Chemistry of Natural Compounds, Vol. 43, No. 5, 2007

COMPONENTS OF CYCLOHEXANE EXTRACT OF *Anthemis triumfetti*

M. Pavlovic,[1] N. Kovacevic,[1] O. Tzakou,[2] and M. Couladis[2]

UDC 547.915;661.732

The cyclohexane extract of the aerial parts of Anthemis triumfetti *(L.) DC. was analyzed by means of GC-MS to determine the fatty acid and sterol composition. Additionally, camphor, trans-phytol, squalene, and* β*-carotene were isolated and identified on the basis of their data.*

Key words: *Anthemis triumfetti*, fatty acid, sterol.

Anthemis L. (Asteraceae) comprises about 210 species that occur in the Mediterranean region, southwest Asia, and eastern Africa [1]. *Anthemis triumfetti* (L.) DC. [syn.: *Cota triumfetti* (L.) Gay], which belongs to the subgenus *Cota*, is a perennial herb, 30–90 cm high, growing in woods and rocky places on mountains [2].

The species of this genus are used in folk medicine as anti-inflammatory, antibacterial, antispasmodic, and sedative agents [3]. The infusion is taken for intestinal and abdominal colic and the decoction is applied topically on sun-burned skin or skin affected by inflammations of various types [4].

Previous phytochemical studies indicated the presence of sesquiterpene lactones, flavonoids, acetylenes, and essential oils in plants of genus *Anthemis* [5–10].

The aim of the present work was to characterize the cyclohexane extract of aerial parts of *A. triumfetti*, since this species was not a subject of research up to now.

In the petroleum ether extract of *A. triumfetti* stigmasterol was identified as the main sterol constituent as well as the following sterols: cholesterol, campesterol, a-sitosterol, and 24-methylene cycloartanol.

The composition of fatty acids of aerial parts of *A. triumfetti* is presented in Table 1. GC-MS analysis revealed the presence of 16 fatty acids varying from C_9 to C_{28} with the exception of C_{11}, C_{13}, C_{19}, C_{21}, C_{23}, and C_{25}. The main constituents were hexadecanoic acid (palmitic acid), 9,12,15-octadecadienoic acid (linolenic acid), and (*Z*,*Z*)-9,12-octadecadienoic acid (linoleic acid), representing 28.8%, 16.3%, and 13.9%, respectively, of the total fatty acids. Odd-chain fatty acids, such as $C_{9:0}$, $C_{15:0}$, and $C_{17:0}$, were present in relatively small concentrations, representing a total of 3.0% of total fatty acids. Saturated fatty acids constituted 61.2% and unsaturated fatty acids contributed to 38.8% of the total fatty acids.

Fractionation of cyclohexane extract by VLC and preparative TLC led to the isolation of β-carotene, camphor, *trans*-phytol, and squalene.

A literature survey did not reveal any data concerning the fatty acid composition, as well as the presence of carotenoids, *trans*-phytol, and squalene in the genus *Anthemis*. Concerning the sterol composition, there has been one previous report of a mixture of β-sitosterol, stigmasterol, and campesterol from *Anthemis melampodina* [11].

1) Department of Pharmacognosy, Faculty of Pharmacy, University of Belgrade, V. Stepe 450, 11000 Belgrade, Serbia and Montenegro; 2) Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, 157 71 Athens, Greece, fax: +30 210 727 4591, e-mail: kouladi@pharm.uoa.gr. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 420-421, September-October, 2007. Original article submitted June 22, 2006.

 0009-3130/07/4305-0512

TABLE 1. Fatty Acids Composition of Aerial Parts of *Anthemis triumfetti*

Fatty acid methyl ester	%*	Fatty acid methyl ester	%*
Nonanoic (9:0)	1.3	(*E*,*E*)-9,12-Octadecadienoic (18:2)	8.6
Decanoic (10:0)	2.7	9,12,15-Octadecatrienioc (18:3)	16.3
Dodecanoic (12:0)	1.0	Octadecanoic (18:0)	7.0
Tetradecanoic (14:0)	4.8	Eicosanoic (20:0)	3.7
Pentadecanoic (15:0)	0.9	Docosanoic (22:0)	3.4
Hexadecanoic (16:0)	28.8	Tetracosanoic (24:0)	3.4
Heptadecanoic (17:0)	0.8	Hexacosanoic (26:0)	2.1
(*Z*,*Z*)-9,12-Octadecadienoic (18:2)	13.9	Octacosanoic (28:0)	1.3

*The results represent the ratio among fatty acids.

EXPERIMENTAL

Plant Material. The aerial parts of *A. triumfetti* were collected on the mountain Bjelasica in Montenegro, during the flowering period, in July 2003. A voucher specimen is deposited in the Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade (Herbarium BEOU; No. 17723).

