Bioactive Secondary Metabolites from *Boesenbergia pandurata* of Myanmar and Their Preferential Cytotoxicity against Human Pancreatic Cancer PANC-1 Cell Line in Nutrient-Deprived Medium

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The chloroform extract of rhizomes of *Boesenbergia pandurata* demonstrated marked preferential cytotoxicity against human pancreatic PANC-1 cancer cells in nutrient-deprived medium. Bioactivity-directed investigation of this extract yielded four new secondary metabolites, geranyl-2,4-dihydroxy-6-phenethylbenzoate (**1**), 2′,4′-dihydroxy-3′-(1″-geranyl)-6′-methoxychalcone (**2**), (1′*R*,2′*S*,6′*R*)-2-hydroxyisopanduratin A (**3**), and (2*R*)-8-geranylpinostrobin (**4**), and twenty known compounds (**5**–**24**). Among the known compounds, (2*S*)-6-geranylpinostrobin (**5**), (±)-6-methoxypanduratin A (**6**), and (2*S*)-7,8-dihydro-5-hydroxy-2-methyl-2-(4″-methyl-3″-pentenyl)-8-phenyl-2*H*,6*H*-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one (**7**) were isolated for the first time from a natural source. The structures of these compounds were elucidated using extensive spectroscopic techniques including CD measurements. All the isolated compounds showed varying degrees of *in vitro* preferential cytotoxicity against PANC-1 cells. Nicolaioidesin B (**11**) and panduratin A (**17**) were most potent, each showing a PC_{100} at 2.5 μM.

Pancreatic cancer is an aggressive disease with the lowest 5-year survival rate of all cancers. It is largely resistant to conventional forms of treatment and the development of more effective treatment is urgently needed. Esumi et al. reported that certain pancreatic cancer cell lines such as PANC-1, AsPC-1, BxPC-1, and KP-3 have remarkable tolerance against extreme nutrient starvation enabling them to survive for prolonged periods of time even in critically low nutrient conditions.[1] Nutrition deprivation is a common consequence in tumors due to depletion of nutrients by the rapidly proliferating tumor cells and low blood supply. Thus, elimination of this tolerance of cancer cells to nutrient starvation might serve as a new biochemical approach in cancer therapy.[2] Under this hypothesis, we have screened medicinal plants from various origins for the discovery of anticancer agents that preferentially eliminate tumor cell capability to survive under low nutrition conditions using the human pancreatic PANC-1 cancer cell line, a new approach termed antiausterity strategy. Under this strategy, we have screened 500 medicinal plants used in Japanese Kampo medicine, and identified arctigenin[3] and angelmarin[4] as compounds having the ability to eliminate tolerance of cancer cells to nutrient starvation.

Boesenbergia pandurata (Robx.) Schltr. (*Syn. Boesenbergia rotunda*, *Kaempferia pandurata*), a perennial herb of the Zingiberaceae family is cultivated in some tropical countries including Myanmar, Indonesia, Malaysia, and Thailand.[5] It is commonly known as *Seik-phoo* in Myanmar, and has been extensively used in the traditional medicinal formulation *Khu-na-pah-hsay-wa-ga-lay* (commonly known as TMF-47) for the treatment of asthma, diarrhea, indigestion, itching, and fever.[6] It is also a popular folk medicine for the treatment of diseases such as ulcer, dry mouth, stomach discomfort, leucorrhea, and dysentery in Indonesia, Malaysia, and Thailand.[7] The fresh rhizomes have a characteristic aroma and are used as a flavoring agent in Thai cuisine.[8] It has also been used as self-medication by AIDS patients in Thailand.[9] Previous investigations on *B. pandurata* reported anti-HIV,[10] antibacterial,[11] anti-inflammatory, analgesic, antipyretic,[7,12,13] antitumor,[14] antioxidant,[15] and insecticidal activities.[16] In our present investigation, we isolated twenty-four compounds including four new compounds (**1**–**4**) and three known ones (**5**–**7**) from a natural source for the first time. In this paper, we also report *in vitro* preferential cytotoxicity of the isolates against the PANC-1 cancer cell line in nutrient-deprived medium (NDM).

Results and Discussion

The 70% EtOH extract of rhizomes of *B. pandurata* showed 100% preferential cytotoxicity (PC_{100}) against PANC-1 cells in NDM at 10 μg/mL. Thus, it was separated into $CHCl_3$ soluble and insoluble fractions by dissolution in $CHCl_3$. Only the $CHCl_3$-soluble fraction exhibited activity (PC_{100} at 10 μg/mL). Thus, it was subjected to series of chromatographic separations which furnished four new secondary metabolites [geranyl-2,4-dihydroxy-6-phenethylbenzoate (**1**), 2′,4′-dihydroxy-3′-(1″-geranyl)-6′-methoxychalcone (**2**), (1′*R*,2′*S*,6′*R*)-2-hydroxyisopanduratin A (**3**), and (2*R*)-8-geranylpinostrobin (**4**)], together with twenty known compounds [(2*S*)-6- geranylpinostrobin (**5**),[17] (±)-6-methoxypanduratin A (**6**),[18] (2*S*)-7,8-dihydro-5-hydroxy-2-methyl-2-(4″-methyl-3″-pentenyl)-8-phenyl-2*H*,6*H*-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one (**7**),[19] flavokawain C (**8**),[20] cardamonin (**9**),[21] (±)-isopanduratin A1 (**10**),[16] (−)-nicolaioidesin B (**11**),[22] (−)-6-geranylpinocembrin (**12**),[17,23] (−)-pinostrobin (**13**),[24] (−)-pinocembrin (**14**),[24] (−)-alpinetin (**15**),[21] (−)-7,4′-dihydroxy-5-methoxyflavanone (**16**),[25] (−)-panduratin A (**17**),[18] (−)-isopanduratin A2 (**18**),[16] (−)-hydroxypanduratin A (**19**),[8] (±)-panduratin C (**20**),[10]boesenbergin A (**21**),[24] boesenbergin B (**22**),[26] tectochrysin (**23**),[27] and 5,6-dehydrokawain (**24**)[20]].

