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Chemical Constituents of Red Mexican Propolis

Cinzia Lotti,[†] Mercedes Campo Fernandez,[‡] Anna Lisa Piccinelli,[†] Osmany Cuesta-Rubio,[‡] Ingrid Márquez Hernández,[‡] and Luca Rastrelli*,[†]

[†]Dipartimento di Scienze Farmaceutiche, University of Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy, and [‡]Instituto de Farmacia y Alimentos (IFAL), Universidad de La Habana, Avenida 23, 21425 La Lisa, Ciudad de La Habana, Cuba CP 13600

Chemical investigation of a red-type Mexican propolis sample has led to the isolation of three new compounds, 1-(3′,4′-dihydroxy-2′-methoxyphenyl)-3-(phenyl)propane (**1**), (*Z*)-1-(2′-methoxy-4′,5′-dihydroxyphenyl)-2-(3-phenyl)propene (**2**) and 3-hydroxy-5,6-dimethoxyflavan (**3**), together with seven known flavanones, isoflavans, and pterocarpans. Structural determination, was accomplished by spectroscopic analysis, particularly 2D NMR and ESI-MS/MS techniques. The present study appears to be the first report on the occurrence of isoflavonoids in Mexican propolis. In addition, the presence of compounds with a 1,3-diarylpropane and 1,3-diarylpropene carbon skeleton were found for the first time in propolis. Isolated compounds **1**−**10** indicated the possible relation between red Mexican propolis and the genus *Dalbergia*.

KEYWORDS: Mexican propolis; 1,3-diarylpropane derivatives; flavonoids and isoflavonoids; pterocarpans; 1D and 2D NMR spectroscopy; ESI-MS/MS

INTRODUCTION

Propolis is a resinous mixture that honeybees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive and contains mostly sticky plant substances, beeswax, and other bee secretions. Propolis is well-known for its potential health benefits and is reported to possess valuable biological activities such as antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, and anticancer activities (*1*, *2*). Recently, it has been extensively marketed by the pharmaceutical industries as an alternative medicine and as a health food in various parts of the world. Propolis chemical composition is qualitatively and quantitatively variable, depending on the season, the species of bee, vegetation, and the area of collection (*3*, *4*). Propolis originating from temperate zones (West Asia, Europe, and North America) possesses a similar chemical composition, the main constituents being phenolic compounds (flavonoids, cinnamic acids, and derivatives). In these regions, exudates of different poplar buds (*Populus* spp.) are the main sources of propolis, together with other trees such as birch, beech, horse chestnut, alder, and various conifers. In tropical regions, because of the difference in vegetation, the chemical composition of propolis is very different. Its color also varies depending on its botanical source, the most common being dark brown, but red propolis has been observed in tropical countries such as Brazil and Cuba (*2*−*4*).

In our previous studies, we reported for the first time the occurrence of isoflavonoids in a red variety of Cuban propolis sample, suggesting new biological potentialities for this natural product (*4*). In fact, dietary intake of isoflavonoids has been associated with lower incidences of hormonally dependent cancers, relief from symptoms of postmenopausal problems, and a reduction in the risk of osteoporosis and cardiovascular disease (*5*, *6*). Recently, Awale and co-workers (*2*, *7*) reported the isolation and cytotoxic activity against human pancreatic cancer cell line of Brazilian red propolis constituents. From both a chemical and biological point of view, few studies have been developed employing Mexican propolis. From Sonoran propolis chrysin, pinocembrin and pinobanksin 3-acetate were identified by HPLC-DAD and MS analyses as the most abundant constituents (*8*). The antibacterial and free-radical scavenging activities and antiproliferative activity on cancer cell lines of propolis collected from different areas of the Sonoran desert have been also evaluated (*9*). On the other hand, about 100 volatile constituents have been identified employing GC and GC/MS techniques from propolis samples from Yucatan state, but nonvolatile constituents have remained unknown so far (*10*). In this study we conducted a chemical investigation of a red-type Mexican propolis sample, collected in the Yucatan region, that led to the isolation of seven known flavanones, isoflavans, and pterocarpans and three new natural products. The present study appears to be the first report on the occurrence of isoflavonoids in Mexican propolis.

