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ISOLATION OF THE DITERPENOID TEUFLIN (6-EPITEUCVIN) FROM *TEUCRIUM VISCIDUM* VAR. *MIQUELIANUM*

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Key Word Index—*Teucrium viscidum* var. *miquelianum*; Labiatae; teuflin; 6-epiteucvin; diterpene; norclerodane; epimerization; ^{13}C NMR spectra.

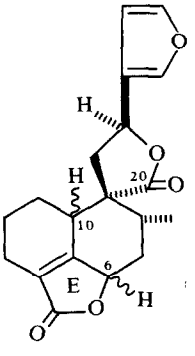
Abstract—The diterpenoid teuflin (6-epiteucvin) has been isolated from *Teucrium viscidum* var. *miquelianum*. Its base catalyzed epimerization into teucvidin was studied under mild conditions and the pathway is discussed.

INTRODUCTION

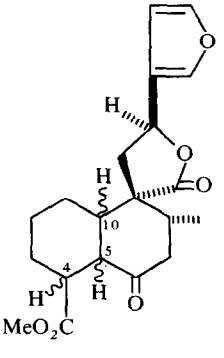
Teucvin (**1**) [1, 2] and teucvidin (**2**) [3, 4] were isolated from the aerial parts of *Teucrium viscidum* var. *miquelianum* and their structures determined. The former was also isolated from *Mallotus rependas* [5, 6] and *Teucrium cubense* [7, 8], and a potent amoebicide activity was reported [7, 8]. The latter was also isolated from *Croton caudatus* [9]. Our further investigation on the minor diterpenoids of *T. viscidum* var. *miquelianum* resulted in the isolation of a crystalline component, which was shown to be the C-6 epimer (**3**) of teucvin by its spectral properties, its chemical conversion into teucvidin (**2**), and an X-ray crystallographic analysis (M. Node, M. Sai, E. Fujita and A. T. McPhail, unpublished results). In the meantime, a paper which reported the isolation of this compound from *T. flavum* and its structure determination by X-ray analysis was published, and it was named teuflin [10].

RESULTS AND DISCUSSION

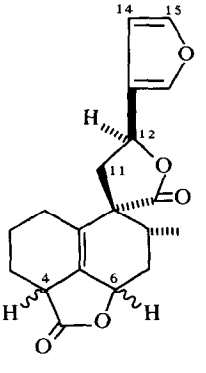
A minor component, $C_{19}H_{20}O_5$, mp 190–192°, isolated from *T. viscidum* var. *miquelianum* was shown to have rings of a β-substituted furan, a γ-lactone, and an αβ-unsaturated γ-lactone, and a secondary methyl group in the molecule (see Experimental). This compound had the same composition and functional groups as those of teucvin (**1**) and teucvidin (**2**) which suggested that it may be their stereoisomer and most probably the C-6 or C-10 epimer of teucvin. The ^1H NMR and ^{13}C NMR spectral data of this new compound, teucvin (**1**), and teucvidin (**2**) were checked in detail. The proton (δ 2.68, *m*) and ^{13}C chemical shifts (δ 43.0) [11] at C-10 were shown to be the same as those of teucvin, which supported the same configuration at C-10 in both compounds. On the other hand, the hydrogen at C-6 (δ 5.72, *m*) showed an unusual paramagnetic shift compared to the C-6 hydrogens of teucvin and teucvidin, which can be attributed to the



1 6β – H, 10β – H
2 6α – H, 10α – H
3 6α – H, 10β – H
8 6β – H, 10α – H



4 4β – H, 5α – H, 10β – H
5 4α – H, 5β – H, 10α – H



6 4α – H, 6α – H
7 4β – H, 6β – H

anisotropic effect due to the lactone carbonyl group at C-20 on a fixed boat-like conformation of the ring B. Thus the C-6 hydrogen must have the configuration opposite to that in teucvin, which is supported by the similarity of the ^{13}C-chemical shifts of C-6 in this compound and teucvidin (**2**).

The chemical evidence for the assignment of the structure 6-epiteucvin to this minor diterpenoid was provided by its chemical conversion into teucvidin (**2**). First of all, methanolysis (Na_2CO_3, MeOH, reflux) was tested on this compound under the same conditions as in the methanolysis of teucvin and teucvidin, but it resulted in the rapid formation of many products, contrary to the production of the methyl esters, **4** and **5**, in the cases of teucvin and teucvidin, respectively [2]. Under the milder conditions (Na_2CO_3, THF, H_2O, room temperature), two isomeric products A and B were obtained. The product A was proved to be identical with the known teucvidin (**2**) by mmp determination and complete identity between their NMR spectra.

