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NEO-CLERODANE DITERPENOIDS FROM *TEUCRIUM POLIUM* SSP. *AURASIANUM*

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Key Word Index—*Teucrium polium* ssp. *aurasianum*; Labiatae; neo-clerodane diterpenoids.

Abstract—Five neo-clerodane diterpenes were isolated from the aerial parts of *Teucrium polium* ssp. *aurasianum*. One was known (teumicropodine) and the others were new: 3-deacetylteumicropodine, 3,20-bis-deacetylteupyreinidine, 6,20-bis-deacetylteupyreinidine and 3,6,20-tri-deacetylteupyreinidine. Their structures and absolute stereochemistry were elucidated by spectral and correlation techniques.

INTRODUCTION

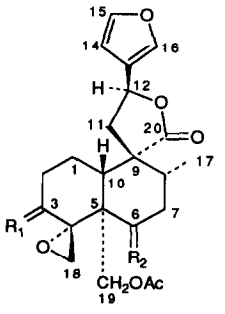
The shrub *Teucrium polium* ssp. *aurasianum* grows widely in many areas of Algeria, Morocco and other Mediterranean countries and in North Africa folk medicine has been used for the treatment of numerous diseases. Our interest in this plant led us to isolate, besides flavones and phenylpropanoid glucosides, five diterpenoids belonging to the neo-clerodane series. A great number of these compounds are known in several species of the family Labiatae and especially in the various subspecies of *Teucrium polium* [1]. However, *T. polium* ssp. *aurasianum* has not been previously studied to our knowledge. Examination of an aqueous extract of the plant led to the isolation of teumicropodine (**1**), a known compound isolated from *T. micropodioides* [2], and four new diterpenes; 3-deacetylteumicropodine (**2**), 3,20-bis-diacetylteupyreinidine (**3**), 6,20-bis-deacetylteupyreinidine (**4**) and 3,6,20-tri-deacetylteupyreinidine (**5**). However, 6,20-bis-deacetylteupyreinidine has been reported previously, although the compound has not been isolated in pure state [3]; we show here that the assigned structure was erroneous and must be corrected to 3,20-bis-deacetylteupyreinidine.

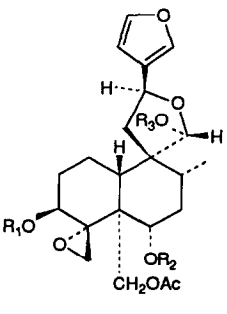
RESULTS AND DISCUSSION

The aqueous extract of the aerial parts of *Teucrium polium* was partitioned with dichloromethane and the diterpenoids contained in the dichloromethane fraction were purified using repeated chromatography on silica gel.

3-Deacetylteumicropodine (**2**) exhibited a $[M]^+$ ion at *m/z* 420 (EI-MS) corresponding to the molecular formula $C_{22}H_{28}O_8$. Its IR spectrum showed the presence of hydroxyl groups (3468 cm^{-1}), a lactone ring (1756 cm^{-1}) and an acetate (1737 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) suggested a structure closely related to teumicropidine (**1**). In fact, the only difference resided in the presence of an hydroxyl group in **2** instead of the acetoxyl group in **1**. The free hydroxyl was located at the C-3β equatorial position as shown by the upfield shift of H-3 (δ4.53, $J = 11.5$, $J' = 5$). Finally, the structure depicted in formula **2** was confirmed by oxidation at C-3 with Dess–Martin reagent [4, 5] yielding the known tafricanin A epoxide (**6**) [6].

3,20-Deacetylteupyreinidine (**3**) had a molecular formula $C_{24}H_{32}O_9$ from the EI-mass spectrum ($[M]^+$ 464) and ^{13}C NMR data. Its IR spectrum revealed the presence of hydroxyl groups (3480 cm^{-1}) and acetoxyl groups (1720 cm^{-1}). The ^1H NMR data (Table 1) were similar to those previously reported for a compound having the structure of 6,20-deacetylteupyreinidine depicted in **4** [3]. However, analysis of the ^1H–^1H and ^1H–^{13}C





1 $R_1 = \alpha H, \beta OAc$; $R_2 = \alpha OH, \beta H$
2 $R_1 = \alpha H, \beta OH$; $R_2 = \alpha OH, \beta H$
6 $R_1 = R_2 = O$
7 $R_1 = O$; $R_2 = \alpha OAc, \beta H$
8 $R_1 = \alpha H, \beta OAc$; $R_2 = O$