Compound **1** was obtained as a pale yellow amorphous solid, and its molecular formula was determined as $C_{25}H_{30}O_4$ by HRFABMS. The IR spectrum of **1** showed absorption bands of hydroxyl (3500 cm^{-1}), carbonyl (1640 cm^{-1}), and phenyl (1600, 1420 cm^{-1}) groups. The ^1H NMR spectrum showed signals due to phenyl ring (δ_H 7.28−7.17), a pair of *meta*-coupled aromatic protons (δ_H 6.30, 6.18, $J = 2.4$ Hz), an oxymethylene (δ_H 4.89), two methylenes (δ_H 3.17, 2.84), and a hydrogen-bonded hydroxyl proton (δ_H 11.80), together with signals ascribable to a geranyl moiety [three vinyl methyls (δ_H 1.72, 1.65, 1.57); two olefinic protons (δ_H 5.45, 5.05); two methylenes (δ_H 2.08−2.00)].[28] The ^{13}C NMR spectrum revealed 25 signals including those for an ester carbonyl carbon (δ_C 171.3), two oxygenated aromatic carbons (δ_C 165.7, 160.2), two quaternary aromatic carbons (δ_C 147.6, 141.8), two aliphatic methylenes (δ_C 38.5, 38.0), and those of the geranyl

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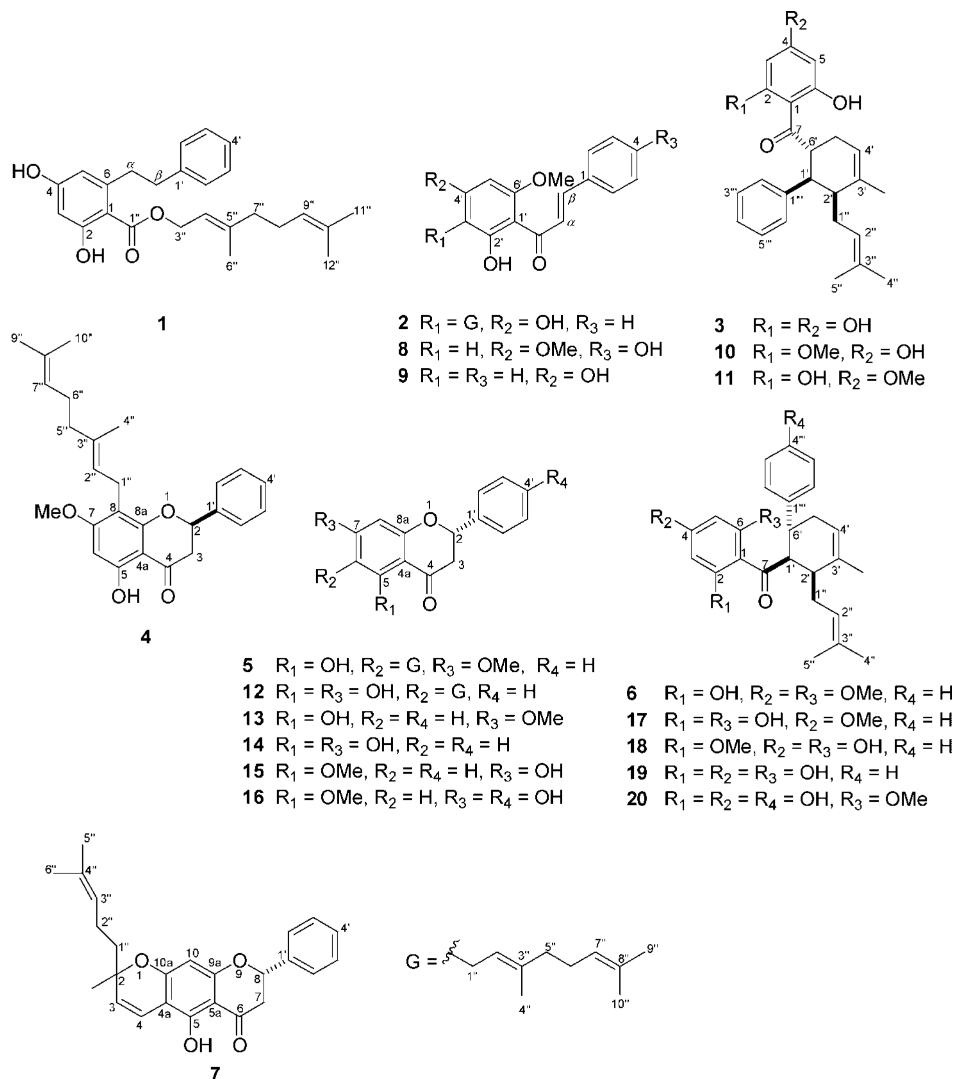

moiety.[28] Analyses of the COSY and HMQC spectra revealed the partial structure (bold lines) as shown in Figure 1a, which were connected based on the long-range correlations observed in the HMBC spectrum. The HMBC correlations between H-α and C-1′ (δ_C 141.8), and H-β and C-2′,6′ (δ_C 128.4) indicated the presence of a phenethyl group. Furthermore, HMBC of H-α with C-1 (δ_C 105.5), C-5 (δ_C 110.9), and C-6 (δ_C 147.6) suggested the connectivity between them at C-6. The hydrogen-bonded OH proton signal at δ_H 11.80 (2-OH) indicated that the ester carbonyl carbon was at C-1. Further, HMBC correlations between 4-OH and C-3, C-4, and C-5, between 2-OH and C-1, C-2, and C-3, between H-3 and C-2 and C-4, and between H-5 and C-1, C-3, and C-4 placed the hydroxyl groups at C-2 and C-4, respectively. Finally, the HMBC correlation between H-3″ (δ_H 4.89) of the geranyl moiety and ester carbonyl carbon (C-1″) indicated that **1** was geranyl-2,4-dihydroxy-6-phenethylbenzoate.

