Enhanced Cytotoxicity in a *Z*-Photoisomer of a Benzopyran Derivative of Propolis

Mikako Hirota,*,† Tetsuya Matsuno,‡ Tsuyoshi Fujiwara,§ Hiroshi Sugiyama,§ and Satoru Mineshita†

Department of Preventive Medicine, Medical Research Institute, Tokyo Medical and Dental University, Kandasurugadai, Chiyoda-Ku, Tokyo 101-0062, Japan, Institute of Cancer Research, Columbia University, New York, New York 10032, and Department of Molecular Design, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Kandasurugadai, Chiyoda-Ku, Tokyo 101-0062, Japan

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(*Z*)-2,2-Dimethyl-8-(3-methyl-2-butenyl)-benzopyran-6-propenoic acid (**1**) was isolated from Brazilian propolis, together with the known benzopyran derivative, (*E*)-2,2-dimethyl-8-(3-methyl-2-butenyl)-benzopyran-6-propenoic acid (**2**). The structure was determined by spectroscopic analyses, which included 1D and 2D ^{1}H and ^{13}C NMR experiments, as well as MS, IR, and UV spectroscopy. Compound **2** rapidly changed to **1** under UV irradiation conditions (365 nm), and the reverse reaction was also observed. The ratio of **1** to **2** reached 2.3 when the reaction began from either **1** or **2**, indicating a photostationary state. Compound **1** displayed an approximate 7-fold stronger cytotoxicity against human lung carcinoma cells (HLC-2) compared with **2**.

Propolis is a resinous material gathered by honeybees from the buds and bark of certain trees and plants.[1,2] It has long been used mainly in Europe as a folk remedy for treating various ailments. Characteristic components of propolis are flavonoids, flavones, flavanones, and phenolic acids, including cinnamic acid, its derivatives, and various trace elements.[3] Several biological activities, including antibacterial,[4−6] antioxidant,[7−9] antiinflammatory,[10−13] antitumor,[14,15] and immunomodulatory[16] properties have been reported for propolis and its constituents. However, a clear correlation between the chemical composition of propolis and any pharmaceutical activity that would explain its purported clinical benefits has not yet been established.

Recently, a new benzopyran derivative, (*E*)-2,2-dimethyl-8-(3-methyl-2-butenyl)-benzopyran-6-propenoic acid (**2**);[17,18] two diterpenoid isomers, 15-oxo-3,13(*Z*)-kolavadien-17-oic acid and 15-oxo-3,13(*E*)-kolavadien-17-oic acid [(13*Z*)- and (13*E*)-symphyoreticulic acid];[19] and 3-[4-hydroxy-3,5-bis-(3-methyl-2-butenyl)phenyl]-2-propenoic acid [3,5-diprenyl-4-hydroxycinnamic acid (artepillin C)],[20] were isolated from Brazilian propolis and shown to have cytotoxic activity.[17,19,20] Compound **2** damaged and retarded the growth of human hepatocellular carcinoma cells (HuH-13), HeLa cells, human epidermoid carcinoma cells (KB), and human lung carcinoma cells (HLC-2).[17] Artepillin C, a compound similar to **2**, has structural features of a cinnamic acid derivative and showed more effective antitumor activity than did 5-fluorouracil.[20] It was reported that **2** is chemically synthesized by cyclization of artepillin C.[17] Compound **2** required a much longer incubation time to induce cell damage than did artepillin C.[17] Thus, the possibility exists that **2** shows a cytotoxic effect after alterations of its structure during incubation.[17] We have attempted to isolate other strongly cytotoxic compounds from Brazilian propolis.

Recently, Boudourova-Krasteva et al. isolated an isomer of **2**,[21] but the biological activities of this isomer have not been reported. We identified a potent photoisomer of **2** from Brazilian propolis during the purification process, and compared its in vitro cytotoxicity toward human lung carcinoma cells (HLC-2) with that of **2**.

