STRUCTURE-ACTIVITY STUDIES OF INDOLE ALKALOID TUMOR PROMOTERS

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Contents

Abbreviations

The following abbreviations are used: BL, Burkitt's lymphoma; CD, circular dichroism; dA-ILV, (-)-2,7-diacetylindolactam V; dansyl-ILV, (-)-7-(2-*N*-dansylaminoethyl)indolactam V; dansyl-ILV-Me, (-)-7-(2-*N*-dansylaminoethyl)-14-*O*-methylindolactam V; dD-ILV, (-)-2,7-didecanoylindolactam V; DEF, *S*,*S*,*S*-tributyl phosphorotrithioate; EBV-EA, Epstein-Barr virus early antigen; EA, early antigen; EIMS, electron ionization mass spectroscopy; EMEM, Eagle's minimum essential medium; FD-ILV, (-)-2-formyl-7-decanoylindolactam V; HL-60, human promyelocytic leukemia; HPLC, high performance liquid chromatography; HR-EIMS, high resolution-EIMS; mansyl-ILV, (-)-7-[2-*N*-(2,6-mansyl)aminoethyl]indolactam V; MeOTs, methyl *p*-toluenesulfonate; NOE, nuclear Overhauser effect; NPC, nasopharyngeal carcinoma; ODC, ornithine decarboxylase; PBS, phosphate-buffered saline; PDBu, phorbol dibutyrate; PKC, protein kinase C; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Abstract

Carcinogenic promoters produced by microorganisms were sought using the Epstein-Barr virus early antigen (EBV-EA) induction test, a short-term promoter test system, and one strain, *Streptoverticillium*

blastmyceticum NA34-17, was found to induce potent EBV-EA. Three biologically-active indole alkaloids, **1**, **2** and **3**, were isolated from the culture broth, and **1** was shown to be (-)-indolactam V, the basic ring-structure of teleocidins. Compound **2** was identified as (-)-14-*O*-acetylindolactam V and **3** as teleocidin B-4.

(-)-Indolactam V is a key compound for structure-activity studies of teleocidin-related compounds because its structure is fundamental to teleocidins and it has various activities itself. The author synthesized about 50 indolactam derivatives, and examined their EBV-EA inducing activity and their binding ability to the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) receptor. The results clarified the structural factors involved in these activities. The hydroxyl group at position 14 of (-)-indolactam V was shown to play an important role in the activities. Large substituents at position 2 or 5 of the indole ring conspicuously lowered the activities, and hydrophobic substituents at position 7 enhanced the activities. Further, the effects of the substituents at position 7 on binding ability to the TPA receptor were analyzed quantitatively using physicochemical substituent parameters and regression analysis. Hydrophobicity of the substituents was found to play a critical role for receptor binding, suggesting that the monoterpenoid moiety of teleocidins was involved in the non-specific hydrophobic interaction with phospholipids in the cell membrane to stabilize the receptor-ligand binding.

In addition to these structure-activity studies, direct identification of the putative receptor sites of tumor promoters using photolabile or fluorescent derivatives is indispensable for revealing the mechanism of tumor promotion. The author synthesized a variety of fluorescent and photolabile indolactam derivatives on the basis of the present structure-activity studies. To screen out more effective fluorescent probes for receptor analysis, the cellular uptake of these fluorescent indolactam derivatives by HeLa cells or C3H 10T1/2 cells was examined. The results indicated that (-)-7-(2-*N*-dansylaminoethyl)indolactam V (dansyl-ILV) and (-)-2-formyl-7-decanoylindolactam V (FD-ILV) would be of practical use for investigating the cellular receptors of tumor promoters in combination with their inactive analogues.

Prologue

Chemical carcinogenesis is nowadays interpreted by "Beremblum's two-stage carcinogenesis theory"[1] of initiation and promotion. Tumor initiators are carcinogens which bind covalently to cellular DNA; tumor promoters are defined as compounds which have no carcinogenic activity by themselves, but drastically enhance tumor yield when applied repeatedly after the treatment of a small amount of an initiator. When Beremblum proposed the two-stage carcinogenesis concept about 50 years ago, polycyclic aromatic hydrocarbons, for example, 7, 12-dimethylbenz[a]anthracene and benzo[a]pyrene, had already been found to be potent initiators. However, the pure tumor-promoting agent was not known, and croton oil, obtained from seeds of *Croton tiglium*, was widely used for two-stage carcinogenesis experiments.

In the latter half of the 1960's, the active principles of the croton oil were isolated by Hecker *et al.* and van Duuren *et al.* independently.[2,3] Among these principles, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was most active. Fullscale studies on tumor promotion began with this as a turning point. In the 1970's, numerous investigations on structure-activity relationships and biological effects *in vivo* and *in vitro* (Table 1) were carried out employing TPA. These revealed that the hydroxyl group at position 20, the β-configuration of the hydroxyl group at position 4 and the ketone at position 3 of TPA played an important role in the appearence of tumor-promoting activity.[20] The long fatty acid chain at position 12 was found to be necessary for enhancing the activity.

Table 1. Biological activities of TPA

Biological activities	References
I Enzymatic reactions	
1) Induction of ornithine decarboxylase (ODC)	4
2) Activation of protein kinase C (PKC)	5
3) Release of arachidonic acid and formation of prostagrandins	6, 7
4) Enhancement of 32,000 and 46,000 protein synthesis	8, 9
5) Induction of plasminogen activator	10
6) Formation of superoxide anion radical	11
II Cellular responses	
1) Irritation of mouse ear	12
2) Inhibition of cell-cell communication	13, 14
3) Adhesion and induction of differentiation of human promyelocytic leukemia (HL-60) cells	15, 16
4) Inhibition of terminal differentiation of Friend erythroleukemia cells	17
5) Induction of Epstein-Barr virus (EBV)	18, 19

The facts that there exist strict structural requirements for activity, and that TPA induces a number of biological activities *in vitro* (Table 1) at doses as low as 10^{-8}~10^{-9}M, indicate the possible existence of TPA receptors which mediate these pleiotropic effects. In 1980, several groups found direct evidence for specific high affinity saturable receptors of phorbol esters in membrane preparations and intact cells by use of [^{3}H]phorbol dibutyrate (PDBu).[21-24]

Nishizuka *et al.* have recently proposed that TPA is a potent activator of Ca^{2+} and phospholipid dependent protein kinase C (PKC).[5,25] Immediately after, several investigators have shown that the specific binding sites of TPA in membrane preparations copurified with PKC.[26-28] These results strongly suggest that the major binding site of TPA is PKC, and that PKC acts as the initial signal tranducers of TPA to cause a number of pleiotropic effects *in vitro*. This mechanism is attractive since several endogenous growth factors and oncogenes may mediate their effects through protein phosphorylation. These findings were epoch-making in the research on tumor promotion. However, not all tumor promotion can be explained by PKC. There exist several tumor promoters which do not activate PKC *in vitro*.[29,30] The existence of specific binding sites in addition to PKC is not completely ruled out. It is also not known whether the target sites of TPA are only the cell membrane, or whether TPA is taken up by cells and binds intracytoplasmic sites.

