Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 5, pp. 453-458, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00047-X

SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW CONFORMATIONALLY FIXED ANALOGUES OF (-)-INDOLACTAM-V, THE CORE STRUCTURE OF TUMOR-PROMOTING TELEOCIDINS

Kazuhiro Irie*, Fumito Koizumi, Yoriko Iwata, Takashi Ishii, Yoshiaki Yanai, Yoshimasa Nakamura, Hajime Ohigashi and Paul A. Wender[a]

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan
[a]Department of Chemistry, Stanford University, Stanford, CA 94305, USA

Abstract: (-)-Indolactam-V (**1**) exists as two stable conformers, the twist and the sofa form, in solution at room temperature. 3-Aza-Cope rearrangement of (-)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**5**) gave new conformationally restricted analogues (**2a** and **3**) along with a normal rearrangement product (**7**). Both **2a** and **3**, whose fixed conformation was the twist form, showed significant biological activities related to tumor promotion.

Tumor-promoting teleocidins[1,2] and their core structure (-)-indolactam-V (**1**)[3,4] exist as two stable conformers, the twist and the sofa form, in solution at room temperature (Figure 1 and 2).[5] To elucidate the common structural features among several TPA-type tumor promoters with different skeletons and quite similar biological activities (teleocidins, phorbol esters and aplysiatoxins),[2] it is indispensable to know the active conformation of teleocidins. Synthesis of conformationally restricted analogues of **1** is one of the most promising approaches. We have previously reported the synthesis of (-)-5-fluoroindolactam-V existing mainly as the sofa conformation, and shown that at least the sofa conformation is not the biologically active form.[6] This communication describes the synthesis and biological activities of novel twist-restricted analogues (**2a** and **3**) along with those of a sofa-restricted analogue, (-)-5-methylindolactam-V (**4**) as a negative control.

The conformational equilibrium of **1** is mainly attributed to a *cis-trans* isomerization of the nine-membered lactam. In the sofa conformation of *trans* amide, the methyl group at position 13 is almost perpendicular to the indole ring, while in the twist conformation of *cis* amide, the methyl group and the indole ring is somewhat on the same plane (Figure 2).[5] To synthesize a twist-restricted analogue of **1**, bridge formation between position 5 and 13 of **1** seems to be effective since this bridge formation increases the strain of the nine-membered lactam to inhibit the formation of the *trans* amide conformation. Recently, Still *et al.* have analyzed systematically the 3-aza-Cope rearrangement of *N*-alkyl-*N*-allylanilines, and shown that *ortho* cyclization products could be obtained when less than a stoichiometric amount of a Lewis acid was used.[7] We examined the 3-aza-Cope rearrangement of (-)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**5**) in order to obtain the twist-restricted analogue of **1**.

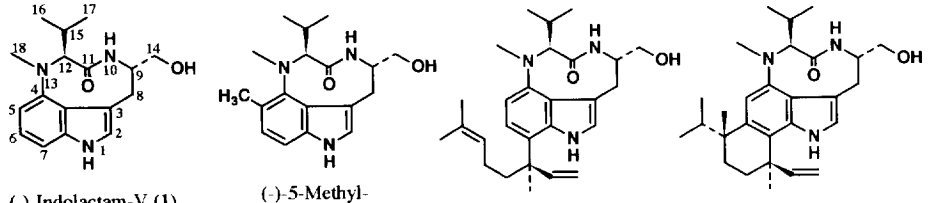

Figure 1. Structure of teleocidin-related compounds.

