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# Antifeedant *neo*-clerodanes from *Teucrium tomentosum* Heyne. (Labiatae)

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**Abstract**

From the acetone extract of *Teucrium tomentosum*, a new antifeedant *neo*-clerodane diterpenoid teuctosin (**1**) was isolated along with teuflin (**2**), teucrin-$H_2$ (**3**), 6β-hydroxyteuscordin (**4**), 6β-acetylteuscordin (**5**) and montanin-D (**6**). The structure of the new compound was elucidated comprehensively using 1D and 2D NMR methods and confirmed by X-ray crystallography. All the compounds showed effective antifeedancy against *Plutella xylostella* and *Spodoptera litura* at 10 μg/cm$^2$ of leaf area.


*Keywords:* Neo-clerodane diterpenoids; *Teucrium tomentosum*; *Plutella xylostella*; *Spodoptera litura*; Antifeedants

## 1. Introduction

The genus *Teucrium* (Labiatae) is a rich source of diterpenoids. More than 200 diterpenoids having the *neo*-clerodane skeleton have been isolated from the aerial parts of about 80 species/subspecies (Piozzi et al., 1998). There is continued interest in these compounds because they have powerful antifeedant activity (Simmonds and Blaney, 1992; Enriz et al., 1994, 2000; De la Torre et al., 1994; Malakov et al., 1994; Rodriguez et al., 1994). In view of the ecotoxicity of synthetic insecticides and resistance developed by the insects, antifeedants offer considerable promise as components of emerging integrated pest management (IPM) due to their capacity to reduce feeding by insects (Alford, 1994). *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), commonly known as the Diamond Back Moth, is one of the most important pests of cruciferous crops throughout the world, and can cause serious economic losses. It can destroy an entire crop even when intensive, but improper, chemical control measures are used (Syed, 1992). *Spodoptera litura* is a destructive pest of subtropical and tropical agriculture.

In continuation of our work on the isolation of insect antifeedants from natural sources, we investigated the aerial parts of *Teucrium tomentosum,* a shrub about 10 cm high, endemic to south India. To our knowledge, there have been no reports on chemical constituents from the plant.

Phytochemical investigation of the $Me_2CO$ extract, led to the isolation of a hitherto unknown compound teuctosin **1** along with known diterpenoids **2**–**6** (Fig. 1). All the compounds were assayed for antifeedant activity against *P. xylostella* and *S. litura*.

## 2. Results and discussion

Six compounds were isolated from the acetone extract. Compound **2** was previously isolated from *Teucrium viscidum* var. *miquelianum* (Node et al., 1981). Compounds **3**–**5** were known from *Teucrium scordium* (Papanov and Malakov, 1981; Papanov et al., 1981). The ambiguity in structure of **6** being either montanin-D (Malakov et al., 1978) or montanin-E (Papanov and Malakov, 1983) was ruled out by performing

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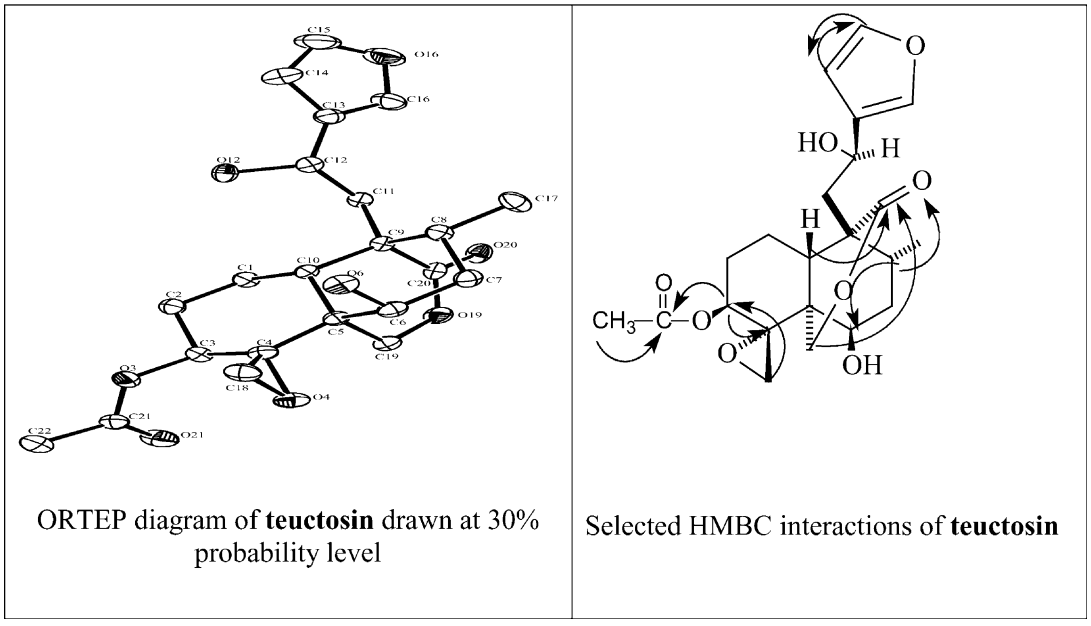


