Panduratins D—I, Novel Secondary Metabolites from Rhizomes of *Boesenbergia pandurata*

Nwet Nwet Win,[a] Suresh Awale,[a] Hiroyasu Esumi,[b] Yasuhiro Tezuka,[a] and Shigetoshi Kadota*,[a]

[a] *Institute of Natural Medicine, University of Toyama; 2630 Sugitani, Toyama 930–0194, Japan: and* [b] *National Cancer Center Research Institute East; 6–5–1 Kashiwa, Chiba 277–8577, Japan.*

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Investigation of the non-polar fraction of *Boesenbergia pandurata* of Myanmar led to the identification of six novel secondary metabolites, panduratins D—I (1—6), together with known diastereomers, panduratins B1 (7) and B2 (8). Their structures were determined based on extensive spectroscopic analysis. The *in vitro* preferential cytotoxicity of all isolates was examined against human pancreatic PANC-1 cancer cells under nutrient-deprived conditions. All exhibited a mild activity.

Key words *Boesenbergia pandurata*; secondary metabolite; preferential cytotoxicity; PANC-1

Boesenbergia pandurata (Roxb.) Schltr. [syn. *B. rotunda* (L.) Mansf. Kulturpfl., *Kaempferia pandurata* Roxb.], belonging to the family Zingiberaceae, is a perennial herb distributed in some tropical countries including Indonesia, Malaysia, Myanmar, and Thailand.[1)] In Myanmar, it is locally known as *Seik-phoo*, and its rhizome 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.[2)] In addition, the rhizome is popular as a folk remedy for the treatment of several conditions such as ulcer, dry mouth, stomach discomfort, leucorrhea, and dysentery, in Indonesia, Malaysia, and Thailand.[3)] The fresh rhizomes have a characteristic aroma and are used as a flavoring agent in Thai cuisine.[4)] It has been commonly used as self-medication by AIDS patients in southern Thailand.[5)] Previous investigations of the chemical constituents of *B. pandurata* reported the anti-HIV,[6)] antibacterial,[7)] anti-inflammatory, analgesic, antipyretic,[3,8,9)] antitumor,[10)] antioxidant,[11)] and insecticidal activities.[12)] In the course of our study to identify biologically active natural products based on the novel screening methodology of the "anti-austerity strategy,"[13—16)] we previously isolated four new secondary metabolites, geranyl-2,4-dihydroxy-6-phenethylbenzoate, 2′,4′-dihydroxy-3′-(1″-geranyl)-6′-methoxychalcone, (1′*R*,2′*S*,6′*R*)-2-hydroxyisopanduratin A, and (2*R*)-8-geranylpinostrobin, together with 20 known ones from *B. pandurata* of Myanmar, and identified panduratin A and nicolaioidesin B as the active principles.[17)] In our continuing study, we have recently isolated six novel secondary metabolites, panduratins D—I (**1**—**6**), along with known diastereomers, panduratins B1 (**7**) and B2 (**8**). In this paper, we report the identification of these new isolates using spectroscopic analysis and their *in vitro* preferential cytotoxicity against human pancreatic PANC-1 cancer cells under nutrient-deprived conditions.

Results and Discussion

Panduratin D (**1**) was isolated as a pale yellow oil with $[\alpha]_D^{25}$ −2.89° ($CHCl_3$) and was assigned the molecular formula $C_{28}H_{30}O_4$ on the basis of high-resolution EI-MS (HR-EI-MS). In the IR spectrum, absorption bands at 3600 (hydroxyl) and 1650 (carbonyl) cm^{-1} were apparent. The ^{1}H-NMR spectrum of **1** (Table 1) displayed signals due to three aliphatic methines [δ 4.62 (H-1′), 2.56 (H-2′), 3.49 (H-6′)], two allylic methylenes [δ 2.13, 2.46 (H_2-5′), 2.13, 2.31 (H_2-1″)], four olefinic methines [δ 4.81 (H-2″), 5.50 (H-4′), 6.85 (H-2), 7.59 (H-3)], three vinyl methyls [δ 1.46 (H_3-4″), 1.48 (H_3-5″), 1.79 (3′-Me)], a monosubstituted phenyl ring [δ 7.09 (H-3‴,5‴), 7.20 (H-2‴,6‴, H-4‴)], an aromatic proton [δ 6.21 (H-5)], a methoxyl group [δ 3.92 (4-OMe)], and a hydrogen-bonded hydroxyl group [δ 13.67 (6-OH)]. These data were similar to those of panduratin A (**9**),[18)] an isolate from the same extract, except for the appearance of additional signals due to a pair of *cis* olefinic protons at δ 6.85 and 7.59 (J=2.2 Hz) in **1**, and the disappearance of one of the aromatic protons of **9**. The ^{13}C-NMR data of **1** (Table 2) were also similar to those of **9**, except for the presence of additional olefinic carbons at δ 104.3 (C-2) and δ 142.2 (C-3). The ^{1}H–^{1}H shift correlation spectroscopy (COSY) correlation between H-2 and H-3 and heteronuclear multiple-bond connectivity (HMBC) correlations of both H-2 and H-3 with C-3a (δ 110.7) and C-7a (δ 154.8), of H-3 with C-4 (δ 158.9), and of H-5 with C-3a (Fig. 1a) suggested the presence of a furan ring at C-3a and C-7a, and the oxygen atom of the furan ring being positioned at C-7a. Thus, compound **1** was determined as a cyclohexenyl furanochalcone derivative. The relative stereochemistry of **1** was determined from the coupling constants and rotating-frame Overhauser enhancement spectroscopy (ROESY) analysis. The small coupling constant between H-1′ and H-2′ (J=5.4 Hz) indicated their *cis* relationship, whereas the large one between H-1′ and H-6′ (J=11.0 Hz) suggested their *trans*-diaxial orientation. In the ROESY spectrum, the correlations of H-1′/H-2′, H-1′/H-2‴, H-2′/3′-Me, H-6′/H-5′β, and H-6′/H-2″ (Fig. 2a) further supported the configuration. Hence, the structure of panduratin D (**1**) was concluded as shown in Chart 1.

