Silphiperfolane Sesquiterpene Acids from *Artemisia chamaemelifolia* Vill.

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Z. Naturforsch. **58c**, 817–819 (2003); received March 14/May 24, 2003

The aerial parts of *Artemisia chamaemelifolia* Vill. afforded, in addition to five known sesquiterpene acids, a new 5-*epi*-cantabrenolic acid (**6**).

Key words: Artemisia chamaemelifolia Vill., Asteraceae, Silphiperfolane Sesquiterpene Acids

Introduction

In a continuation of our study on Bulgarian *Artemisia* species we have now investigated *Artemisia chamaemelifolia* Vill., which grows in the mountains of Southern Europe: Southwestern Alps, Pyrenees, Cordillera Cantabrica and Northwestern Bulgaria (Tutin *et al.*, 1976). A literature survey reveals a few reports concerning the chemical composition of the species and its subspecies. Besides the early investigations on coumarins (Banyukova and Konovalova, 1970) and essential oil content (Banthorpe *et al.*, 1971), there are only two publications dealing with the isolation of silphiperfolane sesquiterpene acids from *A. chamaemelifolia* ssp. *cantabrica* (San Feliciano *et al.*, 1986) and *A. chamaemelifolia* ssp. *chamaemelifolia* (Marco *et al.*, 1996).

Experimental

Plant material

Aerial parts of *A. chamaemelifolia* were collected from West Stara Planina mountain (location Goliama mogila peak, altitude of *ca.* 1560 m). A voucher specimen (SOM-157158) was deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia, Bulgaria.

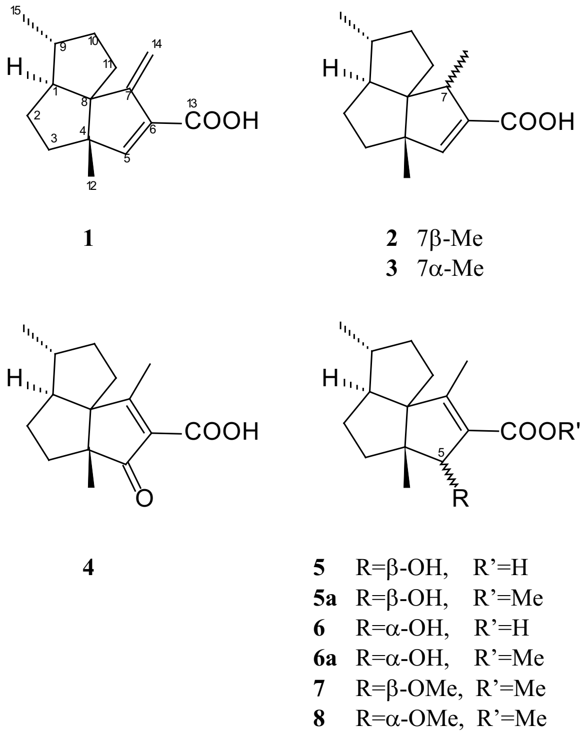
Extraction and isolation

The air-dried plant material (156 g) was extracted with petroleum ether (2 × 200 ml). The crude extract (10 g) was defatted by precipitation with MeOH (2 × 20 ml). After filtration and evaporation of MeOH the resulting residue (6.5 g) was separated into 5 fractions by column chromatography (CC) on silica gel (200 g) using hexane/EtOAc mixtures as eluents. Fraction 2 (1.1 g) was further subjected to CC (silica gel, hexane/EtOAc 4:1 v/v) to give **1** (25 mg) and an unseparable mixture of **2** and **3** (300 mg). Compound **4** (300 mg) crystallized spontaneously from fraction 3 (1.2 g) and then was purified by recrystallization (hexane). The aliquots of **1**–**4** (10 mg) were methylated with ethereal CH_2N_2 affording corresponding methyl esters. A yellow powder from fraction 4 (1.8 g) was separated and recrystallized from hexane/acetone to yield 60 mg of 4′,7-dimethyl-apigenin. The concentrated mother liquors of fraction 4 were subsequently subjected to CC (CH_2Cl_2/MeOH 20:1) and prep. TLC ($CHCl_3$/MeOH/CH_3COOH 25:1:0.1) to give a mixture of **5** and **6** (50 mg). Further methylation (CH_2N_2) and PTLC (hexane/EtOAc, 4:1 v/v) of the reaction mixture allowed to separate **5a** (20 mg) and **6a** (10 mg).

5α-Hydroxy-6-silphiperfolen-13-oic-acid methyl ester (methyl 5-epi-cantabrenolate) (**6a**): Oil. – EIMS: *m/z* (rel. int.) = 264 $[M]^+$ (26), 246 (5), 249 $[M\text{-}CH_3]^+$ (18), 231 (62), 217 (56), 206 (68), 205 $[M\text{-}COOCH_3]^+$ (100), 187 (18), 161 (26), 149 (76), 119 (51), 105 (52), 91 (68), 69 (62), 41 (66). – NMR data in Table I and II.

