Cyclodextrin-Enclosed Substances of Brazilian Propolis

Alaa Mohamed NAFAFY, a Mohamed Ahmed EL-SHANAWANY, b Mahmoud Hamed MOHAMED, c Hashim Abdel-Halim HASSANEAN, b Toshihiro NOHARA, a Hitoshi YOSHIMITSU, a Masateru ONO, a Hiroyuki SUGIMOTO, a Shima DOI, d Ken SASAKI, e and Hirohisa KURODA f

a Faculty of Pharmaceutical Sciences, Kumamoto University; 5-1 Oe-hommachi, Kumamoto, 862-0973, Japan; b Department of Pharmacognosy, Faculty of Pharmacy, Assiut University; Assiut, Egypt; c Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch; Assiut, Egypt; d Faculty of Engineering, Kyushu Kyoritsu University; 1-8 Jiya-gakou, Yahata-nishi-ku, Kitakyushu 807-8585, Japan; e Yamada Apiculture Center, Inc.; 194 Ichiba, Kagamino, Tomata, Okayama 708-0393, Japan; and f Department of Polymer Science & Engineering, Kyoto Institute of Technology; Matsugasaki, Sakyoku, Kyoto 606-8585, Japan. Received January 23, 2003; accepted April 21, 2003

Propolis is a resinous hive product collected by honeybees from various plant sources.1) It is extensively used in food and beverages and in folk medicine for the treatment of different ailments, and is reported to have a broad spectrum of pharmacological activities such as anti-microbial activity, antioxidant, anticancer and as an immune stimulant in addition to other pharmacological effects.2,3)

Concerning the chemical composition of propolis, it turned out to be very complex, and more than 200 compounds have been isolated so far.2) The most important constituent appears to be phenolics, which form more than ca. 50% of the propolis composition.4) Since isolation of the bioactive substances is accompanied by many difficulties owing to their complexity and scarce amounts, we devised a method to obtain the aromatic compounds by using β-cyclodextrin-inclusion. In the present paper we describe the isolation and structural elucidation of some phenolic compounds isolated from Brazilian propolis by using β-cyclodextrin-inclusion as a selective method for the isolation of the phenolic constituents from the propolis extract.

Brazilian propolis was suspended in dist. water containing β-cyclodextrin. After the suspension was subjected to sonication, the insoluble propolis was filtered off. The water-soluble filtrate was concentrated by evaporation under reduced pressure to provide β-cyclodextrin-propolis inclusion, to which ethanol was added and vigorously stirred. The insoluble ethanol portion containing β-cyclodextrin was filtered off to give the ethanol solution, which was then concentrated to give the propolis extract. The propolis extract was subjected to Diaion HP-20, Sephadex LH-20, ODS and HPLC (ODS and TSK gel-120A) to give compounds 1—5. Compounds 1, 2, 4, and 5 were identified with artepillin C, capillartermisin A, aromadendrin and 3,5,7-trihydroxy-4’-methoxyflavanol,5) respectively, by spectroscopic measurements.

Compounds 3 was obtained as a white powder showing [α]D -14.8° (MeOH). It showed a molecular peak at m/z 318 [C9H19O4]+ in the electron impact (EI-MS). In the 1H-NMR spectrum, a singlet at δ 4.62 (2H, d, J = 7.3 Hz), 1.69 and 1.68 each (3H, s). It also showed the presence of two olefinic protons at δ 6.91 and 8.10 each coupled with a large coupling constant (1H, d, J = 15.9 Hz), suggesting it to be a trans-double bond in a side chain. The 13C-NMR spectrum of 3 indicated the presence of 19-carbon atoms composed of four methyls (δ 17.8, 25.8, 2×29.8), three methylenes (δ 25.9, 29.6, 44.4), ten sp2 carbons (δ 116.8, 123.8, 128.3, 127.0, 128.2, 130.1, 131.4, 132.5, 145.3, 156.3) and one carbonyl carbon (δ 169.8). The two dimensional (2D)-NMR spectra of proton–proton chemical shift correlation spectroscopy (1H–1H COSY), heteromolecular multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) were observed for the assignment of 1H and 13C signals. The result of the HMBC spectrum is illustrated in Fig. 1. That is, the structure of 3 was a derivative of 3,5-disubstituted 4-hydroxycinnamic acid, and a tertiary hydroxyl group was attached to C-3’ on the isopropyl moiety. Consequently, 3 was characterized as 3-(3-hydroxy-3-methyl-butyl)-5-prenyl-4-hydroxycinnamic acid, which has not yet been reported.

