Study on the Constituents of Mexican Propolis and Their Cytotoxic Activity against PANC-1 Human Pancreatic Cancer Cells

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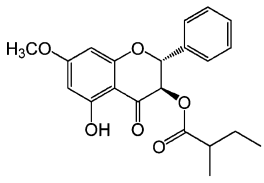
Three new flavonoids, (2*R*,3*R*)-3,5-dihydroxy-7-methoxyflavanone 3-(2-methyl)butyrate (**1**), (7″*R*)-8-[1-(4′-hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]chrysin (**2**), and (7″*R*)-8-[1-(4′-hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]galangin (**3**), together with 41 known compounds (**4**−**44**) were isolated from a methanolic extract of Mexican propolis. Compounds **2** and **3** are unique natural flavones containing a 1-phenylallyl moiety. The in vitro preferential cytotoxicity of all the isolates was evaluated against a PANC-1 human pancreatic cell line. Compound **3** displayed the most potent preferential cytotoxicity (PC_{50} 4.6 μM) in the nutrient-deprived medium (NDM) and triggered apoptosis-like morphological changes in PANC-1 cells.

Propolis, a sticky hive product collected by bees from various plant sources, is well known to possess pharmacological activities such as anticancer,[1,2] antiviral,[3] antifungal,[3,4] antibacterial,[3,5] antioxidant,[6] and anti-inflammatory effects.[7] The use of propolis as a traditional remedy for treatment of various diseases dates back to at least 300 B.C.[8] During the last four centuries, propolis was popular as an official drug in Europe on account of its antibacterial activity.[9] Propolis has been gaining popularity throughout the world as a dietary supplement for disease prevention.[10] As a part of our studies of propolis from various origins,[11−18] we observed strong preferential cytotoxic activity in propolis collected from Brazil and Myanmar against PANC-1 human pancreatic cancer cells (PANC-1) in a nutrient-deprived medium (NDM) and reported their active constituents.[19,20] In our continued study, Mexican propolis also exhibited strong preferential cytotoxicity, PC_{50} value of 13.9 μg/mL, against PANC-1 cells. Our phytochemical investigation on this sample yielded 44 compounds including three new flavonoids (**1**−**3**). We herein report the structure elucidation of **1**−**3** together with the in vitro preferential cytotoxicity of all of the isolates obtained against PANC-1 cells in a NDM .



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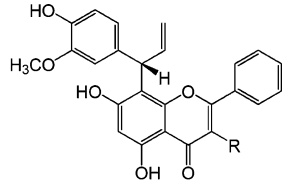

	R
2	H
3	OH

Results and Discussion

Compound **1** was isolated as a yellow, amorphous solid with $[\alpha]^{22}_D$ +31.8 (*c* 0.5, $CHCl_3$), and its molecular formula was determined by HREIMS to be $C_{21}H_{22}O_6$. The IR spectrum indicated the presence of OH (3440 cm^{-1}), ester (1747 cm^{-1}), and conjugated ketone carbonyl (1650 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** (Table 1) displayed signals due to an aromatic ring system (δ_H 7.40−7.48, 5H), a pair of *meta*-coupled aromatic protons (δ_H 6.12, 6.07, d, J = 2.2 Hz), two coupled oxymethines (δ_H 5.86, 5.37, d, J = 12.0 Hz), an *O*-methyl (δ_H 3.82), and a signal typical of a hydrogen-bonded OH (δ_H 11.51). Signals of two methyl (δ_H 0.61, 1.05), a methylene (δ_H 1.30, 1.48), and an acetyl methine (δ_H 2.35) group were also observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of **1** (Table 1) showed signals of 21 carbons including those corresponding to ketone (δ_C 191.9) and ester carbonyl (δ_C 174.9) carbons. These data closely resembled those of pinobanksin 3-(2-methyl)butyrate (**27**),[12] except for the presence of an additional signal ascribable to an OCH_3 group (δ_H 3.82, δ_C 55.8) in **1**. The OCH_3 was deduced to be at C-7 on the basis of HMBC correlations of the signal at δ_H 3.82 (7-OMe) with C-7 (δ_C 168.6) and of a hydrogen-bonded proton at δ_H 11.51 (5-OH) with C-5 (δ_C 164.2), C-6 (δ_C 95.7), and C-10 (δ_C 102.0). Further analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra confirmed the planar structure of **1** (Figure 1a). Finally, the absolute configuration of **1** was determined to be 2*R* and 3*R* by comparing the $[\alpha]_D$ value and CD data with those of compound **27**.[12,21] Therefore, the structure of compound **1** was assigned as (2*R*,3*R*)-3,5-dihydroxy-7-methoxyflavanone 3-(2-methyl)butyrate.

