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Artigo

BAUER-7-EN-3β-YL ACETATE: A MAJOR CONSTITUENT OF UNUSUAL SAMPLES OF BRAZILIAN PROPOLIS

Érica Weinstein Teixeira
Agência Paulista de Tecnologia dos Agronegócios, Secretaria da Agricultura e Abastecimento, CP 07, Pindamonhangaba - SP
Dejair Message
Departamento de Biologia Animal, Instituto de Biociências, Universidade Federal de Viçosa, 36571-000 Viçosa - MG
Giuseppina Negri* e Antonio Salatino
Departamento de Botânica, Universidade de São Paulo, CP 11461, 05422-970 São Paulo - SP

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The pentacyclic triterpenoid bauer-7-en-3β-yl acetate was obtained from the chloroform extract of an unusual sample of propolis from southeast Brazil with the yield of 7%. The compound was identified by comparison of IR, MS and NMR analysis with published data.

Keywords: propolis; triterpenoids; bauer-7-en-3β-yl acetate.

INTRODUCTION

Propolis is a hive product, formed by a complex mixture of beeswax, plant exudates collected by *Apis mellifera* and a suite of other substances[1]. Biological activities, such as anticancer, antioxidant, anti-inflammatory and antibiotic have been reported for propolis and its constituents[2]. More than 200 compounds have been identified in propolis, among which phenolics predominate[1]. In southeast Brazil, in particular in areas dominated by Cerrado - a characteristic savanna vegetation of central and southeast Brazil[3] - "green propolis" is prevalent and has gained the preference of importers of Brazilian propolis. One of the propolis components is beeswax, composed mainly by fatty monoesters and hydrocarbons[4,5]. Another major component is the resin, with constituents obtained from plants. It has been shown that green propolis resin bears predominantly prenylated chromane and phenylpropanoid derivatives[6,7] instead of flavonoids, which predominate in European propolis[1]. Green propolis resin derives mainly from vegetative apices of *Baccharis dracunculifolia* (alecrim plants). However, wide variation detected in the chemical composition suggests contributions from alternative resin plant sources[8]. Predominant components of the resin of green propolis are cinnamic acids, chiefly compounds bearing prenyl groups. Terpenoid compounds, such as sesqui, di and pentacyclic triterpenoids, have been detected in many, but not all, samples investigated[8].

Pentacyclic triterpenoids have been obtained from chloroform extracts of Brazilian propolis[9]. Triterpenic alcohols of amyrin type and cycloartenol have been found in propolis from Brazil and Egypt[10] . Two long-chain alkanoic acid esters of lupeol were isolated together with amyrins, cycloartenol and lupeol from Brazilian propolis produced with resin collected from *Baccharis* plants[11]. Propolis samples with characteristics deviating from "green propolis" occasionally appear in southeast Brazil. In some instances the differences between the unusual and the predominant propolis types lye in high amounts of triterpenoids in the former[8,9]. The triterpenoid bauer-7-en-3β-yl acetate was previously found in trace amounts in a propolis sample from the State of São Paulo[9], being tentatively identified by comparison of EIMS with published data.

A different kind of propolis was noted in the State of Minas Gerais (southeast Brazil), in an area where "green propolis" predominate. Local apiculturists regarded the distinct characteristics of the unusual propolis as being due to its origin, supposedly based on soybean cake collected by bees for propolis production. The present work reports for the first time the isolation from propolis of the triterpenoid bauer-7-en-3β-yl acetate, which was obtained as a major constituent in this sample from southeast Brazil. The sample propolis has a pale cream color and lacks the dark green color of the propolis types that predominate in the area. Instead, it has a brown color with many white punctuations and pulverulent texture, lacking the high hardness and friability of the "green propolis".

EXPERIMENTAL PART

Materials and methods

Propolis sample

Propolis produced by africanized *Apis mellifera* were collected in the dry season (August) in Paula Candido, Minas Gerais, southeast Brazil. Seasonal semideciduous forest fragments with characteristic succession vegetation at the borders characterize the associated flora. The sample collected has a pleasant odor, which diverges from the resinous aroma of the "green propolis".

