Phytochemistry, Vol. 22, No. 3, pp. 723–725, 1983.
Printed in Great Britain.

0031-9422/83/030723-03$03.00/0

6-EPITEUCRIN A, A NEO-CLERODANE DITERPENOID FROM *TEUCRIUM CHAMAEDRYS*

FRANCISCO FERNÁNDEZ-GADEA, CONRAD PASCUAL, BENJAMÍN RODRÍGUEZ* and GIUSEPPE SAVONA†

Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, Madrid-6, Spain; †Istituto di Chimica Organica, Università di Palermo, Archirafi 20, 90123 Palermo, Italy

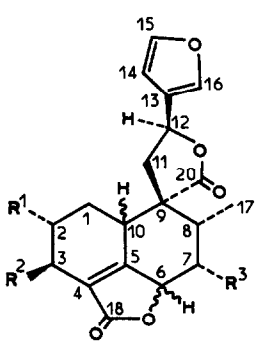
(*Received* 13 *July* 1982)

Key Word Index—*Teucrium chamaedrys*; Labiatae; diterpenoids; neo-clerodane derivatives; teuflin; teuflidin; teucrin E; teuchamaedryn B; new nor-diterpenoid; 6-epiteucrin A.

Abstract—From the aerial part of *Teucrium chamaedrys* a new 19-nor-neo-clerodane diterpenoid, 6-epiteucrin A, has been isolated, besides the previously known diterpenoids teuflin, teuflidin, teucrin E and teuchamaedryn B. The structure of 6-epiteucrin A, (12*S*)-15,16-epoxy-7α-hydroxy-19-nor-neo-cleroda-4,13(16),14-triene-18,6β: 20,12-diolide, was established by spectroscopic means and by correlation with teucrin A.

INTRODUCTION

The diterpenoids of *Teucrium chamaedrys* L. have been the subject of a number of investigations [1–8]. In continuation of our work [7, 8] on this plant we have isolated five neo-clerodane diterpenoids: teucrin E [(12*S*)-15,16-epoxy-6α-hydroxy-neo-cleroda-13(16),14-diene-18,19:20, 12-diolide] [1, 5, 6], teuchamaedryn B [(12*S*)-15,16-epoxy-6β-hydroxy-neo-cleroda-13(16),14-diene-18,19:20,12-diolide] [6] which was found to be identical with teucrin H2 isolated from *T. hyrcanicum* [6, 9], teuflin (**1**) first isolated from *T. flavum* [10] and *T. viscidum* var. *miquelianum* [11, 12], and already found in *T. chamaedrys* (teuchamaedryn A) [6], teuflidin (**2**) [13] also named teucrin H1 [9] isolated from *T. flavum* and *T. hyrcanicum*, respectively, but not previously described as a constituent of *T. chamaedrys* [1–8], and a new diterpenoid, 6-epiteucrin A (**3**), whose structure and absolute configuration have been established.



		R^1	R^2	R^3
1	6α-H, 10β-H	H	H	H
2	6α-H, 10α-H	H	OH	H
3	6α-H, 10β-H	H	H	OH
4	6α-H, 10β-H	H	H	OAc
5	6α-H, 10β-H	OH	H	H
6	6α-H, 10α-H	H	H	H
7	6β-H, 10β-H	H	H	OH
8	6β-H, 10β-H	H	H	H
9	6β-H, 10β-H	H	H	OAc

*To whom correspondence should be addressed.

RESULTS AND DISCUSSION

6-Epiteucrin A (**3**) was found as a minor component of the chromatographic fractions containing chamaedroxide [8] and was purified by transformation into its acetyl derivative, **4**. Elemental analysis and mass spectrometry gave the molecular formula of 6-epiteucrin A acetate (**4**) as $C_{21}H_{22}O_7$. Its IR spectrum was consistent with the presence of a furan ring (3140, 3110, 1503, 880 cm^{-1}), a γ-lactone group (1770 cm^{-1}), an α, β-unsaturated γ-lactone group (1760 cm^{-1}) and an acetate group (1730, 1237 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 nm (log ε, 3.85).