Compound **2** was obtained as a yellowish-brown, amorphous solid with $[\alpha]^{22}_D$ −17.4 (*c* 0.3, $CHCl_3$), and its molecular formula was deduced to be $C_{25}H_{20}O_6$ by HREIMS. The IR spectrum of **2** showed the absorption bands of OH (3175 cm^{-1}) and conjugated ketone carbonyl (1654 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** (Table 1) exhibited signals corresponding to chrysin (**28**), showing a hydrogen-bonded OH (δ_H 12.94), a phenyl group (δ_H 7.44−7.66, 5H), an olefinic singlet (δ_H 6.92, H-3), and an aromatic singlet (δ_H 6.37, H-6),[22] together with signals that were ascribable to a 1,3,4-trisubstituted phenyl group (δ_H 6.77, d, J = 1.6 Hz; δ_H 6.67, d, J = 8.0 Hz; δ_H 6.59, dd, J = 8.0, 1.6 Hz), a methine (δ_H 5.30, d, J = 8.0 Hz, H-7″), a vinyl group (δ_H 6.45, 5.19, 5.16), and an *O*-methyl proton (δ_H 3.57). The presence of a chrysin moiety was supported by ^{13}C NMR data (Table 1). Furthermore, the HMBC correlations of a *meta*-coupled aromatic proton at δ_H 6.77 (H-2″) with two oxygenated aromatic carbons (δ_C 147.4, C-3″; δ_C 144.8, C-4″), C-1″ (δ_C 133.1), C-6″ (δ_C 119.4), and a methine carbon (δ_C 42.8, C-7″) (Figure 1b), together with the NOE correlations between *O*-methyl protons at δ_H 3.57 (3″-OMe) and H-2″, indicated the presence of a 1-(4′-hydroxyl-3′-methoxyphenyl)allyl moiety. The attachment of the phenylallyl moiety to the chrysin moiety was deduced to be via C-7″−C-8 on the basis of HMBC correlations of a methine proton at δ_H 5.30 (H-7″) with C-7 (δ_C 162.2), C-8 (δ_C 108.4), and C-9 (δ_C 154.6) (Figure 1b). Finally, the absolute configuration of **2** was determined by comparing the CD spectrum and $[\alpha]_D$ values to those of the related compounds.

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for Compounds **1**−**3** (*J* values in parentheses)

	1[a]		**2**[b]		**3**[b]	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.37 d (12.0)	81.7		163.2		145.8
3	5.86 d (12.0)	71.9	6.92 s	105.1		136.8
4		191.9		182.1		176.4
5		164.2		159.6		158.9
6	6.12 d (2.2)	95.7	6.37 s	98.9	6.38 s	98.2
7		168.6		162.2		161.8
8	6.07 d (2.2)	94.7		108.4		107.7
9		162.3		154.6		153.5
10		102.0		104.2		103.4
1′		135.2		130.8		130.9
2′ 6′	7.48 m	127.7	7.66 d (7.5)	126.4	7.74 d (7.1)	127.5
3′ 5′	7.40 m	128.7	7.44 t (7.5)	129.0	7.42 m	128.2
4′	7.40 m	129.6	7.54 t (7.5)	131.9	7.42 m	129.7
1″		174.9		133.1		133.1
2″	2.35 qt (7.1, 6.8)	40.8	6.77 d (1.6)	111.3	6.76 d (1.7)	111.3
3″	1.30 m 1.48 m	26.5		147.4		147.3
4″	0.61 t (7.6)	11.0		144.8		144.7
5″	1.05 d (7.1)	16.5	6.67 d (8.0)	115.3	6.67 d (8.0)	115.2
6″			6.59 dd (8.0, 1.6)	119.4	6.58 dd (8.0, 1.7)	119.3
7″			5.30 d (8.0)	42.8	5.29 d (8.0)	42.6
8″			6.45 ddd (16.8, 10.1, 8.0)	138.8	6.45 ddd (16.8, 10.1, 8.0)	138.7
9″			5.19 d (10.1) 5.16 d (16.8)	116.2	5.18 d (10.1) 5.15 d (16.8)	116.0
5-OH	11.51 s		12.94 s		12.48 s	
7-OMe	3.82 s	55.8				
3″-OMe			3.57 s	55.5	3.56 s	55.4