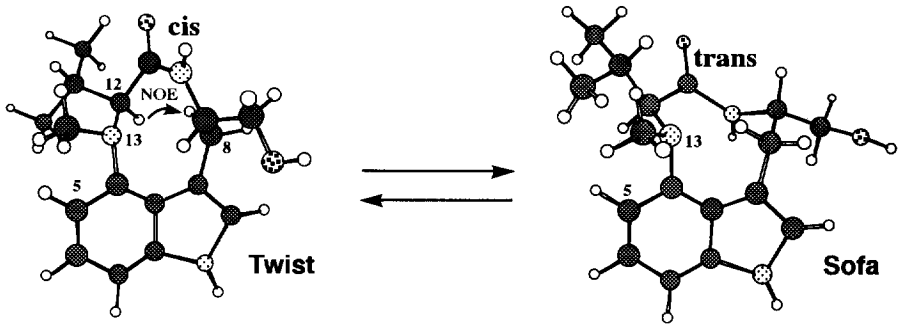

Figure 2. Conformation of (-)-indolactam V (**1**).[5]

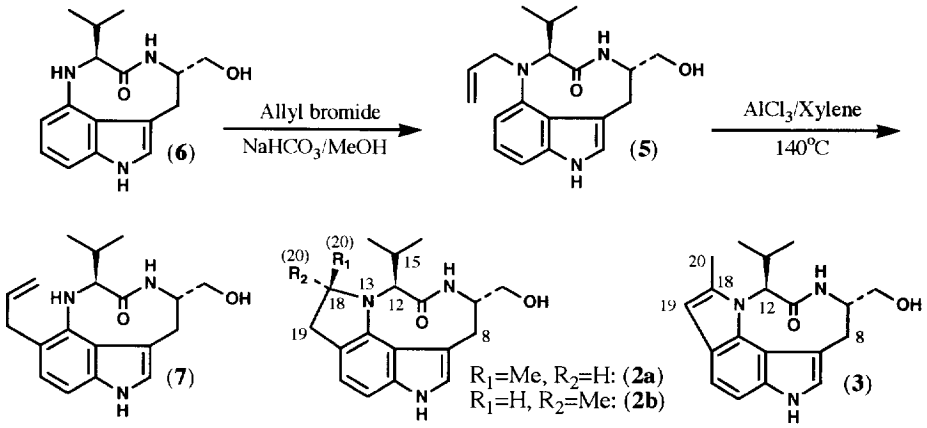

Figure 3. 3-Aza-Cope rearrangement of (-)-N^{13}-desmethyl-N^{13}-allylindolactam-V (**5**).

Synthesis of **5** was done by *N*-allylation of (-)-N^{13}-desmethylindolactam-V (**6**), which was synthesized from L-tryptophan by the method of Nakatsuka *et al.*[8] with slight modifications. Treatment of **6** with allyl bromide in methanol containing sodium hydrogen carbonate gave **5** at 38.2% yield.[9] To get the *ortho* cyclization products of *N*-alkyl-*N*-allylanilines, high concentration of a substrate and less than a stoichiometric amount of a Lewis acid are preferable.[7] First, 0.5M of **5** in xylene was treated with 0.7 equivalent of zinc chloride at 140°C for 2.5 hours in a sealed tube. This reaction gave, however, only the normal [3,3] rearrangement product (**7**) at 26.4% yield.[10] Other Lewis acids reported to promote the rearrangement were attempted. Among the Lewis acids tested, aluminum chloride gave a good result. When 0.5M of **5** in xylene was treated with 0.45 equivalent of aluminum chloride at 140°C for 20 minutes in a sealed tube, desired *ortho* cyclization products (**2a**, **2b** and **3**) along with **7** were obtained at 2.7%, 4.6%, 0.5% and 21.5% yield, respectively.[11] The cyclization products were deduced to be formed from **7** by addition of nitrogen at position 13 to the allyl group. To prove this, **7** was subjected to the same reaction condition of the 3-aza-Cope rearrangement of **5**. Compounds **2a**, **2b** and **3** were obtained at 5.3%, 5.6% and 1.0% yield, respectively.

