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Note

A New Chalcone from Propolis Collected on Jeju Island, Korea

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The new chalcone, 4′-methoxy-bavachromanol (1), was isolated from propolis collected on Jeju, a southern island of Korea together with two known compounds, laserpitin (2) and isolaserpitin (3). The structure of each compound was determined by spectral methods, including mass spectrometry and 2D NMR. The IC_{50} value of 1 against soybean lipoxygenase was 14.6 μM.

Keywords: propolis, Jeju, chalcone, khellactone, 4′-methoxy-bavachromanol, lipoxygenase

Introduction

Propolis, a natural substance collected by honeybees from buds and exudates of certain trees and plants to protect their beehives from enemies, is used in folk medicine in many regions of the world and has been reported to have various biological activities such as antibacterial (Kujumgiev *et al.,* 1999), antiviral (Amoros *et al.,* 1994), anti-inflammatory (Wang *et al.,* 1993), and anticancer (Kimoto *et al.,* 2001) properties. Propolis is extensively used in food and beverages to promote health and prevent diseases such as inflammation, heart disease, diabetes, and cancer (Burdock, 1998).

Propolis usually contains a variety of chemical compounds such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids, steroids, and amino acids. The composition of propolis depends upon the vegetation at the site of collection. Due to the geographical difference, propolis samples from Europe, South America, and Asia have different chemical compositions (Marcucci, 1995; Tazawa *et al.,* 2000; Kumazawa *et al.,* 2004). Propolis from Europe and China contained many kinds of flavonoids and phenolic acid esters (Bankova *et al.,* 2000). In contrast, the major components in propolis of Brazilian origin were terpenoids and prenylated derivatives of *p*-coumaric acids (Marcucci & Bankova, 1999; Tazawa *et al.,* 1999; Kumazawa *et al.,* 2003). However, the detailed components of Korean propolis have not been reported. Therefore, we have studied the components in Korean propolis from different geographic origins (Ahn *et al.,* 2004) and found that propolis from Jeju island has constituents not present in propolis from other regions. Jeju is a southern island of Korea with a subtropical climate.

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We examined the components in propolis collected on Jeju island, and isolated three of these components (1–3) (Fig. 1). Here we describe the structure and soybean lipoxygenase inhibition activity of these compounds.

Materials and Methods

General Experimental Procedure Melting point (mp) data, recorded with a Bibby SMP 3 micro-melting point apparatus, were used without correction. Optical rotation values were determined with a Jasco DIP-1000 digital polarimeter. UV and IR spectra were obtained with a Hitachi U-2000 spectrometer and a Jasco FT/IR-550 spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were measured with a Jeol JNM-α400 (400 and 100 MHz, respectively), using TMS as an internal standard. FAB mass spectra were obtained with a Jeol JMS-700 spectrometer using glycerol as a matrix, and ESI mass spectra were taken with a Thermo Electron LCQ spectrometer.

Extraction and Isolation Dried propolis collected on Jeju island in Korea (40 g) was extracted with 1200 ml of ethanol at room temperature for 3 days, and then concentrated under reduced pressure to give a crude extract. This extract was subjected to silica gel column chromatography (50×450 mm i.d.), using a hexane/EtOAc gradient system for elution. The fractions eluted with hexane-EtOAc=2: 1 and 1: 2 were rechromatographed by preparative HPLC in a 20×250 mm i.d. ODS column (Shiseido Capcell Pak C18, Tokyo) in 10 ml CH_3CN-H_2O (6: 4) to give **1** (90 mg), **2** (49 mg) and **3** (145 mg).

4′-Methoxy-bavachromanol (**1**) Yellow powder; mp 215 ℃; UV (EtOH) λ_{max} (log ε) 347 (4.11) nm; IR ν_{max} (nujol) cm^{-1}: 3420, 2970, 2940, 1680; HRFABMS (high resolution fast atom bombardment mass spectrum) *m/z* 355.1573 (calculated for $C_{21}H_{23}O_5$, 355.1545 $[M+H]^+$); ^{1}H- and ^{13}C-NMR data are shown in Table 1.