MATERIALS AND METHODS

General Experimental Procedure. Optical rotations were determined on a model DIP-1000 polarimeter (Jasco, Mary's Court, Easton, MD) equipped with a sodium lamp (589 nm) and a 10 cm microcell. IR spectra were determined on an IR-230 (Jasco). A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for ^{1}H and at 150.86 MHz for ^{13}C, using the UXNMR software package was used for NMR experiments; chemical shifts are expressed in δ (parts per million) referenced to the solvent peaks δ(H) 3.34 and δ(C) 49.0 for CD_3OD; coupling constants,

*To whom correspondence should be addressed. Tel: 0039 89969766. Fax: 0039 89 969602. E-mail: rastrelli@unisa.it.

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Table 1. ^{1}H and ^{13}C NMR (600 MHz) Data for Compounds **1**−**3** in CD_3OD[a]

	1		2			3	
position	$\delta(^1H)$ (J_{HH})	$\delta(^{13}C)$	$\delta(^1H)$ (J_{HH})	$\delta(^{13}C)$	position	$\delta(^1H)$ (J_{HH})	$\delta(^{13}C)$
1	2.57 (t, 15.0, 7.5)	30.26	3.47 (d, 5.7)	34.82	2	4.48 (d, 6.0)	77.9
2	1.87 (m)	34.56	6.37 (d, 11.7)	132.00	3	3.89 (ddd, 9.2, 5.0, 2.5)	77.4
3	2.65 (t, 15.0, 7.5)	36.70	6.39 (d, 10.2)	137.54	4a	2.48 (dd, 13.8, 9.2)	35.0
1′		128.44		125.30	4b	2.68 (dd, 13.8, 4.0)	35.0
2′		148.28		147.62	5		141.00
3′		139.87	6.55 (s)	112.22	6		149.31
4′		146.32		139.74	7	6.62 (d, 8.5)	121.30
5′	6.52 (d, 8.0)	120.05		141.60	8	6.61 (d, 8.5)	107.81
6′	6.49 (d, 8.0)	111.66	6.55 (s)	121.21	9		141.22
1″		144.26		138.90	10		123.90
2″, 6″	7.20 (d, 7.5)	129.42	7.35 (d, 7.6)	127.22	1′		143.60
3″, 5″	7.27 (t, 1.5, 7.5)	129.03	7.27 (t, 1.5, 7.6)	129.40	2′, 6′	7.42 (d, 7.5)	128.23
4″	7.16 (m)	126.29	7.18 (m)	128.11	3′, 5′	7.37 (t, 1.5, 7.5)	129.14
$-OCH_3$	3.75 (s)	60.61	3.82 (s)	59.03	4′	7.29 (m)	128.50
					$-OCH_3$	3.63 (s)	60.52
					$-OCH_3$	3.80 (s)	56.61

[a] Chemical shift values are in ppm from TMS, and *J* values in Hz are presented in parentheses. All signals were assigned by DQFCOSY, HSQC, and HMBC experiments.

J, are in hertz. DEPT, ^{13}C, DQF-COSY, HSQC, and HMBC NMR experiments were carried out using the conventional pulse sequences as described in the literature. Electrospray ionization mass spectrometry (ESIMS) was performed using a Finnigan LCQ Deca instrument from Thermo Electron (San Jose, CA) equipped with Xcalibur software. Full mass and collision-induced dissociation (CID) MS/MS spectra were acquired in both positive and negative modes. Instrumental parameters were tuned for each investigated compound: the capillary voltage was set at 3 V, the spray voltage at 5.10 kV, and the capillary temperature at 220 °C, and the tube lens offset at −60 V was employed; specific collision energies were chosen at each fragmentation step for all the investigated compounds, and the value ranged from 15 to 33% of the instrument maximum. Data were acquired in the MS1 scanning mode (*m*/*z* 150−700). All compounds were dissolved in MeOH−H_2O (1:1) and infused in the ESI source by using a syringe pump; the flow rate was 5 μL/min. Exact masses were measured by a Q-TOF premier (Waters, Manifold, MA) instrument. Chromatography was performed over Sephadex LH-20 (Pharmacia, Uppsala, Sweden) employing MeOH as solvent. Column chromatography was carried out employing silica gel 60 (0.040−0.063 mm; Carlo Erba) and $CHCl_3$−MeOH gradients. HPLC separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector. The column used was a 250 mm × 10 mm i.d. 10 μm Kromasil RP-18 (Phenomenex, Torrance, CA). TLC analysis was performed with Macherey-Nagel precoated silica gel 60 F_{254} plates.

Propolis. The propolis sample was collected in November 2008 in Champoton, Mexico. Both the sample and the dried methanol extract were stored at 5 °C in the dark.