Table 1. Several characteristic signals of the ^{1}H NMR spectra of **1**, **2a**, **3** and **4** in chloroform-*d* (500MHz)

Position	(-)-Indolactam-V (**1**) twist conformer[a]	(-)-Indolactam-V (**1**) sofa conformer[a]	δ (multiplicity, *J* in Hz) Compound **2a** twist conformer[b]	Compound **3** twist conformer[c]	(-)-5-Methylindolactam-V (**4**) sofa conformer[d]
8a	3.01 (dd, *J*=17.4, 3.8)	2.84 (dd, *J*=14.4, 1.6)	2.92 (dd, *J*=17.1, 4.3)	3.05 (dd, *J*=17.0, 4.1)	2.78 (dd, *J*=14.3, 1.5)
8b	3.20 (br.d, *J*=17.4)	3.11 (dd, *J*=14.4, 4.9)	3.21 (br.d, *J*=17.1)	3.50 (br.d, *J*=17.0)	3.21 (dd, *J*=14.3, 4.3)
10	6.59 (br.s)	4.72 (d, *J*=10.8)	6.48 (br.s)	6.48 (br.s)	4.91 (d, *J*=10.7)
12	4.39 (d, *J*=10.2)	2.99 (d, *J*=10.8)	4.33 (d, *J*=10.1)	5.35 (d, *J*=11.0)	2.99 (d, *J*=10.7)
14a	3.54 (m)	3.44 (m)	3.55 (m)	3.55 (m)	3.37 (m)
14b	3.74 (m)	3.44 (m)	3.71 (m)	3.72 (m)	3.37 (m)

[a]sofa:twist = 1.0:2.6 (0.004M, 27°C). [b]twist only (0.01M, 27°C). [c]twist only (0.003M, 27°C). [d]sofa >98% (0.02M, 27°C).

(-)-5-Methylindolactam-V (**4**), a negative control product, was synthesized by reduction of (-)-14-*O*-acetyl-5-formylindolactam-V with lithium aluminum hydride and aluminum chloride in tetrahydrofuran at 56% yield.[12] (-)-14-*O*-Acetyl-5-formylindolactam-V was obtained as a by-product of the Vilsmeier formylation of 14-*O*-acetate of **1** as reported previously.[13]

The ^{1}H NMR spectrum of **2a** in chloroform-*d* clearly showed that **2a** existed only as a single conformer in solution. The fixed conformation was determined to be the twist on the basis of several characteristic signals and coupling constants of the two conformers of **1** (Table 1). NOE-difference spectrum of **2a** also supported this conformation. Saturation of H-12 (δ4.33) resulted in a strong enhancement (11%) of the H-8b signal (δ3.21) as observed for the twist form of **1**.[5] The absolute configuration at position 18 was proved to be *R* by the NOE-difference spectra. Saturation of H-15 (δ2.65) caused a remarkable enhancement (10%) of the H-18 signal (δ4.04); no NOE enhancement except H-19a (δ2.58) was observed by saturation of H_3-20 (δ1.19). Compound **3** also existed only as the twist conformer (Table 1). Remarkable NOE enhancement between H-8b (δ3.50) and H-12 (δ5.35) of **3** supported this conformation.

On the other hand, **2b**, the C-18 epimer of **2a**, existed as two conformers which were deduced to be neither the sofa nor the twist form because of severe steric interaction between the isopropyl group at position 12 and the methyl group at position 18.[14] (-)-5-Methylindolactam-V (**4**) as previous reported (-)-5-fluoroindolactam-V[6] existed exclusively as the sofa conformer in chloroform-*d* as expected (Table 1).

