Further Furoclerodanes from *Teucrium "maghrebinum"*

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Two more pairs of C-12 epimeric neoclerodanes were isolated from the aerial parts of *Teucrium "maghrebinum"* (*Teucrium polium* subspecies still unidentified). They are the known teukotschyn **1**, the new 12-epi-teukotschyn **2**, the new teughrebin **3** and the new 12-epi-teughrebin **4**. The structures of the new products were elucidated mainly by spectroscopic methods.

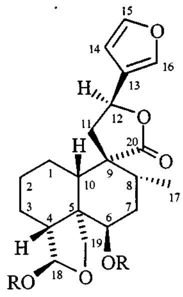
Introduction

Recently we reported[1] the occurrence of several furoclerodanes[2] in the aerial parts of a species of *Teucrium* (Labiatae) growing in Algeria. A preliminary classification indicated the species as *Teucrium maghrebinum* Greuter et Burdet; a more careful study led us now to identify it as a new *Teucrium polium* subspecies.

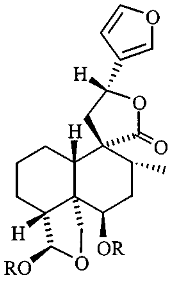
In the previous research,[1] eight neoclerodanes were extracted, three of them being new natural products, whereas the other five had been already isolated from other species of *Teucrium*. The singularity of the result was the fact that the eight products form four pairs of epimers at carbon C-12. The products are: teucjaponin A (12*S* configuration)[3] and the new 12-epi-teucjaponin A (12*R*); montanin D (12*S*)[4] and the new 12-epi-montanin D (12*R*); 19-deacetylteuscorodol (12*S*)[5] and teusalvin C (12*R*);[6] montanin B (12*S*)[4b,7] and the new 12-epi-montanin B (12*R*).

A reinvestigation of some chromatographic fractions containing minor components allowed us to identify four more neoclerodanes, three of them being new natural products, that also form two pairs of epimers at carbon C-12. They are: teukotschyn **1** (12*S* configuration)[8] and the new 12-epi-teukotschyn **2** (12*R*); the new teughrebin **3** (12*S*) and the new 12-epi-teughrebin **4** (12*R*).

Whereas teukotschyn **1** was identified by the reported[8] spectroscopic data of its diacetyl derivative and by direct comparison with an authentic sample, the structures of the three new compounds were elucidated as indicated below.



1 R=H
1a R=Ac



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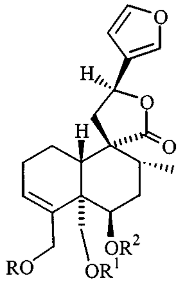
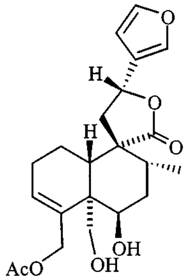
2a R=Ac



3 R=Ac R1=R2=H
5 R=R2=H R1=Ac
6 R=R1=R2=Ac



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Results and Discussion

Chromatographic separation of the extract yielded, besides others, a fraction eluted with petroleum ether/AcOEt (1:4). This fraction was subjected to radial chromatography and gave three subfractions. The third subfraction, whose ^{1}H NMR spectrum was devoid of acetylic methyl signals, was subjected to treatment with Ac_2O/py: a subsequent chromatographic purification gave a solid whose experi-

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mental data (MS, ^1H NMR, ^{13}C NMR, elemental analysis) indicated the presence of two unseparable isomeric products with a molecular formula of $C_{24}H_{30}O_8$, in a 70% to 30% ratio. In fact, almost all the signals were split.

