Terpenoids from *Achillea clusiana*

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Aerial parts of *Achillea clusiana* include two new chrysanthemol derivatives and three new guaianolides in addition to six known sesquiterpenoids. The structures of the new compounds were elucidated by spectroscopic methods.

Introduction

Achillea clusiana Tausch is described in Flora Europea as a strongly aromatic plant occurring in eastern Alps and in the mountains of Bulgaria and Yugoslavia only (Richardson *et al.*, 1976). The limited distribution of this species could be the reason for the total lack of information in the literature about its chemical constituents. In a continuation of our current chemical study on *Achillea* species (Todorova *et al.* 1998; Todorova *et al.* 1998); we now present the results from the phytochemical study of *A. clusiana* and report the isolation of five new terpenoids.

Experimental

Plant material

The above ground parts of *A. clusiana* were collected at the flowering stage in 1996 from Rila mountain. A voucher specimen (SOM 154207) was deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

Extraction and isolation

The air-dried plant material (100 g) was extracted with $CHCl_3$ (2 x 500 ml) at room temperature. After concentration *in vacuo* and working up as described earlier (Todorova *et al.*, 1998), a crude lactone fraction (1.6 g) was obtained. This residue was separated by column chromatography (CC) on silica gel (50 g) using solvent mixture (hexane-acetone) with increasing polarities. Selected fractions were additionally purified by CC and prep. TLC (silica gel, hexane-acetone mixtures) to give **1** (3.4 mg), **2**(4 mg),**3** (7 mg),**4** (18 mg), **5** and **6** (18 mg mixture), **7** (5 mg), **8** (10 mg), **9** (2 mg), **10** (3 mg), and **11** (5 mg).

4-Hydroxy-4,5-dihydro-trans-chrysanthem-5-en-1-acetate (**1**)

Colourless oil, IR ν_{max}^{film} (cm^{-1}): 3600 (OH), 1740, 1250 (OAc); EIMS (70 eV): 212 $[M]^+$ (1), 195 $[M-17]^+$ (55), 153 $[M-18-42]^+$ (3), 135 $[195-60]^+$ (70), 97 (20), 65 (100); ^1H NMR: in Table I.

4-Hydroxy-4,5-dihydro-cis-chrysanthem-5-en-1-acetate (**2**)

Colourless oil, IR ν_{max}^{film} (cm^{-1}): 3600 (OH), 1740, 1250 (OAc); EIMS (70 eV): 212 $[M]^+$ (1), 195 $[M-17]^+$ (50), 153 $[M-18-42]^+$ (5), 135 $[195-60]^+$ (70), 97 (20), 65 (100); ^1H NMR: in Table I.

2α-Hydroxyguaia-3,10(14), 11(13)-trien-12,6α-olide (**9**)

Colourless oil, IR ν_{max}^{film} (cm^{-1}): 3600 (OH), 1770 (γ-lactone); CIMS (NH_3) *m/z* (rel. int.): 264 $[M+NH_4]^+$ (100), 246 $[M]^+$ (45), 228 $[M-18]^+$ (20), ^1H NMR: in Table II.

1α, 4β-Dihydroxy-2,10(14), 11(13)-trien-12,6α-olide (**10**)

Colourless oil, IR ν_{max}^{film} (cm^{-1}): 3600 (OH), 1775 (γ-lactone); CIMS (NH_3) *m/z* (rel. int.) 280 $[M+NH_4]^+$ (100), 262 $[M]^+$ (13), 245 (10), 227 (3); EIMS (70 eV): 262 $[M]^+$ (10), 247 $[M-15]^+$ (100), 244 $[M-18]^+$ (4), 229 (20), 211 (10), 98 (43); ^1H NMR: in Table II.

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2-epi-Chloroklotzchin (**11**)

Colourless oil, IR ν_{max}^{film} (cm^{-1}): 3550 (OH), 1760 (γ-lactone); CIMS (NH_3) *m/z* (rel. int.): 332 $[M+NH_4]^+$ (12), 315 $[M+1]^+$(100), 297 $[M+1-18]^+$ (23), 279 $[297-18]^+$ (10), 261 $[297\text{-}HCl]^+$ (12), 243 $[261-18]^+$ (10); ^1H and ^{13}C NMR: in Table III.

