neoclerodanes from *Teucrium* species have been the subject of many studies during the past few years (1). Recently, these studies have been directed toward the elucidation of the configuration at C-12 by spectroscopic techniques (2,3), since both 12*R* and 12*S* series of isomers have been isolated from related species. The most generally accepted method in this sense is measurement of the nOe of the H-12 signal upon irradiation at the secondary methyl, H-17, as the configuration at C-8 is well established on biogenetic grounds. Thus, only 12*R* isomers give significant nOe (8–12%), although 12*S* isomers do not show this effect (3) as H-12 is remote from C-17. Another satisfactory empirical correlation has been found to be the chemical shifts for carbons C-8 and C-10 in the $^{13}C$ spectra of the epimers (2); this has been attributed to the effect of the furan ring, which is nearer to C-8 in the 12*S* series and to C-10 in the 12*R* series.

As part of our research on bioactive compounds from native plants, we have examined the $CH_2Cl_2$ extract from the tops and leaves of *Teucrium bicolor* L.E.Sm. (Labiatae), a plant with reported antitumor activity (4) and have isolated a new neoclerodane, (12*R*)-*epi*-teuscordonin [**1**] whose structure is proposed on spectroscopic evidence. The known diterpenoids montanin C (3), 12-*epi*-teucvin (3), teucvin (5), teucrin H-2 (6), teupolin I (3), and 12-*epi*-teupolin I (3) were also present in the extract, and their structures were identified by comparison of their spectroscopic and physical properties with those reported in the literature. The *S* configuration at C-12 of teucrin H-2, which has not previously been reported, was established by nOe difference experiments on the corresponding acetate.

Montanin C [**2**], teucvin [**3**] and teucrin H-2 [**4**], the more abundant compounds in the extract, were tested




**1** $R_1$=H, $R_2$=β-furyl
**5** $R_1$=β-furyl, $R_2$=H

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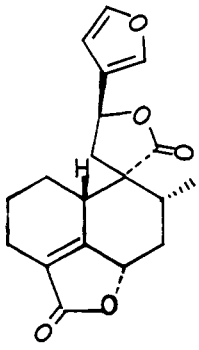

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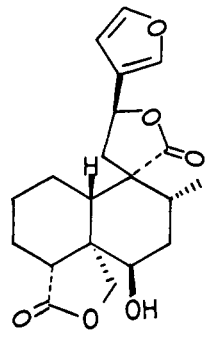

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for toxicity with the brine shrimp bioassay (*Artemia salina*) as a preliminary indication of biological activity (7).

The extract also afforded a minor crystalline compound ($C_{20}H_{22}O_6$) showing spectroscopic features consistent with a furoneoclerodane skeleton. In addition to the furan ring (3125, 1500, 880 $cm^{-1}$), the ir had absorbances associated with a hydroxyl group (3450 $cm^{-1}$) and two γ-lactones (1750 and 1725 $cm^{-1}$), one of them α,β-unsaturated (1665 $cm^{-1}$). The $^1$H-nmr spectrum (Table 1) showed the characteristic resonances for a secondary methyl group, a β-substituted furan ring, a low field olefinic proton (H-3, δ 6.68, dd, $J$ = 5.2, 2.6 Hz), a hydroxymethylene group (AB system δ 3.98 and 3.41, $J$ = 12.6 Hz; the latter sharpening on addition of $D_2O$) and two double doublets at δ 5.45 ($J$ = 10.8, 5.2 Hz, H-12) and δ 5.06 ($J$ = 12.1, 5.9 Hz, H-6) assigned by selective decoupling experiments and the requirement of an oxygen function at C-6, a factor present in all neoclerodanes isolated from *Teucrium* species (2). These structural features, together with the analysis of the $^{13}$C-nmr spectrum (Table 2) led us to a structure similar to teuscordonin [**5**], a diterpenoid previously isolated from *Teucrium scorodonia* (8). A compari-