[a] Measured in $CDCl_3$. [b] Measured in DMSO-d_6.

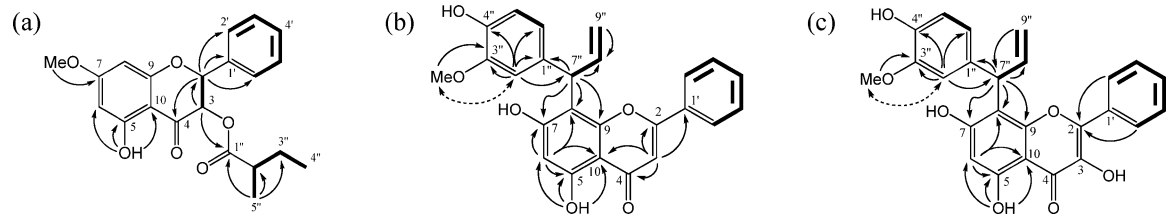

Figure 1. COSY (bold lines) and selected HMBC (solid arrows: ^{1}H→^{13}C) correlations and difference NOE (dashed arrows) in **1** (a), **2** (b), and **3** (c).

Compound **2** showed a positive Cotton effect at 230 nm ([θ]$_{230}$ +1778) and a negative specific rotation, which were similar to those of the known compound (7*R*)-2-dydroxy-4,5-dimethoxydalbergiquinol ([θ]$_{225}$ +1624; [α]$_D$ −47.8), but opposite of those of (7*S*)-2,4-dihydroxydalbergiquinol ([θ]$_{231}$ −3794; [α]$_D$ +34.7) and (7*S*)-3,4-dihydroxydalbergiquinol([θ]$_{229}$ −3119; [α]$_D$ +36.5), isolated from Nepalese propolis, in previous work (shown in the Supporting Information),[17] suggesting that the absolute configuration at C-7″ is *R*. Therefore, **2** was identified as (7″*R*)-8-[1-(4′-hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]chrysin. To the best of our knowledge, this is the first report of a flavone having a phenylallyl moiety from a natural source.

Compound **3** was a dark yellow, amorphous solid having [α]$^{22}_D$ −14.3 (*c* 0.3, CH_3OH) and the molecular formula $C_{25}H_{20}O_7$ (HREIMS). The IR spectrum of **3** indicated the presence of OH and conjugated ketone carbonyl groups. The ^{1}H and ^{13}C NMR data of **3** (Table 1) were similar to those of compound **2**, except for the lack of signal due to an olefinic proton (δ_H 6.92, s, H-3, in **2**). These data together with its molecular formula indicated the presence of an OH group at C-3 in **3**. The absolute configuration of **3** was determined to be the same as that of **2** on the basis of its [α]$_D$ value and the CD spectrum. Thus, **3** was concluded to be (7″*R*)-8-[1-(4′-hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]galangin.