Compound **2** was obtained as yellow oil and its molecular formula was found to be $C_{26}H_{30}O_4$ by HRFABMS. The IR spectrum of **2** showed absorptions due to hydroxyl and carbonyl groups. The ^{1}H NMR spectrum of **2** showed signals due to a phenyl group (δ_H 7.61 and 7.39), a pair of *trans* olefinic protons (δ_H 7.91, 7.78, J = 15.6 Hz), an aromatic proton (δ_H 5.95), a methoxyl group (δ_H 3.91), a hydrogen-bonded hydroxyl proton (δ_H 14.59), and signals due to a geranyl moiety. The ^{13}C NMR spectrum showed 26 signals including those for a ketone carbonyl carbon (δ_C 192.9) and three oxygenated aromatic carbons (δ_C 165.1, 162.4, 161.3). These data closely resembled those of 2′,4′,6′-trihydroxy-3′-(1″-geranyl)-chalcone[23,29] except for the appearance of a methoxyl group (δ_H 3.91) instead of a hydroxyl group (δ_H 9.46).[23,29] Finally, the methoxyl group was determined to be at C-6′ based on the HMBC correlation between the methoxyl protons and C-6′ (Figure 1b). Thus, **2** was concluded to be 2′,4′-dihydroxy-3′-(1″-geranyl)-6′-methoxychalcone.

Compound **3** was isolated as a pale yellow amorphous solid with $[\alpha]^{25}_D$ +7.6. Its molecular formula ($C_{25}H_{28}O_4$) was deduced from HRFABMS. The IR spectrum of **3** indicated the presence of hydroxyl and carbonyl groups. The ^{1}H NMR spectrum displayed signals corresponding to a phenyl group (δ_H 7.14, 7.06), two magnetically equivalent aromatic protons (δ_H 5.80, br s), two olefinic methine protons (δ_H 5.47, 4.80), three aliphatic methines (δ_H 4.77, 3.49, 2.23), two allylic methylenes (δ_H 2.61, 2.04, 2.02–1.85), and three vinyl methyls (δ_H 1.74, 1.57, 1.42). Its ^{13}C NMR spectrum indicated the presence of a ketone carbonyl carbon (δ_H 210.7), 12 aromatic carbons, four olefinic carbons (δ_H 138.5, 131.3, 124.9, 121.4), three methine carbons (δ_H 47.5, 47.0, 44.4), two methylenes (δ_H 31.9, 29.1), and three vinyl methyls (δ_H 26.1, 23.3, 17.9). These data were similar to those of isopanduratin A1 (**10**)[16] except for the absence of signals due to a methoxyl group at C-2 in **10** (δ_H 3.95; δ_C 56.5). Thus, compound **3** was assumed to be 2-hydroxyisopanduratin A, which was confirmed by the HMBC spectrum (Figure 1c). The configuration of **3** was determined from the coupling constant data and the ROESY analysis. The large coupling constant between H-1′ and H-6′ (J = 11.9 Hz) indicated that they should be in *trans*-diaxial orientation and the small coupling constant between H-1′ and H-2′ (J = 5.1 Hz) indicated their *cis* relationship. This was further supported by the

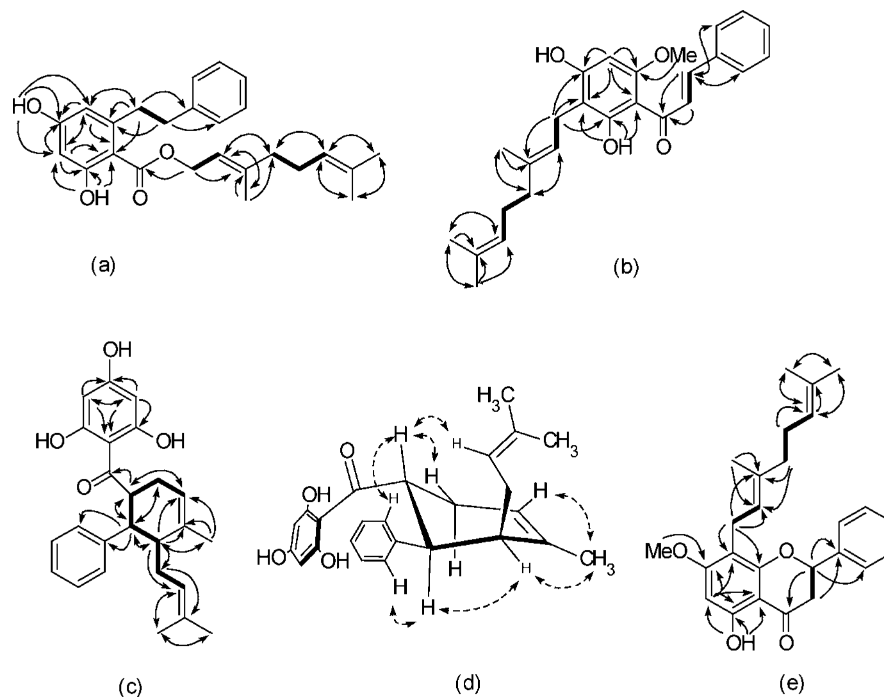

Figure 1. COSY (bold lines) and HMBC ($^1H \rightarrow {}^{13}C$) (arrows) correlations in **1** (a), **2** (b), **3** (c), and **4** (e), and ROESY (dashed arrows) correlations in **3** (d).