However, it was the ^1H NMR spectrum of 6-epiteucrin A acetate that provided the most information and established for this compound the structure and relative configuration depicted in **4**. Effectively, it showed signals for a β-substituted furan ring (ABX system, two α-furan protons at δ 7.49 and 7.47 and one β-furan proton at δ 6.41; H-16, H-15 and H-14, respectively) and for a secondary methyl group (δ 1.05, *d*, J = 7.3 Hz; 3H-17). The signal of the C-8 proton appeared at δ 2.69 as a doublet of quartets ($J_{8,17}$ = 7.3 Hz, $J_{8,7}$ = 8.7 Hz), the signal of the C-7 proton showed a double doublet at δ 4.94 ($J_{7,8}$ = 8.7 Hz, $J_{7,6}$ = 12.5 Hz), whilst the signal of the lactonic C-6 proton appeared at δ 5.85 as a doublet ($J_{6,7}$ = 12.5 Hz) with long-range coupling with the C-3 methylene and C-10 methyne protons ($J_{6,3}$ = 4 Hz, $J_{6,3'}$ = $J_{6,10}$ = 2 Hz). The low field resonance of the C-6 proton showed it was *cis* to the C-20 lactone group [9, 10, 12], and the fact that the C-10 proton of **4** appeared at δ 2.76 (*ddd*, $J_{10,1\alpha}$ = 11 Hz, $J_{10,1\beta}$ = 4 Hz, $J_{10,6}$ = 2 Hz) confirmed it was *trans* to the C-20 lactone function [9, 10,

12, 14], because in compounds with a 10α-H configuration this proton appears at δ 3.24 [9, 12–16]. In agreement with all the above assignments compound **4** possesses 6α-H and 10β-H configurations as in teuflin (**1**) [6, 10–12] and teucrin H4 (**5**) [9], but not 6α-H and 10α-H configurations as in teuflidin (**2**) [9, 13], teucvidin (**6**) [15] and crotocaudin [16], or 6β-H and 10β-H configurations as in teucrin A (**7**) [1–7], teucvin (**8**) [17, 18] and isocrotocaudin [14]. Moreover, the observed coupling values show that H-7 must be β and axial ($J_{6,7}$ = 12.5 Hz) and H-8 must be also β and axial ($J_{7,8}$ = 8.7 Hz) with ring B in a boat conformation (see the molecular model of **4**), in which the 6α-H and the C-20 lactone groups are very close. In agreement with these deductions, a clear NOE (12%) on 7β-H was observed when the 8β-proton was irradiated, and vice versa.

On the other hand, the ^{1}H NMR spectrum of compound **4** showed a singlet of the acetyl group (δ 2.15, 3H) and an ABX system corresponding to the C-11 methylene and C-12 lactonic protons (δ_A 2.72, δ_B 2.39, δ_X 5.40; J_{AB} = 13.8 Hz, J_{AX} = 7.3 Hz, J_{BX} = 10.2 Hz, H-11, H′-11 and H-12, respectively). (All the ^{1}H NMR assignments were confirmed by double resonance experiments.) The coupling values of this ABX system are identical with those found in compounds with 6α-H and 10β-H configurations, such as teuflin (**1**) [10–12] and teucrin H4 (**5**) [9], but very different from those observed in 19-nor-clerodа-4-ene-18,6-olide diterpenoids possessing 6α-H and 10α-H, or 6β-H and 10β-H configurations (J_{AB} = 13–14 Hz, J_{AX} = J_{BX} = 8–8.5 Hz) [1–7, 9, 13–18]. We suppose that in compounds **1**, **4** and **5** the 6α-H–10β-H stereochemistry causes a deformation of the C-20–C-12 lactonic ring, and since a 12*S* configuration for teuflin (**1**) has been established by X-ray diffraction analysis [10, 11], teucrin H4 (**5**) [9] and the new diterpenoid **4** also possess a 12*S* stereochemistry.

The neo-clerodane [19] absolute configuration of 6-epiteucrin A (**3**) was established by the CD curve of its derivative, **4**, which showed Cotton effects of $\Delta\varepsilon_{249}$ + 11.60 and $\Delta\varepsilon_{221}$ − 42.80, identical with those found in teucrin H4 (**5**) [9] and teuflin (**1**) [10–12]. Thus, these three diterpenoids possess a neo-clerodane absolute configuration, because it has been firmly established for teuflin (**1**) by X-ray analysis [10, 11] and by correlation with teucvidin (**6**) [12], whose absolute configuration is also known [20]. Furthermore, the neo-clerodane absolute configuration of teucrin A (**7**) is also known [4], and its CD curve showed Cotton effects of $\Delta\varepsilon_{230}$ + 31.08 and $\Delta\varepsilon_{202}$ − 8.52, in complete agreement with all the above deductions and confirming the stereochemical difference between teucrin A (**7**) and its C-6 epimer (**3**), because the enhanced intensity of the negative Cotton effect in the acetyl derivative of this last compound (**4**) must be ascribed to an increased distortion of the α, β-unsaturated γ-lactone.

A final proof that 6-epiteucrin A acetate has the structure and absolute configuration depicted in **4** was obtained by treating this compound with sodium borohydride [14, 16], which yielded a substance identical in all respects (mp, mmp, $[\alpha]_D$, IR, ^{1}H NMR and mass spectra) with 7-acetyl teucrin A (**9**) [1–4].

