Neoclerodane Diterpenoids from *Teucrium maghrebinum*

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Eight neoclerodane diterpenoids were identified in the extract of the aerial parts of *Teucrium maghrebinum*. Three of these, 12-*epi*-teucjaponin A (**1**), 12-*epi*-montanin D (**2**), and 12-*epi*-montanin B (**3**), are new natural products, whereas five, teucjaponin A, montanin D, 19-deacetylteuscorodol, teusalvin C (**4**), and montanin B, are already known. These eight compounds form four pairs of epimers at carbon C-12.

The number of natural clerodane diterpenoids has grown rapidly in the last years. In 1992, they were estimated at around 650;[1] in 1994, they were believed to be almost 800;[2] and at the present time, there are probably about 1000 such compounds. The genus *Teucrium*, belonging to the Labiatae (Lamiaceae), is one of the richest sources of neoclerodanes, with more than 200 compounds isolated to date from the aerial parts of about 80 species or subspecies.[3−6] Many of these products have shown antifeedant activity against certain insect pests. As a part of our investigation of this genus, we report herein three new (**1**−**3**) and five known neoclerodane diterpenoids from *T. maghrebinum* W. Greuter et Burdet, a species growing in Algeria and Morocco. The plant is called locally "kayatat el gerah" and is used in traditional medicine to treat burns and fevers, as well as having use as an antimicrobial agent.

An acetone-soluble extract of the aerial parts of *T. maghrebinum* was fractionated by column chromatography. Repeated column and radial chromatography led to eight neoclerodane diterpenoids, of which three (**1**−**3**) have not been reported previously. The structures are representative of four pairs of epimers at carbon C-12: the known teucjaponin A[7−9] and the new 12-*epi*-teucjaponin A (**1**); the known montanin D[10,11] and the new 12-*epi*-montanin D (**2**); the known 19-deacetylteuscorodol[12] and the known teusalvin C (**4**);[13] and the known montanin B[11,14] and the new 12-*epi*-montanin B (**3**).

The fraction eluting with EtOAc−petroleum ether (3:2) was subjected to radial chromatography, yielding three fractions A, B, and C. Fraction A was apparently homogeneous, but ^{1}H and ^{13}C NMR spectra showed several split signals, indicating the presence of an unresolvable mixture of two compounds. Elemental analysis and MS proved that the products are isomers with the elemental formula $C_{22}H_{28}O_7$. One of the isomers was identified as teucjaponin A on the basis of its known NMR data.[7,8] The second isomer was elucidated as 12-*epi*-teucjaponin A (**1**) after a careful study of the small differences of NMR chemical shifts observed between teucjaponin A (12*S* absolute configuration) and **1** (12*R* absolute configuration) for certain protons and carbon atoms. Thus, the values for the CH_3-17 protons showed small but consistent differences in the case of the 12*S* versus the 12*R* configuration, and small differences occurred for the chemical shifts of C-8, C-9, and C-10, whereas the other ^{1}H and ^{13}C NMR shifts were otherwise almost identical for the two epimers. Similar observations have been reported previously.[15−17] Accordingly, compound **1** was assigned as the new natural product, 12-*epi*-teucjaponin A.

Fraction B was homogeneous. The ^{1}H and ^{13}C NMR spectra were in full agreement with those reported for montanin D,[10,11] having a 12*S* configuration.

Fraction C was a complex, unresolvable mixture, whose ^{1}H NMR spectrum was devoid of acetate signals. Acetylation of the mixture followed by radial chromatography allowed the isolation of diacetylmontanin D, already described,[14] and another product whose structure was elucidated as diacetyl-12-*epi*-montanin D (**5**). The MS and elemental analysis indicated $C_{24}H_{30}O_8$ as the molecular formula, and comparison of the ^{1}H and ^{13}C NMR spectra of these two acetylated products allowed us to attribute the 12*R* configuration to **5**. Moreover, a NOE experiment on **5** confirmed the 12*R* configuration, as irradiation of the CH_3-17 protons gave a 10% increase in the intensity of H-12. Hence, it was inferred that the original extract of *T. maghrebinum* contained 12-*epi*-montanin D (**2**).