Results and Discussion

An *Achillea clusiana* population occurring in the Rila mountain was the subject of the present study. The $CHCl_3$ extract of the sample was worked up as described bellow to give the corresponding lactone fraction which was subjected to further chromatographic separation yielding eleven compounds. Six of them were assigned, by analogy of their spectral data to those published, as the oxygenated nerolidol derivative **3** (Appendino *et al.*, 1985), the 1,10-secoguaianolides **4** (Huneck *et al*, 1986), **5** and **6** (Tan *et al.*, 1991), and the guaianolides canin (**7**) and artecanin (**8**) (Bohlmann and Zdero, 1982). However, the other five compounds were proved to be new natural terpenoids and their structures elucidated on the basis of their spectroscopic properties.

Table I. ^1H NMR spectral data of **1** and **2** in $CDCl_3$.

H	1	2
1	4.11 dd (11.6, 7.0)	4.10 dd (7.5, 0.8)
1'	4.00 dd (11.6, 8.0)	
2	0.86 ddd (7.0, 8.0, 5.3)	0.98 ddd (7.5, 7.5, 5.0)
3	0.75 dd (9.6, 5.3)	0.75 dd (9.3, 5.0)
4	3.66 br d (9.6)	3.65 d (9.3)
6	4.81 q (1.5)	4.85 q (1.0)
6'	4.95 q (0.9)	4.97 q (1.0)
7	1.80 br s	1.79 br s
9	1.24 s	1.10 s
10	1.15 s	1.11 s
OAc	2.03 s	2.07 s

Compounds **1** and **2** gave rise to identical MS spectra, which exhibited a weak peak at *m/z* 212, in agreement with a molecular formula $C_{12}H_{20}O_3$ and two prominent peaks at *m/z* 195 $[M-17]^+$ and 135 $[M-17-60]^+$. The structural similarity of **1** and **2** was further shown by their ^1H NMR spectra (Table I) which indicated the presence of a cyclopropane ring bearing two gem-methyl groups, an acetoxymethyl and a hydroxyisobutenyl group. These data proved to be very close to those reported for some chrysanthemol hydroperoxides (Zdero *et al.*, 1987; Kastner *et al.*, 1995), thus showing that **1** and **2** were acetylated chrysanthemol derivatives which differed in the stereochemistry at C-2. Thus, the *syn*-orientation of the acetoxymethyl group and one of the methyl groups at C-8 in the *trans*-isomer **1** apparently caused the observed downfield shift of the corresponding signal (δ 1.24) and the nonequivalency of the C-1 methylene protons. Further, the upfield shift of the H-2 signal in **1** was clearly due to the shielding effect of the hydroxyisobutenyl group. Hence, **1** and **2** were confirmed as 4-hydroxy-4,5-dihydro-*trans*-chrysanthem-5-en-1-acetate and 4-hydroxy-4,5-dihydro-*cis*-chrysanthem-5-en-1-acetate, respectively.

Chrysanthemol derivatives appeared not to be common compounds for the genus *Achillea*, as they have been isolated only from *A.nobilis* so far (Kastner *et al.*, 1995).

Compound **9** was assigned molecular formula $C_{15}H_{18}O_3$ on the basis of its CIMS spectrum

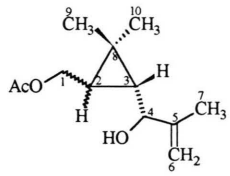
Table II. ^1H NMR spectral data of **9** and **10** in $CDCl_3$.

