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Phytochemical investigation of *Anthemis cotula*

IVAN VUČKOVIĆ[1#], LJUBODRAG VUJISIĆ[1#], VLATKA VAJS[1#], VELE TEŠEVIĆ[2#], PEDJA JANAĆKOVIĆ[3] and SLOBODAN MILOSAVLJEVIĆ2[*#]

[1]Institute for Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, [2]Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade and [3]Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia and Montenegro (e-mail: smilo@chem.bg.ac.yu)

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Abstract: The investigation of roots of *Anthemis cotula* (Asteraceae) from east Serbia revealed, in addition to polyacetylenes previously isolated from the same species, three prenylated 4-hydroxyacetophenones, detected for the first time in any *Anthemis* species. It is possible that they act as phytoalexins in the plant. From the aerial parts, six linear sesquiterpene lactones (four known and two new ones), as well as two known flavones, apigenin and hispidulin, were isolated.

Keywords: Asteraceae, *Anthemis cotula*, polyacetylenes, isopentenylacetophenones, linear sequiterpene lactones, flavones.

INTRODUCTION

The large genus *Anthemis* with more than 130 species is widely distributed over the Mediterranean region. According to the literature, this is one of the best phytochemically investigated genera of the family Asteraceae (syn. Compositae). Polyacetylenes, flavonoids and sesquiterpene lactones are the three main classes of secondary metabolites of the genus.[1–4] *A. cotula* L. is one of nine *Anthemis* species that is to be found in Serbia.[5] It grows on sandy terrains in the eastern part of the country.

EXPERIMENTAL

General

The NMR spectra wre recorded using a Varian Gemini 2000 (^{1}H 200 MHz, ^{13}C 50 MHz) instrument. The mass spectra were obtained on a Finnigan MAT 8230 (EI, 70 eV and DCI, 150 eV, isobutane) and on a VG 70SE (FAB, Cs-ion beam at 20 kV) instruments. The IR spectra were measured on a Perkin Elmer FTIR Spectrometer 1725X.

Serbian Chemical Society active member.

* Corresponding author.

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Silica gel (0.063–0.200 mm) was used for column chromatography (CC) and silica gel (<0.063 mm) was used for dry-column flash chromatography. Silica gel G and silica gel F-254 were used for analytical (0.25 mm) and preparative (0.75 mm) thin layer chromatography (TLC).

Plant material

The plant material was collected during the flowering time near Kladovo in July 2002. A voucher specimen (AC26052002) was deposited in the Herbarium of the Botanic Garden "Jevremovac", Faculty of Biology, University of Belgrade.

Extraction and isolation

The powdered roots (134 g) were extracted twice with petroleum ether–Et_2O (2:1) at room temperature. The crude extract (600 mg) was subjected to CC on silica gel starting elution with petroleum ether and increasing polarity by adding Et_2O up to 50 % to yield 50 fractions ($frs^R_{1–50}$). $Frs^R_{5–8}$ contained 120 mg of **2**, $frs^R_{14–16}$ gave 20 mg of **1**, whereas $frs^R_{21–23}$ yielded 20 mg of a mixture of **3** + **4**. Prep. TLC (toluene–EtOAc 8:2) of $frs^R_{28–29}$ afforded 3.4 mg of **5**. Prep. TLC of $frs^R_{36–40}$ (toluene–EtOAc 8:2) followed by additional prep. TLC (petroleum ether–Et_2O 4:6) afforded **6** (2 mg) and **7** (3 mg).