Fig. 1. ORTEP diagram and HMBC interactions in teuctosin.

an acetylation reaction with $Ac_2O$ and pyridine. Formation of a diacetyl derivative **7**, confirmed **6** to be montanin-D (there are 4 hydroxyl groups in montanin-E). The spectral data of **7** were consistent with the literature data (Bruno et al., 2000).

Compound **1** had the molecular formula $C_{22}H_{28}O_8$. Its IR spectrum showed absorptions corresponding to that of hydroxyl (3427 $cm^{-1}$), furan (2923,1442,889 $cm^{-1}$), γ-lactone (1747 $cm^{-1}$) and acetate (1692,1235 $cm^{-1}$) groups.

$^1$H-NMR showed characteristic signals for a β-substituted furan (*δ* 7.7 *br s*, *δ* 7.58 *t*, *δ* 6.68 *d*), a 4α-18 oxirane (*δ* 3.27, *δ* 3.32 each *d*) and a C-17 secondary methyl group (*δ* 0.93 *d*), identical with those found in *neo*-clerodanes isolated from several species of *teucrium* (Bruno et al., 1995a,b; Camps et al., 1987; Eguren et al., 1981). An additional peak at *δ* 1.89(3H, *s*) was assigned to the acetyl methyl. Signals at *δ* 3.87(1H, *m*), *δ* 5.71(1H, *dd*) and *δ* 5.25 (1H, *m*) indicated methine protons bearing oxygen substitution. HOMOCOSY correlations showed the connectivity between the protons at *δ* 3.87 to C-7 protons (*δ* 1.95 and *δ* 1.65, respectively), suggesting the placement of OH at C-6. From the $^{13}$C-NMR and DEPT experiments, two oxo-methylenes at 52 ppm and 73 ppm were ascribed to the 18-oxirane and 19-$CH_2$, respectively. The carbonyl resonating at 169 ppm was attributed to the acetyl moiety, because of its correlations shown in the HMBC by the acetyl methyl protons. Presence of acetate group at C-3 was confirmed by HMBC (Fig. 1). The proton at *δ* 5.71 showed correlations with that of the acetyl carbonyl at 169 ppm as well as to the C-4 bearing the oxirane moiety. The occurrence of the C19–C20 lactone was detected from the HMBC correlations of 8β-, 10β- and C19- protons with that of C20 carbonyl, ruling out the possibility of C2–C19 lactone occasionally present in some compounds (Tonn et al., 1988).

Finally the structure of the diterpenoid was unequivocally resolved using X-ray diffraction technique (Fig. 1—ORTEP diagram).

All the *neo*-clerodane diterpenoids isolated were tested for their efficacy as insect antifeedants against *P. xylostella* and *S. litura*. Azadirachtin-A, a generous gift of the Late Professor T.R. Govindachari, was used as a standard (Table 1). The dosage of the compounds for our study was fixed at 5 and 10 μg/cm$^2$ for both the insects. Of all the compounds tested, Teuflin **2** was the most potent antifeedant and diacetyl montanin-D **7** was the least effective. Teuflin belongs to a group of 19-*nor neo*-clerodane diterpenoids, which are infrequent. All the other compounds have a comparable antifeedant activity profile.

## 3. Experimental

### 3.1. General experimental methods

Melting points were determined with a Concord melting point apparatus and are uncorrected. Optical rotations were determined on an Autopol apparatus.

Routine $^1$H (200 MHz) and $^{13}$C (50 MHz) NMR spectra were recorded on a Bruker DPX200 spectrometer with TMS as internal standard. HRMS was done on a Micromass ESI-TOF instrument.

