Silphiperfolane Sesquiterpene Acids from *Artemisia chamaemelifolia* Vill.

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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$_2$N$_2$ 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$_2$Cl$_2$/MeOH 20:1) and prep. TLC (CHCl$_3$/MeOH/CH$_3$COOH 25:1:0.1) to give a mixture of 5 and 6 (50 mg). Further methylation (CH$_2$N$_2$) andPTLC (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$^+$] (16), 233 [M-CH$_3$]+ (54), 231 (62), 217 (56), 206 (68), 205 [M-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
(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_{10}H_{24}O_{3} and the highest mass peak at m/z 205 [M-COOCH_{3}]^{+}. The $^{1}$H 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 $\gamma$-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).
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.

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