Recent studies on environmental carcinogenesis have indicated that naturally occurring tumor promoters rather than initiators play a significant role in human cancer : for example, Weber *et al.* have reported that the frequent incidence of esophageal cancer in Curaqao might be ascribable to the habit of chewing *Croton flavens* (Euphorbiaceae), from which phorbol ester type tumor promoters were isolated.[31] Ito *et al.* have also proposed the hypothesis that tumor-promoting substances might be present in the areas where African Burkitt's lymphoma (BL), anaplastic nasopharyngeal carcinoma (NPC) or adult

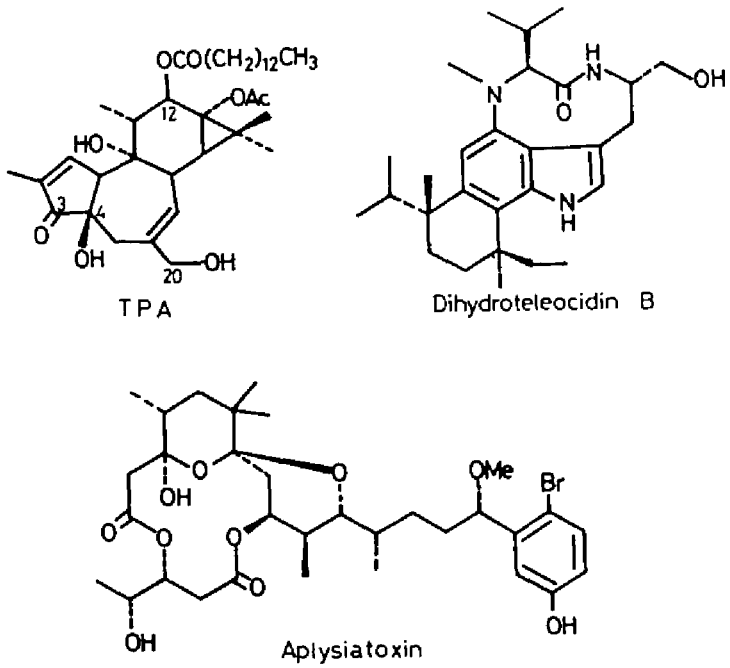


Fig. 1. Structure of naturally occurring potent tumor promoters.

T-cell leukemia are endemic.[32] This activated the search for new potent tumor promoters other than phorbol esters in the environment. The identification of new potent tumor promoters is also a pressing need from the view point of further elucidation of the mechanism of tumor promotion as well as prevention of cancer.

Since the promoter test using mice is a lengthy procedure, quicker promoter test systems have been developed using the biological activities of TPA (Table 1). Fujiki *et al.* had the working hypothesis that other potent tumor promoters existed that were structurally different from TPA in the environment. They subjected numerous skin irritant agents to two short term tests, irritation of mouse ear[12] and induction of ornithine decarboxylase (ODC)[4] in mouse skin, and have recently found teleocidins and aplysiatoxins (Figure 1) as potent tumor promoters comparable to TPA.[33]

From the standpoint of environmental carcinogenesis, microorganisms are noteworthy especially in Japan, where fermented foods are common. Hitherto, a large number of biologically active compounds have been isolated from microorganisms. This prompted the author to search for tumor promoters produced by microorganisms. The Epstein-Barr virus early antigen (EBV-EA) induction test was chosen as a short-term promoter test since crude extracts of culture broth of microorganisms could be tested without purification by this assay system. EBV belongs to the herpes virus group, and is thought to be the cause of BL and NPC.[34] EBVs are under the strict control of the host human lymphoblastoid Raji cells. They are activated, however, by treatment with such chemicals as tumor promoters to produce the early antigen (EA),[18] which can easily be

detected by an indirect immunofluorescence technique.[35] Ito *et al.*[19] suggested that the EBV-EA induction test using Raji cells was useful for the detection of tumor promoters in this environment. Using this test system, the author screened about 5000 actinomycetes, and found one strain, NA34-17, showing potent EBV-EA induction. This strain produced in large quantities a new microbial metabolite, (-)-indolactam V, recently synthesized as the basic ring-structure of teleocidins.[36]

Teleocidins were first isolated as skin irritants from *Streptomyces mediocidicus* by Takashima *et al.*[37~40] They found two types of compounds, and designated them teleocidin A (MW. 437) and teleocidin B (MW. 451). A main component of the teleocidin B type compounds was purified to give "teleocidin B" which was subjected to chemical modifications to yield dihydroteleocidin B monobromoacetate, whose structure was determined by X-ray analysis.[41~43] Later, teleocidin A was shown to consist of two isomers (teleocidin A-1 and A-2)[44]; teleocidin B, four isomers (teleocidin B-1~B-4).[45] "Teleocidin B", which Hirata *et al.* isolated, proved to be identical with teleocidin B-4. In 1979, Moore *et al.* isolated lyngbyatoxin A[46] as a highly inflammatory substance from *Lyngbya majuscula* which was responsible for a severe dermatitis known as "swimmer's itch" in Hawaii.[47,48] The structure of lyngbyatoxin A was closely related to that of teleocidins, and lyngbyatoxin A proved identical with teleocidin A-1.[44] It was in 1981 that Fujiki *et al.* published the tumor-promoting activity of teleocidins.[49] This stimulated a wide range of studies on teleocidins, for example, structure determination of teleocidin-related compounds,[50~55] total synthesis of (-)-indolactam V[36,56,57] and teleocidins,[58,59] and their biological activities *in vivo* and *in vitro*,[60] since the teleocidins were the first potent tumor promoters whose

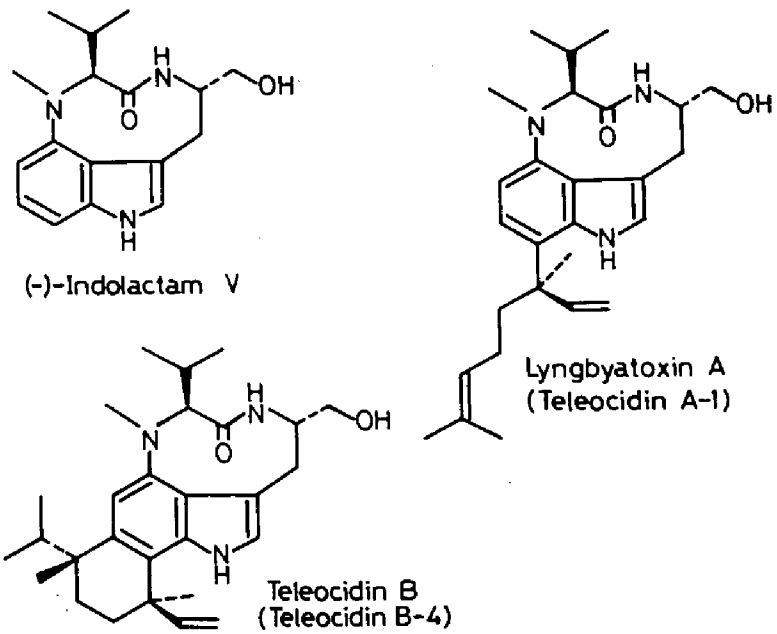


Fig. 2. Structure of naturally occurring teleocidin-related compounds.

structure was quite different from TPA.

