GC-MS Analysis of Propolis Samples from Two Different Regions of Turkey

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Z. Naturforsch. **57 c**, 905–909 (2002); received May 14/June 24, 2002

Propolis, GC/MS, Chemical Composition

Silylated ethanolic extract of two propolis samples from Kazan and Marmaris regions in Turkey were investigated by capillary GC-MS. The compounds were characterized by comparison with library searches. Twenty four compounds from Kazan samples were identified, eight of them were new for propolis. Eighteen compounds from Marmaris samples were identified, two of them were new for propolis.

Introduction

Propolis is a complex resinous hive product and mixture of wax, sugars and plant exudates collected by bees from certain plant sources in the neighborhood. More than 300 constituents have been identified in different propolis samples (Banskota *et al.*, 2001). In general, propolis composition is directly related to that of bud exudates collected by bees from various trees: poplar (*Populus spp.*), birch (*Betula alba*), beech (*Fagus sylvatica*), horse chestnut (*Aesculus hippocastanum*), alder (*Alnus glutinosa*) and various conifers (Mochida *et al.*, 1985; Ghisalberti, 1979; Amoros *et al.*, 1992; Bankova *et al.*, 2000). Literature survey revealed that flavonoids, aromatic acids, diterpenic acids and phenolic compounds appear to be the principal components responsible for the biological activities of propolis samples. The ethanolic extract of propolis has some activities such as antibacterial (Mochida *et al.*, 1985; Ghisalberti, 1979; Velikova *et al.*, 2000; Pepeljnjak *et al.*, 1985), antifungal (Dimov *et al.*, 1991; Schneidewind *et al.*, 1979; Murad *et al.*, 2002), antiviral (Amoros *et al.*, 1992, Amoros *et al.*, 1994), local-anaesthetic (Paintz and Metzner, 1979), anti-inflammatory (Strehl *et al.*, 1993; Miyataka *et al.*, 1997), antioxidant (Sun *et al.*, 2000; Isla *et al.*, 2001), hepatoprotective (Gonzales *et al.*, 1995), immunostimulating (Dimov *et al.*, 1991), and cytostatic (Frenkel *et al.*, 1993; Banskota *et al.,* 2001).

The present study investigated the composition of 70% ethanolic extracts of two propolis samples collected from different regions of Turkey. The variation in composition is related to the constituents of the plant material making up the native vegetation.

Material and Methods

Propolis origin

Propolis samples were collected from two different localities in Turkey Ankara-Kazan in April 1996 (Central Anatolia) and Muğla-Marmaris in May 1996 (Southwestern Anatolia), representing two different regions in Turkey.

Hand-collected propolis samples were kept desiccated in the dark up to their processing. Voucher specimens are deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ankara, Turkey.

Chemicals

Bis-(trimethyl-silyl)trifluoroacetamide (BSTFA) (Merck-10255) and trimethylchlorosilane (TMCS) (Merck-2333) were used as silylation reagents with spectrophotometric grade pyridine (Merck-7460).

Instruments

Gas chromatography-mass spectrometry was carried out on a Fisons GC 8000 gas chromatograph coupled to a Fisons MD 800 mass detector under electron impact ionization (70 eV). The interface temperature 230 °C, and the MS scan range was 35–450 atomic mass units (AMU). The chromatographic column for the analysis was fused silica OV1 capillary column (25 m × 0.25 mm

0939–5075/2002/0900–0905 $ 06.00

i.d.). The carrier gas used was helium at a flow rate of 10 ml/min. Marmaris samples were analysed with the column held initially at 60 °C for 2 min and then increased to 170 °C with a 2 °C/min heating ramp and then kept at 170 °C for 3 min. For the analysis of Kazan samples; the column held initially at 60 °C for 2 min and then increased to 170 °C with a 3 °C/min heating ramp and then kept at 170 °C for 3 min. Finally, temperature was increased to 250 °C with a 3 °C/min heating ramp and the temperature was kept at 250 °C for 120 min. for both samples. The injection was performed in split mode at 220 °C.