Results and Discussion

Both compound **1**, which appeared near the retention time of **2**, and **2**, together with two known diterpenoid isomers, were isolated by chromatographic methods,[17,19] followed by HPLC of the MeOH extract of Brazilian propolis. Compound **2** was isolated from Brazilian propolis and characterized as a new benzopyran derivative, (*E*)-2,2-dimethyl-8-(3-methyl-2-butenyl)-benzopyran-6-propenoic acid.[17,18] The two diterpenoid isomers were previously identified as (13*Z*)- and (13*E*)-symphyoreticulic acid from Brazilian propolis.[19]

The structures of **1** and **2** were determined by ^{1}H and ^{13}C NMR, NOEDS, HMQC, HMBC, and HREIMS, which indicated that both molecular formulas were $C_{19}H_{22}O_3$. Compound **1** was an amorphous and dark brown substance, having its UV maximum at 257 nm with a shoulder at 282 nm. IR detected the presence of alkane, alkene, and carboxylic acid groups (2932, 1688 cm^{-1}); a double bond (1618 cm^{-1}); and an aromatic ring group (1466 cm^{-1}). The ^{1}H NMR resonances and ^{13}C NMR spectra of **1** and **2** are listed in Table 1. The ^{1}H NMR signals of **1** were very similar to those of **2**,[17,18] except for the chemical shifts of two olefinic protons (H-5, H-7) within an aromatic ring system and two additional olefinic protons (H-14, H-15) in the propenoic acid group, which were shifted downfield and upfield by several parts per million, respectively, in comparison with those of **2**. This suggested that **1** is the geometric isomer in the $\Delta^{14,15}$double bond found in **2**. The small coupling constant (J = 12.5 Hz) observed in **1** for the $\Delta^{14,15}$ olefinic protons also showed a cis configuration when compared with **2** (J = 15.9 Hz). Furthermore, the relative stereochemistry of **1** was established by difference NOE spectra (NOEDS). The most useful NOEs in the structure of **1** were observed between H-14 and H-15, H-14 and H-5, H-14 and H-7, H-7 and H-9, and H-9 and H-10. Thus, this experiment revealed that the proton pairs H-14/H-15, H-14/H-5, H-14/H-7, H-7/H-9, and H-9/H-10 of **1** were within NOE proximity, indicating that H-14 and H-15 in **1** are cis (Figure 1).

* To whom correspondence should be addressed. Tel.: (81) 3-5280-8080. Fax: (81) 3-5280-8081. E-mail: mikaprm@mri.tmd.ac.jp.
† Department of Preventive Medicine, Tokyo Medical and Dental University.
‡ Institute of Cancer Research, Columbia University.
§ Department of Molecular Design, Tokyo Medical and Dental University.

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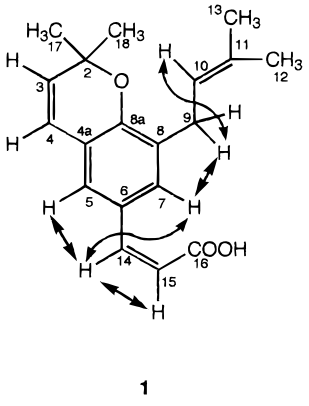
Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds **1** and **2** ($CDCl_3$)

	^{1}H NMR				^{13}C NMR	
	compound **1**		compound **2**		compound **1**	compound **2**
number	δ_H (ppm)	*J* (Hz)	δ_H (ppm)	*J* (Hz)	δ_C (ppm)	δ_C (ppm)
2					76.82 (s)	76.75
3	5.59	(d, 9.8)	5.63	(d, 9.8)	130.50 (d)	131.09
4	6.29	(d, 9.8)	6.37	(d, 9.8)	122.37 (d)	122.03
4a					120.33 (s)	121.04
5	7.34	(d, 4.3)	7.10	(d, 2.0)	126.93 (d)	124.41
6					126.37 (s)	126.29
7	7.34	(d, 4.3)	7.17	(d, 2.0)	132.65 (d)	129.92
8					128.79 (s)	129.81
8a					152.30 (s)	153.28
9	3.24	(d, 7.6)	3.25	(d, 7.3)	28.09 (t)	28.07
10	5.25	(ddd, 7.6, 7.6, 0.9)	5.25	(ddd, 7.3, 7.3, 1.2)	122.27 (d)	121.96
11					132.33 (s)	132.74
12	1.70	(d, 0.9)	1.71	(d, 1.2)	17.81 (q)	17.87
13	1.70	(d, 0.9)	1.73	(d, 1.2)	25.76 (q)	25.80
14	6.85	(d, 12.5)	7.66	(d, 15.9)	146.08 (d)	147.25
15	5.78	(d, 12.5)	6.32	(d, 15.9)	114.81 (d)	113.88
16	not observed		not observed		168.96 (s)	171.87
17	1.40	(s)	1.42	(s)	28.26 (q)	28.24
18	1.40	(s)	1.42	(s)	28.26 (q)	28.24



