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Synthesis and Biological Activities of (−)-6-*n*-Octyl-indolactam-V, a New Potent Analogue of the Tumor Promoter (−)-Indolactam-V

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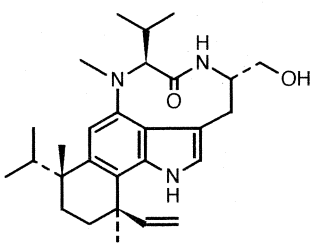
(−)-Indolactam-V (1) without a hydrophobic chain at positions 6 and 7 of the indole ring is a weak tumor promoter compared with teleocidin Bs. To investigate the effects of the hydrophobic substituent at position 6 of teleocidin Bs, we synthesized (−)-6-*n*-octyl-indolactam-V (2) by a palladium-catalyzed coupling reaction from (−)-6-bromo-indolactam-V (7) which had been obtained by microbial conversion with *Streptoverticillium blastmyceticum* NA34-17 as the key step. (−)-7-*n*-Octyl-indolactam-V (3) with potent biological activities comparable to those of teleocidin Bs was similarly synthesized from (−)-7-bromo-indolactam-V as a positive control. Compound 2 showed similar biological activities to those of 3, indicating that the effect of the hydrophobic substituent at position 6 of 1 was identical to that at position 7.

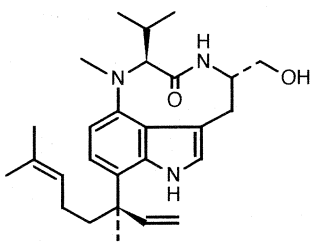
Key words: Epstein-Barr virus; (−)-6-*n*-octyl-indolactam-V; protein kinase C; teleocidin; tumor promoter

Teleocidins (teleocidin As and Bs)[1] are potent skin tumor promoters and activators of protein kinase C (PKC), a crucial enzyme involved in cellular signal transduction.[2] (−)-Indolactam-V (**1**),[3,4] which lacks the hydrophobic moieties at positions 6 and 7 of the indole ring of teleocidin Bs, is the minimal basic structure for exhibiting biological activities related to tumor promotion (Fig. 1). Thus, **1** is a key compound for investigating the structural requirements for tumor promoting activity. Over the past decade, structure-activity studies have been carried out on over 100 indolactam derivatives to determine the structural factors required for activity.[5] The various biological activities of **1** are lower than those of teleocidins, indicating that the monoterpenoid moiety of teleocidins plays an important role in amplifying the activities. To examine the effects of this moiety at each position, we synthesized various 7-substituted indolactams from **1** and we showed that hydrophobic substituents at position 7 enhanced the activities without any steric hindrance at the receptor site. (−)-7-*n*-Octyl-indolactam-V (**3**) exhibited the most potent activity comparable to that of teleocidins.[6] However, we could not synthesize (−)-6-*n*-octyl-indolactam-V (**2**) by electrophilic aromatic substitution of **1** with standard methods to clarify the effects of the substituent at position 6.[7]

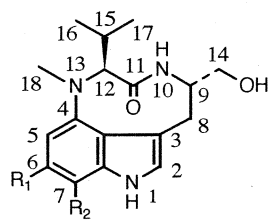
We have recently developed a new strategy to obtain 6-substituted indolactams by the microbial conversion of 6-substituted *N*-methyl-L-valyl-L-tryptophanols with teleocidin-producing *Streptoverticillium blastmyceticum* NA34-17.[8] Although direct introduction of a substituent larger than a methyl group into position 6 proved to be difficult because of the substrate specificity of the cyclization enzyme, (−)-6-bromo-indolactam-V (**7**), a useful starting material for synthesizing **2**, was obtained in a fairly good yield comparable to that of the natural precursor of **1**, *N*-methyl-L-valyl-L-tryptophanol. This prompted us to synthesize (−)-6-*n*-octyl-indolactam-V (**2**) from **7** by the palladium-catalyzed coupling reaction.[9] We describe here the synthesis and biological activities of **2**, together with those of **3** as a positive control.



Teleocidin B-4



Teleocidin A-1



R_1 = H, R_2 = H : (-)-Indolactam-V (**1**)
R_1 = *n*-C_8H_{17}, R_2 = H : (-)-6-*n*-Octyl-indolactam-V (**2**)
R_1 = H, R_2 = *n*-C_8H_{17} : (-)-7-*n*-Octyl-indolactam-V (**3**)

Fig. 1. Structures of Teleocidins and Indolactams (**1–3**).