The more abundant product was easily identified as the already known diacetylteukotschyn **1a**, on the basis of its spectroscopic data. The less abundant component showed signals in the ^1H and ^{13}C NMR spectra almost identical to those of teukotschyn for the majority of the atoms, but with significant differences[1,8,10] for H-17 ($\delta = 1.07$ vs. $\delta = 0.97$), C-8 ($\delta = 35.5$ vs. $\delta = 33.2$), C-9 ($\delta = 51.4$ vs. $\delta = 51.2$) and C-10 ($\delta = 46.7$ vs. $\delta = 47.8$): such differences are typical of the presence of a 12*R* configuration instead of the 12*S* of teukotschyn **1**. Therefore we assigned the product the structure **2a** of 12-epi-diacetylteukotschyn. Hence the original chromatographic fraction contained teukotschin and the unreported 12-epi-teukotschyn **2**.

Table 1. ^1H NMR spectroscopic data[a] of compounds **2a**, **3**, and **4**

H	**2a**	**3**	**4**
3	[b]	5.94 t	5.88 t
6α	5.38 t	4.26 m	4.31 m
11A	[b]	[b]	[b]
11B	[b]	[b]	[b]
12	5.34 t	5.42 t	5.42 t
14	6.38 dd	6.43 dd	6.41 dd
15	7.45 m	7.45 m	7.45 m
16	7.45 m	7.45 m	7.45 m
Me-17	1.07 d	1.03 d	1.12 d
18α	5.70 s	–	–
18A	–	4.70 d	4.76 d
18B	–	4.74 d	4.80 d
19A	4.12 d	3.99 d	3.99 d
19B	4.25 d	4.45 d	4.45 d
Ac	2.10 s	1.98 s	1.98 s
Ac	2.00 s	–	–
$J_{H,H}$ (Hz)	**2a**	**3**	**4**
2α,3	–	3.5	3.5
2β,3	–	3.5	3.5
6α,7α	3.2	[c]	[c]
6α,7β	3.2	[c]	[c]
11A,12	8.9	8.5	8.5
11B,12	8.4	8.5	8.5
11A,11B	[c]	[c]	[c]
14,15	1.6	1.7	1.7
14,16	1.1	1.1	1.1
17,8β	6.4	6.4	7.0
18α,4	0	–	–
18A,18B	–	11.8	11.8
19A,19B	10.7	11.5	11.5

[a] 250MHz, $CDCl_3$ solution. – [b] These signals were not identified. – [c] Overlapped signal.

The first subfraction contained a pure product whose ^1H and ^{13}C NMR spectra were similar to those of the isomeric teuscorodol[9] **5**. Acetylation of the product yielded the same triacetyl derivative **6** obtained from teuscorodol,[9] whose 12*S* configuration is known: hence the 12*S* configuration is proved for our compound. Moreover, a NOE experiment on **3** confirmed the 12*S* configuration, as the irradiation of the 17-CH_3 protons gave a 5% and a 3% enhancement in the intensity of H-14 and H-16, respectively.[10a] We assigned this compound the structure **3** and the name teughrebin. In fact, both teuscorodol and teughrebin have only one acetoxy group: as the 6β-OH is free, the acetylation occurs on C-19 in the case of teuscorodol and on C-18 in the case of teughrebin.

Table 2. ^{13}C NMR spectroscopic data[a] of compounds **2a**, **3**, and **4**

C	**2a**	**3**	**4**
1	22.6 t	19.6 t	19.0 t
2	25.4 t	25.7 t	26.0 t
3	27.0 t	133.9 d	133.9 d
4	44.5 d	139.5 s	140.0 s
5	48.1 s	47.0 s	46.8 s
6	70.3 d	67.4 d	67.4 d
7	31.0 t	33.1 t	33.2 t
8	35.5 d	32.5 d	34.6 d
9	51.4 s	51.9 s	52.3 s
10	46.7 d	44.4 d	42.3 d
11	42.7 t	44.6 t	44.8 t
12	73.4 d	72.0 d	71.6 d
13	125.2 s	125.4 s	125.4 s
14	108.1 d	108.1 d	108.1 d
15	144.2 d	144.1 d	144.1 d
16	139.1 d	139.4 d	139.0 d
17	17.0 q	16.3 q	17.0 q
18	103.1 d	66.1 t[b]	66.1 t[b]
19	71.2 t	64.9 t[b]	64.9 t[b]
20	176.9 s	177.5 s	177.5 s
OAc	171.0 s	170.3 s	170.3 s
	169.9 s		
	21.4 q	21.0 q	21.0 q
	21.4 q		

[a] 62.7 MHz, $CDCl_3$ solution. – [b] These assignments may be reversed.