Treatment of 6-epiteucvin under more mild conditions selectively afforded isomer B accompanied by starting material. The isomer B showed a strong carbonyl absorption at 1770 cm^{-1} in its IR spectrum; the absorptions at 1750 and 1705 cm^{-1} in 6-epiteucvin (**3**) disappeared. The UV absorption showed a hypsochromic shift of the original absorption at 217 nm of **3**; it supported disappearance of the conjugated double bond in **3**. The deuterium exchange experiment [2] has shown incorporations at C-6 and C-10. Accordingly a 5(10)-ene structure (**6**) is easily assumed for B, and this assumption was well supported by the NMR data and the decoupling experiment between 14-H and 15-H, 11-$H_{A,B}$ and 12-H, and 11-H_A and 11-H_B.† Furthermore teucvin (**1**) has been partially converted into the 5(10)-ene derivative C [2], whose spectral data were very similar with those of the isomer B. Consideration of these facts led to the reasonable assignment of the structures **6** and **7** to the compounds B and C, respectively.

The compound **6** is thus an intermediate in the base-catalyzed isomerization of 6-epiteucvin (**3**) to teucvidin (**2**), formed by the migration of the C-10-H. The $\beta\sigma$-bond between C-10 and hydrogen is more labile than the $\alpha\sigma$-bond between C-6 and hydrogen in **3**, because of the maximum overlapping of the former with the C-4, C-5 π-bond.

Thus the base catalyzed isomerizations of 6-epiteucvin (**3**) and teucvin (**1**) are represented as shown in Figs. 1 and 2.

The stereomodel of compound **3** suggests it to be unstable, because of the big strain on ring E. In fact, deprotonation of the 10-H occurs easily under mild basic conditions to give an enolate anion, whose protonation under kinetic controlled conditions occurs preferentially at C-4 to yield compound **6**. It is transformed into more stable teucvidin (**2**) under thermodynamic control (see Fig. 1).

On the other hand, teucvin (**1**) is known to be most stable‡ among all the isomers; it forms an enolate anion under strong basic conditions. Protonation at C-4 occurs only partially; almost all of the enolate anion protonates at C-10 to recover the stable teucvin (**1**). In this case, the formation of compound **8** cannot be observed, probably because of its unstable nature (see Fig. 2).

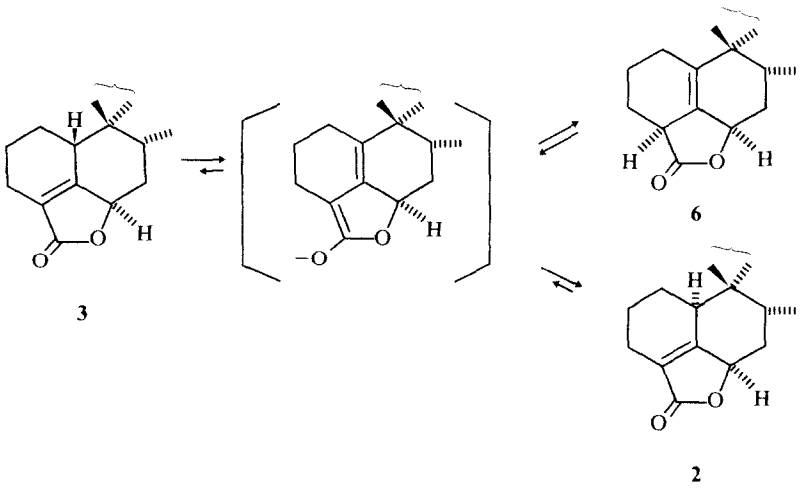


Fig. 1. Base catalyzed isomerization of 6-epiteucvin (**3**) under mild conditions.

The enol lactone structure (having a double bond between (C-5 and C-6) is excluded on the basis of the IR data and the presence of proton signal at δ 5.00 assignable to 6-H in the ^1H NMR spectrum. The *trans*-isomer between 4-H and 6-H is also excluded based on the unfavorable non-bonded interaction because of the boat conformation of the rings A and B in the molecule.

