Botanical Sources of the Biomarkers of Mediterranean Propolis

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Introduction

Propolis is a bee product that has been proven to play a significant role in bees' social immunity, contributing to the overall good health of honeybee colonies.[1] Since bees collect propolis from resinous plant materials, propolis from different geographic regions might be of very specific chemical composition due to the specificity of the local flora. During the last decade, numerous studies have demonstrated the existence of a new European propolis type: Mediterranean, which is characterised by high diterpene concentration and remarkable antibacterial activity.[2] Based on the identified diterpenes, the source plant has been suggested to be some conifer species, most probably of the Cupressaceae family, in which the flora of the Mediterranean Region is very rich.[3] However, Pinus species could not be disregarded as resin sources.[4] In this study we report on the identification of the source of the most abundant and important diterpenes in Mediterranean propolis: the resin of the common cypress, *Cupressus sempervirens*.

Methodology

Propolis samples were collected from apiaries at 14 different locations in Malta and at 3 locations on the island of Gozo. The vegetative material and external resin of *C. sempervirens* (2 samples) and *Pinus halepensis* (2 samples) were collected in Malta from the Buskett area.

GC–MS analysis was performed with a Hewlett–Packard gas chromatograph 5890 series II Plus linked to a Hewlett–Packard 5972 mass spectrometer system equipped with a 30 m long, 0.25 mm i.d., and 0.5 μm film thickness HP5-MS capillary column.

The identification of the compounds was performed using commercial libraries and comparison of mass spectra and retention times of reference compounds. In the cases of lack of the corresponding reference compounds, the structures were proposed on the basis of their general fragmentation and using reference literature spectra where possible. The semiquantification of the main compounds was carried out by internal normalisation with the area of each compound.

Results

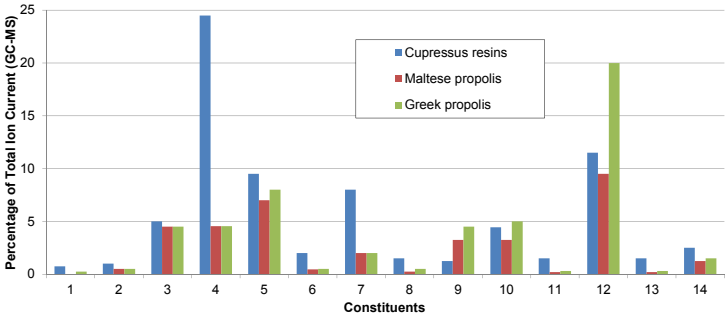

Figure 1: Diterpenic profile of Maltese and Greek propolis[4] , and of *C. sempervirens* resin (mean values).1) ferruginol, 2) sempervirol, 3) communic acid, 4) totarol + 13-epitorulosal, 5) pimaric acid + imbricataloic acid, 6) 13-epi-torulosol, 7) 13-epi-cupressic acid, 8) ferruginolon, 9) isoagatholal, 10) agathadiol + imbricatoloic acid, 11) totarolon, 12) isocupressic acid, 13) 6/7-hydroxyferruginol, 14) acetylisocupressic acid.

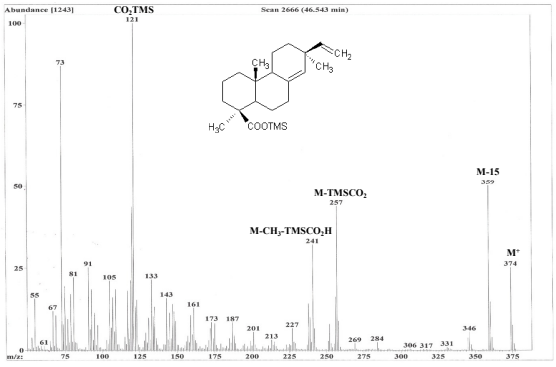

Figure 2: Mass spectra of silylated pimarane diterpene, pimaric acid (9.5% and 7% GC-MS TIC of *C. sempervirens* resin and Maltese propolis, respectively).

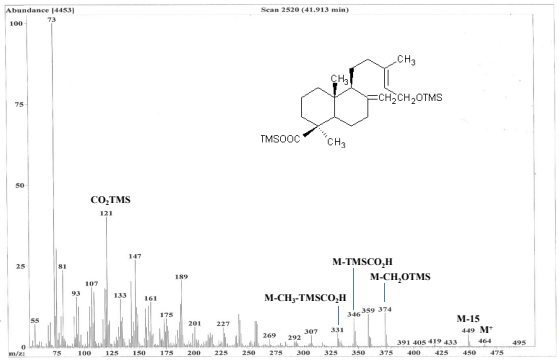

Figure 3: Mass spectra of silylated labdane diterpene, isocupressic acid (11% and 9.5% GC-MS TIC of *C. sempervirens* resin and Maltese propolis, respectively).

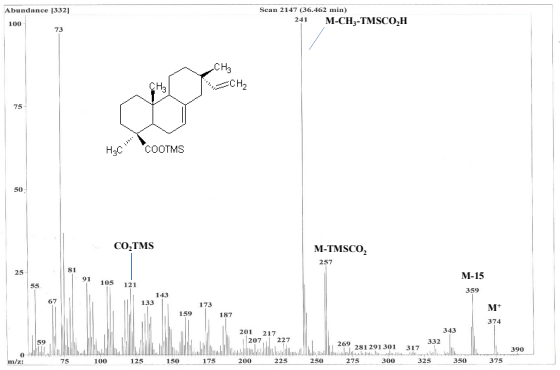

Figure 4: Mass spectra of silylated pimarane diterpene, isopimaric acid identified in *P. halepensis* (15% GC-MS TIC) but not in *C. sempervirens* and Maltese propolis.

Conclusions

The diterpenic profile of Maltese propolis was close to the one of propolis from South-Eastern Greece.[3] They both displayed significant similarity to the profile of the resin of *C. sempervirens* (Fig. 1). The most important diterpenic constituents, 19 individual compounds, were the same in all three materials. The resin of *P. halepensis* did not contain any of these compounds. The major constituents of the latter were abietic, dehydroabietic, neoabietic, isopimaric and palustric acids. Of them, isopimaric and palustric acids were not identified in propolis, and only traces of the acids with abietane skeleton were detected. This allowed us to exclude *P. halepensis* as a plant source for Mediterranean propolis. Our data thus support the hypothesis that the diterpene-rich Mediterranean propolis originates mainly from the resin of the common cypress *C. sempervirens*.

References

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4. Cox RE, Yamamoto S, Otto A, Simoneit BRT. (2007) Oxygenated di- and tri-cyclic diterpenoids of southern hemisphere conifers. Biochemical Systematics and Ecolgy 35, 342-362.