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TEUGIN, A *NEO*-CLERODANE DITERPENOID FROM *TEUCRIUM FRAGILE*

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Key Word Index—*Teucrium fragile*; Labiatae; diterpenoid; new *neo*-clerodane derivative; teugin; 15,16-epoxy-2β,6β-dihydroxy-*neo*-cleroda-3,13(16),14-triene-18,19:20,12*S*-diolide.

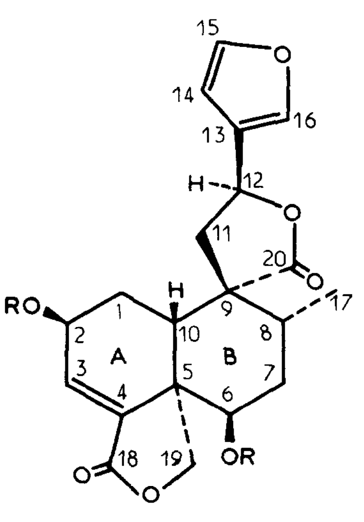
Abstract—From the aerial part of *Teucrium fragile* a new *neo*-clerodane diterpenoid, teugin, has been isolated. Its structure, 15,16-epoxy-2β,6β-dihydroxy-*neo*-cleroda-3,13(16),14-triene-18,19:20,12*S*-diolide, was established mainly by spectroscopic means.

INTRODUCTION

Continuing our studies on diterpenic compounds from *Teucrium* species [1–4], we have now investigated *T. fragile*, a species which grows only in the high mountains of southern Spain. From the aerial part of this plant a new diterpenoid (**1**) has been isolated, for which we propose the name teugin. Its structure and absolute configuration were established on the basis of spectroscopic evidence and by comparison with closely related compounds.

RESULTS AND DISCUSSION

Combustion analysis and mass spectrometry indicated the molecular formula $C_{20}H_{22}O_7$ for teugin (**1**). Its IR spectrum was consistent with the presence of a furan ring, an olefinic double bond, two lactone groups (one of them α,β-unsaturated) and OH groups (see Experimental). The presence of two hydroxyl groups was confirmed by the formation, on treatment with Ac_2O-pyridine, of a diacetate (**2**), the IR spectrum of which showed no OH absorption. The presence of an α,β-unsaturated lactone group was also revealed by the UV spectrum (λ_{max}^{EtOH} 240 nm, $\varepsilon = 2000$).



1 R = H
2 R = Ac

However, it was the ^1H NMR spectrum of teugin (**1**) that provided the most information. It showed signals of a secondary methyl group at δ 1.05 (*d*, $J = 6$ Hz), of a β-substituted furan ring (ABX system, two α-furan protons having their resonance at 7.82 and 7.70, and one β-furan proton at 6.59), and of an olefinic proton at 7.11 (*d*, $J = 6.5$ Hz), which can be assigned to an olefinic β-proton of an α,β-unsaturated lactone group. The following signals due to five protons on carbon atoms bearing oxygen atoms could also be seen: 5.63 (1 H, *t*, $J = 7.5$ Hz), 4.84 and 4.23 (AB system, $J = 10$ Hz), 4.73 (1 H, *dd*, $J_1 = 6.5$ Hz, $J_2 = 3.5$ Hz) and 4.46 (1 H, *t*, $J = 3$ Hz). The signals at 4.73 and 4.46 were assigned to the two protons at the carbon atoms bearing the OH groups, as these signals were downfield shifted in the ^1H NMR spectrum of the diacetyl derivative **2**, both appearing at about 5.40. In addition, the ^1H NMR spectrum of teugin (**1**) showed two one-proton double doublets, one at 3.93 ($J_1 = 3$ Hz, $J_2 = 13.5$ Hz) and the other at 2.79 ($J_1 = 7.5$ Hz, $J_2 = 13.5$ Hz), as well as a doublet of triplets at 1.67 (1 H, $J_1 = 3.5$ Hz, $J_2 = 13.5$ Hz).

In order to establish the relative arrangement of the protons, a series of proton-decoupling experiments was carried out. As a result of irradiation at δ 4.73, the olefinic proton doublet at 7.11 collapsed into a singlet and at the same time the doublet of triplets at 1.67 was converted into a triplet ($J = 13.5$ Hz). Irradiation at 1.67 caused modifications of the signals at 3.93 (now appearing as a doublet, $J = 3$ Hz) and at 4.73 (now also a doublet, $J = 6.5$ Hz). Furthermore, by irradiating at 5.63, the double doublet at 2.79 was converted into a doublet ($J = 13.5$ Hz).