Known compounds isolated in this study were (*E*)-cinnamyl benzoate (**4**),[23] benzyl (*E*)-cinnamate (**5**),[24] (*E*)-cinnamyl (*E*)-cinnamate (**6**),[25] (2*S*)-pinostrobin (**7**),[26] tectochrysin (**8**),[27] (2*R*,3*R*)-alpinone-3-acetate (**9**),[28] izalpinin (**10**),[29] benzyl (*E*)-3,4-dimethoxycinnamate (**11**),[30] (*E*)-cinnamyl (*E*)-3,4-dimethoxycinnamate (**12**),[31] 3,3-dimethylallyl (*E*)-ferulate (**13**),[32] isopent-3-enyl (*E*)-ferulate (**14**),[32] benzyl (*E*)-ferulate (**15**),[33] (*E*)-cinnamyl (*E*)-isoferulate (**16**),[31] (*E*)-cinnamic acid (**17**),[34] (*E*,*E*)-cinnamylideneacetic acid (**18**),[34] benzoic acid (**19**),[35] (2*R*,3*R*)-alpinone (**20**),[28,36] galangin (**21**),[37] (2*S*)-pinocembrin (**22**),[38] benzyl (*E*)-*p*-coumarate (**23**),[33] (2*R*,3*R*)-pinobanksin 3-acetate (**24**),[39] (2*R*,3*R*)-pinobanksin 3-propanoate (**25**),[21] (2*R*,3*R*)-pinobanksin 3-isobutyrate (**26**),[21] (2*R*,3*R*)-pinobanksin 3-(2-methyl)-butyrate (**27**),[12,21] chrysin (**28**),[22] vanillin (**29**),[40] (2*R*,3*R*)-3,7-dihydroxy-5-methoxyflavanone (**30**),[41] (*E*)-3,4-dimethoxycinnamic acid (**31**),[30] (*E*)-ferulic acid (**32**),[42] (*E*)-cinnamyl (*E*)-*p*-coumarate (**33**),[31] (2*R*,3*S*)-8-[4-phenylprop-2-en-1-one]-4′,7-dihydroxy-3′,5-dimethoxyflavan-3-ol (**34**),[43] 2-acetyl-1,3-di-(*E*)-feruloylglycerol (**35**),[44] 2-acetyl-1-(*E*)-*p*-coumaroyl-3-(*E*)-feruloylglycerol (**36**),[14] 2-acetyl-1,3-di-(*E*)-*p*-coumaroylglycerol (**37**),[14] 2-acetyl-3-(*E*)-caffeoyl-1-(*E*)-*p*-coumaroylglycerol (**38**),[45] 3-acetyl-1-(*E*)-*p*-coumaroylglycerol (**39**),[46] 4′,5,7-trihydroxy-3,3′-dimethoxyflavone (**40**),[47] (*E*)-*p*-coumaric acid (**41**),[48] 4′,7-dihydroxy-3-methoxyflavone (**42**),[49] (2*S*)-naringenin (**43**),[38] and apigenin (**44**),[38] which were identified by comparison of NMR data with reported data.

Among the isolated compounds, flavanones such as pinocembrin (**22**) and pinobanksin 3-acetate (**24**) and flavones such as tectochrysin (**8**), galangin (**21**), and chrysin (**28**) were major constituents reported from *Populus nigra*.[50−53] Other isolates such as aromatic acids (**17**, **19**, **23**, **32**, **41**) and their esters (**4**, **6**, **13**−**16**, **33**) together with phenylpropanoid glycerides (**35**−**39**) were also reported as characteristic constituents of *Populus* spp.[54−57] Therefore, the botanical origin of this Mexican propolis sample is assumed to be the genus *Populus*.

Table 2. Preferential Cytotoxicity of Compounds **1**−**44** on PANC-1 Cells in NDM

compound	PC_{50} (μM)[a]	compound	PC_{50} (μM)[a]
1	44.8	**28**	88.7
2	12.7	**32**	66.6
3	4.6	**33**	38.2
9	75.3	**34**	22.4
11	58.8	**35**	25.8
12	98.9	**36**	32.7
13+**14**	66.3	**37**	38.8
15	17.0	**38**	17.4
16	31.7	**40**	59.8
20	74.2	**41**	77.0
21	22.1	**44**	89.5
22	46.5	**4**−**8**, **10**, **17**−**19**	>100
23	23.8	**29**−**31**, **39**, **42**, **43**	>100
24	36.6	5-fluorouracil[b]	>100
25	18.2	paclitaxel[b]	>100
26	19.8	arctigenin[c]	0.51
27	17.5		

[a] The concentration at which 50% of cells died preferentially in NDM. [b] Conventional anticancer drug in clinical use. [c] Positive control.

