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TERPENOIDS FROM *ACHILLEA CLYPEOLATA*

MILKA N. TODOROVA,* MARIA L. KRASTEVA, MIGLENA M. MARKOVA,
ELENA T. TSANKOVA, RILKA ML. TASKOVA† and DIMITAR R. PEEV†

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences,
1113 Sofia, Bulgaria and †Institute of Botany, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

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Key Word Index—*Achillea clypeolata*; Asteraceae; sesquiterpenoids; eudesmane alcohol clypeotriol; guaianolides.

Abstract—A new sesquiterpene alcohol named clypeotriol and characterised as eudesm-4(15)-en-3α,7α, 11-triol was isolated from *Achillea clypeolata* collected in south-western Bulgaria. Populations of the same taxon growing in the eastern regions of Bulgaria afforded only sesquiterpene lactones two of which were the novel guaianolides 3α,4α-epoxyrupicolin-A and 3α,4α-epoxyrupicolin-B. The structures of the new compounds were elucidated on the basis of spectral data. © 1998 Published by Elsevier Science Ltd. All rights reserved

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INTRODUCTION

Achillea clypeolata Sibth.et Sm. is among those *Achillea* species which have not received much attention. This is most probably due to the fact that its distribution is restricted to the Balkan peninsula extending northwards to south-east Romania [1]. Hitherto, there are only three papers dealing with *A. clypeolata*. These report on the epicuticular flavonoids in Bulgarian population [2], the essential oil composition of taxa of Romanian origin [3] and diterpenes in the roots of Jugoslavian populations [4]. It should be noted that the aerial parts of the latter have not been found to contain any terpenoids apart from β-sitosterol and stigmasterol [4].

In continuation of our chemical investigations of Asteraceae species used in folk medicine, we have now studied several Bulgarian populations of *A. clypeolata* and the results are discussed in this paper.

RESULTS AND DISCUSSION

Six *A. clypeolata* populations occuring in different regions of Bulgaria were subject of the present study. The plant samples were collected in north-east Bulgaria (samples 1–4), south-east Bulgaria (sample 5) and south-west Bulgaria (sample 6). The air-dried aerial parts of each sample were extracted with chloroform and the total extracts obtained, E_1–E_6, were subjected to TLC comparison using some sesquiterpene lactones as references. The extracts E_1–E_5 turned out to be practically identical regarding the main constituents, which were lacking almost completely in E_6. The absence of sesquiterpene lactones in E_6 was further confirmed by the absence of the typical IR absorption at 1750–1775 cm^{-1}. Working-up of E_6 as described in the Experimental led to isolation of a new sesquiterpene alcohol **1** for which we propose the name clypeotriol. The molecular formula of **1** was established as $C_{15}H_{26}O_3$ (CIMS: *m/z* 272 $[M + NH_4]^+$). The IR absorption at 1650 and 3650 cm^{-1} suggested the presence of a double bond and a hydroxyl group. The base peak in the EIMS spectrum at *m/z* 195 $[M - 59]^+$ obtained by loss of a hydroxyl-bearing isopropyl group $[(CH_3)_2C{=}OH]$ and the prominent peaks at *m/z* 178 and 159, together with the signals at δ 73.5 *d*, 75.4 *s* and 76.4 *s* in the ^{13}C NMR spectrum (Table 1) indicated the presence of one secondary and two tetriary hydroxyl groups. This was in good agreement with the ^{1}H NMR spectrum (Table 1) which exhibited a single carbinol proton at δ 4.30. Furthermore, the signals for three methyl groups and an exocyclic methylene group (δ 4.57 and 4.96) suggested the eudesmane skeleton for **1** and a structure closely related to β-eudesmol. The second tetriary hydroxyl group was assumed to be attached to C-7, as the H-15/H-5/H-6 connectivity was deduced from the COSY spectrum and the

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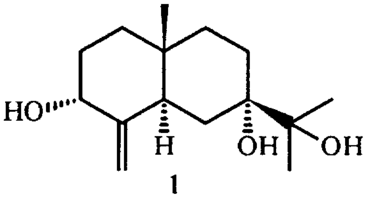
*Author to whom correspondence should be addressed.