The fraction that eluted with EtOAc−MeOH (19:1) was subjected to radial chromatography, yielding two further fractions, D and E. Fraction D gave ^{1}H and ^{13}C NMR spectra, which showed the typical split spectra of an unresolvable mixture. The MS and elemental analysis data indicated a $C_{20}H_{26}O_6$ elemental formula, and the two isomers present were identified as deacetylteuscorodol, having a 12*S* configuration, and teusalvin C (**4**), having a 12*R* configuration, on the basis of their NMR data. The ^{13}C NMR chemical shifts of **4**, not previously reported, helped to discriminate the two epimeric configurations.

Fraction E was shown by its NMR spectra to be a mixture, and MS and elemental analysis data indicated a $C_{19}H_{24}O_5$ molecular formula. One compound had NMR data in agreement with those reported for montanin B[11,14] with a 12*S* configuration. The NMR data of the second compound were consistent with the structure of 12-*epi*-montanin B (**3**) with a 12*R* configuration. Acetylation of the mixture of montanin B and **3** also yielded an inseparable mixture of the two acetyl derivatives **6** and **7**. Diacetyl-

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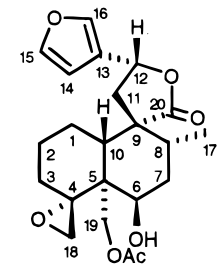
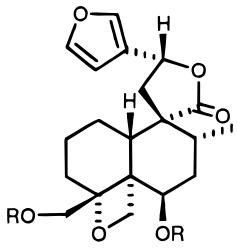
montanin B (**7**) has been synthesized previously[14] but its NMR data have not been reported.



1



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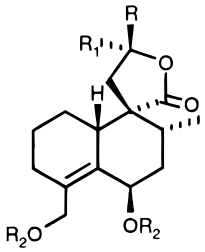
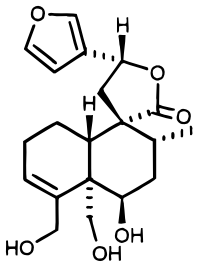
5 R = Ac



3 R = H; R_1 = β-furyl; R_2 = H
6 R = H; R_1 = β-furyl; R_2 = Ac
7 R = β-furyl; R_1 = H; R_2 = Ac



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The co-occurrence of a pair of epimers in the same plant is not unusual, but in the case of *T. maghrebinum* four pairs were present. For the *Teucrium* species previously investigated, two pairs of diterpene epimers were reported from *T. kotschyanum*.[17]

Experimental Section

General Experimental Procedures. 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 $CDCl_3$ or pyridine-d_5 solution using a Bruker AC 250E instrument at 250 MHz, and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.27) or pyridine (δ 7.21, 7.57, 8.72) solvent signals. ^{13}C NMR spectra were recorded in $CDCl_3$, pyridine-d_5, or $CDCl_3$−DMSO-d_6 solution on the same apparatus at 62.7 MHz, and chemical shifts are reported with respect to solvent signals, [δ_C 77.00 ($CDCl_3$), δ_C 123.5, 135.5, 149.5 (pyridine-d_5)]. ^{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 Si gel (70−230 mesh), deactivated with 15% H_2O, was used for column chromatography. Radial chromatography was performed on a Harrison Chromatotron 7924 T apparatus using Merck Si 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 voucher specimen is deposited in the Herbarium of the Institut National d'Agronomie (INA), El-Harrach, Algeria.

Extraction and Isolation. Dried and finely powdered aerial parts of *T. maghrebinum* (270 g) were extracted with Me_2CO (3 × 5 L) at room temperature for 1 week. After filtration, the solvent was evaporated at low temperature (35 °C), yielding a gum (25 g) that was chromatographed over a Si gel dry column with a solvent gradient from 100% petroleum ether (bp 50−70 °C) to 100% EtOAc, and finally with EtOAc−MeOH (19:1, 9:1). The fraction that eluted with petroleum ether−EtOAc (2:3) (180 mg) was subjected to radial chromatography, using $CHCl_3$−MeOH (24:1) as eluent, to afford, in order of increasing polarity, a mixture (19 mg) of teucjaponin A and 12-*epi*-teucjaponin A (**1**), montanin D (11 mg), and a complex mixture (120 mg) containing also montanin D and 12-*epi*-montanin D (**2**). The fraction that eluted with EtOAc−MeOH (19:1) (150 mg) was purified by radial chromatography (CH_2Cl_2−MeOH, 19:1) to give, in order of increasing polarity, a mixture (27 mg) of 19-deacetylteuscorodol and teusalvin C (**4**) and a mixture (41 mg) of montanin B and 12-*epi*-montanin B (**3**). Previously known compounds were identified by their [α]$_D$, IR, ^{1}H NMR, ^{13}C NMR, and mass spectra.[7,8,10−14]