‡ The base catalyzed epimerization [9] of teucvidine (**2**) into teucvin (**1**) supports this.

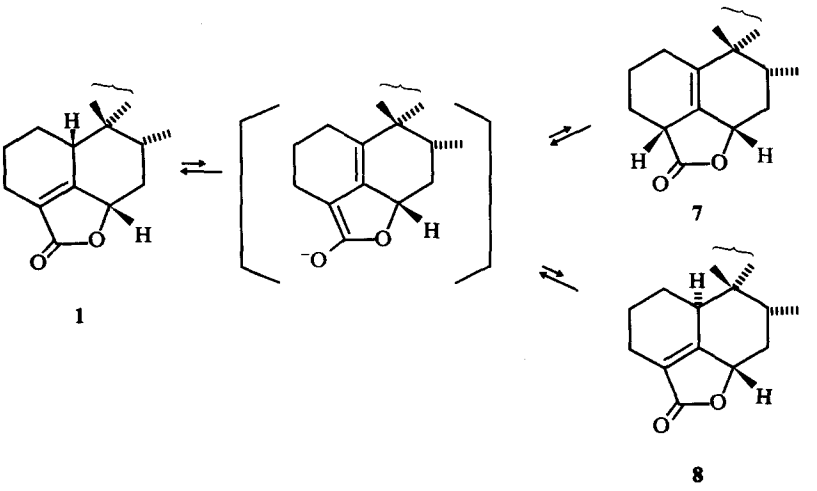


Fig. 2. Base catalyzed isomerization of teucvin (**1**) under strong conditions.

As described above, 6-epiteucvin (**3**) was shown to be abnormally unstable to the base and to be easily convertible to teucvidin (**2**). Thus, it is possible that teucvidin may be an artifact produced from the original 6-epiteucvin (**3**) because sodium carbonate was used in the acid-removing step in the previous work [2]. A direct ethereal extract (obtained without treatment with alkali or acid) from a new plant was then checked by HPLC and the extract was proved to contain teucvidin in addition to 6-epiteucvin and teucvin. Hence it seems unlikely that teucvidin is an artifact produced in the extraction procedure.

EXPERIMENTAL

Mps were taken on a micro hot-stage apparatus and are uncorr. ^{1}H NMR spectra were recorded in $CDCl_3$ with TMS as int. standard.

Isolation of teuflin (*6-epiteucvin*). Dried aerial part (0.75 kg) of *T. viscidum* var. *miquelianum* was extracted with Et_2O at room temp. and the solvent was evapd to leave a residue (20 g) which was dissolved in MeOH and treated with charcoal (20 g) at 70° for 30 min. The residue obtained after filtration and evaporation was chromatographed on a Si gel column to give teuflin (6-epiteucvin) (68 mg) by elution with CH_2Cl_2–*n*-hexane (7:3). It was crystallized as prisms (26 mg) from MeOH, mp 190–192°. $[\alpha]_D^{25}$ + 18.5° (*c* = 0.1, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 218 (3.92). IR ν_{max}^{KBr} cm^{-1}: 1750, 1740 (sh), 1700, 1600, 1505, and 880. ^{1}H NMR δ: 1.22 (3H, *d*, *J* = 7.1 Hz, 8-Me), 2.67 (1H, *dd*, *J* = 13.7 and 7.3 Hz, 11-H_A), 2.39 (1H, *dd*, *J* = 13.7 and 9.9 Hz, 11-H_B), 2.68 (1H, *m*, 10-H), 5.72 (1H, *m*, 6-H), 5.38 (1H, *dd*, *J* = 9.9 and 7.3 Hz, 12-H), 6.41 (1H, *m*, 14-H), and 7.46 (2H, *m*, 15-H, 16-H). ^{13}C NMR δ: 17.6, 18.7, 23.3, 23.7, 31.8, 35.8, 42.8, 43.0, 51.0, 71.6, 76.6, 107.8, 123.7, 124.3, 139.8, 144.2, 166.2, 173.5, and 175.9. MS *m/e* 328 (M^+) (Calc. for $C_{19}H_{20}O_5$: M^+ 328.131). (Found: C, 65.45; H, 6.65. $C_{19}H_{20}O_5 \cdot 1\frac{1}{2}$MeOH requires: C, 65.41; H, 6.96%).