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Results and Discussion

As already mentioned, direct introduction of an *n*-octyl group into position 6 of the indole ring of **1** is almost impossible since the several electrophilic aromatic substituents in **1** are exclusively at position 7.[7] However, our recent method using teleocidin-producing actinomycete circumvented this problem;[8] bromination of 14-*O*-acetyl-*N*-methyl-8-oxo-L-valyl-L-tryptophanol (**4**) resulted in substitution at position 6, and the *seco*-compound of **7** (**6**) was efficiently converted to (−)-6-bromo-indolactam-V (**7**). First, we prepared a sufficient amount of **7** by microbial conversion. The *seco*-compound (**6**) was synthesized in 9 steps from 50 g of L-tryptophan by the method reported previously.[8] The overall yield of **6** was 0.3% (278 mg). The feeding experiment of **6** was performed in a 30-liter jar fermenter to afford **7** in a 28.8% yield. This conversion yield (28.8%) is significantly higher than that of the previous small-scale experiment (8.1%) with 500-ml flasks.[8] This suggests that a high agitation rate and aeration of the jar fermenter increased the metabolic efficiency of the actinomycete.

The introduction of an *n*-octyl group at position 6 of **7** was accomplished by the method of Endo *et al.*[9] with slight modifications as shown in Fig. 2. After protecting the hydroxyl group of **7** with a *tert*-butyldimethylsilyl (TBDMS) group (99.7%), treatment of the TBDMS derivative (**8**) with 1-octene in the presence of palladium acetate and tri-*o*-tolylphosphine in triethylamine and acetonitrile exclusively afforded (−)-6-(*trans*-1-octenyl)-14-*O*-TBDMS-indolactam-V (**9**) in a 58.8% yield. Catalytic hydrogenation of **9** gave (−)-6-*n*-octyl-14-*O*-TBDMS-indolactam-V (**10**) in a 72.6% yield. Deprotection of the TBDMS group then gave **2** in a 61.0% yield. The overall yield of **2** from **7** was 22.5%. (−)-7-*n*-Octyl-indolactam-V (**3**) was similarly synthesized from (−)-7-bromo-14-*O*-TBDMS-indolactam-V, which had been prepared in a 40.2% yield by a method similar to that reported by Moreno and Kishi.[10] The overall yield of **3** was 10.3%.

The ^{1}H-NMR spectrum of (−)-6-*n*-octyl-indolactam-V (**2**) in deuterochloroform shows that **2** existed as two stable conformers due to *cis-trans* isomerization of the amide bond, the active twist and inactive sofa form,[11,12] at room temperature as has been observed for **1** and **3**.[13] However, the conformational ratio of **2** (twist:sofa=3.3:1 at 9 mM) is slightly different from that of **3** (1.4:1 at 6 mM). This difference is deduced to have been the result of the electron-donating effect of the *n*-octyl group.[7] The lone-pair electrons on the nitrogen atom at position 13 of **1** are delocalized on the aromatic ring in the twist conformation, but relatively localized on the nitrogen atom in the sofa form. The electron-donating *n*-octyl group at the *para* position in **3** seems to have hindered the resonance to disfavor formation of the twist conformation. In contrast, the lone-pair electrons at position 13 of **2** would have been delocalized like those of **1**, since the *n*-octyl group is located at the *meta* position in **2**. The conformational ratio of **2** is almost the same as that of **1** (2.6:1 at 4 mM).

The biological activities of **2**, together with those of **1** and **3** were examined by three *in vitro* bioassays related to *in vivo* tumor promotion: binding to the protein kinase C (PKC) regulatory domain,[12,14] Epstein-Barr virus early antigen (EBV-EA)-inducing ability,[15,16] and superoxide (O_2^-) generation-inducing ability.[17,18] Tables 1 and 2 summarize the results of these assays.

