Phytochemistry, Vol. 22, No. 12, pp. 2787–2789, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

CLERODANE DITERPENOIDS FROM *TEUCRIUM MONTANUM* SUBSP. *SKORPILII*

GEORGI Y. PAPANOV and PETER Y. MALAKOV

Department of Organic Chemistry, Plovdiv University, 24 Tsar Assen Street, 4000 Plovdiv, Bulgaria

(*Revised received* 7 *March* 1983)

Key Word Index—*Teucrium montanum*; Labiatae; diterpenoid; new clerodane derivative; montanin E; montanin F; teucjaponin A.

Abstract—From the aerial part of *Teucrium montanum* subsp. *skorpilii* a new clerodane diterpenoid, montanin E, has been isolated and its structure was established by spectroscopic and chemical means and by partial synthesis from 19-acetylgnaphalin. In addition, a second compound, montanin F, was shown to be identical to the known diterpenoid teucjaponin A.

INTRODUCTION

Recently, we described the structure and stereochemistry of four novel diterpenoids furolactones from the acetone extract of *Teucrium montanum* subsp. *skorpilii* [1–4]. Continuing our studies on diterpenic compounds from the *Teucrium* species growing in Bulgaria, we isolated two minor furanoid lactones from *Teucrium montanum* subsp. *skorpilii* designated as montanin E and montanin F. Montanin E was also isolated from *Teucrium polium* and *Teucrium scordium*.

RESULTS AND DISCUSSION

Montanin E (**1**) was the most polar compound of the bitter fraction isolated from *T. montanum* and *T. polium*. Elemental analysis and high resolution mass spectrometry gave the molecular formula of montanin E as $C_{20}H_{28}O_7$. Its IR spectrum was consistent with the presence of a furan ring (3160, 1600, 1510 and 872 cm^{-1}), a lactone group (1755 cm^{-1}) and a strong broad band for one or more hydoxyl groups (3280–3400 cm^{-1}). The number and nature of the hydroxyl groups was established by acetylation of **1** with acetic anhydride–pyridine leading to a triacetate (**2**). The IR spectrum of **2** showed a strong band for a tertiary hydroxyl group (3550 cm^{-1}) and strong bands for ester groups (1725–1735 and 1245 cm^{-1}). The ^{1}H NMR spectrum of **1** (see Table 1), showed signals for a β-substituted furan ring (α-furan protons at δ7.67 and 7.82 and one β-furan proton at δ6.64) and a secondary methyl group (δ1.14, 3H, J = 7 Hz). The following signals due to six protons on carbon atoms bearing oxygen atoms were present: δ 5.57 (1H, *t*, J = 8 Hz, C-12), 4.67 and 4.11 (2H, *d*, J = 11.5 Hz, C-18), 5.51 and 4.36 (2H, *d*, J = 12.5 Hz, C-19) and 5.47 (1H, *t*, J = 3 Hz, C-6). According to the small coupling constant the proton at C-6 is equatorial and the hydroxyl group is an axial one. The equatorial 6α-configuration of the C-6 proton was also confirmed by comparing the ^{1}H NMR data of **1** with those reported for related compounds [4–8]. The ^{1}H NMR spectrum of **2** (Table 1) clearly showed three singlets at δ 2.28 (3H), 2.10 (3H) and 2.09 (3H) due to the acetate methyls. Two of the acetylated hydroxyls were primary and the third one was attached to a methine group (δ 5.67, *d*). The signal of the proton at the carbon of the fourth hydroxyl group appeared as a doublet at δ 3.70 (J = 3 Hz), which disappeared after addition of D_2O. The double doublet at δ 2.80 was assigned to the C-10 β axial proton, which is strongly deshielded by the 1,3-diaxial interaction with the tertiary

Table 1. ^{1}H NMR spectral data of **1** and **2** and ^{13}C NMR chemical shifts (δ values from internal TMS) of **1** (400 MHz)