Panduratin E (**2**), a pale yellow oil with $[\alpha]_D^{25}$ −3.29°, was assigned the molecular formula $C_{31}H_{36}O_4$ based on HR-EI-MS. The ^{1}H-NMR data of **2** (Table 1) were similar to those of **9**[18)] except for the appearance of a pair of *cis* olefinic protons at δ 5.44 (H-3) and 6.58 (H-4) (J=10.0 Hz) and two methyl resonances at δ 1.53 (6H, s, H_3-9, H_3-10), with the disappearance of one of the aromatic protons of **9**. The ^{13}C-NMR data of **2** (Table 2), on the other hand, revealed five additional carbon signals, which were identified as two olefinic methines [δ 124.3 (C-3), 116.8 (C-4)], two methyls [δ 27.9

* To whom correspondence should be addressed. e-mail: kadota@inm.u-toyama.ac.jp

Table 1. ^{1}H-NMR Data of **1**—**4** in $CDCl_3$

Position	**1**	Position	**2**	Position	**3**	**4**
2	6.85 (1H, d, 2.2)	3	5.44 (1H, d, 10.0)	3	5.33 (1H, d, 10.0)	5.44 (1H, d, 10.0)
3	7.59 (1H, d, 2.2)	4	6.58 (1H, d, 10.0)	4	6.61 (1H, d, 10.0)	6.66 (1H, d, 10.0)
5	6.21 (1H, s)	6	5.91 (1H, s)	6		5.95 (1H, s)
4-OMe	3.92 (3H, s)	9	1.53 (3H, s)	8	5.88 (1H, s)	
6-OH	13.67 (1H, s)	10	1.53 (3H, s)	2-Me	1.38 (3H, s)	1.57 (3H, br s)
1′	4.62 (1H, dd, 5.4, 11.0)	5-OMe	3.83 (3H, s)	5-OH	14.16 (1H, d, 1.9)	
2′	2.56 (1H, dd, 5.4, 10.6)	7-OH	13.77 (1H, br s)	7-OH		13.72, 13.69 (each 1H, s)
4′	5.50 (1H, br s)	1′	4.72 (1H, dd, 5.3, 11.7)	7-OMe	3.90 (3H, s)	
5′α	2.13 (1H, m)	2′	22.65 (1H, dd, 5.3, 10.5)	5-OMe		3.82 (3H, br s)
5′β						
6′	3.49 (1H, ddd, 6.6, 10.7, 11.0)	4′	5.43 (1H, br s)	1′	2.05[a] (2H, m)	1.94 (2H, m)
3′-Me	1.79 (3H, s)	5′α	2.09 (1H, m)	2′	2.05[a] (2H, m)	2.15 (2H, m)
		5′β	2.41 (1H, dt, 1.9, 6.1, 18.6)			
1″	2.13, 2.31 (each 1H, m)	6′	3.42 (1H, ddd, 6.1, 10.7, 11.7)	3′	5.08 (1H, t, 7.3)	5.07 (1H, m)
2″	4.81 (1H, t, 7.6)	3′-Me	1.78 (3H, s)	5′	1.66 (3H, s)	1.68 (3H, br s)
4″	1.46 (3H, s)	1″	2.01, 2.29 (each 1H, m)	6′	1.57 (3H, s)	1.60 (3H, br s)
5″	1.48 (3H, s)	2″	4.90 (1H, t, 6.8)	1″	4.49 (1H, dd, 4.6, 11.0)	3.59 (1H, dd, 4.6, 11.7)
2‴,6‴	7.20 (2H, m)	4″	1.54 (3H, s)	2″	2.48 (1H, dd, 4.6, 10.3)	2.15 (1H, m)
3‴,5‴	7.09 (2H, m)	5″	1.54 (3H, s)	4″	5.41 (1H, br s)	5.38 (1H, br s)
4‴	7.20 (1H, m)	2‴,6‴	7.18 (2H, m)	5″α	2.05[a] (1H, m)	2.12 (1H, m)
				5″β	2.41 (1H, dt, 1.9, 6.6, 18.5)	2.61 (1H, m)
		3‴,5‴	7.08 (2H, m)	6″	3.41 (1H, ddd, 6.6, 10.5, 11.0)	4.76 (1H, ddd, 6.1, 11.3, 11.7)
		4‴	7.18 (1H, m)	3″-Me	1.78 (3H, s)	1.77 (3H, br s)
				1‴	2.22—2.30 (2H, m)	1.96 (2H, m)
				2‴	4.85 (1H, t, 6.8)	4.66 (1H, m)
				4‴	1.50 (3H, s)	1.49 (3H, br s)
				5‴	1.50 (3H, s)	1.33 (3H, br s)
				2⁗,6⁗	7.18 (2H, m)	7.17 (2H, m)
				3⁗,5⁗	7.08 (2H, m)	7.08 (2H, m)
				4⁗	7.18 (1H, m)	7.17 (1H, m)