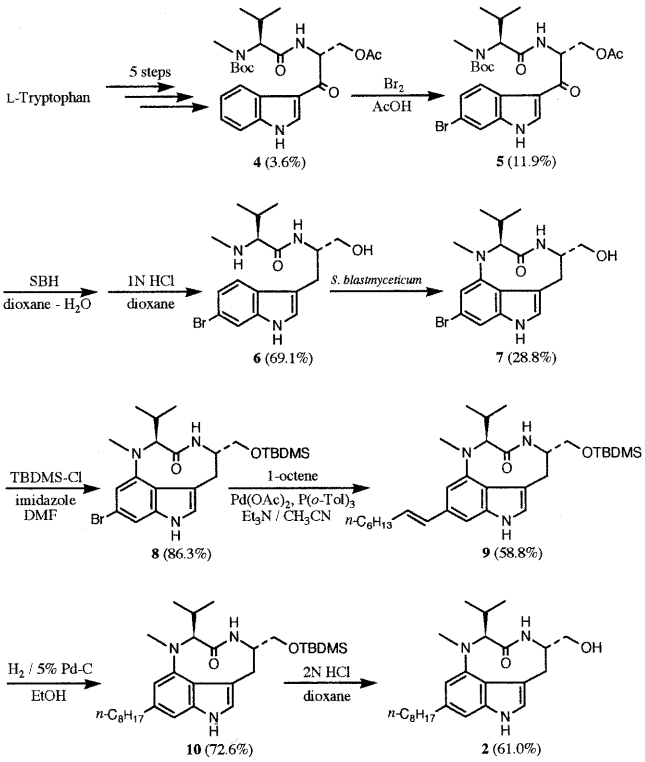

Fig. 2. Synthetic Scheme for (−)-6-*n*-Octyl-indolactam-V (**2**).

The binding affinity to the PKC regulatory domain was evaluated by inhibition of the specific binding of [^{3}H]phorbol-12,13-dibutyrate (PDBu) to the conventional PKC mixture (PKCα, βI/βII, and γ) and is expressed as the concentration required to cause 50% inhibition (IC_{50}) that was calculated with a computer program (Statistical Analysis System, SAS) by the probit procedure.[19] EBVs are under the strict control of the host human lymphoblastoid Raji cells. They are activated by tumor promoters to produce the early antigen (EA).[15,16] The EBV-EA-inducing activity is expressed as the percentage of EA-positive cells. Under our experimental conditions, teleocidin B-4 exhibited maximum induction between 30% and 40% at 10^{-7} M. Superoxide (O_2^-) generation is triggered by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter comparable to teleocidin Bs, in epithelial cells and leukocytes through the xanthine oxidase[20] and NADPH oxidase systems,[21] respectively. Therefore, we selected the O_2^- generation-inducing ability to evaluate the activity of the *n*-octyl derivatives (**2** and **3**) together with **1**. The ability is expressed as the level of O_2^- production, and under our experimental conditions, TPA produced 1.32 nmol/ml/min of O_2^- at 10^{-7} M.

The PKC binding ability of **2** was far stronger than that of **1**, and comparable to that of **3**. Similar tenden-

Table 1. PKC-binding and EBV-EA-inducing Activities of Indolactam Derivatives

Compound	Inhibition of specific [^{3}H]PDBu binding[a] pIC_{50} (log 1/M)	EBV-EA induction test[b] % of EA-positive cells					
		10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
(−)-Indolactam-V(**1**)	6.58 (0.06)[c]			1.3 (0.3)	15.5 (3.4)	37.6 (3.5)	30.4 (0.3)
(−)-6-*n*-Octyl-indolactam-V(**2**)	8.57 (0.01)	1.6 (0.5)	13.9 (1.4)	30.3 (0.8)	28.1 (1.4)	20.9 (0.5)	
(−)-7-*n*-Octyl-indolactam-V(**3**)	8.24 (0.08)	2.0 (0.4)	10.6 (2.4)	29.8 (4.0)	34.6 (3.6)	22.1 (0.2)	

[a] This assay was performed by the polyethylene glycol precipitation method,[12,14] using a native conventional PKC mixture.
[b] This assay was performed by the method reported previously.[15,16] The viability of the cells exceeded 70% in all experiments.
[c] Standard deviation.

Table 2. Superoxide Generation-inducing Activities of Indolactam Derivatives

Compound	O_2^- generation[a] (nmol/ml/min)					
	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
(−)-Indolactam-V(**1**)			0.04 (0.01)[b]	0.12 (0.03)	1.23 (0.03)	1.30 (0.01)
(−)-6-*n*-Octyl-indolactam-V(**2**)	0.07 (0.04)	1.28 (0.05)	1.17 (0.15)	1.32 (0.05)		
(−)-7-*n*-Octyl-indolactam-V(**3**)	0.06 (0.01)	1.24 (0.06)	1.36 (0.01)	1.31 (0.03)		

[a] This assay was performed by the method of Murakami *et al.*[18] with slight modifications.
[b] Standard deviation.

cies were observed in both the EBV-EA and superoxide generation induction test. Both **2** and **3** showed potent EBV-EA-inducing activity at 10^{-8} M and superoxide generation induction at 10^{-9} M, while **1** showed these activities only at 10^{-6} M. Although **2** and **3** existed as two stable conformers in different ratios, these ratios were found not to affect the biological activities because of the small difference in free energy between the two conformers (<1 kcal/mol). These results clearly indicate that the hydrophobic substituent at position 6 of **1** played a similar role to that at position 7.