Extraction and Isolation Procedure of Compounds 1−10. The propolis sample (30 g) was extracted with methanol (100 mL x 6) for a total of 3 h; after filtration, the methanol extract was taken to dryness under reduced pressure to yield a dark red gum (24.1 g). A portion of this extract (9 g) was fractionated over a Sephadex LH-20 column (100 × 5 cm) using methanol as solvent to furnish five fractions (1−5). Fraction 3 (358.4 mg) was purified by RP-HPLC (45% CH_3CN) to give **5** (4.3 mg). Fraction 4 (794 mg) was purified by RP-HPLC (40% CH_3CN) to give **7** (14.7 mg) and **8** (9.3 mg), respectively. Fraction 2 (1 g) was purified by column chromatography on silica gel using $CHCl_3$−MeOH gradients and afforded seven subfractions (A1−A7). Fraction A1 (90.4 mg) was subjected to RP-HPLC (70% MeOH) to afford **1** (8.5 mg), **2** (8.1 mg), **9** (4.3 mg), **10** (2.9 mg), **4** (3.8 mg), and **6** (2.5 mg). Fraction A3 (44.0 mg) was purified by HPLC (55% MeOH) to afford **3** (2.6 mg).

1-(3′,4′-Dihydroxy-2′-methoxyphenyl)-3-(phenyl)propane (*1*). Brown residue. IR: ν_{max} (KBr) 3380, 2940, 1600, 1515 cm^{-1}. HRESIMS: *m*/*z* 257.1188, calcd for CHO $[M + H]^+$ 257.1178. ^{1}H and ^{13}C NMR: see **Table 1**.

(Z)-1-(2′-Methoxy-4′,5′-dihydroxyphenyl)-2-(3-phenyl)propene (*2*). Brown residue. IR: ν_{max} (KBr) 3330, 2890, 2870, 1645, 1493 cm^{-1}. HRESIMS: *m*/*z* 255.2318, calcd for CHO $[M + H]^+$, 255.2324. ^{1}H and ^{13}C NMR: see **Table 1**.

3-Hydroxy-5,6-dimethoxyflavan (*3*). White residue. $[\alpha]_D^{25}$ = +1.17° (*c* = 0.19, CH_3OH). IR: ν_{max} (KBr) 3365, 3010, 1614, 1584, 1460 cm^{-1}. HRESIMS: *m*/*z* 287.1270, calcd for CHO $[M + H]^+$, 287.1342. ^{1}H and ^{13}C NMR: see **Table 1**. ESI-MS (positive mode): *m*/*z* 287 $[M + H]^+$, MS/MS: *m*/*z* (relative abundance) 269 (100.0), 255 (1.6), 237 (1.2), 167 (81.4), 155 (1.3), 133 (5.1), 107 (1.0).

(−)-*7-Hydroxyflavanone* (*4*). $[\alpha]_D^{25}$ = −10.14° (*c* = 0.07, CH_3OH). ^{1}H NMR (CD_3OD): δ 7.76 (1H, d, *J* = 8.7, H-5), 7.54 (1H, d, *J* = 7.3, H-2′,6′), 7.45 (1H, t, *J* = 7.3, H-3′,5′), 7.39 (1H, m, H-4′), 6.53 (1H, dd, *J* = 2.0−8.7, H-6), 6.40 (1H, d, *J* = 2.0, H-8), 5.53 (1H, dd, *J* = 2.8−12.7, H-2), 3.06 (1H, dd, *J* = 12.9−16.9, H-3a), 2.79 (1H, dd, *J* = 12.9−16.9, H-3b). ^{13}C NMR (CD_3OD): δ 193.0 (C-4), 165.6 (C-7), 156.4 (C-9), 141.4 (C-1′), 129.7 (C-5), 129.6 (C-3′,5′), 129.4 (C-4′), 127.90 (C-2′,6′), 112.4 (C-10), 112.0 (C-6), 103.6 (C-8), 80.9 (C-2), 44.8 (C-3). ESI-MS (positive mode): *m*/*z* 239 $[M + H]^+$. MS/MS: *m*/*z* (relative abundance) 242 (8.7), 241 (100.0), 240 (2.6), 227 (1.5), 226 (37.9), 225 (1.8), 224 (2.1.), 223 (30.7), 213 (4.4), 199 (11.1), 195 (16.3), 171 (1.5), 163 (14.0), 137 (14.3), 131 (29.1), 103 (1.3).

(+)-*Pinocembrin* (*5*). $[\alpha]_D^{25}$ = +6.46° (*c* = 0.14, CH_3OH). ^{13}C NMR data were consistent with those previously reported (*11*). ESI-MS (negative mode): *m*/*z* 255 $[M - H]^-$. MS/MS: *m*/*z* (relative abundance) 255.1, 213.1, and 151.0 (*12*).