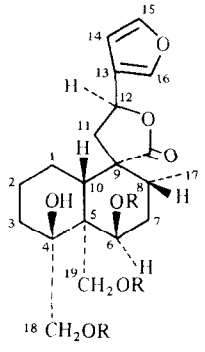
1 (Pyridine-d_5)		**2** ($CDCl_3$)		**1** C (ppm)	
1α-H	1.60 *dddd*	1.57 *dddd*		C-1	21.78 *t*
1β-H	—	–		C-2	22.01 *t*
6α-H	5.47 *t*	5.67 *dbr*		C-3	31.02 *t*
10β-H	3.19 *dd*	2.80 *dd*		C-4	78.42 *s*
11-H	2.54 *d*	2.58 *dd*		C-5	47.30 *s*
11-H		2.47 *dd*		C-6	67.82 *d*
12-H	5.57 *dd*	5.41 *dd*		C-7	35.40 *t*
14-H	6.64 *dd*	6.40 *dd*		C-8	33.03 *d*
15-H	7.67 *dd*	7.44 *dd*		C-9	52.16 *s*
16-H	7.82 *br s*	7.46 *dd*		C-10	43.62 *d*
18-H	4.67 *d*	4.46 *d*		C-11	45.91 *t*
18′-H	4.11 *d*	3.72 *d*		C-12	71.08 *d*
19-H	5.51 *d*	5.35 *d*		C-13	124.61 *s*
19′-H	4.36 *d*	4.51 *d*		C-14	108.41 *d*
OH	—	3.70 *d*		C-15	144.38 *d*
		2.28 *s*		C-16	140.01 *d*
OAc		2.10 *s*		C-17	16.96 *q*
		2.09 *s*		C-18	68.32 *t*
				C-19	60.50 *t*
				C-20	178.06 *s*

J(Hz) **1**: 1α, 1β = 1α, 10β = 13; 6α, 7α = 6α, 7β = 3; 11, 12 = 11′, 12 = 8; 18, 18′ = 11; 19, 19′ = 12.

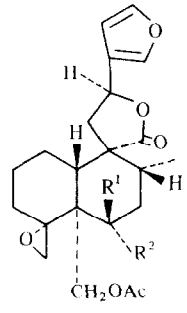
J(Hz) **2**: 1α, 1β = 1α, 10β = 12; 6α, 7α = 6α, 7β = 4β-OH, 10β-H = 3; 11, 11′ – 8.5; 11, 12 = 11′,12 = 8; 18, 18′ = 11.5; 19, 19′ = 12.5.

hydroxyl group at C-4 [8]. On the other hand, on irradiation at 2.80 (10β-proton) the doublet at 3.70 was transformed into a singlet, which showed unambiguosly that the proton of the hydroxyl group at C-4 interacted with the proton of C-10. According to the small spin constant (J = 3 Hz), the hydroxyl group at C-4β and the proton at C-10β were axially orientated.

The stereochemistry at the other chiral centres and the *trans*-junction of rings A and B were deduced from the spin decoupling and by comparing the ^{13}C NMR chemical shift data of **1** (Table 1) with those reported for related compounds [4].



1 R=H
2 R=Ac



3 R^1=OH, R^2=H
4 R^1=OAc, R^2=H
5 R^1=R^2=O
6 R^1=H, R^2=OH

Montanin F (**3**) (*Teucjaponin A*)

Montanin F was a colourless crystalline compound with mp 143–146°. The elementary analysis and mass spectrometry indicated the molecular formula $C_{22}H_{28}O_7$. Its IR spectrum showed characteristic absorptions for a furan ring (3135, 1600, 1505 and 875 cm^{-1}), a γ-lactone (1740 cm^{-1}), ester group (1725 and 1250 cm^{-1}) and a hydroxyl group (3480 cm^{-1}). The presence of this last function was confirmed because acetic anhydride-pyridine treatment of compound **3** yielded a diacetyl derivative (**4**).

The ^1H NMR spectrum of **3** (Table 2) showed signals for a secondary methyl group at δ 1.02 (3H, *d*, J = 6.5 Hz) and for a β-substituted furan ring (two α-furan protons, 7.46 *dd* and 7.44 *br s* and one β-furan proton at δ 6.40 *dd*). The signal at δ 5.37 (1H, *dd*, J = 8 Hz) was assigned to the lactonic C-12 proton. The furan ring and γ-lactone are in the same position as in the other clerodane diterpenoids [4]. The singlet at δ 2.11 for 3H was assigned to the acetyl group. In addition to the signals at δ 4.98 and 4.91 (2H, AB system, J = 12 Hz, C-19) and 3.78 and 2.28 (2H, J_{gem} = 5.5 Hz, $J_{18,3ax}$ = 1.5 Hz, C-18), the ^1H NMR spectrum showed a signal at δ 4.22 (1H, *t*, J = 3 Hz), which was assigned to the equatorial 6α-proton [4, 5]. The secondary nature of the hydroxyl group in **3** was established by the shift of the triplet from δ 4.22 to 5.13 (J = 3 Hz) after acetylation. According to the ^1H–^1H couplings, H-6 in **3** and **4** was equatorial. The structure **3** was confirmed also by ^{13}C NMR data which are presented in Table 2. The structure and stereochemistry of **3** was deduced by spin decoupling and also by correlation with 19-acetyl-

