Phytochemistry, Vol. 28, No. 12, pp. 3539–3541, 1989.
Printed in Great Britain.

0031 9422/89 $3.00+0.00

NEO-CLERODANE DITERPENOIDS FROM *TEUCRIUM CANADENSE*

MAURIZIO BRUNO, FRANCO PIOZZI, GIUSEPPE SAVONA, MARÍA C. DE LA TORRE* and BENJAMÍN RODRÍGUEZ*

Dipartimento di Chimica Organica, Università di Palermo, Archirafi 20, 90123 Palermo, Italy; *Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

(*Received* 3 *May* 1989)

Key Word Index—*Teucrium canadense*; Labiatae; diterpenoids; *neo*-clerodane derivatives.

Abstract—From the aerial parts of *Teucrium canadense* two new *neo*-clerodane diterpenoids, 18-acetylmontanin D and isoteuflin, have been isolated, besides the previously known diterpenes teucvidin, teuflin, teucvin, 12-epiteupolin II, teuscorodal and (12*R*)-teupolin I. The structures of 18-acetylmontanin D (18-acetoxy-4α,19;15,16-diepoxy-6β-hydroxy-*neo*-cleroda-13(16),14-dien-20,12*S*-olide) and isoteuflin (15,16-epoxy-19-nor-*neo*-cleroda-5(10),13(16),14-triene-18,6β;20,12*S*-diolide) were established by chemical and spectroscopic means. The isolation of isoteuflin from *T. canadense* is of biogenetic importance, since it was previously postulated as the intermediate in the biosynthesis of teucvidin and until now only known as a synthetic compound.

INTRODUCTION

The *neo*-clerodane diterpenoids of *Teucrium* species (Labiatae) have recently attracted considerable attention [1]. In continuation of our studies on the diterpenoids of these plants [1–3] we have examined the aerial parts of *Teucrium canadense* L. (American germander), a species in which some aspects of the metabolism of indole-3-acetic acid [4] and the oligosaccharide constituents [5] have previously been studied.

Extraction of the dried plant with acetone and chromatography of the residue on silica gel afforded the already known *neo*-clerodane diterpenoids teucvidin (**1**) [1], teuflin (**2**) [1], teucvin (**3**) [1], 12-epiteupolin II [6], teuscorodal [1], and (12*R*)-teupolin I [1, 7], also named 12-epiteupolin I [8]. In addition, two new diterpenoids, 18-acetylmontanin D (**4**) and isoteuflin (**5**), have also been obtained from the same source and their structures established mainly by spectroscopic means. Isoteuflin (**5**), previously obtained [9] by basic treatment of teuflin (**2**), has been postulated as an intermediate in the biosynthesis of the diterpenoids belonging to the H-10α-19-nor-*neo*-clerodane series, such as teucvidin (**1**) [9].

RESULTS AND DISCUSSION

The first of the new diterpenoids isolated from *T. canadense*, 18-acetylmontanin D (**4**), had a molecular formula $C_{22}H_{28}O_7$ and its ^{1}H NMR spectrum (Table 1) was almost identical with that of montanin D (**6**) [10], the only differences being consistent with the presence in the former of an acetoxyl group at the C-18 position (δ2.04, 3H, *s*; C-18 methylene protons at δ4.88 and 5.06, both doublets, J_{gem} = 11.6 Hz) instead of the primary alcohol of compound **6** (C-18 methylene protons at δ3.75 *d* and 4.62 *d*, J_{gem} = 12.3 Hz) [10], since the geminal proton of the secondary hydroxyl group at the C-6 position (H-6α) appeared at almost identical field in both compounds (δ4.87 in **4** and δ4.98 in **6**, see Table 1 and ref. [10]).

NOE experiments on compound **4** (Table 2) established that its C-17 methyl group and the β-substituted furan ring are on the same side of the plane defined by the C-20, C-12 lactone ring, because irradiation of the C-17 methyl protons caused NOE enhancement in the signals of the H-14 (δ6.63, 3.4% NOE enhancement) and H-16 (δ7.86, 2%) protons and no effect was observed in the signal of the C-12 proton (δ5.57) [7, 11]. Thus, diterpenoid **4** possessed the same C-12 stereochemistry as compound **6** [10, 11] and teucroxide (**7**) [11, 12], and it is the C-18 acetyl derivative of montanin D (**6**) or its enantiomer.

