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Phytochemical study of *Anthemis rumelica* (Velen.) Stoj. & Acht.

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1. Subject and source

The genus *Anthemis* (tribe Anthemideae, family Asteraceae) is widely distributed not only over Europe, but also in West, Southwest, and Central Asia, as well as in North Africa. About 62 species are reported for the European flora (Fernandes, 1976), 24 of which are found in Bulgaria (Andreev et al., 1992). *Anthemis rumelica* (synonym *A. tenuiloba* var. *rumelica* (Velen.) Stoj. & Acht.) and some other species are endemic for the Bulgarian region (Fernandes, 1976). This is a relatively new species with limited distribution in Bulgaria, discovered by the Bulgarian botanists Prof. N. Stojanov and B. Achtarov, in 1936. We herein report the isolation and identification of two flavonoids and four sesquiterpene lactones, two of which are new natural compounds. In addition the place of *A. rumelica* in the chemotaxonomic scheme proposed earlier is discussed (Staneva et al., 2008).

2. Previous work

Numerous studies on sesquiterpene lactones and flavonoids in many *Anthemis* species have been conducted and summarized by Staneva et al. (2008) and Gonenc et al. (2011), respectively. Nevertheless, there are no phytochemical reports on the endemic plant *A. rumelica*.

3. Present study

3.1. Plant material

The aerial parts of *A. rumelica* were collected in the Danube Plain, in sandy herbaceous places, south of the village of Dabovan, district of Gulyantsi, 24 June 2010, in full flowering stage. The voucher specimen (SOM-165932) was deposited in the Herbarium of the Institute for Biodiversity and Ecosystem Research, BAS.

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3.2. Extraction and isolation

The air-dried flower heads (56 g) were extracted exhaustively with chloroform. The crude extract (1.5 g) was dissolved in 50% aqueous ethanol and defatted with petroleum ether (500 ml). The ethanol was removed under vacuum. The water layer was subsequently extracted with chloroform (300 ml) and ethyl acetate (200 ml). The IR spectra of these fractions showed, that only the chloroform extract contained an absorption band at 1760 cm^{-1}, characteristic for γ-lactones. The crude lactone fraction was subjected to column chromatography (silica gel, $CHCl_3$ – Acetone with increasing polarity) and 10 fractions (A-1 – A-10) were collected. β-Sitosterol, α- and β-amyrin as well as taraxerol were detected in fraction A-2, while β-sitosterol glucoside was found in fraction A-10, by TLC comparison with authentic samples. Fraction A-3 was purified on prep. TLC (petroleum ether/diethyl ether, 1:1) to give β-sitosterol (3 mg) and 2 mg of tanacin (**1**) (Yunusov et al., 1976). Prep. TLC (hexane/diethyl ether, 1:1 × 2) of the fraction A-5 afforded a flavonoid (2 mg) identified as centaureidin, **5** (Barbera et al., 1988). One more flavonoid, santin, **6** (3 mg) (Barbera et al., 1988) and two lactones – hanphyllin (**2**) (4 mg) (Sigstad et al., 1991) and compound **3** (1.5 mg) were isolated from fr. A-7 by prep. TLC (hexane/diethyl ether/acetone, 1:1:0.2). Under the same conditions, 2 mg of compound **4** was isolated from fr. A-9. Hanphyllin (**2**) and centaureidin (**5**) have been isolated recently from *Anthemis ruthenica* (Hajdú et al., 2010). In addition this flavonoid has been detected in *A. altissima* and *A. melanolepis* (Gonenc et al., 2011). Furthermore, santin (**6**) has been found in *A. chia* and *A. dumetorum* (Williams et al., 2001). Lactone **1** is new for the genus, while guaianolides **3** and **4** are new natural compounds. The structures of the isolated compounds (**1–6**) are presented in Fig. 1.