(−)-*Mucronulatol* (*6*). $[\alpha]_D^{25}$ = −18.49° (*c* = 0.54, CH_3OH). ^{1}H NMR (CD_3OD): δ 6.89 (1H, d, *J* = 8.2, H-5), 6.72 (1H, d, *J* = 8.5, H-6′), 6.63 (1H, d, *J* = 8.5, H-5′), 6.35 (1H, dd, *J* = 1.9−8.2, H-6), 6.27 (1H, bs, H-8), 4.20 (1H, bdd, *J* = 10.3, H-2eq), 3.94 (1H, bt, *J* = 10.3, H-2ax), 3.85 (OCH_3-2′,4′), 3.45(1H, m, H-3), 2.92 (1H, bt, *J* = 15.5, H-4ax), 2.80 (1H, dd, *J* = 4.9−15.5, H-4eq). ^{13}C NMR (CD_3OD): δ 157.6 (C-7), 156.5 (C-9), 147.8 (C-2′), 146.9 (C-4′), 139.9 (C-3′), 131.0 (C-5), 128.5 (C-1′), 117.5 (C-6′), 114.2 (C-10), 108.9 (C-6), 108.2 (C-5′), 103.9 (C-8), 71.2 (C-2), 60.9 (OCH_3-2′), 56.4 (OCH_3-4′), 32.8 (C-3), 31.9 (C-4). ESI-MS (positive mode): *m*/*z* 303 $[M + H]^+$. MS/MS: *m*/*z* (relative abundance) 305 (1.0), 303 (17.0), 181 (9.1), 167 (100.0), 163 (25.3), 137 (27.0).

(−)-*Arizonicanol A* (*7*). $[\alpha]_D^{25}$ = −85.60° (*c* = 0.86, CH_3OH). ^{1}H NMR (CD_3OD): δ 6.90 (1H, d, *J* = 8.2, H-5), 6.77 (1H, d, *J* = 8.5, H-6′), 6.58 (1H, d, *J* = 8.5, H-5′), 6.34 (1H, dd, *J* = 1.8−8.2, H-6), 6.26 (1H, bs, H-8), 4.26 (1H, bdd, *J* = 10.0, H-2eq), 3.98 (1H, bt, *J* = 10.0, H-2ax), 3.84 (3H, s, H-4′), 3.50 (1H, m, H-3), 2.97 (1H, dd, *J* = 11.0, 15.5, H-4ax), 2.81 (1H, dd, *J* = 4.9−15.5, H-4eq). ^{13}C NMR (CD_3OD): δ 157.4 (C-7), 156.1 (C-9), 147.8 (C-4′), 144.3 (C-2′), 135.1 (C-3′), 130.8 (C-5), 128.3 (C-1′), 117.6 (C-6′), 113.7 (C-10), 109.3 (C-6), 104.2 (C-8), 103.6 (C-5′), 71.4 (C-2), 56.0 (OCH_3-4′), 33.0 (C-3), 31.6 (C-4). ESI-MS (positive mode): *m*/*z* 289 $[M + H]^+$. MS/MS: *m*/*z* (relative abundance) 291 (1.2), 289 (15.2), 179 (3.1), 167 (9.2), 154 (2.9), 153 (100.0), 149 (29.0), 123 (29.9).

(+)-*Vestitol* (***8***). $[\alpha]_D^{25} = +34.57°$ (c = 0.74, CH_3OH). ^{13}C NMR data were consistent with those previously reported (*4*). ESI-MS (positive mode): m/z 273 $[M + H]^+$. MS/MS: m/z 163, 137, 123 (*13*).