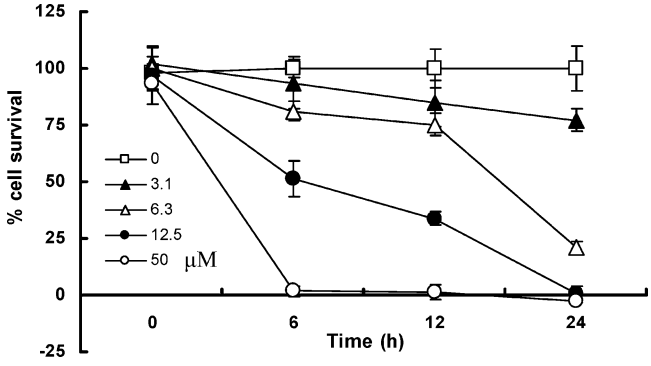

Figure 2. Survival rate of PANC-1 cells in NDM within 0, 6, 12, and 24 h treatment by 0−50 μM of **3**. Data expressed as mean ± SD, $n = 3$.

The constituents of propolis from various regions differ in composition because of differences in the local vegetation.[12,14,17−20] The composition of propolis may also depend on the bee species that produced it, due to their preferences for particular plants.[21,58−61] The propolis sample in this study was collected from a hive of Africanized (*Apis mellifera*) honey bees. *Apis mellifera* bees in Europe tend to gather bud exudates of poplar trees,[21,61] while those in Brazil tend to gather propolis from bud exudates of the poplar trees, *Hyptis divaricata*, and *Baccharis dracunculifolia*.[62]

Pancreatic cancer cells, such as PANC-1 cells, have a high tolerance to nutrition starvation that enables them to survive under hypovascular conditions (austerity). Thus, discovery of agents that retard the cancer cells' tolerance to nutrition starvation is regarded as a novel antiausterity strategy in anticancer drug discovery.[63] This strategy is based on the search for agents that preferentially kill cancer cells in nutrient-deprived conditions, without having toxicity under normal conditions.[64] Following this antiausterity strategy, all the isolates (**1**−**44**) were evaluated for in vitro preferential cytotoxicity against PANC-1 cells in a NDM. The results are summarized in Table 2. Compound **3** showed the most potent activity, with a PC_{50} at 4.3 μM. As shown in Figure 2, **3** displayed preferential cytotoxicity in a concentration- and time-dependent manner. Furthermore, microscopic observation of the dying PANC-1 cells displayed typical apoptosis-like morphological changes such as membrane bleb, nuclear condensation, and fragmentation by treatment of **3** within 24 h at 12.5 μM (Figure 3). By way of contrast, the conventional anticancer drugs paclitaxel and 5-fluorouracil, used as negative controls, were inactive ($PC_{50} > 100$ μM). Arctigenin, the positive control, displayed preferential cytotoxicity

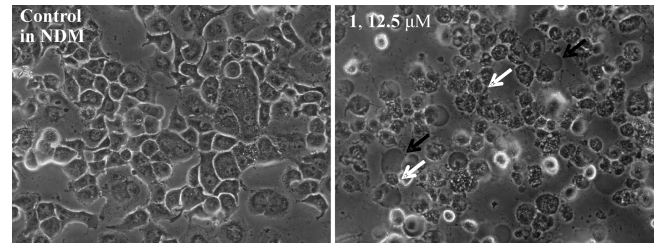

Figure 3. Morphological changes of PANC-1 cells (white arrow: nucleus fragmentation and condensation; black arrow: membrane bleb) in NDM after 24 h exposure with 12.5 μM of **3**.

at a PC_{50} of 0.51 μM. Compound **3**, which showed the most potent activity against PANC-1 cells, was also tested for its toxic effect against normal TIG-3 human fetal lung fibroblast cells using the same protocol. Interestingly, **3** was noncytotoxic up to 100 μM against TIG-3 cells, indicating its selectivity against PANC-1 cells.