Compound **3** showed in the HR-EIMS a molecular ion at *m/z* 346.1404 $[M]^+$ calculated for $C_{19}H_{22}O_6$. The ^1H NMR spectrum (Table 1) revealed a guaianolide structure with one isolated disubstituted C-2/C-3 and one tetrasubstituted C-1/C-10 double bond. This statement was based on the two doublets at δ 6.10 and 6.33 with $J_{2,3} = 5.8$ Hz, and a 3-proton doublet ($J_{5,14}$ 2.1 Hz) at δ 1.97 for C-10 methyl group. Two downfield doublets at δ 5.49 and 6.24 as well as IR absorption at 1770 cm^{-1} supported the presence of α-methylene-γ-lactone. The fragments at *m/z* 286 $[M\text{-}CH_3COOH]^+$ and 226 $[M\text{-}2xCH_3COOH]^+$, the two 3H singlets at δ 2.08 and 2.18, as well as the IR absorption at 1725 cm^{-1} required two acetoxy groups. Their position at C-4 and C-9 were attributed from the chemical shift of C-4 methyl group and COSY experiment. Cross peaks of the proton geminal to the acetyl residue (δ 5.52) and H-7 (δ 3.21) with the protons of the single CH_2 group (at C-8) in the structure of **3**, showed that the ester side chain is attached at C-9. COSY and NOESY (Fig. 2) experiments showed the proposed structure and relative configuration. Thus, compound **3** was identified as 4α, 9α-diacetoxyguaia-2,1(10),11(13)-trien-12,6α-olide.

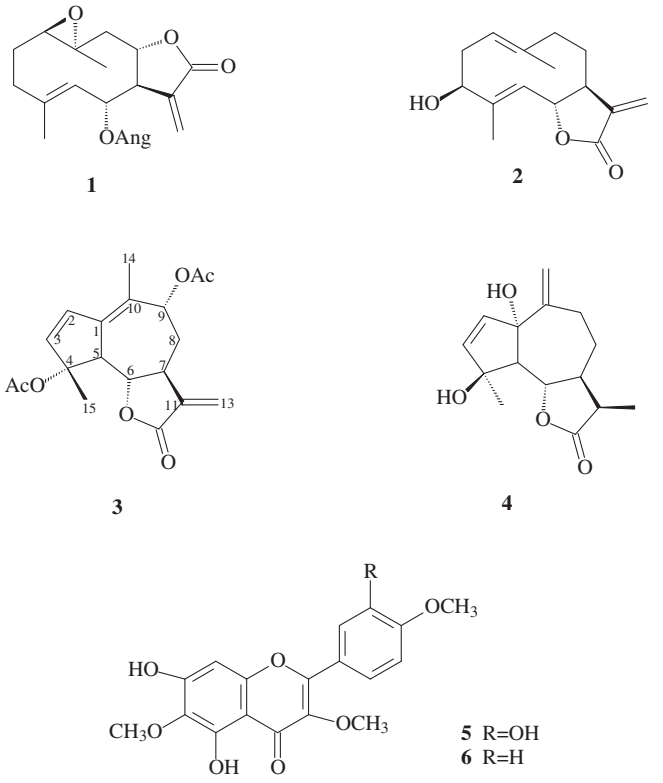


Fig. 1. Chemical structures of compounds **1–6** isolated from *Anthemis rumelica*.

Table 1
^{1}H NMR spectroscopic data (250 MHz, $CDCl_3$, *J* in Hz) for compounds **3** and **4**.

Position	**3**	**4**
2	6.10 d (5.8)	5.89 d (5.7)
3	6.33 d (5.8)	6.11 d (5.7)
5	3.19 dq (2.1. 11.3)[a]	2.10 d (11.3)
6	4.02 dd (10.1, 11.3)	4.60 dd (10.2, 11.3)
7	3.21 m[a]	2.28 m
8	2.42 ddd (2.1, 4.8, 14.6)	1.75–2.05
8′	1.69 ddd (2.2, 11.5, 14.6)	1.75–2.05
9	5.52 dd (2.2, 4.8)	2.45 m
9′	–	2.75 m
11	–	2.64 dq (7.5, 7.5)
13	5.49 d (3.2)	1.23 d (7.5)
13′	6.24 d (3.4)	–
14	1.97 d (2.1)	1.59 s
15	1.46 s	4.87 dd (0.9,2.5)
15′	–	4.92 dd (0.9, 2.5)
2 × Ac	2.08 s and 2.17 s	–

[a] Overlapping signals.