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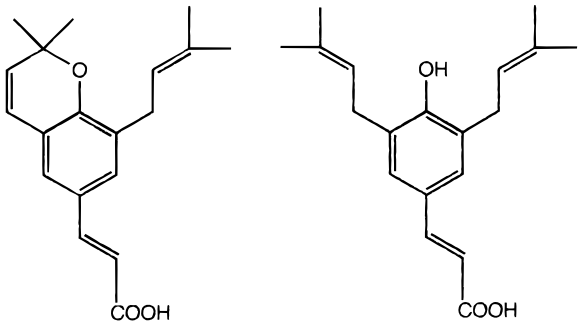

2 artepillin C

Figure 1. Critical NOEDS correlations for assigning stereochemistry in compound **1**, and the structures of compound **2** and artepillin C.

This assumption was confirmed as follows. The ^{13}C NMR spectra, together with the information from a DEPT spectra of **1** and **2**, revealed the presence of 18 signals corresponding to 19 carbon atoms (two carbons have the same chemical shift),[17,18] while the ^{13}C NMR spectrum of **1** showed a phenolic carbon at δ 152.30 (C-8a), an oxygenated carbon at δ 76.82 (C-2), and a carbonyl carbon at δ 168.96 (C-16) (Table 1). The spectroscopic similarity between **1** and **2** also suggested that the two compounds are isomers. The carbon skeletons of **1** and **2** were established by the 2D NMR experiments, HMQC, and HMBC. In the HMBC spectrum, the olefinic proton signal at δ 6.85 (H-14) in **1** showed correlations with a carbonyl carbon signal at δ 168.96 (C-16), while two phenolic carbon signals at δ 126.93 (C-5) and 132.65 (C-7) and a separate olefinic proton signal at δ 6.29 (H-4) correlated with three phenolic carbon signals at δ 152.30 (C-8a), 120.33 (C-4a), and 126.93 (C-5) and an oxygenated carbon signal at δ 76.82 (C-2) (Figure 2). Other significant long-range correlations are shown in Figure 2. Thus, the cis geometry of **1** was also determined, and **1** was characterized as (*Z*)-2,2-dimethyl-8-(3-methyl-2-butenyl)-benzopyran-6-propenoic acid.

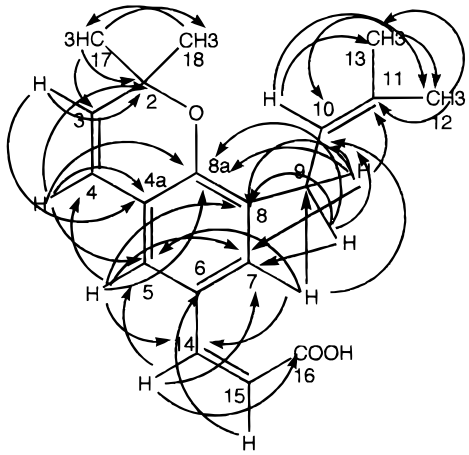

Figure 2. HMBC correlations of compound **1**.

The structures of **1** and **2** were further confirmed by their photoisomeric properties. The UV maxima of **2** were observed at 305, 271, and 263 nm, whereas the UV maximum of **1** was found only at 257 nm, indicating that the UV maximum of **1** disappears at the long wavelength end of the spectrum. Thus, the possibility exists that **1** and **2** are geometrical isomers, given that **2** has structural features of a cinnamic acid derivative that shows photoisomerization.[22]

In methanolic solutions of both **1** and **2** that had been irradiated by a transilluminator for 1 min at 365 nm (4900 μW/cm^2), HPLC analyses indicated that the two experiments showed very similar profiles and that the ratio of **1** to **2** reached 2.3 when the reaction began from either **1** or **2**, which corresponded to a photoequilibration and indicated that a photostationary state was achieved (Figure 3).[22] After being left to stand for 7 days in MeOH and exposed

