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12-EPITEUPOLIN II, A NEO-CLERODANE DITERPENOID FROM *TEUCRIUM LAMIIFOLIUM*

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Key Word Index—*Teucrium lamiifolium*; Labiatae; diterpenoids; neo-clerodane derivatives; 12-epiteupolin II; teuscordinon; teuflin; montanin C; 19-acetylgnaphalin.

Abstract—From the aerial parts of *Teucrium lamiifolium*; a new neo-clerodane diterpenoid, 12-epiteupolin II, has been isolated, together with the previously known diterpenes teuscordinon, teuflin, montanin C and 19-acetylgnaphalin.

The structure of 12-epiteupolin II (6α-acetoxy-19-hydroxy-4α, 18:15, 16-diepoxy-neo-clerodane-13 (16),14-dien-20,12*R*-olide) was established by chemical and spectroscopic means and by correlation with montanin C.

INTRODUCTION

In continuation of our studies on the diterpenes from *Teucrium* species [1–9] we have now investigated *T. lamiifolium* D'urv., a species which grows in southeastern Bulgaria. From the aerial parts of this plant we have isolated four previously known neo-clerodane diterpenoids, teuscordinon [10], teuflin [11], montanin C [8], 19-acetylgnaphalin [12], and a new diterpenoid, 12-epiteupolin II (**1**), whose structure and absolute configuration have been established on the basis of spectroscopic evidence, chemical transformations and correlation with montanin C (**3**).

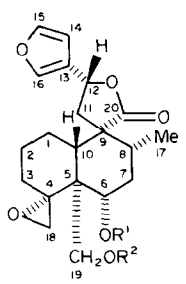
RESULTS AND DISCUSSION

12-Epiteupolin II (**1**), $C_{22}H_{28}O_7$, had an IR spectrum which was consistent with the presence of a hydroxyl group (3630 cm^{-1}), a furan ring (3140, 1505, 1600 and 875 cm^{-1}), an ester group (1740, 1240 cm^{-1}) and a γ-lactone (1760 cm^{-1}). The ^1H NMR spectrum of the new diterpenoid (**1**, Table 1) revealed the existence of a secondary methyl group (δ1.12, *d*, J = 6.5 Hz), a β-substituted furan ring (two α-furan protons at δ7.45 and one β-furan proton at δ6.37), an α,α-disubstituted oxirane ring (two protons forming an AB system at 2.26 and 2.90 J = 4 Hz), and an acetoxyl group which must be placed at the C-6 position one proton double doublet at δ4.80, J_1 = 15, J_2 = 4 Hz). The presence of a primary hydroxyl group in **1** was revealed by the ^1H NMR spectrum of its derivative obtained by treatment with chloroacetylisocyanate. The resonance of the corresponding NH proton appeared at δ8.43 as a singlet, while the triplet at 4.06 (J = 12 Hz) (H-19A, OH) was now a doublet at 4.42 (J = 13 Hz) and the doublet of H-19B at 4.76 was paramagnetically shifted at 5.65 (J = 13 Hz), thus confirming that the free alcohol group of **1** was a primary one. Moreover, the ^1H NMR spectrum of compound **1** (Table 1) was almost identical with that of teupolin II (**2**) a diterpenoid previously isolated from *T. polium* [2, 5]. In fact, the difference between the ^1H NMR spectra of **1** and **2** was only in the chemical shift of the C-17 methyl protons, which appeared at a slightly lower field in **1** (δ1.12) than in **2** (0.99) (Table 1). On the other hand, comparison between the ^{13}C NMR spectra of 12-epiteupolin II (**1**) and teupolin II (**2**) (Table 2) showed a significant difference in the C-8 (Δδ +2.8) and C-10 (Δδ −2,1) chemical shifts.

These data clearly established that the structural difference between compounds **1** and **2** must be only the configuration of its C-12 centre [13, 14]. Moreover, the (12 *R*)-configuration of **1** was also in agreement with NOE experiments, since irradiation of the Me-17 protons of 12(-epiteupolin II caused a 7% NOE enhancement of the H-12 signal (δ5.40) [13, 15, 16].

Final proof that 12-epiteupolin II has the structure and absolute configuration depicted in formula **1** was obtained by acetic anhydride-pyridine treatment of compound **1**. This gave a diacetyl derivative (**3**) identical in all respects ($[\alpha]_D$, ^1H NMR, ^{13}C NMR and mmp) with natural montanin C [8, 13].

12-Epiteupolin II, montanin C [8, 13] and montanin G [16, 17] are the only neo-clerodane-20, 12-olides isolated from Bulgarian *Teucria* so far possessing the infrequent (12*R*)-configuration.