ROESY correlations H-1′/H-2′, H-1′/H-2‴, H-6‴, H-2′/H$_3$-3′, H-6′/H-5′b, H-6′/H-2″, and H-6′/H-2‴, H-6‴ (Figure 1d). The absolute configuration of **3** was elucidated by applying the exciton chirality rule. Compound **3** showed negative Cotton effects ($[\theta]_{285}$ −3052, $[\theta]_{235}$ +2442) suggesting the absolute chirality between the phenyl chromophore at C-1′ and the 2,4,6-trihydroxy-benzoyl chromophore at C-6′ was in *M* helical twist (Supporting Information, Figure S3).[30,31] Therefore, the structure of compound **3** was assigned as (1′*R*,2′*S*,6′*R*)-2-hydroxyisopanduratin A.

Compounds **4** and **5** were obtained as yellow oils with $[\alpha]^{25}_D$ −35.3 and −5.3, respectively. The HRFABMS indicated that they had the same molecular formula ($C_{26}H_{30}O_4$). Their ^1H and ^{13}C NMR data were also similar, and showed signals for a methylene and an oxymethine characteristic of flavanones, a hydrogen-bonded hydroxyl group, a geranyl group, a methoxyl group, an aromatic singlet, and a phenyl group. These data closely resembled those of 6-geranylpinocembrin (**12**),[17,23] except for the presence of one methoxyl group in both **4** and **5** (δ_H 3.85, s; δ_C 55.9), suggesting that these are flavanone derivatives having geranyl side chains. Analysis of the HMBC spectra indicated the methoxyl group to be at C-7 in **4** and **5**, and the geranyl unit to be at C-8 in **4** and at C-6 in **5**, respectively (Figures 1e and S2a). The literature survey indicated **4** to be a new compound, while **5** was reported as a semisynthetic product by Johannes et al.[17] However, the reported ^1H NMR data ($\delta_{H\text{-}4''}$ 1.57, $\delta_{H\text{-}9''}$ 1.80, and $\delta_{H\text{-}10''}$ 1.65) should be revised as $\delta_{H\text{-}4''}$ 1.76, $\delta_{H\text{-}9''}$ 1.64, and $\delta_{H\text{-}10''}$ 1.54. Finally, the absolute configuration of both **4** and **5** was determined by analyses of the CD spectra. Compounds **4** and **5** displayed opposite Cotton effects (**4**: $[\theta]_{288}$ +1740, $[\theta]_{276}$ −269; **5**: $[\theta]_{288}$ −580, $[\theta]_{276}$ +510), indicating the absolute configuration at C-2 to be *R* in **4** and *S* in **5**.[32] Thus, the structures of **4** and **5** were concluded to be (2*R*)-8-geranylpinostrobin and (2*S*)-6-geranylpinostrobin, respectively.

Compound **7** was isolated as a pale yellow amorphous solid with molecular formula $C_{25}H_{26}O_4$. Analysis of NMR data indicated **7** to be 7,8-dihydro-5-hydroxy-2-methyl-2-(4″-methyl-3″-pentenyl)-8-phenyl-2*H*,6*H*-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one, previously synthesized by Bandaranayake et al.[19] However, we isolated **7** as a natural product for the first time and assigned its ^1H and ^{13}C NMR data through 2D NMR analysis (Figure S2c). Compound **7** displayed negative Cotton effect at 290 nm, indicating the configuration at C-2 to be *S*.[32]

Table 1. Preferential Cytotoxicity of Compounds **1**–**24** on Human Pancreatic PANC-1 Cancer Cells in Nutrient-Deprived Medium

compound	PC_{100} [μM][a]
1, **2**, **3**, **18**, **19**	16
4, **5**	128
6, **7**, **14**, **21**, **22**	64
10, **12**	8
8, **9**, **13**, **15**, **16**, **20**, **23**, **24**	>256
11, **17**	2.5
Taxol	>256
Arctigenin[b]	1

[a] The concentration at which 100% cancer cell death occurred preferentially in nutrient-deprived medium (NDM). [b] Positive control.

Among the compounds isolated from the rhizomes of *B. pandurata* of Myanmar, **1**–**4** were new and **5**–**7** were isolated for the first time from a natural source. Preferential cytotoxicity of all isolated compounds was evaluated against the human pancreatic cancer (PANC-1) cell line and they showed activity in a concentration-dependent manner (Table 1). Compounds **11** and **17** were the most potent, each showing a PC_{100} value at 2.5 μM. Activities of the other compounds were observed in the following order: **10**, **12** > **1**, **2**, **3**, **18**, **19** > **6**, **7**, **14**, **21**, **22** > **4**, **5** > **8**, **9**, **13**, **15**, **16**, **20**, **23**, **24**. In general, the cyclohexenyl chalcone derivatives were more active than the other compounds, with the exception of **20**. The presence of a methoxyl group at C-4 and hydroxyls at C-2 and C-6 in cyclohexenyl chalcones seem to be important for activity (e.g., **11**, **17** >> **3**, **18**, **19**). Among the flavonoids, the compounds possessing a geranyl moiety showed stronger activity (e.g., in the chalcone, **2** >> **8**, **9**; in the flavanone **4**, **5** >> **13**, **15**, **16**). Arctigenin, an antiausterity-based anticancer agent[3] was used as a positive control in this study (PC_{100} at 1 μM). Paclitaxel (Taxol), a well known anticancer agent, was virtually inactive against PANC-1 cells (PC_{100} > 256 μM), consistent with our earlier observations (PC_{100} > 3000 μg/L).[2]

Nicolaioidesin B (**11**) had been reported for quinone reductase activity with cultured Hepa lacla7 mouse hepatoma cells.[22] On the other hand, panduratin A (**17**) had been reported for its anti-inflammatory activity in RAW 264.7 cells,[12] cytotoxicity against androgen-independent human prostate cancer cells PC3 and DU 145,[14] cytotoxicity induced by *t*-BHP in human hepatoma cells,[33] inhibitory activity against dengue-2 Virus N93 protease,[34] and anti-HIV-1-protease activity.[10] In this study we found cytotoxic activity against human PANC-1 cancer cells. These results suggest that cyclohexenyl chalcone derivatives warrant further study against pancreatic cancer in animal models.