TABLE 1. $^1$H-nmr Spectral Data of Compounds **1** and **5**.[a]

| Proton | Compound | |
|---|---|---|
| | **1**[b] | **5**[c] |
| H-3 | 6.68 dd (5.2, 2.6) | 6.72 dd (5.7, 2.8) |
| H-6 | 5.06 dd (12.1, 5.9) | 5.02 dd (11.1, 5.6) |
| H-7α | 2.13 ddd (13.3, 2.9, 5.9) | |
| H-7β | 1.74 m[d] | |
| H-8 | 2.40 m[d] | |
| $H_A$-11 | 2.39 dd (10.8, 12.7) | 2.70 dd (8.8, 13.0) |
| $H_B$-11 | 2.24 dd (5.2, 12.7) | 2.29 dd (2.6, 13.0) |
| H-12 | 5.45 dd (10.8, 5.2) | 5.57 ddd (8.8, 2.6, 1.0) |
| H-14 | 6.38 brs | 6.36 dd (1.8, 0.9) |
| H-15 | 7.48 brs | 7.47 t (1.7) |
| H-16 | 7.44 t (1.5) | 7.44 m |
| Me-17 | 1.28 d (7.8) | 1.09 d (7.3) |
| $H_A$-19 | 3.93 d (12.5) | 3.97 d (12.2) |
| $H_B$-19 | 3.41 brd (12.5) | 3.49 brd (12.2) |

[a]Chemical shifts are in δ values from TMS ($J$ in Hz).
[b]At 250 MHz, $CDCl_3$ solution.
[c]At 270 MHz, $CDCl_3$ solution.
[d]These assignments were confirmed by double resonance experiments.

TABLE 2. $^{13}$C-nmr Chemical Shifts of Compounds **1** and **5**.

| Carbon | Compound | | |
|---|---|---|---|
| | **1**[a] | **5**[b] | Δδ[c] |
| C-1 | 17.9 t | 17.6 t | +0.3 |
| C-2 | 25.0 t | 24.6 t | +0.4 |
| C-3 | 136.5 d | 134.9 d | +1.6 |
| C-4 | 136.5 s | 139.7 s | −3.2 |
| C-5 | 44.8 s | 45.6 s | −0.8 |
| C-6 | 76.8 d | 77.4 d | −0.6 |
| C-7 | 30.8 t | 31.2 t | −0.4 |
| C-8 | 35.0 d | 37.0 d | −2.0 |
| C-9 | 49.9 s | 48.2 s | +1.7 |
| C-10 | 42.0 d | 45.5 d | −3.5 |
| C-11 | 46.2 t | 44.3 t | +1.9 |
| C-12 | 71.1 d | 71.9 d | −0.8 |
| C-13 | 122.6 s | 127.0 s | −4.4 |
| C-14 | 108.1 d | 108.7 d | −0.6 |
| C-15 | 144.2 d | 144.7 d | −0.5 |
| C-16 | 140.3 d | 139.7 d | +0.6 |
| C-17 | 16.9 q | 17.6 q | −0.7 |
| C-18 | 168.6 s | 169.3 s | −0.7 |
| C-19 | 63.0 t | 63.4 t | −0.4 |
| C-20 | 178.5 s | 177.3 s | +1.2 |

[a]In δ values from TMS, at 62.5 MHz, $CDCl_3$.
[b]In δ values from TMS, at 20.15 MHz, in pyridine-$d_5$. Data are from Marco *et al.* (8).
[c]$\Delta\delta = \delta_{12R} - \delta_{12S}$.

son of the reported $^1$H- and $^{13}$C-nmr data (Tables 1 and 2), however, showed differences that could not be attributed to solvent effects alone, particularly the resonances assigned to H-11, H-17 in the $^1$H-nmr and C-8, C-9, C-10, and C-11 in the $^{13}$C-nmr spectra. The observed differences could originate either from a change in configuration at C-12 or C-6 or both. A change at C-6 would necessitate a change in the conformation of ring B (from chair to boat) in order to have an axial (β) H-6 ($J$ = 12.1, 5.9 Hz). Irradiation at δ 1.28 ppm (H-17) resulted in 14.2% nOe enhancement of the H-6 and 2.8% nOe enhancement of the H-12 signals. These results confirmed the boat conformation of ring B with H-6α and a 12*R* configuration, because only this case, as shown in structure **1**, places the relevant groups very close to the secondary methyl group. The small value of the nOe enhancement of H-12 (2.8%) is also a consequence of the boat conformation of ring B.