(−)-*Melilotocarpan A* (***9***). $[\alpha]_D^{25} = -9.43°$ (c = 0.06, CH_3OH). ^1H NMR (CD_3OD): δ 7.22 (1H, d, J = 8.4, H-7), 7.00 (1H, d, J = 8.4, H-1), 6.75 (1H, d, J = 8.4, H-2), 6.49 (1H, dd, J = 1.8, 8.2, H-8), 6.41 (1H, d, J = 1.8, H-10), 5.56 (1H, d, J = 8.4, H-11a), 4.36 (1H, m, H-6β), 3.89 (OCH_3-3), 3.77 (OCH_3-9), 3.61 (1H, m, H-6a), 3.60 (1H, m, H-6α). ^{13}C NMR (CD_3OD): δ 162.5 (C-9), 162.1 (C-10a), 149.6 (C-3), 145.6 (C-4a), 135.6 (C-4), 126.5 (C-7), 122.4 (C-1), 120.7 (C-6b), 115.4 (C-11b), 107.4 (C-8), 107.2 (C-2), 97.8 (C-10), 80.1 (C-11a), 68.1 (C-6), 56.5 (OCH_3-3), 55.8 (3H, OCH_3-9), 40.6 (C-6a). ESI-MS (positive mode): m/z 301 $[M + H]^+$, MS/MS: m/z (relative abundance) 302 (5.0), 301 (26.9), 300 (15.7), 285 (2.7), 283 (1.0), 282 (1.4), 274 (1.2), 273 (2.6), 270 (1.1), 269 (11.8), 193 (3.4), 177 (8.0), 161 (10.0), 153 (100.0), 137 (4.5).

(−)-*Melilotocarpan D* (***10***). $[\alpha]_D^{25} = -18.6°$ (c = 0.04, CH_3OH). ^1H NMR (CD_3OD): δ 7.07 (1H, d, J = 8.4, H-1), 6.80 (1H, d, J = 8.4, H-8), 6.75 (1H, d, J = 8.4, H-2), 6.55 (1H, d, J = 8.4, H-7), 5.57 (1H, d, J = 6.0, H-11a), 4.37 (1H, m, H-6β), 3.90 (OCH_3-3), 3.84 (OCH_3-9), 3.60 (1H, m, H-6a), 3.61 (1H, m, H-6α). ^{13}C NMR (CD_3OD): δ 150.9 (C-9), 149.9 (C-3), 148.5 (C-10a), 146.0 (C-4a), 136.2 (C-4), 132.5 (C-10), 122.9 (C-6b), 122.1 (C-1), 115.7 (C-7), 115.0 (C-11b), 106.7 (C-2), 106.0 (C-8), 80.4 (C-11a), 67.7 (C-6), 56.6 (OCH_3-9), 56.5 (OCH_3-3), 41.4 (C-6a). ESI-MS (positive mode): m/z 317 $[M + H]^+$. MS/MS: m/z (relative abundance) 318 (1.6), 317 (28.4), 316 (34.4), 301 (8.1), 299 (1.8), 285 (12.5), 281 (2.1), 260 (1.4), 210 (1.2), 197 (2.5), 177 (11.3), 153 (100.0), 138 (1.5).

RESULTS AND DISCUSSION

The MeOH-soluble fraction of red-type propolis sample was fractionated by gel filtration on a Sephadex LH-20 column and by RP-HPLC, giving three new compounds (**1**−**3**), together with seven known compounds, identified as the flavanones (−)-7-hydroxyflavanone (**4**) and (+)-pinocembrin (**5**), the isoflavans (−)-mucronulatol (**6**), (−)-arizonicanol A (**7**), and (+)-vestitol (**8**), and the pterocarpans (−)-melilotocarpan A (**9**) and (−)-melilotocarpan D (**10**), by comparison of their spectroscopic data, especially NMR, with those in the literature (**Figure 1**).

Compound **1** was isolated as a brown oily substance. The HR-ESIMS of **1** gave an $[M - H]^-$ ion at m/z 257.1188, indicating a molecular formula of $C_{16}H_{18}O_3$, in good agreement with the observation of three methylene, seven methine, one methoxyl group, and five quaternary carbon resonances in its ^{13}C NMR spectrum (**Table 1**). The IR spectrum showed absorptions at ν_{max} 3380−2940, 1600, and 1515 cm^{-1} due to hydroxy and aromatic groups, respectively. Analysis of the 1D and 2D NMR spectra with homo- and heteronuclear direct or long-range correlations allowed assignment of ^1H and ^{13}C NMR signals, as given in **Table 1**. The ^1H NMR spectrum of **1** showed signals at δ 2.65 (2H, t, J = 1.5, 7.5 Hz), 2.57 (2H, t, J = 1.5, 7.5 Hz), and 1.87 (2H, m), corresponding to three methylene protons, together with a methoxyl group at δ 3.75 (3H, s), in addition to five protons in the aromatic region between δ 6.49 and 7.27 including a monosubstituted benzene ring: δ 7.20 (2H, d, J = 7.5 Hz, H-2″,6″), 7.27 (2H, t, J = 1.5, 7.5 Hz, H-3″,5″), and 7.16 (1H, m, H-4″). The concerted interpretation of the ^1H NMR and COSY spectra allowed us to assemble the three methylene groups as a 1,3-propane chain and to link this unit to a monosubstituted benzene ring and a ortho-substituted phenyl group, as evidenced by the observation of H-1/H-2 and H-2/H-3 vicinal couplings and H-5′/H-6′ ortho coupling. The location of the three oxygen functions on the benzene ring was deduced from HMBC correlations; thus, cross-peaks were observed between methylene protons (δ 2.57; H_2-1) and C-2, C-3, C-2′, and C-6′, methoxyl protons and C-2′, H-6′ and C-2′ and C-4′, H-5′ and C-1′ and C-3′. These data closely resembled those of broussonin A and B (phytoalexins with a 1,3-diarylpropane carbon skeleton) isolated from *Broussonetia papyrifera* except for the appearance of the signals due to a monosubstituted benzene ring instead of a para-substituted benzene ring (*14*). Therefore, the structure of **1** was established as 1-(3′,4′-dihydroxy-2′-methoxyphenyl)-3-(phenyl)propane (**1**).

