Volatile Constituents of Propolis

V. Bankova, R. Christov, S. Popov
Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev str. bl. 9, 1113 Sofia, Bulgaria

O. Pureb
Institute of Chemistry, Mongolian Academy of Sciences, Ulan Bator, Mongolia

and

G. Bocari
Study Institute of Traditional Medicine and Medicinal Plants, Tirana, Albania

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Volatile oils from Albanian, Bulgarian and Mongolian propolis have been investigated.

Introduction

Propolis (bee glue) is a resinous hive product, collected by bees. It possesses versatile biological activity – antibacterial, antiviral, fungicidal, antiulcer, immunostimulating, hypotensive, cytostatic, *etc.* [1]. The chemical composition of propolis appeared to be very complex – at least 156 propolis constituents have been identified so far [2, 3]. Most important constituents of propolis appeared to be phenolics, which constitute more than 50% of its total weight and are probably connected to a substantial part of its biological activity. For this reason most of the investigations on the chemical composition of propolis have been connected with its phenolic constituents. In few publications the chemical composition of volatile oils from propolis was described [4–8]. More than 30 constituents of these oils have been identified but a substantial part of the GC/MS peaks remained unidentified. The phenolic composition of most investigated propolis samples appeared to be similar and this could be explained with their common origin from poplar bud exudates. Contrary to the phenolics, the composition of volatile oils differed significantly in all investigated samples. Different groups of compounds have been found in volatile oils from propolis. These oils consist mainly of terpenoids. In this group sesquiterpenoid alcohols and hydrocarbons predominate, accompanied by some monoterpenes, mainly alcohols. Another part of the volatiles appeared to be alcohols, mainly aromatic, phenols, aldehydes, ketones, acids (from acetic to stearic acid), esters (together with terpenoids the largest group of constituents of volatile oils from propolis). A series of alkanes have been found, accompanied by alkylated benzenes, and surprisingly by naphthalene. Two vinyl ethers have been found, also.

Till now, no comparison between the volatile oils of different propolis samples have been made, with the exception of Hungarian samples [7]. In this work we present the investigation on the volatile oils from propolis samples from three countries, which differ in their plant origin. In Bulgaria the main source of propolis was established to be *Populus nigra* buds and partially *P. italica* buds [9]. In Mongolia the sole source of propolis appeared to be *P. suaveolens,* the only poplar growing in this country [9]. In Albania propolis is collected partially from *P. nigra* buds, but there are other, unidentified sources, too [10]. The data obtained will be compared with these for other propolis samples, investigated earlier in order to make a characterization of volatile constituents of propolis, similar to that for phenolics.

Experimental

Propolis

The propolis samples were collected in July in Albania in the region of Tirana, in North Bulgaria near the city of Russe, in Mongolia near Ulan Bator.

Reprint requests to Prof. S. Popov.

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Sample preparation

The propolis samples were grated after cooling and subjected to steam distillation for 4 h. The collected distillates were extracted with ethyl ether/*n*-pentane 1 : 1, the extracts dried over Na_2SO_4, evaporated and submitted to GC/MS analysis. The volatile oil content of the Albanian propolis sample was 0.27%, of the Bulgarian −0.30%, and of the Mongolian −0.60% from the weight of dry propolis.

Gas chromatography-mass spectrometry

For the GC/MS analysis a 30 m SPB-1 silica capillary column was used in a JEOL JGC-20 K gas chromatograph directly coupled to a JEOL JMS-D 300 mass spectrometer. The samples were introduced *via* an all-glass injector working in the split mode (split ratio 1 : 80), with helium as the carrier gas and a temperature programme 60–280 °C at 6 °C/min. The mass spectrometer was run in the electron impact mode, the ionization potential was 70 eV, ionization current 100 μA and the ion source temperature 100 °C.

The components were identified by a comparison with library spectra.