Sample preparation

5 gr Propolis samples were extracted twice (30 min.) in an ultrasonic bath (Sonicor; SC-50–22) with 100 ml of 70% ethanol, at room temperature to obtain the extract. After filtration; the extracts were combined and evaporated to dryness under vacuum at 50 °C. 1 mg of dry extract was reacted with 50 μl pyridine + 100 μl bis-(trimethylsilyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube for 30 min at 100 °C to prepare samples for gas chromatography (Greenaway *et al.*, 1988). Sample volumes of 1 μl were injected and analysed by GC-MS.

Identification of compounds

Peaks were identified by computer searches in commercial reference libraries. Good spectral matches for some compounds could be found in the Wiley and National Bureau of Standards (NBS) mass spectral library.

Results and Discussion

Different substance combinations will be essential for the biological activity of different propolis samples. Analyzing the ethanolic extract of propolis samples by a quick screening method will help to know its composition and promote biological properties. The identification of mixtures of natural products by mass spectral analysis alone is rather difficult, because of the number of isomers and minor differences can be observed in their mass spectra (Pereira *et al.*, 2000). Therefore; caffeic acids were indicated as isomers in the Tables.

The compounds of propolis samples are identified and listed in Table I. The peak numbers in the Table I are given according to the retention time only to the major peaks. The results showed that the complex composition of samples and main types of compounds were identified as listed in Table II. The following compounds were identified for the first time in propolis: 1H-3A,7-Methanoazulene, 2,3,4,7,8,8A-hexahydro-3,6,8,8-tetramethyl-[3*R*-(3α,3aβ,7β, 8aα)]; 8-βH-cedran-8-ol; 1,4-anhydroglucitol; 1-naphthalenemethanol, decahydro-1,10-dimethyl-6-methenyl-5-(5-hydroxy-3-pentene); 3-α,5-β-pregnan-20-one; androstan-1,17-dimethyl-17-hydroxy-3-one; thunbergol and docosa-8,14-diyn-*cis*-1,22-diol in Kazan samples, 4-βH,5α-eremophi1–1(10)-ene and 1-(5-ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethanol in Marmaris samples. Benzoic acid, glycerol, butanedioic acid, malic acid, mannose, cinnamic acid, α-D-mannopyranose, hexadecanoic acid, ferulic acid, caffeic acid, oleic acid, and isopimaric acid were detected in both samples.

The variability of constituents of propolis in two samples showed that they were collected by the honeybee from different plants depending on the geographic location. Flavonoid aglycones were not detected in two samples as the typical compounds of poplar propolis. The main compounds were isopimaric acid, androstan-1,17-dimethyl-17-hydroxy-3-one, docosa-8,14-diyn-*cis*-1,22-diol and thunbergol of the Kazan and the plant source of these compounds remains unknown. Caffeic acid isomers, abietic acid, dehydroabietic acid and isopimaric acid were major compounds of the Marmaris probably originates from the bud exudate of *Pinus brutia* L. Presence of steroid compounds and long-chain fatty alcohol in Kazan propolis indicated that there could be another plant source for propolis which needs more investigation.

Table I. Identified compounds of Kazan and Marmaris propolis samples. (The peak numbers in the table are given according to the retention time only to the major peaks).