The present study, taken with previous structure-activity studies, reveals the effects of hydrophobic substituents at all positions of the indole ring of **1**. Introduction of a substituent at position 1 decreased the activity[22] and that at position 2 cancelled the activity by steric hindrance with the receptor.[6] A substituent at position 5 caused the conformational change to lose activity; 5-substituted indolactams existed only as the inactive sofa conformer.[12] On the other hand, introduction of a hydrophobic substituent at positions 6 and 7 markedly increased the activity by hydrophobic interaction at the receptor site without any steric hindrance. These observations will contribute to elucidate the binding mode of **1** and teleocidins with such putative receptors as PKCs.

Experimental

General methods. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; $[\alpha]_D$, Jasco DIP-1000; ^{1}H-NMR, Bruker ARX500 (reference to TMS); HPLC, Waters model 600E with model 484 UV detector; (HR) EI-MS, Jeol JMS-DX300 and JMS-600H. HPLC was carried out in YMC-packed AQ-323 (ODS, 10 mm I.D. × 250 mm) and SH-342-5 (ODS, 20 mm I.D. × 150 mm) columns (Yamamura Chemical Laboratory). Wakogel C-100 and C-200 (silica gel, Wako Pure Chemical Industries) were used for column chromatography. [^{3}H]PDBu (21.0 Ci/mmol) and the native conventional PKC mixture from rat brain were purchased from NEN Research Products and Boehringer Mannheim, respectively. All other chemicals and reagents were purchased from chemical companies and used without further purification.

Synthesis of (−)-*6-n-octyl-indolactam-V* (**2**)

(2S-(2R*, 5R*))-10-Bromo-5-(*tert*-butyldimethylsilyloxymethyl)-1,2,4,5,6,8-hexahydro-1-methyl-2-(1-methylethyl)-3H-pyrrolo(4, 3, 2-gh)-1, 4-benzodiazonin-3-one [(−)-6-bromo-14-*O*-TBDMS-indolactam-V] (**8**). Compound **7** (79.5 mg, 209.2 μmol) was obtained from **6** (278.0 mg, 727.7 μmol) by a feeding experiment[8] in a 30-liter jar containing 20 liters of medium consisting of 2% glucose, 1% polypeptone, 1% meat extract, and 0.5% NaCl (pH 6.6) in a 28.8% yield. Compound **7** (31.6 mg, 83.4 μmol) was dissolved in anhydrous DMF (1.0 ml). To this solution were added TBDMS chloride (76.0 mg, 500 μmol) and imidazole (68.0 mg, 1.0 mmol), and the reaction mixture was stirred at room temperature for 2 h. The mixture was extracted with EtOAc, and the EtOAc layer was dried over Na_2SO_4. After evaporating, the residue was purified by column chromatography on Wakogel C-200 with EtOAc and hexane (2:8) to give **8** (35.4 mg, 72.0 μmol) in an 86.3% yield. Compound **8**, amorphous, IR ν_{max} ($CHCl_3$) cm^{-1}: 3478, 1655, 1599, 1491, 1260, 1103, 1053, 839, 750. ^{1}H-NMR (500 MHz, $CDCl_3$, 0.058 M, 27°C, twist:sofa=6.7:1) δ ppm for the twist conformer: 0.02 (3H, s), 0.05 (3H, s), 0.63 (3H, d, J=6.8 Hz), 0.87 (9H, s), 0.93 (3H, d, J=6.3 Hz), 2.61 (1H, m), 2.89 (1H, dd, J=17.5, 3.5 Hz), 2.90 (3H, s), 3.12 (1H, br.d, J=17.5 Hz), 3.47 (1H, dd, J=10.1, 9.5 Hz), 3.63 (1H, dd, J=10.1, 4.4 Hz), 4.16 (1H, m), 4.33 (1H, d, J=10.2 Hz), 6.15 (1H, br.s, NH-10), 6.58 (1H, d, J=1.5 Hz), 6.84 (1H, s), 7.04 (1H, d, J=1.3 Hz), 8.10 (1H, br.s, NH-1). EIMS m/z (%): 493 (M^+, 100), 450 (29), 407 (36), 348 (11).