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 in $CHCl_3$. 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. FABMS and HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer and glycerol was used as matrix. Column chromatography was performed with normal-phase silica gel (Silica Gel 60N, Spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc). Analytical and preparative TLC was carried out on precoated silica gel $60F_{254}$ and RP-$18F_{254}$ plates (Merck, 0.25 or 0.50 mm thickness). HPLC was performed with a SUPELCO Discovery C-18 column (25 × 10 mm, 5 μm) using Shimadzu LC-6AD, Jasco-OR-2090-Plus Chiral detector.

Plant Material. Rhizomes of *Boesenbergia pandurata* (Roxb.) Schult. were collected from Pindaya Township, Shan State, Myanmar in November 2004. A voucher specimen (TMPW 251155) was deposited at the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Japan.

Extraction and Isolation. The rhizomes of *B. pandurata* (150 g) were extracted with 70% EtOH under sonication (800 mL, 90 min, 3×) at room temperature and the solvent was evaporated under reduced pressure to give 18 g of extract, which was again sonicated with $CHCl_3$ (25 mL, 90 min, 3×) to give $CHCl_3$ soluble (10 g) and insoluble (8 g) fractions.

The $CHCl_3$ soluble fraction was chromatographed on silica gel with a EtOAc–hexane solvent system to give seven fractions [1: EtOAc–hexane (15:85) eluate, 3.88 g; 2: EtOAc–hexane (30:70) eluate, 2.16 g; 3: EtOAc–hexane (40:60) eluate, 0.35 g; 4: EtOAc–hexane (50:50) eluate, 0.38 g; 5: EtOAc–hexane (75:25) eluate, 0.32 g; 6: EtOAc–hexane (100:0) eluate, 1.24 g; 7: MeOH eluate, 1.11 g].

Fraction 1 (3.88 g) was rechromatographed on silica gel with EtOAc–hexane to give three subfractions [1–1: EtOAc–hexane (5:95) eluate, 2.23 g; 1–2: EtOAc–hexane (10:90) eluate, 302 mg; 1–3: EtOAc–hexane (15:85) eluate, 1.35 g]. Subfraction 1–2 (302 mg) was subjected to normal-phase preparative TLC with hexane–Me_2CO (3:1), followed by reverse-phase preparative TLC with Me_2CO–H_2O (7:1), to give geranyl–2,4-dihydroxy-6-phenethylbenzoate (**1**, 4.8 mg), (2*R*)-8-geranylpinostrobin (**4**, 30 mg), (2*S*)-6-geranylpinostrobin (**5**, 3.9 mg), (±)-6-methoxypanduratin A (**6**, 3.3 mg), (2*S*)-7,8-dihydro-5-hydroxy-2-methyl-2-(4′′-methyl-3′′-pentenyl)-8-phenyl-2*H*,6*H*-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one (**7**, 6.2 mg), flavokawain C (**8**, 1.3 mg), (−)-6-geranylpinocembrin (**12**, 4.9 mg), boesenbergin A (**21**, 3.2 mg), and boesenbergin B (**22**, 2.5 mg). Subfraction 1–3 (1.35 g) [EtOAc–hexane (15:85) eluate] was left overnight, which gave the crystals of (−)-pinostrobin (**13**, 1 g). The mother liquor (100 mg) was subjected to normal-phase preparative TLC with C_6H_6–Me_2CO (9:1), followed by reverse-phase preparative TLC with Me_2CO–H_2O (3:1), to give 2′,4′-dihydroxy-3′-(1′′-geranyl)-6′-methoxychalcone (**2**, 1.9 mg) and (−)-pinocembrin (**14**, 20 mg).

Fraction 2 (2.16 g) was rechromatographed on silica gel with EtOAc–hexane (1:4) to afford four subfractions [2–1, 129 mg; 2–2, 134 mg; 2–3, 587 mg; 2–4, 1.31 g]. Subfraction 2–3 was further purified by repeated preparative HPLC with CH_3CN–H_2O–trifluoroacetic acid (TFA) (80:20:0.2) [column: Discovery C-18, Supelco; flow rate: 5 mL/min] to give tectochrysin (**23**, 30 mg; t_R 2.4 min), (−)-panduratin A (**17**, 95 mg; t_R 8 min), (±)-isopanduratin A1 (**10**, 20 mg; t_R 8.5 min), and (−)-nicolaioidesin B (**11**, 19 mg; t_R 8.8 min).

Fraction 3 (0.35 g) was rechromatographed on silica gel with EtOAc–hexane (1:2) to afford four subfractions [3–1, 56 mg; 3–2, 150 mg; 3–3, 35 mg; 3–4, 20 mg]. Subfraction 3–2 on recrystallization in hexane–CH_2Cl_2 (7:3), gave (−)-isopanduratin A2 (**18**, 85 mg).

Fraction 4 (0.38 g) was dissolved in $CHCl_3$–hexane (3:7) and left overnight to give 5,6-dehydrokawain (**24**, 180 mg). After separation of the crystals, the mother liquor (200 mg) was purified by preparative HPLC with CH_3CN–H_2O–TFA (80:20:0.2) solvent system [column: Discovery C-18, Supelco; flow rate: 5 mL/min] to give (−)-hydroxylpanduratin A (**19**, 29.8 mg; t_R 58 min) and (1′*R*,2′*S*,6′*R*)-2-hydroxyisopanduratin A (**3**, 18 mg; t_R 60.8 min).