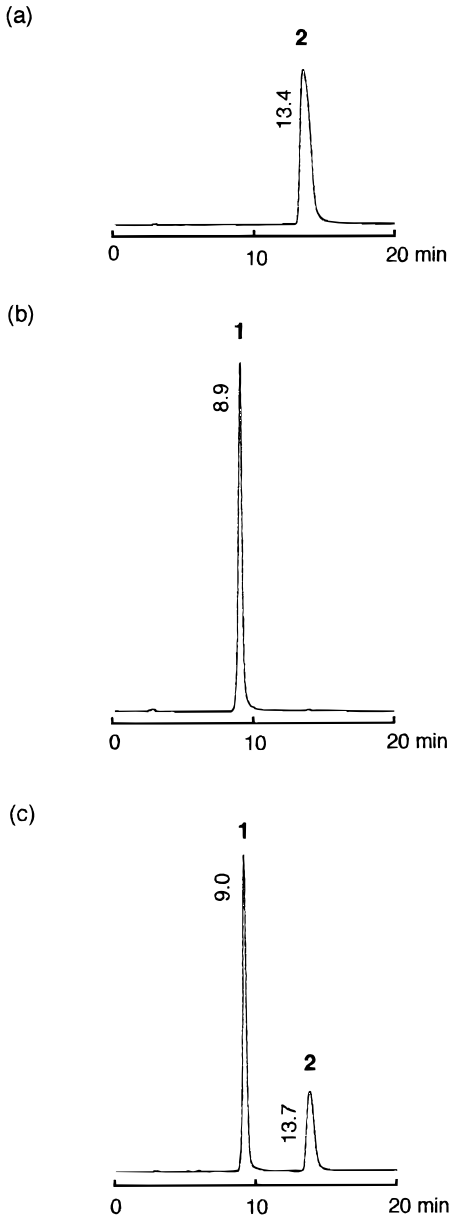

Figure 3. Photoequilibration: Analytical reversed-phase HPLC chromatograms, (a) pure compound **2**, (b) pure compound **1**, (c) two photoisomers **1** and **2**. Methanolic solution of both **1** and **2** irradiated by transilluminator for 1 min at 365 nm, HPLC analysis indicated that the ratio of **1** to **2** reached 2.3 when the reaction began from either **1** or **2**. Mobile phase: CH_3CN−50 mM ammonium formate (50:50). UV detection at 254.0 nm. Flow rate of 1 mL/min. ULTRON S-C_{18} 250 × 4.6 mm column.