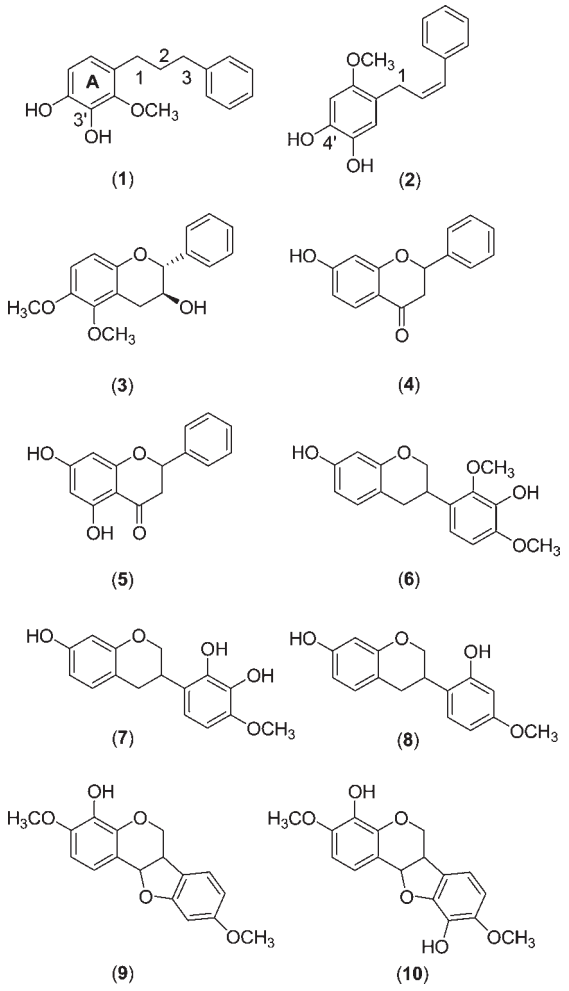

Figure 1. New compounds (**1**−**3**) and flavanones, isoflavans, and pterocarpans (**4**−**10**) identified from red-type Mexican propolis.

Compound **2** was isolated as a brown oily substance; its HR-ESIMS gave an $[M - H]^-$ ion at m/z 255.2318, indicating a molecular formula of $C_{16}H_{16}O_3$, in good agreement with the observation of one methylene, nine methine, one methoxyl group, and five quaternary carbon resonances in its ^{13}C NMR spectrum (**Table 1**). The IR spectrum showed absorptions at ν_{max} 3330, 2890, 1645, and 1493 cm^{-1} due to hydroxy and aromatic groups, respectively.

Compound **2** showed 1D and 2D NMR features similar to those of compound **1**, except for the presence of two vinyl protons at δ 6.37 and δ 6.39 coupled between them in a cis relationship (both d, J = 11.7 Hz, H-2 and H-3, respectively) and of only one methylene group at δ 3.47 (2H, d, J = 5.7 Hz, H-1). Moreover, in the ^1H NMR spectrum of **2** the signal at 6.55 (2H, s, H-3′ and H-6′) suggested the presence of a 1,2,4,5-tetrasubstituted aromatic ring (**Table 1**). The $^1H-^1H$ COSY spectrum established the proton sequences of H-1/H-2/H-3 and H-3′/H-6′ and allowed us to assemble a 1,3-propene unit with two benzene rings. All the above proton resonances of **2** were associated unambiguously with the relevant carbon atom using the HSQC spectrum, while HMBC cross-peaks were observed between the methylene protons (δ 3.47; H_2-1) and C-3, C-2′, and C-6′ and between the H-3 and C-1, C-2″, and C-6″, establishing that CH_2-1 was attached to tetrasubstituted ring A and the vinyl group at the monosubstituted ring B.