Fraction 5 (0.32 g) was dissolved in $CHCl_3$–MeOH (99:1) and left overnight to give crystals of cardamonin (**9**, 150 mg). After separation of the cardamonin, the mother liquor (150 mg) was subjected to preparative HPLC with CH_3CN–H_2O–TFA (80:20:0.2) [column: Discovery C-18, Supelco; flow rate: 2 mL/min] to give (±)-panduratin C (**20**, 10 mg; t_R 15.58 min).

(−)-Alpinetin (**15**, 80 mg) and (−)-7,4′-dihydroxy-5-methoxyflavanone (**16**, 30 mg) were obtained directly from fraction 6 (EtOAc eluent).

Geranyl-2,4-dihydroxy-6-phenethylbenzoate (1): pale yellow amorphous solid; IR ν_{max} 3500, 1640, 1600, 1500, 1420, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.80 (1H, s, 2-OH), 7.28 (2H, m, H-3′,5′), 7.17 (3H, m, H-2′,6′; H-4′), 6.30 (1H, d, J = 2.4 Hz, H-3), 6.18 (1H, d, J = 2.4 Hz, H-5), 5.45 (1H, t, J = 7.3 Hz, H-4′′), 5.21 (1H, s, 4-OH), 5.05 (1H, t, J = 7.3 Hz, H-9′′), 4.89 (2H, d, J = 7.3 Hz, H-3′′), 3.17 (2H, m, H-α), 2.84 (2H, m, H-β), 2.08–2.00 (4H, m, H-7′′, H-8′′), 1.72 (3H, s, CH_3-6′′), 1.65 (3H, s, CH_3-11′′), 1.57 (3H, s, CH_3-12′′); ^{13}C NMR ($CDCl_3$) δ171.3 (C-1′′), 165.7 (C-2), 160.2 (C-4), 147.6 (C-6), 143.5 (C-5′′), 141.8 (C-1′), 131.9 (C-10′′), 128.4 (C-2′,6′; C-4′), 125.9 (C-3′,5′), 123.6 (C-9′′), 117.7 (C-4′′), 110.9 (C-5), 105.5 (C-1), 101.7 (C-3), 62.2 (C-3′′), 39.5 (C-7′′), 38.5 (C-α), 38.0 (C-β), 26.2 (C-8′′), 25.6 (C-11′′(C-12′′ (C-6′′); HRFABMS *m/z* 395.2220 (calcd for $C_{25}H_{31}O_4$, 395.2222).

2′,4′-Dihydroxy-3′-(1′′-geranyl)-6′-methoxychalcone (2): yellow oil; IR ν_{max} 3500, 1620, 1500, 1420, 1260, 1200, 1050, 920 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 14.59 (1H, s, 2′-OH), 7.91 (1H, d, J = 15.6 Hz, H-α), 7.78 (1H, d, J = 15.6 Hz, H-β), 7.61 (2H, m, H-3, 5), 7.39 (3H, m, H-2, 6; H-4), 6.12 (1H, br s, 4′-OH), 5.95 (1H, s, H-5′), 5.21 (1H, t, J = 6.6 Hz, H-2′′), 5.06 (1H, t, J = 6.6 Hz, H-7′′), 3.91 (3H, s, 6-′OCH_3), 3.43 (2H, d, J = 7.3 Hz, H-1′′), 2.17−2.10 (4H, m, H_2-5′′, H_2-6′′), 1.82 (3H, s, CH_3-4′′), 1.69 (3H, s, CH_3-9′′), 1.60 (3H, s, CH_3-10′′); ^{13}C NMR ($CDCl_3$) δ 192.9 (C=O), 165.1 (C-2′), 162.4 (C-4′), 161.3 (C-6′), 142.0 (C-β), 139.9 (C-3′′), 135.7 (C-1), 132.2 (C-8′′), 129.9 (C-4), 128.9 (C-2, 6), 128.3 (C-3, 5), 127.9 (C-α), 123.7 (C-7′′), 121.6 (C-2′′), 106.2 (C-3′′), 101.9 (C-1′), 91.3 (C-5′), 55.8 (6′-OMe), 39.7 (C-5′′), 26.3 (C-6′′), 25.6 (C-9′′), 21.6 (C-1′′), 17.7 (C-10′′), 16.3 (C-4′′); HRFABMS *m/z* 407.2223 (calcd for $C_{26}H_{31}O_4$, 407.2222).