a) Overlapping resonances.

Table 2. ^{13}C-NMR Data of **1**—**4** in $CDCl_3$

Position	**1**	Position	**2**	Position	**3**	**4**
2	104.3	2	77.9	2	80.5	80.86
3	142.2	3	124.3[a]	3	123.9	122.7; 122.8
3a	110.7	4	116.8	4	116.7	117.4; 117.5
4	158.9	4a	102.6	4a	102.9	102.5; 102.6
5	94.9	5	160.5	5	162.3[a]	160.9
6	166.2	6	92.6	6	106.3	92.5; 92.6
7	103.1	7	166.9	7	162.2[a]	166.9; 167.0
7a	154.8	8	106.7	8	90.9	105.9; 106.1
4-OMe	55.6	8a	155.1	8a	159.8	155.7; 155.8
1′	53.2	9	27.9[b]	2-Me	27.2	26.9
2′	42.4	10	27.6[b]	5-OMe		55.7
3′	137.0	5-OMe	55.7	7-OMe	55.7	
4′	121.2	1′	54.2	1′	41.7	41.8; 41.9
5′	35.5	2′	42.9	2′	22.7	23.5; 23.6
6′	36.8	3′	137.1	3′	123.9	123.6; 123.7
3′-Me	22.8	4′	121.1	4′	131.8	132.0; 132.1
1″	28.9	5′	36.6	5′	25.7	25.7; 25.8
2″	123.9	6′	37.1	6′	17.6	17.6; 17.7
3″	132.0	3′-Me	22.8	1″	54.2	45.9; 46.1
4″	25.6	1″	28.8	2″	42.7	46.6; 46.7
5″	17.9	2″	124.4[a]	3″	137.3	139.2; 139.6
1‴	146.7	3″	131.7	4″	120.9	118.7; 119.0
2‴,6‴	127.1	4″	25.7	5″	35.9	31.4; 31.6
3‴,5‴	128.4	5″	17.9	6″	37.1	43.4; 43.7
4‴	125.7	1‴	147.0	3″-Me	22.9	23.3; 23.4
C=O	203.83	2‴,6‴	127.0	1‴	28.9	29.9; 30.0
		3‴,5‴	128.4	2‴	124.2	130.3; 130.5
		4‴	125.6	3‴	131.8	124.9; 125.0
		C=O	206.6	4‴	25.7	25.5; 25.6
				5‴	17.9	17.3; 17.4
				1⁗	147.2	143.4; 143.5
				2⁗,6⁗	127.0	128.0; 128.1
				3⁗,5⁗	128.3	128.0; 128.1
				4⁗	125.5	125.6; 125.7
				C=O	206.2	209.1; 209.2

a, *b*) Interchangeable within the same column.

(C-9), 27.6 (C-10)], and an oxygenated quaternary carbon [δ 77.9 (C-2)]. The COSY correlation between H-3 and H-4 and the HMBC correlations of H-3, H-4, H_3-9, and H_3-10 with C-2 suggested the presence of a 2,2-dimethylchromene ring in **2** (Fig. 1b). The key HMBC correlations of H-4 with C-5 and C-8a and of H-6 with C-4a, C-5, C-7, and C-8 indicated that the 2,2-dimethylchromene ring anellated to the aryl acyl moiety at C-4a and oxygenated quaternary carbon C-8a. This orientation of the chromene ring was further supported by the difference NOE experiments that showed enhancements of H-6 (8%) and H-4 (2%) on irradiation of 5-OCH_3 (Fig. 2b). The relative configuration of **2** was found to be the same as that of **9** based on the coupling constants ($J_{H\text{-}1',H\text{-}2'}$=5.3 Hz, $J_{H\text{-}1',H\text{-}6'}$=11.7 Hz) and the ROESY correlations (Fig. 2b). Thus, the structure of panduratin E (**2**) was concluded as shown in Chart 1.