Peak No	Rt (minutes)	KAZAN	Peak No	Rt (minutes)	MARMARİS
	7.407	benzoic acid		8.456	benzoic acid
(1)	8.306	glycerol		10.056	glycerol
	8.773	butanedioic acid		10.500	α-Terpineol
	9.779	1H-3A,7-Methanoazulene, 2,3,4,7,8,8A-hexa-hydro-3,6,8,8-tetramethyl-[3R-(3α,3aβ,7β, 8aα)]	(1)	10.869	Butanedioic acid
	11.389	malic acid	(2)	15.799	malic acid
	12.295	8-βH-Cedran-8-ol		20.498	4-βH,5α-Eremophi1–1(10)-ene
	13.146	dodecanoic acid		20.706	1-(5-Ethenyltetrahydro-5-methyl-2-furanyl) –1-methylethanol
	14.703	β-D-Galactofuranose	(3)	24.839	mannose
(2)	15.270	D-Fructose		25.063	cinnamic acid
	15.537	1,4-Anhydroglucitol	(4)	26.901	α-D-Mannopyranose
(3)	16.058	mannose		27.121	hexadecanoic acid
	16.210	cinnamic acid		27.834	ferulic acid
	16.513	1-Naphthalenemethanol, Decahydro-1,10-dimethyl-6-methenyl-5-(5-hydroxy-3-pentene)	(5)	28.994	caffeic acid isomer-1
(4)	17.075	α-D-Mannopyranose	(6)	30.164	oleic acid
	17.256	hexadecanoic acid	(7)	31.764	isopimaric acid
	17.631	ferulic acid	(8)	32.722	dehydroabietic acid
(5)	17.930	farnesol	(9)	32.827	abietic acid
	18.211	caffeic acid	(10)	33.283	caffeic acid isomer-2
(6)	18.853	oleic acid			
(7)	20.297	3-α,5-β-Pregnan-20-one			
(8)	20.680	androstan-1,17-dimethyl-17-hydroxy-3-one			
(9)	21.127	thunbergol			
(10)	21.594	docosa-8,14-diyn-cis-1,22-diol			
(11)	22.016	isopimaric acid			

Table II. Chemical composition of ethanol extracts of Kazan and Marmaris propolis samples (% of total ion current, GC-MS)*.

Compound	Kazan	Marmaris
Aliphatic acids		
Butanedioic acid	0.19	1.12
Malic acid	0.11	1.58
Dodecanoic acid	0.04	–
Hexadecanoic acid	0.73	0.29
Oleic acid	4.72	2.79
Aromatic acids		
Benzoic acid	0.34	0.20
Cinnamic acid	0.08	0.10
Ferulic acid	0.09	0.29
Caffeic acid	0.32	–
Caffeic acid isomer-1	–	1.90
Caffeic acid isomer-2	–	18.54
Monoterpenes		
α-Terpineol	–	0.23
Sesquiterpenes		
1H-3A,7-Methanoazulene, 2,3,4,7,8,8A-hexahydro-3,6,8,8-tetramethyl-[3R-(3α,3aβ,7β, 8aα)][a]	0.06	–
8-βH-Cedran-8-ol[a]	0.09	–
4-βH,5α-Eremophi1 – 1(10)-ene[a]	–	0.52
Farnesol	1.57	–
Diterpenes		
1-Naphthalenemethanol, decahydro-1,10-dimethyl-6-methenyl-5-(5-hydroxy-3-Pentene)[a]	0.19	–
Thunbergol[a]	7.82	–
Isopimaric acid	26.88	11.17
Dehydroabietic acid	–	10.61
Abietic acid	–	11.39
Sugars		
β-D-Galactofuranose	0.05	–
D-Fructose	1.11	–
α-D-Mannopyranose	1.04	0.62
Mannose	1.04	0.52
Steroid compounds		
3-α,5-β-Pregnan-20-one[a]	2.28	–
Androstan-1,17-dimethyl-17-hydroxy-3-one[a]	12.71	–
Others		
Glycerol	1.47	0.38
1,4-Anhydroglucitol[a]	0.12	–
Docosa-8,14-diyn-cis-1,22-diol[a]	12.05	–
1-(5-Ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethanol[a]	–	0.40

* The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.
[a] For the first time in propolis.

Amoros M., Simoes C. M. O., Girre L., Sauvager F. and Cormier M. (1992), Synergistic effect of flavones and flavonols against *Herpes simplex* virus type 1 in cell culture. Comparison with the antiviral activity of propolis. J. Nat. Prod. **55**, 1732–1740.

Amoros M., Lurton E., Boustie J., Girre L., Sauvager F. and Cormier M. (1994), Comparison of the anti-*Herpes simplex* virus activities of propolis and 3-methylbut-2-enyl caffeate. J. Nat. Prod. **57**, 644–647.