Teleocidins have the special advantage of smaller molecular weight and higher stability than the other potent tumor promoters, phorbol esters and aplysiatoxins. This allows teleocidins to be useful for studying the mechanism of tumor promotion. This is especially true for (-)-indolactam V, whose structure is fundamental to teleocidin-related compounds, and is a key compound for investigating structure-activity relationship because it has tumor-promoting activity itself.[61] Structure-activity studies using a variety of indolactam derivatives are important since they contribute to the elucidation of the structural requirement for tumor-promoting activity and provide a basis for designing TPA antagonists and new probes for receptor analysis of tumor promoters, which are useful not only for chemoprevention of cancer, but also for investigating the mechanism of tumor promotion. Recently, TPA, teleocidins and aplysiatoxins have been shown to bind to the same high affinity receptor,[62-64] and the structural similarity of these three potent tumor promoters is being investigated by computer graphics.[65,66] The structure-activity studies on a wide range of indolactam derivatives will also contribute to the elucidation of this structural similarity.

Since the author has succeeded in obtaining large quantities of (-)-indolactam V from natural sources, he has made the most of the above-mentioned characteristics of (-)-indolactam V. This paper describes isolation of (-)-indolactam V, structure-activity studies of teleocidin, synthesis of biologically active fluorescent and photolabile indolactam derivatives, and cellular uptake studies of these fluorescent probes.

I. Isolation of (-)-Indolactam V, the Fundamental Structure of Teleocidins

Carcinogenic promoters produced by microorganisms were sought using the EBV-EA induction test, and one strain, NA34-17, was found to induce potent EBV-EA. Two biologically active compounds, **1** and **2**, along with a small amount of teleocidin B-4 (**3**)[41,45] were, isolated from the culture broth of this microorganism. This section deals with the isolation and structure of these compounds.

NA34-17 was cultured by deep aerated fermentation for 70 hr, and the filtered broth (40 l) was extracted with ethyl acetate. The ethyl acetate extract was chromatographed on silica gel with acetone-toluene mixtures to give three active fractions : 20, 30 and 40% acetone eluates. The 40% acetone eluate was purified by ODS column chromatography to give **1** (700 mg). Fractionation of the 20 and 30% acetone eluates by ODS column chromatography, followed by preparative high performance liquid chromatography (HPLC), led to the isolation of **2** (8.2 mg) and teleocidin B-4 (**3**, 9.2 mg), respectively.

The molecular formula of **1** was established to be $C_{17}H_{23}N_3O_2$ by high-resolution electron ionization mass spectroscopy (HR-EIMS) : observed *m/z*, 301.17907 ; calculated *m/z*, 301.17903. The presence of the indole chromophore in **1** was revealed by its UV spectrum [λ_{max}^{EtOH} nm (ε) : 301 (7900), 229 (28,100)], together with IR absorption bands at 1610, 1565, 1500 cm^{-1} and eight aromatic carbons in the ^{13}C NMR spectrum of **1**. Acetylation of **1** with acetic anhydride-pyridine gave the monoacetate whose ^1H NMR spectrum showed an AB quartet ascribable to hydroxymethyl shifted upfield compared

with those of **1**. The IR spectrum of **1** exhibited an amide carbonyl absorption at 1650 cm^{-1}. Acid hydrolysis of **1** gave the lactone amine (**4**) (IR ν_{max}^{KBr} cm^{-1} : 1730), which was easily reversed back to **1** by alkaline treatment. This fact indicates that a primary alcohol and an amide bond in **1** are located near each other. The mass spectrum of **1** showed the fragment ions at m/z : 301 (M^+), 270 (M^+-CH_2OH) and 258 (M^+-C_3H_7), supporting the presence of a primary alcohol and a propyl moiety.

The ^1H NMR spectrum of **1** indicated the presence of an *N*-substituted methyl at δ 2.92 (3H, s) ; an isopropyl at δ 2.59 (1H, m), 0.63 (3H, d) and 0.93 (3H, d) ; a hydroxymethyl at δ 3.57 (1H, m) and 3.74 (1H, m) ; a methylene at δ 3.05 (1H, dd) and 3.17 (1H, br. d) ; two methines at δ 4.31 (1H, br. s) and 4.40 (1H, d) ; four aromatic protons at δ 6.50 (1H, d), 6.89 (1H, s), 6.90 (1H, d) and 7.06 (1H, t) ; and two NH protons at δ 7.37 (1H, br. s) and 8.02 (1H, br. s). The spectrum was very similar to that of lyngbyatoxin A[46] (teleocidin A-1,[44] **5**). In the ^1H NMR spectrum of **1**, all the proton signals of **5**, except for the protons of the monoterpenoid moiety at position 7, were observed. Furthermore, the ^1H NMR spectrum of **1** exhibited four well-separated aromatic protons at δ 6.50 (1H, d, J=7.6 Hz), 6.89 (1H, s), 6.90 (1H, d, J= 7.6 Hz) and 7.06 (1H, t, J=7.6 Hz), which indicated that the monoterpenoid moiety at position 7 of lyngbyatoxin A (**5**) was replaced by a hydrogen in **1**. This assignment was supported by the ^{13}C NMR spectrum of **1**.

Compound **1** has two asymmetric centers and is optically active. The circular dichroism (CD) spectrum of **1** was similar to that of dihydroteleocidin B(**6**),[41,46] suggesting that **1** and **6** have the same absolute configuration in the nine-membered lactam ring. This is also supported by the presence of teleocidin B-4 (**3**) in the culture broth of NA 34-17 since **1** seems to be biosynthesized through the same pathway as that of teleocidin

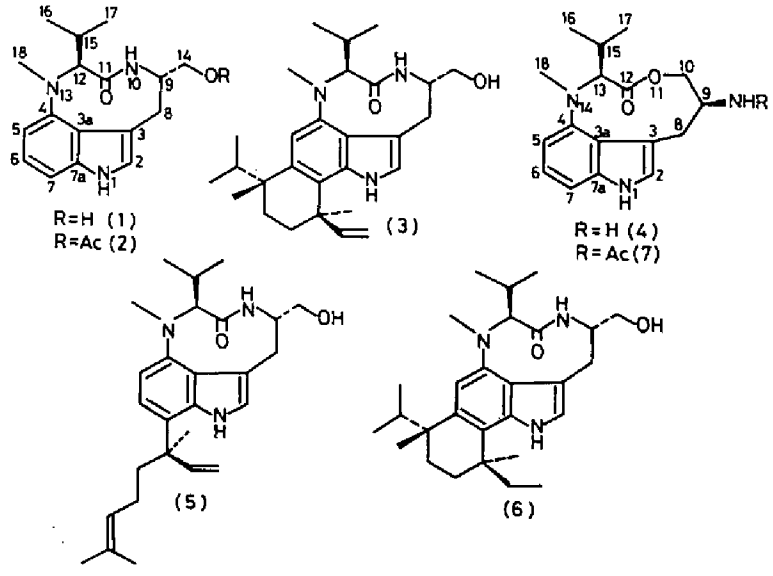


Fig. 3. Naturally occurring teleocidin-related compounds and their derivatives.