The tumor-promoting activity of these compounds was examined by two *in vitro* bioassays closely related to *in vivo* tumor promotion: binding affinity to the protein kinase C regulatory domain and the Epstein-Barr virus early antigen (EBV-EA) inducing ability. Table 2 summarizes the results of these assays. The binding affinity of these compounds was evaluated by inhibition of the specific binding of [^{3}H]phorbol-12,13-dibutyrate (PDBu) to the regulatory domain of rat brain protein kinase C γ.[15-17] Binding affinity was evaluated by the concentration required to cause 50% inhibition of the specific binding of [^{3}H]PDBu, IC_{50}. The binding affinity of the sofa-restricted (-)-5-methylindolactam-V (**4**) was under the detection level, while the twist-restricted **2a** and **3** showed significant binding affinity comparable to that of (-)-indolactam-V (**1**).

Table 2. Biological activities of the indolactam derivatives (**1**, **2a**, **2b**, **3** and **4**)

Compound	Inhibition of specific [^{3}H]PDBu binding[a]	Epstein-Barr virus early antigen (EBV-EA) inducing activity[b]		
		Percentage of EA-positive cells at the indicated concentration		
	pIC_{50} (log1/M)	10^{-7}M	10^{-6}M	10^{-5}M
(-)-Indolactam-V (**1**)	5.56 (0.27)[c]	7.7 (2.5)	19.0 (4.1)	35.0 (1.8)
Compound **2a**	5.90 (0.18)	1.2 (1.1)	14.1 (2.8)	16.0 (2.1)
Compound **2b**	4.62 (0.23)	0.2 (0.3)	0.8 (0.4)	17.8 (0.7)
Compound **3**	5.00 (0.11)	12.7 (4.7)	34.0 (0.2)	36.1 (2.5)
(-)-5-Methylindolactam-V (**4**)	<4.00	0.3 (0.4)	1.4 (0.5)	9.4 (2.1)

[a]This assay was carried out by the polyethylene glycol precipitation method[15] using the model peptide containing the second cysteine-rich sequence of rat brain protein kinase C γ regulatory domain (amino acids 101-151).[16,17] The assay solution (250μl) consisted of 50mM Tris-HCl (pH 7.4), 3mg/ml bovine γ-globulin, 20nM[^{3}H]PDBu, 50μg/ml phosphatidylserine dioleoyl, 100nM the peptide and various concentrations of an inhibitor. [b]This assay was done by the method reported previously[18] with slight modifications.[19] Sodium *n*-butyrate (3mM) was added to all samples to enhance the sensitivity of Raji cells. The viability of the cells exceeded 70% in all experiments. Final dimethyl sulfoxide concentration was 0.5%. [c]Standard deviation.

A similar tendency was observed in the EBV-EA induction test. EBVs are under the strict control of the host human lymphoblastoid Raji cells. They are activated by treatment with tumor promoters to produce the early antigen (EA).[20] The EBV-EA-inducing activity is expressed as the percentage of EA-positive cells; in our experimental condition using a potent tumor promoter teleocidin B-4,[19] maximum induction between 35 and 40% was observed at 10^{-7}M. (-)-Indolactam-V (**1**) and the twist-restricted **2a** and **3** showed significant EBV-EA induction at 10^{-6}M. By contrast, the sofa-restricted **4** scarcely showed any induction at 10^{-6}M. Compound **2b**, whose conformation was deduced to be neither sofa nor twist, showed weak activity in both assays. It is noteworthy that **3** is a more potent EBV-EA inducer than **1** though **3** shows lower binding affinity to protein kinase C γ regulatory domain than **1**. Since protein kinase C plays a significant role in the induction of EBV-EA,[21] this disagreement would probably due to the diversity of protein kinase C isozymes.

These results clearly show that the active conformation of (-)-indolactam-V (**1**) is close to the twist form, not the sofa form. Although a similar conclusion has been recently reported by Kozikowski *et al.*[22] and Ohno *et al.*[23] using newly designed benzolactams, indolactam analogues without the pyrrole moiety, this is the first time to demonstrate the active conformation using the intact indolactam skeleton. Strictly speaking, there still remains a possibility of conversion from the twist form to other putative conformers[24,25] in **2a** and **3**. However, the present results remarkably reduce the number of active conformation candidates because of severe conformational restriction in **2a** and **3**. Bridge formation between position 5 and 13 of (-)-indolactam-V (**1**) is one of the most effective methods to fix the molecule to the active conformation and to develop new protein kinase C activators with high isozyme selectivity.