Panduratin F (**3**) was obtained as a pale yellow oil with $[\alpha]_D^{25}$ −7.5°. HR-EI-MS of **3** gave a molecular ion peak [M^+] at *m/z* 540.3220, corresponding to the molecular formula $C_{36}H_{44}O_4$. The IR absorptions at 1630 and 3600 cm^{-1} indicated the presence of carbonyl and chelated hydroxyl groups. Its ^{1}H- and ^{13}C-NMR data (Tables 1, 2) closely resembled those of panduratins B1 and B2 (**7** and **8**), isolates from the same extract,[19,20] and displayed signals due to a substituted cyclohexene ring, a phenyl ring, an aromatic singlet, a methoxyl singlet, a hydrogen-bonded hydroxyl broad singlet, and those for a 2-methyl-2-(4-methylpent-3-enyl)chromene ring. However, they slightly differed in ^{13}C-NMR resonances [**3**: δ 162.3 (C-5), 162.2 (C-7), 159.8 (C-8a); **7**: δ 160.5 (C-5), 166.9 (C-7), 155.5 (C-8a)], suggesting the possibility of differences in the arrangement of the methoxyl, hydroxyl, and chromene ring on the aryl acyl unit. The HMBC correla-

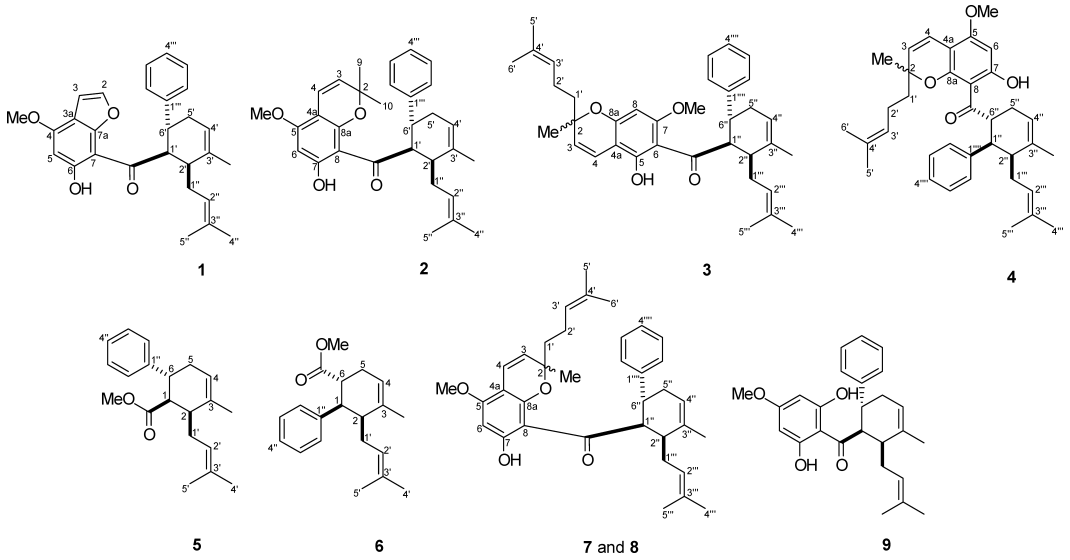

Chart 1

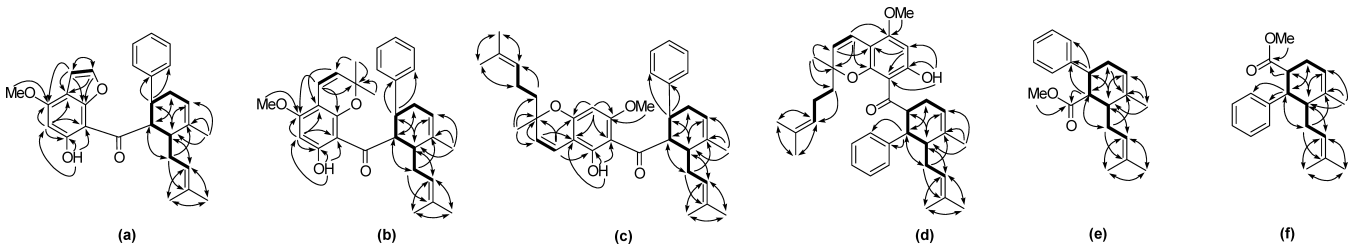

Fig. 1. COSY (Bold Lines) and HMBC ($^{1}H \rightarrow ^{13}C$) (Arrows) Correlations in **1** (a), **2** (b), **3** (c), **4** (d), **5** (e), and **6** (f)