B-4. From the above data, **1** is shown to have the structure **1**, which was quite recently synthesized by Endo *et al.* and termed (-)-indolactam V.[36,67] Compound **1** had the same CD spectrum as that of (-)-indolactam V.[67] Compound **2**, a new metabolite, was identified as the 14-*O*-acetate of **1** by its comparison with the spectral data of **2** derived from **1**.

Moore *et al.*[46] have reported that several signals in the ^{1}H NMR spectrum of lyngbyatoxin A (**5**) were doubled in a 5 : 1 ratio in chloroform-d. They performed a temperature study and suggested that the doubling of signals could be due to two conformations of the toxin. They could not exclude, however, the possibility that lyngbyatoxin A (**5**) was a mixture of two structural isomers in which the side chain was substituted at position 5 or 7 of the indole ring.

Though in the ^{1}H NMR spectrum of **1**~**4** the signals were doubled, those of the 9-*N*-acetate of **4** (**7**) were not doubled. This strongly suggests that the doubling signals can be ascribed to the conformers of the nine-membered lactam ring and not to the structural isomers. A temperature study of **1** at 90 MHz in deuterated dimethyl sulfoxide and pyridine was attempted at first. Although some signal changes were observed in both solvents, definite evidence of the existence of two conformers could not be obtained. Subsequently, **1** was analyzed by HPLC using a μ-Porasil column with a mixture of chloroform and 2-propanol (95 : 5). When the column and solvent were cooled below -10°C, **1** separated into two peaks. Each peak was collected at room temperature. Both the ^{1}H NMR spectra coincided with that of I, confirming that **1** was present as two conformational isomers at room temperature. Recently, Endo *et al.* have proposed the structure of the two conformers, conformer A of the SOFA type and B of the TWIST type, on the basis of the nuclear Overhauser effect (NOE) and exhaustive examination of molecular models, followed by empirical force-field calculation.[68,69]

A numbering system based on (-)-indolactam V (**1**) would be pertinent. Hitherto, the monoterpenoid moiety on the indole ring has taken precedence in numbering over the substituents on the nine-membered lactam ring in teleocidins.[46] But in the new numbering system proposed, the substituents on the nine-membered lactam ring take precedence over the monoterpenoid moiety on the indole ring because (-)-indolactam V (**1**) is the

Table 2. EBV-EA inducing activity of teleocidin-related compounds[a]

Compound number	Percentage of EA-positive cells at the indicated concentration (μg/ml)					
	0.008	0.04	0.2	1	5	25
1	14.1	26.3	31.1	20.6	16.4	
2		11.1	21.6	29.6	15.6	
3	20.2	32.0	28.0	18.1	11.0	
4		2.1	5.7	16.4	12.1	12.0
7		0.1	0.1	7.1	0.1	0.1

[a] Sodium *n*-butyrate was added to all samples to enhance the sensitivity of Raji cells. Only 0.1% EA-induction was demonstrated at 4 mM. The viability of the cells exceeded 60% in each experiment.

fundamental structure in teleocidin-related compounds. The new numbering system has been introduced here to discuss the contribution of the functional groups of teleocidin-related compounds to activity.

The EBV-EA inducing activity of I and its related compounds is summarized in Table 2. The EBV-EA inducing activity is expressed as the percentage of EA-positive cells, and evaluated in terms of this percentage, and of the concentration of the chemical required to induce maximal EA production. The percentage of EA-positive cells decreased at a superoptimum concentration, and this has previously been attributed to cytotoxic effects of chemicals.[70] Compound **1** showed potent EBV-EA inducing activity although it was less active than teleocidin B-4 (**3**). Compound **2**, the 14-*O*-acetate of **1**, also induced EBV-EA while **4** had only a little activity, and **7** had no activity. Some part of the activity of **4** appeared to be caused by **1** formed by the isomerization of **4**. These indicate that the nine-membered lactam ring is essential for the EBV-EA induction. This ring moiety has also been found to be important for the induction of ODC, the adhesion of human promyelocytic leukemia (HL-60) cells, the transformation of BALB 3T3 (A31-1-1) cells, and the binding ability to the TPA receptor, by using four synthetic indolactam V diastereomers.[71] Furthermore, (-)-indolactam V(**1**) acted as a tumor promoter in a two-stage carcinogenesis experiment.[61] These results indicate that (-)-indolactam V (**1**) is highly suitable as the starting material for synthesizing a number of teleocidin-related compounds for structure-activity studies. The detailed structure-activity relationship of **1** and its analogues is summarized in Chapter IV.

Experimental

General remarks. Melting points are not corrected. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200: ORD, Jasco Model J-5; ^{1}H NMR, Hitachi Model R-22 (90 MHz, ref. TMS) and JEOL GX 400 (400 MHz, ref. TMS); ^{13}C NMR, JEOL GX 400 (100 MHz, ref. TMS); IR, Shimadzu Model 435; HPLC, Waters Model M6000A with a UV detector; CD, Jasco Model J-500; MS, JEOL JMS-DX 300 (70 eV, 300 μA).

HPLC was carried out on YMC packed S-343 and A-311 column (Yamamura Chemical Laboratory), μ-Porasil, semi preparative μ-Bondapak C_{18} and NOVA-PAK C_{18} (Waters Associates). Wako C-100 and C-200 gel (Wako Pure Chemical Industries) and YMC I-40/64 gel (Yamamura Chemical Laboratory) were used for column chromatography.

Isolation and cultivation of microorganisms. Test strains were isolated from soil by conventional methods. Each of about 5000 isolates of actinomycetes was inoculated into test tubes (2.4 cm × 20 cm) with a medium (10 ml) consisting of 1% glucose, 1% polypeptone (Daigo Eiyo Kagaku), 1% meat extract (Wako Pure Chemicals) and 0.5% NaCl (pH 7.0). After shaking at 30°C for 3 to 4 days, the culture broth was filtered. The filtrate was used for the assay of EBV-EA induction at concentrations of 10, 2 and 0.4 μl/ml.

Strain NA34-17 kept on Waksman's medium was transferred to a 500 ml shaking flask containing 100 ml of the medium mentioned above, and the flask was shaken at 30°C for 50 hr. The seed culture thus obtained was transferred to a 30-liter jar fermenter (Marubishi type MSJ-U2) containing 20 liters of a medium. The conditions of the cultivation were as follows: medium, 2% glucose, 1% polypeptone, 1% meat extract, 0.5% NaCl, 0.05% adekanol; initial pH, 7.0; temperature, 30°C; aeration, 22 liters/min; agitation, 400 rpm.