Acknowledgement

We thank Dr. J. Oda and Ms. K. Omine of the institute for Chemical Research at Kyoto University, Mr. R. Imamura of the Faculty of Science at Kyoto University for the ^{1}H NMR measurements. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas No.06240228 and for Encouragement of Young Scientists No.06760109 from the Ministry of Education, Science and Culture, Japan.

References and Notes

1. Takashima, M.; Sakai, H. *Bull. Agric. Chem. Soc. Jpn.* **1960**, *24*, 647.
2. Fujiki, H.; Sugimura, T. *Adv. Cancer Res.* **1987**, *49*, 223.
3. Endo, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1982**, *30*, 3457.
4. Irie, K.; Hirota, M.; Hagiwara, N.; Koshimizu, K.; Hayashi, H.; Murao, S.; Tokuda, H.; Ito, Y. *Agric. Biol. Chem.* **1984**, *48*, 1269.
5. Endo, Y.; Shudo, K.; Itai, A.; Hasegawa, M.; Sakai, S. *Tetrahedron* **1986**, *42*, 5905.
6. Okuno, S.; Irie, K.; Suzuki, Y.; Koshimizu, K.; Nishino, H.; Iwashima, A. *BioMed. Chem. Lett.* **1994**, *4*, 431.
7. Beholz, L. G.; Stille, J. R. *J. Org. Chem.* **1993**, *58*, 5095.
8. Nakatsuka, S.; Masuda, T.; Sakai, K.; Goto, T. *Tetrahedron Lett.* **1986**, *27*, 5735.
9. (-)-N^{13}-Desmethyl-N^{13}-allylindolactam-V (**5**): $[\alpha]_D$ -32.1° (*c*=0.28, MeOH, 21°C). UV λ_{max} (EtOH) nm (ε): 293 (7,000), 226 (25,000). ^{1}H NMR δ ($CDCl_3$, 0.045M, 27°C, sofa:twist=1.0:1.1) ppm: twist conformer; 0.70 (3H, d, *J*=6.8Hz, H_3-16 or 17), 0.93 (3H, d, *J*=6.3Hz, H_3-16 or 17), 2.59 (1H, m, H-15), 2.69 (1H, br.s, OH-14), 3.06 (1H, dd, *J*=17.4, 3.8Hz, H-8a), 3.15 (1H, br.d, *J*=17.4Hz, H-8b), 3.56 (1H, m, H-14a), 3.75 (1H, m, H-14b), 3.88 (1H, m, H-18a), 4.18 (1H, dd, *J*=15.8, 7.3Hz, H-18b), 4.39 (1H, d, *J*=10.0Hz, H-12), 4.45 (1H, m, H-9), 5.04 (1H, d, *J*=10.2Hz, H-20a), 5.22 (1H, d, *J*=17.1Hz, H-20b), 5.72 (1H, m, H-19), 6.54 (1H, d, *J*=7.8Hz, H-5), 6.89 (1H, s, H-2), 6.90 (1H, d, *J*=7.7Hz, H-7), 7.03 (1H, t, *J*=7.9Hz, H-6), 7.10 (1H, br.s, NH-10), 8.01 (1H, br.s, NH-1): sofa conformer; 0.94 (3H, d, *J*=6.5Hz, H_3-16 or 17), 1.28 (3H, d, *J*=6.6Hz, H_3-16 or 17), 1.48 (1H, br.s, OH-14), 2.38 (1H, m, H-15), 2.84 (1H, dd, *J*=14.6, 1.4Hz, H-8a), 3.02 (1H, d, *J*=10.9Hz, H-12), 3.18 (1H, dd, *J*=14.6, 4.8Hz, H-8b), 3.42 (2H, m, H_2-14), 3.52 (1H, dd, *J*=15.2, 6.7Hz, H-18a), 3.88 (1H, m, H-18b), 4.45 (1H, m, H-9), 4.77 (1H, d, *J*=10.0Hz, H-20a), 4.83 (1H, br.d, *J*=10.5Hz, NH-10), 4.84 (1H, d, *J*=18.7Hz, H-20b), 5.72 (1H, m, H-19), 7.04 (1H, s, H-2), 7.05 (1H, dd, *J*=7.0, 0.7Hz, H-5), 7.15 (1H, t, *J*=7.7Hz, H-6), 7.27 (1H, d, *J*=7.7Hz, H-7), 8.35 (1H, br.s, NH-1). HR-EIMS *m/z*: 327.1943 (M^+, calcd. for $C_{19}H_{25}N_3O_2$, 327.1948).
10. Compound **7**: $[\alpha]_D$ -182.4° (*c*=0.13, MeOH, 23°C). UV λ_{max} (EtOH) nm (ε): 281 (6,200), 229 (29,900), ^{1}H NMR δ ($CDCl_3$, 0.015M, 27°C) ppm: 1.06 (3H, d, *J*=6.8Hz, H_3-16 or 17), 1.24 (3H, d, *J*=6.5Hz, H_3-16 or 17), 2.23 (1H, m, H-15), 2.54 (1H, br.s, OH-14), 2.88 (1H, dd, *J*=15.4, 9.4Hz, H-8a), 3.08 (1H, dd, *J*=15.4, 6.9Hz, H-8b), 3.40-3.56 (5H, m, H_2-18, H-12, H-14a, NH-13), 3.74 (1H, m, H-14b), 4.97 (1H, dd, *J*=17.1, 1.7Hz, H-20a), 5.07 (1H, dd, *J*=10.2Hz, H-20b), 5.42 (1H, br.s, H-9), 5.83 (1H, d, *J*=10.2Hz, NH-10), 6.03 (1H, m, H-19), 6.80 (1H, s, H-2), 6.95 (1H, d, *J*=8.2Hz, H-6 or 7), 7.03 (1H, d, *J*=8.2Hz, H-6 or 7), 7.94 (1H, br.s, NH-1). HR-EIMS *m/z*: 327.1946 (M^+, calcd. for $C_{19}H_{25}N_3O_2$, 327.1948).
11. Compound **2a**: $[\alpha]_D$ -333.9° (*c*=0.078, MeOH, 20°C). UV λ_{max} (EtOH) nm (ε): 319 (8,300), 288.5 (5,900), 235 (22,900). ^{1}H NMR δ ($CDCl_3$, 0.01M, 27°C, twist only) ppm: twist conformer; 0.72 (3H, d, *J*=6.6Hz, H_3-16 or 17), 1.00 (3H, d, *J*=6.3Hz, H_3-16 or 17), 1.19 (3H, d, *J*=6.4Hz, H_3-20), 2.05 (1H, br.s, OH-14), 2.58 (1H, dd, *J*=15.3, 3.7Hz, H-19a), 2.65 (1H, m, H-15), 2.92 (1H, dd, *J*=17.1, 4.3Hz, H-8a), 3.21 (1H, br.d, *J*=17.1Hz, H-8b), 3.55 (1H, m, H-14a), 3.63 (1H, dd, *J*=15.3, 9.8Hz, H-19b), 3.71 (1H, m, H-14b), 4.04 (1H, m, H-18), 4.25 (1H, br.s, H-9), 4.33 (1H, d, *J*=10.1Hz, H-12), 6.48 (1H, br.s, NH-10), 6.71 (1H, d, *J*=8.1Hz, H-6 or 7), 6.85 (1H, s, H-2), 6.91 (1H, d, *J*=8.1Hz, H-6 or 7), 7.82 (1H, br.s, NH-1). HR-EIMS *m/z*: 327.1944 (M^+, calcd. for $C_{19}H_{25}N_3O_2$, 327.1948).