### 3.2. Plant material, extraction and isolation

The aerial parts of *Teucrium tomentosum* were collected from Kolli hills, Tamilnadu, India and were

Table 1
Antifeedant activity of diterpenoids against *Plutella xylostella* and *Spodoptera litura*[a]

| Compounds | Antifeedant activity (%) | | | |
|---|---|---|---|---|
| | *Spodoptera litura* (μg/cm$^2$) | | *Plutella xylostella* (μg/cm$^2$) | |
| | 10 | 5 | 10 | 5 |
| Teuctosin (**1**) | 71.5±2.0ab | 63.2±2.4ab | 77.4±2.1cd | 71±2.1c |
| Teuflin (**2**) | 80.2±2.1c | 73±1.6d | 84.2±2.9e | 76±2.4c |
| Teucrin-$H_2$ (**3**) | 72.0±2.7b | 67±2.0bc | 78.7±2.3de | 63±2.6b |
| 6β-Hydroxyteuscordin (**4**) | 73.0±3.86b | 70±1.7cd | 80±2.5de | 72±1.8c |
| 6β-Acetylteuscordin (**5**) | 73±1.9b | 64.5±1.9abc | 72±2.0bc | 60.5±1.7ab |
| Montanin-D (**6**) | 73±2.6b | 67±2.7bc | 70±1.9b | 61.3±2.0ab |
| Diacetyl montanin-D (**7**) | 67±1.6a | 59±2.5a | 62±2.1a | 56±2.6a |
| Azadirachtin-A (**8**)[b] | 79±2.1 | | 71±2.4 | |

[a] Values are mean±S.D. Values followed by a common letter within a column are not significantly different at $P<0.05$. 25 insects (five replicates with five individuals each) were used.
[b] Treatment at 0.5 μg/cm$^2$ for both insects.

identified by Dr. M.V. Rao, School of Biological Sciences, Bharathidasan University, Tiruchirapalli, India. A voucher specimen (RHT 640) is deposited in the herbarium of the Bharathidasan University.

The shade-dried, powdered aerial parts (8.4 kg) of *T. tomentosum* were exhaustively percolated with acetone (3 × 35 l). The extract was then dried in a rotary evaporator to yield the residue (146 g).

A portion (55 g) of the residue was subjected to column chromatography using silica gel (70–325) and eluted with $CHCl_3$–MeOH (1–100%) to yield a total of 69 fractions. Frs 31–38 gave a solid that was identified as **4** (300 mg).

Frs 28–38 were further chromatographed over silica gel (70–325) and eluted with *n*-hexane–EtOAc (1–100%) to yield **1** (75 mg), **2** (170 mg), **3** (125 mg), **6** (25 mg) and **5** (20 mg) (Fig. 2).

### 3.2.1. Teuctosin (*1*)

White solid, mp 198–200 °C, $[\alpha]_D = +88$ ($c = 0.25$, MeOH), IR $\nu_{max}^{KBr}$ cm$^{-1}$ 3427, 2923, 1442, 889, 1747, 1692, 1235, $^1$H-NMR; (values in $\delta$, pyridine-$d_5$) (Table 2),$^{13}$C-NMR; (values in $\delta$, pyridine-$d_5$) (Table 2), HRMS; calc. (M+ $Na^+$); 443.1682, observed—443.1667.

### 3.3. Insect rearing and bioassay

The test insects *P. xylostella* and *S. litura* were reared in the laboratory on *Brassica oleracea var. capitata* (Cabbage) and *Ricinus communis* (Castor) leaves at 25±2 °C, respectively. Bioassay was conducted by no choice method. Fresh leaf discs (22 cm$^2$ for *P. xylostella* and 180 cm$^2$ for *S. litura*) were cut and kept in Petri dishes. For *P. xylostella*, 110 μg of individual compounds was dissolved in 500 μl of methanol to give 5 μg/cm$^2$ and 200 μg was dissolved in 500 μl to give 10 μg/cm$^2$ concentrations. In the case of *S. litura*, 900 μg and 1800 μg of individual compounds were dissolved in 2 ml methanol, respectively, for 5 and 10 μg/cm$^2$ concentrations. The test solutions were painted on both sides of the leaf using a pipette. Controls were treated with methanol alone and air-dried. Freshly moulted 3rd instar larvae (of the respective insects) were taken from the culture and starved for 1 h prior to testing. Five