Isolation of 1, 2 and 3. The culter filtrate (40 liters) was concentrated to 4 liters *in vacuo* at 40°C and then extracted with 10 liters of EtOAc. The EtOAc layer was dried over sodium sulfate and

evaporated *in vacuo* to give a brown oily syrup (13 g). The residue was chromatographed on Wako C-200 gel (500 g), eluting with toluene containing increasing amounts of acetone to give three active fractions: I (20% acetone, 1.26 g), II (30% acetone, 1.18 g) and III (40% acetone, 5.66 g).

Fraction I was chromatographed on 160 g of YMC I-40/64 gel with 1 liter of a linear gradient of MeOH-water, starting with 50% MeOH and ending with 100% MeOH. The active fraction (42.6 mg) eluted with the MeOH-water mixture (at 600 ml~735 ml elution) was further purified by HPLC on semi prep. μ-Porasil with 30% EtOAc in hexane, followed by HPLC on semi prep. μ-Bondapak C_{18} with 60% MeOH in water to give **2** (8.2 mg) as a colorless powder (mp 100~110°C). $[\alpha]_D^{26}$-57° (c=0.315, EtOH). UV λ_{max} (EtOH) nm (ε): 295 (7200), 227 (27,800). IR ν_{max} (KBr) cm^{-1}: 3350, 2950, 1735, 1660, 1605, 1500, 1365, 1240, 1040, 745. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 2.6; conformer B, 0.64 (3H, d, $J_{15.16}$=6.7Hz, H_3-16), 0.93 (3H, d, $J_{15.17}$=6.1Hz, H_3-17), 2.09 (3H, s, Ac), 2.61 (1H, m, H-15), 2.93 (3H, s, H_3-18), 3.09 (1H, dd, J_{gem}=17.7Hz, $J_{8a.9}$=4.0Hz, Ha-8), 3.24 (1H, br. d, J_{gem}=17.7Hz, Hb-8), 3.99 (1H, dd, J_{gem}=11.5Hz, $J_{9.14a}$=8.5Hz, Ha-14), 4.20 (1H, dd, J_{gem}=11.5Hz, $J_{9.14b}$=3.5Hz, Hb-14), 4.36 (1H, d, $J_{12.15}$=10.4Hz, H-12), 4.51 (1H, br.s, H-9), 6.01 (1H, br.s, NH-10), 6.53 (1H, d, $J_{5.6}$=7.6Hz, H-5), 6.90 (1H, s, H-2), 6.91 (1H, d, $J_{6.7}$=7.6Hz, H-7), 7.07 (1H, t, $J_{5.6}$=7.6Hz, $J_{6.7}$=7.6Hz, H-6), 8.03 (1H, br.s, NH-1); conformer A, 0.94 (d, J=6.1Hz), 1.24 (d, J=6.7Hz), 2.02 (s), 2.40 (m), 2.75 (s), 2.78 (d, J=14.5Hz), 2.98 (d, J=10.7Hz), 3.12 (dd, J=14.5Hz, 5.5Hz), 3.85 (m), 4.65 (br.d, J=12.0Hz), 6.98 (br.s), 7.04 (d, J=7.5Hz), 7.16 (t, J=7.5Hz), 7.28 (d, J=7.5Hz), 8.30 (br.s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 343.19023 (M^+, calcd. for $C_{18}H_{25}N_3O_3$, 343.18959).

Fraction II was fractionated by column chromatography on Wako C-200 gel (30 g) with toluene (200 ml) and EtOAc (200 ml) by gradient elution to give an active fraction (42.2 mg), which was further purified by HPLC on Radialpak μ-Bondapak C_{18} with 80% MeOH in water to yield **3** (9.2 mg). Colorless rods from MeOH, mp 212~215°C, $[\alpha]_D^{25}$-150° (c=0.273, EtOH). CD $[\theta]_{309}$+3200, $[\theta]_{290}$ 0, $[\theta]_{252}$-24,900, $[\theta]_{237}$-31,600, $[\theta]_{224}$-38,300, $[\theta]_{205}$ 0 ($c=8.4\times10^{-5}$, MeOH). UV λ_{max} (EtOH) nm (ε): 286 (9100), 233 (29,500). IR ν_{max} (KBr) cm^{-1}: 3440, 3375, 3075, 1648, 1600, 1548, 1503, 1465, 1450, 1408, 1372, 1055, 978, 913. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 4.5; conformer, B, 0.54 (3H, d, J=6.7Hz), 0.69 (3H, d, J=6.7Hz), 0.92 (3H, d, J=6.4Hz), 1.01 (3H, d, J=6.4Hz), 1.35 (3H, s), 1.44 (2H, m), 1.51 (3H, s), 1.90 (2H, d, J=10.1Hz), 2.25 (1H, m), 2.62 (1H, m), 2.90 (3H, s), 2.99 (1H, dd, J=17.7Hz, 3.4Hz), 3.13 (1H, br.d, J=17.7Hz), 3.53 (1H, m), 3.72 (1H, m), 4.31 (1H, d, J=10.1Hz), *ca.* 4.3 (1H, br.m), 5.25 (1H, d, J=10.7Hz), 5.41 (1H, d, J=17.7Hz), 6.16 (1H, dd, J=17.7Hz, 10.7Hz), 6.51 (1H, s), 6.78 (1H, s), 7.26 (1H, br.s) 8.67 (1H, br.s); conformer A, 0.61 (d, J=6.7Hz), 0.93 (d, J=6.4Hz), 1.03 (d, J=6.7Hz), 1.25 (d, J=6.7Hz), 1.50 (s), 2.73 (s). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 451 (M^+). Anal. Calcd. for $C_{28}H_{41}N_3O_2$: C, 74.46; H, 9.15; N, 9.30. Found: C, 74.64; H, 9.35; N, 9.19.