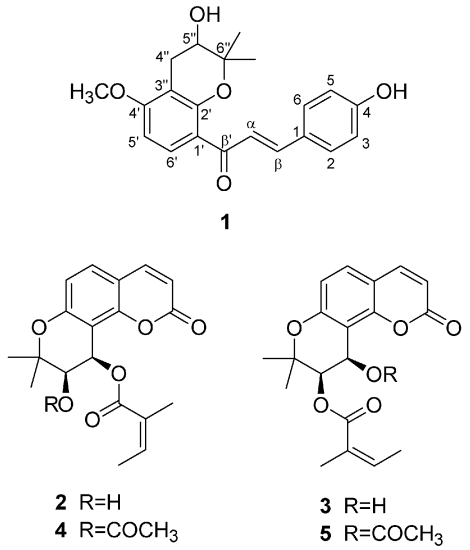

Fig. 1. Structures of 4′-methoxy-bavachromanol (**1**), laserpitin (**2**) and isolaserpitin (**3**).

Acetylation of **2** *and* **3** A crude mixture (20 mg) of **2** and **3**, acetic anhydride (3 ml) and anhydrous pyridine (3 ml) were kept at room temperature for 1 h. The excess acetic anhydride was decomposed with MeOH, and the solvent was removed under reduced pressure. The acetylated **2** and **3** were purified by preparative HPLC to give the monoacetates **4** (10 mg) and **5** (5 mg), respectively.

3′-acetyl-4′-(2″-methyl-2″-butenoxy) khellactone (**4**) White amorphous; ESIMS (electrospray ionization mass spectrum) *m/z* 409 $[M+Na]^+$; ^{1}H-NMR (in acetone-d_6): δ 1.47 (s, 6H), 1.85 (s, 3H), 1.95 (d, 3H, J=7.2 Hz), 2.06 (s, 3H), 5.33 (d, 1H, J=4.8 Hz), 6.05 (q, 1H, J=7.2 Hz), 6.23 (d, 1H, J=9.6 Hz), 6.59 (d, 1H, J=4.8 Hz), 6.86 (d, 1H, J=8.4 Hz), 7.62 (d, 1H, J=8.4 Hz), 7.90 (d, 1H, J=9.6 Hz); ^{13}C-NMR (in acetone-d_6): δ15.7, 20.6, 20.7, 22.3, 25.5, 61.0, 71.2, 78.0, 108.1, 113.5, 113.6, 115.0, 128.6, 130.8, 137.7, 144.7, 155.0, 157.0, 160.0, 167.3, 170.1; $[\alpha]_D^{27}$ +28.6° (*c* 0.75, MeOH).

4′-acetyl-3′-(2″-methyl-2″-butenoxy) khellactone (**5**) White amorphous; ESIMS *m/z* 409 $[M+Na]^+$; ^{1}H-NMR (in acetone-d_6): δ1.49 (s, 6H), 1.86 (s, 3H), 1.94 (d, 3H, J=7.2 Hz), 2.06 (s, 3H), 5.40 (d, 1H, J=4. Hz), 6.18 (q, 1H, J=7.2 Hz), 6.24 (d, 1H, J=9.6 Hz), 6.56 (d, 1H, J=4.8 Hz), 6.87 (d, 1H, J=8.4 Hz), 7.61 (d, 1H, J=8.4 Hz), 7.91 (d, 1H, J=9.6 Hz); ^{13}C-NMR (in acetone-d_6): δ15.9, 20.6, 20.7, 23.0, 25.1, 61.7, 70.8, 78.4, 107.9, 113.5, 113.6, 114.9, 128.0, 130.6, 139.8, 144.8, 155.0, 157.5, 160.1, 166.8, 170.0; $[\alpha]_D^{27}$ −24.3° (*c* 0.25, MeOH).

Soybean lipoxygenase inhibitory assay Soybean lipoxygenase activity was measured by a spectrophotometric method (Komoda *et al.,* 1995). In the standard assay, 20 μl of a sample MeOH solution and 2 ml of a 0.2 M borate buffer at pH 9.0, were used. In the control test, the same volume of MeOH was used instead of the sample solution. An enzyme solution (1000 units in 25 μl of a 0.2 M borate buffer) was then added, and preincubation was carried out for 5 min at room temperature. Next, 50 μl of a linoleic acid solution (4.18 mM in EtOH) was added, and the increase in absorbance at 234 nm was recorded. This increase in absorbance was compared with that obtained in the control test.

Table 1. NMR Data for Compound 1[a].

