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PHYTOCHEMICAL STUDY OF LEAVES AND ROOTS OF *GYPSOPHILA TRICHOTOMA* WEND. USING GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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Summary. Volatile constituents of fresh leaves and roots as well as butanol fractions from the roots of *Gypsophila trichotoma* Wend. were analysed by GC/MS. Different groups of compounds were found: esters, sterols, terpens, alcohols, acids, hydrocarbons, etc. The butanol fractions were characterized by high content of sterols (from 0.5% to 33.7%). The main volatile compounds were octadecenoic acid in the leaves (14.2%) and dihydroxynaphthalene in the roots (66.4%).

Key words: *Gypsophila trichotoma*, Caryophyllaceae, GC/MS, volatiles, sterols

ФИТОХИМИЧНО ИЗСЛЕДВАНЕ НА ЛИСТА И КОРЕНИ ОТ GYPSOPHILA TRICHOTOMA WEND. ЧРЕЗ ГАЗ ХРОМАТОГРАФИЯ/МАССПЕКТРОМЕТРИЯ

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Резюме. Летливи вещества в свежи листа и корени, както и бутанолни фракции от корени на *Gypsophila trichotoma* Wend. са изследвани чрез ГХ/МС. Различни групи вещества са идентифицирани като: естери, стероли, терпени, алкохоли, киселини, въглеводороди и др. Бутанолните фракции съдържат висок процент стероли (от 0.5 до 33.7%). Главните летливи вещества са октадеценова киселина в листата (14.2%) и дихидроксинафтален – в корените (66.4%).

Ключови думи: *Gypsophila trichotoma*, Caryophyllaceae, ГХ/МС, летливи вещества, стероли

Indroduction

Gypsophila trichotoma Wend. (Caryophyllaceae) is a perennial herb, distributed in Southeast Europe (from East Bulgaria to Southeast Russia). The plant is spread in the Northeast region of the Black Sea coast in Bulgaria [8]. Previously oleanane type triterpene saponins have been isolated from the roots of *G. trichotoma* [1, 4, 5, 6, 7]. Our earlier research on this species resulted in the identification of three new sulfated saponins [3], sterols and flavonoids [2]. Continuing our studies on the constituents of *G. trichotoma*, we investigated butanol fractions and volatiles from the roots and leaves by GC/MS.

Experimental

Plant material

The plant material of *G. trichotoma* (dried roots, fresh leaves and roots) was collected in the Northeast region of the Black Sea coast, locality "Zelenka", near Balgarevo village, Bulgaria. The voucher specimen (SO 103887) was deposited in the Herbarium of Sofia University, Bulgaria.

Extraction and fractionation of butanol extract

The dried roots (800 g) were exhaustively extracted with 80% MeOH and the extract was evaporated under reduced pressure to allow for obtaining the aqueous residue, which was partitioned between CH_2Cl_2, 1-BuOH and water. The 1-BuOH extract was evaporated to dryness and chromatographed on a Sephadex LH-20 column, eluting with MeOH to give four fractions (Fr. I, II, III and IV). Fr. III was further separated by flash chromatography over silica gel, eluting with $CHCl_3$-MeOH-H_2O (18:11:2) to afford Fr. IIIa. Fr. IV was purified by column chromatography over Sephadex LH-20 column, eluting with MeOH to give Fr. IVa. After acid hydrolysis (10% HCl for 4 h), fractions IIIa and IVa were analysed by GC/MS.

Extraction of volatiles

Fresh materials – leaves (20 g) and roots (30 g) – have been subjected to simultaneous distillation-extraction using a Licken-Nickersson apparatus for 4 h. The volatiles were extracted with diethyl ether and analysed by GC/MS. The yield of the total volatiles was 5.4 mg from leaves and 3.9 mg from roots.

Analysis conditions

The samples were analysed by GC/MS via Hewlett Packard gas chromatograph 6890 equipped with a Hewlett Packard 6890 + MS 5973 detector (Hewlett Packard, Palo Alto, CA, USA). A HP5-MS capillary column was used (30 m x 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Wilmington, Delaware, USA). Helium was used as a carrier gas and the temperature program was 40ºC to 280ºC at 6ºC/min and a 10 min hold. The ion source was set at 250ºC and the ionisation voltage was 70 eV.

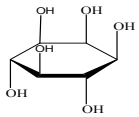
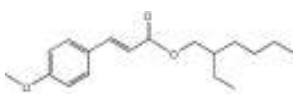
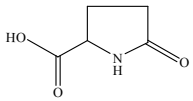
The GC/MS investigation was based on the interpretation of the mass spectral fragmentation followed by comparison of the spectra obtained and those of authentic samples. Computer searches in a HP Mass Spectral Library NIST98 were also applied. Only the clearly identified compounds are reported in tables.