Compound **4** showed in its HR-EIMS a molecular ion at *m/z* 264.1356 $[M]^+$, which was in accordance with the molecular formula $C_{15}H_{20}O_4$. The IR spectrum showed absorption bands typical for OH (3550 cm^{-1}) and a γ-lactone C═O (1765 cm^{-1}) groups. The ^{1}H NMR spectrum (Table 1) is very similar to those of 1α,4β-dihydroxyguaia-2,10(14),11(13)-trien-12,6α-olide isolated earlier from *Achillea clusiana* (Todorova et al., 1999). Thus, doublets for two isolated olefinic protons at δ 5.89 and 6.11 were observed. Their coupling constant ($J = 5.7$ Hz) supported the presence of a double bond in a 5-membered ring. The only position of the later was C-2/C-3. The presence of two dd at δ 4.87 and 4.92, corresponded to exomethylene protons and the cross peak in COSY spectrum of these signals with C-9 protons showed their place at C-10. The structure of the new lactone differed from the previously isolated in the signals for H-13. Instead of signals for methylene protons of the lactone ring a doublet at δ 1.23 for C-11 methyl group and dq at δ 2.64 for H-11 were observed. The β orientation of the methyl group at C-11 clearly followed from the value of the vicinal coupling $J_{7,11}$ (7.5 Hz). The structure and stereochemistry of the isolated compound was determined on the basis of the chemical shifts and coupling constants in ^{1}H NMR, compared with the known analog compounds. Accordingly, compound **4** is 1α,4β-dihydroxy-11αH-guaia-2,10(14)-dien-12,6α-olide. 4,11-Diastereoisomer of the isolated lactone has been obtained by reduction of 1α-hydroxy-4α-hydroperoxy-11βH-guaia-2,10(14)-dien-6α,12-olide, isolated from *Artemisia adamsii* (Bohlmann et al., 1985).

4α,9α-Diacetoxyguaia-2,1(10), 11(13)-trien-12,6α-olide (3): yellowish oil; IR (film) ν_{max} 1770, 1725 cm^{-1}; ^{1}H NMR see Table 1. HR-EIMS *m/z* 346.1404 $[M]^+$ (calcd for $C_{19}H_{22}O_6$ 346.1411).

1α,4β-Dihydroxy-11αH-guaia-2,10(14)-dien-12,6α-olide (4): yellowish oil; IR (film) ν_{max} 3550, 1765 cm^{-1}; ^{1}H NMR see Table 1. HR-EIMS *m/z* 264.1356 $[M]^+$ (calcd. for $C_{15}H_{20}O_4$ 264.1356).

4. Chemotaxonomic significance

According to the botanical classification presented in Flora Europaea (1976) the species from the *Anthemis* genus are divided into three subgenera. Each of them is separated into sections. *A. rumelica* is classified in sect. *Hiorthia* of subgenus *Anthemis*. A chemotaxonomical scheme of genus *Anthemis* based on sesquiterpene lactones as chemotaxonomic markers was discussed in our recent review (Staneva et al., 2008). The present phytochemical study of the Bulgarian endemic *A. rumelica* exhibited that this species synthesized sesquiterpene lactones of germacrane and guaiane skeletal type. Guaianolides and germacranolides were found to be characteristic for sect. *Hiorthia*. Thus, the obtained results are in accordance with its place in the botanical taxonomical scheme.

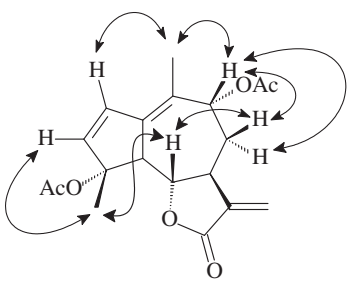


Fig. 2. NOESY correlations of **3**.

Flavonoids santin and centaureidin have been found in *Anthemis* species classified in different sections and subgenera (Gonenc et al., 2011). Obviously, they could not be used as chemotaxonomic markers according to currently available data.

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