(2S-(2R*, 5R*))-5-(*tert*-Butyldimethylsilyloxymethyl)-

1,2,4,5,6,8-hexahydro-1-methyl-2-(1-methylethyl)-10-(*trans*-1-octeny)-3H-pyrrolo(4,3,2-gh)-1,4-benzodiazonin-3-one [(−)-14-*O*-TBDMS-6-(*trans*-1-octenyl)-indolactam-V] (**9**). A mixture of **8** (21.2 mg, 43.0 μmol), 1-octene (15 μl, 86.0 μmol), palladium acetate (9.7 mg, 43.0 μmol), tri-*o*-tolylphosphine (19.6 mg, 64.5 μmol) and triethylamine (1.0 ml, 8.6 mmol) in acetonitrile (1.2 ml) was heated at 120°C in a sealed tube for 2.5 h and then filtered. After removing the solvent under reduced pressure, the residue was diluted with EtOAc, and the EtOAc layer was dried over Na_2SO_4. After evaporating, the residue was purified by column chromatography on Wakogel C-200 with EtOAc and hexane (1:1), this being followed by HPLC on YMC SH-342-5 with 95% MeOH to give **9** (13.3 mg, 25.3 μmol) in a 58.8% yield. Compound **9**, amorphous, IR ν_{max} ($CHCl_3$) cm^{-1}: 3481, 1647, 1541, 1489, 1260, 1105, 1053, 839, 752. ^{1}H-NMR (500 MHz, $CDCl_3$, 0.008 M, 27°C, twist:sofa=6.3:1) δ ppm for the twist conformer: 0.02 (3H, s), 0.04 (3H, s), 0.64 (3H, d, J=6.8 Hz), 0.87 (9H, s), 0.91 (3H, t, J=5.4 Hz), 0.92 (3H, d, J=6.3 Hz), 1.25–1.39 (6H, m), 1.48 (2H, m), 2.21 (2H, m), 2.62 (1H, m), 2.87 (1H, dd, J=17.5, 3.5 Hz), 2.94 (3H, s), 3.13 (1H, br.d, J=17.5 Hz), 3.46 (1H, dd, J=10.2, 9.3 Hz), 3.63 (1H, dd, J=10.2, 4.4 Hz), 4.19 (1H, m), 4.35 (1H, d, J=10.2 Hz), 6.14 (1H, br.s, NH-10), 6.19 (1H, m), 6.40 (1H, d, J=15.7 Hz), 6.54 (1H, s), 6.83 (1H, br.s), 6.84 (1H, s), 7.88 (1H, br.s, NH-1). EIMS m/z (%): 525 (M^+, 100), 482 (26), 439 (16), 380 (13).

(2S-(2R*, 5R*))-5-(*tert*-Butyldimethylsilyloxymethyl)-1,2,4,5,6,8-hexahydro-1-methyl-2-(1-methylethyl)-10-octyl-3H-pyrrolo(4,3,2-gh)-1,4-benzodiazonin-3-one [(−)-14-*O*-TBDMS-6-*n*-octyl-indolactam-V] (**10**). A mixture of **9** (5.8 mg, 11.0 μmol) and 10% Pd-charcoal (1.0 mg) in EtOH (10 ml) was stirred under H_2 at room temperature for 15 min and then filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography on Wakogel C-100 by using toluene and increasing amounts of acetone, this being followed by HPLC on YMC SH-342-5 with 97% MeOH to give **10** (3.7 mg, 7.0 μmol) in a 63.8% yield. Compound **10**, amorphous, IR ν_{max} ($CHCl_3$) cm^{-1}: 3482, 1647, 1541, 1509, 1260, 1105, 1049, 839, 749. ^{1}H-NMR (500 MHz, $CDCl_3$, 0.006 M, 27°C, twist:sofa=10:1) δ ppm for the twist conformer: 0.03 (3H, s), 0.05 (3H, s), 0.63 (3H, d, J=6.8 Hz), 0.88 (9H, s), 0.88 (3H, t, J=5.5 Hz), 0.92 (3H, d, J=6.4 Hz), 1.23–1.28 (8H, m), 1.30 (2H, m), 1.64 (2H, m), 2.63 (3H, m), 2.88 (1H, dd, J=17.4, 3.5 Hz), 2.92 (3H, s), 3.13 (1H, br.d, J= 17.4Hz), 3.48 (1H, dd, J= 10.9, 5.5Hz), 3.63 (1H, dd, J=10.9, 4.3 Hz), 4.22 (1H, m), 4.35 (1H, d, J=10.1 Hz), 6.13 (1H, s, NH-10), 6.34 (1H, s), 6.69 (1H, s), 6.80 (1H, br.s), 8.07 (1H, br.s, NH-1). EIMS m/z (%): 527 (M^+, 100), 484 (25), 441 (17), 382 (12).