R¹ R²
1 Ac H
3 Ac Ac

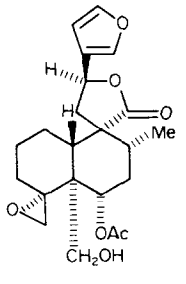


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Table 1. ^{1}H NMR data of compounds **1** and **2** (250 MH, $CDCl_3$ solution, TMS as int. standard)*

H	1	2	Δppm
3α	†	2.50 *m*	–
3β	†	1.09 *m*	–
6β	4.80 *dd*	4.86 *dd*	−0.06
7β	1.59 *m*	1.61 *m*	−0.02
8β	†	2.00 *m*	–
11A	2.35 *d*	2.38 *d* (8.2Hz)	
11B	2.15 *d*		
12	5.40 *t*	5.35 *t*	−0.05
14	6.37 *m*	6.39 *m*	−0.02
15	7.45 *m*	7.44 *m*	+0.01
16			
17	1.12 *d*	0.99 *d*	+0.13
18A	2.90 *dd*	2.92 *dd*	−0.02
18B	2.26 *d*	2.23 *d*	+0.03
19A	4.06 *t*‡	4.00 *t*‡	+0.06
19B	4.76 *d*	4.71 *d*	+0.5
COMe	2.12 *s*	2.045 *s*	+0.08

J(Hz) **1**,2: 6β,7β = 4; 6β,7α = 15; 11A,11B = 14; 11A, 12 = 11B, 12 = 8; 17, 8β = 6.5; 18A,18B = 4; 18Δ,3α = 2; 19A,19B = 13; 19A,OH = 12.

*All these assignments have been confirmed by double resonance experiments.

†Overlapped signal.

‡Collapsed into a *d* after addition of D_2O.

Table 2. ^{13}C NMR chemical shifts of compounds **1** and **2** ($CDCl_3$, TMS as int. standard).

C	1	2	Δδ
1	22.1 *t*	22.8 *t*	−0.7
2	24.9 *t*	24.9 *t*	0.0
3	31.9 *t**	31.8 *t**	+0.1
4	65.5 *s*	65.2 *s*	+0.3
5	46.2 *s*	46.3 *s*	−0.1
6	73.9 *d*	73.7 *d*	+0.2
7	32.7 *t**	32.4 *t**	+0.3
8	40.7 *d*	37.9 *d*	+2.8
9	51.2 *s*	50.8 *s*	+0.4
10	50.7 *d*	52.8 *d*	−2.1
11	43.6 *t*	43.2 *t*	+0.3
12	71.4 *d*	71.5 *d*	−0.1
13	125.3 *s*	125.1 *s*	+0.2
14	107.9 *d*	108.1 *d*	+0.2
15	144.2 *d*	144.2 *d*	0.0
16	139.1 *d*	139.6 *d*	−0.5
17	16.8 *q*	16.5 *q*	+0.3
18	47.4 *t*	47.3 *t*	+0.1
19	61.5 *t*	60.8 *t*	+0.7
20	176.1 *s*	176.3 *s*	−0.2
OAc	169.6 *s*	169.5 *s*	+0.1
	21.3 *q*	21.2 *q*	+0.1

*Assignments bearing the same sign may be reversed.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ soln with TMS as int. standard. Plant materials were collected in July 1984 near Malko Tirnovo, Bulgaria.

Extraction and isolation of the diterpenoids. Dried and finely powdered *T. lamiifolium* aerial parts (1.28 kg) were extracted with Me_2CO (30 l) at room temp. for a week. After evpan of the solvent, the residue was treated as in refs [18, 19]. The $CHCl_3$ extract (29 g) was chromatographed over a silica gel column (Merck, 7734, deactivated with 10% H_2O, 500 g) eluted with petrol, CH_2Cl_2–petrol mixtures and pure CH_2Cl_2. Elution with CH_2Cl_2–petrol (2:8) gave teuflin (60 mg) and teuscordinon (40 mg); elution with CH_2Cl_2 gave 19-acetylgnaphalin (38 mg), montanin C (400 mg) and 12-epiteupolin II (**1**) (80 mg).

The previously known diterpenoids, teuflin, teuscordinon, 19-acetylgnaphalin and montanin C, were identified by their physical (mp. $[\alpha]_D$ and spectroscopic (IR, ^{1}H NMR, MS) data and by comparison with authentic samples (mmp, TLC).

12-Epiteupolin II (**1**) Mp 173–175° (from Me_2CO–petrol); $[\alpha]_D^{20}$ +14° (acetone; *c* 0.33). IR ν_{max}^{KBr} cm^{-1}: 3630, 3140, 2995, 2860, 1760, 1740, 1505, 1420, 1380, 1350, 1240, 1180, 1145, 1030, 970, 920, 875, 850, 805, 655, 605. ^{1}H NMR and ^{13}C NMR (see Tables 1 and 2). EIMS (direct inlet) 75 eV: *m/z* (rel. int.): 404 $[M]^+$ (18), 312 (38), 369 (22), 220 (28), 218 (28), 187 (30), 159 (21), 157 (19), 96 (98), 95 (88), 94 (20), 91 (40), 81 (48), 43 (100). (Found: C, 65.77; H, 7.12%. $C_{22}H_{28}O_7$ requires: C, 65.41; H, 7.01%)

Acetylation of **1** *to give* **3**. A soln of 25 mg of 12-epiteupolin II (**1**) in 0.5 ml of pyridine and 0.2 ml of Ac_2O was allowed to stand overnight at room temp. Usual work-up and recrystallization from EtOAc–Et_2O gave 22 mg of **3**, identical in all respects (mmp, $[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR, MS, TLC) with natural montanin C.