Fraction III was chromatographed on 160 g of YMC I-40/64 gel with 60% MeOH in water to give **1** as a yellow powder (700 mg), which was recrystallized from MeOH to yield **1** as pale yellow rods (mp 112~115°C). $[\alpha]_D^{27}$-170° (c=0.499, EtOH). CD $[\theta]_{304}$+8700, $[\theta]_{291}$ 0, $[\theta]_{242}$-22,100, $[\theta]_{221}$-47,900, $[\theta]_{207}$ 0 ($c=1.04\times10^{-2}$, MeOH). IR ν_{max} (KBr) cm^{-1}: 3370, 2950, 1650, 1610, 1565, 1500, 1275, 1040, 780, 740. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 4.4; conformer B, 0.63 (3H, d, $J_{15.16}$=6.7Hz, H_3-16), 0.93 (3H, d, $J_{15.17}$=6.4Hz, H_3=17), 2.59 (1H, m, H-15), 2.92 (3H, s, H_3-18), 3.05 (1H, dd, J_{gem}=17.4Hz, $J_{8a.9}$=3.7Hz, Ha-8), 3.17 (1H, br.d, J_{gem}=17.4Hz, Hb-8), 3.57 (1H, m, Ha-14), 3.74 (1H, m, Hb-14), 4.31 (1H, br.s, H-9), 4.40 (1H, d, $J_{12.15}$=10.4Hz, H-12), 6.50 (1H, d, $J_{5.6}$=7.6Hz, H-5), 6.89 (1H, s, H-2), 6.90 (1H, d, $J_{6.7}$=7.6Hz, H-7), 7.06 (1H, t, $J_{5.6}$=7.6Hz, $J_{6.7}$=7.6Hz, H-6), 7.37 (1H, br.s, NH-10), 8.02 (1H, br.s, NH-1); conformer A, 0.93 (d, J=6.4 Hz), 1.25 (d, J=6.7Hz), 2.75 (s). Other peaks had weak intensities and overlapped those of the major conformer. ^{13}C NMR δ ($CDCl_3$) ppm: conformer B, 19.4 (q), 21.5 (q), 28.4 (d), 32.9 (q), 33.8 (t), 56.0 (d), 64.6 (t), 70.9 (d), 104.3 (d), 106.1 (d), 113.8 (s), 117.9 (s), 121.7 (d), 122.4 (d), 139.4 (s), 147.5 (s), 174.6 (s); conformer A, 19.4 (q), 24.4 (d), 28.4 (t), 35.7 (q), 55.0 (d), 62.0 (t), 76.5 (d), 107.6 (s), 110.5 (d), 125.8 (d), 127.9 (s), 145.0 (s), 173.0 (s). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 301 (M^+, 36.6), 270 (3.7), 258 (9.8), 215 (41.5), 185 (19.5), 171 (100), 155 (37.8), 143 (13.9), 130 (33.7), 115 (15.9).

Anal. Calcd. for $C_{17}H_{23}N_3O_7CH_3OH$: C, 64.84 ; H, 8.16 ; N, 12.60. Found : C, 64.63 ; H, 8.25 ; N, 12.39.

Acetylation of 1. Compound 1 (14.1 mg) was dissolved in dry pyridine (0.5 ml) and acetic anhydride (0.5 ml), and the solution was allowed to stand at room temperature for two days. After adding crushed ice, the reaction mixtrue was extracted with EtOAc. This extract was purified by HPLC on semi prep. μ-Bondapak C_{18} with 65% MeOH in water. Compound 2 (8.6 mg) was obtained as a colorless powder (mp 98~110°C).

Acid hydrolysis of 1. An EtOH solution of 1 (43.2 mg) was put into a test tube and evaporated *in vacuo*. To the tube, 0.3 ml of 6N HCl was added and the tube was sealed and heated at 120°C for 4 hr. The resultant solution was evaporated *in vacuo* to dryness and dissolved in a small amount of EtOH. Careful addition of EtOAc to the EtOH solution gave fine crystals which were filtered off and dissolved in water. To this solution, a small amount of $NaHCO_3$ was added and the solution was extracted two times with EtOAc. The EtOAc layer was washed with water, dried over sodium sulfate, and evaporated *in vacuo* to dryness. The EtOAc extracts were purified by column chromatography on Wako C-200 gel (1.8 g), eluting with $CHCl_3$ containing increasing amounts of MeOH. The eluates with 10.0, 12.5, 15.0 and 17.5% MeOH were combined at *ca.* 0°C and evaporated *in vacuo* at *ca.* 5°C to give colorless needles (19.3 mg). The needles were washed with water and dried over sodium sulfate to yield 4 (12 mg, mp 149~152°C). $[\alpha]_D^{27}$-35° (c=0.130, EtOH). UV λ_{max} (EtOH) nm (ε) : 292.5 (5900), 286 (6100), 226 (26,900). IR ν_{max} (KBr) cm^{-1} : 3620, 3400, 3340, 3170, 2960, 2800, 1730, 1615, 1600, 1500, 1390, 1080, 1020, 745. ^1H NMR δ ($CDCl_3$+CD_3OD) ppm : Major conformer, 1.10 (3H, d, J=6Hz), 1.15 (3H, d, J=6Hz), 2.40 (1H, m), 2.80 (3H, s), 3.40 (1H, d, J=8Hz), 2.90~3.70 (3H, m), 3.90 (2H, m), 7.00~7.20 (4H, m) ; minor conformer, 0.83 (d, J=6Hz), 1.33(d, J=6Hz). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z : 301.17946 (M^+, calcd. for $C_{17}H_{23}N_3O_2$, 301.17903).

Acetylation of 4. Compound 4 (10 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) were mixed and left to stand overnight at room temperature. The reaction mixture was purified by column chromatography on Wako C-200 gel (1 g), eluting with toluene containing increasing amounts of acetone, and followed by HPLC on semi prep. μ-Bondapak C_{18} with 65% MeOH to give 7 as a colorless powder (mp 110~115°C). $[\alpha]_D^{27}$-35° (c=0.259, EtOH). UV λ_{max} (EtOH) nm (ε) : 287 (6100), 225 (28,400). IR ν_{max} (KBr) cm^{-1} : 3390, 3290, 2970, 1725, 1655, 1535, 1500, 1265, 1185, 1025, 745. ^1H NMR δ ($CDCl_3$) ppm : 1.05 (3H, d, J=6Hz), 1.18 (3H, d, J=6.5Hz), 2.05 (3H, s), 2.33 (1H, m), 2.84 (3H, s), 2.96 (1H, dd, J=14Hz, 6Hz), 3.19 (1H, dd, J=14Hz, 10.5Hz), 3.34 (1H, d, J=9.5Hz), 3.81 (1H, dd, J=12.5Hz, 5.5Hz), 4.06 (1H, dd, J=12.5Hz, 2Hz), 4.46 (1H, br.s), 6.60 (1H, br.d, J=9Hz), 6.95~7.20 (4H, m), 8.58 (1H, br.s). HR-EIMS m/z : 343.19025 (M^+, calcd. for $C_{19}H_{25}N_3O_3$, 343.18959).

Temperature study of 1. In deuterated dimethyl sulfoxide at 140°C, two doublets of the comformer B at δ 0.50 and 0.78, and those of the conformer A at δ 0.78 and 1.10 overlapped to give two doublets at δ 0.75 and 0.82. In deuterated pyridine at 110°C, two doublets of conformer B at δ 0.66 and 1.05, and those of conformer A at δ 0.92 and 1.20 changed to one doublet at δ 0.98 and one broad singlet at δ 0.84, respectively.