3 $R_1 = H$; $R_2 = OAc$; $R_3 = H$
4 $R_1 = OAc$; $R_2 = H$; $R_3 = H$
5 $R_1 = R_2 = R_3 = H$
9 $R_1 = R_2 = R_3 = OAc$

Table 1. ^{13}C (75 MHz) and ^{1}H (300 MHz) NMR data for **2–5** (in $CDCl_3$)

	2*		3		4		5	
Position	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)
1	22.7		21.9	2.10 *m*	22.8	2.05 *m*	22.4	2.10 *m*
				2.75 *m*		2.65 *m*		2.40 *m*
2	34.9		33.4	1.30 *m*	32.9	1.12 *m*	34.0	1.45 *m*
				2.35 *m*		2.25 *m*		2.40 *m*
3	64.8	4.53 *dd* (11.5, 4)	70.7	4.12 *dd* (12, 5)	67.9	5.35 *dd* (11, 4)	66.4	4.12 *dd* (11.5, 4)
4	70.0		68.7		67.5		69.6	
5	46.0		45.3		46.7		45.6	
6	73.8	3.96 *dd* (12, 4.5)	66.2	4.93 *dd* (12, 5)	71.4	3.75 *dd* (11.5, 4.5)	71.1	3.70 *dd* (11, 4.5)
7	34.6	1.90 *m*	34.8	1.35 *m*	36.5	1.30 *m*	36.1	1.70 *m*
		2.40 *m*						
8	38.4	1.65 *m*	41.0	1.50 *m*	42.1	1.50 *m*	41.8	1.25 *m*
9	49.8		51.7		54.0		53.5	
10	52.0		51.5	1.70 *m*	51.5	1.80 *m*	51.3	1.70 *m*
11	43.4	2.35 *d* (8.5)	42.9	1.90 *m*	44.0	1.65 *m*	44.5	1.80 *m*
				2.25 *m*		2.25 *m*		2.45 *m*
12	72.0	5.60 *dd* (10.5, 7)	72.3	5.18 *dd* (10, 6.5)	74.1	5.26 *dd* (10, 6.5)	74.4	5.15 *dd* (10.5, 7)
13	126.3		125.1		126.1		125.6	
14	109.1	6.61 *m*	108.8	6.45 *m*	109.8	6.48 *m*	109.3	6.55 *m*
15	140.9	7.71 *m*	143.4	7.40 *m*	144.3	7.45 *m*	143.8	7.42 *m*
16	145.0	7.87 *m*	139.4	7.43 *m*	140.4	7.45 *m*	139.9	7.37 *m*
Me-17	17.2	1.05 *d* (7)	17.2	1.01 *d* (6.5)	18.3	1.02 *d* (7)	17.8	0.98 *d* (7)
18	43.2	3.22 *d* (5)	44.0	2.84 *d* (3.5)	44.9	2.86 *d* (3.5)	43.6	3.05 *d* (3.5)
		3.41 *d* (5)		2.97 *d* (4)		3.14 *d* (3.5)		3.15 *d* (3.5)
19	62.8	4.79 *d* (13)	62.4	4.66 *d* (13)	63.5	4.40 *d* (13)	63.7	4.45 *d* (13)
		5.71 *d* (13)		4.79 *d* (13)		5.01 *d* (13)		4.70 *d* (13)
20	177.0		99.9	5.35 *d* (3)	100.9	5.28 *d* (2.5)	100.4	5.25 *d* (3)
$MeCO_2$	170.6		170.0		170.4		172.0	
$MeCO_2$			171.1		172.8			
$MeCO_2$	21.4	1.82 *s*	21.1	2.01 *s*	21.7	2.10 *s*	21.7	2.12 *s*
$MeCO_2$			21.2	2.15 *s*	21.8	2.20 *s*		
OH-20				3.50 *d* (3)		3.85 *d* (2.5)		3.99 *d* (3)

Assignments of protons and protonated carbons based on ^{1}H–^{1}H and ^{1}H–^{13}C COSY.
*In pyridine-d_5.

COSY spectra indicated that the assignments of H-3α and H-6β had to be reversed. H-3 (δ4.12, $J=12$, $J'=5$) clearly resonated upfield compared to H-6 (δ4.93, $J=12$, $J'=5$) and thus was geminal to an hydroxyl group, whereas H-6 was geminal to an acetate. This was confirmed by simultaneous oxidation of the hemiacetal and OH-3 by Dess–Martin reagent, which led to tafricanin B epoxide (**7**) identical with the reported product [6].