Results and Discussion

Propolis samples have been powdered and distilled with water for 4 h. The collected distillates were extracted with diethyl ether/pentane 1 : 1, the extracts dried, evaporated and subjected to GC/MS investigation. The data obtained were summarized in Table I. In the same table the data from the literature about the volatile oils of other propolis samples are presented. The identification of the constituents of the volatile oils is based on the comparison with the library mass spectra of authentic samples. In the cases when such spectra have not been available only the structural type of the corresponding component was proposed on the basis of the mass spectral fragmentation observed. The quantitation of the components of volatile oils is based on the ion currents generated by different compounds. It must be kept in mind that the ion current generated depends on the characteristics of the compound concerned and is not a true quantitation. The results are almost quantitative only inside a group of structurally related compounds, because of the identity of their fragmentation. Independently of these limitations, volatile constituents of different samples of propolis can be compared, because the same method of quantitation was used in all cases [11].

According to Petri *et al.* [7] propolis could be separated into two types (I and II), the first one characterized by the presence of substantial amounts of β-eudesmol, while in type II the main volatile constituent appeared to be benzyl benzoate. According to our results, propolis from Mongolia is type II, while this from Bulgaria is type I. Probably type II propolis does not originate from pine trees, as Petri *et al.* [7] suggested, because all the data available about the phenolic composition of Hungarian propolis indicate that its main source are *P. nigra* buds [12–14]. Till now, it is not proved that pine trees can be a source of propolis, and in no one case volatiles from propolis contained typical pine terpenoids, for example pinenes.

In the propolis sample investigated we found representatives of most of the structural groups, identified till now in the volatile oils from propolis. The smallest number of volatile compounds have been found in Mongolian propolis, which has only one source *(P. suaveolens)* while Bulgarian and Albanian propolis originate from different poplar species (mainly *P. nigra)* and eventually from other tree buds.

Analogously to all other propolis samples investigated till now, the largest amount of volatile constituents appeared to be sesquiterpenoids. Most of them have been identified in Bulgarian propolis. From Table I it is evident that in the investigated propolis samples a substantial part of the identified sesquiterpenoids have not been found earlier in other propolis samples. Besides the identified sesquiterpenoids, a large number unidentified representatives of this group of terpenoids have been found. They are hydrocarbons (12 substances) and alcohols (11 substances). Most of them are constituents of Mongolian propolis, which showed more substantial differences from the other samples. This can be explained with its specific source – *P. suaveolens.*

It can be concluded that terpenoid composition of Albanian and Bulgarian propolis is similar, which is in agreement with their common origin. Similarity was found also in the hydrocarbon composition of the above mentioned two propolis samples.

Table I. Composition of propolis volatile oils[a] (% total ion current[b]).

Substance	Alban.	Bulg.	Mong.	Others
Ketones, alcohols, phenols				
Methoxyacetophenone	9.0	3.3	1.7	–
Methoxyacetophenone (isomer)	–	0.6	–	–
4-Phenyl-3-buten-2-one	1.0	1.1	–	–
2-Phenylethanol	0.9	–	–	+ [8]
Isoeugenol	1.1	0.8	–	–
Esters				
Unidentified ester of 2-phenylethanol	–	1.6	–	–
Benzyl acetate	–	1.6	1.0	+ [4, 7, 8]
Benzyl benzoate	1.7	–	8.6	+ [4, 7]
Terpenoids				
δ-Cadinen	1.0	5.3	–	–
Guaiol	1.3	2.9	–	+ [4, 6]
α-Copaen	–	0.9	–	+ [6, 8]
Caryophylen	–	1.2	–	+ [4, 6]
β-Selinen	–	1.2	–	+ [4]
α-Elemen	–	2.3	–	–
Calamenen	–	2.2	2.6	+ [6]
α-Muurolen	0.9	2.0	1.2	–
γ-Muurolen	–	4.7	1.8	–
Cadinen (isomer)	10.5	3.4	–	–
β-Eudesmol	–	8.8	–	+ [4, 7]
Bulnesol	–	2.3	–	–
Hydrocarbons				
3-Methylinden	0.8	–	–	–
Alkylbenzol ($M^+ = 162$)	–	0.6	–	+ [4]
Heneicosane (C-21)	3.6	–	–	+ [4, 6, 14]
Tricosane (C-23)	4.8	4.9	1.4	+ [6, 14]
Pentacosane (C-25)	4.1	4.4	–	+ [6, 14]
Heptacosane (C-27)	6.6	2.7	–	+ [14]
Nonacosane (C-29)	5.4	–	–	+ [14]
Hentriacontane (C-31)	4.0	–	–	+ [14]
Unidentified sesquiterpene hydrocarbons				
$M^+ = 200$	–	–	2.4	
$M^+ = 202$	–	–	2.8	
$M^+ = 204$	1.2	–	–	
$M^+ = 204$	2.0	–	–	
$M^+ = 204$	2.4	–	15.4	
$M^+ = 204$	–	2.0	–	
$M^+ = 204$	–	1.9	–	
$M^+ = 204$	–	–	2.0	
$M^+ = 204$	–	–	6.8	
$M^+ = 204$	–	–	2.0	
$M^+ = 204$	–	–	2.4	
$M^+ = 204$	–	–	1.8	
$M^+ = 204$	–	–	3.0	