Table 1. ^{1}H NMR Spectral Data of Compounds **1** and **3**−**7**

proton	**1**[a]	**3**[a]	**4**[b]	**5**[a]	**6**[a]	**7**[a]
3				5.91 (t)		
6α	4.17 (m)	5.00 (m)	*c*	6.66 (t)	6.01 (t)	5.98 (t)
11A	*c*		*c*	2.41 (dd)		2.44 (dd)
		2.52 (d)			2.49 (d)	
11B	*c*		*c*	2.49 (dd)		2.53 (dd)
12	5.37 (t)	5.42 (t)	5.57 (t)	5.38 (t)	5.38 (t)	5.38 (t)
14	6.37 (m)	6.42 (m)	6.62 (m)	6.41 (m)	6.42 (m)	6.41 (m)
15	7.44 (m)	7.47 (m)	7.66 (m)	7.47 (m)	7.44 (m)	7.44 (m)
16	7.44 (m)	7.47 (m)	7.81 (m)	7.47 (m)	7.44 (m)	7.44 (m)
Me-17	1.09 (d)	1.12 (d)	1.15 (d)	1.04 (d)	1.09 (d)	0.98 (d)
18A	2.26 (d)	4.00 (d)	4.48 (d)	4.04 (d)	4.68 (d)	4.68 (d)
18B	3.78 (m)	4.33 (d)	4.94 (d)	4.11 (d)	4.83 (d)	4.83 (d)
19A	4.90 (d)		4.44 (d)	4.16 (d)		
19B	4.98 (d)		5.24 (d)	4.92 (d)		
Ac	2.08 (s)			2.10 (s)	2.05 (s)	2.05 (s)
Ac				2.08 (s)	2.03 (s)	2.03 (s)
$J_{H,H}$ (Hz)						
3,2α			3.4			
3,2β	*d*		3.4			
6α,7α	*d*	*d*	*c*	2.6	2.6	2.6
6α,7β	*d*	*d*	*c*	2.6	2.6	2.6
11A,12	8.4	8.6	8.7	8.8	8.6	8.6
11B,12	8.4	8.6	8.7	8.1	8.6	8.6
11A,11B	*c*	*d*	*c*	13.9	*d*	14
17,8β	6.6	6.6	6.9	6.9	6.6	6.6
18A,18B	5.9	12.2	11.4	12.1	12.2	12.2
19A,19B	12.9		11.1	8.0		

[a] $CDCl_3$ solution. [b] Pyridine-d_5 solution. [c] Not observed. [d] Overlapped signal.

Mixture of teucjaponin A and 12-*epi*-teucjaponin A (1): amorphous solid; IR (KBr) ν_{max} 3480, 3146, 2960, 2935, 2880, 1760, 1735, 1505, 1450, 1385, 1320, 1255, 1180, 1155, 1117, 1026, 920, 875, 800 cm^{-1}; ^{1}H NMR (250 MHz), see Table 1; ^{13}C NMR (62.7 MHz), see Table 2; EIMS *m*/*z* [M]$^+$ absent, 386 (1) [M − H_2O]$^+$, 344 (5) [M − HOAc]$^+$, 331 (24), 313 (35), 269 (7), 253 (9), 222 (14), 191 (14), 179 (14), 161 (36), 133 (32), 105 (53), 95 (90), 81 (80), 55 (46), 43 (100); *anal.* C 65.15%, H 6.81%, calcd for $C_{22}H_{28}O_7$, C 65.53%, H 6.98%.

Acetylation of the Mixture of Montanin D and 12-*epi*-Montanin D (2). The unresolved mixture (120 mg) containing montanin D and compound **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 Si gel (petroleum ether−EtOAc 1:1) yielded 37 mg of diacetylmontanin D and 14 mg of 12-*epi*-diacetylmontanin D (**5**).

12-*epi*-diacetylmontanin D (5): amorphous solid; [α]$^{20}_D$ −22° (*c* 0.64 MeOH); IR (KBr) ν_{max} 3140, 2956, 2891, 1760, 1735, 1500, 1442, 1375, 1247, 1161, 1033, 980, 872, 802 cm^{-1}; ^{1}H NMR (250 MHz), see Table 1; ^{13}C NMR (62.7 MHz), see Table 2; EIMS *m*/*z* 446 (23) [M]$^+$, 386 (15) [M − HOAc]$^+$, 373 (15), 297 (15), 274 (35), 214 (21), 106 (22), 94 (50), 81 (15), 43 (100); *anal.* C 64.40%, H 6.66%, calcd for $C_{24}H_{30}O_8$, C 64.56%, H 6.77%.

