New Bioactive Chalcones in Propolis from El Salvador
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2’,3’-Dihydroxy-4,4’-dime thox ychalcone (1) and 2’,3’,4-trihydroxy-4’-methoxy-chalcone,
two new chalcones, were isolated from propolis from El Salvador. The compounds showed
significant antibacterial ana antifungac activity and moderate toxicity to Artemia salina
nauplii.

Introduction

Propolis (bee glue) is a resinous hive product collected by bees from plant exudates. It is widely
used in medicine, cosmetics and food industry (Marcucci 1995; Matsuda 1994), due to its versatile
biological activities. These include antimicrobial, fungicidal, antiviral, immunostimulating, cytostatic
and radical-scavenging activities (Marcucci, 1995; Krol et al., 1996). For a long period, investigations
on propolis were focused on samples originating from the temperate zones (Ghisalberti, 1979; Mar­
cucci, 1995). However, the chemical composition of bee glue depends on the flora at the site of col­
lection and varies at different geographic locations (Bankova et al., 2000). For this reason the com­
position of tropical propolis is quite different form propolis from temperate regions. Recently, tropi­
cal propolis has become a subject of increasing interest for chemists and biologists (Banskota et al.,
2000a, Marcucci and Bankova, 1999; Valcic et al., 1999, Bankova et al., 1998). It turned out to be a
source of new biologically active compounds (Banskota et al., 2000b; Claus et al., 2000, Hirota
et al., 2000; Kimoto et al., 1998). In this paper we report the isolation and characterization of two
new chalcones from propolis originating from El Salvador, Central America, and their biological
activity.

Experimental

Propolis was collected in the eastern region of

Extraction of propolis

Propolis (50 g) was cut into small pieces and ex­
tacted with 70% ethanol (1:10, w:v) at room tem­
perature for 24 h. A small part of this extract
(10 ml) was evaporated to dryness and used in bio­
logical tests. The ethanol extract was concentrated
in vacuo and extracted successively with n-hexane
(3 times) and with ethyl ether (3 times). The hex­
ane extract was evaporated to give 9.1 g dry resi­
due and the ethyl ether extract gave 13.0 g dry resi­
due after evaporation.

Isolation of compounds

The ethyl ether extract was subjected to column
chromatography on silica gel with an acetone – n­
hexane gradient to produce several fractions. Af­
ter repeated column chromatography and prepara­
tive TLC on silica gel, n-hexane – methylethyl
ketone as the mobile phase, compounds 1 and 2
were isolated.

2’,3’-dihydroxy-4,4’-dimethox ychalcone (1)
51 mg, M.p. 143–145 °C. UV (MeOH) λmax
294, 308, 366 nm. EIMS (70 eV) m/z 300 (M+,
100%), 285 ([M-15]+, 7%), 166 (A1+,
57%), 161 (B3+,

8%), 138 ([A_2 - CO]^+, 59%) 134 (B_{1^+}, 78%), 121 (B_{4^+}, 17%). For ^1H and ^13C NMR – see Table I.

2',3',4-trihydroxy-4'-methoxycalcone (2). 153 mg. M.p. 179–184 °C. UV (MeOH) \( \lambda_{max} \) 305, 372 nm. EIMS (70 eV) \( m/z \) 286 (M^+, 100%), 271 ([M-15]^+, 9%), 167 (A_{2^+}, 69%), 138 ([A_2 - CO]^+, 63%), 120 (B_{1^+}, 26%). For ^1H and ^13C NMR – see Table I.

**Cytotoxicity assay**

*Artemia salina* (nauplii) lethality (Soils et al., 1993) was determined using caffeic acid phenethyl ester (CAPE) as active reference compound. Concentrations of 1000, 100, 10 and 1 ppm were used (3.33, 0.33, 0.033 and 0.0033 nM for 1 and 3.5, 0.35, 0.035 and 0.0035 nM for 2). 10 *A. salina* per concentration plus control. The activity of the total extract and of the individual compounds was measured.