The observed differences in the chemical shifts of C-8, C-9, C-10, and C-11 for **1** and **5** (Table 2) did not follow the expected pattern for C-12 epimers (3); however, the correlations described in the literature are reported only for compounds having ring B in the chair conformation where the spatial relationship between C-8, C-10, and the furan ring is different from that of the boat conformation. There are no further examples in the literature that could further support our observations.

Montanin C [**2**] was the only compound that exhibited toxicity against *A. salina* ($LC_{50}$ 66.4 ppm, confidence interval 90.0–49.3 ppm), while teucvin [**3**] and teucrin H-2 [**4**] gave values over 500 ppm. The observed toxicity of montanin C parallels well with the antifeedant activity reported for teucjaponin A, the C-6, C-12 epimer of **2**, isolated from *Teucrium japonicum* (9).

## EXPERIMENTAL

GENERAL PROCEDURES.—Nmr spectra were obtained with a Bruker WM-250 spectrometer. Mass spectral analyses were performed on VG Instruments MM 16F and 7070 EHF mass spectrometers. Ir spectra were recorded on a Perkin-Elmer 700 spectrophotometer. Melting points are uncorrected and were determined on a Fisher hot stage apparatus.

PLANT MATERIAL AND ISOLATION.—Flowering specimens of *T. bicolor* were collected in Cuesta Zapata (IV Region, Chile) in November 1984. A specimen is deposited in the Faculty of Sciences (Universidad de Chile) Herbarium. Air-dried aerial parts (4.8 kg) were extracted at room temperature with $CH_2Cl_2$ (30 liters). The extract (150 g) was repeatedly chromatographed over Si gel using a flash column eluted with petroleum ether or $CH_2Cl_2$ with increasing amounts of EtOAc (0→100%) yielding the following compounds in order of elution: teucvin (650 mg), 12-*epi*-teucvin (112 mg), teucrin H-2 (370 mg), (12*R*)-*epi*-teuscordonin (65 mg), and teupolin I and 12-*epi*-teupolin I as a mixture (70 mg). The previously known compounds were identified by their physical and spectroscopic data (mp, ir, nmr, ms) and by comparison with those reported in the literature (3–6). The configuration at C-12 of teucrin H-2 was determined by $^1$H nOe mea-

surements at 500 MHz by the FT difference method with the decoupler operating in the gated mode (3% H-14 and 2% H-16).

BIOASSAY PROCEDURES.—Bioassays with *A. salina* were performed as described in the literature (7). Toxicities of compounds were tested at 10, 50, 100, 250, 500, and 1000 ppm in 10-ml sea-water solutions. Ten one-day nauplii were used in each test and survivors counted after 24 h. Five replications were used at each concentration, and confidence intervals were calculated statistically by Finney's method (10). Teucrin H-2 and teucvin gave $LC_{50}$ values >1000 ppm.

*(12R)*-epi-*Teuscordonin* [**1**].—Mp 199–203° (petroleum ether/EtOAc); ir $\nu$ max (KBr) 3450 (hydroxyl), 3125, 1500, 880 (furan ring), 1750 ($\gamma$-lactone), 1725, 1665 $cm^{-1}$ ($\alpha,\beta$-unsaturated $\gamma$-lactone); $^1H$ nmr (250 MHz, $CDCl_3$) see Table 1; $^{13}C$ nmr ($CDCl_3$) see Table 2; eims (probe) (70 eV) *m/z* (%) $[M]^+$ 358 (3.3), 328 (15.9), 310 (12.5), 234 (25.2), 215 (17.9), 148 (20.0), 95 (100); hrms (70 eV) *m/z* 358.1414 (calcd 358.1454 for $C_{20}H_{22}O_6$).

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