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LIGNANS AND OTHER CONSTITUENTS OF PROPOLIS FROM THE CANARY ISLANDS

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Key Word Index—Propolis; lignans; carbohydrates; volatiles; GC-MS.

Abstract—Two propolis samples, collected at different location on Gran Canaria (Canary Islands) were analysed by TLC and GC-MS. In volatiles sesquiterpenoids predominated, while alcoholic extracts mainly contain furofuran lignans and carbohydrates. Partial structures of two new lignans are proposed.

INTRODUCTION

Propolis (bee glue) is a resinous hive product, collected by bees from plant exudates. It has wide application in medicine, cosmetics and food industry [1] due to its versatile biological activities. These include antimicrobial, fungicidal, antiviral, antiulcer, immunostimulating, hypotensive and cytostatic activities [2].

In temperate zones, from Arizona to Mongolia, and even in New Zealand, propolis originates from the bud exudate of *Populus* species. Thus propolis from these regions contains the typical poplar bud phenolics: mainly flavonoid aglycones and esters of aromatic acids [3–7]. In tropical regions there are no poplars, but nevertheless bees collect propolis there. Obviously they have to find new plant sources of bee glue and so tropical propolis might have a chemical composition quite different from that of temperate zone propolis. This proposal is in agreement with data published on chemical constituents of propolis from Brazil and Venezuela [8–10]. Tropical samples from different locations showed significant qualitative differences to propolis from temperate zones.

For this reason it is interesting to investigate the chemical composition of propolis from tropical regions. We chose the Canary Islands because of their climatic differences from Europe and tropical South America, as well as because of the absence of poplars in this area, which implies other resource(es) of propolis.

RESULTS AND DISCUSSION

Both samples were collected at the Canary Islands. Preliminary analysis by TLC showed a significant similarity in their chemical composition: only quantitative differences existed. The extract with 70% alcohol, the so called ''propolis balsam'' usually applied in medicine, was investigated by GC-MS. Besides some low molecular mass acids and phosphoric acid, characteristic for propolis from different regions, the investigated samples contained mainly carbohydrates and phenolics (Table 1). Sample K-1 was very rich in carbohydrates: pentoses, hexoses and disaccharides. The main compound was identified as mannose. Glucose, fructose and sucrose, characteristic for honey and propolis, were also found in significant amounts. Some polyalcohols such as xylitol and mio-inositol were also detected (Table 1). The same compounds occur in sample K-2 in lower concentrations.

Phenolic compounds in both samples appeared to be identical, their concentrations in K-2 being much higher. Unlike bee glue samples from temperate zones, the typical ''propolis phenolics'' were absent. Instead, two of the significant components of sample K-2 were identified as episesamin **1** and methyl xanthoxylol **2** using computer search on commercial libraries (identity of the spectra 97%). These substances are lignans, and are both of the furofuran type (2,6-diaryl-3,7-dioxabicyclo[3,3,0]octanes). The mass spectral fragmentation of furofuran lignans produces a few very typical fragments [11], as shown in Fig. 1. Careful analysis of the mass spectra allowed us to propose the tentative structures of 11 other members of this class of compounds, **3**–**13**, present in both propolis samples from Canary Islands.The molecular mass and the masses of fragment ions shown in Fig. 1 enabled us to determine the type and number of substituents in every aromatic nucleus, but not their exact positions. The fragmentation can give no information about the stereochemistry of the molecule, either. So substances

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Table 1. Chemical composition (%)* of ethanol extracts of propolis from Canary Islands (Gran Canaria)