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TRITERPENOIDS FROM *AGRIMONIA PILOSA*

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Key Word Index—*Agrimonia pilosa*; Rosaceae; triterpenoid; 19α-hydroxyursolic acid.

Abstract—Two new triterpenoids have been isolated as a methylester from the whole plant of *Agrimonia pilosa*, along with 2α,19α-dihydroxyursolic acid (28-1)β-D-glucopyranoside from the roots of this plant. On the basis of chemical and spectral evidence, the structures were established as 1β,2α,3β,19α-tetrahydroxyurs-12-en-28-oic acid and 1β,2β,3β,19α-tetrahydroxyurs-12-en-28-oic acid.

INTRODUCTION

Agrimonia pilosa Ledeb is widely distributed in Asia. The chemical components of this plant have been extensively examined and agrimonolide [1], luteolin 7-*O*-β-D-glucoside, apigenin 7-*O*-β-D-glucoside [2, 3], agrimol A, B and D [4] agrimophol [5] and tannins [6, 7] were obtained. The antitumour activity of the extracts from the roots of this plant was also reported [8]. We report now on the constituents of *Agrimonia pilosa*.

RESULTS AND DISCUSSION

The methanol extract of the aerial parts of *A. pilosa* was fractioned by the usual procedure (Experimental) to afford a triterpenoid fraction. This was treated with diazomethane in methanol because of the difficulty of separation, and compounds **1** (45 mg) and **2** (112 mg) were isolated. The ^{1}H NMR spectrum of compound **1** $C_{31}H_{50}O_6$ (EIMS, *m/z* 518) showed the characteristic broad singlet at δ2.57, together with the tertiary methyl [δ0.68, 0.83, 1.02 (2Me), 1.22 (2 Me)], the secondery methyl (0.94, *d*, *J* = 6.4 Hz), the ester methyl (3.60) and the olefinic (5.35, *t*, *J* = 3.4 Hz) protons, all of which suggested a 19α-hydroxyurs-12-en type of triterpenoid. The olefinic carbon signals (δ130.0, C-12; 137.3, C-13) in the ^{13}C NMR spectrum of **1** also indicated that **1** had an urs-12-en skeleton [9]. Although the hydroxy methine protons were not obvious in the ^{1}H NMR spectrum of **1**, an acetate (**1a**) of **1** showed the signals of three acetoxyls and three acetoxy methine groups. The latter of which exhibited two doublets (δ4.79, *J* = 10.6 Hz; 4.88, *J* = 9.3 Hz) and a double doublet (δ5.22, *J* = 10.6 and 9.3 Hz), and were assignable to C-1 (or 3), C-3 (or 1) and C-2, respectively. Three hydroxy methine carbons were also indicated in the ^{13}C NMR spectrum of **1** (δ74.6, 74.9, 79.9). As the *J*-values of these signals indicated trans-diaxial correlated protons, the three acetoxyls must be equatorial. It was concluded that compound **1** was 1β,2α,3β,19α-tetrahydroxyurs-12-en-28-oate, and that the natural compound should be originally 1β,2α,3β,19α-tetrahydroxyurs-12-en-28-oic acid (**1b**).

The ^{1}H NMR spectrum of compound **2** ($C_{31}H_{50}O_6$; EIMS, *m/z* 518) showed a broad singlet at δ2.59 similar to that of **1**. As the other signals of the ^{1}H NMR and the ^{13}C NMR spectra of **2** were quite similar to those of **1**, compound **2** was considered to posses the same skeleton as **1**. An acetate (**2a**) of **2** showed in the ^{1}H NMR spectrum the signals of three acetoxyls and three acetoxy methine protons. These methine protons appeared at δ4.70 (*d*, *J* = 3.7 Hz, C-1 or 3), 4.75 (*d*, *J* = 3.7 Hz, C-3 or 1) and 5.44 (*t*, *J* = 3.7 Hz, C-2) compared to those of **1**, and these coupling constants were accommodated on an axial–equatorial–axial correlation for these protons. These findings indicated **2** is 1β,2β,3β,19α-tetrahydroxyurs-12-en-28-oate, and that the naturally occurring compound should be 1β,2β,3β,19α-tetrahydroxyurs-12-en-28-oic acid (**2b**).

An acetonide reaction of **2** with acetone and *p*-toluene sulphonic acid yielded two compounds **2d** and **2e** (trace), although the same reaction for **1** gave only the starting

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