Cross-peaks were also observed between H-3′, C-1′, and C-5′ and H-6′, C-1, C-2′, and C-4′, confirming the location of the three oxygen functions on the benzene ring at the C-2′, C-4′, and C-5′ positions. Furthermore, the HMBC spectrum allowed us to confirm the position of the methoxyl groups, showing a correlation between ^{1}H signals at δ 3.82 and C-2′ (δ 147.6). From all these data, the structure of **2** was established as (*Z*)-1-(2′-methoxy-4′,5′-dihydroxyphenyl)-2-(3-phenyl)propene (**2**).

Compound **3** was isolated as a white solid. From the HR-ESIMS and HSQC and HMBC NMR data, a molecular formula of $C_{17}H_{18}O_4$ was deduced. The complete structure of **3** was elucidated by 1D and 2D NMR experiments at 600 MHz. The ^{1}H NMR spectrum of **3** (**Table 1**) displayed two ortho-coupled doublets located at δ 6.62 (1H, d, J = 8.5 Hz) and 6.61 (1H, d, J = 8.5 Hz) and ascribable to H-7 and H-8, of ring A, on the basis of HMBC cross-peaks observed for H-8 with C-6, C-9, and C-10 and for H-7 with C-5 and C-10 and proton signals at δ 7.42 (2H, d, J = 7.5, 4.0 Hz, H-2′,6′), 7.37 (2H, t, J = 1.5, 7.5 Hz, H-3′, 5′), and 7.29 (1H, m, H-4′) indicative of the monosubstituted ring B.

A methylene signal at 35.0 ppm in the ^{13}C NMR spectrum (C-4) along with signals at δ 77.9 (C-2) and 77.43 (C-3) ppm are typical of a flavan-3-ol structure (*15*), further corroborated by the presence of characteristic methine protons for H-2 and H-3 at δ 4.48 (1H, d, J = 6.0 Hz), δ 3.89 (1H, ddd, J = 9.2, 5.0, 2.5 Hz) and methylene protons for 2H-4 at δ 2.68 (1H, dd, J = 13.8, 4.0 Hz) and 2.48 (1H, dd, J = 13.8, 9.2 Hz) in the ^{1}H NMR spectrum (*16*). The magnitude of the coupling constants of H-2 and H-3 suggests a 2,3-*trans* geometry and the diaxial orientation of the H-2 and H-3 in **3** (*17*). The ^{1}H NMR spectrum also showed two methoxyl groups at δ 3.63 (3H, s) and 3.80 (3H, s). The HSQC spectrum established all of the correlations between protons and carbons of **3** (**Table 1**), whereas the HMBC spectrum showed connectivities for H-2/C-4, C-9, C-2′, and C-6′, H-3/C-10 and C-1′, and H-4/C-2, C-5, and C-9. The HMBC cross-peaks observed for a singlet due to the methoxyl group at δ 3.63 and protons at positions 4 and 7 with C-5 indicated the position of the first methoxyl group at position 5; moreover, cross-peaks observed for a further methoxyl group at δ 3.80 and H-8 with C-6 indicated the position of the second further methoxyl group at the C-6 position. In addition, the lack of correlation of H-7 and H-8 with 2H-4 in a ROESY spectrum confirmed the positions of two methoxyl groups at C-5 and C-6. The ^{1}H NMR and ^{13}C NMR spectra of **3** were almost superimposable with those of 3,6-dihydroxy-5-methoxyflavan isolated from a Nepalese propolis sample except for the presence of one additional methoxyl group, consistent with the molecular formula (*18*). On the basis of these data, the structure of a new compound **3** was elucidated as 3-hydroxy-5,6-dimethoxyflavan.

Compounds **4** and **5** were identified as (2*S*)-7-hydroxyflavanone and (2*S*)-5,7-dihydroxyflavanone (pinocembrin), respectively, from comparison of their spectroscopic data with those reported in the literature (*11*, *12*). Many of the biological actions of pinocembrin have been reported since the 1980s, including antimicrobial, antioxidant, anti-inflammatory, and endothelium-relaxation effects and a neuroprotective effect on ischemia (*19*−*21*).