Finally, it is important to note that acetylation of 6-epiteucrin A (**3**) is very easy (acetic anhydride–pyridine, 5 hr at room temperature), but teucrin A (**7**) requires more vigorous conditions (acetyl chloride–*N*,*N*-dimethylaniline, 5 hr at 100°) [2]. This difference is due to the fact that **3** possesses ring B in a boat conformation, in which the C-7α alcohol function is equatorial, whereas teucrin A possesses ring B in a chair conformation, in which the C-7α hydroxyl group is axial and forms a hydrogen bond with the C-20 oxygen atom.

EXPERIMENTAL

Mps are uncorr. For general details on exptal see refs. [7, 8]. Plant materials were collected in July 1981, near Ciruelos del Pinar (Guadalajara, Spain) and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy (Madrid 'Complutense' University).

Extraction and isolation of the diterpenoids. Dried and finely powdered *T. chamaedrys* L. aerial parts (2.19 kg) were extracted with Me_2CO as previously described [7]. The chromatographic fractions (1.7 g) obtained after elution of β-sitosterol and before elution of teugin [7, 21], were repeatedly chromatographed over Si gel columns eluted with *n*-hexane–EtOAc (3:1), yielding the following compounds in order of elution: teuflin (**1**, 60 mg) [6, 10–12], teucrin E (86 mg) [1, 5, 6], teuflidin (**2**, 156 mg) [9, 13], teuchamaedryn B (102 mg) [6, 9], chamaedroxide (150 mg) [8] and a 1:1 mixture (38 mg) of this last compound and 6-epiteucrin A (**3**).

The previously known diterpenoids were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, UV, ^{1}H NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

Preparation and purification of 6-*epiteucrin A acetate* (**4**). The 1:1 mixture (37 mg) of chamaedroxide [8] and 6-epiteucrin A (**3**) was treated with Ac_2O–pyridine (3 ml, 1:1) for 5 hr at room temp. Work-up in the usual manner yielded 40 mg of a mixture of **4** and acetyl chamaedroxide [8], from which pure **4** (17 mg, less polar component) was obtained after prep. TLC on Si gel plates (Merck, No. 5554) eluted with EtOAc. Compound **4** crystallized from Me_2CO–*n*-hexane, mp 195–198°; $[\alpha]_D^{20}$ + 96.2° ($CHCl_3$; *c* 0.185); IR ν_{max}^{KBr} cm^{-1}: 3140, 3110, 1503, 880 (furan ring), 1770 (γ-lactone), 1760 (α, β-unsatd γ-lactone), 1730, 1237 (acetate), 2980, 2950, 2925, 2865, 1450, 1375, 1355, 1335, 1305, 1180, 1160, 1130, 1035, 1015, 993, 970, 815, 795, 760, 740; UV λ_{max}^{EtOH} nm (log ε): 218 (3.81) (furan ring), 224 (3.85) (α, β-unsatd-γ lactone); CD: $\Delta\varepsilon_{265}$ 0, $\Delta\varepsilon_{249}$ + 11.60, $\Delta\varepsilon_{241}$ 0, $\Delta\varepsilon_{221}$ − 42.80, $\Delta\varepsilon_{215}$ − 35.02 (MeOH; *c* 0.496); ^{1}H NMR (270 MHz, $CDCl_3$): see Results and Discussion; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): $[M]^+$ absent, 326 $[M-HOAc]^+$ (36), 311 (6), 308 (5), 232 (76), 217 (7), 201 (8), 200 (11), 187 (72), 166 (15), 160 (26), 138 (34), 107 (17), 105 (15), 95 (40), 94 (34), 91 (22), 81 (31), 79 (30), 77 (28), 69 (22), 65 (16), 53 (20), 51 (14), 43 (100). (Found: C, 65.34; H, 5.66. $C_{21}H_{22}O_7$ requires: C, 65.27; H, 5.74%.)