The powdered air-dried aerial parts (1000 g) were extracted twice with petroleum ether–Et_2O–MeOH (1:1:1) at room temperature and then reextracted with MeOH.[6] The crude extract (~ 30 g) was fractionated by dry-column flash chromatography using petroleum ether–Et_2O–MeOH with increasing polarity to yield 20 fractions ($frs^A_{1–20}$). $Frs^A_{6–7}$ (3.18 g) were purified by CC (toluene–EtOAc grad.) to yield 30 subfractions ($sfrs_{1–30}$). $Srfs_{8–10}$ (1.06 g) were subjected to CC (petroleum ether–Et_2O 1:2) to afford 120 mg of **8**. CC (CH_2Cl_2–MeOH 95:5) of $sfrs_{11–13}$ (0.72 g) yielded 47 mg of **11**. $Frs^A_{8–9}$ (1.23 g) were subjected to CC (toluene–EtOAc grad.) and 50 subfractions ($sfrs_{1–50}$) were obtained. A combination of CC (petroleum ether–Et_2O 1:3) and prep. TLC (CH_2Cl_2–MeOH 95:5) of $sfrs_{35–39}$ resulted in the isolation of **10** (14 mg) and **12** (9 mg). $Frs^A_{11–12}$ were partly soluble in CH_2Cl_2. The soluble part, after CC (petroleum ether–Et_2O grad.) and prep. TLC (CH_2Cl_2–MeOH 95:5, two developments) yielded **9** (26 mg) and **13** (3.7 mg). Prep. TLC of the precipitate (CH_2Cl_2–MeOH 95:5, two developments) furnished **14** (4 mg) and **15** (3 mg).

(5*R*,6*R*+5*S*,6*S*)-Anthecotuloide-5,6-oxide (**12**): colourless oil; IR film: ν_{max}: 1764, 1684, 1619, 1270, 817 cm^{-1}; FAB MS *m*/*z* (rel. int.): 265 [MH^+] (100); ^{1}H-NMR in Table I; ^{13}C-NMR in Table II.

(6*R*+6*S*)-6-Hydroxy-4,5-dehydro-5,6-dihydroanthecotuloide (**13**): colourless oil; IR film: ν_{max}: 3532, 1765, 1674, 1615, 1115 cm^{-1}; EIMS *m*/*z* (rel. int.): 264 [M^+] (5), 249 [M-15]$^+$ (5), 98 (20), 83 (100); ^{1}H-NMR in Table I.

RESULTS AND DISCUSSION

The four acetylenes (**1** – **4**) (Fig. 1) isolated from the roots of *A. cotula* were already known constituents of the roots of the species.[7,8] Generally, dehydromatricaria ester (**1**) and its thioether derivatives (*e.g.* **2** – **4**) are characteristic for *Anthemis* species.[1] Dehydromatricaria ester exhibits significant antimycobacterial[9] and allelopathic activities.[10]

In addition to polyacetylenes, three prenylated acetophenones **5** – **7** were isolated from the roots. Their structures were established by comparison of their spectral data with those reported.[11–13] These compounds, common in Asteraceae, have not hitherto been isolated from any *Anthemis* species. A previous investigation showed the presence of a compound with a similar structure, 7-methoxy-6-acetyl-2,2-dimethylchromene, in the aerial parts of *A. cotula*.[14] Isopentenylaceto-