On the basis of these results, the following assignment for the ring A protons could be made, which are in agreement with structure **1** for teugin. The signal at δ 4.73 was assigned to the 2α-proton ($J_{2\alpha,3} = 6.5$ Hz, $J_{1\alpha,2\alpha} = 3.5$ Hz, $J_{1\beta,2\alpha} \simeq 0$ Hz), the signal at 1.67 to the 1α-proton ($J_{1\alpha,1\beta} = J_{1\alpha,10\beta} = 13.5$ Hz, $J_{1\alpha,2\alpha} = 3.5$ Hz) and the double doublet at 3.93 to the 1β-proton ($J_{1\beta,1\alpha} = 13.5$ Hz, $J_{1\beta,10\beta} = 3$ Hz, $J_{1\beta,2\alpha} \simeq 0$ Hz). A value close to zero for $J_{1\beta,2\alpha}$ is consistent with a H-1β–C–C–H-2α dihedral angle of *ca* 90°, as can be seen in the Dreiding model of teugin (**1**). On the other hand, the ^1H NMR spectrum of compound **2** showed the 1β-proton signal at 3.05 (*dd*, $J_{1\beta,1\alpha} = 14.1$ Hz, $J_{1\beta,10\beta} = 2.1$ Hz, $J_{1\beta,2\alpha} \simeq 0$ Hz) and on irradiation at 2.05 (10β-proton) it was transformed into a doublet ($J = 14.1$ Hz). These assignments are supported by the ^{1}H NMR data of the diterpenoid articulin [5], which has an identical ring A to that of teugin (**1**).

The signals at δ 5.63 and 2.79 were assigned to the proton at C-12 and one of the protons at C-11, respectively, by analogy with the results obtained from several diterpenoids previously isolated from *Teucrium* species [1, 3], the structures of which have been firmly established by X-ray analysis [2, 4].

Owing to the low solubility of teugin, it was more convenient to run the ^{13}C NMR spectrum of its diacetyl derivative (**2**). This spectrum confirmed all the above results and provided information on the position of the hydroxyl group in ring B of teugin (**1**). The ^{1}H NMR spectrum of teugin had already indicated that this hydroxyl group had an axial configuration, since the signal of its geminal proton, at δ 4.46, appeared as a triplet ($J_{ee'} = J_{ea'} = 3$ Hz). The only positions available for this OH group were C-6 and, less probably, C-7. The chemical shift value of the C-17 carbon atom in the diacetyl derivative **2** (16.1 ppm) excluded the axial position at C-7 for the OAc group, since, in this case, a chemical shift value between 10.6 and 12.0 ppm would be expected for the C-17 carbon atom [2, 6]. Therefore, only the axial 6β-position can be occupied by this OH group in teugin (**1**). This was further confirmed by the fact that the chemical shift values of the C-8, C-9 and C-17 carbon atoms in compound **2** (34.4, 49.8 and 16.1 ppm, respectively) were practically identical to those of the corresponding carbon atoms in a clerodane-like diterpenoid possessing a 6β-oxygen function (34.0, 49.9 and 16.2 ppm, respectively) [3] as opposed to those observed in a 6α-substituted compound (38.1, 50.8 and 16.4 ppm, respectively) [4]. This conclusion was also supported by the fact that neither of the two protons at C-19 in **1** and **2** showed any long-range coupling in their ^{1}H NMR spectra. The requirement for the existence of such a long-range coupling [7, 8], which has been observed ($J = 2.5$ Hz) in some clerodan-18,19-olides lacking a substituent at C-6 [5, 6, 9], is the existence of an axial proton at C-6.

The equatorial 8α-configuration of the C-17 secondary methyl group and the *trans*-junction of rings A and B were also established by comparing the ^{13}C chemical shift data of **2** (see the Experimental) with those reported for related compounds [2–4, 9–12].

Finally, the negative Cotton effect ($\Delta\varepsilon_{248} = -8.73$) shown by the α,β-unsaturated γ-lactone group indicated [5, 6, 9] that teugin (**1**) had the same absolute configuration as *neo*-clerodane [13].

As a result of all the above data and in accordance with the terminology recently suggested by Rogers *et al.* [13], teugin can be assigned as 15,16-epoxy-2β,6β,-dihydroxy-*neo*-cleroda-3,13(16),14-triene-18,19:20,12*S*-diolide (**1**). However, taking into account that *neo*-cleordanes are biogenetically related to *ent*-labdanes, in which C-20 is an α-substituent, while *ent*-*neo*-clerodanes are biogenetically related to normal labdanes, in which C-20 is a β-substituent, there might be some risk of confusion by using this new nomenclature. We would therefore prefer to consider teugin (**1**) as *ent*-15,16-epoxy-2α,6α-dihydroxy-cleroda-3,13(16),14-triene-18,19:20,12*S*-diolide.

EXPERIMENTAL

Mps were determined on a Kofler apparatus and are uncorr. Elemental analyses were carried out in Madrid with the help of an automatic analyser. ^{1}H NMR and ^{13}C NMR spectra were measured at 90 and 25.2 MHz, respectively, in pyridine-d_5 or $CDCl_3$ soln with TMS as internal standard. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Plant materials were collected in June 1979, in Sierra de Cázulas (Granada, Spain), and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy (Madrid 'Complutense' University).