(2S-(2R*, 5R*))-1,2,4,5,6,8-Hexahydro-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-10-octyl-3H-pyrrolo (4,3,2-gh)-1,4-benzodiazonin-3-one [(−)-6-*n*-octyl-indolactam-V] (**2**). Compound **10** (6.7 mg, 12.7 μmol) was dissolved in dioxane (1.0 ml). To this solution was added 2N HCl (0.5 ml) while stirring. After stirring for 15 min at room temperature, the mixture was extracted with EtOAc, and the EtOAc layer was dried over Na_2SO_4. After evaporating, the residue was purified by column chromatography on Wakogel C-100 by using toluene and increasing amounts of acetone, this being followed by HPLC on YMC SH-342-5 with 87% MeOH to give **2** (3.2 mg, 7.7 μmol) in a 61.0% yield. Compound **2**, amorphous, $[\alpha]_D$ −169° (c=0.15, MeOH, 21.2°C). UV λ_{max} (MeOH) nm(ε): 294 (9,500), 231 (37,000). IR ν_{max} ($CHCl_3$) cm^{-1}: 3478, 1655, 1545, 1509, 1262, 1098, 1051, 810, 749. ^{1}H-NMR (500 MHz, $CDCl_3$, 0.009 M, 27°C, twist:sofa=3.3:1) δ ppm for the twist conformer: 0.63 (3H, d, J=6.7 Hz, H_3-16 or 17), 0.87 (3H, t, J=5.1 Hz, H_3-26), 0.93 (3H, d, J=6.3 Hz, H_3-16 or 17), 1.25–1.31 (10H, m, H_2-21, 22, 23, 24, 25), 1.64 (2H, m, H_2-20), 2.16 (1H, br.m, OH-14), 2.62 (3H, m, H-15, H_2-19), 2.92 (3H, s, H_3-18), 2.98 (1H, dd, J=17.2, 3.0 Hz, H-8a), 3.17 (1H, br.d, J=17.2 Hz, H-8b), 3.53 (1H, m, H-14a), 3.74 (1H, m, H-14b), 4.30 (1H, m, H-9), 4.37 (1H, d, J=10.2 Hz, H-12), 6.34 (1H, s, H-5), 6.70 (1H, s, H-7), 6.74 (1H, br.s, NH-10), 6.82 (1H, s, H-2), 7.84 (1H, br.s, NH-1); δ ppm for the sofa conformer: 0.89 (3H, t, J=5.1 Hz, H_3-26), 0.95 (3H, d, J=6.7 Hz, H_3-16 or 17), 1.25 (3H, d, J=6.3 Hz, H_3-16 or 17), 1.25–1.31 (10H, m, H_2-21, 22, 23, 24, 25), 1.32 (1H, br.m, OH-14), 1.64 (2H, m, H_2-20), 2.40 (1H, m, H-15), 2.62 (1H, m, H-19), 2.73 (3H, s, H_3-18), 2.80 (1H, br.d, J=14.5 Hz, H-8a), 2.97 (1H, d, J=10.6 Hz, H-12), 3.09 (1H, dd, J=14.5, 4.7 Hz, H-8b), 3.45 (2H, m, H-14a, H-14b), 4.44 (1H, m, H-9), 4.78 (1H, d, J=11.1 Hz, NH-10), 6.88 (1H, s, H-5), 6.96 (1H, br.d, J=2.1 Hz, H-2), 7.08 (1H, s, H-7), 8.12 (1H, br.s, NH-1). ^{13}C-NMR (125 MHz, 0.010 M, 27°C) δ ppm for the twist conformer: 14.10 (C-26), 19.43 (C-16 or C-17), 21.57 (C-16 or C-17), 22.68 (C-25), 28.54 (C-15), 29.32 (C-21, C-22 or C-23), 29.39 (C-21, C-22 or C-23), 29.57 (C-21, C-22 or C-23), 31.85 (C-20), 31.91 (C-24), 32.99 (C-18), 34.08 (C-8), 36.27 (C-19), 55.61 (C-9), 65.18 (C-14), 71.07 (C-12), 103.20 (C-7), 107.76 (C-5), 114.47 (C-3), 116.01 (C-3a), 120.62 (C-2), 138.14 (C-6), 139.72 (C-7a), 147.43 (C-4), 173.74 (C-11), these assignments being derived from ^{1}H-^{1}H COSY, HMQC, and HMBC spectra. HR-EIMS m/z: 413.3042 (M^+, calcd. for $C_{25}H_{39}N_3O_2$, 413.3042).