(1′*R*,2′*S*,6′*R*)-2-Hydroxyisopanduratin A (3): pale yellow amorphous solid; $[\alpha]^{25}_D$ +7.6 (*c* 4.6, MeOH); IR ($CHCl_3$) ν_{max} 3500, 3400–3100, 1630, 1160 cm^{-1}; CD (*c* 2.549 × 10^{-4} M, EtOH) $[\theta]_{285}$ −3052, $[\theta]_{235}$ +2442; ^{1}H NMR (CD_3OD) δ 7.14 (2H, m, H-2′′′,6′′′), 7.06 (3H, m, H-3′′′,5′′′; H-4′′′), 5.80 (2H, br s, H-3,5), 5.47 (1H, br s, H-4′), 4.80 (1H, t, J = 7.0 Hz), 4.77 (1H, ddd, J = 6.0, 11.9, 11.9 Hz, H-6′), 3.49 (1H, dd, J = 5.1, 11.9 Hz, H-1′), 2.61 (1H, dt, J = 1.9, 6.0, 17.3 Hz, H-5′b), 2.23 (1H, dd, J = 5.1, 9.8 Hz, H-2′), 2.04 (1H, ddd, J = 1.9, 11.9, 17.3 Hz, H-5′a), 2.02–1.85 (2H, m, H-1′′), 1.74 (3H, s, 3′-CH_3), 1.57 (3H, s, CH_3-4′′), 1.42 (3H, s, CH_3-5′′); ^{13}C NMR (CD_3OD) δ 210.7 (C-7), 165.9 (C-2,6; C-4), 144.9 (C-1′′′), 138.5 (C-3′), 131.3 (C-3′′), 129.4 (C-3′′′,5′′′), 128.9 (C-2′′′,6′′′), 126.6 (C-4′′′), 124.9 (C-2′′), 121.4 (C-4′), 106.1 (C-1), 95.9 (C-3), 95.8 (C-5), 47.5 (C-1′), 47.0 (C-2′), 44.4 (C-6′), 31.9 (C-5′), 29.1 (C-1′′), 26.1 (C-4′′), 23.3 (3′-CH_3), 17.9 (C-5′′); HRFABMS *m/z* 393.2056 (calcd for $C_{25}H_{29}O_4$, 393.2066).

(2*R*)-8-Geranylpinostrobin (4): pale yellow oil; $[\alpha]^{25}_D$ −35.3 (*c* 0.626, $CHCl_3$); IR ν_{max} 3500, 1630, 1510, 1430, 1200, 1050, 930 cm^{-1}; CD (*c* 2.461 × 10^{-4} M, EtOH) $[\theta]_{288}$ +1740, $[\theta]_{276}$ −269; ^{1}H NMR ($CDCl_3$) δ 12.12 (1H, s, 5-OH), 7.42 (5H, m, Ph-H), 6.09 (1H, s, H-6), 5.41 (1H, dd, J = 3.0, 12.7 Hz, H-2), 5.14 (1H, t, J = 7.1 Hz, H-2′′), 5.06 (1H, t, J = 6.8 Hz, H-7′′), 3.85 (3H, s, 7-OCH_3), 3.25 (2H, d, J = 7.1 Hz, H-1′′), 3.04 (1H, dd, J = 12.7, 17.1 Hz, H-3_{ax}), 2.81 (1H, dd, J = 3.0, 17.1 Hz, H-3_{eq}), 2.02 (2H, m, H-6′′), 1.94 (2H, m, H-5′′), 1.64 (3H, s, CH_3-9′′), 1.61 (3H, s, CH_3-4′′), 1.56 (3H, s, CH_3-10′′); ^{13}C NMR ($CDCl_3$) δ 196.3 (C-4), 165.8 (C-7), 162.7 (C-5), 158.8 (C-8a), 138.9 (C-1′), 134.9 (C-3′′), 131.2 (C-8′′), 128.8 (C-3′, 5′), 128.5

(C-4′), 126.2 (C-2′, 6′), 124.4 (C-7″), 122.4 (C-2″), 109.2 (C-8), 103.0 (C-4a), 92.5 (C-6), 78.7 (C-2), 55.9 (7-OCH_3), 43.5 (C-3), 39.8 (C-5″), 26.7 (C-6″), 25.7 (C-9″), 21.7 (C-1″), 17.6 (C-10″), 15.9 (C-4″); HRFABMS *m/z* 429.2069 (calcd for $C_{26}H_{30}O_4Na$, 429.2042).

(2*S*)-6-Geranylpinostrobin (5): pale yellow oil; $[\alpha]^{25}_D$ −5.3 (*c* 0.74, $CHCl_3$); IR ν_{max} 3500, 1630, 1510, 1430, 1200, 1050, 930 cm^{-1}; CD (*c* 2.461 × 10^{-4} M, EtOH) $[\theta]_{288}$ −580, $[\theta]_{276}$ +510; ^{1}H NMR ($CDCl_3$) δ 12.04 (1H, s, 5-OH), 7.42 (5H, m, Ph-H), 6.09 (1H, s, H-8), 5.41 (1H, dd, *J* = 3.0, 13.2 Hz, H-2), 5.18 (1H, t, *J* = 7.1 Hz, H-2″), 5.07 (1H, t, *J* = 7.1 Hz, H-7″), 3.85 (3H, s, 7-OCH_3), 3.27 (2H, d, *J* = 7.1 Hz, H-1″), 3.09 (1H, dd, *J* = 13.2, 17.1 Hz, H-3_{ax}), 2.81 (1H, dd, *J* = 3.0, 17.1 Hz, H-3_{eq}), 2.04 (2H, m, H-6″), 1.96 (2H, m, H-5″), 1.76 (3H, s, CH_3-4″), 1.64 (3H, s, CH_3-9″), 1.54 (3H, s, CH_3-10″); ^{13}C NMR ($CDCl_3$) δ 195.8 (C-4), 165.6 (C-7), 161.4 (C-8a), 160.4 (C-5), 138.6 (C-1′), 135.1 (C-3″), 131.2 (C-8″), 128.9 (C-3′,5′), 126.2 (C-2′,6′; C-4′), 124.5 (C-7″), 122.2 (C-2″), 110.2 (C-6), 102.9 (C-4a), 91.0 (C-8), 79.4 (C-2), 55.9 (7-OCH_3) 43.5 (C-3), 39.9 (C-5″), 26.7 (C-6″), 25.7 (C-9″), 21.0 (C-1″), 17.7 (C-10″), 16.1 (C-4″); HRFABMS *m/z* 407.2209 (calcd for $C_{26}H_{31}O_4$, 407.2222).

