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2β-HYDROXYTEUCVIDIN FROM *TEUCRIUM WEBBIANUM*

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Key Word Index—*Teucrium webbianum*; Labiatae; diterpenoids; neo-clerodane derivatives; teuflidin; teucrin A; new nor-diterpenoid; 2β-hydroxyteucvidin.

Abstract—A new 19-nor-neo-clerodane diterpenoid, 2β-hydroxyteucvidin, has been isolated from the aerial parts of *Teucrium webbianum*, besides the previously known diterpenoids teuflidin and teucrin A. The structure of 2β-hydroxyteucvidin, (12*S*)-15,16-epoxy-2β-hydroxy-19-nor-10α-neo-clerodane-4,13(16),14-triene-18,6β;20,12-diolide, was established mainly by spectroscopic means.

INTRODUCTION

In continuation of our studies of the diterpenoid compounds from *Teucrium* spp. [1–4], we have now investigated *T. webbianum* Boiss. From the aerial parts of this plant we have isolated three neo-clerodane diterpenoids, two of which are the already known teuflidin (**1**) [5] (also named teucrin H1 [6]) and teucrin A (**2**) [7–10], and the third is a new substance, 2β-hydroxyteucvidin (**3**), whose structure and absolute configuration have now been established.

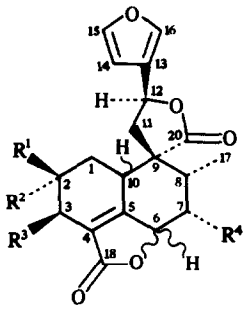
RESULTS AND DISCUSSION

2β-Hydroxyteucvidin (**3**) was purified by transformation into its acetyl derivative **4**. Elemental analysis and mass spectrometry gave the molecular formula of compound **4** as $C_{21}H_{22}O_7$. Its IR spectrum was consistent with the presence of a furan ring (3150, 3120, 1505, 880 cm^{-1}), a γ-lactone group (1760 cm^{-1}), an α,β-unsaturated-γ-lactone group (1750 cm^{-1}) and an acetate group (1735, 1245 cm^{-1}). The presence in **4** of an α,β-unsaturated-γ-lactone moiety was also confirmed by its UV absorption at λ_{max} 224.5 nm (log ε 4.01).

However, it was the ^{1}H NMR spectrum of 2β-hydroxyteucvidin acetate (Table 1) that provided the most information and established for this compound the structure and relative configuration depicted in **4**. This ^{1}H NMR spectrum was almost identical with that reported for teucvidin (**5**, see Table 1), a 19-nor-neo-clerodane diterpenoid previously isolated from *T. viscidum* var. *Miquelianum* and whose structure is well known from an X-ray diffraction analysis [11, 12]. In fact, the ^{1}H NMR spectra of compound **4** and teucvidin (**5**) were almost identical in the chemical shifts and coupling values corresponding to the H-6α, H-7α, H-7β, H-10α, H_A-11, H_B-11, H-12, H-14, H-15, H-16 and Me-17 protons (see Table 1). The only difference was the presence in the former of an acetoxyl group (δ2.07, 3H, *s*) whose geminal proton appeared as a multiplet ($W_{1/2}$ = 10 Hz) at δ5.32.

Moreover, a comparison of the ^{13}C NMR spectra of compounds **4** and **5** [5] (Table 2) showed that the C-5–C-9, C-11–C-18 and C-20 carbon atom chemical shifts and SFORD multiplicities were identical in both compounds and very different from those reported for other 19-nor-neo-clerodane-18,6;20,12-diolides, such as teucvin (**6**) [5, 13, 14], teuflin (**7**) [15], teucrin H4 (**8**) [6] and isoteuflidin (**9**) [16], which possess an H-10β and an H-6α (**7** and **8**) or H-6β (**6** and **9**) configuration. Furthermore, the ^{13}C NMR spectrum of teuflidin (**1**) [5, 6], which has an H-10α and H-6α arrangement, also showed identical chemical shifts for its C-6–C-9, C-11–C-18 and C-20 carbon atoms to those for compound **4** and teucvidin (**5**, Table 2).