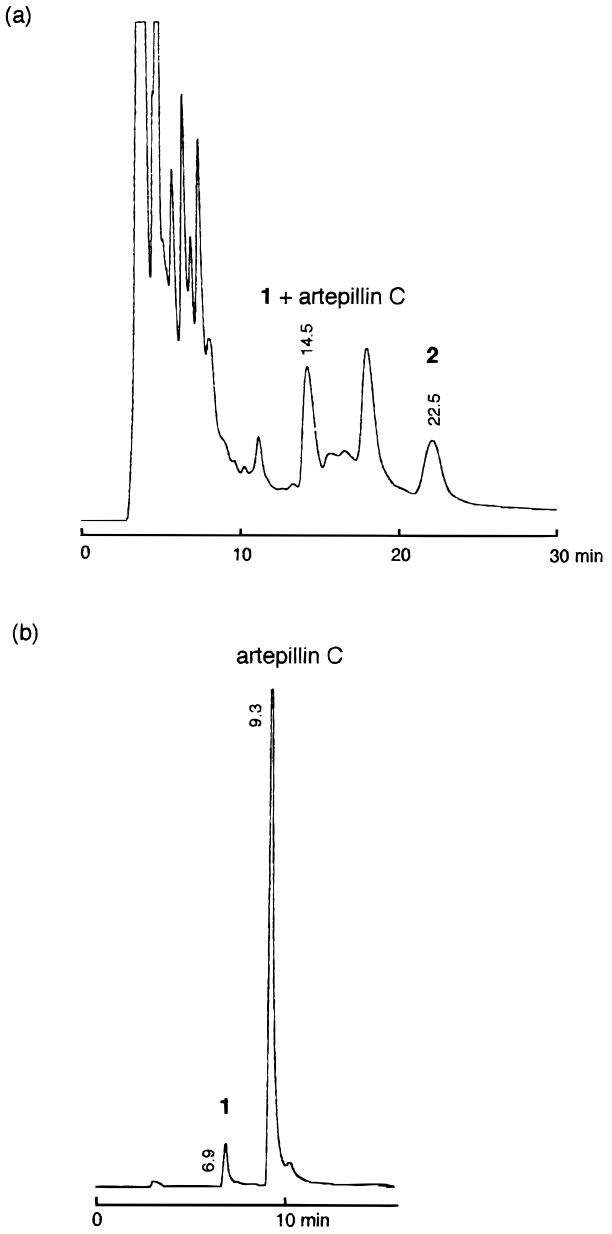

Figure 4. Quantitation of compound **1** in Brazilian propolis: **(**a) Analytical reversed-phase HPLC profiles of the extract with 70% MeOH obtained from Brazilian propolis without exposure to light at −20 °C. The peaks were labeled **1** + artepillin C and **2** in the chromatogram, respectively, (the area ratio, **1** + artepillin C−**2** 1.4:1.0). Mobile phase: CH_3CN−50 mM ammonium formate (35:65). UV detection at 254.0 nm. Flow rate of 1 mL/min. ULTRON S-C_{18} 250 × 4.6 mm column. (b) Analytical normal-phase HPLC profiles of **1** and artepillin C (the area ratio, **1**−artepillin C 1.0:12.9). Mobile phase: $CHCl_3$(100%). UV detection at 254.0 nm. Flow rate of 1 mL/min. Senshu PEGASIL Silica 60-5 250 × 4.6 mm column.

to visible light, **2** underwent ca. 71% constant conversion to **1**, which corresponded to a quantitative conversion, and indicated that it had also reached a state of photoequilibration.

Thus, **1** is a (*Z*)-photoisomer and **2** is an (*E*)-photoisomer. It is interesting to note that the two photoisomers (**1** and **2**) appear to be the photodynamically more stable substances without exposure to light, whereas the pure compounds **1** and **2** rapidly change to **2** and **1**, respectively, under UV or visible irradiation conditions.

Given this relationship, the ratio of **1** to **2** in the crude extract from Brazilian propolis was analyzed by HPLC without exposure to light. HPLC chromatograms for the ratio of **1** to **2** are shown in Figure 4. In HPLC profiles of the extract with 70% MeOH obtained from Brazilian propolis without exposure to light at −20 °C, the area ratio of **1** and artepillin C to **2** was 1.4 to 1.0, while the area ratio of **1** to artepillin C was 1.0 to 12.9. Thus, the ratio of **1** to **2** was 1.0:8.3, whereas the ratio of **1** to artepillin C was 1.0:51.5 (Figure 4). This implies that a minute amount of **1** was contained in the crude extract from Brazilian propolis compared with **2**.

The two photoisomers (**1** and **2**) were tested for in vitro cytotoxicity toward human lung carcinoma cells (HLC-2). The IC_{50} values of **1** and **2** on day 3 were determined to be 34 and 230 μM, respectively. On day 6, **1** and **2** showed cytotoxic activity for HLC-2 cells, with IC_{50} values of around 10 and 100 μM, respectively, when a low density of cells was inoculated in each well. Compound **1** also showed stronger cytotoxicity against human hepatocellular

carcinoma cells (HuH-13) than did **2** (data not shown). The comparison of **1** with **2**, which differed only in the stereochemistry of the $\Delta^{14,15}$double bond in the propenoic acid, revealed a strong enhancement of the cytotoxic activity when the configuration of the propenoic acid was cis. Compounds **2** and **1** readily underwent conversion to **1** and **2**, respectively, upon exposure to light. The finding of a previous study whereby **2** required a much longer incubation time to induce cell damage in contrast to the similar compound, artepillin C, suggested the involvement of photoisomerization of **2**.[17] This possibility is being investigated in relation to their chemical structures. Analogous enhancement of cytotoxic activity has previously been observed when the configuration is cis.[23] Careful attention should be paid to the light when biological activity assays are performed.