HPLC analysis of 1. Compound 1 was analyzed by HPLC to give a single peak on five columns with various solvents at room temperature. The conditions were as follows (column, column size, solvent, flow rate, t_R) : 1) Cosmosil (5Ph) packed column (Nakarai Chemicals, Ltd.), 4.6 mm i.d. × 150 mm, 60% MeOH in water, 0.4 ml/min, 14.5 min ; 2) Cosmosil (5TMS) packed column (Nakarai Chemicals, Ltd.), 4.6 mm i.d. × 150 mm, 40% MeOH in water, 1.0 ml/min, 20.0 min ; 3) Cosmosil ($5C_8$) packed column (Nakarai Chemicals, Ltd.), 4.6 mm i.d. × 150 mm, 70% MeOH in water, 0.4 ml/min, 9.4 min ; 4) YMC packed A311 column, 6.0 mm i.d. × 100 mm, 60% MeOH in water, 1.0 ml/min, 8.5 min ; 5) μ-Porasil, 4.0 mm i.d. × 300 mm, 60% EtOAc in hexane, 1.0 ml/min, 24.6 min ; and 6) μ-Porasil, 4.0 mm i.d. × 300 mm, 5% 2-PrOH in $CHCl_3$, 1.0 ml/min, 7.4 min.

Compound 1, however, gave two peaks (t_R=5.2, 7.6 min) by HPLC using μ-Porasil with 5% 2-PrOH in $CHCl_3$ (flow rate, 1.0 ml/min) at -17°C. Each peak was collected under the following conditions : column, μ-Porasil ; column size, 4.0 mm i.d. × 300 mm ; solvent, 4% 2-PrOH in $CHCl_3$; flow

rate, 1.0 ml/min; temperature of the column and solvent, *ca.* -15°C. Four runs of collection and concentration gave fraction 1 (t_R=5.6~7.2 min, 43.2 mg) and fraction 2 (t_R=9.4~11.1 min, 6.2 mg).

EBV-EA induction test. See Chapter IV.

II. Identification of the Microorganism NA34-17 Which Produces (-)-Indolactam V

As mentioned in Chapter I, the strain NA34-17 produced (-)-indolactam V (1) in large quantities and its 14-*O*-acetate (2) in small quantities along with some teleocidin B-4 (3) in the culture broth. This section states the identification of this strain and the

Table 3. Physiological characteristics of strain NA34-17

Nitrate reduction	Negative
Starch hydrolysis	Positive
Milk coagulation	Negative
Milk peptonization	Positive
Melanin production	Positive
Gelatin liquefaction	Positive
H_2S production	Weakly positive
Temperature range for growth	12~37°C
optimum at	27~30°C
pH Range for growth	4.5~9.5
optimum at	5~7
Cell wall type	LL-DAP
Carbon utilization*: D-glucose, +; sucrose, −; glycerol, +; D-xylose, −; D-fructose, +; lactose, −; maltose, +; rhamnose, −; raffinose, ±; D-galactose, +; L-arabinose, −; D-mannose, +; D-trehalose, −; inositol, +; mannitol, −; inulin, −; cellulose, −; salicin, −; chitin, −; sodium citrate, ±; sodium succinate, ±; sodium acetate, −.	

*+, utilization; ±, doubtful utilization; −, no utilization.

Table 4. Cultural characteristics of strain NA34-17

Medium	Aerial mass color	Reverse side color	Soluble Pigment
Yeast-malt extract (ISP-2)	Greenish gray	Light brown	None
Oatmeal agar (ISP-3)	Grayish white	Colorless	None
Inorganic salts-starch agar (ISP-4)	Beige	Light brown	None
Glycerol-asparagine agar (ISP-5)	Grayish white	Pale yellowish brown	None
Peptone-yeast extract-iron agar (ISP-6)	None	Brown	Brown
Tyrosine agar (ISP-7)	Grayish white	Pale yellowish brown	None
Glucose-asparagine agar	Grayish white	Pale yellowish brown	None
Nutrient agar	None	Pale yellow orange	None
Sucrose-nitrate agar	None	Colorless	None
Potato-dextrose agar	Greenish gray	Grayish yellow brown	None

cultural conditions for the production of (-)-indolactam V (1) and teleocidin B-4 (3).

Taxonomic studies on this strain were conducted according to the procedures of the International Streptomyces Project (ISP),[72] Waksman[73] and Bergey's Manual of Determinative Bacteriology, 8th Ed.,[74] and colors were described according to the Color Standard (Nihon Shikisai Co., Ltd.). Strain NA34-17 showed moderate growth on various agar media. The mature spores formed chains and exhibited typical whorl formation. The spores were cylindrical ($0.4\sim0.6\times1\sim1.5$ μm) with smooth surfaces. The physiological and cultural characteristics of strain NA34-17 are listed in Tables 3 and 4, respectively. Cell wall analysis of strain NA34-17 according to the methods described by Becker *et al.*[75] and Yamaguchi[76] revealed the presence of LL-diaminopimelic acid.

From the properties described above, strain NA34-17 was deduced to belong to the

Table 5. Effect of carbon sources on the probuction of (-)-indolactam V and teleocidin B-4[a]

Carbon sources	Concn. (%)	(-)-Indolactam V[b] (mg/liter)	Teleocidin B-4 (mg/g of mycelia)	Wt. of mycelia (g/liter)	Final pH
Glucose	2	12.3	1.5	42	7.6
Glycerol	2	2.3	2.2	49	6.3
Soluble starch	2	0	2.2	29	6.4
Na-citrate	2	0	3.5	4.7	7.7
Glucose	0	0	2.7	6.6	8.0
	1	3.0	1.4	27	7.6
	2	12.3	1.5	42	7.6
	3	2.4	1.8	55	7.2
	4	0	2.7	47	6.3

[a] Each carbon source was added to a medium containing 1% meat extract (Wako Pure Chemicals), 1% polypepton (Daigo Eiyo Kagaku) and 0.5% NaCl (pH 7.0). The strain was inoculated into 100 ml of the medium per 500-ml volume shaking flask and cultured at 30°C for 4 days on a reciprocal shaker.
[b] (-)-Indolactam V and teleocidin B-4 were quantified by the HPLC procedure.

Table 6. Effect of nitrogen sources on the production of (-)-indolactam V and teleocidin B-4[a]

Carbon sources	Concn. (%)	(-)-Indolactam V (mg/liter)	Teleocidin B-4 (mg/g of mycelia)	Wt. of mycelia (g/liter)	Final pH
Meat extract	2	7.2	0.9	36	7.4
Polypepton	2	0	0.1	15	5.9
Casamino acids	2	0	33	1.1	6.4
$NaNO_3$	2	0	0	0.3	4.4
$(NH_4)_2SO_4$	1.6	0	0	0.8	5.0
NH_4Cl	1.2	0	0	1.0	4.8
Meat extract	Each 1	12.3	1.5	42	7.6
+Polypepton	Each 1.5	12.8	0.9	47	7.9
	Each 2	10.4	1.2	41	7.7

[a] Each nitrogen source was added to a medium containing 2% glucose and 0.5% NaCl (pH 7.0). See the legend of Table 5 for the cultivation conditions.

genus *Streptoverticillium*. Among known species of the genus *Streptoverticillium*, strain NA34-17 is considered to be closely related to *Streptoverticillium blastmyceticum* Locci, Baldacci and Petrolini Baldan (1969).[76] Therefore, *S. blastmyceticum* IFO 12747 and strain NA34-17 were compared. Strain NA34-17 showed good agreement with *S. blastmyceticum* IFO 12747 except for H_2S production. This difference does not seem sufficient to distinguish strain NA34-17 from *S. blastmyceticum*. Strain NA34-17 was, thus, identified as *Streptoverticillium blastmyceticum* NA34-17.