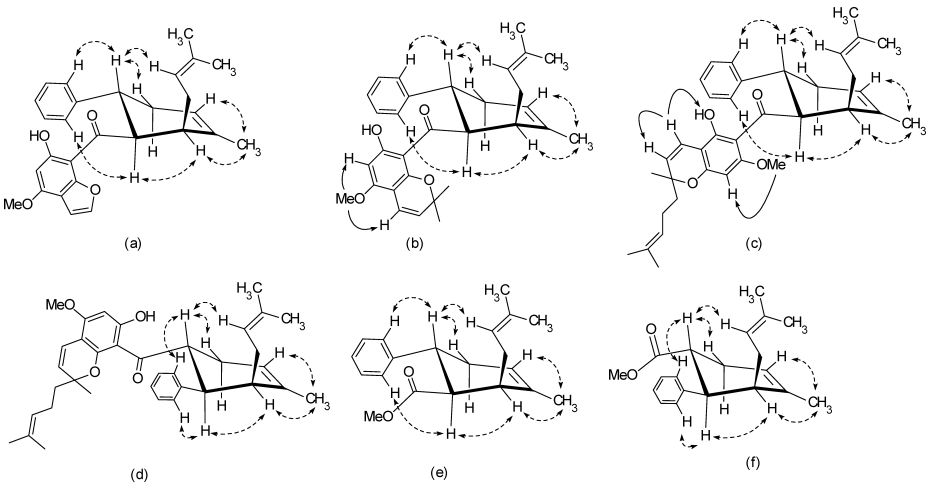

Fig. 2. ROESY Correlations (Dashed Arrows) and NOEs in Difference NOE Spectra (Solid Arrows) Correlations in **1** (a), **2** (b), **3** (c), **4** (d), **5** (e), and **6** (f)

tions of H-4 with C-5 and C-8a indicated that the chromene ring anellated at C-4a and oxygenated at C-8a (Fig. 1c). This was further supported by difference NOE experiments. Irradiation of 7-OCH_3 enhanced H-8 (6%) and irradiation of H-4 enhanced H-3 (5%) and 5-OH (1%) (Fig. 2c). The coupling constants and ROESY correlations (Fig. 2c) suggested **3** to have the same relative configuration as **9** in the cyclohexenyl chalcone unit. Accordingly, panduratin F (**3**) was elucidated as shown in Chart 1.

Panduratin G (**4**) was isolated as a pale yellow oil and showed a single spot on TLC using various solvent systems. HR-EI-MS identified the molecular formula of $C_{36}H_{44}O_4$. The ^{1}H- and ^{13}C-NMR spectra of **4** (Tables 1, 2) resembled those of known diastereomers, panduratins B1 (**7**) and B2 (**8**).[19,20] However, **4** showed a pair of signals (1 : 1) in the ^{13}C-NMR spectrum, suggesting a mixture of diastereomers. The significant differences in ^{1}H-NMR data were an upfield shift of H-1″ (δ 3.59, dd, J=4.6, 11.7 Hz) and downfield shift of H-6″ (δ 4.76, ddd, J=6.1, 11.3, 11.7 Hz), suggesting the positioning of the aryl acyl group at C-6″ and the phenyl ring at C-1″ on the cyclohexenyl ring, as in nicolaioidesin B.[21] This was further confirmed by the COSY and HMBC corre-

Table 3. ^{1}H- and ^{13}C-NMR Data of **5** and **6** in $CDCl_3$

Position	**5**		**6**	
	δ_H	δ_C	δ_H	δ_C
1	3.09 (1H, dd, 5.4, 11.2)	49.2	3.33 (1H, dd, 5.6, 11.2)	46.3
2	2.45 (1H, dd, 5.4, 10.2)	42.7	2.21 (1H, dd, 5.6, 10.2)	44.6
3		136.3		137.7
4	5.42 (1H, br s)	121.1	5.45 (1H, br s)	119.5
5α	2.04 (1H, m)	35.1	2.34 (1H, m)	29.5
5β	2.34 (1H, dt, 1.9, 6.0, 18.3)		2.43 (1H, dt, 1.9, 6.1, 18.0)	
6	3.19 (1H, ddd, 6.0, 10.0, 11.2)	37.2	3.18 (1H, ddd, 6.1, 9.6, 11.2)	39.9
3-Me	1.77 (3H, s)	22.6	1.74 (3H, s)	22.9
1′	2.26, 2.18 (2H, m)	29.2	1.93 (2H, m)	27.7
2′	5.06 (1H, t, 6.8)	123.0	4.80 (1H, t, 7.6)	123.4
3′		131.9		131.0
4′	1.67 (3H, s)	25.9	1.56 (3H, s)	25.8
5′	1.59 (3H, s)	17.9	1.34 (3H, s)	17.7
1″		145.9		142.4
2″,6″	7.15 (2H, m)	127.1	7.15 (2H, m)	128.2
3″,5″	7.25 (2H, m)	128.3	7.25 (2H, m)	126.2
4″	7.15 (1H, m)	125.9	7.15 (1H, m)	128.2
$COO\underline{C}H_3$	3.40 (3H, s)	51.0	3.49 (3H, s)	51.5
$\underline{C}OOCH_3$		173.7		176.5

lations (Fig. 1d). Analysis of the ROESY data established the relative configuration of the cyclohexenyl unit to be the same as in **3**, **7**, and **8** (Fig. 2d). Thus, panduratin G (**4**) was concluded as a mixture of diastereomers differing in stereochemistry at C-2.

