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New sesquiterpenoids from *Achillea clypeolata*

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Abstract

The aerial parts of *Achillea clypeolata* afforded, in addition to clypeotriol (**5**), four new polyoxigenated sesquiterpenoids of eudesmane and cyperane type. The structures were established on the basis of their spectral properties as 7α-hydroxycarisson (**1**), 7α-hydroxyisopterocarpolone (**2**), 3α-dehydroxy-3α-hydroperoxy-clypeotriol (**3**) and 4-oxo-cyperan-3α,7α,11-triol (**4**). © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Achillea clypeolata; Asteraceae; Sesquiterpenoids

1. Introduction

We have reported previously that collections of *Achillea clypeolata* Sibth. et Sm. growing in different regions of Bulgaria differ in their chemical constituents to such an extent that the existence of chemotypes might be suggested (Ahmed & Jakupovic, 1990). This prompted us to extend our investigation to another collection of the same taxon from south-western Bulgaria and the results are described in this paper.

2. Results and discussion

The extract from the aerial parts of *A. clypeolata* was repeatedly chromatographed to afford three new eudesmane derivatives (**1**–**3**), a new ketotriol of cyperane type (**4**), and the previously isolated alcohol clypeotriol (**5**) (Todorova et al., 1998). Sesquiterpene lactone were not detected even in traces.

Compound **1** was assigned the molecular formula $C_{15}H_{24}O_3$ by CI-mass spectrum and ^{13}C-NMR data (see Experimental). The presence of a keto group conjugated with a tetrasubstituted double bond was deduced from the UV absorption at 251 nm and the chemical shift of the corresponding carbon atoms (δ 198.58 *s*, 131.58 *s*, 159.73 *s*). These data placed the double bond at C-4/C-5 which was in good accordance with the chemical shift of the angular methyl (δ 1.21). The ^{1}H NMR spectrum of **1** (see Experimental) also exhibited signals for the vinylic methyl (δ 1.79), and gem-dimethyl of a hydroxyisopropyl group (δ 1.30, 6H), together with four downfield shifted signals assigned to the methylene protons at C-2 (δ 2.40 and 2.45) and C-6 (δ 2.25 and 2.90). All the NMR data were very similar to those of carissone (Raharivelomanana, Bianchini, Cambon, Azzaro & Fayre, 1995; Wang, Kuoh & Wu, 1996) except for the signal at δ 77.84 for a quaternary carbon atom bearing an OH group. The only place for the latter was obviously at C-7, the relative stereochemistry of which was derived from the observed shift of C-9 in the higher field (δ 37.28), when compared to carissone (Wang et al., 1996). The Drieding model shows that such an upfield shift is possible only if the hydroxyl group at C-7 is α-axial. Therefore, compound **1** was shown to be 7α-hydroxycarissone.

Compound **2** gave rise to a CI-mass spectrum which was nearly identical with that of **1** ($C_{15}H_{24}O_3$, $[M+NH_4]^+$ at *m*/*z* 270). The UV maximum at 243 nm again indicated the presence of an unsaturated carbonyl group. However, the ^{1}H NMR spectrum (see

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Table 1
NMR data of compound **4** in $CDCl_3$

H/C	^{1}H NMR (400 MHz)	^{13}C NMR (100 MHz)
1	1.72 *ddd*	37.55 *t*
	1.45 *ddbr*	
2	2.35 *dddd*	32.98 *t*
	1.84 *dddbr*	
3	4.45 *dd*	77.56 *d*
4	–	212.40 *s*
5	–	65.99 *s*
6	2.22 *d*	29.58 *t*
	1.93 *dd*	
7	–	73.58 *s*
8	1.96 *m*	26.76 *t*
	1.58 *ddd*	
9	1.55 *m*	34.26 *t*
	1.22 *ddd*	
10	–	43.14 *s*
11	–	75.65 *s*
12	1.29 *s*[a]	24.98 *q*[a]
13	1.30 *s*[a]	24.58 *q*[a]
14	0.95 *s*	22.98 *q*
15	2.17 *s*	29.83 *q*

[a] Assignment may be interchanged; *J*(Hz): 1,1′ = 1,2 = 12.0; 1,2′ = 1′,2 = 2,3 = 8.0; 2,2′ = 13.0; 2′3 = 6.0; 6,6′ = 9,9′ = 15.0; 6′,8 = 2.0; 8,8′ = 8,9 = 12.5; 8,9′ = 3.0.