Table I. (Continued).

Substance	Alban.	Bulg.	Mong.	Others
Unidentified sesquiterpene alcohols				
$M^+ = 220$	0.9	3.6	–	
$M^+ = 220$	–	2.3	–	
$M^+ = 220$	–	–	2.4	
$M^+ = 220$	–	–	4.3	
$M^+ = 222$	9.0	–	–	
$M^+ = 222$	18.5	–	–	
$M^+ = 222$	3.4	–	15.4	
$M^+ = 222$	–	5.3	–	
$M^+ = 222$	–	15.5	–	
$M^+ = 222$	–	–	8.6	
$M^+ = 222$	–	–	15.7	
Unidentified compounds				
$M^+ = 136$	1.4	–	–	
$M^+ = 148$	0.8	1.8	1.6	
$M^+ = 256$	2.7	0.9	–	
$M^+ = 270$	0.9	–	–	

[a] Compounds, found in volatile oils of other propolis samples by other authors but absent in our samples, are not included in this table.
[b] The total ion current generated depends on the characteristics of the compound concerned and is not a true quantitation.

They contain much more hydrocarbons than Mongolian propolis. Higher homologues (C_{27}, C_{29} and C_{31}) were found in Albanian propolis. Unexpectedly we found 3-methylinden in Albanian propolis. This is not a natural product and could be an artefact, formed by steam distillation.

The number and concentrations of esters are low and they are mainly in Mongolian propolis. Some specific ketones have been found. Till now *p*-methoxyacetophenone was identified in propolis volatiles. Now its *o*- and *m*-isomers have been identified in Bulgarian propolis. One of them was found also in Albanian and Mongolian propolis. Both isomers can not be distinguished because of the absence of authentic samples but no one of them appeared to the *p*-methoxyacetophenone.

4-Phenyl-3-buten-2-one was found for the first time in nature in Albanian and Bulgarian propolis. Its structure is close to this of cinnamic acid, whose derivatives are important propolis constituents.

From the results obtained it is evident that the differences between the composition of volatile oils from propolis from different locations are higher than in their phenolic composition. There are specific constituents, characterizing every propolis sample. In the propolis samples investigated we identified some sesquiterpene alcohols, which might possess antimicrobial and other biological activities [6]. This shows that the well recognized biological activity of propolis must be attributed not only to phenolics. The essential oil of propolis is known to possess antibacterial activity [7].

The obtained substantial differences in the composition of volatiles from propolis of different locations are an indication that volatile oils from propolis originate from tree buds analogously to phenolics and observed differences are due to the differences in the origin of propolis. Future investigations on volatile oils from different tree buds will be performed in order to solve this problem.

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