Volatile Components of Some Rutaceae Species

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The volatile components of the aerial parts of \textit{Ruta graveolens} and \textit{Haplophyllum suaveolens}, as well as of leaves of \textit{Zanthoxylum limoncello}, \textit{Z. panamense} and \textit{Z. setulosum} have been studied by GC/MS analysis.

\textit{Key words}: Volatiles, \textit{Ruta graveolens}, \textit{Haplophyllum suaveolens}, \textit{Zanthoxylum limoncello}, \textit{panamense} and \textit{setulosum}

\textbf{Introduction}

The genera \textit{Ruta} L., \textit{Haplophyllum} Juss., and \textit{Zanthoxylum} L. belong to the family Rutaceae. The plants of these genera find application in the traditional and contemporary medicine of many countries (Petit-Paly \textit{et al.}, 1988; Charlton, 1998; Ahsan \textit{et al.}, 2000; Shim \textit{et al.}, 2001). The volatile oil from \textit{R. graveolens} possesses phototoxical, bacteriostatical and anthelminthical activities (Petit-Paly \textit{et al.}, 1988). The anti-inflammatory, anesthetic and hypotensive activities of the essential oil from the fruits of \textit{Z. rhesta} (Ahsan \textit{et al.}, 2000) and the repellent activity of the volatiles from \textit{Z. piperitum} are also reported (Shim \textit{et al.}, 2001).

The presence of coumarins, alkaloids, terpenes and flavonoids in \textit{Ruta graveolens} has been reported (Kostova \textit{et al.}, 1999). Data exist about the chemical composition of leaf, fruit and root oils from \textit{R. graveolens} of German origin, as well as of the essential oil from the aerial parts of \textit{R. graveolens} grown in Italy (Kubeczka, 1971, 1974; De Feo \textit{et al.}, 2002). The total content of the essential oil in different Bulgarian populations of \textit{R. graveolens} was established (Vitkova and Philipov, 1999). However, the composition of the essential oil of the Bulgarian \textit{R. graveolens} has not been studied yet.

\textit{Haplophyllum suaveolens} contains quinoline alkaloids, flavonoids, lignans and coumarins (Kostova \textit{et al.}, 2000; Ivanova \textit{et al.}, 2001b). Preliminary GC/MS investigations of the petroleum ether extract of the aerial parts of this plant species revealed the presence of aliphatic acids, phosphoric acid, glycerol and its esters, sesquiterpenes and sterols (Ivanova \textit{et al.}, 2001a).

The genus \textit{Zanthoxylum} is a rich source of alkaloids, coumarins, fucoquinarins, terpenoids and other metabolites (Stermitz and Sharifi, 1977). The volatile components of \textit{Z. limoncello}, \textit{Z. panamense} and \textit{Z. setulosum} have not been investigated.

The aim of the present study is to analyze the volatile components of the aerial parts of \textit{R. graveolens} and \textit{H. suaveolens} of Bulgarian origin, as well as of the leaves of \textit{Z. limoncello}, \textit{Z. panamense} and \textit{Z. setulosum}, growing in Costa Rica.

\textbf{Experimental}

\textbf{Plant material}

Aerial parts of \textit{Haplophyllum suaveolens} (DC.) G. Don fil. were collected in July 1998 in Ognyanovo (the region of Plovdiv, Bulgaria) and identified by Dr. A. Vitkova. A voucher specimen was deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

\textit{Ruta graveolens} L. cultivated in the region of Gorna Orjahoviza, collected in 1996 and purchased from Bilkocoop Ltd. (Sofia, Bulgaria) was investigated.

The leaves of the three \textit{Zanthoxylum} species were collected in May 2000 in the following regions of Costa Rica: \textit{Zanthoxylum limoncello} (Planch & Oerst) – San Rafael, Heredia; \textit{Zan-
the species were authenticated by Dr. Hernan R. Navas and voucher specimens deposited at the Institute of Botany, National University, Heredia, Costa Rica.