The ^{1}H NMR spectra of **6**−**8** showed signals at δ 3.83−3.98 (1H, t, J = 10 Hz, H-2ax), 4.17−4.26 (1H, dd, J = 10, 3 Hz, H-2eq), 3.40−3.50 (1H, m, H-3ax), 2.81−2.97 (1H, dd, J = 15.7, 10.5 Hz, H-4ax), and 2.66−2.81 (1H, dd, J = 15.7, 5.1 Hz, H-4eq), assigned to the heterocyclic protons of the isoflavan moiety and confirmed also by signals in the ^{13}C NMR spectrum (δ 70.9−71.4 for C-2, 32.7−33.0 for C-3, and 31.0−31.9 for C-4). Isoflavans **6**−**8** were identified by a detailed analysis of 1D and 2D NMR experiments (DQF-COSY, HSQC, and HMBC), as mucronulatol (**6**), arizonicanol A (**7**),and the widely known vestitol (**8**), respectively.

Mucronulatol (**6**) is one of the most cytotoxic substances present in Caribbean propolis. Mucronulatol exerts cytotoxicity in cancer cell lines by targeting the control of cell cycle progression, indicating that the mechanism of action of this compound involves interference with the cell cycle machinery (*22*). Mucronulatol was also detected in red Brazilian propolis, and it exhibited potent activity against LLC (IC50, 8.38 lM) and A549 (IC50, 9.9 lM) cancer cell lines; these activity data were comparable to those of the clinically used anticancer drugs 5-fluorouracil and doxorubicin against the tested cell lines, suggesting that **6** is a good candidate for future anticancer drug development (*7*).

In the ^{1}H spectra of compounds **9** and **10**, a characteristic set of four protons due to hydrogens at C-6, C-6a, and C-11a suggested that the compounds have a pterocarpan skeleton. The presence of hydroxyl and methoxyl groups and their locations were established on the basis of the ^{13}C NMR spectra and HMBC data. The structures of **9** and **10** were deduced from a detailed analysis of the ^{1}H and ^{13}C NMR data aided by 2D NMR experiments (DQF-COSY, HSQC, and HMBC) and identified as melilotocarpan A and D. Pterocarpans constitute the second largest group of natural isoflavonoids, and they have been gaining considerable interest due to their wide range of biological effects. Many of them are phytoalexins possessing high antifungal and antibacterial activity (*23*), and several of them have been reported to inhibit HIV-1 reverse transcriptase and the cytopathic effect of HIV-1 in cell cultures (*24*), as well as inhibitory effects on protein tyrosine phosphatase 1B (*23*).

The isolation of flavanones, isoflavans, and pterocarpans was in agreement with the chemical profiles of the Cuban and Brazilian red propolis. 7-Hydroxyflavanone (**4**) was previously reported as a chemical constituent of red Brazilian propolis (*2*), whereas pinocembrin (**5**) is one of the flavonoids at the highest concentration in propolis from different sources, including the Leguminosae family, and was reported from plants of the *Dalbergia* species (*D. odorifera*, *D. louvelii*, *D. ecastophyllum*, and *D. parviflora*) (*12*, *19*, *25*, *26*). Mucronulatol (**6**) was isolated from different *Dalbergia* species (Leguminosae) (*27*) and reported from Caribbean (*22*) and Brazilian (*13*) propolis, whereas arizonicanol A (**7**) was reported from *Sophora* spp. and (*28*) *Smirnowia iranica*, and in the trunk exudates of *Dalbergia sissoo* (*29*) both belonging to the Leguminosae family. Vestitol (**8**) was reported from plants of the genus *Dalbergia* frequently and detected in Cuban and Brazilian red propolis samples (*4*, *7*). Melilotocarpan A (**9**) and D (**10**) were previously isolated from the heartwood of *Dalbergia odorifera* (*30*). Although isoflavans and pterocarpans appear common in the red varieties of propolis, this is the first report of arizonicanol A (7) and melilotocarpan A (**9**) and D (**10**). Therefore compounds **7**, **9**, **10** might also be useful taxonomic markers for the red Mexican propolis.

Also, the isolation of compounds with 1,3-diarylpropane and 1,3-diarylpropene carbon skeletons (**1** and **2**) indicated the close relation between the red Mexican propolis and the genus *Dalbergia*. Similar compounds were obtained from the heartwood of *D. louvelii* (*25*) and *D. candenatensis* (*31*) and from the stem bark of *D. cultrate* (*32*).

In conclusion, the characteristic compounds of red Mexican propolis have a very restricted distribution in the plant kingdom and occur almost exclusively in the Leguminosae family; moreover, our chemical study supported that the botanical origin of the reddish propolis is *Dalbergia* genus and that the botanical sources of this *red* type probably belong to this genus (*25*).

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