H	9	12[a]	10	13[b]
1	2.90 dd (3.4, 8.0)	3.15 dd (6.0, 9.0)		
2	4.78 brd (3.4)	4.73 brd (6.2)	5.92 d (5.7)	5.61 d (5.5)
3	5.71 dq (1.7, 1.0)	5.72 dq (2.0, 1.0)	6.13 d (5.7)	5.94 d (5.5)
5	3.07 dd (10.0, 8.0)	2.68 brt (9.0)	2.20 d (11.0)	2.42 d (11.0)
6	4.02 dd (9.0, 10.0)	4.23 dd (9.0, 10.0)	4.41 dd (9.8, 11.0)	4.12 dd (11.0, 9.0)
7	2.83 m	2.84 m	2,69 m	3.29 m
13	6.23 d (3.5)	6.20 d (3.5)	5.51 d (3.2)	5.56 d (3.0)
13'	5.51 d (3.2)	5.48 d (3.0)	6.23 d (3.4)	6.25 d (3.5)
14	4.92 brs	5.08 brs	4.90 d (2.4)	4.75 brs
14'	4.83 brs	6.01 brs	4.94 d (2.4)	4.96 brs
15	1.93 brs	1.95 d (1.0)	1.60	1.34 s

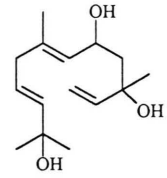
[a] See Zdero and Bohlmann (1989).
[b] See Jakupovic *et al.* (1991).

($[M+NH_4]^+$ at *m/z* 264). The structure followed from the ^1H NMR data (Table II). All the signals could be assigned by spin decoupling and the stereochemistry of the annelation of the hydroazulene system and the lactone ring could be deduced from the corresponding coupling constants $J_{1,5}$=8.0 Hz and $J_{6,7}$=9.0 Hz. The only remaining problem was the stereochemistry at C-2. Comparison of the ^1H NMR of **9** with those of **12** (Zdero and Bohlmann, 1989) revealed a significant similarity (see Table II.). However, the observed downfield shift of H-5 (δ 3.07) and upfield shift of H-6 (δ 4.02) compared to the corresponding signals in **12** (δ 2.68 and 4.23, respectively) could only be due to a change in the relative stereochemistry at C-2. The suggestion that the newly isolated lactone **9** is the C-2 epimer of **12** was further confirmed by the difference in the vicinal coupling of the carbinolic proton (see Table II). Therefore, **9** was assigned as 2α-hydroxyguaia-3,10(14), 11(13)-trien-12,6-olide.

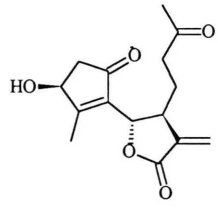
Compound **10** had the composition $C_{15}H_{18}O_4$, as judged by the CIMS – $[M+NH_4]^+$ at *m/z* 280. Its ^1H NMR spectrum (Table II) was very similar to those reported for the guaianolide **13** (Jakupovic *et al.*, 1991) suggesting that both compounds were epimers. Thus, the H-15 and H-6 signals in



1 2αH
2 2βH



3



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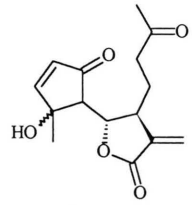
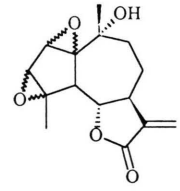
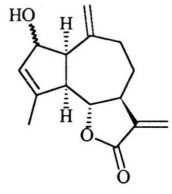