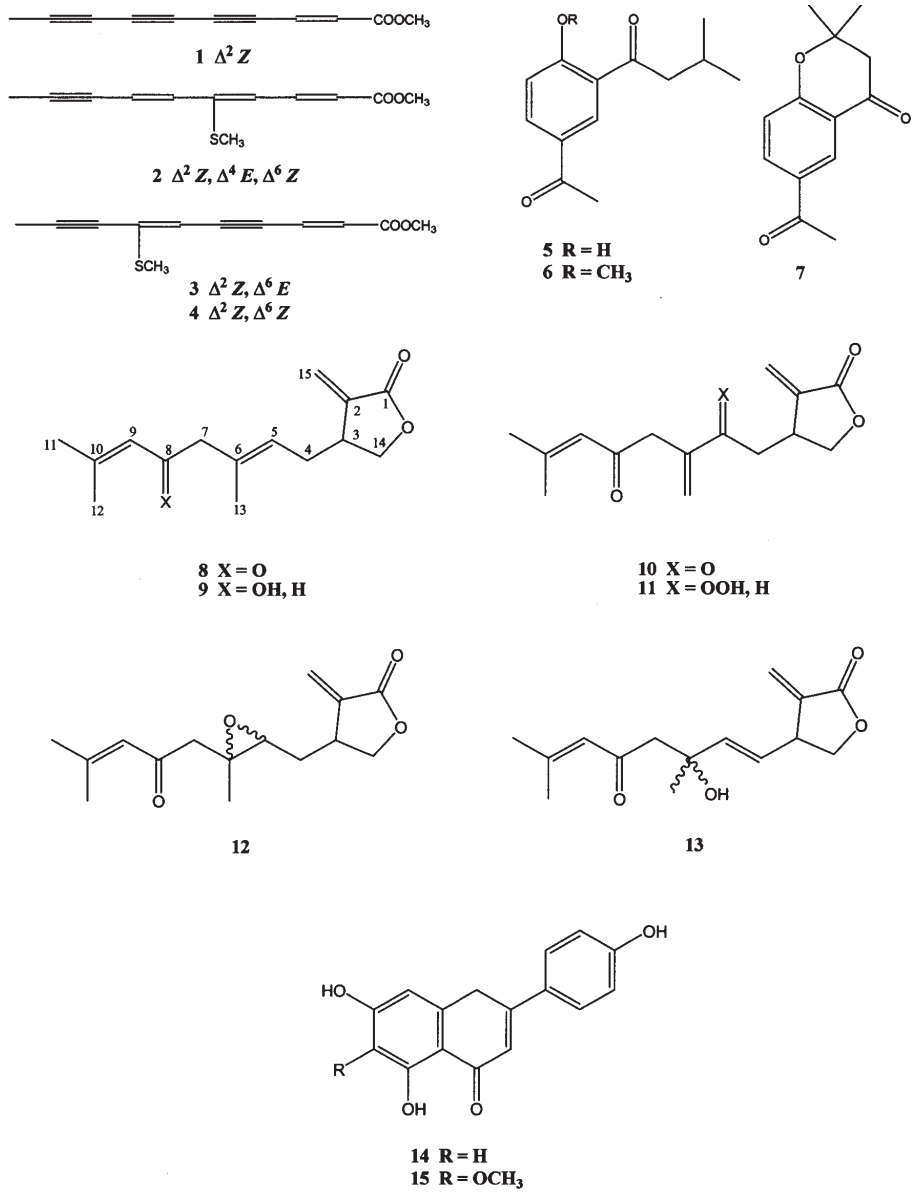

Fig. 1. **1.** (2*Z*)-8-dehydromatricaria ester (methyl dec-2*Z*-en-4,6,8-triynoate); **2.** methyl 5-(methylthio)deca-2*Z*,4*E*,6*Z*-trien-8-ynoate; **3.** methyl 7-(methylthio)deca-2*Z*,6*E*-dien-4,8-diynoate; **4.** methyl 7-(methylthio)deca-2*Z*,6*Z*-dien-4,8-diynoate; **5.** 4-hydroxy-3-isovalerylacetophenone; **6.** 4-methoxy-3-isovalerylacetophenone;
7. 2,2-dimethyl-6-acetylchromanone; **8.** anthecotuloide; **9.** 8-*O*-dihydroanthecotuloide;
10. 5-oxo-6,13-dehydro-5,6-dihydroanthecotuloide;
11. 5-hydroperoxy-6,13-dehydro-5,6-dihydroanthecotuloide; **12.** anthecotuloide-5,6-oxide (new compound); **13.** 6-hydroxy-4,5-dehydro-5,6-dihydroanthecotuloide (new compound);
14. apigenin; **15.** hispidulin.

phenones, especially benzopyrans (chromenes) and benzofurans, have considerable chemotaxonomic and biological significance.[15] Several phenylated acetophenones, among them **5** and **7**, showed anti-inflammatory activities[16] and growth-inhibitory activities toward the larvae of the yellow mealworm *Tenebrio molitor*.[17] Additionally, the prenylated acetophenones could act as phytoalexins, which are low-molecular-weight antimicrobial compounds which accumulate in plants as a result of infection or stress.[18] They can be chemically diverse: phenylpropanoids, (iso)flavonoids, sesquiterpenes and polyketides.[19] Acetophenone-related phytoalexins are rare; only a single species from the family Asteraceae, *Polymnia sonchifolia*, exhibits this type of phytoalexins. It has been shown that 4-hydroxy-3-isovalerylacetophenone (**5**) and two similar acetophenone derivatives are synthesized in the tubers of *Polymnia sonchifolia* after inoculation with *Pseudomonas cichorii*.[20] Since previous investigations of the root of *A. cotula* from other localities did not show the presence of acetophenones **5** – **7**, it is possible that their biosynthesis was induced by some external endangering factor, *i.e.*, that they act as phytoalexins. Further investigation is required to confirm this assumption.