Inspection of structures and activity data led to the following generalizations. The aromatic esters displayed stronger activity than aromatic acids (**11**, **12** > **31**; **13**+**14**, **15** > **32**; **23**, **33** > **41**). The presence of an OH group on the phenyl ring significantly increased activity (**23** ≫ **5**, **33** ≫ **6**; **41** ≫ **17**). A *meta* or *para* OH group on the phenyl ring is more favorable than a OCH_3 group for activity (**15** > **11**; **16** > **12**; **32** > **31**). In phenylpropanoid glycerides, an increase in the number of OCH_3 or OH groups on a phenyl ring or phenylpropanoid group enhances the activity (**35** > **36**; **38** > **37**; **37** > **39**). A *meta*-substituted OH group is preferable for activity to that of a OCH_3 group (**38** > **36**). Among the flavones and flavanones, a phenylpropanoid group at C-8 significantly enhances the activity (**2** > **28**; **3** > **21**), while at C-7, a OH group is more favorable than a OCH_3 group (**22** > **7**; **24** > **9**; **28** > **8**). Similarly, an OH group at C-3 increases the activity (**3** > **2**; **20** > **7**; **21** > **28**). However, the presence of a conjugated double bond between C-2 and C-3 seems to diminish the activity (**10** < **20**; **28** < **22**; **43** < **44**).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. CD measurements were carried out on a JASCO J-805 spectropolarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer. NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in δ values (ppm). EIMS and HREIMS measurements were carried out on a JEOL JMS-700T spectrometer. Column chromatography (CC) utilized silica gel (silica gel 60N, spherical, neutral, 40−50 μm, Kanto Chemical Co., Inc.) and reversed-phase silica gel (Cosmosil $75C_{18}$-OPN, Nacalai Tesque Inc.). Medium-pressure liquid chromatography (MPLC) was performed using a Buchi double-pump module C-605 system. Preparative TLC was carried out on precoated silica gel $60F_{254}$ and RP-$18F_{254}$ plates (Merck, 0.25 or 0.50 mm thickness).

Biological Material. Crude propolis from a hive of *Apis mellifera* was collected at Caborca, Sonora state, Mexico, in May 1999, and was stored at −40 °C. A voucher specimen (TMPW 26808) was deposited at the Museum of Materia Medica, Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Japan.

Extraction and Isolation. Propolis (40.0 g) was extracted with MeOH under sonication (90 min, ×3) at room temperature, and the solvent was evaporated under reduced pressure to give 15.8 g of extract. The MeOH extract (15.0 g) was chromatographed on silica gel with MPLC using a hexane and then MeOH−$CHCl_3$ solvent system to give six fractions [1, hexane eluate, 2.8 g; 2, $CHCl_3$ eluate, 254 mg; 3, MeOH−$CHCl_3$ (3:96) eluate, 805 mg; 4, MeOH−CH_2Cl_2 (6:92) eluate, 4.5 g; 5, MeOH−CH_2Cl_2 (10:90) eluate, 2.4 g; 6, MeOH−CH_2Cl_2 (30:70) eluate, 1.9 g].

Fraction 2 (254 mg) was separated by TLC with EtOAc−hexane (1:9), followed by TLC with C_6H_6−hexane (3:7), to give **4** (6.8 mg), **5** (32 mg), **6** (24 mg), and **7** (10 mg). Fraction 3 (805 mg) was rechromatographed on silica gel using an EtOAc−hexane gradient

system to give five subfractions (3-1: 27 mg; 3-2: 315 mg; 3-3: 108 mg; 3-4: 103 mg; 3-5: 53 mg). Subfraction 3-2 (315 mg) yielded crystals of **8** (246 mg). The mother liquor was subjected to PTLC with acetone−C_6H_6 (5:95) to give **8** (13 mg), **9** (13 mg), and **10** (21 mg). Subfraction 3-3 (108 mg) was purified by TLC with acetone−C_6H_6 (5:95), then by RP-TLC with H_2O−MeOH−CH_3CN (1:2:2), to give **1** (1 mg), **11** (3 mg), **12** (4 mg), and a 1:1 mixture (4 mg) of **13** and **14**. Subfraction 3-4 (103 mg) was subjected to TLC with 7% acetone−C_6H_6 to give **15** (76 mg) and **16** (8 mg). Subfraction 3-5 (53 mg) was separated by RP-TLC with H_2O−MeOH−CH_3CN (1:1:1) to give **17** (5 mg) and **18** (32 mg).