Position	δ_C		δ_H
1	128.03	C	
2	130.75	CH	7.55 (1H, d), *J*=8.0 Hz
3	116.76	CH	6.92 (1H, d), *J*=8.0 Hz
4	160.33	C	
5	116.76	CH	6.92 (1H, d), *J*=8.0 Hz
6	130.75	CH	7.55 (1H, d), *J*=8.0 Hz
1′	123.16	C	
2′	154.18	C	
3′	110.18	C	
4′	161.86	C	
5′	103.07	CH	6.64 (1H, d), *J*=8.0 Hz
6′	125.63	CH	7.56 (1H, d), *J*=8.0 Hz
4″	27.32	CH_2	2.60 (1H, dd), *J*=8.8, 7.7 Hz
			2.95 (1H, dd), *J*=8.8, 7.2 Hz
5″	68.90	CH	3.85 (1H, dd), *J*=8.8, 7.2 Hz
6″	78.59	C	
α	130.65	CH	7.58 (1H, d), *J*=15.0 Hz
β	141.43	CH	7.57 (1H, d), *J*=15.0 Hz
β′	190.31	C	
CH_3	20.95	CH_3	1.36 (3H, s)
CH_3	25.97	CH_3	1.43 (3H, s)
OCH_3	56.11	CH_3	3.90 (3H, s)

[a] Measured in acetone-d_6 at 400 MHz for ^{1}H and at 100 MHz for ^{13}C.

Results and Discussion

Compound **1** was obtained as a yellow powder. The molecular formula of **1** was determined to be $C_{21}H_{22}O_5$ by HRFABMS. The IR spectrum of **1** indicated the presence of hydroxyl and carbonyl functions. The ^{1}H-NMR spectrum of **1** in acetone-d_6 gave two sharp singlets at δ1.36 and 1.43 for geminal dimethyl protons. A triplet at δ3.85, which was coupled with methylene protons at δ2.60 and 2.95, indicated the presence of a methine proton attached to a carbon bearing a secondary hydroxyl group. There were two *ortho*-coupled doublets at δ6.64 and 6.92 integrating for one and two protons, respectively. Multiplet signals between δ7.54 and 7.59 integrating for five protons were also observed. The ^{13}C-NMR spectrum of **1** contained 21 carbon signals. The signals in the ^{1}H- and ^{13}C-NMR spectra were assigned from the ^{1}H-^{1}H COSY, HSQC and HMBC data. In the HMBC spectrum of **1**, correlations supporting the chalcone structure were observed. Figure 2 shows the key HMBC correlations (H to C) for **1**. Based on these results, compound **1** was determined to be 4,5″-dihydroxy-4′-methoxy-6″,6″-dimethyldihydropyrano-(2″,3″ : 2′,3′)-chalcone, a new chalcone 4′-methoxy-bavachromanol. However, the configuration of 5″-position is unknown. Bavachromanol, containing the 4′-OH group, has been reported from the seed of *Psoralea corylifolia* (Suri *et al.,* 1980).

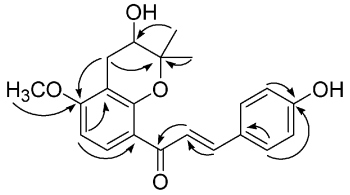

Fig. 2. Key HMBC correlations (H to C) for **1**.

Compounds **2** and **3** easily interconverted with each other when dissolved in alcoholic solvent. Thus the structure of these compounds was determined by using the acetyl derivatization. Compounds **2** and **3** were stabilized by acetylation for spectroscopic analysis such as MS and NMR by acetylation. By comparison with literature data, **2** and **3** were identified to be laserpitin and isolaserpitin, respectively, which are khellactone derivatives. Laserpitin and isolaserpitin are reported to have been isolated from the plants, *Musineon divaricatum* (Swager *et al.,* 1985) and *Angelica keiskei* (Akihisa *et al.,* 2003). However, this is the first report of the isolation of **2** and **3** from propolis.

Compound **1** was evaluated for their soybean lipoxygenase inhibitory activity. The results showed that **1** had strong enzyme-inhibitory activity with IC_{50} values of 14.6μM. The IC_{50} value of the well-known lipoxygenase inhibitor, kojic acid, was 19.5μM in the present assay.

Lipid peroxidation is one of the major factors in deterioration during the storage and processing of foods. The results presented here show that compound **1** was a potent inhibition of lipoxygenase. This result suggests that propolis collected on Jeju Island may be used as a source for antioxidant food additives.

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