Teughrebin **3** is the main component of the mixture contained in the second subfraction: after spectroscopic examination of the mixture, the minor compound turned out to be similar to **3**, although the signals for H-17, C-8, C-9 and C-10 indicate the occurrence of the 12*R* configuration. Therefore the product is 12-epi-teughrebin with structure **4**. Both **3** and **4** are new natural products. A method based on the chemical shift differences of H-17, C-8, C-9 and C-10 has previously been shown[1,8,10] to be reliable for distinguishing the *R* and *S* absolute configurations in the peculiar case of the C-12 epimers of the 20,12-γ-lactones in the neo-clerodane series. The values for the pairs **2a** and **1a**, and **4** and **3** are shown in Table 3.

Table 3. Chemical shift differences $\Delta(\delta_{12R} - \delta_{12S})$ (in ppm) observed for H-17, C-8, C-9 and C-10 between **2a** and **1a**, and **4** and **3**

	2a vs. **1a**	**4** vs. **3**
H-17	+0.1	+0.09
C-8	+2.3	+2.1
C-9	+0.2	+0.4
C-10	−1.1	−2.1

The results reported here show that this species of *Teucrium* contains six pairs of epimers at C-12. This is very unusual as in only one out of the almost 100 species of *Teucrium* investigated has the co-occurrence of pairs of epimers at C-12 been observed: *Teucrium kotschyanum* contained (besides pure teukotschyn) the two pairs teucvidin/12-epi-teucvidin and teuflin/12-epi-teuflin.

Experimental Section

General: Optical rotations were measured on a Perkin−Elmer 141 polarimeter. IR spectra (KBr) were obtained on a Perkin−Elmer 1310 spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ solution using a Bruker AC 250E instrument at 250 MHz, and chemical shifts are reported with respect to residual $CHCl_3$ ($\delta = 7.27$). ^{13}C NMR spectra were recorded in $CDCl_3$ solution on the same apparatus at 62.7 MHz, and chemical shifts are reported with respect to solvent signals ($CDCl_3$: $\delta_C = 77.00$). ^{13}C NMR assignments were determined by DEPT spectra. MS were recorded on a Finnigan TSQ70 instrument (70 eV, direct inlet). Elemental analysis was carried out with a Perkin−Elmer 240 apparatus. Merck silica gel (70−230 mesh), deactivated with 15% H_2O, was used for column chromatography. Radial chromatography has been performed on a Harrison Chromatotron 7924 T apparatus using Merck silica gel PF_{254} 60 as plate adsorbent.

Plant Material: The aerial parts of *T. maghrebinum* were collected at Oum El-Hdjel, Ferdjioua, near Wadi Mila, Algeria, in May 1998. A typical specimen has been deposited in the Herbarium of the Institut National d'Agronomie (INA), El-Harrach, Algeria.

Extraction and Isolation: The dried and finely powdered aerial parts of *T. maghrebinum* (270 g) were extracted with Me_2CO (3 × 5 L) at room temperature for one week. The extract (25 g) was chromatographed over a silica gel dry column with a solvent gradient from 100% petroleum ether (b.p. 50−70 °C) to 100% EtOAc and finally with EtOAc/MeOH (19:1, 9:1). The fraction eluted with petroleum ether/EtOAc (1:4) (160 mg) was subjected to radial chromatography, with $CHCl_3$/MeOH (23:2) as eluent, to afford, in order of increasing polarity, three subfractions: teughrebin (**3**, 7 mg), a mixture in the ratio 7:3 (20 mg) of teughrebin (**3**) and 12-*epi*-teughrebin (**4**) and a complex mixture (40 mg) containing teukotschyn (**1**) and 12-*epi*-teukotschyn (**2**).