Isolation of volatile compounds

A part of the chloroform extracts of the studied plant species (260.0 mg from \textit{R. graveolens}; 236.0 mg from \textit{H. suaveolens}; 249.0 mg from \textit{Z. limoncello}; 280.0 mg from \textit{Z. panamense} and 230.0 mg from \textit{Z. setulosum}) was subjected to a four-hour distillation-extraction in Licknes-Nickerson apparatus (Hendriks et al., 1981). The volatile compounds were extracted from the distillate with diethyl ether. Yields: \textit{R. graveolens} – 26.8 mg (10.3\% of the lipophylic extract); \textit{H. suaveolens} – 43.0 mg (18.2\% of the lipophylic extract); \textit{Z. limoncello} – 34.4 mg (13.8\% of the lipophylic extract); \textit{Z. panamense} – 33.0 mg (11.8\% of the lipophylic extract) and \textit{Z. setulosum} – 31.6 mg (13.7\% of the lipophylic extract).

Analysis and identification of volatile compounds by GC/MS

The samples were investigated by analytical GC/MS on Hewlett-Packard Gas Chromatograph 6890 equipped with a Hewlett-Packard MS 5973 detector. HP5-MS capillary column (30 m × 0.25 μm film thickness, Agilent Technologies, Wilmington, Delaware, USA) was used. The temperature was programmed from 40°C to 280°C at a rate of 6°C·min⁻¹. Helium was used as a carrier gas at 9 ml·min⁻¹. The ion source was set at 250°C and the ionization voltage was 70 eV. The identification of components was accomplished by computer searches in the HP Mass Spectral Library NIST98 (Hewlett-Packard, Palo Alto, California, USA).

Results and Discussion

The volatile compounds in \textit{R. graveolens}, \textit{H. suaveolens}, \textit{Z. limoncello}, \textit{Z. panamense} and \textit{Z. setulosum} were analyzed by GC/MS. The results are summarized in Table I. Aliphatic acids, alcohols and ketones, hydrocarbons, mono- and sesquiterpenoids, and coumarins are the main groups of the compounds identified.

The presence of aliphatic acids is a characteristic feature of all samples under investigation. The biggest amount of aliphatic fatty acids is found in \textit{Z. panamense} (17.2\%), followed by \textit{R. graveolens} (16.5\%), \textit{Z. limoncello} (14.7\%), \textit{H. suaveolens} (9.2\%) and \textit{Z. setulosum} (5.3\%). Pentanoic, hexanoic, octanoic and nonanoic acids are common compounds in the volatiles of the five species. The branched fatty acids, 2-methylbutanoic and 3-methylbutanoic acid, are detected in trace amount only in \textit{R. graveolens} and \textit{H. suaveolens}. The C₃–C₇ fatty acids are in low amounts in all species investigated.

Aliphatic alcohols are present in \textit{R. graveolens} and \textit{Z. limoncello}, while aliphatic ketones are found only in \textit{R. graveolens}.

Hydrocarbons are observed in the volatiles of \textit{R. graveolens}, \textit{Z. limoncello} and \textit{Z. setulosum} – 1.8\%, 3.1\% and 6.3\%, respectively.

Monoterpenoids are only detected in \textit{H. suaveolens} (\textit{α}-terpineol, myrtenol), whereas sesquiterpenoids are observed in different quantitative and qualitative ratios in the volatile components of all species. The biggest amount of sesquiterpenoids is found in \textit{Z. panamense} (21.1\%), followed by \textit{H. suaveolens} (19.0\%) and \textit{Z. setulosum} (16.0\%).

The coumarin xanthotoxin is found in the volatiles of \textit{R. graveolens}, while 4,4,5,7,8-pentamethyldihydrocoumarin is identified in \textit{H. suaveolens} and \textit{Z. setulosum}.