Mixture of 19-deacetylteuscorodol and teusalvin C (4): amorphous solid; IR (KBr) ν_{max} 3450, 3335, 3220, 2960, 2940, 2900, 1755, 1654, 1560, 1508, 1460, 1330, 1185, 1170, 1155, 1125, 1110, 1050, 1040, 1025, 960, 940, 910, 875, 805 cm^{-1}; ^{1}H NMR (250 MHz), see Table 1; ^{13}C NMR (62.7 MHz), see Table 2; EIMS *m*/*z* 362 (6) [M]$^+$, 344 (2) [M − H_2O]$^+$, 314 (7), 296 (32), 269 (3), 251 (11), 228 (17), 197 (17), 187 (19), 169 (21), 157 (29), 129 (30), 119 (44), 105 (55), 95 (100), 81 (68), 69

Table 2. ^{13}C NMR Spectral Data of Compounds **1** and **3**−**7**

carbon	**1**[b]	**3**[a]	**4**[c]	**5**[b]	**6**[b]	**7**[b]
1	22.2 t	22.2 t	19.4 t	21.7 t	21.1 t	20.6 t
2	24.6 t	25.9 t	26.5 t	16.1 t	25.9 t	25.5 t
3	33.2 t	28.0 t	129.4 d	29.1 t	28.6 t	28.5 t
4	62.9 s	134.4 s[d]	146.0 s	86.3 s	131.6 s	131.7 s
5	45.7 s	135.0 s[d]	48.5 s	46.2 s	134.3 s	134.1 s
6	65.9 d	63.4 d	66.6 d	73.4 d	68.7 d	68.6 d
7	35.6 t	36.0 t	34.8 t	29.9 t	34.6 t	34.5 t
8	34.4 d	34.0 d	35.3 d	34.8 d	35.2 d	33.4 d
9	52.0 s	53.6 s	52.9 s	52.6 s	53.8 s	53.4 s
10	44.4 d	38.7 d	43.6 d	36.0 d	39.6 d	41.4 d
11	45.3 t	40.6 t	45.6 t	41.2 t	40.5 t	40.8 t
12	71.7 d	71.4 d	71.7 d	72.2 d	71.6 d	71.8 d
13	125.4 s	125.5 s	126.6 s	125.3 s	125.6 s	125.5 s
14	108.1 d	108.2 d	109.0 d	108.1 d	108.1 d	108.1 d
15	144.1 d	143.9 d	144.7 d	144.2 d	144.1 d	144.1 d
16	139.1 d	139.5 d	140.1 d	139.4 d	139.5 d	139.4 d
17	17.1 q	17.2 q	17.5 q	16.8 q	17.1 q	16.9 q
18	52.4 t	60.9 t	65.4 t	67.1 t	64.1 t	63.9 t
19	64.1 t		66.9 t	72.5 t		
20	177.1 s	176.9 s	178.0 s	177.3 s	176.5 s	176.6 s
OAc	171.0 s			171.0 s	170.9 s	170.9 s
				169.9 s	169.9 s	169.9 s
	21.1 q			21.4 q	21.4 q	21.4 q
				20.9 q	20.9 q	20.9 q

[a] $CDCl_3$−DMSO-d_6 (5:1) solution. [b] $CDCl_3$ solution. [c] Pyridine-d_5 solution. [d] These assignments may be reversed.

(61), 55 (47); *anal.* C 66.39%, H 7.29%, calcd for $C_{20}H_{26}O_6$, C 66.28%, H 7.23%.

Mixture of montanin B and 12-*epi*-montanin B (3): amorphous solid; IR (KBr) ν_{max} 3465, 3360, 2950, 2920, 2880, 1745, 1597, 1507, 1190, 1160, 1018, 990, 875 cm^{-1}; ^1H NMR (250 MHz), see Table 1; ^{13}C NMR (62.7 MHz), see Table 2; EIMS *m/z* $[M]^+$ absent, 314 (48) $[M - H_2O]^+$, 297 (10), 269 (16), 251 (4), 233 (18), 220 (33), 197 (25), 187 (26), 175 (46), 161 (45), 136 (61), 121 (70), 103 (97), 91 (100), 79 (78), 71 (61), 58 (98); *anal.* C 68.56%, H 7.15%, calcd for $C_{19}H_{24}O_5$, C 68.65%, H 7.28%.