Bankova V. S., De Castro S. L. and Marcucci M. C. (2000), Propolis: recent advances in chemistry and plant origin. Apidologie **31**, 3–15.

Banskota A. H., Tezuka Y. and Kadota S. (2001), Recent progress in pharmacological research of propolis. Phytoth. Res. **15**, 561–571.

Dimov V., Ivanovska N., Manolova N., Bankova V., Nikolov N. and Popov S. (1991), Immunomodulatory action of propolis. Influence on anti-infectious protection and macrophage function. Apidologie **22**, 155–162.

Frenkel K., Wei H., Bhimani R., Ye J., Zadunaisky J. A., Huang Mou-Tuan, Ferraro T., Conney A. H. and Grunberger D. (1993), Inhibition of tumor promoter-mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. Cancer Res. **53**, 1255–1261.

Ghisalberti E. L. (1979), Propolis: A review. Bee World **60**, 59–84.

Gonzales R., Corcho I., Remirez D., Rodriguez S., Ancheta O., Merino N., Gonzales A. and Pascual C. (1995), Hepatoprotective effects of propolis extract on carbon tetrachloride-induced liver injury in rats. Phytoth. Res. **9**, 114–117.

Greenaway W., Scaysbrook T. and Whatley F. R. (1988), Composition of propolis in Oxfordshire, U. K. and its relation to poplar bud exudate. Z. Naturforsch. **43c**, 301–305.

Isla M. I., Moreno M. I. N., Sampietro A. R. and Vattuone M. A. (2001), Antioxidant activity of Argentina propolis extracts. J. Ethnopharm. **76**, 165–170.

Miyataka H., Nishiki M., Matsumoto H., Fujimoto T., Matsuka M. and Satoh T. (1997), Evaluation of propolis. I. Evaluation of Brazilian and Chinese propolis by enzymatic and physico-chemical methods. Biol. Pharm. Bull. **20**, 496–501.

Mochida S., Haga M. and Takino Y. (1985), Chemical constituents and antimicrobial activity of Japanese propolis. Apimondia. The XXXth Intern. Apicultural Congr. Nagoya-Japan, pp.455–456.

Murad J. M., Calvi S. A., Soares A. M. V. C., Bankova V. and Sforcin J. M. (2002), Effect of propolis from Brazil and Bulgaria on fungucidal activity of macrophages against *Paracoccidioides brasiliensis*. J. Ethnopharm. **79**, 331–334.

Paintz M. and Metzner J. (1979), Zur Lokalanästhetischen Wirkung von Propolis und einigen Inhaltsstoffen. Pharmazie **34**, 839–841.

Pepeljnjak S., Jalsenjak I. and Maysinger D. (1985), Flavonoid content in propolis extracts and growth inhibition of *Bacillus subtilis*. Pharmazie **40**, 122–123.

Pereira A. S., Norsell M., Cardoso J. N. and Aquino Neto F. R. (2000), Rapid screening of polar compounds in Brazilian propolis by high-temperature high-resolution gas chromatography-mass spectrometry. J. Agric. Food. Chem. **48**, 5226–5230.

Schneidewind E.-M., Büge A., Kala H., Metzner J. and Zschunke A. (1979), Identification of an antimicrobially active constituent isolated from propolis. Pharmazie **34**, 103–106.

Strehl E., Volpert R. and Elstner E. F. (1993), Biochemical activities of propolis extracts III. Inhibition of dihydrofolate reductase. Z. Naturforsch. **49c**, 39–43.

Sun F., Hayami S., Haruna S., Ogiri Y., Tanaka K., Yamada Y., Ikeda K., Yamada H., Sugimoto H., Kawai N. and Kojo S. (2000), *In vivo* antioxidative activity of propolis evaluated by the interaction with vitamin C and vitamin E and the level of lipid hydroperoxides in rats. J. Agric. Food Chem. **48**, 1462–1465.

Velikova M., Bankova V., Tsvetkova I., Kujumgiev A. and Marcucci M. C. (2000), Antibacterial ent-kaurene from Brazilian propolis of native stingless bees. Fitoterapia **71**, 693–696.