TABLE I. ^{1}H-NMR spectral data of lactones **11** – **13** (200 MHz, $CDCl_3$) (chemical shift in ppm, multiplicity, coupling constant in Hz)

H	**11**	**12**	**13**
3	3.22 *m*	3.35 *m*	3.68 *m*
4	1.70–1.90	1.60–2.00	5.60/5.56 *dd* (15.0, 7.2)
4’			–
5	4.46/4.44 *dd* (10.0, 5.0)	2.84/2.81 *dd* (6.2, 1.6/4.8, 2.0)	5.74/5.77 *d* (15.0)
7	3.31 *s*	2.98/2.93 *d* (16.4)	2.74 *d* (17.2)
7’		2.39/2.35 *d* (16.4)	2.62 *d* (17.2)
9	6.18 *m*	6.06 *m*	6.01 *m*
11	1.96 *br s*	1.91 *d* (1.4)	1.92 *br s*
12	2.20 *br s*	2.16 *d* (2.8)	2.15 *br s*
13	5.39 *s*	1.34 *s*	1.30/1.31 *s*
13’	5.16 *s*	–	–
14	4.56/4.54 *dd* (9.0, 8.0)	4.61 *dd* (9.2, 8.0)	4.50/4.490 *dd* (9.0, 9.0)
14’	4.05/4.02 *dd* (9.0, 6.0)	4.14 *dd* (9.2, 6.0)	3.98 *dd* (9.0, 7.6)
15	6.29/6.23 *d* (2.8)	6.34/6.22 *d* (2.8)	6.28/6.29 *d* (3.0)
15’	5.65/5.66 *d* (2.6)	5.14/5.68 *d* (2.6)	5.53/5.56 *d* (2.6)
–OH	–	–	4.71/4.68 *s*
–OOH	11.02/11.01 *s*	–	–

From the aerial parts of *A. cotula*, six linear sesquiterpene lactones were isolated. The known compounds were identified by comparison of their ^{1}H NMR spectral data with those reported as: anthecotuloide (**8**),[21] 8-*O*-dihydroanthecotu-

loide (**9**),[14] 5-oxo-6,13-dehydro-5,6-dihydroanthecotuloide (**10**)[14] and 5-hydroperoxy-6,13-dehydro-5,6-dihydroanthecotuloide (**11**).[22] The structures of **8** – **11** were also confirmed by ^{13}C NMR spectroscopy (Table II). Anthecotuloide (**8**) is considered to be one of the most potent contact allergens.[23]

The ^{1}H-NMR spectrum of compound **12** was rather similar to that of **8**. The most significant difference between the spectra of these lactones was the absence of a low-field resonance of a vinyl proton (H-5), occurring in the spectrum of **8** at δ 5.21. Instead of this, a one-proton signal was observed at $\delta \sim 2.8$ ppm in ^{1}H NMR spectrum of **12** (Table I), indicating the presence of an epoxy function. The FAB mass spectrum exhibiting the $[MH]^+$ ion at *m*/*z* 265, compatible with the molecular formula $C_{15}H_{20}O_4$, was also in accordance with an additional (epoxy) oxygen in **12**. The ^{13}C NMR spectrum (Table II) confirmed the latter. The observed doubling of the signals led to the conclusion that **12** was a (5*R*,6*R* + 5*S*,6*S*) diastereomeric mixture in a ratio of *ca*. 1:1, which could be formed by epoxidation of the Δ^5 double bond of anthecotuloide. Thus, the structure of anthecotuloide-5,6-oxide could be assigned to **12**.