6,20-bis-Deacetylteupyreinidine (**4**) exhibited a $[M]^+$ ion at *m/z* 464 (EI-MS) corresponding to the same molecular formula $C_{24}H_{32}O_9$ as the preceding compound. The IR showed the characteristic absorptions of an hydroxyl at 3450 cm^{-1} and an acetoxyl group at 1720 cm^{-1}. The 1D and 2D NMR spectra (Table 1) revealed that the difference between the two isomers **3** and **4** was located at C-3 and C-6. Thus the axial H-3α (δ5.35, $J=11$, $J'=4$) was geminal to an acetoxyl group and H-6β (δ3.75, $J=11.5$, $J'=4.5$) was geminal to an hydroxyl group. Finally, the NOEs observed between Me-17 and H-20, H-14, H-16 in a NOE difference experiment indicated the same relative configuration at C-20 as that of **3**. The structure depicted in **4** was confirmed by simultaneous oxidation of the hemiacetal and OH functions, which gave the known **8** [7].

3,6,20-tri-Deacetylteupyreinidine (**5**) had a molecular formula $C_{22}H_{30}O_8$ from the EI-mass spectrum ($[M]^+$ 422) and ^{13}C NMR data. Its IR spectrum revealed the presence of hydroxyl groups (3495 cm^{-1}) and an acetate group (1722 cm^{-1}). Comparison of the ^{1}H and ^{13}C NMR spectra (Table 1) with those of **3** clearly showed an identical hydrocarbon skeleton and oxygenated pattern in both compounds and established that **5** possessed only one acetate located in the C-19 position and free hydroxyl substituents at the C-3β and C-6α equatorial positions (H-3α, δ4.12, $J=11.5$, $J'=4$; H-6β, δ3.70, $J=11$, $J'=4.5$). A NOE experiment revealed, in the same way as for **4**, an endospatial relationship between Me-17 and H-20. Attempts to correlate **5** (as well as **3**) with teupyreinidine (**9**) [8] showed that acetylation of the hemiacetal function was not easily achieved. Acetic anhydride treatment in

the presence of DMAP–pyridine afforded an unresolved mixture of teupyreinidine and another compound, which was not fully characterized and could be an isomer at position 12 and/or 20. In contrast, correlation with tafricanin A epoxide (**6**) by Dess–Martin oxidation was straightforward.

All the diterpenes isolated from *Teucria* have been found to possess the same absolute configuration, whenever known, i.e. they belong to the neo-clerodane series. It is reasonable to assume that the diterpenes isolated here also have the same absolute stereochemistry. This has been previously established for teumicropodine (**1**), and was definitively proven in the present work for **2**, **3** and **5** through the transformation of **2** and **5** into **6** and of **4** into **8**. Thus, the absolute stereochemistry of tafricanine A epoxide (**6**) has been established by correlation with tafricanin A, a natural diterpene isolated from *Teucrium tafricanum*, whose absolute stereochemistry has been determined by X-ray crystallography [8]. In the same way, **8** has been previously correlated to tafricanine A [9]. In the case of **3**, this diterpene has been transformed into tafricanine B epoxide, a compound correlated to natural tafricanine B, isolated from the same plant as tafricanine A, though correlation of both tafricanines has not been formally achieved [6].

Antifeedant properties have been reported for neo-clerodane diterpenoids [1, 9]. However, teumicropodine and teupyreinidine appeared to be devoid of such biological activities.

EXPERIMENTAL

General. Mp: uncorr; optical rotations: 20°; EI-MS: 70 eV.

Plant material. Aerial parts of *Teucrium polium* ssp. *aurasianum* Maire were collected in May 1993 at El Kouahi, Ain M'lila, Algeria. Voucher specimen are deposited in the Herbarium of the Institut National d'Agronomie (INA), El-Harrach, Algeria, under the reference AC-AS3.

