Chemical Composition of Egyptian Propolis

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Propolis, Polyphenols, GC/MS

A sample from Egyptian propolis was investigated by TLC and GC/MS. 39 compounds were identified, 8 being new for propolis. Partial structures of four new esters of caffeic acid have been proposed.

Introduction

Propolis (bee glue) is a resinous hive product collected by bees from plant exudates. It performs defensive functions in the hive and has recently found wide application in medicine and cosmetics, due to its versatile biological activities: antibacterial, antiviral, fungicidal, antiulcer, immunostimulating, hypotensive, cytostatic, etc. (Marcucci, 1995). Recently, propolis is extensively used also in beverages and foods intended to maintain or improve human health (Matsuda, 1994).

The chemical composition of bee glue is extremely complex and more than 180 constituents have been identified so far (Marcucci, 1995). The most important ones appeared to be polyphenols, which are the main biologically active propolis components (Ghisalberti, 1979; Marcucci, 1995).

In the temperate zone, the main plant source of propolis are poplar buds and propolis samples from these regions possess similar chemical composition: flavonoids (mainly flavanones), phenolic acids and their esters. Recently, some studies were published on tropical propolis from Brazil (Aga *et al.,* 1994, Bankova *et al.,* 1995; Bankova *et al.,* 1996) and Venezuela (Tomas-Barberan *et al.,* 1993), where no poplars grow. As expected, bees have found other sources of propolis (at least 2–3 plants) in this region and bee glue from different locations shows significant differences in its qualitative composition. In general it differs from European propolis by the lower content of flavonoids, and absence of esters of phenolic acids. Instead, dihydrocinnamic acid in high concentrations was identified, together with prenylated p-coumaric acids, some diterpenes, etc. (Aga *et al.,* 1994; Bankova *et al.,* 1992; Bankova *et al.,* 1996; Boudourova-Krasteva, 1997).

Unill now, there are only some preliminary investigations on the composition and biological activity of African propolis, performed in Egypt (Hegazi *et al.,* 1993; Abd El-Hady, 1994; Abd El-Hady and Hegazi, 1994). In this country there are poplars, but the subtropical and tropical climate and the connected with it specific flora could affect the chemical composition of Egyptian propolis. This assumption is in agreement with the above mentioned reports. For this reason we performed a detailed investigation on the composition of the so called "balsam" (extract with 70% alcohol) from Egyptian propolis.

Experimental

Propolis

Propolis was collected in Bani Swaief near Giza.

Extraction and sample preparation

Propolis (2 g), cut into small pieces, was extracted with 20 ml 70% ethanol (twice x 24h) at room temperature. The extract was evaporated to dryness (0.77 g) and analyzed by TLC. About 2.5 mg of the alcohol extract was dissolved in 20 µl dry pyridine, 30 µl N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were added and the mix-

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ture heated at 80 °C for 20 min in a screw-cap vial. The sample was diluted with 100 µl dry pyridine and analyzed by GC-MS.

TLC analysis

For TLC analyses Alufolien Kieselgel F_{254} Merck was used, mobile phases chloroform-ethyl acetate 7:3 and n-hexane-acetone 2:1.

GC/MS analysis

For the GC/MS analyses a 30 m x 0.25 mm ID, HP-5 fused silica capillary column was used (film thickness 25 µm), in a Hewlett-Packard 5890 gas chromatograph with a Hewlett-Packard 5972 series mass selective detector, with He as a carrier gas, split ratio 1:100, temperature program 80–240 °C at 8 deg.min^{-1}, 240–300 °C at 12 deg.min^{-1} and a 20 min hold at 300 °C; injector temperature 300 °C, detector temperature 300 °C.

Identification of compounds

The identification was accomplished using computer searches on commercial libraries. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. Reference compounds were co-chromatographed where possible to confirm GC retention times. (See Table I.)

Results and Discussion

Propolis sample have been cut into small pieces and extracted with 70% ethanol. Alcoholic extract was subjected to preliminary investigation by thin layer chromatography (TLC). It showed similarity with European propolis: the spots of flavonoids and esters of phenolic acids have been observed, but the number of the esters was much larger than in European samples.

In order to investigate the chemical composition of the alcoholic extract as completely as possible, it was silylated and subjected to GC/MS analysis. The results obtained are summarised in Table I. Literature data concerning a Bulgarian sample, originaiting from *Populus nigra* (Bankova *et al.*, 1992) and a British sample, originating from various *Populus* species (Greenaway *et al.*, 1988) are displayed as well, to compare Egyptian and European propolis. From the results obtained it is evident that Egyptian propolis has a complex chemical composition and few groups of compounds

Table I. Chemical composition (% TIC)[a] of 70% ethanolic extract of propolis from Egypt, compared to European samples.