Table 2. ^1H NMR spectral data of **3** and **4** and ^{13}C NMR chemical shifts (ppm from internal TMS) of **3** and **6** (400 MHz)

	3(CDCl$_3$)	4 (CDCl$_3$)		3	6
1α-H	1.67 *dddd*	—	C-1	23.43 *t*	22.66 *t*
1β-H	1.88 *br d*	—	C-2	25.03 *t*	24.96 *t*
2α-H	2.05 *br d*	—	C-3	32.81 *t*	31.35 *t*
2β-H	—	—	C-4	64.24 *s*	66.56 *s*
3α-H	1.05 *ddd*	—	C-5	46.24 *s*	45.31 *s*
3β-H	—	—	C-6	64.90 *d*	73.45 *d*
6α-H	4.22 *br d*	5.13 *br d*	C-7	33.72 *t*	33.88 *t*
7α-H	2.37 *ddd*	2.20 *ddd*	C-8	35.94 *d*	38.27 *d*
7β-H	1.54 *ddd*	1.84 *ddd*	C-9	52.51 *s*	51.17 *s*
8β-H	2.17 *dd q*	—	C-10	53.10 *d*	52.42 *d*
10β-H	—	—	C-11	45.24 *t*	43.52 *t*
11′-H	2.51 *dd*	2.51 *dd*	C-12	72.06 *d*	71.49 *d*
11-H	2.41 *dd*	2.42 *dd*	C-13	126.40 *s*	125.22 *s*
12-H	5.37 *dd*	5.39 *dd*	C-14	108.91 *d*	108.06 *d*
14-H	6.40 *dd*	6.39 *dd*	C-15	144.67 *d*	144.20 *d*
15-H	7.46 *dd*	7.46 *dd*	C-16	140.49 *d*	139.61 *d*
16-H	7.44 *br s*	7.44 *dd*	C-17	16.74 *q*	15.52 *q*
17-H	1.02 *d*	0.99 *d*	C-18	46.36 *t*	48.56 *t*
18-H	3.78 *dd*	2.97 *dd*	C-19	62.32 *t*	61.75 *t*
18′-H	2.28 *d*	2.27 *dd*	C-20	178.03 *s*	176.04 *s*
19-H	4.98 *d*	5.03 *d*	Ac–Me–	21.13 *q*	21.23 *q*
19′-H	4.91 *d*	4.95 *d*	Ac–CO–	170.92 *s*	170.64 *s*
OH	1.19 *d*	2.08 *s*			
OAc	2.11 *s*	2.09 *s*			

J (Hz) 1α,1β = 1α,2β = 12; 1α,2α = 1β,2β = 1β,10β = 4; 7α,7β = 7α,8β = 15; 7β,8 = 4; 6α,7α = 6α,7β = 3; 11,11′,12 = 8; 18,18′ = 5.5; $18,3_{ax}$ = 1.5; 19,19′ = 12.

gnaphalin [5,9] described as a natural clerodane type diterpene occuring in *Teucrium hyrcanicum* and *Teucrium gnaphalodes*. Treatment of **3** with chromium trioxide in dry pyridine at room temperature for 20 hr gave **5** [10]. Therefore montanin F is a diastereoisomer of teupolin I (**6**) [11] at C-6. The ^{1}H NMR and mass spectral data of montanin F were in good agreement with those of teucjaponin A, already reported as a constituent of *Teucrium japonicum* [12].

EXPERIMENTAL

Mps were determined on a Kofler apparatus and are uncorr. ^{1}H NMR and ^{13}C NMR spectra were obtained in pyridine-d_5 or $CDCl_3$ soln with TMS as int. standard. The ^{13}C NMR spectra were recorded at 100.6 MHz. Plant material was collected in June 1980 at Rhodopes mountain, Bulgaria.