Extraction and isolation of the diterpenoids. The dried and powdered plant (300 g) was extracted with H_2O (3 × 1 l) for 6 hr. The aq. extract was concd and partitioned with CH_2Cl_2. The organic layer was evapd yielding a crude extract (2.15 g), which was repeatedly chromatographed on silica gel (Merk 7736). The diterpenoids were eluted in the following order: teumicropodine (**1**) (31 mg), CH_2Cl_2–MeOH (49:1) 3-deacetylteumicropodine (**2**) (15 mg) CH_2Cl_2–MeOH (49:1); 3,6,20-trideacetylteupyreinidine (**5**) (76 mg) CH_2Cl_2–MeOH (49:1); 3,20-bis-deacetylteupyreinidine (**3**) (114 mg) (i) CH_2Cl_2–MeOH (24:1), (ii) hexane–Me_2CO (7:3); 6,20-bis-deacetylteupyreinidine (**4**) (32 mg) (i) CH_2Cl_2–MeOH (24:1), (ii) hexane–Me_2CO (7:3). Identification of teumicropodine (**1**) was carried out by comparison of its physical and spectral data with lit. values [2].

3-Deacetylteumicropodine (**2**). Amorphous solid, $[\alpha]_D$ +32° ($CHCl_3$; *c* 0.2); IR ν_{max}^{KBr} cm^{-1}: 3468, 1756, 1737, 1256; EI-MS *m/z* (rel. int.): 420 $[M]^+$ (2.7), 348 (27), 330 (36), 312 (27), 93 (100); ^1H and ^{13}C NMR: Table 1.

3,20-bis-Deacetylteupyreinidine (**3**). Gum, $[\alpha]_D$ +10° ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3480, 1720, 1240; EI-MS *m/z* (rel. int.): 464 $[M]^+$ (1.5), 419 (18), 417 (18), 404 $[M-60]^+$ (3), 302 (86), 297 (100); ^1H and ^{13}C NMR: Table 1. The ^1H NMR spectrum is similar to the one reported previously for a compound (obtained as a mixt. with 3-deacetylteupyreinidine), which was assigned structure **4**.

6,20-bis-Deacetylteupyreinidine (**4**). Gum, $[\alpha]_D$ +17° ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3450, 1720, 1240; EI-MS *m/z* (rel. int.): 464 $[M]^+$ (1.5), 418 (8), 404 $[M-60]^+$ (4), 339 (68); ^1H and ^{13}C NMR: Table 1.

3,6,20-tri-Deacetylteupyreinidine (**5**). Gum, $[\alpha]_D$ +12° ($CHCl_3$; *c* 1); IR ν_{max}^{KBr} cm^{-1}: 3495, 1722. EI-MS *m/z* (rel. int.): 422 $[M]^+$ (3), 376 (25), 362 $[M-60]^+$ (12), 297 (91), 95 (100); ^1H and ^{13}C NMR: Table 1.

Tafricanine A epoxide (**6**): (a) *from 3-deacetylteumicropodine* (**2**). A soln of **2** (13 mg, 0.032 mmol) in CH_2Cl_2 (1 ml) was added to a stirred suspension of Dess–Martin reagent (12-I-5-triacetoxyperiodinane prepd according to refs [4, 5]) (25 mg, 0.06 mmol) in CH_2Cl_2 (1 ml). After 20 min, more reagent was added (50 mg, 0.12 mmol), and the same addition was repeated ×4 within 3.5 hr until completion of the reaction (TLC monitoring). To the mixt. diluted with Et_2O (10 ml) was added a satd soln of $NaHCO_3$ (5 ml) containing of $Na_2O_3S_2$ (1.25 g). The mixt. was stirred for 5 min. The Et_2O layer was washed with a satd soln of $NaHCO_3$, H_2O and finally a satd soln of NaCl and then dried over Na_2SO_4. Removal of the solvent under vacuum gave **6** (11 mg), $[\alpha]_D$ +72° ($CHCl_3$; *c* 1) (lit. +112°), spectral data (^1H NMR and IR) identical to those reported [6]. (b) *from* 3,6,20-*tri-deacetylteupyreinidine* (**5**). Compound **5** (36 mg) treated as described above for **2** gave **6** (15 mg) having identical physical and spectral data.

Tafricanine B epoxide (**7**) *from* 3,20-*bis-deacetylteupyreinidine* (**3**). Compound **3** (36 mg) treated as described above for **2** yielded **7** (18 mg), $[\alpha]_D$ −3.5° ($CHCl_3$; *c* 1) (lit. −5.4°), spectral data (^1H NMR) identical to those reported [6].

Compound **8** *from* 6,20-*bis-deacetylteupyreinidine* (**4**). Compound **4** (20 mg) treated as described above for **2** yielded **7** (16 mg), $[\alpha]_D$ +32° ($CHCl_3$; *c* 1) (lit. +36°6), spectral data (IR, ^1H NMR and MS) identical to those reported [7].

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