**Antibacterial activity**

For the investigation of the antibacterial activity, the agar cup method (Spooner and Sykes, 1972) was used with test strains *Staphylococcus aureus* 209 (obtained from the Bulgarian Type Culture Collection, Institute for State Control of Drugs, Sofia) and *Escherichia coli* W F+ (obtained from the Collection of ZIM ET, Central Institute of Microbiology and Experimental Therapy, Jena). An inhibitory zone with a diameter less than 10 mm corresponds to lack of activity (10 mm is the diameter of the cup). 0.1 ml of test solution containing 0.4 mg of each substance in ethanol was applied to every cup.

**Antifungal activity**

For the investigation of the antifungal activity the agar cup method was used (Spooner and Sykes, 1972). As test microorganism, *Candida albicans* 562 (obtained from the Bulgarian Type Culture Collection, Institute for State Control of Drugs, Sofia) was used. The antifungal activity was measured as diameter of the inhibitory zones. An inhibitory zone with a diameter less than 10 mm corresponds to lack of activity (10 mm is the diameter of the agar cup). 0.1 ml of test solution containing 0.5 mg of each substance in ethanol was applied to every cup. Solvents showed no activity.

**Results and Discussion**

The ether fraction from the ethanolic extract of the investigated propolis sample was subjected to repeated column chromatography and preparative TLC on silica gel and afforded two yellow crystalline compounds (1 and 2). Their structures were determined by UV, EIMS, ^1H and ^13C NMR spectra, DEPT, HSQC, HMQC, HMBC.

![Chemical structures of compounds 1 and 2](image)

The UV spectrum of compound 1 was characteristic for chalcones (Mabry et al., 1970), with a dominant band I absorption at 366 nm, a shoulder at 308 nm and a relatively minor band II (294 nm). This compound afforded a M^+ ion peak at \( m/z \) 300, according to EIMS. Additional peaks at 166 (A_{2^+}), 161 (B_{5^+}), 138 ([A_2 - CO]^+), and 134 (B_{1^+}, 78%) suggested the presence of methoxylated ring B and ring A with two hydroxy and one methoxy group. Analysis of the 1D and 2D NMR spectra with homo- and heteronuclear direct or long-range correlation allowed assignment of ^1H and ^13C NMR signals, as listed in Table I. The ^1H NMR revealed 6 aromatic proton signals, belonging to two aromatic nuclei. The two doublets at \( \delta 7.02 \) and \( 7.87 \) (2H each, \( J = 8.8 \) Hz) could be attributed to a 1,4-disubstituted benzene ring, and the doublets at \( \delta 6.48 \) and \( 7.96 \) (1H each, \( J = 9 \) Hz) to a 1,2,3,4-tetrasubstituted one. Two more singlet signals at \( \delta 7.80 \) and \( 7.81 \) were observed in this spectrum; they were assigned to H-α and H-β by analysis of the HMBC data. Correlations between these protons and C-2, C-6, and the conjugated carbonyl carbon (\( \delta 192.2 \)), showed that they belonged to the E-double bond of the chalcone unit. The coupling constant \( J_E = 16 \) Hz, indicative of the E-configuration of the double bond, could only be observed in the HSQC spectrum without decoupling. Cross peak was observed also between H-6' and the carbonyl carbon. The two methoxy groups were positioned at C-4' and C-4 because the methyl protons at these groups (\( \delta 3.73 \) and 3.82)
Table I. ^1H and ^13C NMR data of chalcones 1 and 2.a