Extraction and isolation of the diterpenoids. Dried and finely powdered *T. montanum* aerial parts (1 kg) were extracted with Me_2CO (10 l.) at room temp. for 1 week. After evaporation the residue was treated as in ref. [13]. The $CHCl_3$ extract (8 g) was passed through a silica gel column (400 g) Merck 0.05–0.2 mm (deactivated with 10% H_2O). Elution with petrol–$CHCl_3$ (2:8) gave a mixture of montanins A, C and F (teucjaponin A) (6 g). Further elution with $CHCl_3$, $CHCl_3$–MeOH (99:1) gave a mixture of montanin D and B (1.8 g). The elution with $CHCl_3$–MeOH (90:10) yielded montanin E (0.120 g). The mixture of montanins A, C and F was easily separated on a silica gel (0.063–0.200,/200 g/Merck, deactivated with 10% H_2O) column. Elutcion with C_6H_6 yielded montanin A. Elution with petrol–$CHCl_3$ (3:7) gave montanin C and montanin F (petrol–$CHCl_3$, 1:9; 0.9 g).

Montanin E (**1**). Mp 219–223°C; $[\alpha]_D^{28}$ +23.5° (*c* 0.136, Me_2CO); IR ν_{max}^{KBr} cm^{-1}: 3280–3400, 3160, 2960, 2920, 2880, 1755, 1600, 1510, 1480, 1440, 1390, 1330, 1275, 1220, 1180, 1150, 1140, 1110, 1050, 1040, 1030, 990, 980, 930, 940, 895, 872, 850, 792, 750. MS (75 eV, direct inlet) *m/z* (rel. int.): 380 $[M]^+$ (22), 362 $[M-H_2O]^+$ (10), 349 $[M-CH_2OH]^+$ (22), 344 $[362-H_2O]^+$ (20), 331 $[349-H_2O]^+$ (26), 326 $[344-H_2O]^+$ (12), 302 (18), 301 (80), 255 (14), 232 (22), 219 (27), 201 (13), 199 (14), 189 (16), 161 (28), 145 (24), 133 (26), 119 (34), 105 (44), 96 (48), 95 (100), 94 (62), 91 (50), 81 (76), 55 (50). (Found: C, 63.11; H, 7.29. $C_{20}H_{28}O_7$ requires: C, 63.01; H, 7.37%.) ^{1}H NMR and ^{13}C NMR spectral data: see Table 1.

Acetylation of **1** *to* **2**. Acetylation of **1** (40 mg) with Ac_2O (0.7 ml) and pyridine (1–3 drops) yielded crude acetate (30 mg) which on recrystallization from Me_2CO–Et_2O yielded **2**, mp 152–157°. IR ν_{max}^{KBr} cm^{-1}: 3560, 3130, 2975, 2880, 1750, 1730, 1725, 1510, 1240, 1150, 1030, 872. MS (75 eV, direct inlet) *m/z* (rel. int.): 488 $[M-H_2O]^+$ (1.2), 464 $[M-\text{ketene}]^+$ (1.3), 446 $[M-AcOH]^+$ (7), 386 $[446-AcOH]^+$ (41), 373 $[346-CH_2OAc]^+$ (90), 368 $[386-H_2O]^+$ (10), 313 (32), 301 (22), 274 (92), 232 (18), 225 (20), 218 (30), 214 (34), 176 (40), 173 (38), 159 (44), 157 (22), 145 (29), 133 (30), 132 (36), 131 (26), 119 (43), 106 (42), 105 (38), 96 (34), 95 (100), 94 (96), 81 (78). ^{1}H NMR spectral data: see Table 2.

Montanin F (**3**) (*Teucjaponin A*). Mp 143–146°. $[\alpha]_D^{28}$ +46° (*c* 0.178, Me_2CO). IR ν_{max}^{KBr} cm^{-1}: 3480, 3135, 2940, 2860, 1750, 1725, 1600, 1505, 1460, 1400, 1380, 1250, 1160, 1100, 1040, 980, 920, 875, 740. MS (75 eV, direct inlet) *m/z* (rel. int.): 404 $[M]^+$ (1.3), 386 $[404-H_2O]^+$ (1.6), 362 $[M-\text{ketene}]^+$ (0.3), 344 $[404-AcOH]^+$ (14), 331 (38), 326 (11), 344 $[362-H_2O]^+$ (19), 314 (32), 313 (40), 232 (12), 220 (14), 204 (13), 191 (12), 179 (30), 161 (28), 159 (22), 148 (20), 136 (19), 123 (20), 121 (19), 105 (29), 96 (66), 95 (100), 94 (40), 81 (58). (Found: C, 65.72; H, 7.10. $C_{22}H_{28}O_7$ requires: C, 65.41; H, 6.98%.) ^{1}H NMR and ^{13}C NMR spectral data: see Table 2.