The absolute configuration of 18-acetylmontanin D (**4**) was not ascertained due to the small sample available (see Experimental). However, it is reasonable to assume that compound **4** possesses a *neo*-clerodane configuration as do the other previously known diterpenoids co-occurring in the same species. Moreover, all the diterpenoids until now isolated from plants belonging to the *Teucrium* genus, including montanin D itself (**6**), have a *neo*-clerodane absolute configuration [1–3, 6–12]. The almost identical $[\alpha]_D$ values of compounds **4** and **6** (−5.0° and −5.7° [10], respectively) further supported this point.

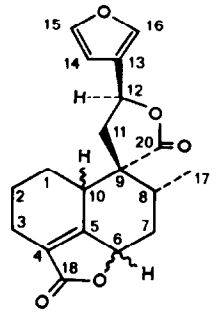
Isoteuflin (**5**, $C_{19}H_{20}O_5$) was the major constituent (48.2%) isolated from the diterpene fraction of *T. canadense*. The IR, UV and ^{1}H NMR spectra of this compound (**5**, see Experimental and Table 1) were identical with those reported [9] for a synthetic diterpenoid obtained by base catalysed isomerization of teuflin (**2**) under mild conditions. In our hands, mild basic treatment of both diterpenoids, teuflin (**2**) and isoteuflin (**5**), yielded the same 2:1 mixture of isoteuflin (**5**) and teucvidin (**1**), respectively (see Experimental).

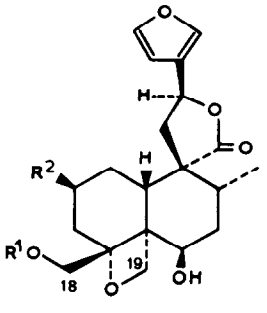
Although compound **5** is already known as a synthetic substance [9], its complete physical and spectroscopic data have not been reported in the literature [9] and they are included in Tables 1, 2 and 3 and in the experimental part of this communication. These additional data (NOE experiments, Table 2; ^{13}C NMR spectrum, Table 3) clearly revealed the close structural relationship between isoteuflin (**5**) and the acetyl derivative of isoteucrin H4 (**8**), another natural 19-nor-*neo*-clerodan-5(10)-ene diterpenoid recently isolated by us from *T. kotschyanum* [2].

Table 1. ^{1}H NMR data of compounds **4** and **5***

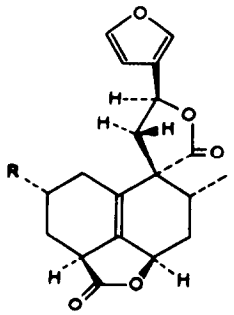
	4†	5‡
H-3β	§	1.25 *dddd*
H-4α	—	3.17 *br dd*¶
H-6α	4.87 *t*	4.98 *br ddt*
H-10β	2.58 *dd*	—
H-11α	2.49 *dd*‖	2.88 *dd*
H-11β	2.40 *dd*‖	2.23 *dd*
H-12	5.57 *br t*	5.59 *ddd*
H-14	6.63 *dd*	6.36 *dd*
H-15	7.71 *t*	7.47 *t*
H-16	7.86 *m*	7.44 *m*
Me-17	1.03 *d*	1.09 *d*
H_A-18	4.88 *d*	—
H_B-18	5.06 *d*	—
H_A-19	4.36 *d*	—
H_B-19	5.06 *d*	—
OAc	2.04 *s*	—
J (Hz)		
3β,3α	§	13.4
3β,2α	§	10.7
3β,2β	§	2.8
3β,4α	—	10.5
4α,3α	—	4.9
4α,6α	—	1.7
6α,7α	3.0	6.9
6α,7β	3.0	6.9
6α,1α	0	2.0
6α,1β	0	<0.4
8β,17	6.4	7.3
10β,1α	13.0	—
10β,1β	5.3	—
11α,11β	14.0	13.5
11α,12	8.6‖	9.1
11β,12	9.0‖	3.4
12,16	<0.3	1.2
14,15	1.7	1.7
14,16	0.9	0.9
15,16	1.7	1.7
18A,18B	11.6	—
19A,19B	7.4	—

*All these assignments have been confirmed by double resonance experiments.
†In pyridine-d_5 solution.
‡In deuteriochloroform solution.
§Overlapped signal.
‖These assignments may be interchanged.
¶ $J_{4\alpha,1\alpha}+J_{4\alpha,1\beta}+J_{4\alpha,6\alpha}\simeq 3.8$ Hz.