Isolation of teugin (**1**). Dried and finely powdered *T. fragile* Boiss aerial parts (200 g) were extracted with Me_2CO (2 l.) at room temp. for 1 week. After filtration the solvent was evapd yielding a gum (6 g) which was subjected to dry-column chromatography over Si gel (200 g, Merck No. 7734, deactivated with 15 % H_2O). Elution with EtOAc–*n*-hexane (4:1) gave teugin (**1**, 76 mg after crystn from EtOAc): mp 243–245°; $[\alpha]_D^{22}$ $-170.4°$ (*c* 0.115, pyridine); IR ν_{max}^{KBr} cm^{-1}: 3440, 3150, 3140, 3040, 2965, 2930, 2890, 1765, 1750, 1720, 1600, 1505, 1470, 1355, 1330, 1270, 1205, 1165, 1140, 1030, 990, 970, 925, 880, 860, 815, 805, 780, 710. UV λ_{max}^{EtOH} nm (ε): 215 (7000), 240 (2000). CD (EtOH, *c* 0.36): $\Delta\varepsilon_{300} = 0$; $\Delta\varepsilon_{248} = -8.73$; $\Delta\varepsilon_{230} = -3.78$.

^{1}H NMR (pyridine-d_5): see discussion of results. MS (75 eV, direct inlet) m/z (rel. int.): 374 (M^+, 6), 356 (11), 338 (10), 326 (8), 310 (7), 293 (8), 284 (17), 262 (58), 244 (36), 239 (29), 219 (65), 178 (90), 133 (40), 123 (37), 115 (34), 105 (51), 95 (100), 94 (94), 91 (90), 82 (95), 81 (95), 77 (71). (Found: C, 64.23; H, 5.89. $C_{20}H_{22}O_7$ requires: C, 64.16; H, 5.92 %.)

Teugin diacetate (**2**). Ac_2O–pyridine treatment of compound **1** (65 mg) gave the derivative **2** (60 mg, after crystallization from MeOH): mp 207–209°; $[\alpha]_D^{20}$ $-129.5°$ (*c* 0.875, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3145, 3135, 3045, 2970, 2945, 2930, 1780, 1765, 1750, 1730, 1600, 1505, 1470, 1440, 1375, 1365, 1240, 1195, 1170, 1040, 1020, 990, 930, 880, 860, 820, 780, 760, 745. UV λ_{max}^{EtOH} nm (ε): 218 (6100), 245 (1200). ^{1}H NMR ($CDCl_3$): δ 7.52 (2 H, *m*, H-15 and H-16), 6.88 (1 H, *d*, $J = 6.6$ Hz, H-3), 6.46 (1 H, *dd*, $J_{14,15} = 2$ Hz, $J_{14,16} = 0.9$ Hz, H-14), 5.40 (3 H, complex signal, H-2, H-6 and H-12), 4.66 and 3.97 (AB system, $J = 10$ Hz, 2 H-19), 3.05 (1 H, *dd*, $J_{1\beta,1\alpha} = 14.1$ Hz, $J_{1\beta,10\beta} = 2.1$ Hz, $J_{1\beta,2\alpha} \simeq 0$ Hz, H-1β), 2.13 and 2.03 (3 H each, *ss*, two –OAc), 1.65 (1 H, *ddd*, $J_{1\alpha,1\beta} = J_{1\alpha,10\beta} = 14.1$ Hz, $J_{1\alpha,2\alpha} = 3.3$ Hz, H-1α), 1.08 (3 H, *d*, $J = 6$ Hz, 3 H-17). ^{13}C NMR ($CDCl_3$) ppm: 26.6 *t* (C-1), 65.0 *d* (C-2), 130.6 *d* (C-3), 140.9 *s* (C-4), 49.7† *s* (C-5), 69.4 *d* (C-6), 31.8 *t* (C-7), 34.4 *d* (C-8), 49.8† *s* (C-9), 36.9 *d* (C-10), 41.0 *t* (C-11), 71.8 *d* (C-12), 124.3 *s* (C-13), 107.8 *d* (C-14), 144.2 *d* (C-15), 139.8 *d* (C-16), 16.1 *q* (C-17), 166.9 *s* (C-18), 70.6 *t* (C-19), 176.8 *s* (C-20); $\underline{C}H_3COO$: 20.8 *q*, 20.8 *q*; Me$\underline{C}$OO: 169.4 *s*, 169.2 *s* (signals marked † may be interchanged). MS (75 eV, direct inlet) m/z (rel. int.): 458 (M^+, 1.5), 398 (100), 356 (2), 340 (2), 338 (1), 309 (6), 219 (9), 199 (8), 178 (15), 149 (12), 135 (12), 105 (11), 95 (44), 94 (22), 91 (15), 81 (23). (Found: C, 63.81; H, 5.80. $C_{24}H_{26}O_9$ requires: C, 63.87; H, 5.72 %.)

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