Results and discussion

Purification of the 1-BuOH-soluble extract of the roots of G. trichotoma by column chromatography over Sephadex LH-20 and silica gel yielded purified fractions, which were analysed after acid hydrolysis by GC/MS. Twenty compounds from different groups were identified: sterols, alcohols, acids, esters, etc. (table 1). The butanol fraction IIIa is characterized by a high sterol content from 0.5 to 33.7%. Previously, we identified some of the sterols in ethyl acetate fractions of G. trichotoma (Krasteva et al., 2008). The content of acids, esters and alcohols was lower – from traces to 1.8%.

The results of GC/MS analysis of the volatiles are presented in Table 2. Thirteen compounds were identified and two main compounds were found: octadecenoic acid (14.2%) in the leaves and dihydroxynaphtalene (66.4%) in the roots.

Table 1. *Composition of butanol fractions (% of the total compounds) in the roots*

№	compounds	structure	amount
1	lauric acid (dodecanoic acid)	$CH_3(CH_2)_{10}COOH$	0.3
2	palmitic acid (n-hexadecanoic acid)	$CH_3(CH_2)_{14}COOH$	0.5
3	levulic acid (4-oxopentanoic acid)	$CH_3COCH_2CH_2COOH$	0.8
4	benzoic acid	C_6H_5COOH	1.8
5	chlorobenzoic acid	ClC_6H_4COOH	trace
6	salicylic acid	$C_6H_4(OH)COOH$	trace
7	methoxybenzoic acid	$CH_3OC_6H_4COOH$	0.4
8	nitrophenol	$HOC_6H_4NO_2$	trace
9	glycerol	$C_3H_5(OH)_3$	trace
10	inositol		trace
11	octyl methoxycinnamate (Parsol MCX)		0.3
12	5-oxoproline		0.4

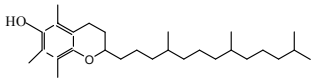
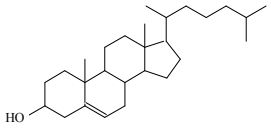
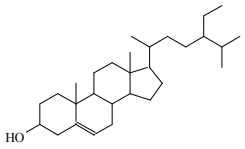
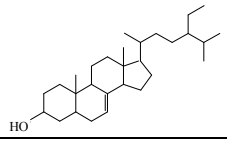
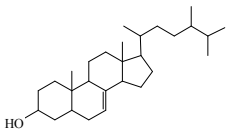
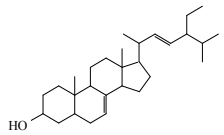
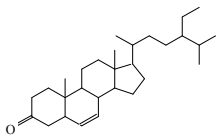
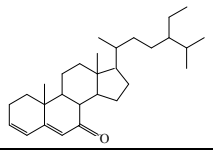
13	vitamin E		trace
14	cholesterol		0.5
15	β-sitosterol		28.3
16	ergost-7-en-3-ol		5.7
17	stigmast-7-en-3-ol		33.7
18	spinasterol		31.6
19	22,23-dihydrospinasteron		3.1
20	stigmasta-3,5-dien-7-on		8.6

Table 2. *Composition of the volatile substances (% of the total volatiles) in the leaves and roots*

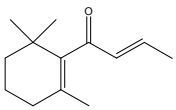
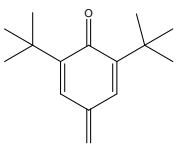
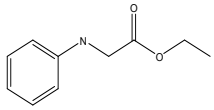
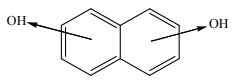
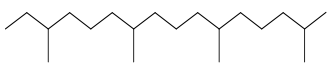
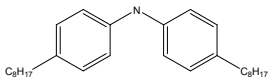
№	**compound**	**structure**	**leaves**	**roots**
1	β-damascone		0.1	trace
2	2,6-di-(t-butyl)-4-methylene-2,5-cyclohexadiene-1-one		0.1	trace
3	N-phenylglycine ethyl ester		1.4	5.3

Table 2. *Continuation*

4	dihydroxynaphthalene		9.6	66.4
5	phytan		1.8	trace
6	p,p'-dioctyldiphenylamine (Vanlube 81)		1.5	trace
7	isopropyl tetradecanoate	$CH_3(CH_2)_{12}COOCH(CH_3)_2$	1.0	trace
8	isopropyl hexadecanoate	$CH_3(CH_2)_{14}COOCH(CH_3)_2$	7.4	trace
9	heneicosane	$CH_3(CH_2)_{19}CH_3$	2.0	trace
10	tricosane	$CH_3(CH_2)_{28}CH_3$	1.1	trace
11	9-octadecenoic acid	$CH_3\ (CH_2)_{16}COOH$	14.2	trace
12	methyl octadecanoate	$CH_3(CH_2)_{16}COOCH_3$	trace	4.6
13	methyl hexadecanoate	$CH_3(CH_2)_{14}COOCH_3$	trace	0.1

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