Experimental

Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter (l = 5 cm). EI-MS was obtained using a JEOL JMS-DX300. NMR spectra were measured in pyridine-d, on a JEOL α-500 spectrometer, and chemical shifts were referenced to tetramethylsiline (TMS). Column chromatography was carried out with silica gel 60 (230—400 mesh, Merck), Sephadex LH-20 (25—100 mm, Pharmacia Fine Chemicals), MCI gel CHP-20P (75—150 mm, Mitsubishi Chemical Co.), Chromatorex ODS (30—50 mm, Fuji Silysia Chemical, Ltd.), and TLC was performed on precoated silica gel 60F254 (0.2 mm, Merck) and RP-18 F254S (Merck). HPLC was performed on ODS gel (TSK gel-120A, Tosoh Co., Ltd., Φ = 7.7 mm, L = 300 mm).

Isolation of Compounds 1—5 from Brazilian Propolis Using β-Cyclodextrin-Inclusion

Brazilian propolis (10 g), which is regarded to be

* To whom correspondence should be addressed. e-mail: none@gpo.kumamoto-u.ac.jp © 2003 Pharmaceutical Society of Japan
collected mainly from *Baccharis dracunculifolia*, Compositae, in the Minas Gerais region in Brazil, was suspended in 11 of dist. water, which contained 10 g of β-cyclodextrin. After the suspension was subjected to sonication for 4 h, the insoluble propolis was filtered off. The water-soluble filtrate was concentrated by evaporation under reduced pressure to provide β-cyclodextrin-propolis inclusion, to which 700 ml of ethanol was added and vigorously stirred. The insoluble ethanol portion containing β-cyclodextrin was filtered off to give the ethanol solution, which was then concentrated to give the propolis extract. The propolis extract (1.054 g) was subjected to Sephadex LH-20 column chromatography using methanol to give two main fractions. Fraction 2.1 was chromatographed through ODS column chromatography using MeOH–H2O (50, 60%) to give 2 fractions. Fraction 2.1.1 was further chromatographed by HPLC (ODS) using 50% MeOH–H2O (50, 60, 70%, successively) to give 2 fractions. Fraction 2.1.2 was also chromatographed through ODS column chromatography using MeOH–H2O systems (60, 70, 80%) gradiently to give 2 fractions. Fractions 2.1.1 and 2.1.2 were further chromatographed through Sephadex LH-20 using MeOH to give two main fractions. Fraction 3.2 was chromatographed through ODS column chromatography using the MeOH–H2O system (60, 70, 80%) to give Compound 2 (21.3 mg). Fraction 2.2 was further chromatographed by HPLC (ODS) using 60% MeOH–H2O (50, 60%) to give 2 fractions. Fraction 2.2.1 was further chromatographed by HPLC (ODS) using 50% MeOH–H2O to give Compound 1 (21.3 mg). Fraction 2.2.2 was also chromatographed through ODS column chromatography using the MeOH–H2O system (60%) to give Compound 2 (21.3 mg). Fraction 2.2.2 was further chromatographed by HPLC (ODS) using 50% MeOH–H2O to give Compound 4 (11.2 mg). Fraction 3.1 (0.128 g) was further chromatographed through Sephadex LH-20 using MeOH to give 2 main fractions. Fraction 3.1 was chromatographed by ODS column chromatography and MeOH–H2O systems (60, 70, 80%) gradiently to give 2 fractions. Fractions 3.1.1 and 3.1.2 were further chromatographed through HPLC [column TSK gel-120A] using a 70% MeOH–H2O system to give compounds 2 (13.4 mg) and 3 (10.4 mg), respectively. Fraction 3.2 was chromatographed through ODS column chromatography using the MeOH–H2O system (60, 70%) to give fraction 3.2.1, which was further chromatographed through HPLC (ODS) using a 60% MeOH–H2O system to give compound 5 (9.0 mg).