Panduratin H (**5**) was obtained as a pale yellow oil. The molecular formula of $C_{20}H_{26}O_2$ was deduced by HR-EI-MS and IR spectra showed the absorption band of ester carbonyl at 1720 cm^{-1}. The ^{1}H-NMR spectrum revealed signals due to three aliphatic methines [δ 3.19 (H-6), 3.09 (H-1), 2.45 (H-2)], two allylic methylenes [δ 2.04, 2.34 (H_2-5), 2.26, 2.18 (H_2-1′)], two olefinic methines [δ 5.42 (H-4), 5.06 (H-2′)], three vinyl methyls [δ 1.77 (3-Me), 1.67 (H_3-4′), 1.59 (H_3-5′)], a phenyl ring [δ 7.15 (H-2″,6″, H-4″), 7.25 (H-3″,5″)], and a methoxyl singlet (δ 3.42). On the other hand, the ^{13}C-NMR spectrum of **5** indicated 20 carbon signals including six aromatic carbons, three methines, two methylenes, two olefinic methines, three methyls, two olefinic quaternary carbons, one methoxyl, and one ester carbonyl carbon (Table 3). These data were similar to those of panduratin A (**9**).[18] However, the ^{13}C-NMR spectrum of **5** did not reveal signals due to a substituted aryl group. Moreover, the signal of the carbonyl carbon appeared in the upfield (δ 173.7), ascribable to an ester carbonyl carbon. Based on this evidence, **5** was assumed to be a cyclohexenyl methyl ester derivative, which was confirmed by the HMBC spectrum. The partial connectivity between C-4–C-5–C-6–C-1–C-2–C-1′–C-2′ was deduced from COSY analysis. The HMBC correlations of H-1 with the ester carbonyl carbon and C-1′, of H-2′ with C-2, and of H-6 with C-1″ and C-2″,6″ (Fig. 1e) suggested the carboxymethyl, isoprenyl, and phenyl groups to be at C-1, C-2, and C-6, respectively. The small coupling constant (5.4 Hz) observed for $J_{H\text{-}1,H\text{-}2}$ suggested their *cis* relationship, while the large one (11.2 Hz) for $J_{H\text{-}1,H\text{-}6}$ indicated their *trans*-diaxial orientation. Further, ROESY correlations of H-1/H-2, H-1/H-2″,6″, H-2/3-Me, H-4/3-Me, and H-6/H-5β (Fig. 2e) supported the relative configuration. Accordingly, the structure of **5** was concluded as shown in Chart 1.

Panduratin I (**6**) was obtained as a pale yellow oil. Its molecular formula, $C_{20}H_{26}O_2$, was deduced by HR-EI-MS and found to be the same as that of **5**. The ^{1}H- and ^{13}C-NMR spectral data of **6** (Table 3) were also similar to those of **5**, except for the downfield shift of H-1 (**5**: δ 3.09; **6**: δ 3.33) and upfield shift of H-6 (**5**: δ 3.19; **6**: δ 3.18) Thus, the positions of the phenyl and carboxymethyl groups were considered to have been reversed, which was confirmed by the HMBC correlations (Fig. 1f). Accordingly, the coupling constants and ROESY correlations (Fig. 2f) indicated **6** to have the same relative configuration as **5**. Thus, the structure of **6** was determined as shown in Chart 1.

Compounds **1**—**6** did not show a clear Cotton effect in the CD spectra. Therefore, the absolute configuration of newly isolated compounds could not be determined. All the isolated compounds (**1**—**8**) were examined for their *in vitro* preferential cytotoxicity under nutrient-deprived conditions using human pancreatic PANC-1 cancer cells by the previously described method.[13] Compounds **1**, **2**, and **4**—**8** exhibited mild activity with PC_{100} (the concentration at which 100% cancer cell death occurred preferentially in NDM) values of 128 μM, whereas panduratin F (**3**) showed a weaker activity (PC_{100} 256 μM). On comparison of the activity of all the constituents isolated from *B. pandurata*, panduratin A (**9**) and nicolaioidesin B[17] were found to be the most potent constituents, showing a PC_{100} of 2.5 μM.[17] The present results are consistent with our previous observation, that the presence of a methoxyl group at C-4 and hydroxyl groups at C-2 and C-6 are important structural requirements for the strong activity, and any modification leads to a decrease in activity. In the present study, the presence of additional rings in **1**—**4**, **7**, and **8** and the absence of a substituted aryl group in **5** and **6**, were found to lead to a weaker activity, consistent with our previous findings.[17]