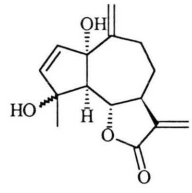
5 4αOH
6 4βOH



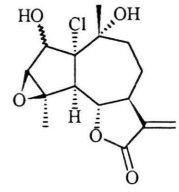
7 β-epoxides
8 α-epoxides



9 2αOH
12 2βOH



10 4αOH
13 4βOH



11 2αOH
14 2βOH

Fig. 1.
1: 4-hydroxy-4,5-dihydro-*trans*-chrysanthem-5-en-1-acetate;
2: 4-hydroxy-4,5-dihydro-*cis*-chrysanthem-5-en-1-acetate;
3: (*E,E*)-3,7,11-trimethyl-1,6,9-dodecatriene-3,5,11-triol;
4: iso-seco-tanapartholide, **5:** seco-tanapartholide-A, **6:** seco-tanapartholide-B;
7: canin, **8:** artecanin, **9:** 2α-hydroxyguaia-3, 10(14), 11(13)-trien-12,6α-olide
10: 1α, 4α-dihydroxy-2, 10(14), 11(13)-trien-12,6α-olide; **11:** 2-epi-chloroklotzchin;
12: 2β-hydroxyguaia-3,10(14), 11(13)-trien-12,6α-olide;
13: 1α, 4β-dihydroxy-2,10(14), 11(13)-trien-12,6α-olide, **14:** chloroklotzchin.

10 were shifted to lower field compared to **13**, whereas the signals for H-5 and H-7 appeared at higher field. This indicated that the OH group at C-4 in **10** is rather situated in close proximity with H-6 than with H-5 and H-7, as is the arrangement in **13**. In addition, the spatial proximity of the C-4 methyl group with the lactone ring resulted in the downfield shift of H-15. Accordingly, compound **10** is the C-4-epimer of **13**.

Compound **11** had a molecular formula of $C_{15}H_{19}O_5Cl$ as determined from CIMS and ^{13}C NMR spectra (Table III). The CIMS (NH_3) spectrum exhibited, in addition to the base peak at *m/z* 315 $[M+H]^+$ peaks at *m/z* 297, 279, 261 and 243 due to subsequent elimination of two molecules of H_2O and HCl. From the results of COSY and HMBC correlation (Table III), it was concluded that **11** was a C-1 chlorinated guaianolide with three oxygen containing functionalities – an oxirane and two hydroxyl groups at C-2 and C-10. Comparison of the ^1H and ^{13}C NMR spectral data of **11** with those of chloroklotzchin (**14**) (Mata *et al.*, 1985) showed a significant similarity, the only difference being the chemical shifts of the signals for H-2, H-3 and H-14. In an attempt to determine the relative stereochemistry at the chiral centres of **11**, the 2D NOESY was measured. It revealed the *syn*-α-orientation of H-7, H-5 and the C-4 methyl group, and also the spatial proximity of H-2 and the C-10 methyl group. The α-orientation of the C-2 hydroxyl group apparantly coused the observed upfield shift of the H-14 signal to δ 1.23 when compare with that in chloroklotzchin (δ 1.49). Additionally, the anti-orientation of H-2 and H-3 obviously affected the magnitude of the coupling constant between them, causing the observed decrease to 0.8 Hz, when compare to chloroklotzchin ($J_{2,3}$=2 Hz), (Mata *et al.*, 1985). Therefore, the lactone **11** must be 2-*epi*-chloroklotzchin.

Table III. ^1H and ^{13}C NMR spectral data of **11** ($CDCl_3$, 250/62.9 MHz).

Position	δ_H (*J* in Hz)	δ_C	HMBC
1	–	73.2 s	H-3, H-5, H-9 (δ 1.92), H-14
2	3.86 d (0.8)	75.8 d	
3	4.10 d (0.8)	64.1 d	H-15
4	–	65.8 s	H-2
5	2.80 d (10.9)	49.8 d	H-3, H-15
6	4.37 dd (10.9, 9.7)	78.5 d	H-8 (δ 2.33)
7	3.60 m	43.3 d	H-5, H-9 (δ 1.92), 2H-13
8	2.33 m 1.65 m[a]	22.9 t	H-6
9	1.92 dd (5.6, 9.1) 1.65 m[a]	33.6 t	H-14
10	–	81.2 s	H-8 (δ 1.65), H-5, H-2
11	–	140.0 s	
12	–	170.0 s	2H-13
13	5.45 d (3.3) 6.19 d (3.6)	119.2 t	
14	1.23 s	23.4 q	H-9 (δ 1.92)
15	1.56 s	24.0 q	H-5

[a] Overlapping signals.

The chloro containing terpenoids are nowadays accepted as naturally occurring products, but the presence of a halogenated sesquiterpene lactone has no precedent in the *Achillea* genus.

Acknowledgements

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