**Compound 1** 1H-NMR (in pyridine-d$_5$): δ: 1.71 (6H, s, H$_3$-5, 5$'$), 1.74 (6H, s, H$_2$-4, 4$'$), 3.65 (2H, d, J=7.3 Hz, H2-1, 1$'$), 5.62 (2H, t-like, H-2, 2$'$), 6.86 (1H, d, J=15.9 Hz, H-8), 7.67 (2H, s, H-2, 6), 8.15 (1H, d, J=15.9 Hz, H-7).

**Compound 2** 1H-NMR (in pyridine-d$_5$): δ: 1.68 (6H, s, H$_3$-4, 4$'$), 1.90 (3H, s, H$_3$-5, 5$'$), 3.68 (2H, d, J=6.7 Hz, H$_2$-1, 1$'$), 3.79 (2H, d, J=7.3 Hz, H$_2$-1, 1$'$), 4.32 (2H, s, H$_2$-4, 4$'$), 5.56 (1H, t, H-2), 6.14 (1H, t, H-2), 6.88 (1H, d, J=15.9 Hz, H-8), 7.55 (2H, d, J=2.4 Hz, H-2, 6), 8.14 (1H, d, J=15.9 Hz, H-7).

**Compound 3** 1H-NMR (in pyridine-d$_5$): δ: 14.1 (C-3'), 17.8 (C-5'), 25.7 (C-4'), 29.3, 29.6 (C-1', 1$'$), 67.8 (C-4'), 117.1 (C-8), 123.1 (C-2'), 123.8 (C-2), 127.1 (C-1), 128.1, 128.3 (C-2, 6), 129.9, 130.0 (C-3, 5), 145.2 (C-7), 169.8 (C-9). Compound 2 was identified with capillartemisin A.$^{[6]}$

**Compound 4** 1H-NMR (in pyridine-d$_5$): δ: 4.73 (1H, d, J=11.6 Hz, H-3), 5.28 (1H, d, J=11.6 Hz, H-2), 5.87 (1H, d, J=2.4 Hz, H-6), 5.90 (1H, d, J=2.4 Hz, H-8), 7.25 (2H, d, J=8.5 Hz, H-3', 5'), 7.74 (2H, d, J=8.5 Hz, H-2', 6'). 13C-NMR (in pyridine-d$_5$): δ: 84.6, 73.3, 198.9, 165.1, 97.5, 168.8, 96.2, 163.1, 101.7, 128.9, 130.2, 116.0, 159.6, 128.9, 130.2. Compound 4 was identified with aromadendrin.$^{[7-9]}$

**Compound 5** 1H-NMR (in pyridine-d$_5$): δ: 4.98 (1H, d, J=11.0 Hz, H-3), 5.45 (1H, d, J=11.0 Hz, H-2), 6.38 (1H, d, J=1.8 Hz, H-6), 6.52 (1H, d, J=1.8 Hz, H-8), 7.06 (2H, d, J=7.9 Hz, H-3', 5'), 7.74 (2H, d, J=8.5 Hz, H-2', 6'). 13C-NMR (in pyridine-d$_5$): δ: 84.3, 73.3, 198.5, 165.1, 97.5, 168.8, 96.3, 163.8, 101.6, 130.5, 130.0, 114.3, 160.5, 130.0, 55.3. Compound 5 was identified with 3,5,7-trihydroxy-4′-methoxyflavanol.$^{[10]}$

**References**