Experimental

General Experimental Procedures Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$. NMR spectra were assessed

using a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in δ values. HR-EI-MS measurements were carried out on a JEOL JMS-700T spectrometer. Column chromatography was performed with silica gel (Silica Gel 60N, Spherical, neutral, 40—50 μm, KANTO CHEMICAL CO., INC., Japan). Analytical and preparative TLC were carried out on precoated silica gel 60F_{254} or RP-18F_{254} plates (Merck, 0.25 or 0.50 mm thickness). HPLC was performed using a Shimadzu LC-6AD with a 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 a 70% EtOH extract (18 g). The 70% EtOH extract 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 an EtOAc–hexane solvent system to give seven fractions (Fr. 1—Fr. 7).[17]

Fr. 1-1 (2.23 g) was rechromatographed on silica gel with hexane–Me_2CO (50 : 1) to afford three subfractions (fr. 1-1-1, 548 mg; fr. 1-1-2, 995 mg; fr. 1-1-3, 250 mg). Subfraction 1-1-1 was a mixture of fatty substances, as indicated by the NMR spectrum. Subfraction 1-1-2 (995 mg) was subjected to normal-phase preparative TLC with hexane–Me_2CO (20 : 1), followed by reverse-phase preparative TLC with Me_2CO–H_2O (9 : 1), to give panduratins D (**1**, 2.2 mg), E (**2**, 2.5 mg), F (**3**, 2.0 mg), H (**5**, 15.5 mg), and I (**6**, 8.7 mg). Subfraction 1-1-3 was subjected to normal-phase preparative TLC with C_6H_6–hexane (2 : 1), followed by reverse-phase preparative TLC using Me_2CO–H_2O (9 : 1) to give a mixture of panduratin B (43 mg) and panduratin G (**4**, 5.9 mg). Panduratin B was further purified by semipreparative HPLC with CH_3CN–H_2O (9 : 1) (column: Supelco Discovery C-18, 250×10 mm, 5 μm; flow rate: 5 ml/min] to give panduratins B1 (**7**, 4.9 mg; t_R 33 min) and B2 (**8**, 6 mg; t_R 35 min).

Panduratin D (**1**): Pale yellow oil. $[\alpha]_D^{25}$ −2.89° (c=0.50, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3600, 1650, 1600, 1460, 1390, 1090. HR-EI-MS *m/z*: 430.2139 [Calcd for $C_{28}H_{30}O_4$: 430.2144 (M^+)]. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Panduratin E (**2**): Pale yellow oil. $[\alpha]_D^{25}$ −3.29° (c=0.55, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3600, 1650, 1600, 1460, 1380, 1100. HR-EI-MS *m/z*: 472.2614 [Calcd for $C_{31}H_{36}O_4$: 472.2614 (M^+)]. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Panduratin F (**3**): Pale yellow oil. $[\alpha]_D^{25}$ −7.5° (c=0.65, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3600, 1630, 1420, 1200, 1050, 930. HR-EI-MS *m/z*: 540.3220 [Calcd for $C_{36}H_{44}O_4$: 540.3240 (M^+)]. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Panduratin G (**4**): Pale yellow oil. $[\alpha]_D^{25}$ 0° (c=1.55, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3600, 1640, 1600, 1450, 1370, 1250, 920. HR-EI-MS *m/z*: 540.3268 [Calcd for $C_{36}H_{44}O_4$: 540.3240 (M^+)]. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Panduratin H (**5**): Pale yellow oil. $[\alpha]_D^{25}$ +3.85° (c=0.80, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1720, 1450, 950. HR-EI-MS *m/z*: 298.1904 [Calcd for $C_{20}H_{26}O_2$: 298.1933 (M^+)]. ^{1}H- and ^{13}C-NMR: see Table 3.

Panduratin I (**6**): Pale yellow oil. $[\alpha]_D^{25}$ +3.27° (c=0.80, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1720, 1450, 1400, 1100, 920. HR-EI-MS *m/z*: 298.1955 [Calcd for $C_{20}H_{26}O_2$: 298.1933 (M^+)]. ^{1}H- and ^{13}C-NMR: see Table 3.