Experimental Section

General Experimental Procedures. EIMS and HREIMS spectra were obtained on a JEOL JMS-AX505HA instrument at 70 eV. ^{1}H and ^{13}C NMR spectra were measured using a JEOL ALPHA-500 spectrometer equipped with a 5-mm H5X-FG2 (^{1}H) and tunable (^{13}C) probe using $CDCl_3$ as the solvent and TMS as the internal standard, operated at 500 MHz for ^{1}H NMR and at 125.65 MHz for ^{13}C NMR. The HMBC experiment was carried out applying delays for a long-range coupling constant of 8 Hz. IR and UV spectra were recorded using a JASCO FT/IR-7000 and a Hitachi U-3200 spectrophotometer, respectively. Wakogel C-200 (Wako Pure Chemical Co., Ltd., Osaka, Japan) was used for Si gel column chromatography. Preparative reversed-phase HPLC was carried out by a Tosoh-Tokyo CCPP-M system equipped with a UV-8011 spectrometer and a Tosoh ODS 80 T_M 55 × 300 mm column with a flow rate of 20 mL/min. Semipreparative normal-phase HPLC and reversed-phase HPLC separations were carried out using a Millipore-Waters system (600) equipped with a Waters 484 spectrometer and an Inertsil YMC-Pack SIL 250 × 10.0 mm column with a flow rate of 7 mL/min, and a Tosoh-Tokyo CCPS system equipped with a UV-820 spectrometer and a CHEMCOBOND 5-ODS-H 10 × 150 (W) column with a flow rate of 3 mL/min, respectively. Analytical normal-phase and reversed-phase HPLC were performed using a Shimadzu LC-6A system equipped with a SPD-6A spectrometer and a Senshu PEGASIL Silica 60-5 250 × 4.6 mm column with a flow rate of 1 mL/min, and a Tosoh-Tokyo CCPS system equipped with a UV-820 spectrometer and a ULTRON S-C_{18} 250 × 4.6 mm column with a flow rate of 1 mL/min, respectively. 3UV Transilluminator (Funakoshi, Tokyo, Japan) was used for irradiation. TLC was performed on precoated sheets [Kieselgel 60F_{254}, 0.25 mm (Merck, Darmstadt, Germany)], with detection provided by UV light (254 nm) and then developed via an iodine-color reaction.

Material. Propolis was a mixture of samples collected from hives located in various districts of Brazil, including São Paulo, Paraná, and Santa Catarina, among other areas.

Extraction and Isolation. Brazilian propolis was homogenized and extracted by stirring at room temperature with MeOH. Water [10%(v/v)] was added to the extract, and the resulting precipitate was removed by low speed centrifugation. An equal volume of ethyl acetate and a half volume of distilled water were added to the supernatant, which was then mixed. The upper layer was collected, followed by evaporation of the solvent by a rotary evaporator. The extract was dissolved in MeOH and filtered through a nylon membrane (Type MNYL, 0.2 μm, Whatman, England) and used for isolation by means of preparative reversed-phase HPLC where the mobile phase was a linear gradient of MeOH [70−100%(v/v)] with UV detection at 210 nm.[17] The pooled fraction (eluted by ca. 95% MeOH, retention time: ca. 120 min) (3 L) was concentrated in vacuo at 30 °C to produce a residue, which was dissolved in ether and $CHCl_3$, adsorbed onto Si gel, and rechromatographed over Si gel with a continuous gradient of increasing polarity from *n*-hexane−EtOAc (10:1) to EtOAc to MeOH. Eluted fractions were pooled based on TLC [$CHCl_3$−MeOH (95:5)] using **2** as an authentic marker to yield 12 fractions. Fraction 4 [eluted by *n*-hexane−EtOAc (10:1)] (14.3 mg), which contained **2**, was combined with fractions 5 (31.4 mg) and 6 (28.5 mg), which exhibited similar TLC profiles. The combined extracts were chromatographed on a semipreparative normal-phase HPLC column (Inertsil YMC-Pack SIL) run isocratically using 99% $CHCl_3$−1% MeOH with UV detection at 254 nm. Combined fractions from the 8.6-min peak and the 13.7-min peak yielded (*Z*)-**1**- and/or (*E*)-**2**-isomers (15.5 mg), whereas the 29.6-min peak and the 32.7-min peak yielded (13*Z*)-symphyoreticulic acid (8.3 mg) and (13*E*)-symphyoreticulic acid (4.9 mg), respectively. The mixture of **1** and **2** was added to MeOH and then irradiated by a transilluminator for 10 min at 365 nm, and the resulting ratio of **1** to **2** was then determined to be 2.2. The mixture of isomers (**1** and **2**) were purified on a reversed-phase HPLC column (CHEMCOBOND 5-ODS-H) run isocratically using 50% CH_3CN−50% 50 mM ammonium formate with UV detection at 254 nm, without exposure to light. The CH_3CN-soluble portion was evaporated and the remaining aqueous layer extracted with CH_2Cl_2 and dried under vacuum, without exposure to light. Combined fractions from the 10.4-min peak and the16.4-min peak yielded the (*Z*)-**1**-isomer (1.1 mg) and the (*E*)-**2**-isomer, respectively.