Acetylation of **3**. Ac_2O–pyridine treatment of compound **3** (25 mg) gave the derivative **4** (18 mg, after recrystallization from Me_2CO–Et_2O, Mp 154–157°. IR ν_{max}^{KBr} cm^{-1}: 2900, 1750, 1730, 1600, 1500, 1442, 1370, 1255, 1090, 1020, 870, 800. MS (75 eV, direct inlet) *m/z* (rel. int.): 446 $[M]^+$ (0.3), 386 $[M-AcOH]^+$ (3), 373 $[M-CH_2OAc]^+$ (4.2), 358 (2.1), 344 $[386-\text{ketene}]^+$ (7.2), 331 (12), 326 $[344-H_2O]^+$ (13), 314 (34), 313 (13), 298 (14), 220 (19), 204 (32), 199 (16), 191 (18), 187 (22), 159 (42), 156 (48), 149 (40), 139 (44), 135 (45), 119 (30), 105 (38), 96 (98), 95 (100), 91 (38), 81 (68), 69 (58). ^{1}H NMR spectral data: see Table 2.

Oxidation of **3** *to* **5**. Treatment of **3** (40 mg) in dry pyridine (2 ml) with chromium trioxide (80 mg) after work up afforded crude crystals (23 mg), which on recrystallization from MeOH–CH_2Cl_2 yielded **5** (mp 225–228°). MS: *m/z* 402 $[M]^+$. (Calc. for $C_{22}H_{26}O_7$: C, 65.66; H, 6.58. Found: C, 65.57; H, 6.60%.) IR ν_{max}^{KBr} cm^{-1}: 1746, 1725, 1710, 1600, 1505, 1490, 1250 and 872. ^{1}H NMR (100 MHz, $CDCl_3$): δ 3.51 (1H, *t*, *J* = 14 Hz, H-7); 2.42 (1H, *d*, *J* = 8.5 Hz, H-11); 5.42 (1H, *t*, *J* = 8.5 Hz, H-12); 5.03 and 5.47 (2H, AB *q*, *J* = 12 Hz, H-19); 1.06 (3H, *d*, *J* = 6.5 Hz, H-17); 6.40 (1H, *m*, H-14); 7.45 (2H, *m*, H-15, H-16) and 2.06 (3H, *s*, OAc). Identical in all respects (mp, mmp, IR, ^{1}H NMR, MS, TLC) with the natural 19-acetylgnaphalin (**5**).

Acknowledgements—We thank Dr. F. Bohlmann, Dr. J. Ziesche and T. V. Beshin for the 400 MHz ^{1}H NMR spectra and useful discussion.

REFERENCES

1. Malakov, P. Y., Papanov, G. Y. and Mollov, N. M. (1978) *Tetrahedron Letters* **23**, 2025.
2. Malakov, P. Y., Papanov, G. Y., Mollov, N. M. and Spassov, S. L. (1978) *Z. Naturforsch. Teil* B **33**, 789.
3. Malakov P. Y., Papanov G. Y., Mollov, N. M. and Spassov, S. L. (1978) *Z. Naturforsch. Teil B* **33**, 1142.
4. Gacs-Baitz, E., Kajtar, M., Papanov, G. Y. and Malakov, P. (1982) *Heterocycles*, Vol. 19, No 3, 539.
5. Gacs-Baitz, E., Radics, L., Oganessian, G. and Mnatsakanian, V. (1978) *Phytochemistry* **17**, 1967.
6. Bruno, M., Savona, G., Pascual, C. and Rodriguez, B. (1981) *Phytochemistry* **20**, 2259.
7. Papanov, G. and Malakov, P. (1982) *Z. Naturforsch. Teil B* **37**, 519.
8. Savona, G., Garcia-Alvarez, M. and Rodriguez, B. (1982) *Phytochemistry* **21**, 721.
9. Savona, G., Paternostro, M., Piozzi, F. and Rodriguez, B. (1979) *Tetrahedron Letters* **4**, 379.
10. Papanov, G. and Malakov, P. (1981) *Z. Naturforsch. Teil B* **36**, 112.
11. Malakov, P., Papanov, G. and Mollov, N. (1979) *Z. Naturforsch. Teil B* **34**, 1570.
12. Toshio, M., Hiroko, K., Tadataka, N., Akira, U., Seigo, F. and Tsunematsu, T. (1981) *Chem. Pharm. Bull.* **29**, 3561.
13. Popa, D. and Reinbold, A. (1972) *Khim. Prir. Soedin.* **1**, 57.