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Essential oils of flowers and fruits of Athamanta haynaldii Borb. et Üchtr. (Apiaceae)

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The essential oils of flowers and unripe fruits of *Athamanta haynaldii* were found to contain, appreciable amounts of a psychotropic aromatic ether myristicin (ca. 39% of oil) in addition to monoterpene and sesquiterpene hydrocarbons. In the essential oil of ripe fruits of *A. haynaldii* the main component was β-pinene (71.7% of oil).

Genus *Athamanta* (9 species) occurs commonly throughout south-eastern Europe. In Serbia one can find only one species, i.e. *A. haynaldii*, in rocky limestone alpine or subalpine areas [1]. This species was analysed with respect to essential oils of flowers and (ripe and unripe) fruits. The plant material originated from the slopes of mountain Kablar (310 m height above sea level) in Ovčar banja gorge (Western Serbia), and was collected during June, July and August 1993.

All essential oil samples were prepared from 10 g air-dried ground flowers and (un)ripe fruits using Likens-Nickerson device for the isolation of volatiles by simultaneous steam distillation/extraction (CH_2Cl_2) [2]. The samples were analysed on a capillary GC and GC/MS and most constituents were identified by comparison of their mass spectra to those from the MS library [3], taking into account the relative retention times. The structure of myristicin was also verified by ^1H NMR data (not shown) of the compound isolated using preparative GC. The results are summarized in the Table.

Table: Constituents of essential oils of *Athamanta haynaldii*

Compound (identified by GC/MS)	Composition [%]		
	Flowers	Unripe fruits	Ripe fruits
1 α-Pinene[a]	1.1	2.2	4.6
2 Camphene	tr	tr	tr
3 n-Hexanal	0.3	0.2	tr
4 β-Pinene[a]	17.3	39.1	71.7
5 Sabinene		tr	0.3
6 β-Myrcene	1.1	1.8	3.4
7 n-Heptanal		0.2	tr
8 Limonene	0.4	0.3	0.6
9 β-Phellandrene		tr	0.2
10 1,8-Cineole		0.3	tr
11 2-Hexenal (E)	1.4	0.3	
12 γ-Terpinene	0.9	0.3	1.1
13 Styrene	0.2	0.1	
14 p-Cymene	0.2	0.2	0.5
15 α-Terpinolene	2.8		
16 2-Heptenal (Z)		0.5	
17 n-Hexanol	0.2		tr
18 3-Hexen-1-ol (Z)	0.2		
19 α-Copaene	0.9	0.3	0.2
20 β-Bourbonene	0.8	0.8	0.3
21 β-Elemene	0.3	0.3	0.1
22 Caryophyllene[a]	2.0	2.8	3.8
23 α-Humulene[a]	1.2	2.1	1.9
24 $C_{15}H_{24}$	21.1	6.4	4.7
25 $C_{15}H_{24}$	2.3	0.3	0.3
26 δ-Cadinene	1.2	0.3	tr
27 Elemicin		tr	
28 Myristicin[b]	39.6	39.1	3.6

tr = trace (<0.1%)
[a] The structure also confirmed by the identity of retention time with that of the authentic sample (coinjection technique). [b] Isolated by preparative GC and identified by ^1H NMR and MS.

All samples contained the same main constituents (monoterpenic and sesquiterpenic hydrocarbons and the aromatic ether myristicin), but in different relative amounts. In addition, small amounts of linear C_6- and C_7-aldehydes and alcohols were detected. While flowers and unripe fruits yielded similar quantities of essential oils (0.04%, calc. per weight of dried plant material), the concentration in ripe fruits was much higher (0.28%). This is mainly due to the highly increased amount of β-pinene (71.7% of oil), the main component among monoterpenes in all samples.

The oil originating from flowers also contained a considerable amount of a sesquiterpene hydrocarbon ($C_{15}H_{24}$, compound 24, Table) exhibiting a mass spectrum almost identical to that of β-cubebene [3, 4]. However, the relative retention of this sesquiterpene, i.e. elution after caryophyllene and α-humulene, was not in favour of β-cubebene structure whose Kovats index (on a polar column) was reported to be smaller in comparison to that of caryophyllene and α-humulene [4].

The presence of aromatic ether myristicin, i.e. 4-methoxy-6-(2-propenyl)-1,3-benzdioxole, the major constituent of the higher boiling fractions of nutmeg and mace oils, was not unusual since it was detected previously in some genera belonging to *Apiaceae* [5]. The biological activities of myristicin, such as psychotropic activity [6] and insecticidal properties [7, 8], have received considerable attention so far. A related aromatic ether elemicin, i.e. 1,2,3-trimethoxy-5-(2-propenyl)-benzene, frequently co-occurring with myristicin was detected (as a trace) in the oil sample originating from the unripe fruits od *A. haynaldii*.

3. Experimental

3.1. Isolation of the essential oils

The analysed essential oils were isolated from 10 g of air-dried ground flowers and (un)ripe fruits of *A. haynaldii* by means of Likens-Nickerson device [2], using 150 ml H_2O and 7 ml CH_2Cl_2 in course of 2 h. The oil samples, obtained as CH_2Cl_2 solutions were analysed directly by means of GC and GC/MS.

3.2. Analytical GC

A Varian model 3400 gas chromatograph, equipped with a split/splittless injector (250 °C) and fused silica capillary column (i.d. 0.25 mm; length, 60 m; Supelcowax) and FID (300 °C), was used for GC and GC/MS measurements. Working conditions: oven temp., starting with isothermal conditions at 50 °C (3 min) and then programming to 220 °C at 10°/min; carrier gas 3 ml H_2/min. Peak areas were calculated by a data station Varian DS-604.

3.3. GC/MS

The gas chromatograph was connected via an open split interface and a fused silica capillary (at 250 °C) to the EI ion source of a Finnigan MAT 8230 spectrometer, equipped with a PDP 11/74 computer. Working conditions: carrier gas, 2 ml He/min; other GC conditions as in 3.2; MS: ion source, 70 eV. Mass spectral identifications were based on a comparison of the measured spectra to those from the MS library [3].

3.4. Preparative GC

The isolation of myristicin from the essential oil was performed using a Varian Aerograph 920 instrument equipped with TCD (210 °C) and a glass column (i.d. 4 mm, length, 2 m) packed with 10% Carbowax 20M on Chromosorb W (60–80 mesh). Working conditions: injector temp. 220 °C oven temp. 170 °C (isothermal conditions); carrier gas, 25 ml H_2/min. Samples (ca. 50 µl of concentrated CH_2Cl_2 solutions) were repeatedly injected and fractions collected at the outlet of the detector in ice-cooled U-tubes.

3.5. ^1H NMR Spectrum of myristicin

^1H NMR spectrum of myristicin was measured in $CDCl_3$ solution on a Varian FT 80A NMR spectrometer at 80 MHz.

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