From all the above data, it is clear that the new diterpenoid has a structure of 15,16-epoxy-19-nor-10α-



		R¹	R²	R³	R⁴
1	H-6α, H-10α	H	H	OH	H
2	H-6β, H-10β	H	H	H	OH
3	H-6α, H-10α	OH	H	H	H
4	H-6α, H-10α	OAc	H	H	H
5	H-6α, H-10α	H	H	H	H
6	H-6β, H-10β	H	H	H	H
7	H-6α, H-10β	H	H	H	H
8	H-6α, H-10β	H	OH	H	H
9	H-6β, H-10β	H	H	OH	H

Table 1. ^{1}H NMR data of compounds **4** and **5** ($CDCl_3$, TMS as internal standard)

	4*	5†
H-1α	2.15 *dt*	§
H-1β	1.69 *ddd*	§
H-2α	5.32 *m*	§
H-3α‡	2.46 *dddd*	§
H-3β‡	2.57 *dddd*	§
H-6α	5.06 *br dd*	5.00 *br dd*
H-7α	2.37 *ddd*	2.33 *ddd*
H-7β	1.49 *ddd*	1.49 *ddd*
H-8β	2.21 *ddq*	§
H-10α	3.43 *br t*	3.24 *m*
H_A-11	1.97 *dd*	1.91 *dd*
H_B-11	2.60 *dd*	2.60 *dd*
H-12	5.49 *t*	5.35 *t*
H-14	6.35 *dd*	6.36 *m*
H-15	7.44 *t*	7.34 *m*
H-16	7.45 *m*	7.34 *m*
Me-17	1.36 *d*	1.35 *d*
OAc	2.07 *s*	—
J (Hz)		
1α,1β	14.1	§
1α,10α	7.0	§
1β,10α	6.6	§
1α,2α	7.0	§
1β,2α	3.1	§
2α,3α	4.8	§
2α,3β	4.5	§
3α,3β	17.4	§
3α,6α	2.7	§
3β,6α	2.2	§
3α,10α	2.7	§
3β,10α	2.2	§
6α,7α	6.9	7
6α,7β	12.1	10.7
6α,10α	≃ 0.4	§
7α,7β	12.4	12.7
7α,8β	3.1	3
7β,8β	4.0	4
8β,17	7.4	7
11A,11B	14.1	14
11A,12	7.3	8
11B,12	8.4	8
14,15	1.7	§
14,16	1.1	§
15,16	1.7	§

*Spectral parameters were obtained by first order approximation. All these assignments have been confirmed by double resonance experiments.

†Taken from ref. [11].

‡These assignments may be interchanged.

§Values not given in ref. [11].

Table 2. ^{13}C NMR chemical shifts of compounds **4** and **5** ($CDCl_3$ solution, δ values from TMS)

C	4	5*
1	27.8 *t*†	21.4 *t*‡
2	66.8 *d*	23.3 *t*‡
3	25.5 *t*	20.1 *t*‡
4	123.8 *s*	127.7 *s*
5	161.8 *s*	162.2 *s*
6	76.0 *d*	76.1 *d*
7	35.9 *t*	35.8 *t*
8	38.5 *d*	38.8 *d*
9	52.2 *s*	52.1 *s*
10	33.7 *d*	35.8 *d*
11	38.8 *t*	39.0 *t*
12	71.8 *d*	71.8 *d*
13	125.1 *s*	125.2 *s*
14	107.8 *d*	108.0 *d*
15	144.4 *d*	144.2 *d*
16	139.6 *d*	139.5 *d*
17	14.4 *q*	14.3 *q*
18	171.9 *s*	172.5 *s*
20	177.2 *s*	177.6 *s*
OAc	170.4 *s*	—
	21.2 *q*	—

*Taken from ref. [5].

†SFORD multiplicity.

‡These assignments may be interchanged.

neo-clerodane-4,13(16),14-triene-18,6β;20,12-diolide with an acetylated secondary hydroxyl group that must be placed at the C-2 position. This conclusion, together with the 2β-axial configuration of the acetoxyl group, the 12*S* stereochemistry and the neo-clerodane absolute configuration of compound **4**, was firmly supported by the following facts.

Double resonance experiments showed that on irradiation of the multiplet at δ5.32 the one-proton signals at δ1.69, 2.15, 2.46 and 2.57 were affected, the coupling constants of 3.1, 7.0, 4.8 and 4.5 Hz, respectively, disappearing. This established that the secondary acetoxyl group was placed between two methylene groups and had an axial configuration. For a C-2 axial acetoxyl group two possibilities can be considered: a C-2α configuration with ring A in a conformation in which H-1β and H-3β are both axially oriented, or a C-2β configuration in which H-1β and H-3β are pseudoequatorial and ring A possesses a $B_{3,10}$ conformation. Since the coupling values between the H-10α and the C-1 methylene protons were 7.0 and 6.6 Hz (Table 1), it is evident that the second possibility is the relevant one, because otherwise the *J* value for the coupling between the *trans*-diaxial H-1β and H-10α protons would be larger than 12 Hz [3]. The Dreiding molecular model reveals the differences in the coupling constants for the two ring A conformations that confirm the above conclusion.