Fraction 4 (4.5 g) was chromatographed on silica gel using an EtOAc−hexane gradient system to afford seven subfractions (4-1, 152 mg; 4-2, 292 mg; 4-3, 1.2 g; 4-4, 908 mg; 4-5, 800 mg; 4-6, 950 mg; 4-7, 156 mg). Purification of subfraction 4-2 by TLC with 6% MeOH−CH_2Cl_2 and then by RP-TLC with H_2O−MeOH−CH_3CN (1:1:1) gave **8** (20 mg), **19** (102 mg), and **20** (6 mg). Subfraction 4-3 (1.2 g) in $CHCl_3$ gave crystals of **21** (251 mg). The mother liquor was separated by reversed-phase MPLC with an H_2O−CH_3CN system (5:5 → 4:6 → 3:7 → 2:8 → 1:9) to obtain five fractions (4-3-1, 24.1 mg; 4-3-2, 345 mg; 4-3-3, 330 mg; 4-3-4, 124 mg; 4-3-5, 20.2 mg). Fractions 4-3-2 and 4-3-4 were identified as **22** and **15**, respectively. Fraction 4-3-3 was subjected to PTLC with EtOAc−hexane (1:4), followed by RPTLC with H_2O−CH_3CN−acetone (1:1:1), to give **21** (50 mg), **23** (34 mg), **24** (18 mg), **25** (21 mg), **26** (34 mg), and **27** (11 mg). Subfraction 4-4 (908 mg) gave crystals of **28** (341 mg), and the remaining solution was separated by repeated TLC using acetone−C_6H_6 (1:9) to afford **16** (48 mg), **18** (32 mg), **24** (218 mg), and **29** (48 mg). Subfraction 4-5 (800 mg) on crystallization in MeOH gave **28** (733 mg). Subfraction 4-6 (950 mg) was separated by RP-MPLC, with an H_2O−CH_3CN−acetone system (3:1:1 → 2:1:1 → 1:1:1 → 1:2:2), to obtain four fractions (4-6-1, 124 mg; 4-6-2, 245 mg; 4-6-3, 170 mg; 4-6-4, 254 mg). Fraction 4-6-1 was subjected to RP-TLC with H_2O−acetone (4:3) to give **30** (45 mg) and **31** (17 mg). Fraction 4-6-2 (245 mg) and fraction 4-6-3 (170 mg) gave crystals of **31** (102 mg). PTLC of the mother liquors using acetone−C_6H_6 (1:9) afforded **31** (32 mg), **32** (12 mg), **33** (20 mg), and a mixture (7 mg). Separation of the mixture by RP-TLC with H_2O−CH_3CN−acetone (3:2:2) yielded **34** (3 mg), **2** (2 mg), and **3** (1 mg). Fraction 4-6-4 (254 mg) was subjected to TLC with acetone−C_6H_6 (1:9), followed by RP-TLC with H_2O−CH_3CN−acetone (1:1:1), to give **35** (47 mg) and **36** (68 mg).

