Isolation and Structures of Two New Compounds from the Essential Oil of Brazilian Propolis

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Two new and seven known compounds, including terpenoids and aromatic compounds, were isolated from the essential oil of Brazilian propolis. The structures of the new compounds were elucidated as 2,2-dimethyl-8-prenyl-6-vinylchromene (1) and 2,6-diprenyl-4-vinylphenol (2) on the basis of spectroscopic analyses.

Key words propolis; steam distillation; essential oil; 2,2-dimethyl-8-prenyl-6-vinylchromene; 2,6-diprenyl-4-vinylphenol

Propolis (bee glue) is a complex resinous material that honey bees (*Apis mellifera*) produce from the exudates of various plants, beeswax, and bee-secretion in a beehive, and has been used in folk medicines, foods, and beverages with the intention of preserving or improving human health since ancient times in many parts of the world.[1)]

In addition, propolis is now known to be a health food which has antibacterial,[2)] antitumoral,[3)] antioxidative,[4)] immunomodulatory,[5)] antifungal,[6)] and other beneficial activities, according to the results of investigations on the chemical composition of Brazilian propolis started within last 5—6 years.

At this time, we conducted the isolation and characterization of components of essential oil obtained from Brazilian propolis, and have reported on the isolation and structures of two new compounds, 2,2-dimethy-8-prenyl-6-vinylchromene (**1**) and 2,6-diprenyl-4-vinylphenol (**2**), together with seven known compounds.

Results and Discussion

The essential oil of Brazilian propolis, prepared by means of steam distillation described in the Experimental section, was chromatographed by silica gel column chromatography (CC) to give 4 fractions. Each fraction was repeatedly separated by a combination of different chromatographies to give two new compounds, **1** and **2**, together with seven known compounds, 2,2-dimethyl-6-vinylchromene (**3**),[7)] acetophenone (**4**), 2-prenyl-4-vinylphenol (**5**),[8)] 3,4-dimethoxy-styrene (**6**),[9)] 3,4-dimethoxy-allylbenzene (**7**),[9)] 4-hydroxy-3,5-diprenylbenzaldehyde (**8**),[10)] and (−)-spathulenol (**9**).[11)]

Compound **1** was isolated as colorless oil. The UV spectrum of **1** showed characteristic absorptions at λ_{max} 249 and 272 nm due to a conjugated aromatic ring. The molecular formula was deduced as $C_{18}H_{22}O$ by high resolution (HR)-FAB-MS and ^{1}H- and ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those of 2,2-dimethyl-6-vinylchromene (**3**), except for signals at ^{1}H-NMR ($CDCl_3$) δ: 1.72 (3H, s), 1.73 (3H, s), 3.27 (2H, d, J=7.6 Hz), 5.29 (1H, t, J=7.5 Hz), and ^{13}C-NMR ($CDCl_3$) δ: 28.3 (t), 122.7 (d), 131.9 (s), 17.9 (q), 25.8 (q), as shown in Table 1.

These additional signals indicated the presence of a prenyl moiety in **1**. Therefore, the structure of **1** was suggested to be the prenyl derivative of 2,2-dimethyl-6-vinylchromene (**3**). The position of the prenyl moiety was determined by the aid of the 2-dimensional NMR experiments. The heteronuclear multiple bond connectivity (HMBC) spectrum of **1** exhibited correlations from methylene hydrogens of the prenyl group [δ 3.27 (2H, d, J=7.5 Hz)] to aromatic carbons [δ 129.9 (s), 127.5 (d), 129.2 (s)]. Futhermore, the nuclear Overhauser effect spectroscopy (NOESY) spectrum of **1** showed consecutive correlations from dimethyls of the prenyl moiety [δ 1.72 (3H, s), 1.73 (3H, s)] through aromatic hydrogens and vinyl hydrogens to other dimethyls [δ 1.41 (6H, s)], as shown in Fig. 2.

Thus, the prenyl moiety was substituted at the C-8 position of 2,2-dimethyl-6-vinylchromene. Accordingly, the structure of **1** was concluded to be 2,2-dimethyl-8-prenyl-6-vinylchromene.

Compound **2** was isolated as an amorphous powder. The IR spectrum of **2** showed absorptions due to phenol (3612, 3087, 908 cm^{-1}) and alkene (1662 cm^{-1}) groups. The molecular formula of **2** was deduced to be $C_{18}H_{24}O$ by HR-FAB-MS and NMR data. The ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H detected single quantum coherence (HSQC) spectra of **2** suggested the presence of two prenyl [δ 1.77 (6H, s), 3.33 (4H, d, J=7.6 Hz), 5.29 (2H, m)], one vinyl [δ 6.60 (1H, dd, J=17.6, 10.7 Hz), 5.56 (1H, dd, J=17.6, 0.9 Hz), 5.07 (1H, dd, J=10.7, 0.9 Hz)] and a trisubstituted phenol [δ 5.36 (1H, s), 7.03 (2H, s)] moieties. Furthermore, the ^{1}H- and ^{13}C-NMR data revealed the symmetric structure of **2**. The connectivities of these functional moieties were examined by HMBC. Since the principal long-range correlations were as depicted in Fig. 3, the structure of **2** was concluded to be 2,6-diprenyl-4-vinylphenol.