    Compound **2b**: $[\alpha]_D$ +27.4° (*c*=0.11, MeOH, 20°C). UV λ_{max} (EtOH) nm (ε): 317 (6,500), 280 (4,300), 235 (20,200). ^{1}H NMR δ ($CDCl_3$, 0.037M, -10°C, major:minor=1.1:1.0) ppm: major conformer; 1.05 (3H, d, *J*=6.5Hz, H_3-16 or 17), 1.14 (3H, d, *J*=6.4Hz, H_3-16 or 17), 1.48 (3H, d, *J*=6.2Hz, H_3-20), 2.51 (1H, d, *J*=15.3Hz, H-8a), 2.57 (1H, m, H-15), 2.77 (1H, dd, *J*=14.5, 10.1Hz, H-19a), 3.22 (2H,

m, H-9, H-19b), 3.58-3.80 (3H, m, H-8b, H_2-14), 4.31 (1H, m, H-18), 4.62 (1H, d, *J*=10.5Hz, H-12), 6.50 (1H, br.s, NH-10), 6.65 (1H, d, *J*=7.9Hz, H-6 or 7), 6.86 (1H, d, *J*=2.1Hz, H-2), 6.89 (1H, d, *J*=7.9Hz, H-6 or 7), 8.03 (1H, br.s, NH-1): minor conformer; 0.37 (3H, br.s, H_3-16 or 17), 0.96 (3H, d, *J*=6.9Hz, H_3-16 or 17), 1.30 (3H, d, *J*=5.9Hz, H_3-20), 2.15 (1H, br.s, H-15), 2.65 (1H, m, H-8a), 2.99 (1H, br.d, *J*=13.5Hz, H-8b), 3.06 (1H, dd, *J*=20.9, 10.0Hz, H-19a), 3.22 (1H, m, H-19b), 3.58-3.80 (4H, m, H-12, H_2-14, H-18), 3.91 (1H, br.s, OH-14), 4.22 (1H, br.s, H-9), 6.91 (1H, d, *J*=2.0Hz, H-2), 6.99 (1H, d, *J*=8.1Hz, H-6 or 7), 7.03 (1H, d, *J*=8.1Hz, H-6 or 7), 7.65 (1H, br.d, *J*=10.4Hz, NH-10), 8.25 (1H, br.s, NH-1). HR-EIMS *m/z*: 327.1952 (M^+, calcd. for $C_{19}H_{25}N_3O_2$, 327.1948).

Compound **3**: UV λ_{max} (EtOH) nm (ε): 314 (2,000), 301 (2,700), 282.5 (5,500), 272 (6,000), 246.5 (27,000), 243 (26,800). ^1H NMR δ ($CDCl_3$, 0.003M, twist only) ppm: twist conformer; 0.20 (3H, d, *J*=6.7Hz, H_3-16 or 17), 1.04 (3H, d, *J*=6.3Hz, H_3-16 or 17), 1.72 (1H, br.s, OH-14), 2.58 (3H, d, *J*=0.9Hz, H_3-20), 2.94 (1H, m, H-15), 3.05 (1H, dd, *J*=17.0, 4.1Hz, H-8a), 3.50 (1H, br.d, *J*=17.0Hz, H-8b), 3.55 (1H, m, H-14a), 3.72 (1H, m, H-14b), 3.91 (1H, m, H-9), 5.35 (1H, d, *J*=11.0Hz, H-12), 6.38 (1H, d, *J*=0.9Hz, H-19), 6.48 (1H, br.s, NH-10), 6.97 (1H, s, H-2), 7.11 (1H, d, *J*=8.4Hz, H-6 or 7), 7.29 (1H, d, *J*=8.4Hz, H-6 or 7), 8.09 (1H, br.s, NH-1). HR-EIMS *m/z*: 325.1780 (M^+, calcd. for $C_{19}H_{23}N_3O_2$, 325.1790).