Fraction 5 (2.4 g) was chromatographed by RP-MPLC, using H_2O−acetone (9:1 → 7:3 → 5:5 → 3:7 → 1:9), to afford five subfractions (5-1, 235 mg; 5-2, 282 mg; 5-3, 323 mg; 5-4, 212 mg; 5-5, 805 mg). Subfraction 5-4 was identified as **37**. Subfraction 5-1 (235 mg) was separated by RPTLC with H_2O−MeOH (2:1), followed by TLC with acetone−C_6H_6 (2:3), to give **39** (8 mg), **40** (10 mg), and **41** (19 mg). Subfractions 5-2 (282 mg) and 5-3 (323 mg) were dissolved in $CHCl_3$−MeOH (9:1) and left overnight to give crystals of **40** (53 mg) and **28** (123 mg), respectively. The mother liquors were combined and separated by TLC with MeOH−CH_2Cl_2 (8:92), followed by RPTLC with H_2O−MeOH−CH_3CN (1:1:1), to afford **30** (23 mg), **38** (13 mg), **40** (22 mg), **42** (3 mg), **43** (6 mg), and **44** (3 mg).

(2*R*,3*R*)-3,5-Dihydroxy-7-methoxyflavanone 3-(2-methyl)butyrate (1): yellow, amorphous solid; $[\alpha]^{22}_D$ +31.8 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3440 (br), 1747, 1650, 1630, 1460 cm^{-1}; CD (*c* 2.7 × 10^{-4} M, EtOH) $[\theta]_{325}$ +13 506, $[\theta]_{279}$ −29 674, $[\theta]_{231}$ +18 738; ^1H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 370.1403 (calcd for $C_{21}H_{22}O_6$, 370.1416).

(7″*R*)-8-[1-(4′-Hydroxy-3′-methoxyphenyl)prop-2-en-1-yl-]chrysin (2): yellow-brown, amorphous solid; $[\alpha]^{22}_D$ −17.4 (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3175 (br), 1654, 1614, 1586, 1513, 1452, 1423 cm^{-1}; CD (*c* 2.4 × 10^{-4} M, EtOH) $[\theta]_{230}$ +1778; ^1H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 416.1232 (calcd for $C_{25}H_{20}O_6$, 416.1260).

(7″*R*)-8-[1-(4′-Hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]galangin (3): yellow, amorphous solid; $[\alpha]^{22}_D$ −14.3 (*c* 0.3, CH_3OH); IR (KBr) ν_{max} 3315 (br), 1648, 1604, 1561, 1517, 1448, 1424 cm^{-1}; CD (*c* 2.3 × 10^{-4} M, EtOH) $[\theta]_{231}$ +2193; ^1H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 432.1211 (calcd for $C_{25}H_{20}O_7$, 432.1209).

Preferential Cytotoxicity in Nutrient-Deprived Medium (NDM). Preferential cytotoxicity of the propolis extract and of the isolated compounds was determined by a procedure described previously.[63] Briefly, PANC-1 human pancreatic cancer cells were seeded in 96-well plates (2 × 10^4 per well) and incubated in fresh Dulbecco's modified Eagle's medium (DMEM; Nissui Pharmaceuticals, Tokyo, Japan) at 37 °C under 5% CO_2 and 95% air for 24 h. After the cells were washed with PBS (Nissui Pharmaceuticals, Tokyo, Japan), the medium was changed to either DMEM or NDM,[64] and serial dilutions of the test samples were added. Cell viability in each medium was then measured after 24 h of incubation. For time-dependent preferential cytotoxicity, cells were incubated with the test compounds for 0, 6, 12, and 24 h. At the end of incubation, the morphological changes were recorded by photomicrograph using a phase-contrast microscope under 200× magnification (Olympus D-340 L/C-840 L Digital Camera, Tokyo, Japan). Then, the cells were washed with PBS, and 100 μL of DMEM containing 10% WST-8 (Dojindo; Kumamoto, Japan) was added to the wells. After 3 h incubation, the absorbance at 450 nm was measured. Cell viability was calculated from the mean values of data from three wells using the following equation:

(%) Cell viability = $[(Abs_{(test\ sample)} - Abs_{(blank)})/(Abs_{(control)} - Abs_{(blank)})] \times 100$

The preferential cytotoxicities were expressed as PC_{50} (the concentration at which 50% cells died preferentially in NDM) values.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**3**, CD data and $[\alpha]_D$ values of **2**, **3**, and related compounds, and structures of the known compounds (**4**−**44**) isolated from Mexican propolis. This information is available free of charge via the Internet at http://pubs.acs.org.

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