		R^1	R^2
1	H-6α, H-10α		
2	H-6α, H-10β		
3	H-6β, H-10β		
4		Ac	H
6		H	H
7		H	OH



5 R = H
8 R = OAc

From a biosynthetic point of view it is of interest to note that the occurrence in nature of compounds such as **5** and **8** supports previous hypothesis [9] on the biogenesis of the 19-nor-*neo*-clerodane derivatives belonging to the H-10α series, like teucvidin (**1**). The possibility that isoteuflin (**5**) could be an artefact can be ruled out, since the procedures used in its extraction and isolation were very mild (see Experimental). Furthermore, isoteuflin (**5**) is the most abundant of the diterpenoids found in *T. canadense* and it has not previously been detected in other *Teucrium* species containing teuflin (**2**) and/or teucvidin (**1**) [1–3, 9].

Table 2. NOE experiments on compounds **4** and **5**

	Irradiation δ (proton)	Observed NOE enhancement (%)						
		H-4α	H-6α	H-11β	H-12	H-14	H-16	Me-17
4	1.03 (Me-17)	—	0	*	0	3.4	2	
5	1.09 (Me-17)	0	13	2	0	2	2	
	4.98 (H-6α)	6	—	0	0	0	0	1.8

*Not measured.

Table 3. ^{13}C NMR chemical shifts of compounds **5** and **8** ($CDCl_3$, TMS as int. standard)

C	5	8	C	5	8
1	22.43 *t**†	29.27 *t*	11	40.56 *t*	40.08 *t*
2	23.33 *t*	66.69 *d*	12	71.97 *d*	71.93 *d*
3	21.23 *t*†	26.54 *t*	13	126.36 *s*	126.17 *s*
4	39.61 *d*	36.02 *d*	14	108.09 *d*	107.75 *d*
5	131.84 *s*	132.21 *s*	15	144.28 *d*	144.52 *d*
6	74.83 *d*	74.72 *d*	16	138.80 *d*	138.66 *d*
7	31.69 *t*	31.76 *t*	17	15.28 *q*	15.50 *q*
8	35.48 *d*	35.27 *d*	18	176.34 s‡	176.05 s‡
9	51.16 s	50.68 s	20	176.88 s‡	175.94 s‡
10	129.10 *s*	125.78 *s*	OAc	—	170.43 *s*
				—	21.28 *q*

*SFORD multiplicity.
†‡These assignments may be interchanged.

EXPERIMENTAL

Mps: uncorr. Plant materials were collected in June 1987, near London, Ontario (Canada), and voucher specimens were deposited in the Herbarium of the Agriculture Canada University (London, Ontario).

Extraction and isolation of the diterpenoids. Dried and finely powdered *T. canadense* aerial parts (390 g) were extracted 2 × with Me_2CO (2 l) at room temp. for a week. The extract (4 g) was chromatographed on a silica gel column (Merck, No. 7734, deactivated with 15% H_2O, 150 g) eluted with *n*-hexane and *n*-hexane–EtOAc mixtures giving three fractions which, in turn, were rechromatographed (silica gel, the same eluent mixtures) yielding the following compounds in order of increasing chromatographic polarity: isoteuflin (**5**, 1.05 g), teucvidin (**1**, 120 mg) [1], teuflin (**2**, 40 mg) [1], 12-epiteupolin II (7 mg) [6], teucvin (**3**, 900 mg) [1], teuscorodal (50 mg) [1], (12*R*)-teupolin I (6 mg) [1, 7] and 18-acetylmontanin D (**4**, 5 mg).

The previously known natural diterpenoids [teucvidin, teuflin, 12-epiteupolin II, teucvin, teuscorodal and (12*R*)-teupolin I] were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^1H NMR, MS) data and by comparison (mmp, TLC) with authentic samples.

18-Acetylmontanin D (**4**). Colourless thick oil, $[\alpha]_D^{21}$ −5.0° ($CHCl_3$; *c* 0.257); IR ν_{max}^{NaCl} cm^{-1}: 3400 (hydroxyl), 3150, 3130, 1508, 875 (furan ring), 1760 (γ-lactone), 1730, 1260 (acetate), 1460, 1380, 1020; ^1H NMR (300 MHz, pyridine-d_5): see Table 1; EIMS (direct inlet) *m/z* (rel. int.): 404 $[M]^+$ (3), 386 (3), 344 (2), 331 (7), 95 (33), 91 (17), 83 (11), 81 (23), 55 (31), 43 (100). (Found: C, 65.51; H, 7.11. $C_{22}H_{28}O_7$ requires: C, 65.33; H, 6.98%.).