Experimental) contained signals for an olefinic proton (δ 5.90) and the corresponding vinylic methyl (δ 1.90) which, together with the singlet at δ 2.27 due to the isolated methylene group α to the CO group, suggested the incorporation of an α,β-unsaturated ketone system as shown in structure **2**. Further, signals for the angular methyl (δ 0.86) and the gem-dimethyl of a hydroxyisopropyl group (δ 1.29, 6H) were also present. On the basis of the above data, compound **2** was found to be structurally related to isopterocarpolone (Kumar, Ravindranath & Seshadri, 1974) and its 6α-hydroxy derivative (Pascual, Bellido & Gonzalez, 1980). However, the second OH group in **2** should be placed at a quaternary carbon atom, as no signal for a carbinol proton was present in the ^{1}H NMR spectrum. Of the two possible places, C-5 and C-7, the former was excluded, as the spectral properties of **2** were not consistent with those described for 5α-hydroxyisopterocarpolone (Hu, Bai & Jia, 1996). This was further supported by the complex signal at δ 2.86 which was attributable to H-5 only. The proposed relative stereochemistry shown in formula **2** was based on both biogenetic cosiderations and the lack of NOE between H-5 and the methyls at C-10 and C-11. Therefore, compound **2** was identified as 7α-hydroxyisopterocarpolone.

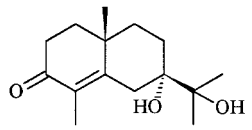
The molecular formula $C_{15}H_{26}O_4$ for compound **3** was established from its EI-mass spectrum which displayed a weak $[M]^+$ at *m*/*z* 270. Moreover, the intense fragment peaks at *m*/*z* 211 $[M-59]^+$, 195 $[211-16]^+$ and 178 $[211-33]^+$ suggested the presence of a hydroxyisopropyl group (C_3H_7O) and a hydroperoxy function. The attachment of the OOH group at C-3 and its α-orientation followed from the observed small couplings of H-3 (δ 4.43, *t*, *J* = 2.8 Hz). The ^{1}H NMR spectrum of **3** (see Experimental) was remarkably similar to that of clypeotriol (**5**), the structure and stereochemistry of which were recently established (Todorova et al., 1998). However, **3** contained an additional signal at δ 8.55 associated with the OOH group. Accordingly, compound **3** was 3α-dehydroxy-3α-hydroperoxy-clypeotriol.

The EI-mass spectrum of compound **4** showed a $[M]^+$ peak at *m*/*z* 270 ($C_{15}H_{26}O_4$), two fragments indicative of the sequential loss of two molecules of water (*m*/*z* 252 and 234) and a peak at *m*/*z* 211 due to loss of a hydroxyisopropyl group $[M-C_3H_7O]^+$. The molecular formula was further confirmed by the ^{13}C and ^{1}H NMR data (Table 1.). Alongside the signals due to the angular methyl (δ_H 0.96, δ_C 22.98), the methyl ketone (δ_H 2.17, δ_C 29.83) and gem-dimethyl of a hydroxyisopropyl group (δ_H 1.29/1.30, δ_C 24.98/24.58), the NMR spectra indicated the presence of two more hydroxyl groups. One of them was attached to a quaternary carbon atom (δ 73.58 *s*), but the other was obviously placed at a secondary carbon (δ 77.56 *d*) the geminal proton of which absorbed at δ 4.45. All the above data were accommodated most readily by the carbocyclic cyperane skeleton bearing a keto group at C-4 and three hydroxyl groups at C-3, C-7 and C-11. Comparison with the spectral data of related compounds of cyperane type (Hikino, Suzuki & Takemoto, 1967; El-Ghazouly, El-Sebakhy, El-Din, Zdero & Bohlmann, 1987; Rustaiyan, Jakupovic, Chau-Thi, Bohlmann & Sadjadi, 1987; Ceccherelli, Curini, Marcotullio & Menghini, 1988; Ahmed & Jakupovic, 1990) supported the structural assignment. The relative stereochemistry at C-3, C-5 and C-7 was derived from the observed spectral data and NOE measurements. Thus, the chemical shift of the angular methyl (δ_C 22.98) required a *cis*-fused hydrindane system (Eggert & Djerassi, 1973) which was supported by the NOE (ca 4%) between H-14 and H-15. The OH group at C-7 was assumed to be α-oriented by analogy to the other eudesmanes **1**–**3**. The ^{13}C NMR spectrum supported this suggestion, as the signal of C-9 appeared at higher field (δ_C 34.26) than in other cyperolone derivatives. (El-Ghazouly et al., 1987; Ahmed & Jakupovic, 1990), this being related to the γ-gauche effect of the 7-OH group. Finally, the α-position of the secondary OH group was assigned on the basis of the observed couplings of H-3 (*J* = 6.0 and 8.0 Hz) and its downfield shift (δ 4.45) caused by the neighbouring methyl ketone group. The *syn*-β-disposition of the latter and H-3 was further confirmed by the significant

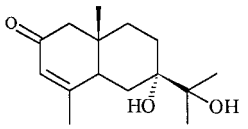
NOE (ca 14%) between them. From the above data, compound **4** was identified as 4-oxo-cyperan-3α,7α,11-triol.