Extraction and isolation of the triterpenoid

The sample was cut in small pieces and 5 g were treated for 3 h, first with methanol and then with chloroform in Soxhlet. The chloroform extract was concentrated and the residue treated with hot methanol. The resultant solution was filtered still hot. Abundant white crystals were obtained upon cooling, the crystals being separated by filtration. Thin layer chromatography with silicagel (F_{254}, Merck) and chlorofom: ethyl ether: ethyl acetate (3:4:4), using lupeol and ursolic acid as references and the Carr-Price reagent for visualization, suggested that the crystals corresponded to a triterpenoid. Purification was achieved by recrystalization from chloroform: methanol (5:2).

*e-mail: gnegri@terra.com.br

The chloroform extract was fractionated in chromatographic columns (4.5 x 40 cm), using silica gel (Merck, 20-70 mesh) and mixtures of petroleum ether, chloroform and methanol (Merck, analytical grade) as mobile phase. The obtained fractions were treated with diazomethane for methylation and analyzed by GC/MS using the same conditions described below for the analysis of bauerenyl acetate.

Identification – NMR and MS analysis

Gas chromatography revealed that the substance obtained was composed by a single compound, which was identified as bauer-7-en-3β-yl acetate (bauerenyl acetate) by EIMS, IR, ^{1}H and ^{13}C NMR spectroscopy. EIMS data were obtained with a Hewlett Packard gas chromatograph 5890 Series II Plus, interfaced with a Hewlett Packard 5989B spectrometer operating at an ionization voltage of 70 eV. 1 μL of an ethyl ether solution was injected into the gas chromatograph. An HP-5 fused silica capillary column (30 m x 0.25 mm), mass selective detector, He as carrier gas at 32 cm min^{-1} and split ratio 1:10 were used. Oven temperatures ranged from 100 to 300 °C at 10 °C min^{-1}, ending with an isothermal period of 15 min. Injector and detector temperatures were 300 °C. Infra-red spectra were obtained with a RX IFT-IR Perkin-Elmer spectrophotometer, in KBr tablet. ^{1}H NMR and ^{13}C NMR ($CDCl_3$ – 500 MHz) spectra were obtained with a Bruker Avance DPX-500 spectrometer. Identification of the compound was based on comparison with data of mass[12] and ^{13}C and ^{1}H NMR spectra[13,14].

RESULTS AND DISCUSSION

Bauerenyl acetate (Figure 1) was isolated as white crystals. Among the data consistent with its identification are the molecular formula $C_{32}O_2H_{52}$, the fragments at *m/z* 289 (90%) and peak base at *m/z* 229 (100%) in the mass spectrum[12] and the infrared band at 1732.79 cm^{-1} (carboxylic ester group); the ^{1}H NMR signals at δ 5.40 ppm (1H,t, *J* = 4.0 Hz, H-7, double bond), δ 4.51 (1H, dd, *J* = 10.5, 5.0 Hz, H-3α) and δ 2.05 (3H, s, (CH_3) 32 linked to C=O group), and the ^{13}C NMR signals at δ 171.03 (C=O), δ 145.45 (C - 8), δ 116.24 (C - 7) and δ 81.15 (C –3)[13,14]. The high amount of bauerenyl acetate and possibly other low polar compounds account for the different characteristics of the sample analyzed in comparison with "green propolis".

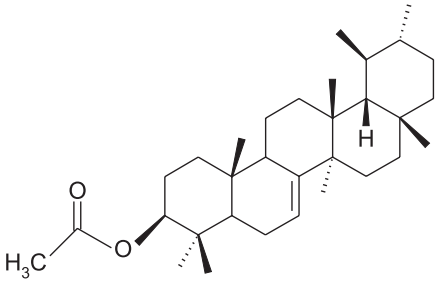

Figure 1. *Bauer-7-en-3β-yl acetate or 3β-acetoxy-D:C-friedours-7-ene*

The abundance of bauerenyl acetate in this propolis sample suggested that the composition of the pale propolis is much simpler than the common types of Brazilian propolis, such as the "green propolis" that is widely produced in the same area wherefrom the analyzed samples stemmed. Benzoic and cinnamic acid derivatives that may be obtained also from chloroform extracts of "green propolis"[7] were not detected in the analyzed sample, using the same CG/MS conditions to analyze the chloroform extract and the isolated baurenyl acetate. This is additional evidence that factors other than local flora influence propolis chemical composition and is indicative that detailed searches for propolis constituents is prone to reveal diverse propolis chemical patterns, even in restricted geographic areas. Hence, it seems that much endeavor is necessary to reach a thorough understanding of propolis chemical profiles.

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