Panduratin B1 (**7**): Pale yellow oil. $[\alpha]_D^{25}$ −9.49° (c=1.25, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 1.48 (3H, s, Me-2), 1.54 (6H, s, H_3-4‴, H_3-5‴), 1.58 (3H, s, H_3-6′), 1.67 (3H, s, H_3-5′), 1.78 (3H, s, Me-3″), 1.90 (2H, m, H_2-1′), 2.02 (1H, m, H-5″a), 2.10 (1H, m, H-1‴), 2.26 (2H, m, H-2′), 2.27 (1H, m, H-1‴), 2.40 (1H, dt, J=1.9, 6.4, 18.3 Hz, H-5″b), 2.62 (1H, dd, J=4.4, 11.4 Hz, H-2″), 3.41 (1H, ddd, J=6.4, 11.4, 11.7 Hz, H-6″), 3.76 (3H, s, OMe-5), 4.75 (1H, dd, J=4.4, 11.7 Hz, H-1″), 4.89 (1H, t, J=6.8 Hz, H-2‴), 5.10 (1H, t, J=7.1 Hz, H-3′), 5.39 (1H, d, J=10.0 Hz, H-3), 5.44 (1H, br s, H-4″), 5.91 (1H, s, H-6), 6.62 (1H, d, J=10.0 Hz, H-4), 7.08 (2H, m, H-3‴, 5‴), 7.17 (3H, m, H-2‴, 6‴, H-4‴), 13.74 (1H, s, OH-7). ^{13}C-NMR ($CDCl_3$) δ 17.7 (C-6′), 17.9 (C-5‴), 22.8 (3″-Me), 23.3 (C-2′), 25.7 (C-4‴, C-5′), 26.6 (2-Me), 28.8 (C-1‴), 36.6 (C-5″), 36.9 (C-6″), 41.7 (C-1′), 43.3 (C-2″), 54.0 (C-1″), 55.7 (5-OMe), 80.7 (C-2), 92.4 (C-6), 102.1 (C-4a), 106.4 (C-8), 117.3 (C-4), 121.3 (C-4″), 122.7 (C-3), 123.6 (C-3′), 124.3 (C-2‴), 125.5 (C-4‴), 127.1 (C-2‴, 6‴), 128.4 (C-3‴, 5‴), 131.7 (C-3‴), 132.2 (C-4′), 136.8 (C-3″), 147.0 (C-1‴), 155.5 (C-8a), 160.5 (C-5), 166.9 (C-7), 206.6 (C=O).

Panduratin B2 (**8**): Pale yellow oil. $[\alpha]_D^{25}$ −11.72° (c=1.05, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 1.48 (3H, s, Me-2), 1.54 (6H, s, H_3-4‴, H_3-5‴), 1.60 (3H, s, H_3-6′), 1.69 (3H, s, H_3-5′), 1.75 (3H, s, Me-3″), 1.94 (2H, m, H_2-1′), 2.02 (1H, m, H-5″a), 2.10 (1H, m, H-1‴), 2.16 (2H, m, H-2′), 2.29 (1H, m, H-1‴), 2.39 (1H, dt, J=1.9, 6.1, 18.0 Hz, H-5″b), 2.62 (1H, dd, J=4.6, 11.3 Hz, H-2″), 3.41 (1H, ddd, J=6.1, 11.3, 11.7 Hz, H-6″), 3.79 (3H, s, OMe-5), 4.72 (1H, dd, J=4.6, 11.7 Hz, H-1″), 4.89 (1H, t, J=6.3 Hz, H-2‴), 5.10 (1H, t, J=6.4 Hz, H-3′), 5.43 (1H, d, J=10.0 Hz, H-3), 5.44 (1H, br s, H-4″), 5.91 (1H, s, H-6), 6.63 (1H, d, J=10.0 Hz, H-4), 7.08 (2H, m, H-3‴, 5‴), 7.17 (3H, m, H-2‴, 6‴, H-4‴), 13.69 (1H, s, OH-7). ^{13}C-NMR ($CDCl_3$) δ 17.8 (C-6′), 18.0 (C-5‴), 22.6 (3″-Me), 23.4 (C-2′), 25.7 (C-4‴, C-5′), 26.4 (2-Me), 28.7 (C-1‴), 36.5 (C-5″), 37.3 (C-6″), 41.8 (C-1′), 43.0 (C-2″), 54.2 (C-1″), 55.7 (5-OMe), 80.7 (C-2), 92.5 (C-6), 102.5 (C-4a), 106.8 (C-8), 117.4 (C-4), 121.2 (C-4″), 122.9 (C-3), 123.7 (C-3′), 124.4 (C-2‴), 125.6 (C-4‴), 127.1 (C-2‴, 6‴), 128.4 (C-3‴, 5‴), 131.6 (C-3‴), 132.1 (C-4′), 136.9 (C-3″), 147.0 (C-1‴), 155.4 (C-8a), 160.4 (C-5), 166.8 (C-7), 206.7 (C=O).

***In Vitro* Preferential Cytotoxicity under Nutrient-Deprived Conditions** The *in vitro* preferential cytotoxicity (PC_{100}) of **1**—**8** was determined by the procedure previously described by Izuishi *et al.*[13] 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.*[13] 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 (256 to 0.125 μM) 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. Cell viability was calculated from the mean values of data from three wells using the following equation:

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

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20) In the present study, diastereomers of panduratin B, panduratins B1 (**7**) and B2 (**8**) were obtained as pure isolates by HPLC and fully assigned their ^{1}H- and ^{13}C-NMR data by 2D-NMR analysis. The ^{1}H-NMR resonances of H-6″ in reference 19 should be revised as [**7**: 3.41 (1H, ddd, J=6.4, 11.4, 11.7 Hz); **8**: 3.41 (1H, ddd, J=6.1, 11.3, 11.7 Hz)] and that of H-2″ should be added as [**7**: 2.61 (1H, dd, J=4.8, 11.4 Hz); **8** 2.63 (1H, dd, J=4.8, 11.3 Hz)].
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