Results and Discussion

The aerial parts of *A. chamaemelifolia* were worked-up as described in the Experimental to give six closely related compounds with silphiperfolane framework. Cantabradienic (**1**), silphiperfol-5-en-13-oic (**2**), 7-*epi*-silphiperfol-5-en-13-oic



1

2 7β-Me
3 7α-Me

4

5 R=β-OH, R'=H
5a R=β-OH, R'=Me
6 R=α-OH, R'=H
6a R=α-OH, R'=Me
7 R=β-OMe, R'=Me
8 R=α-OMe, R'=Me

Fig. 1. **1**: Cantabradienic acid; **2**: silphiperfol-5-en-13-oic acid; **3**: 7-*epi*-silphiperfol-5-en-13-oic acid; **4**: cantabrenonic acid; **5**: cantabrenolic acid; **5a**: methyl cantabrenolate; **6**: 5-*epi*-cantabrenolic acid; **6a**: methyl 5-*epi*-cantabrenolate; **7**: methyl O-methyl cantabrenolate; **8**: methyl O-methyl-5-*epi*-cantabrenolate.

(**3**) and cantabrenonic (**4**) acids were identified by comparison of the spectral data of their methyl esters with those published in the literature (San Feliciano *et al.*, 1986; Marco *et al*, 1996).

Compounds **5** and **6** were purified as the corresponding methyl esters **5a** and **6a.** Spectral data of **5a** were identical with those of the known methyl ester of 5β-hydroxy-6-silphiperfolen-13-oic-acid (methyl cantabrenolate) (San Feliciano *et al.*, 1986). Compound **6a** showed the same mass spectral behavior as that of **5a**: a molecular ion at *m/z* 264, corresponding to a molecular formula $C_{16}H_{24}O_3$ and the highest mass peak at *m/z* 205 $[M-COOCH_3]^+$. The ^1H NMR spectrum (Table I) of **6a** was remarkably similar to that of **5a**. Slight differences in the chemical shift values of some proton signals could be explained by the presence of C-5 epimer of **5a**. This assumption was further confirmed by NOE difference experiments. Thus, irradiation of the H-5 signal in **5a** produced clear NOEs at H-12, H-3 and H-3′, while in **6a** strong NOE was only observed between H-5 and H-12. Furthermore, the ^{13}C NMR spectral data (Table II) of **6a** were also consistent with the proposed structure. The upfield shift in the signal of C-3 is obviously due to the shielding γ-effect of the 5α-OH group. On the contrary, the downfield shift in the signal of C-14 derives from disappearance of this shielding effect. All these spectral features of **5a** and **6a** are strongly reminiscent of those observed for the known C-5 epimeric pair **7** and **8** found in *A. chamaemelifolia* ssp. *chamaemelifolia* (Marco *et al.*, 1996).

Table I. ^1H NMR data of **5a** and **6a** in $CDCl_3$ (250 MHz).

H	**5a**	**6a**
1	1.76 m	1.76 m
2	1.40 m	1.49 m
2′	1.73 m	1.68 ddd (14.5, 9.0, 6.5)
3	1.51 m*	1.89 m
3′	1.55 m*	1.39 m
5	4.54 brs	4.49 brs
9	1.62 m*	1.55 m
10	1.30 m	1.25 m*
10′	1.83 m	1.84 m*
11	1.59 m	1.83 m*
11′	1.66 m*	1.30 m*
12	1.05 s	1.11 s
14	2.08 d (1.2)	2.07 d (1.4)
15	1.00 d (6.6)	1.00 d (6.7)
OCH_3	3.77 s	3.77 s

* Overlapped signal.

Table II. ^{13}C NMR data of **5a** and **6a** in $CDCl_3$ (250 MHz).

C	**5a**	**6a**
1	58.2	58.7
2	29.8	30.3
3	39.9	35.8
4	53.9	52.8
5	81.4	83.4
6	126.6	126.3
7	166.7	166.7
8	73.1	73.1
9	42.1	41.7
10	36.9	36.8
11	31.4	30.4
12	16.9	23.0
13	164.1	164.1
14	13.6	13.6
15	19.5	19.6
$COOCH_3$	51.19	51.12

In addition, 4′,7-dimethylapigenin was isolated from the extract and identified by comparison its UV and ^{1}H NMR spectral data with those reported (Bandoni *et al.*, 1978).

The above described results demonstrate a close relationship between the investigated sample and the two subspecies of *A. chamaemelifolia* of Spanish origin. All of them produce sesquiterpene acids with silphiperfolane skeleton and seems to be very important from chemotaxonomical point of view since these compounds have not been detected in any *Artemisia* species so far.

Acknowledgements

The authors are grateful for the financial support of project X-911 provided by the Bulgarian National Research Foundation.

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