Synthesis of (−)-*7-n-octyl-indolactam-V* (**3**)

(2S-(2R*, 5R*))-5-(*tert*-butyldimethylsilyloxymethyl)-1,2,4,5,6,8-hexahydro-1-methyl-2-(1-methylethyl)-9-(*trans*-1-octeny)-3H-pyrrolo(4,3,2-gh)-1,4-benzodiazonin-3-one [(−)-14-*O*-TBDMS-7-(*trans*-1-octenyl)-indolactam-V]. Compound **1** (21.6 mg, 71.8 μmol), which had been isolated from the culture broth of *Streptoverticillium blastmyceticum* NA34-17, was treated in a manner similar to that described for the synthesis of **8** to give (−)-14-*O*-TBDMS-indolactam-V (35.4 mg, 72.0 μmol) in a 99.7% yield. (−)-14-*O*-TBDMS-indolactam-V (67.2 mg, 162.0 μmol) was dissolved in anhydrous DMF (1.0 ml). To this solution was added NBS (34.6 mg, 194.4 μmol) in anhydrous DMF (2 ml) at 0°C, and the reaction mixture was stirred at 0°C for 1.5 h. The mixture was extracted with EtOAc, and the EtOAc layer was dried over Na_2SO_4. After evaporating, the residue

was purified by column chromatography on Wakogel C-200 with EtOAc and hexane (2:8), this being followed by HPLC on YMC SH-342-5 with 90% MeOH to give (−)-7-bromo-14-*O*-TBDMS-indolactam-V (32.0 mg, 64.6 μmol) in a 40.3% yield. (−)-7-Bromo-14-*O*-TBDMS-indolactam-V (14.1 mg, 28.5 μmol) was treated in a manner similar to that described for the synthesis of **9** to give (−)-7-(*trans*-1-octenyl)-14-*O*-TBDMS-indolactam - V (5.8 mg, 11.0 μmol) in a 38.8% yield. (−)-7-(*trans*-1-Octenyl)-14-*O*-TBDMS-indolactam-V, amorphous, ^{1}H-NMR (500 MHz, $CDCl_3$, 0.013 M, 27°C, twist:sofa=7.4:1) δ ppm for the twist conformer: 0.02 (3H, s), 0.04 (3H, s), 0.63 (3H, d, *J*=6.8 Hz), 0.88 (9H, s), 0.88 (3H, t, *J*=5.4 Hz), 0.92 (3H, d, *J*=6.4 Hz), 1.02-1.39 (6H, m), 1.49 (2H, m), 2.26 (2H, m), 2.61 (1H, m), 2.71 (1H, dd, *J*=17.4, 3.6 Hz), 2.91 (3H, s), 2.91 (1H, dd, *J*=17.4, 3.6 Hz), 3.63 (1H, dd, *J*=10.1, 4.2 Hz), 4.20 (1H, m), 4.34 (1H, d, *J*=10.1 Hz), 6.11 (1H, br.s), 6.13 (1H, s, NH-10), 6.49 (1H, d, *J*=8.0 Hz), 6.52 (1H, d, *J*=15.9 Hz), 6.89 (1H, br.s), 7.06 (1H, d, *J*=8.0 Hz), 8.21 (1H, br.s, NH-1). EIMS *m/z* (%): 525 (M^+, 100), 482 (15), 439 (13), 380 (6).

(2S-(2R*, 5R*))-5-(*tert*-Butyldimethylsilyloxymethyl)-1,2,4,5,6,8-hexahydro-1-methyl-2-(1-methylethyl)-9-octyl-3H-pyrrolo(4,3,2-gh)-1,4-benzodiazonin-3-one [(−)-14-*O*-TBDMS-7-*n*-octyl-indolactam-V]. (−)-7-(*trans*-1-Octenyl)-14-*O*-TBDMS-indolactam-V (5.8 mg, 11.0 μmol) was treated in a manner similar to that described for the synthesis of **10** to give (−)-7-*n*-octyl-14-*O*-TBDMS-indolactam-V (3.7 mg, 7.0 μmol) in a 63.8% yield. (−)-7-*n*-Octyl-14-*O*-TBDMS-indolactam-V, amorphous, ^{1}H-NMR (500 MHz, $CDCl_3$, 0.014 M, 27°C, twist:sofa=3.8:1) δ ppm for the twist conformer: 0.03 (3H, s), 0.05 (3H, s), 0.64 (3H, d, *J*=6.8 Hz), 0.88 (9H, s), 0.88 (3H, t, *J*=5.7 Hz), 0.92 (3H, d, *J*=6.3 Hz), 1.27-1.31 (8H, m), 1.39 (2H, m), 1.71 (2H, m), 2.62 (1H, m), 2.71 (2H, t, *J*=8.3 Hz), 2.91 (1H, dd, *J*=17.4, 3.7 Hz), 2.91 (3H, s), 3.15 (1H, br.d, *J*=17.4 Hz), 3.46 (1H, t, *J*=9.3 Hz), 3.64 (1H, dd, *J*=10.1, 4.2 Hz), 4.26 (1H, m), 4.31 (1H, d, *J*=10.1 Hz), 6.13 (1H, br.s, NH-10), 6.46 (1H, d, *J*=8.1 Hz), 6.87 (1H, d, *J*=8.1 Hz), 6.88 (1H, s), 7.91 (1H, br.s, NH-1). EIMS *m/z* (%): 527 (M^+, 100), 484 (24), 441 (17), 382 (8).