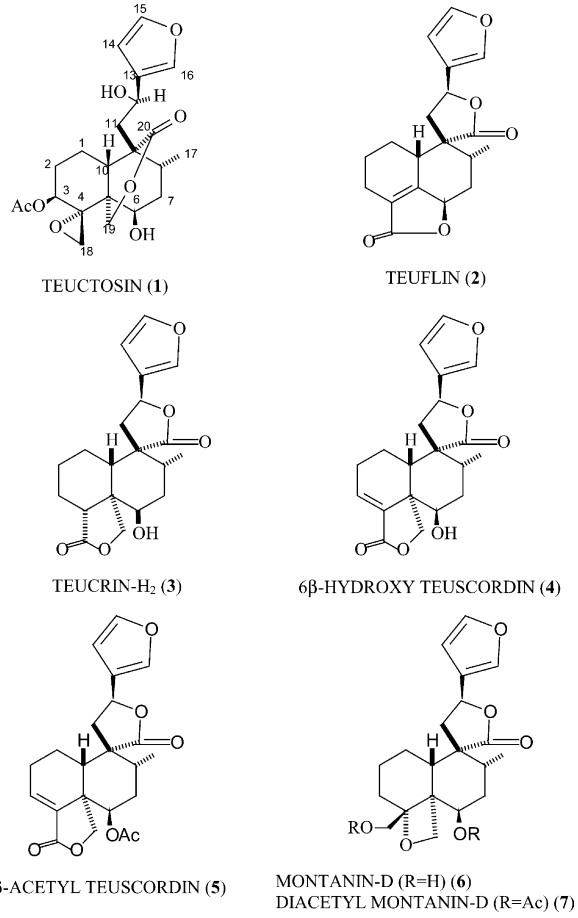


Fig. 2. Compounds isolated from *Teucrium tomentosum*.

Table 2
NMR spectral data of compound **1** ($\delta$ in ppm, $J$ in Hz) (200 and 50 Mhz, pyridine-$d_5$)

| Atom | δH | δC |
|---|---|---|
| 1 | | 23.1 *t* |
| 2 | | 29.5 *t* |
| 3 | 5.71 *dd* (12.1, 5.0) | 62.0 *d* |
| 4 | | 60.0 *s* |
| 5 | | 49.9 *s* |
| 6 | 3.87 *m* | 68.1 *d* |
| 7 | 1.95 *m* | 37.2 *t* |
| | 1.65 *m* | |
| 8 | | 36.9 *d* |
| 9 | | 42.0 *s* |
| 10 | 3.72 *dd* (13.3, 3.9) | 29.6 *d* |
| 11 | | 36.9 *t* |
| 12 | 5.25 *m* | 68.4 *d* |
| 13 | | 132.5 *s* |
| 14 | 7.7 *br s* | 109.2 *d* |
| 15 | 7.58 *t* (1.6) | 138.6 *d* |
| 16 | 6.68 *d* (1.6) | 143.3 *d* |
| 17 | 0.93 *d* (6.8) | 16.2 *q* |
| 18 | 3.27 *d* (5.1) | 49.7 *t* |
| | 3.82 *d* (5.1) | |
| 19 | 4.19 *d* (13) | 71.5 *t* |
| | 5.03 *d*(13) | |
| 20 | | 172.1 *s* |
| $\underline{C}OCH_3$ | | 169.4 *s* |
| $CO\underline{CH_3}$ | 1.89 *s* | 20.1 *q* |

larvae were placed in each Petri dish containing a leaf disc with a small piece of wet cotton to prevent desiccation. Five replicates were maintained for all treatments. The set-up was undisturbed for 24 h, after which the leaf discs were removed for analysis. The discs were placed on $\Delta T$ leaf area meter to determine leaf area consumed in treated versus control.

The antifeedant activity percentage was calculated by the following method

$$\%AF = 100 - (\text{Treated/control})^{*}100$$

Azadirachtin-A was tested at 0.5 μg/cm$^2$ concentration for both the insects.

### 3.3.1. Statistical analysis

The data were subjected to analysis of variance (ANOVA) in a completely randomized block design. The means were subjected to Duncan's multiple range test (DMRT) (Duncan, 1955) to prove their significance.

## 3.4. X-ray crystallography of teuctosin

Needle-shaped crystals of the compound were obtained from a mixture of methanol and chloroform in the ratio 1:4. The compound crystallizes in orthorhombic system with space group $P2_12_12_1$.The unit cell parameters are $a = 7.723(5)$ Å, $b = 19.062(9)$ Å, $c = 13.890(13)$ Å. The volume of the unit cell is 2045.1(3) Å$^3$. There are four molecules in the unit cell.

The crystal of dimensions $0.20 \times 0.20 \times 0.25$ mm was selected for data collection. The data were collected on a charge couple device (CCD), using graphite monochromatized Mo/$K_\alpha$ radiation. A total of 4749 reflections were measured, of which 4302 were unique reflections. The structure was solved using SHELXS-97 (Sheldrix, 1997) with direct methods and was refined using SHELXL-97 [9]. All the hydrogen atoms were fixed geometrically. The final $R$ index: $R = 0.048$, $(\Delta/\sigma)_{max} = 0.06$, $(\Delta\rho)_{min} = -0.21$ e/Å$^3$, $(\Delta\rho)_{max} = 0.48$ e/Å$^3$, the goodness of fit (S) = 1.131. Lists of atomic coordinates, thermal parameters, structural factors, bond lengths and bond angles of **1** are deposited as a supplementary material at the Cambridge Crystallographic Data Centre (CCDC No. 205654).

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