Teughrebin (3): Amorphous solid. − $[\alpha]_D^{20} = -30.2$ ($c = 0.57$, $CHCl_3$). − IR (KBr): $\tilde{\nu}_{max}$ = 3400, 3130, 2975, 1760, 1740, 1660, 1450, 1380, 1250, 1150, 925, 875 cm^{-1}. − ^{1}H NMR (250 MHz), see Table 1. − ^{13}C NMR (62.7 MHz), see Table 2. − EIMS: *m/z* (%) = 404 (1) $[M]^+$, 386 (1) $[M - H_2O]^+$, 344 (2) $[M - CH_3COOH]^+$, 326 (7) $[M - H_2O - CH_3COOH]^+$, 313 (5), 281 (2), 179 (4), 115 (3), 105 (4), 91 (7), 81 (5), 43 (100). − $C_{22}H_{28}O_7$ (404.46): calcd. C 65.33, H 6.98; found C 65.20, H 7.02.

Mixture of Teughrebin (3) and 12-epi-Teughrebin (4): Amorphous solid. − IR (KBr): $\tilde{\nu}_{max}$ = 3400, 3130, 2975, 1760, 1740, 1660, 1450, 1380, 1250, 1150, 925, 875 cm^{-1}. − ^{1}H NMR (250 MHz), see Table 1. − ^{13}C NMR (62.7 MHz), see Table 2. − EIMS: *m/z* (%) = 404 (1) $[M]^+$, 386 (1) $[M - H_2O]^+$, 344 (2) $[M - CH_3COOH]^+$, 326 (8) $[M - H_2O - CH_3COOH]^+$, 313 (8), 281 (3), 179 (7), 115 (9), 105 (4), 91 (7), 81 (10), 43 (100). − $C_{22}H_{28}O_7$ (404.46): calcd. C 65.33, H 6.98; found C 65.20, H 7.02.

Acetylation of Teughrebin (3): Teughrebin (**3**, 4 mg) was dissolved in 2 mL of Ac_2O/pyridine (2:1) and maintained at room temperature for 24 h. The reaction mixture was diluted with H_2O, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, and dried with anhydrous Na_2SO_4. Purification by radial chromatography (CH_2Cl_2/MeOH, 49:1) yielded 3.5 mg of compound **6**. This compound showed identical $[\alpha]_D$, ^{1}H NMR, IR and MS values to those of the compound previously synthesized from teuscorodol (**5**).

Acetylation of the Mixture Containing Teukotschyn (1) and 12-*epi*-Teukotschyn (2): The unresolved mixture (40 mg) containing teukotschyn (**1**) and 12-epi-teukotschyn (**2**) was dissolved in 3 mL of Ac_2O/pyridine (2:1) and maintained at room temperature for 24 h. The reaction mixture was diluted with H_2O, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, and dried with anhydrous Na_2SO_4. Column chromatography on silica gel (petroleum ether/EtOAc, 1:1) yielded 25 mg of a mixture (7:3) of diacetylteukotschyn (**1a**) and 12-*epi*-diacetylteukotschyn (**2a**) as an amorphous solid. − IR (KBr): $\tilde{\nu}_{max}$ = 3140, 2950, 2850, 1760, 1745, 1730, 1450, 1380, 1250, 875 cm^{-1}. − ^{1}H NMR (250 MHz), see Table 1. − ^{13}C NMR (62.7 MHz), see Table 2. − EIMS: *m/z* (%) = 446 (7) $[M]^+$, 403 (36) $[M - CH_3CO]^+$, 387 (98) $[M - CH_3COO]^+$, 360 (29) $[M - 2(CH_3CO)]^+$, 343 (32) $[M - CH_3CO - CH_3COOH]^+$, 340 (35), 327 (8) $[M - CH_3COO - CH_3COOH]^+$, 298 (58), 204 (47), 159 (47), 105 (14), 96 (100), 81 (40), 55 (17), 43 (73). − $C_{24}H_{30}O_8$ (446.49): calcd. C 64.56, H 6.77; found C 64.70, H 6.74.

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