TABLE II. ^{13}C NMR spectral data of lactones **9** –**12** (50 MHz, $CDCl_3$)

C	**8**	**9**	**10**	**11**	**12**
1	170.1	170.8	170.4	170.5	~170
2	137.4	137.9	137.6	138.0/137.1	137.8
3	37.9	38.6	42.1	35.5/36.8*	37.4/36.8
4	31.4	31.9	34.2	35.4/34.9*	32.4/32.3
5	123.5	122.6	196.7	86.1/87.4	60.7/60.4
6	133.8	135.7	143.1	140.4/140.7	58.1/58.4
7	54.4	47.9	46.6	46.1	53.2/53.3
8	197.9	66.2	198.3	200.4	197.1
9	122.3	127.5	122.5	122.5/122.3*	123.5
10	155.5	135.2	157.2	160.0	157.3
11	26.9	25.7	27.7	28.0	27.8
12	20.0	18.1	20.8	21.3	20.9
13	16.1	16.6	127.5	121.3/121.4*	17.6
14	69.9	70.5	71.4	71.9/70.7	70.6/70.9
15	121.5	122.1	122.9	122.8	122.3/122.7

* The assignments can be interchanged.

The molecular formula of **13**, derived from the EIMS, was identical to that of **12** ($C_{15}H_{20}O_4$). The doubling of the signals in the ^{1}H NMR spectrum (Table I) showed the presence of two diastereomeric forms, this time in the ratio of *ca.* 1:1.4. The relative intensity of the low-field signals in the range of δ 5.45 – 5.82 indicated the presence of three vinyl protons, one of them assigned as H-15'. A low-field pair

of one-proton doublets (J = 15.0 Hz), at δ 5.74 and 5.77, in the ratio of 1.4/1, respectively, could be assigned as H-5, coupled vicinally to the *E*-positioned vinyl proton (H-4) resonating at a slightly higher field (*m*, δ *ca.*, 5.45 – 5.70). The assignment of the signal of H-4 was based on its simplification upon irradiating a multiplet (δ 3.68) identified as H-3. In addition, the signals of the allylic methyl (H_3-14), observed in **8** at δ 1.62, were replaced by a pair of three-proton singlets (δ 1.30 and 1.31, in the ratio of 1/1.4, respectively) typical for methyls attached to a carbinol carbon (C-6). This, together with the similarity of the remaining ^{1}H NMR signals (Table I) to those of **8**, led to the structure of 6-hydroxy-4,5-(*E*)-dehydro-5,6-dihydroanthecotuloide for this lactone, consisting of a (6*R* + 6*S*)-epimeric mixture.

In addition, two flavones, apigenin (**14**) and hispidulin (**15**) were isolated from the aerial parts of *A. cotula*. This type of flavones is characteristic for the members of the genus *Anthemis*.[2]

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ИЗВОД

ФИТОХЕМИЈСКО ИСПИТИВАЊЕ БИЉНЕ ВРСТЕ *Anthemis cotula*

ИВАН ВУЧКОВИЋ[1], ЉУБОДРАГ ВУЈИСИЋ[1], ВЛАТКА ВАЈС[1], ВЕЛЕ ТЕШЕВИЋ[2], ПЕЂА ЈАНАЋКОВИЋ[3] и СЛОБОДАН МИЛОСАВЉЕВИЋ[2]

[1]Институт за хемију, технологију и металургију, Његошева 12, 11000 Београд, [2]Хемијски факултет, Универзитет у Београду, Студентски трг 16, 11000 Београд и [3]Биолошки факултет, Универзитет у Београду, Студентски трг 16, 11000 Београд

Ново испитивање корена биљке *A. cotula* (Asteraceae) показало је, поред четири већ изолована полиацетилена, и присуство три пренилована 4-хидроксиацетофенона који до сада нису били изоловани из овог рода. Из надземног дела биљке изоловано је шест линеарних сесквитерпенских лактона, од који су два нова, док су преостали раније нађени у истој биљци. Такође су у надземном делу идентификована два позната флавона, апигенин и хиспидулин.

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