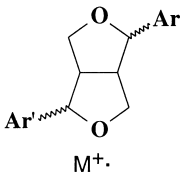
Compound	K-1	K-2	Compound	K-1	K-2
Acids			Sugar alcohols and acids		
Palmitic acid	0.9	0.5	erytriol†	0.1	0.1
Stearic acid	0.1	0.1	xylitol†	0.1	<0.1
Oleic acid	1.1	1.0	inositol†	0.2	0.1
Methylmalonic acid†	<0.1	<0.1	*myo*-inositol†	0.1	0.1
Lactic acid	0.3	0.3	erytraric acid†	0.1	—
Malic acid	0.2	0.1	2-deoxyerytropentaric acid†	0.1	0.1
Dimethyoxybenzoic acid	0.1	—	tetronic acid†	<0.1	—
Phosphoric acid	1.5	0.9	glucuronic acid†	0.3	0.1
Sugars			Lignans		
D-ribofuranose	0.5	0.1	isosesamin **1**†	2.0	7.4
D-xylopiranose†	0.2	0.1	methyl xantoxylol **2**†	3.1	13.5
D-mannopyranose†	13.0	2.0	**3**†	0.1	0.6
D-sorbopyranose	9.5	2.1	**4**†	0.1	0.4
D-galactose†	1.2	0.4	**5**†	0.2	1.1
D-fructose	5.6	1.7	**12**†	4.5	20.3
β-D-glucopyranose	10.4	2.0	**6**†	0.4	6.4
Sucrose	1.6	0.7	**13**†	1.4	6.4
Lactose†	0.5	—	**7**†	0.2	1.0
Maltose†	2.4	0.3	**8**†	2.8	13.5
Melibiose†	0.5	0.2	**9**†	1.8	7.4
			10†	0.1	0.4
			11†	0.1	0.4
			Others		
			diterpene acid	0.1	0.1

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

12 and **13** possess mass spectra identical to **5** and **6** respectively but have different R_t. Probably they are diastereoisomers or positional isomers of **5** and **6**.

According to the mass spectra, substance **10** has two methoxy groups in one of the aromatic nuclei and two hydroxy groups in the other one; while in **11** one of the nuclei bears three methoxy groups, and the other two hydroxy groups. To the best of our knowledge, furofurans with such substituents have not been isolated from natural sources until now. We are not able to give their exact constitution and stereochemistry, but evidently they are new natural compounds and we are going to try to isolate and characterize them.

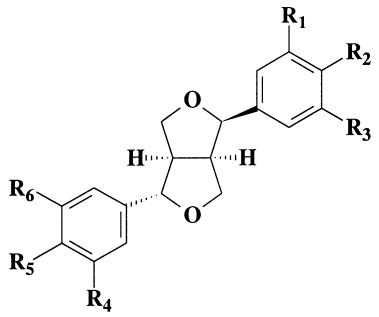
Until now, only one lignan has been found in propolis [12], belonging to the benzofuran type. The discovery of lignans can give information about the origin of propolis from Canary Islands. The source has to be a plant species producing resinous exudate rich in lignans of the furofuran type. According to the data



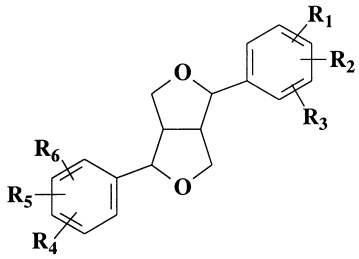
$ArCHO^{+}\cdot$ $ArCO^{+}$ $ArCH{=}CHCH_2^{+}$ $ArCH_2^{+}$

$Ar'CHO^{+}\cdot$ $Ar'CO^{+}$ $Ar'CH{=}CHCH_2^{+}$ $Ar'CH_2^{+}$

Fig. 1. Main mass spectral fragments produced from furofuran lignans.



	R1	R2	R3	R4	R5	R6
1	OCH_2O		H	OCH_2O		H
2	OMe	OMe	H	OCH_2O		H



	R1	R2	R3	R4	R5	R6
3	OMe	OMe	H	OMe	OMe	H
4	OH	OH	H	OCH_2O		H
5, 12	OMe	OMe	OMe	OCH_2O		H
6, 13	OMe	OMe	H	OMe	OMe	OMe
7	OMe	OH	H	OMe	OMe	OMe
8	OMe	OMe	OMe	OMe	OMe	OMe
9	OMe	OMe	OMe	OCH_2O		OMe
10	OMe	OMe	H	OH	OH	H
11	OMe	OMe	OMe	OH	OH	H

obtained, there could be a second plant source from which most sugars, besides glucose and fructose, originate.

Volatile compounds are in low concentrations in propolis, but their aroma and significant biological activity [13, 14] makes them of importance for the characterization of propolis. Also, their composition can give valuable information about the origin of propolis.