Extraction and Isolation. Air-dried aerial parts of *A. triumfetti* (487.3 g) were extracted with a mixture of solvents CH_2Cl_2–MeOH (1:1, v/v); the solvent was removed under reduced pressure and the residue was partitioned between MeOH (10%) and cyclohexane (90%) to afford 7.0 g of oily residue in the cyclohexane extract.

Cyclohexane extract (1 g) was saponified with KOH solution (50%, w/v) and extracted with petroleum ether (0.4 g). After removal of the solvent one part of the petroleum ether extract (0.1 g) was dissolved in pentane and used for analysis of sterols using gas chromatography. The petroleum ether extract was further subjected to preparative TLC (silica gel, Merck Art. 5715; petroleum ether–ether–HCOOH, 25:25:0.25) to obtain phytol (6.5 mg). The aqueous phase remaining after extraction with petroleum ether was used for the analysis of fatty acids after hydrolysis and esterification of free fatty acids with anhydrous MeOH and conc. H_2SO_4.

Furthermore, cyclohexane extract (6.0 g) was fractionated by vacuum liquid chromatography (VLC) over silica gel (silica gel 60H, Merck Art. 7736) using cyclohexane–EtOAc mixtures of increasing polarity as eluents to give 9 fractions (F1-F9), which were combined on the basis of their TLC profiles. Fraction F4 consisted of squalene (2.1 mg). Preparative TLC (silica gel, Merck Art. 5715; CH_2Cl_2–cyclohexane, 7:3) of fraction F5 gave camphor (4.3 mg) and b-carotene (0.8 mg).

Preparation, Analysis, and Identification of Fatty Acid Methyl Esters. The aqueous solution remaining after extraction with petroleum ether was heated to dissolve soaps, acidified with conc. HCl, and extracted with ether (3×400 mL). After evaporation of the solvent, the fatty acids were converted into methyl esters by heating with 1 mL conc. H_2SO_4 and 150 mL anhydrous MeOH for 30 min at 80°C under reflux. After that, 100 mL H_2O was added and extracted with petroleum ether (3×250 mL). The solvent was removed under reduced pressure and the extract was dried (0.25 g), dissolved in petroleum ether, and submitted to GC-MS analysis.

GC-MS analysis was performed on a Hewlett Packard 6890-5973 GC-MS system, operating in the EI mode at 70 eV, equipped with a split/splitless injector (200°C). The transfer line temperature was 250°C. Helium was used as carrier gas (1 mL/min) and the capillary column used was HP 5MS (30 m × 0.25 mm; film thickness 0.25 μm). The temperature program was 60°C to 280°C at a rate of 3°C/min; split ratio 1:10. The injected volume was 1.0 mL.

The fatty acids were identified by using the Wiley and NIST/NBS MS libraries and by comparison of their retention times with those of authentic compounds from Sigma Chemical Co. (St. Louis, MO, USA). The relative amounts of the fatty acids were calculated from peak areas.

Analysis of Sterols. Petroleum ether extract was dissolved in pentane and analyzed by GC-FID. GC analysis was performed on a Perkin Elmer 8500 gas chromatograph fitted with an FID detector and 3% SE-30 (2 m × 1/8in) column; He_2 was used as the carrier gas. The column temperature was 210°C; the injector temperature 250°C and the detector temperature

300°C. The identification of sterols was performed by comparison of their retention times with those of standards from Sigma Chemical Co. (St. Louis, MO, USA).

Identification of Compounds. Camphor was identified by GC-MS and by co-chromatography with an authentic from sample Sigma Chemical Co. (St. Louis, MO, USA). The structure of squalene was determined using GC-MS and by ^{1}H-NMR and confirmed by comparison with literature data [12]. β-Carotene was identified by co-TLC with an authentic sample, on silica plates in three solvent systems (petroleum ether-benzene, 1:1, R_f 0.94; $CHCl_3$–EtOAc, 4:1, R_f 0.97; hexane–acetone, 7:3, R_f 0.90) and by UV-VIS analyses in two solvents, hexane and chloroform [13]. Phytol was identified by GC-MS and by ^{1}H-NMR [14]. ^{1}H-NMR spectra were taken on a Bruker DRX 400 spectrometer, using $CDCl_3$; UV-VIS spectra were recorded using a Shimadzu UV-160A spectrophotometer.

ACKNOWLEDGMENT

The authors are grateful to Dr. Dmitar Lakusic (Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade) for identification of the plant material. This work was supported by the Greek State Scholarship Foundation (I.K.Y.) and by the Ministry of Science and Environmental Protection of Serbia (Grant No. 1568).

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