To our knowledge, both compounds **1** and **2** are new com-

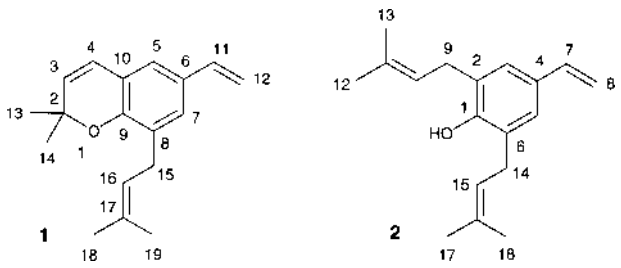

Fig. 1. Compounds **1** and **2**

Assignments were aided by ^{1}H–^{1}H COSY, HSQC, and distortionless enhancement by polarization transfer (DEPT) spectra.

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Table 1. ^{1}H- and ^{13}C-NMR Data of **1**—**3** in $CDCl_3$

Atom	**1**		**2**		**3**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	—	—	5.36 (1H, s, ϕ-OH)	152.6 (s)	—	—
2	—	76.2 (s)	—	127.2 (s)	—	76.4 (s)
3	5.61 (1H, d, J=9.8 Hz)	130.8 (d)	7.03 (1H, s)	125.8 (d)	5.62 (1H, d, J=10.0 Hz)	131.0 (d)
4	6.31 (1H, d, J=9.8 Hz)	122.6 (d)	—	130.0 (d)	6.31 (1H, d, J=10.0 Hz)	122.2 (d)
5	6.90 (1H, d, J=1.8 Hz)	127.9 (d)	7.03 (1H, s)	125.8 (d)	7.04 (1H, d, J=2.5 Hz)	124.0 (d)
6	—	129.9 (s)	—	127.2 (s)	—	130.5 (s)
7	7.02 (1H, d, J=1.8 Hz)	127.5 (d)	6.60 (1H, dd, J=10.8, 17.6 Hz)	136.7 (d)	7.16 (1H, dd, J=2.5, 8.5 Hz)	127.1 (d)
8	—	129.2 (s)	5.07 (1H, dd, J=10.8 Hz) 5.56 (1H, dd, J=17.6 Hz)	111.1 (t)	6.73 (1H, d, J=8.5 Hz)	116.3 (d)
9	—	152.9 (s)	3.33 (2H, d, J=7.3 Hz)	29.7 (t)	—	152.9 (s)
10	—	120.9 (s)	5.31 (2H, m)	122.0 (d)	—	121.1 (s)
11	6.62 (1H, dd, J=10.8, 17.5 Hz)	136.3 (d)	—	134.3 (s)	6.61 (1H, dd, J=11.0, 18.0 Hz)	136.3 (d)
12	5.08 (1H, dd, J=0.9, 10.8 Hz) 5.54 (1H, dd, J=0.9, 17.5 Hz)	111.1 (t) —	1.77 (6H, s)	25.8 (q)	5.10 (1H, dd, J=1.0, 11.0 Hz) 5.58 (1H, dd, J=1.0, 18.0 Hz)	111.4 (t) —
13	1.41 (3H, s)	28.0 (q)	1.77 (6H, s)	17.8 (q)	1.42 (3H, s)	28.0 (q)
14	1.41 (3H, s)	28.0 (q)	3.33 (2H, d, J=7.3 Hz)	29.7 (t)	1.42 (3H, s)	28.0 (q)
15	3.27 (2H, d, J=7.6 Hz)	28.3 (t)	5.31 (2H, m)	122.0 (d)		
16	5.29 (1H, t, J=7.5 Hz)	122.7 (d)	—	134.3 (s)		
17	—	131.9 (s)	1.77 (6H, s)	25.8 (q)		
18	1.73 (3H, s)	25.8 (q)	1.77 (6H, s)	17.8 (q)		
19	1.72 (3H, s)	17.9 (q)				

Values are in (δ) ppm. Figures in parentheses are coupling constants (J) in Hz.