12. (-)-5-Methylindolactam-V (**4**): $[\alpha]_D$ +86.2° (*c*=0.11, MeOH, 17°C). UV λ_{max} (EtOH) nm (ε): 290 (6,300), 226 (24,900). ^1H NMR δ ($CDCl_3$, 0.022M, sofa > 98%) ppm: sofa conformer; 0.97 (3H, d, *J*=6.5Hz, H_3-16 or 17), 1.29 (3H, d, *J*=7.0Hz, H_3-16 or 17), 2.46 (3H, s, H_3-19), 2.65 (1H, m, H-15), 2.73 (3H, s, H_3-18), 2.78 (1H, dd, *J*=14.3, 1.5Hz, H-8a), 2.99 (1H, d, *J*=10.7Hz, H-12), 3.21 (1H, dd, *J*=14.3, 4.3Hz, H-8b), 3.37 (2H, m, H_2-14), 4.47 (1H, m, H-9), 4.91 (1H, d, *J*=10.7Hz, NH-10), 7.01 (1H, d, *J*=2.5Hz, H-2), 7.08 (1H, d, *J*=8.2Hz, H-6 or 7), 7.18 (1H, d, *J*=8.2Hz, H-6 or 7), 8.22 (1H, br.s, NH-1). HR-EIMS *m/z*: 315.1933 (M^+, calcd. for $C_{18}H_{25}N_3O_2$, 315.1948).

13. Irie, K.; Okuno, S.; Koizumi, F.; Koshimizu, K.; Nishino, H.; Iwashima, A. *Tetrahedron* **1993**, *49*, 10817.
14. The conformation of **2b** is under investigation.
15. Sharkey, N. A.; Blumberg, P. M. *Cancer Res.* **1985**, *45*, 19.
16. Miller, B. L. Ph. D. dissertation, Stanford University, 1994.
17. Wender, P. A.; Irie, K.; Miller, B. L. *Proc. Natl. Acad. Sci. USA*, in press.
18. Ito, Y.; Yanase, S.; Fujita, J.; Harayama, T.; Takashima, M.; Imanaka, H. *Cancer Lett.* **1981**, *13*, 29.
19. Murakami, A.; Tanaka, S.; Ohigashi, H.; Hirota, M.; Irie, R.; Takeda, N.; Tatematsu, A.; Koshimizu, K. *Biosci. Biotech. Biochem.* **1992**, *56*, 769.
20. zur Hausen, H.; Bornkamm, G. W.; Schmidt, R.; Hecker, E. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 782.
21. Lazdins, J.; Zompetta, C.; Grimaldi, S.; Barile, G.; Venanzoni, M.; Frati, L.; Faggioni, A. *Int. J. Cancer* **1981**, *28*, 125.
22. Kozikowski, A. P.; Ma, D.; Pang, Y-P.; Shum, P.; Likic, V.; Mishra, P. K.; Macura, S.; Basu, A.; Lazo, J. S.; Ball, R. G. *J. Am. Chem. Soc.* **1993**, *115*, 3957.
23. Ohno, M.; Endo, Y.; Hirano, M.; Itai, A.; Shudo, K. *Tetrahedron Lett.* **1993**, *34*, 8119.
24. Kawai, T.; Ichinose, T.; Endo, Y.; Shudo, K; Itai, A. *J. Med. Chem.* **1992**, *35*, 2248.
25. Kawai, T.; Ichinose, T.; Takeda, M.; Tomioka, N.; Endo, Y.; Yamaguchi, K.; Shudo, K.; Itai, A. *J. Org. Chem.* **1992**, *57*, 6150.

(*Received in Japan* 5 *December* 1994; *accepted* 10 *January* 1995)