Compound **4** is a new representative of the relatively small group of natural sesquiterpenes bearing a cyperane skeleton. The co-occurrence of the eudesmanes **1**–**3** and **5**, and the cyperane **4** in the same plant is suggestive of their biogenetic inter-relationship, which is corroborated by the in vivo achieved rearrangement of 4,5-epoxy-eudesmanes into cyperanes (Hikino, Kohama & Takemoto, 1969).

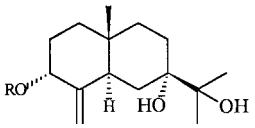
The results of the present study, together with those published previously (Todorova et al., 1998) revealed a clear chemical diversification within the *Achillea clypeolata* species. With respect to the chemical constituents, two well defined chemotypes should be considered. The first one which includes collections from eastern Bulgaria is characterized by sesquiterpene lactones, mainly guaianolides. The collections from western Bulgaria form the other chemotype which contains highly oxygenated sesquiterpenes, mainly eudesmanes, but no sesquiterpene lactones. One may speculate that the latter chemotype might be related to the collection from south-east Serbia which is reported to be also free of lactones (Aljancic et al., 1996).



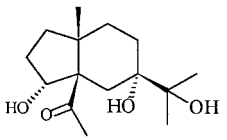
1



2



3 R=OH
5 R=H



4

3. Experimental

3.1. Plant material

The aerial parts of *A. clypeolata* were collected from Pirin mountain (south-western Bulgaria) in July 1996. The plant material was identified by Mrs. R. Taskova and a voucher specimen (SOM 153961) was deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

3.2. Extraction and isolation

The air-dried plant material (71 g) was extracted with CH_2Cl_2 to give, after evaporation of the solvent under red. pres., a brownish gum (3.3 g). It was then separated into 7 frs. by CC on silica gel (100 g) using CH_2Cl_2–Me_2CO mixtures as eluents. Each of the frs. was subjected further to repeated CC and/or prep.TLC to yield **1** (8 mg), **2** (8 mg), **3** (3 mg), **4** (7 mg) and **5** (10 mg).

3.2.1. 7α-Hydroxycarissone (*1*)

Gum, CIMS (NH_3): *m/z* (rel. int.): 270 $[M+NH_4]^+$ (7), 253 $[M+H]^+$ (100), 235 $[M+H-H_2O]^+$ (3); λ_{max}^{MeOH} 251 nm; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.21 (3H, *s*, H-14), 1.30 (6H, *s*, H-12 and H-13), 1.79 (3H, *d*, $J_{15,6}=1.4$ Hz, H-15), 2.25 (1H, *dd*, $J_{6,6'}=15.0$, $J_{6,15}=1.4$ Hz, H-6), 2.40 (1H, *ddd*, $J_{2,2'}=16.8$, $J_{2,1}=5.0$, $J_{2,1'}=3.4$ Hz, H-2), 2.45 (1H, *ddd*, $J_{2',2}=16.8$, $J_{2',1}=13.4$, $J_{2',1'}=5.5$ Hz, H-2′), 2.90 (1H, *dd*, $J_{6',6}=15.0$, $J_{6',8}=2.7$ Hz, H-6′); ^{13}C NMR (62.9 MHz, $CDCl_3$, C-1–C-15) δ: 36.96 *t*, 33.47 *t*, 198.58 *s*, 131.58 *s*, 159.73 *s*, 33.72 *t*, 77.84 *s*, 26.83 *t*, 37.28 *t*, 35.48 *s*, 75.00 *s*, 24.75 *q* (2C), 11.13 *q*, 21.99 *q*.

3.2.2. 7α-Hydroxyisopterocarpolone (*2*)

Gum, CIMS (NH_3): *m/z* (rel. int.):270 $[M+NH_4]^+$ (26), 253 $[M+H]^+$ (100), 235 $[M+H-H_2O]^+$ (5); λ_{max}^{MeOH} 243 nm; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 0.86 (3H, *s*, H-14), 1.29 (6H, *s*, H-12 and H-13), 1.90 (3H, *t*, $J_{15,3}=J_{15,5}=1.4$ Hz, H-15), 2.27 (2H, *s*, 2H-1), 2.86 (1H, *ddbr*, $J_{5,6}=13.0$, $J_{5,6'}=4.5$ Hz, H-5), 5.90 (1H, *q*, $J_{3,15}=1.4$ Hz, H-3).