(±)-6-Methoxypanduratin A (6): pale yellow amorphous solid; $[\alpha]^{25}_D$ 0 (*c* 11.2, $CHCl_3$); IR ν_{max} 3500, 1620, 1520, 1420, 1200, 1050, 930 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 13.94 (1H, s, 2-OH), 7.19 (3H, m, H-2‴,6‴; H-4‴), 7.09 (2H, m, H-3‴,5‴), 5.98 (1H, d, *J* = 2.4 Hz, H-3), 5.94 (1H, d, *J* = 2.4 Hz, H-5), 5.42 (1H, br s, H-4′), 4.86 (1H, t, *J* = 7.1 Hz, H-2″), 4.49 (1H, dd, *J* = 4.6, 11.2 Hz, H-1′), 3.91 (3H, s, 6-OCH_3), 3.79 (3H, s, 4-OCH_3), 3.42 (1H, ddd, *J* = 6.4, 11.2, 11.2 Hz, H-6′), 2.50 (1H, m, H-2′), 2.40 (1H, m, H-5′b), 2.24 (1H, m, H-1″b), 2.10 (1H, m, H-5′a), 2.06 (1H, m, H-1″a), 1.77 (3H, s, 3′-CH_3), 1.55 (3H, s, CH_3-4″), 1.51 (3H, s, CH_3-5″); ^{13}C NMR ($CDCl_3$) δ 206.3 (C-7), 168.0 (C-2), 165.4 (C-4), 162.1 (C-6), 147.1 (C-1‴), 137.3 (C-3′), 131.8 (C-3″), 128.3 (C-3‴,5‴), 127.0 (C-2‴,6‴), 125.6 (C-4‴), 124.2 (C-2″), 120.9 (C-4′), 106.7 (C-1), 93.9 (C-3), 90.9 (C-5), 55.7 (6-OCH_3), 55.5 (4-OCH_3), 54.2 (C-1′), 42.6 (C-2′), 37.1 (C-6′), 35.8 (C-5′), 28.9 (C-1″), 25.7 (C-4″), 22.9 (3′-CH_3), 17.9 (C-5″); HRFABMS *m/z* 421.2378 (calcd for $C_{27}H_{33}O_4$, 421.2379).

(2*S*)-7,8-Dihydro-5-hydroxy-2-methyl-2-(4″-methyl-3″-pentenyl)-8-phenyl-2*H*,6*H*-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one (7): pale yellow amorphous solid; $[\alpha]^{25}_D$ −12.1 (*c* 1.10, $CHCl_3$); IR ν_{max} 3500, 1630, 1450, 1380, 1100, 900 cm^{-1}; CD (*c* 2.562 × 10^{-4} M, EtOH) $[\theta]_{320}$ +34, $[\theta]_{290}$ −2813; ^{1}H NMR ($CDCl_3$) δ 12.28 (1H, s, 5-OH), 7.40 (5H, m, Ph-H), 6.66 (1H, d, *J* = 10.2 Hz, H-4), 5.96 (1H, s, H-10), 5.44 (1H, d, *J* = 10.2 Hz, H-3), 5.41 (1H, dd, *J* = 3.2, 13.0 Hz, H-8), 5.08 (1H, t, *J* = 7.2 Hz, H-3″), 3.07 (1H, dd, *J* = 13.0, 17.1 Hz, H-7_{ax}), 2.81 (1H, dd, *J* = 3.2, 17.1 Hz, H-7_{eq}), 2.08 (2H, dd, *J* = 7.2, 15.9 Hz, H-2″), 1.75 and 1.62 (each 1H, m, H-1″), 1.66 (3H, s, CH_3-5″), 1.57 (3H, s, CH_3-6″), 1.40 (3H, d, *J* = 2.4 Hz, 2-CH_3); ^{13}C NMR ($CDCl_3$) δ 195.7 (C-6), 162.6 (C-9a), 162.4 (C-10a), 158.4 (C-5), 138.5 (C-1′), 131.9 (C-4″), 128.9 (C-3′,5′), 126.1 (C-2′,6′ and C-4′), 125.1 (C-3), 123.8 (C-3″), 115.8 (C-4), 102.9 (C-4a), 102.8 (C-5a), 96.0 (C-10), 80.9 (C-2), 79.1 (C-8), 43.4 (C-7), 41.8 (C-1″), 27.3 (2-CH_3), 25.7 (C-5″), 22.6 (C-2″), 17.6 (C-6″); HRFABMS *m/z* 391.1880 (calcd for $C_{25}H_{27}O_4$, 391.1909).

***In vitro* Preferential Cytotoxicity under Nutrient-Deprived Condition.** *In vitro* preferential cytotoxicity (PC_{100}) of crude extracts and isolated compounds was determined by the procedure previously described by Izuishi et al.[1] 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. The nutrient-deprived medium (NDM) was prepared following the procedure described by Izuishi et al.[1] After the cells were washed with PBS (Nissui Pharmaceuticals), the medium was changed to either DMEM or NDM and serial dilutions of the test samples were added. After 24 h incubation, the cells were washed with PBS, and 100 μL of DMEM containing 10% WST-8 cell counting kit (Dojindo; Kumamoto, Japan) solution was added to the wells. After 2 h incubation, the absorbance at 450 nm was measured. The crude extracts were tested at 10, 50, 100, and 200 μg/mL concentrations, while the pure isolates were tested ranging from 1 to 256 μM. Cell viability was calculated from the mean values of data from three wells by using the following equation:

$$(\%)\ \text{Cell viability} = [(\text{Abs}_{(\text{test samples})} - \text{Abs}_{(\text{blank})})/(\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})})] \times 100$$

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Supporting Information Available: Figure S1 showing structures of known compounds **21**–**24**, Figure S2 for COSY (bold lines) and HMBC (^{1}H → ^{13}C) (arrows) correlations in **5** (a), **6** (b), and **7** (c), and Figure S3 showing the CD spectra of **3**. This information is available free of charge via the Internet at http://pubs.acs.org.

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