(2S-(2R*, 5R*))-1,2,4,5,6,8-Hexahydro-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-9-octyl-3H-pyrrolo (4,3,2-gh)-1,4-benzodiazonin-3-one [(−)-7-*n*-octyl-indolactam-V] (**3**). (−)-7-*n*-Octyl-14-*O*-TBDMS-indolactam-V (3.7 mg, 7.0 μmol) was treated in a manner similar to that described for the synthesis of **2** to give **3** (1.2 mg, 2.9 μmol) in a 41.5% yield. Compound **3**, amorphous, UV λ_{max} (MeOH) nm(ε): 287 (8,500), 227 (28,000). ^{1}H-NMR (500 MHz, $CDCl_3$, 0.006 M, 27°C, twist:sofa=1.4:1) δ ppm for the twist conformer: 0.65 (3H, d, *J*=6.8 Hz), 0.88 (3H, t, *J*=5.1 Hz), 0.92 (3H, d, *J*=6.1 Hz), 1.24-1.38 (8H, m), 1.40 (2H, m), 1.65 (1H, m, OH-14), 1.74 (2H, m), 2.61 (1H, m), 2.71 (2H, t, *J*=7.7 Hz), 2.91 (3H, s), 2.99 (1H, dd, *J*=17.2, 3.7 Hz), 3.20 (1H, br.d, *J*=17.2 Hz), 3.52 (1H, m), 3.74 (1H, m), 4.33 (1H, m), 4.33 (1H, d, *J*=10.1 Hz), 6.33 (1H, s, NH-10), 6.46 (1H, d, *J*=7.8 Hz), 6.87 (1H, d, *J*=7.8 Hz), 6.90 (1H, s), 7.92 (1H, br.s, NH-1); δ ppm for the sofa conformer: 0.87 (3H, t, *J*=5.5 Hz), 0.94 (3H, d, *J*=5.9 Hz), 1.24 (3H, d, *J*=6.7 Hz), 1.25-1.38 (8H, m), 1.40 (2H, m), 1.65 (1H, m, OH-14), 1.73 (2H, m), 2.38 (1H, m), 2.74 (3H, s), 2.79 (1H, t, *J*=7.7 Hz), 2.83 (1H, br.d, *J*=14.6 Hz), 2.97 (1H, d, *J*=10.7 Hz), 3.10 (1H, dd, *J*=14.6, 4.8 Hz), 3.43-3.47 (2H, m), 4.45 (1H, m), 4.72 (1H, d, *J*=11.5 Hz, NH-10), 6.97 (1H, d, *J*=7.7 Hz), 6.99 (1H, d, *J*=7.7 Hz), 7.03 (1H, d, *J*=1.9Hz), 8.16 (1H, br.s, NH-1). HR-EIMS *m/z*: 413.3056 (M^+, calcd. for $C_{25}H_{39}N_3O_2$, 413.3042).

Inhibition of specific [^{3}H]PDBu binding to a native conventional PKC mixture. Inhibition of the specific [^{3}H]PDBu binding to a native conventional PKC mixture was achieved by the method reported previously[12] under the following conditions: 50 mM Tris-HCl (pH 7.4 at 25°C), 100 μM $CaCl_2$, 2 nM cPKC mixture, 10 nM [^{3}H]PDBu (21.0 Ci/mmol), 100 μg/ml 1,2-di(*cis*-9-octadecenoyl)-*sn*-glycero-3-phospho-L-serine, and 3 mg/ml bovine γ-globulin.

Epstein-barr virus early antigen induction test. The EBV-EA induction test was carried out in the Raji cell (nonproducer) system with sodium *n*-butyrate (3 mM) by the method reported previously.[15,16]

Superoxide generation test. The superoxide generation test was carried out by the method reported previously[17,18] without inhibitors.

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