Volatiles were obtained from both investigated samples and analysed by GC-MS. The results are summarised in Table 2. The composition of volatiles is more or less similar to that of propolis from other regions. The main components appeared to be terpenoids. Their concentrations were significantly higher in sample K-2. This is an indication that the plant source of these compounds might be the same one that gives the furofuran lignans, which predominate in the alcohol extract of sample K-2. Most of the terpenes were sesquiterpene hydrocarbons and alcohols, analogously to all other propolis samples investigated, while monoterpenoids were in low concentrations. The characteristic for Brazilian propolis spatulenol appeared to be the main sesquiterpene in Canary Island samples. Benzyl benzoate, but not β-eudesmol, was discovered in both samples, which is an indication that Canary Islands propolis belongs to the benzyl benzoate type [14], analogously to Brazilian propolis [15]. Other aromatic compounds were found in low concentrations (Table 2).

The pesticide Vanguard BT, as well as *m*-methylstirol and 2-methylnaphthalene evidently are due to the pollution and confirm our suggestion that propolis could be used as a bioindicator of pollution.

Table 2. Chemical composition (%)* of volatiles from propolis from Canary Islands (Gran Canaria)

Compounds	K-1	K-2	Compounds	K-1	K-2
Acids and Esters			Sesquiterpenes		
Myristic acid	1.3	0.7	aromadendrene	0.3	2.8
Cinnamic acid	3.6	0.5	ledol	1.6	3.8
methyl palmitate†	0.7	0.4	spatulenol	3.2	8.4
Ethyl palmitate	4.3	1.1	isospatulenol†	1.2	0.8
Ethyl oleate	6.5	2.5	palustrol†	0.2	0.8
Benzyl benzoate	0.7	1.2	*β*-cayophillene	2.4	1.7
Ethyl dihydrocinnamate	0.3	0.2	α-humulene	0.2	1.1
Aldehydes			Aliphatic hydrocarbons		
Benzaldehyde	0.4	0.2	nonane	0.3	0.2
Piperonal†	0.4	0.2	decane	0.7	0.2
Monoterpenes					
Linalyl propionate†	—	0.5	undecane	1.4	0.6
Geraniol†	—	0.2	dodecane	1.9	0.6
Sesquiterpenes					
Nerolidol	3.2	11.0	tridecane	1.4	0.5
δ-cadinene	0.9	2.5	tetradecane	1.0	0.3
α-muurolene	0.7	0.9	hexadecane	1.4	1.2
α-calakorene	0.5	0.7	heptadecane	1.0	0.6
T-muurolol	1.2	2.2			
β-selinene	0.2	0.8	octadecane	0.6	0.5
Germacrene d†	0.2	0.5	nonadecane	1.5	0.7
α-copaene	0.5	0.2	henicosane	1.4	0.9
Ledene†	1.5	1.3	docosane	1.3	0.9
			tricosane	1.1	1.2
			Aromatic hydrocarbons		
			2-methylnaphthalene	0.5	0.2
			m-methylstirol	—	0.4
			Others		
			Vanquard BT (pesticide)	3.7	1.5
			dodecaniene-1-ol†	2.0	0.8

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

EXPERIMENTAL

Propolis

Propolis samples were collected from the Canary Island of Gran Canaria as follows: Sample K-1 near San Mateo, Sample K-2 near Telde.

Extraction and sample preparation

Propolis, grated after cooling, was extracted twice (×24 h) with 70% EtOH, at room temp. The extract was evaporated to dryness. About 5 mg of the residue was mixed with 40 μl dry pyridine and 60 μl BSTFA, heated at 80°C for 20 min and analysed by GC-MS.

Isolation of volatiles

The propolis samples were grated after cooling and subjected to hydrodistillation for 4 h using a Likens-Nickerson apparatus [16]. The volatiles were collected in a mixture *n*-pentane–Et_2O (1:1) and dried over anhydrous Na_2SO_4.

GC-MS analysis of ethanol extracts

For the GC-MS analysis, a 30 m × 0.25 mm ID HP-5, film thickness 25 μm, fused silica capillary column was used, mass selective detector, with He as a carrier gas, linear velocity 32 cm min^{-1}, split ratio 1:10, temp. program 80–240°C at 8°C min^{-1}, 240–300°C at 12°C min^{-1}, injector temp. 300°C.

GC-MS analysis of volatiles

The same column and carrier gas were used, temp. program 50–200°C at 5°C min^{-1}, 200–300°C at 10°C min^{-1}, injector temp. 300°C.

Identification of compounds

Identification was accomplished using computer searches in 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.

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