Photoequilibration. A methanolic solution containing both **1** (1 mM) and **2** (1 mM) was irradiated by a transilluminator for 1 min at 365 nm. Two photoisomers (**1** and **2**) were analyzed on an analytical reversed-phase HPLC column (ULTRON S-C18) run isocratically using 50% CH_3CN−50% 50 mM ammonium formate with UV detection at 254 nm, without exposure to light.

Pure compound **2** (10.71 mg) in MeOH (1 L) was converted into **1** by exposure to light for 12 days. The solvent was evaporated in vacuo. Compound **1** was purified on a reversed-phase HPLC column (CHEMCOBOND 5-ODS-H) run isocratically using 50% CH_3CN−50% 50 mM ammonium formate with UV detection at 254 nm, without exposure to light, to yield 7.39 mg of **1**.

Quantitation of Compound 1 in Brazilian Propolis. Brazilian propolis (0.1419 g) was homogenized and extracted by stirring with 70% MeOH (5 mL) at −20 °C for 48 h, without exposure to light. A mixture of **1** + artepillin C and **2** was isolated on an analytical reversed-phase HPLC column (ULTRON S-C_{18}) run isocratically using 35% CH_3CN−65% 50 mM ammonium formate with UV detection at 254 nm, without exposure to light. The mixture of **1** and artepillin C was analyzed on an analytical normal-phase HPLC column (Senshu PEGASIL Silica 60-5), using 100% $CHCl_3$ with UV detection at 254 nm, without exposure to light [**1**: UV (EtOH) (ϵ) 254.0 nm (20 577), **2**: UV (EtOH) (ϵ) 254.0 nm (23 971), artepillin C: UV (EtOH) (ϵ) 254.0 nm (5163)].

Cytotoxicity Assay. Colorimetric assay using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt; Dojindo Laboratories, Kumamoto, Japan] was performed in 96-well plates (Falcon 3072, Falcon, Franklin Lakes, NJ). The assay was based on the reduction of WST-8 by the mitochondrial dehydrogenase of viable cells to yield a formazan product that can be measured spectrophotometrically. Human lung carcinoma cells (HLC-2) (5×10^3 cells) were inoculated in each well using 50 μL/well of Eagle's MEM-α medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum (JRH BIOSCIENCES, Australia) and 100 μU/mL penicillin G−100 μg/mL streptomycin sulfate (Gibco BRL) kept at 37 °C in a humidified atmosphere of 5% CO_2, and the cells were then maintained in logarithmic growth. One day later, the medium was changed, and compounds **1** and **2**, which had been dissolved in DMSO (Sigma Chemical, St. Louis, MO), were added at various concentrations to the culture medium (100 μL), without exposure to light. The final concentration of DMSO did not exceed 0.1%. On day 3, 10 μL of WST-8 solution per well was added to each cultured medium without exposure to light. After a further 1 or 2 h of incubation, optical density

measurements were made using a microplate reader (Tosoh MPR-A4I, Tokyo, Japan) at two wavelengths (450 and 600 nm). The IC_{50} values were calculated by linear interpolation, with values selected above and below the 50% mark. The cytotoxicity assay was performed in triplicate.

(*Z*)-2,2-Dimethyl-8-(3-methyl-2-butenyl)-benzopyran-6-propenoic acid (1): obtained as an amorphous substance; UV (EtOH) λ_{max} (log ϵ) 257 nm (4.32), 282 nm (sh) (3.92); IR ν_{max} (KBr) 2932, 1688, 1618, 1466, 1379, 1209, 1141, 955, 888, 822 cm^{-1}; EIMS *m*/*z* (rel int) 298 $[M]^+$ (21.0), 283 $[M - CH_3]^+$ (100.0); HREIMS 298.1552 (calcd for $C_{19}H_{22}O_3$ 298.1550); ^1H and ^{13}C NMR data, see Table 1.

(*E*)-2,2-Dimethyl-8-(3-methyl-2-butenyl)-benzopyran-6-propenoic acid (2): obtained as an amorphous substance; UV (EtOH) λ_{max} (log ϵ) 305 (4.20), 271 (4.49), 263 (4.49) nm; IR ν_{max} (KBr) 2962, 1684, 1626, 1597, 1468, 1439, 1377, 1361, 1336, 1276, 1207, 1151, 1122, 996, 951, 907 cm^{-1}; EIMS *m*/*z* (rel int) 298 $[M]^+$ (20.9), 283 $[M - CH_3]^+$ (100.0); HREIMS 298.1563 (calcd for $C_{19}H_{22}O_3$ 298.1550); ^1H and ^{13}C NMR data, see Table 1.