3.2.3. 3α-Dehydroxy-3α-hydroperoxy clypeotriol (*3*)

Gum,: EIMS (70 eV) *m/z* (rel. int.): 270 $[M]^+$ (1), 252 $[M-H_2O]^+$ (3), 234 $[M-2H_2O]^+$ (5), 219 $[234-CH_3]^+$ (10), 211 $[M-C_3H_7O]^+$ (100), 195 $[211-16]^+$ (34), 178 [211-33]$^+$ (48), 159 (57), 133 (34), 119 (33), 93 (35), 59 (74); ^{1}H NMR (250 MHz, $CDCl_3$) δ: 0.69 (3H, *s*, H-14), 1.26 (6H, *s*, H-12 and H-13), 1.50 (1H, *dd*, $J_{6,6'}=16.0$, $J_{6,5}=11.5$ Hz, H-6), 1.60 (1H, *dd*, $J_{6',6}=16.0$, $J_{6',5}=5.0$ H-6′), 1.82 (1H, m, H-2), 1.90 (1H, m, H-2′), 2.62 (1H, *dbr*, $J_{5,6}=11.5$, $J_{5,6'}=5.0$ Hz, H-5), 4.43 (1H, *tbr*, $J_{3,2}=J_{3,2'}=2.8$ Hz, H-3), 4.78 (1H, *t*, $J_{15,3}=J_{15,5}=1.6$ Hz, H-15), 5.08 (1H, *t*, $J_{15',5}=J_{15',3}=1.2$ Hz, H-15′), 8.55 (1H, *br*, OOH).

3.2.4. 4-Oxo-cyperan-3α,7α,11-triol (*4*)

Gum, CIMS (NH_3): *m/z* (rel. int.): 288 $[M+NH_4]^+$ (15), 271 $[M+H]^+$ (11), 253 $[M+H-H_2O]^+$ (100); 235 $[M+H-2H_2O]^+$ (33) EIMS (70 eV): *m/z* (rel. int.): 270 $[M]^+$ (17), 252 (10), 234 (22), 211 (64), 193

(46), 177 (25), 151 (60), 137 (51), 133 (35), 123 (33), 109 (29), 93 (31), 59 (50), 43 (100); ^{1}H- and ^{13}C NMR: in Table 1.

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References

Ahmed, A. A., & Jakupovic, J. (1990). *Phytochemistry*, *29*, 3658.

Aljancic, I., Macura, S., Juranic, N., Andjelkovic, S., Randjelovic, N., & Milosavljevic, S. (1996). *Phytochemistry*, *43*, 169.

Ceccherelli, P., Curini, M., Marcotullio, M. C., & Menghini, A. (1988). *Journal of Natural Products*, *51*, 1006.

Eggert, H., & Djerassi, C. (1973). *Journal of Organic Chemistry*, *38*, 3788.

El-Ghazouly, M. G., El-Sebakhy, N. A., El-Din, A. A. S., Zdero, C., & Bohlmann, F. (1987). *Phytochemistry*, *26*, 437.

Hikino, H., Suzuki, N., & Takemoto, T. (1967). *Chemical Pharmaceutical Bulletin (Tokio)*, *15*, 1395.

Hikino, H., Kohama, T., & Takemoto, T. (1969). *Tetrahedron*, *25*, 1037.

Hu, J-F., Bai, S-P., & Jia, Z-J. (1996). *Phytochemistry*, *43*, 815.

Kumar, N., Ravindranath, B., & Seshadri, T. R. (1974). *Phytochemistry*, *13*, 633.

Pascual, J., Bellido, I. S., & Gonzalez, M. S. (1980). *Tetrahedron*, *36*, 371.

Raharivelomanana, P., Bianchini, J. P., Cambon, A., Azzaro, M., & Fayre, R. (1995). *Magnetic Resonance in Chemistry*, *33*, 233.

Rustaiyan, A., Jakupovic, J., Chau-Thi, T. V., Bohlmann, F., & Sadjadi, A. (1987). *Phytochemistry*, *26*, 2603.

Todorova, M., Krasteva, M., Markova, M., Tsankova, E., Taskova, R., & Peev, D. (1998). *Phytochemistry*, *49*, 2371.

Wang, C-C., Kuoh, C-S., & Wu, T-S. (1996). *Journal of Natural Products*, *59*, 409.