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ_H</td>
<td>δ_C</td>
<td>δ_H</td>
</tr>
<tr>
<td>1</td>
<td>127.3</td>
<td>127.0</td>
</tr>
<tr>
<td>2</td>
<td>7.87 d (8.8)</td>
<td>131.2</td>
</tr>
<tr>
<td>3</td>
<td>7.02 d (8.8)</td>
<td>114.6</td>
</tr>
<tr>
<td>4</td>
<td>161.7</td>
<td>160.4</td>
</tr>
<tr>
<td>5</td>
<td>7.02 d (8.8)</td>
<td>114.6</td>
</tr>
<tr>
<td>6</td>
<td>7.87 d (8.8)</td>
<td>131.2</td>
</tr>
<tr>
<td>C=O</td>
<td>192.2</td>
<td>192.2</td>
</tr>
<tr>
<td>Α</td>
<td>7.81 s</td>
<td>118.6</td>
</tr>
<tr>
<td>Β</td>
<td>7.80 s</td>
<td>144.1</td>
</tr>
<tr>
<td>1'</td>
<td>134.9</td>
<td>134.8</td>
</tr>
<tr>
<td>2'</td>
<td>158.7</td>
<td>158.6</td>
</tr>
<tr>
<td>3'</td>
<td>113.8</td>
<td>113.8</td>
</tr>
<tr>
<td>4'</td>
<td>157.6</td>
<td>157.3</td>
</tr>
<tr>
<td>5'</td>
<td>6.48 d (9)</td>
<td>108.2</td>
</tr>
<tr>
<td>6'</td>
<td>7.96 d (9)</td>
<td>127.2</td>
</tr>
<tr>
<td>OMe (C-4)</td>
<td>3.82 s</td>
<td>55.5</td>
</tr>
<tr>
<td>OMe (C-4')</td>
<td>3.73 s</td>
<td>59.9</td>
</tr>
</tbody>
</table>

a ^1H and ^13C NMR were measured at 250 and 62.9 MHz, respectively, in DMSO-d_6 and coupling constants (parentheses) are in Hz.

showed diagnostic HMBC correlations to C-4' and C-4, respectively. Other significant long-range correlations are shown in Fig. 1. and thus 1 was characterized as 2',3'-dihydroxy-4,4'-dimethoxychalcone.

The spectral characteristics of 2 were similar to those of 1. The difference between the two compounds appeared to be the presence of only one methoxy group in the molecule of 2. This was obvious from the comparison of NMR and MS spectral data. The signals corresponding to the methoxy group at C-4 (at δ 3.82 in the ^1H NMR spectrum and at δ 55.5 in the ^13C NMR spectrum) were absent in the spectra of 2. The mass spectrum of 2 indicated the presence of a hydroxy group instead of a methoxy in ring B. The fragments originating from ring A were the same in the mass spectra of 1 and 2. Based on these data, the structure of 2 was elucidated as 2',3',4-trihydroxy-4'-methoxychalcone.

To the best of our knowledge, 1 and 2 are new natural compounds. Both compounds showed good activity against *Staphylococcus aureus* and *Candida albicans*, which was found significantly higher than that of the total extract (Table II). No activity against *Escherichia coli* was observed. The new chalcones exhibited moderate activity against *Artemia salina* nauplii (Table II). Obviously, 1 is partially responsible for the toxicity of the ethanol extract.

The plant origin of the chalcones 1 and 2 in the investigated bee glue is unclear. The identification of new natural compounds in propolis samples provides suitable markers because the identification of 1 and 2 in some plant exudate will be an indication that this exudate is one of the sources of propolis in El Salvador.

Table II. Biological activity of propolis extract and isolated compounds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cytotoxicity assay LC50±SD (mg/ml) a</th>
<th>Antibacterial and antifungal activity Diameter of the inhibitory zone ± SD (mm)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol extract</td>
<td>39±9</td>
<td>12±1</td>
</tr>
<tr>
<td>1</td>
<td>38±8</td>
<td>29±3</td>
</tr>
<tr>
<td>2</td>
<td>327±47</td>
<td>23±1</td>
</tr>
<tr>
<td>CAPE</td>
<td>0.45±0.05</td>
<td>–</td>
</tr>
</tbody>
</table>

a Mean of three measurements.

**Acknowledgements**

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Krol W., Scheller S., Czuba Z., Matsuno T., Zydovicz G., Shani J. and Mos M. (1996), Inhibition of neutrophils chemiluminescence by ethanol extract of propolis (EEP) and its phenolic constituents. J. Ethnopharmacol. 55, 19–25.