Isoteuflin (**5**). An amorphous solid which melted at 70–80°; $[\alpha]_D^{21}$ +121.7° ($CHCl_3$; *c* 1.370); IR ν_{max}^{KBr} cm^{-1}: 3140, 3120, 1505, 875 (furan ring), 1770 (γ-lactones), 1453, 1340, 1180, 1160, 1140, 1130, 970; UV λ_{max}^{MeOH} nm (log ε): 214 (3.68); ^1H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (50.3 MHz, $CDCl_3$): see Table 3; EIMS (direct inlet) *m/z* (rel. int.): 328 $[M]^+$ (2), 234 (81), 206 (40), 161 (33), 94 (24), 91 (12), 85 (45), 83 (100). (Found: C, 69.36; H, 6.21. Calc. for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14%.) IR, UV and ^1H NMR spectra identical with those reported [9] for the synthetic compound.

Isomerization of teuflin (**2**) *into isoteuflin* (**5**) *and teucvidin* (**1**). Teuflin (**2**, 30 mg) was treated in THF (20 ml) with a soln of Na_2CO_3 (30 mg) in 1 ml of H_2O at room temp. for 7 days. The reaction was diluted with H_2O, extracted with $CHCl_3$, dried (Na_2SO_4) and evapd to dryness. The residue was subjected to CC (silica gel, *n*-hexane–EtOAc 4:1 as eluent) yielding [9] isoteuflin (**5**, 15 mg, less polar constituent) and teucvidin (**1**, 8 mg), which were characterized by their mp and IR, ^1H NMR and mass spectra, and by comparison (mmp, TLC) with authentic samples.

Isomerization of isoteuflin (**5**) *into teucvidin* (**1**). Treatment of isoteuflin (**5**, 200 mg) as above yielded 100 mg of the starting material (**5**) and 70 mg of a compound identical in all respects (mp, mmp, $[\alpha]_D$, IR, ^1H NMR, MS, TLC) with natural teucvidin (**1**) [1, 9].

Acknowledgements—The authors thank Dr T. T. Lee and Dr A. N. Starratt (London Research Centre, Agriculture Canada University, London, Ontario, Canada) for the collection and botanical classification of the plant material. This work was supported by funds from the Spanish DGICYT (Grant No. PB87-0418), the Italian CNR and MPI, and the "Proyecto Conjunto CSIC-CNR" (No. 3.3). One of us (M. C. de la T.) thanks the CSIC, Spain, for a fellowship.

REFERENCES

1. Piozzi, F., Rodríguez, B. and Savona, G. (1987) *Heterocycles* **25**, 807.
2. Simoes, F., Rodríguez, B., Piozzi, F., Savona, G., Bruno, M. and Arnold, N. A. (1989) *Heterocycles* **28**, 111.
3. Simoes, F., Rodríguez, B., Bruno, M., Piozzi, F., Savona, G. and Arnold, N. A. (1989) *Phytochemistry* **28**, 2763.
4. Lee, T. T. and Starratt, A. N. (1986) *Phytochemistry* **25**, 2457.
5. Wild, G. M. and French, D. (1952) *Proc. Iowa Acad. Sci.* **59**, 226.
6. Boneva, I. M., Malakov, P. Y. and Papanov, G. Y. (1988) *Phytochemistry* **27**, 295.
7. Fayos, J., Fernández-Gadea, F., Pascual, C., Perales, A., Piozzi, F., Rico, M., Rodríguez, B. and Savona, G. (1984) *J. Org. Chem.* **49**, 1789.
8. Gács-Baitz, E., Papanov, G. Y., Malakov, P. Y. and Szilagyi, L. (1987) *Phytochemistry* **26**, 2110.
9. Node, M., Sai, M. and Fujita, E. (1981) *Phytochemistry* **20**, 757.
10. Malakov, P. Y., Papanov, G. Y., Mollov, N. M. and Spassov, S. L. (1978) *Z. Naturforsch.* **33B**, 1142.
11. Pascual, C., Fernández, P., García-Alvarez, M. C., Marco, J. L., Fernández-Gadea, F., de la Torre, M. C., Hueso-Rodríguez, J. A., Rodríguez, B., Bruno, M., Paternostro, M., Piozzi, F. and Savona, G. (1986) *Phytochemistry* **25**, 715.
12. García-Alvarez, M. C., Lukacs, G., Neszmelyi, A., Piozzi, F., Rodríguez, B. and Savona, G. (1983) *J. Org. Chem.* **48**, 5123.