Preparation of teucrin A acetate (**9**) *from* 6-*epiteucrin A acetate* (**4**). Compound **4** (8 mg) was dissolved in MeOH (2 ml) and the soln was cooled in ice. Excess $NaBH_4$ was added. After 15 min the soln was acidified by dropwise addition of dilute H_2SO_4 and the reaction mixture was extracted with $CHCl_3$. Work-up in the usual manner yielded **9** as the major product (5 mg after prep. TLC and crystallization from Me_2CO–*n*-hexane): mp 185–188°; $[\alpha]_D^{29}$ + 74.2° ($CHCl_3$; *c* 0.232); IR ν_{max}^{KBr} cm^{-1}: 3155, 3130, 1505, 880, (furan ring), 1775 (γ-lactone), 1755 (α, β-unsatd γ-lactone), 1735, 1250 (acetate). ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.45 (1H, *m*, $W_{\frac{1}{2}}$ = 3 Hz, H-16), 7.40 (1H, *m*, $W_{\frac{1}{2}}$ = 3 Hz, H-15), 6.40 (1H, *m*, $W_{\frac{1}{2}}$ = 4 Hz, H-14), 5.56 (1H, *dd*, J_1 = 4.8 Hz, J_2 = 2.4 Hz, H-7), 5.43 (1H, *t*, *J* = 8.5 Hz, H-12), 4.87 (1H, *m*, $W_{\frac{1}{2}}$ = 11 Hz, H-6), 2.63 (1H, *dd*, J_1 = 13 Hz, J_2 = 8.5 Hz, H-11), 2.52 (1H, *dd*, J_1 = 13 Hz, J_2 = 8.5 Hz, H′-11), 2.03 (3H, *s*, OAc), 1.08 (3H, *d*, *J* = 7 Hz, 3H-17); EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 386 $[M]^+$ (2), 326 (30), 232 (48), 187 (52), 95 (38), 94 (30), 43 (100). (Found: C, 65.12, H, 5.68. Calc. for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74%.) Identical in all

respects with an authentic sample of acetyl teucrin A (**9**) [2, 3] obtained from teucrin A [7].

Acknowledgements—We thank Dr. J. Borja, Department of Botany, Faculty of Pharmacy, Madrid, for the botanical classification of the plant material. This work was supported in part by the National Research Council (CNR), Rome, and in part by the 'Comisión Asesora de Investigación Científica y Técnica' (grant No. 11/1981), Madrid.

REFERENCES

1. Popa, D. P. and Reinbol'd, A. M. (1972) *Khim. Prir. Soedin.* 67.
2. Popa, D. P. and Reinbol'd, A. M. (1973) *Khim. Prir. Soedin.* 31.
3. Popa, D. P., Reinbol'd, A. M. and Rezvukhin, A. I. (1973) *Khim. Prir. Soedin.* 169.
4. Popa, D. P. and Reinbol'd, A. M. (1973) *Khim. Prir. Soedin.* 321.
5. Reinbol'd, A. M. and Popa, D. P. (1974) *Khim. Prir. Soedin.* 589.
6. Papanov, G. Y. and Malakov, P. Y. (1980) *Z. Naturforsch. Teil B* **35**, 764.
7. Savona, G., García-Alvarez, M. C. and Rodríguez, B. (1982) *Phytochemistry* **21**, 721.
8. Eguren, L., Perales, A., Fayos, J., Rodríguez, B., Savona, G. and Piozzi, F. *J. Org. Chem.* **47** (in press).
9. Gács-Baitz, E., Radics, L., Oganessian, G. B. and Mnatsakanian, V. A. (1978) *Phytochemistry* **17**, 1967.
10. Savona, G., Paternostro, M., Piozzi, F., Hanson, J. R., Hitchcock, P. B. and Thomas, S. A. (1979) *J. Chem. Soc. Perkin Trans.* 1, 1915.
11. Node, M., Sai, M., Fujita, E. and McPhail, A. T. (1981) *J. Chem. Res.* 32.
12. Node, M., Sai, M. and Fujita, E. (1981) *Phytochemistry* **20**, 757.
13. Savona, G., Paternostro, M., Piozzi, F., Hanson, J. R., Hitchcock, P. B. and Thomas, S. A. (1978) *J. Chem. Soc. Perkin Trans.* 1, 1080.
14. Chatterjee, A., Banerjee, A. and Bohlmann, F. (1978) *Phytochemistry* **17**, 1777.
15. Uchida, I., Fujita, T. and Fujita, E. (1975) *Tetrahedron* **31**, 841.
16. Chatterjee, A., Banerjee, A. and Bohlmann, F. (1977) *Tetrahedron* **33**, 2407.
17. Fujita, E., Uchida, I., Fujita, T., Masaki, N. and Osaki, K. (1973) *J. Chem. Soc. Chem. Commun.* 793.
18. Fujita, E., Uchida, I. and Fujita, T. (1974) *J. Chem. Soc. Perkin Trans.* 1, 1547.
19. Rogers, D., Unal, G. G., Williams, D. J., Ley, S. V., Sim, G. A., Joshi, B. S. and Ravindranath, K. R. (1979) *J. Chem. Soc. Chem. Commun.* **97**.
20. Uchida, I., Fujita, E., Taira, Z. and Osaki, K. (1974) *Cryst. Struct. Commun.* **3**, 569.
21. Bruno, M., Savona, G., Pascual, C. and Rodríguez, B. (1981) *Phytochemistry* **20**, 2259.