Epimerization of teuflin (**3**) *into teucvidin* (**2**) *and the* 5(10)*-ene derivative* (**6**). Teuflin (2.5 mg) was treated in THF (2 ml) with a soln of Na_2CO_3 (3.0 mg) in 11 drops H_2O at 24° for 5 days. The mixture was poured onto the brine and extracted with CH_2Cl_2. The organic layer, after drying over Na_2SO_4, was evaporated under the red. pres. at 18° to leave a residue, which was subjected to prep. TLC to give teucvidin (**2**) (1 mg) and the 5(10)-ene derivative (**6**) (1.5 mg). Pure teucvidin (**2**) was obtained by chromatography on a Si gel column followed by recrystallization: mp 214° (from MeOH). The mmp with an authentic sample (mp 214.5–216°) showed 214.5–216°. Its ^{1}H NMR spectrum was identical with that of the authentic sample. Compound **6** was also purified by chromatography on a Si gel column to give a solid.

Epimerization of teufin (**3**) *into teucvidin* (**2**). Teuflin (4.5 mg) was treated with Na_2CO_3 (4.5 mg) in abs. MeOH (2 ml) at room temp. for 37 min. The mixture was poured onto brine and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and the solvent was evaporated under red. pres. at room temp. The same procedure was repeated 3× to give total 4.7 mg of teucvidin, which was purified by Si gel CC mp and mmp with authentic sample 214.5–216° (from MeOH), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1690, 1600, 1505, and 875, ^{1}H NMR δ: 7.44 (2H, *m*, 15-H), 6.36 (1 H, *m*, 14-H), 5.35 (1 H, *t*, *J* = 8 Hz, 12-H), 4.99 (1 H, *dd*, *J* = 10 and 7 Hz, 6-H), 3.26 (1H, *m*, 10-H), 2.58 (1H, *dd*, *J* = 14 and 8 Hz, 11-H), and 1.36 (3H, *d*, *J* = 7 Hz, 8-Me).

Epimerization of teuflin (**3**) *into the* 5(10)*-ene derivative* (**6**) (*isomer B*). Teuflin (6.7 mg) was treated in THF (2.5 ml) with a soln of Na_2CO_3 (5.5 mg) in H_2O (10 drops) for 6.3 days. The mixture was poured onto the brine and extracted with CH_2Cl_2. After drying over Na_2SO_4, the solvent was evaporated under red. pres. at 18° to leave a residue, which was subjected to preparative TLC to separate a product (2.7 mg: 40.3%) from the starting material (40.3% recovered). The product was purified by chromatography on a Si gel column to give the 5(10)-ene derivative (**6**) as a solid. UV λ_{max}^{MeOH} nm (log ε): 210 (3.96). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1503, 1030, and 875. ^{1}H NMR δ: 7.45 (2H, *m*, 15-H, 16-H), 6.36 (1H, *m*, 14-H), 5.59 (1H, *dd*, *J* = 9 and 4.2 Hz, 12-H), 5.00 (1 H, *br*, *t*, 6-H), 3.16 (1 H, *br*, *s*, 4-H), 2.86 (1 H, *dd*, *J* = 13.5 and 9.3 Hz, 11-H), 2.22 (1H, *dd*, *J* = 13.5 and 3.4 Hz, 11-H), and 1.09 (3H, *d*, *J* = 7.1 Hz, 8-Me). MS *m/e* 328 (M^+) (Calc. for $C_{19}H_{20}O_5$, 328).

HPLC of the ether extract of Teucrium viscidum *var.* miquelianum. The aerial part of *Teucrium viscidum* var. *miquelianum* was extracted with Et_2O at room temp. and treated with active charcoal. The residue (2 mg) was dissolved in *i*-PrOH (0.2 ml) and checked with HPLC. Standard solns of teucvin,

teucvidin, and teuflin in *i*-PrOH (1 μg/μl) were prepared. A JASCOSIL SS-05 4.5 mm ϕ × 250 mm column was used. The column was eluted with *i*-PrOH–*n*-hexane (2:1) at the rate of 2 ml/min under the pressure 60 kg/cm^2. The sample soln (1 μl) was injected and detected at 217 nm. The R_ts were 3.3, 7.6, and 12.2 min for teucvidin, teuflin, and teucvin, respectively. The sample showed three peaks at the same retention times as those of the standard samples.

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