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References and Notes

(1) Ghisalberti, E. L. *Bee World* **1979**, *60*, 59−84.
(2) König, B. *Bee World*. **1985**, *66*, 136−139.
(3) Greenaway, W.; May, J.; Scaybrook, T.; Whatley, F. R. *Z. Naturforsch.* **1991**, *46c*, 111−121.
(4) Aga, H.; Shibuya, T.; Sugimoto, T.; Kurimoto, M.; Nakajima, S. *Biosci. Biotech. Biochem*. **1994**, *58*, 945−946.
(5) Grange, J. M.; Davey, R. W. *J. Royal Soc. Med*. **1990**, *83*,159−160.
(6) Metzner, J.; Bekemeier, H.; Paintz, M.; Schneidewind, E. *Pharmazie* **1979**, *34*, 97−102.
(7) Scheller, S.; Wilczok, T.; Imielski, S.; Krol, W.; Gabrys, J.; Shani, J. *Int. J. Radiat. Biol.* **1990**, *57*, 461−465.
(8) Pascual, C.; Gonzalez, R.; Torricella, R. G. *J. Ethnopharmacol.* **1994**, *41*, 9−13.
(9) Matsuno, T.; Chen, C.; Basnet, P. *Med. Sci. Res.* **1997**, *25*, 583−584.
(10) Wang, L.; Mineshita, S.; Ga, I.; Shigematsu, T.; Matsuno, T. *Jpn. J. Pharmacol. Ther.* **1993**, *24*, 223−224.
(11) Krol, W.; Scheller, S.; Czuba, Z.; Matsuno, T.; Zydowics, G.; Shani, J.; Mos, M. *J. Ethnopharmacol.* **1996**, *55(1)*, 19−25.
(12) Mirzoeva, O. K.; Calder, P. C. *Prostaglandins Leukotrienes Essent. Fatty Acids* **1996**, *55(6)*, 441−9.
(13) Natarajan, K.; Singh, T. R., Jr.; Grunberger, D.; Aggarwal, B. B. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93(17)*, 9090−9095.
(14) Mitamura, T.; Matsuno, T.; Sakamoto, S.; Maemura, M.; Kudo, H.; Suzuki, S.; Kuwa, K.; Yoshimura, S.; Sassa, S.; Nakayama, T.; Nagasawa, H. *Anticancer Res.* **1996**, *16*, 2669−2672.
(15) Jaiswal, A. K.; Venugopal, R.; Mucha, J.; Carothers, A. M.; Grunberger, D. *Cancer Res.* **1997**, *57(3)*, 440−446.
(16) Natarajan, K.; Singh, T. R., Jr.; Grunberger, D.; Aggarwal, B. B. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93(17)*, 9090−9095.
(17) Matsuno, T.; Saito, M.; Matsumoto, Y.; Morikawa, J. *Z. Naturforsch.* **1998**, *53c*, 1037−1039.
(18) Tazawa, S.; Warashina, T.; Noro, T.; Miyase, T. *Chem. Pharm. Bull.* **1998**, *46(9)*, 1477−1479.
(19) Matsuno, T.; Matsumoto, Y.; Saito, M.; Morikawa, J. *Z. Naturforsch.* **1997**, *52c(9−10)*, 702−704.
(20) Matsuno, T.; Jung, S. K.; Matsumoto, Y.; Saito, M.; Morikawa, J. *Anticancer Res.* **1997**, *17*, 3565−3568.
(21) Boudourova-Krasteva, G.; Bankova, V.; Sforcin, J. M.; Nikolova, N.; Popov, S. *Z. Naturforsch.* **1997**, *52c*, 676−679.
(22) Fessenden, R. J.; Fessenden, J. S. *Organic Chemistry, 4th ed.*; Brooks/Cole, 1990; pp 867−872.
(23) Queiroz, E. F.; Roblot, F.; Figadere, B.; Laurens, A.; Duret, P.; Hocquemiller, R.; Cave, A.; Serani, L.; Laprevote, O.; Cotte-Laffitte, J.; Quero, A. M. *J. Nat. Prod.* **1998**, *61(1)*, 34−39.

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