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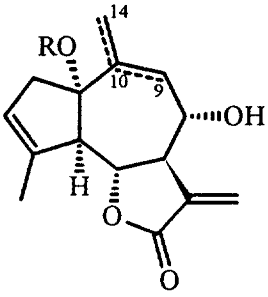
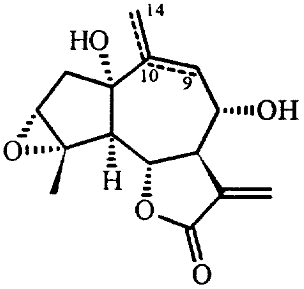


R
2 H, $\Delta^{9,10}$
3 H, $\Delta^{10,14}$
4 OH, $\Delta^{9,10}$
5 OH, $\Delta^{10,14}$



6 $\Delta^{9,10}$
7 $\Delta^{10,14}$

complex doublet at δ 2.71 was attributable to H-5 only. The proposed stereochemistry at C-7 was based on both biogenetic considerations and the observed shift of C-5 and C-9 in the higher fields when compared with β-eudesmol [5] and its 3α-hydroxy derivative [6]. The Dreiding model shows that such an upfield shift is possible only if the hydroxyl group at C-7 is axial. Finally, the location of the secondary hydroxyl group at C-3 followed from the ^1H-^1H COSY spectrum and its α-disposition was demonstrated by the coupling constants of its geminal proton ($J_{3,2} = J_{3,2'} = 2.8$ Hz). Consequently, the new sesquiterpene alcohol **1** was identified as eudesm-4(15)-en-3α,7α,11-triol. It is noteworthy to remark that chenopotriol and 3-*epi*-chenopotriol from *Chenopodium botrys* (Salsolaceae) [7] and longilobol from *Artemisia longiloba* (Asteraceae) [8] are the only three other dihydroxylated β-eudesmols reported so far.

Table 1. NMR data of compound **1** in $CDCl_3$

H/C	^1H NMR (250 MHz)	^{13}C NMR (62.9 MHz)
1	1.25–1.48 *m**	35.2 t^a
2	1.80 *d* (2.8)**	29.7 *t*
3	4.30 *t* (2.8)	73.5 *d*
4	–	151.8 *s*
5	2.71 *dm* (11.5)	38.3 *d*
6	1.50 *m***	28.9 *t*
7	–	75.4 s^b
8	1.25–1.48 *m**	26.5 *t*
9	1.25–1.48 *m**	35.9 t^a
10	–	35.4 *s*
11	–	76.4 s^b
12	1.25 *s*	24.6 q^c
13	1.26 *s*	24.7 q^c
14	4.57 *t* (1.6) 4.96 *t* (1.4)	108.8 *t*
15	0.68 *s*	14.5 *q*

* Overlapping signals with intensity for 6 protons.
** Assignment based on decoupling and COSY experiment.
a, b, c Assignment may be interchanged.

Since the extracts E_1–E_5 showed identical TLC spots, only E_5 was subjected to chromatoraphic separation. Relevant fractions were further separated by prep. TLC to afford seven sesquiterpene lactones. By analogy of their spectral data to those published, five were assigned as rupicolin-A, **2**, and rupicolin-B, **3** [9], 1-desoxy-1α-peroxy-rupicolin-A, **4**, and 1-desoxy-1α-peroxy-rupicolin-B, **5** [10], and ridentin-B [9]. However, the remaining two lactones, **6** and **7**, were shown to be new natural products. Their structures were deduced as 3α,4α-epoxides of rupicolin-A and -B, respectively, from the following spectroscopic data.

The CIMS spectra of **6** and **7** gave molecular peak at *m/z* 296 $[M + NH_4]^+$ corresponding to the molecular formula $C_{15}H_{18}O_5$. The ^1H and ^{13}C NMR data (Table 2) indicated guaianolide structure similar to **2** and **3** but showed that the Δ^3-double bond was replaced by an oxirane. Selective ^1H decoupling, as well as ^1H–^1H- and ^1H–^{13}C COSY

Table 2. ^1H NMR and ^{13}C NMR data of **6** and **7** in $CDCl_3$

H/C	^1H NMR (250 MHz)		^{13}C NMR (62.9 Hz)	
	6	**7**	**6**	**7**
1	–	–	80.6 *s*	81.9 *s*
2	1.93 *dd*	1.93 *d*	42.2 *t*	41.0 *t**
2′	2.48 *d*	2.17 *brd*		
3	3.54 *brs*	3.57 *brs*	63.1 *d*	64.3 *d*
4	–	–	67.4 *s*	71.1 *s*
5	2.55 *d*	2.30 *d*	60.5 *d*	61.3 *d*
6	3.70 *dd*	3.89 *dd*	75.0 *d*	75.2 *d*
7	3.05 *dddd*	2.88 *dddd*	51.8 *d*	50.3 *d*
8	4.13 *brd*	3.92 *ddd*	70.7 *d*	71.1 *d*
9	5.43 *q*	2.48 *dd*	126.5 *d*	38.9 *t**
9′	–	2.33 *dd*		
10	–	–	137.4 *s**	141.6 *s*
11	–	–	136.2 *s**	137.0 *s*
12	–	–	169.1 *s*	169.1 *s*
13	6.37 *dd*	6.28 *dd*	125.5 *t*	123.4 *t*
13′	6.33 *dd*	6.16 *dd*		
14	1.95 *dd*	5.10 *d*	24.8 *q*	116.9 *t*
14′		5.57 *d*		
15	1.72 *s*	1.66 *s*	19.7 *q*	18.5 *q*