The 12*S*-configuration of compound **4** was in agreement with the chemical shift of its C-8 carbon atom (δ38.5, Table 2), as in the case of teucvidin (**5**, δ38.8) [5] and teuflidin (**1**, δ38.6) [5, 6]. These almost identical values clearly indicate [17] that compounds **1**, **4** and **5** all possess the same 12*S*-configuration, which has been firmly established for teucvidin (**5**) [11, 12] and teuflidin (**1**) [5] by X-ray diffraction analyses. Moreover, NOE experiments showed that, when the Me-17 protons of compound **4**

(δ1.36) were irradiated, NOE enhancements were observed in the signals of the H-6α (δ5.06, 12%), H-7α (δ2.37, 7%), H-8β (δ2.21, 8%), H-10α (δ3.43, 7%) and H-16 (δ7.45, 1%) protons, thus confirming a 1,3-diaxial relationship between the Me-17 group and the H-6α and H-10α protons [11], and that the furan ring moiety and the Me-17 group are on the same side of the plane defined by the C-20,C-12 γ-lactone ring [18].

Finally, the neo-clerodane [19] absolute configuration of 2β-hydroxyteucvidin (**3**) was established by the CD curve of its derivative **4**, which showed a Cotton effect of $\Delta\varepsilon_{230} - 14.0$, identical with that found in teucvidin (**5**) [11] and teuflidin (**1**) [5, 6], whose neo-clerodane absolute configuration is well known.

EXPERIMENTAL

Mp is uncorr. For general details on methods, see refs [1–4, 10, 16, 17]. Plant materials were collected in July 1985 at Sierra de Segorbe (Castellón, Spain), and voucher specimens were deposited in the Herbarium of the 'Dipartimento di Biologia' of the University of Milan, Italy.

Extraction and isolation of the diterpenoids. Dried and finely powdered *T. webbianum* Boiss. aerial parts (130 g) were extracted with Me_2CO (2 l.) at room temp. for a week. After filtration, the extract (9 g) was chromatographed on a silica gel (Merck No. 7734, deactivated with 10% H_2O, 300 g) column eluted with *n*-hexane, *n*-hexane–EtOAc mixtures and pure EtOAc, yielding the following compounds in order of elution: teuflidin (**1**, 100 mg) [5, 6], a complex mixture of several diterpenoids (130 mg), in which 2β-hydroxyteucvidin (**3**) was the main constituent, and teucrin A (**2**, 70 mg) [7–10].

The previously known diterpenoids, teuflidin (**1**) [5, 6] and teucrin A (**2**) [7–10] were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, UV, ^{1}H NMR, MS) data and by comparison with authentic samples.

Preparation and purification of 2β-hydroxyteucvidin acetate (**4**). The mixture of diterpenoids (130 mg, see above) was treated with Ac_2O–pyridine (3 ml, 1:1) for 48 hr at room temp. Work-up in the usual manner yielded a residue (140 mg) which was subjected to dry-CC over silica gel. Elution with *n*-hexane–EtOAc (2:1) gave pure **4** (90 mg, after crystallization from EtOAc–*n*-hexane), mp 201–203°; $[\alpha]_D^{20}$ − 10.3° ($CHCl_3$; *c* 0.136); CD nm (Δε): 210 (0), 230 (− 14.0), 260 (0) (MeOH; *c* 0.008); UV λ_{max}^{EtOH} nm (log ε): 224.5 (4.01); IR ν_{max}^{KBr} cm^{-1}: 3150, 3120, 3005, 2950, 2910, 1760, 1750, 1735, 1700, 1505, 1425, 1375, 1315, 1245, 1220, 1180, 1085, 1030, 1003, 965, 930, 880, 800, 750; ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (75.4 MHz, $CDCl_3$): see Table 2; EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 386 $[M]^+$ (92), 326 (10), 232 (13), 189 (24), 176 (94), 133 (19), 105 (30), 95 (57), 94 (100), 91 (21), 81 (19), 77 (18), 43 (66). (Found: C, 65.19; H, 5.68. $C_{21}H_{22}O_7$ requires: C, 65.27; H, 5.74%.)

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