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Scientific note

A scientific note on the high toxicity of propolis that comes from *Myroxylon balsamum* trees

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Propolis samples show significant chemical differences among locations within the tropics and among locations between temperate and tropical zones. For this reason, tropical bee glue has recently become a subject of increasing interest to chemists and biologists (Bankova et al., 2000) and has turned out to be a source of new biologically active compounds (Banskota et al., 2000; Claus et al., 2000; Hirota et al., 2000; Kimoto et al., 1998). In our laboratory we performed a preliminary screening for cytotoxicity of 18 propolis samples from El Salvador, Central America, using the brine shrimp *Artemia salina* (Arthropoda, Crustaceae) lethality test (Soils et al., 1993). The most active sample, originating from the Western region of the country was subjected to chemical analysis.

The extract with 70% ethanol (the so called "balsam") was analyzed by GC-MS after silylation. This analysis revealed that benzyl benzoate (6%)** and benzyl cinnamate (10%) were among the major constituents of the balsam. It also contained significant amounts of benzoic acid (7%), cinnamic acid (7%), benzyl alcohol (1%), benzaldehyde (1%), benzyl ferulate (2%) and benzyl isoferulate (4%). These compounds are characteristic for the Balsam of Peru, an exudate from the tree *Myroxylon balsamum* (Fabaceae) formed when bark is injured, and used in medicine for healing of wounds, skin diseases, cough and bronchitis (Wollenweber et al., 1990). The propolis sample was collected from hives placed in a *M. balsamum* tree forest. This fact and the chemical composition of the sample allowed us to suggest that *M. balsamum* exudate was the plant source of propolis in our study. Until now, this plant material was not known to be a propolis source.

The propolis "balsam" was subsequently extracted with light petroleum, chloroform and butanol. The three extracts were tested for their toxicity and the light petroleum extract was found to be the most active (Tab. I). From the latter extract, 4 compounds were isolated by column and thin layer chromatography and their structures determined by MS, UV and NMR spectra. They were identified as benzyl cinnamate, benzyl benzoate, α-amyrin and α-amyrin acetate by comparison of their spectra with those of authentic samples, as well as by direct TLC or GC comparison with authentic samples. The main components, benzyl cinnamate and benzyl benzoate, are known propolis constituents (Wollenweber et al., 1990). α-Amyrin and its acetate, microcomponents of the extract, are new for propolis; only β-amyrin was found earlier in bee glue (Marcucci et al., 1998). The toxicity of the main constituents to *A. salina* nauplius larvae was measured (Tab. I). Benzyl cinnamate and benzyl benzoate showed remarkable toxicity, greater than that of caffeic acid phenylethyl ester (CAPE), the standard substance with well-known cytotoxicity (Grundberger et al., 1988). It is notable that both CAPE and benzyl cinnamate are esters of acids with the cinnamate

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** The figures correspond to the percent of total ion current (GC-MS). The ion current generated depends on the characteristics of the compound concerned and is not a true quantification.

Table I. Toxicity assay of propolis extracts and isolated compounds on nauplius larvae of *Artemia salina*.

Sample	% deaths at 1000 μg/ml	% deaths at 100 μg/ml	% deaths at 10 μg/ml	% deaths at 1 μg/ml	LC_{50}[a] + SD[b] (μg/ml)
Propolis balsam (extract with 70% ethanol)	100	100	97	47	1.8 ± 0.9
Light petroleum extract	100	100	100	30	1.7 ± 0.8
Chloroform extract	56	13	3	0	900 ± 450
Butanol extract	100	90	67	10	23 ± 9
Benzyl cinnamate	100	100	100	100	< 0.4
Benzyl benzoate	100	100	100	97	< 0.4
CAPE	100	100	83	63	0.45 ± 0.05

[a] Lethal concentration for 50% of the *Artemia salina* nauplii.
[b] Mean of three measurements (10 nauplii per concentration plus control in one measurement; dead nauplii were counted).

carbon skeleton. Benzyl cinnamate and benzyl benzoate are of particular interest for further investigation of their cytotoxicity to tumor cells on appropriate models in vitro and in vivo.

Note scientifique sur la forte toxicité de la propolis issue des arbres *Myroxylon balsamum*.

Eine wissenschaftliche Notiz über die hohe Toxizität des von *Myroxylon balsamum* Bäumen stammenden Propolis.

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