* Assignment may be interchanged; *J* [Hz]: **6**: 2,2′ = 15.0; 2,3 = 1.0; 5.6 = 11.7; 6,7 = 8.5; 7,8 = 11.4; 7,13 = 3.4; 7,13′ = 3.0; 9,14 = 1.3; 8,14 = 1.0; 13,13′ = 1.0; **7**: 2,2′ = 15.3; 5,6 = 11.0; 6,7 = 7,8 = 9.2; 7,13 = 7,13′ = 3.3; 9,9′ = 14.7; 8,9 = 2.7; 8,9′ = 6.0; 13,13′ = 0.6; 14,14′ = 1.7

experiments led to the assignment of all the signals. The trans-disposition of H-5/H-6/H-7/H-8 in both **6** and **7** followed from the corresponding coupling constants and NOE experiments. Since the H-6 signal was not deshielded when compared with those of related lactones with different geometry at C-4, the α-orientation of the epoxide ring was assumed. This was further supported by the observed NOE between the C-4 methyl group and H-6. Accordingly, the new lacctones **6** and **7** are the 3α,4α-epoxyrupicolin-A and -B, respectively.

Guaianolides appeared to characterize *Achillea* species [11] and the cooccurance of rupicolin-A and -B as main constituents, together with their derivatives **4–7** in the collections under study was not unexpected. Only the collection from south-western Bulgaria (sample 6) was not found to contain any sesquiterpene lactones. Similarly, the collection from south-eastern Serbia was reported to be free of lactones [4].

EXPERIMENTAL

Plant material

The aerial parts of *A. clypeolata* were collected in July 1996 from wild growing plants in the following locations: Kaliakra, Balchik, Varna, Jailite (north-east Bulgaria, samples 1–4), Stranga mountain (south-east Bulgaria, sample 5) and Slavjanka mountain (south-west Bulgaria, sample 6). Voucher specimens (SOM 153306-153311) were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

Extraction and isolation

The dried aerial parts of each sample (30–150 g) were extracted at room temperature with $CHCl_3$ to give after evaporation of the solvent *in vacuo* the crude extracts E_1–E_6 in amounts of 2.0, 0.8, 3.2, 1.0, 14.0 and 14.3 g, respectively.

E_6 (14.3 g) was dissolved in EtOH–H_2O (1:1) and precipitated with aq. soln. of $Pb(OAc)_2$. Filtration, concentration *in vacuo* to remove EtOH, followed by extraction with $CHCl_3$ and removal of the solvent *in vacuo* furnished a gum residue (2.8 g) which was subjected to CC on silica gel using a gradient of $CHCl_3$–Me_2CO (up to 10% Me_2CO). Frs. from $CHCl_3$–Me_2CO (15:1) were combined and purified by repeated prep. TLC ($CHCl_3$–Me_2CO, 5:1) to give **1** (10 mg).

E_5 (14.0 g) was worked-up by vacuum liquid chromatography over silica gel (70 g) using $CHCl_3$ and a gradient of $CHCl_3$–Me_2CO (up to 100% Me_2CO). Frs from $CHCl_3$–Me_2CO (5:1) afforded **4** (4 mg), **5** (5 mg), **6** (5 mg), and **7** (3 mg) after further prep. TLC purification. Frs. from Me_2CO gave **2** (10 mg), **3** (12 mg), and ridentin-**B** (15 mg).

Eudesm-4(15)-ene-3,7,11-triol (clypeotriol), **1**

Colourless gum, IR ν_{max}^{film} cm^{-1}: 3640, 1640, 1120, 1050, 905; EIMS *m/z* (rel. int.): 236 $[M-18]^+$ (1), 218 (5), 195 (100), 178 (43), 159 (62), 145 (9), 133 (19), 119 (24), 93 (20), 84 (23), 59 (339), 49 (62).; ^1H- and ^{13}C-NMR: in Table 1.

3α,4α-*Epoxyrupicolin-A*, **6**

Gum, IR ν_{max}^{film} cm^{-1}: 3550, 1760, 1660; CIMS (NH_3) *m/z* (rel. int.): 296 $[M + NH_4]^+$ (100), 279 (8), 261 (10), 243 (5), 215 (3); ^1H- and ^{13}C-NMR: in Table 2.

3α,4α-*Epoxyrupicolin-B*, **7**

Gum, IR ν_{max}^{film} cm^{-1}: 3560, 1760, 1660, 1640; CIMS (NH_3) *m/z* (rel. int.): 296 $[M + NH_4]^+$ (100), 279 (7), 261 (12), 243 (5), 215 (4); ^1H- and ^{13}C-NMR: in Table 2.

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