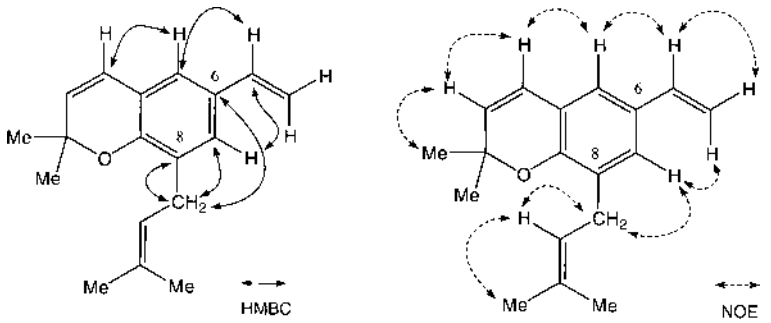

Fig. 2. The Principal HMBC and NOE Correlations of **1**

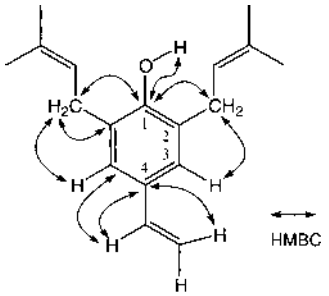

Fig. 3. The Principal HMBC Correlations of **2**

pounds. Compound **1** must be biosynthesized from **2** by cyclization between the prenyl moiety and phenol function. The biological activities of **1** and **2** will be examined.

Experimental

UV spectra were recorded on a JASCO U-best 30 spectrometer. IR spectra were taken on an FT/IR-410 spectrometer (JASCO). ^{1}H- and ^{13}C-NMR spectra were recorded on anVarian Unity 500 (500 MHz) spectrometer. Positive HR-FAB-MS spectra were measured on a JMS-S×102 A/JMA-DA 6000 (JEOL). Normal and reverse phase TLC were performed with Silica gel 60 F_{254} and RP-8 F_{254} (Merck), respectively. CC was carried out with BW-300 (Fuji silysia), LiChroprep RP-8 (Merck) and YMC.GEL.ODS-AM120-S50 (YMC).

Materials Brazilian propolis glue obtained from the virgin forests of Alecrim in the Southern part of Minas Gerais State in Brazil, was supplied by Yamada Apiculture Center, Inc.

Isolation of Components from Propolis Propolis glue (550 g) was subjected to steam distillation for 5 h according to the guidelines of the Japanese pharmacopoeia. The distillate was extracted with diethyl ether, and the extracts were dried over Na_2SO_4 and evaporated to give essential oil (1.87 g, 0.34%). The essential oil was subjected to silica gel CC with petroleum ether, then MeOH, to give 4 fractions [fr. 1 (453.7 mg), fr. 2 (**3** : 2,2-dimethyl-6-vinylchromene, 8.8 mg), fr. 3 (**1**, 1.7 mg), fr. 4 (1.27 g). Fraction 4 was chromatographed on a silica gel CC with petroleum ether–diethyl ether with a linear gradient to give 6 fractions [fr. 5 (247.6 mg), fr. 6 (56.7 mg), fr. 7 (155.3 mg), fr. 8 (21.6 mg), fr. 9 (80.2 mg), fr. 10 (301.3 mg)]. Fraction 6 was successively subjected to silica gel CC with *n*-hexane : MeOH (49 : 1), reversed phase CC with 70% MeOH/H_2O, and preparative TLC with *n*-hexane : MeOH (9 : 1) to give **2** (27.0 mg). Fraction 7 was chromatographed on a silica gel CC with *n*-hexane : MeOH (19 : 1) and preparative TLC with *n*-hexane : MeOH (9 : 1) to give **4** (acetophenone, 70.3 mg). Fraction 8 was chromatographed on a silica gel CC with *n*-hexane : MeOH (9 : 1) and preparative TLC with *n*-hexane: MeOH (7 : 1) and reversed phase CC with 70% MeOH/H_2O to give **5** (2-prenylstyrene, 0.23 mg), **6** (3,4-dimethoxystyrene, 0.38 mg), **7** (3,4-dimethoxyallylbenzene, 5.74 mg), and **8** (4-hydroxy-3,5-diprenylbenzaldehyde, 0.19 mg). Fraction 9 was purified in the same manner as for **5** to give **9** [(−)-spathulenol, 4.60 mg].

Compound **1**: Colorless oil. HR-FAB-MS Found *m/z*: 254.1701 (Calcd for $C_{18}H_{22}O$: 254.1671); UV λ_{max} (*n*-hexane) nm (ε) 249 (34200), 272 (7600);

IR ($CHCl_3$) cm^{-1} 3045, 1626, 902; ^1H- and ^{13}C-NMR ($CDCl_3$): See Table 1.

Compound **2**: Amorphous powder. HR-FAB-MS Found *m/z*: 256.1764 (Calcd for $C_{18}H_{24}O$: 256.1827); UV λ_{max} (*n*-hexane) nm (ε): 215 (30740), 262 (14000). IR (CCl4) cm^{-1}: 3612, 3087, 1662, 908. ^1H- and ^{13}C-NMR ($CDCl_3$): See Table 1.

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