Our results show a high content of aliphatic acids, alcohols and ketones in \textit{R. graveolens}. Pentanoic, hexanoic, octanoic and nonanoic acids, 2-undecanol and 2-undecanone are the main components. The identified 2-undecanol, 2-dodecanol and 2-tridecanol are biogenetic precursors of 2-undecane, 2-dodecanone and 2-tridecanone. 2-Undecane and 2-undecanol have been also found in the leaf and fruit oils of \textit{R. graveolens} of German origin (Kubeczka, 1971), as well as in the root oil of \textit{R. graveolens}, grown in Italy (De Fco et al., 2002). No terpenes and only one sesquiterpenoid (\textit{β}-eudesmol) have been detected in the volatiles of the aerial parts from the Bulgarian \textit{R. graveolens}. The presence of terpenoids (elemol, myrcene, pregeijerene and geijerene) has also been reported in the essential oil of \textit{R. graveolens} of German origin (Kubeczka, 1974).
Table I. GC/MS analysis of the volatile components of *Ruta graveolens* (R. g.), *Haplophyllum suaveolens* (H. s.), *Zanthoxylum limoncello* (Z. l.), *Z. panamense* (Z. p.) and *Z. setulosum* (Z. s.) (in % of total ion current<sup>a</sup>).

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<td>–</td>
</tr>
<tr>
<td>β-Selinene</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.1</td>
<td>–</td>
</tr>
<tr>
<td>Selina-4(14),7(11)-diene</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.6</td>
<td>–</td>
</tr>
<tr>
<td>Farnesol</td>
<td>–</td>
<td>–</td>
<td>0.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nerolidol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6.7</td>
</tr>
<tr>
<td><strong>Diterpenes</strong></td>
<td></td>
<td></td>
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<tr>
<td>Phytol</td>
<td>0.6</td>
<td>0.6</td>
<td>2.0</td>
<td>6.1</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Aromatic alcohols</strong></td>
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<td></td>
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<tr>
<td>Phenylethyl alcohol</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>Benzyl alcohol</td>
<td>–</td>
<td>0.5</td>
<td>tr</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Coumarins</strong></td>
<td>0.6</td>
<td>3.2</td>
<td>–</td>
<td>–</td>
<td>1.8</td>
</tr>
<tr>
<td>4,4,5,7,8-Pentamethyl-dihydrocoumarin</td>
<td>–</td>
<td>3.2</td>
<td>–</td>
<td>–</td>
<td>1.8</td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>1.1</td>
<td>2.6</td>
<td>2.6</td>
<td>5.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Dihydroactinidiolide</td>
<td>0.9</td>
<td>1.6</td>
<td>0.4</td>
<td>1.8</td>
<td>4.8</td>
</tr>
<tr>
<td>2-Propenoic acid, 3-(4-methoxyphenyl), 2-ethylhexyl ester</td>
<td>0.2</td>
<td>1.0</td>
<td>0.7</td>
<td>0.5</td>
<td>0.8</td>
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<tr>
<td>Isopropylpalmitate</td>
<td>–</td>
<td>–</td>
<td>1.5</td>
<td>2.8</td>
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</table>

*a* The ion current generated depends on the characteristics of the compounds and is not true quantitation.

*b* tr – trace.

Monoterpenoids and coumarins are specific for *H. suaveolens*. Spathulenol (12.4%) is the main volatile component. The hexanoic, octanoic, dodecanoic, tetradecanoic and hexadecanoic acids and the sesquiterpenes spathulenol and β-bourbonene have already been found in the petroleum ether extract of the same plant (Ivanova et al., 2001a).

The presence of aliphatic acids, hydrocarbons, sesquiterpenoids, diterpenes and coumarins is established for *Zanthoxylum* species. The content of these structural classes varies for the studied plants. Hexadecanoic acid and its ethyl ester are the main components of *Z. panamense* (3.6% and 6.6%, respectively), while 2-nonenioic (3.4%) and nonanoic (2.3%) acid and the ethyl ester of hexadecanoic acid (3.2%) are those of *Z. limoncello*. The sesquiterpenoids caryophyllene oxide (5.0%), spathulenol (3.9%), γ-cadinene (3.4%) and selina-4,7-diene (2.6%) are major compounds in *Z. pana-
mense; nerolidol (6.7%), spathulenol (4.4%) and caryophyllene oxide (3.4%) are in big amount in Z. setulosum.

Acknowledgements
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