Compound	Egy	Bg[b]	Brit[c]
Acids (aliphatic)			
Palmitic acid[e]	3.0	<1	-
Stearic acid[e]	0.9	tr	-
Oleic acid[e]	4.0	-	-
Tetracosanoic acid[e]	1.6	-	-
Succinic acid[e]	0.3	-	-
Lactic acid[e]	1.3	-	-
Piruvic acid[e,g]	0.3	-	-
Acids (aromatic)			
Benzoic acid[e]	0.2	-	2.7
trans-p-coumaric acid[e]	0.5	<1	6.1
Caffeic acid[d]	0.3	2	2.9
Ferulic acid[d]	0.2	<1	0.1
Dimethoxycinnamic acid[e]	0.4	<1	0.6
Esters			
Ethyl palmitate[e]	0.5	-	-
Ethyl oleate[e,g]	1.2	-	-
Isopentenyl caffeate[d]	0.9	5	-
Dimethylallyl caffeate[d]	1.3	6	7.1
Dodecyl caffeate[f,g]	1.1	-	-
Tetradecyl caffeate[f,g]	3.1	-	-
Tetradecenyl caffeate[f,g]	0.3	-	-
Hexadecyl caffeate[f,g]	4.7	-	-
Benzyl caffeate[e]	0.6	3	6.9
Phenylethyl caffeate[e]	-	7	2.1
Sugars			
D-glucose[e]	6.1	-	7.7
Sorbose[e]	3.1	-	-
Fructose[e]	3.1	-	7.0
Sucrose[e]	1.6	-	0.5
Mannitol[e]	0.2	-	-
Flavonoids			
Pinocembrin[d]	1.1	23	11.8
Galangin[d]	0.7	6	5.0
Chrysin[d]	0.8	4	4.8
Pinostrobin[e]	0.6	tr	-
Pinobanksin[e]	0.3	7	-
3-O-acetylpinobanksin[e]	1.1	6	-
Triterpenic alcohols			
Lanosterol[e]	1.2	-	-
Cycloartenol[e,g]	7.1	-	-
Triterpenic alcohol of amyrine type[f]	4.8	-	-
β-amyrine[e,g]	4.7	-	-
Others			
Phosphoric acid[e]	2.7	-	-
Tricosane[e]	0.5	-	-
Glycerol octadecyl ether[e,g]	1.8	-	-

[a] The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.
[b] Data from Bankova *et al.*, 1992.
c Data from Greenaway *et al.*, 1988.
[d] Identified by comparison with authentic samples (RT, mass spectrum).
[e] Idntified by mass spectra (computer surches on commercial libraries).
[f] Tentatively identified by analysis of mass spectrum.
[g] For the first time in propolis.

were identified in it. Analogous to European propolis the main components appeared to be phenolics: phenolic acids, their esters and flavonoids. Phenolic acids concentrations were lower than these of their esters, as it was found in European samples. Most of the identified acids and some of their esters are characteristic for European bee glue: benzoic acid, p-coumaric, 3,4-dimethoxycinnamic, ferulic and caffeic acid, as well as three esters of caffeic acid: isopentenyl caffeate, dimethylallyl caffeate and benzyl caffeate. The main components of this group appeared to be four new compounds, tentatively identified as esters of caffeic acid with long-chain alcohols: dodecyl, tetradecyl, tetradecenyl and hexadecyl caffeates. The exact structures of the alcohols remain unknown and their determination needs a further isolation of the esters in pure state.

The flavonoid composition of Egyptian propolis resembles that of the European one. In the both cases flavanones predominated, but their amount is significantly lower in the Egyptian sample.

Between the main components of the sample investigated appeared to be phosphoric acid, palmitic acid and oleic acid, accompanied by stearic acid and tetracosanoic acid in average concentrations. Ethyl esters of palmitic and oleic acids, observed in lower concentrations, probably are artefacts, formed in the ethanolic solution of propolis. 1-Octadecylglycerol was identified for the first time in propolis

Contrary to European propolis the Egyptian sample contained some alcohols in notable amounts.. Two of the significant propolis components possess very similar spectra and evidently are isomeric pentacyclic triterpeneic alcohols from the amyrine type, one of them identified as widely-spread in plants β-amyrine. Analogous compounds have been found recently in Brazilian propolis (Bankova *et al.,* unpublished results), but never in European samples.

In European propolis, some phytosterols have been identified (Maciejewicz *et al.,* 1982; Marcucci, 1995), which are normal for higher plants. Surprisingly in Egyptian propolis we did not found the above mentioned sterols. Instead, we found their biogenetic precursors: lanosterol (in low concentration) and cycloartenol, the latter being one of the main propolis constituents. Cycloartenol was found for the first time in propolis.

In Egyptian propolis we found high concentrations of carbohydrates. Besides the main compounds from this group: glucose, fructose and sucrose, which are characteristic for hives, we identified sorbose and some pentoses, tentatively identified as xylose, ribose and arabinose. Mannitol was also identified.

The comparison of the chemical composition of the investigated sample with this of earlier studied Egyptian propolis (Abd El-Hady and Hegazi, 1994; Abd El-Hady, 1994) showed significant differences. We did not find any of the phenolics reported earlier. Only three compounds in both samples possessed identical molecular masses but entirely different mass spectral fragmentation. Both samples contained different flavonoids and in the sample, investigated by us, no chalcones were present. These results confirm the variability of the chemical composition of tropical propolis, known for South American bee glue (Tomas-Barberan *et al.,* 1993; Aga *et al.,* 1994; Bankova *et al.,* 1996). The explanation could be analogous: the complex origin of Egyptian propolis, which must be gathered from more than one plant source. One of the plant sources has to be some poplar species, probably the most widely distributed in Egypt and especially at the collection site poplar *P. nigra.* This is indicated by the high concentrations of esters of phenolic acids, the presence of pentenyl caffeates and the typical flavanones. The origin of the long chain caffeates remains unclear, investigation of poplar bud exudates from Egypt is necessary to find out if they are poplar metabolites. The presence of substances unusual for poplar buds, such as sterol precursors, amyrines, some alcohols, are an indication that there could be another plant source of propolis in Egypt. In order to solve this problem, propolis from different regions of Egypt has to be investigated, especially these without poplars in the vicinity of the hives. Also, Egyptian plants possessing resinous exudates must be studied as probable sources of propolis.

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