Acetylation of the Mixture of Montanin B and 12-*epi*-Montanin B (3). The mixture (20 mg) containing montanin B and 12-*epi*-montanin B (**3**) 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 Si gel (petroleum ether−EtOAc, 1:1) yielded 18 mg of an inseparable mixture of 12-*epi*-diacetylmontanin B (**6**) and diacetylmontanin B (**7**).

Mixture of 12-*epi*-diacetylmontanin B (6) and diacetylmontanin B (7): amorphous solid; IR (KBr) ν_{max} 3145, 2960, 2930, 2860, 1760, 1735, 1510, 1370, 1320, 1250, 1185, 1155, 1020, 950, 875 cm^{-1}; ^1H NMR (250 MHz), see Table 1; ^{13}C NMR (62.7 MHz), see Table 2; EIMS *m/z* $[M]^+$ absent, 356 (8) $[M - HOAc]^+$, 314 (55), 296 (43), 269 (13), 251 (23), 228 (22), 187 (24), 169 (25), 157 (56), 143 (45), 117 (51), 95 (81), 81 (73), 43 (100); *anal.* C 66.21%, H 6.82%, calcd for $C_{23}H_{28}O_7$, C 66.33%, H 6.78%.

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References and Notes

(1) Merritt, A. T.; Ley, S. V. *Nat. Prod. Rep.* **1992**, 243−287.
(2) Hagiwara, H.; Inome, K.; Uda, H. *Tetrahedron Lett.* **1994**, *35*, 8189−8192.
(3) Piozzi, F. *Heterocycles* **1981**, *15*, 1489−1503.
(4) Piozzi, F.; Savona, G.; Rodriguez, B. *Heterocycles* **1987**, *25*, 807−841.
(5) Piozzi, F. *Heterocycles* **1994**, *37*, 603−626.
(6) Piozzi, F.; Bruno, M.; Rosselli, S. *Heterocycles* **1998**, *48*, 2185−2203.
(7) Miyase, T.; Kawasaki, H.; Noro, T.; Ueno, A.; Fukushima, S.; Takemoto, T. *Chem. Pharm. Bull.* **1981**, *29*, 3561−3564.
(8) Papanov, G. Y.; Malakov, P. Y. *Phytochemistry* **1983**, *22*, 2787−2789.
(9) Savona, G.; Piozzi, F.; Servettaz, O.; Fernandez-Gadea, F.; Rodriguez, B. *Phytochemistry* **1984**, *23*, 611−613.
(10) Malakov, P. Y.; Papanov, G. Y.; Mollov, N. M.; Spassov, S. L. *Z. Naturforsch.* **1978**, *33b*, 1142−1144.
(11) Gacs-Baitz, E.; Kajtàr, M.; Papanov, G. Y.; Malakov, P. Y. *Heterocycles* **1982**, *19*, 539−550.
(12) de la Torre, M. C.; Fernandez-Gadea, F.; Michavila, A.; Rodriguez, B.; Piozzi, F.; Savona, G. *Phytochemistry* **1986**, *25*, 2385−2387.
(13) de la Torre, M. C.; Pascual, C.; Rodriguez, B.; Piozzi, F.; Savona, G.; Perales, A. *Phytochemistry* **1986**, *25*, 1397−1403.
(14) Malakov, P. Y.; Papanov, G. Y.; Mollov N. M. *Tetrahedron Lett.* **1978**, 2025−2026.
(15) Fayos, J.; Fernandez-Gadea, F.; Pascual, C.; Perales, A.; Piozzi, F.; Rico, M.; Rodriguez, B.; Savona, G. *J. Org. Chem.* **1984**, *49*, 1789−1793.
(16) Pascual, C.; Fernandez, P.; Garcia-Alvarez, M. C.; Marco, J. L.; Fernandez-Gadea, F.; de la Torre, M. C.; Hueso-Rodriguez, J. A.; Rodriguez, B.; Bruno, M.; Paternostro, M.; Piozzi, F.; Savona, G. *Phytochemistry* **1986**, *25*, 715−718.
(17) Simoes, F.; Rodriguez, B.; Bruno, M.; Piozzi, F.; Savona, G.; Arnold, A. N. *Phytochemistry* **1989**, *28*, 2763−2768.

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