To determine the most suitable carbon and nitrogen sources for the production of (-)-indolactam V (1), various compounds were examined. The results shown in Table 5 indicate that glucose is most effective for the production of **1**, and that the production

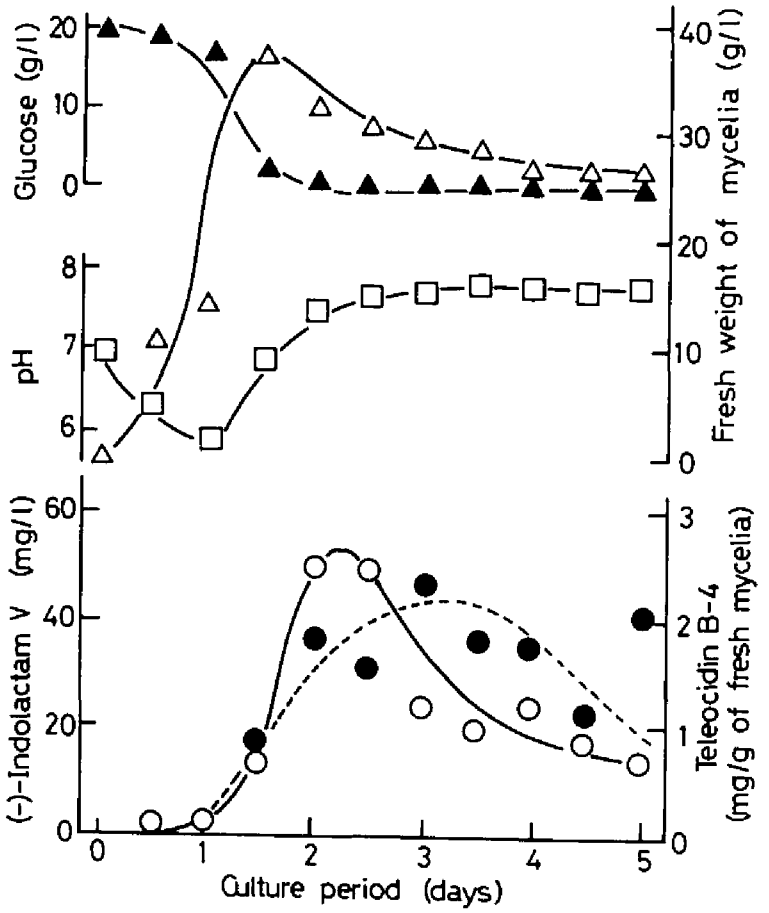


Fig. 4. Time course study on (-)-indolactam V (1) and teleocidin B-4 (3) production. *Streptoverticillium blastmyceticum* NA34-17 was cultured in 100 ml of the medium containing 2% glucose, 1% meat extract, 1% polypeptone and 0.5% NaCl (pH 7.0) in 500 ml shaking flasks. ○ (-)-indolactam V (mg/l) ; ● teleocidin B-4 (mg/g of fresh mycelia) ; △ cell growth (fresh weight, g/l) ; ▲ glucose (g/l) ; □ pH of the medium.

is dependent on the glucose concentration. The optimum concentration for the production of (-)-indolactam V (**1**) was 2% and a higher concentration inhibited the production. On the other hand, the amount of teleocidin B-4 (**3**) in the mycelia increased from 1.5 to 2.7 mg/g fresh mycelia as the glucose concentration increased from 2 to 4 %. The amounts of **1** in the mycelia cultured in the 2 and 4% glucose media were found to be very small, suggesting that the glucose concentration influenced not the release of **1** into the culture broth, but rather the direct production of **1**.

The effects of nitrogen sources were also tested. As shown in Table 6, meat extract was necessary for the production of **1**, and **1** was produced in a high amount in the medium containing meat extract and polypepton (each 1~1.5%). In the medium containing casamino acids, this strain grew poorly but accumulated much more teleocidin B-4 (**3**) in the mycelia.

A time course study of (-)-indolactam V (**1**) and teleocidin B-4 (**3**) was done in a medium consisting of 2% glucose, 1% meat extract, 1% polypeptone and 0.5% NaCl (pH 7.0). As shown in Figure 4, when the glucose was almost used up after 36 hr of cultivation, the fresh weight of the mycelia reached the highest value, and the production of **1** in the culture broth and the accumulation of **3** in the mycelia began. The amount of **1** reached the maximum after 50 hr of cultivation, and that of **3** , after 70 hr.

On the basis of these results, *S. blastmyceticum* NA34-17 was cultured for 48 hr in a medium consisting of 2% glucose, 1% meat extract, 1% polypeptone and 0.5% NaCl (pH 7.0) to obtain (-)-indolactam V (**1**). From about 800 l of the culture broth, *ca.* 15 g of **1** was obtained.

III. Derivation of (-)-Indolactam V : Substitution Reaction on the Indole Ring

(-)-Indolactam V (**1**) is a key compound for structure-activity studies of teleocidin-related compounds since its structure is fundamental to teleocidins and it has various activities itself : for example, induction of EBV-EA, induction of ODC, adhesion of HL-60 cells, binding to the TPA receptor, activation of PKC and tumor-promoting activity.[6,7]

Teleocidins consist of a hydrophilic region of position 1, 10 and 14 and a hydrophobic region of position 6, 7 and 12, and are amphiphilic, suggesting that the hydroxyl group at position 14 of teleocidins plays an important role in activity. To examine the contribution of this hydroxyl group to activity, a series of 14-*O*-acyl and alkyl derivatives of **1** (**8**~**16**) was prepared. The series of 14-*O*-acyl derivatives (**8**~**13**) was obtained by acylation of **1** with an acid anhydride and pyridine, and the series of 14-*O*-alkyl derivatives (**14**~**16**), by Williamson synthesis of **1** with an alkyl *p*-toluenesulfonate and sodium in toluene or sodium hydride in benzene. To investigate in detail the role of this functional group for activity, (-)-14-dehydroxyindolactam V (**17**) and (-)-14-chloroindolactam V (**18**) were also derived from **1** by conventional methods (Figure 5).

The various activities of (-)-indolactam V (**1**) are lower than those of teleocidin B-4 (**3**)[7] which carries a monoterpenoid moiety at positions 6 and 7 of the indole ring, suggesting that the monoterpenoid moiety in **3** acts to increase the number of activities. To

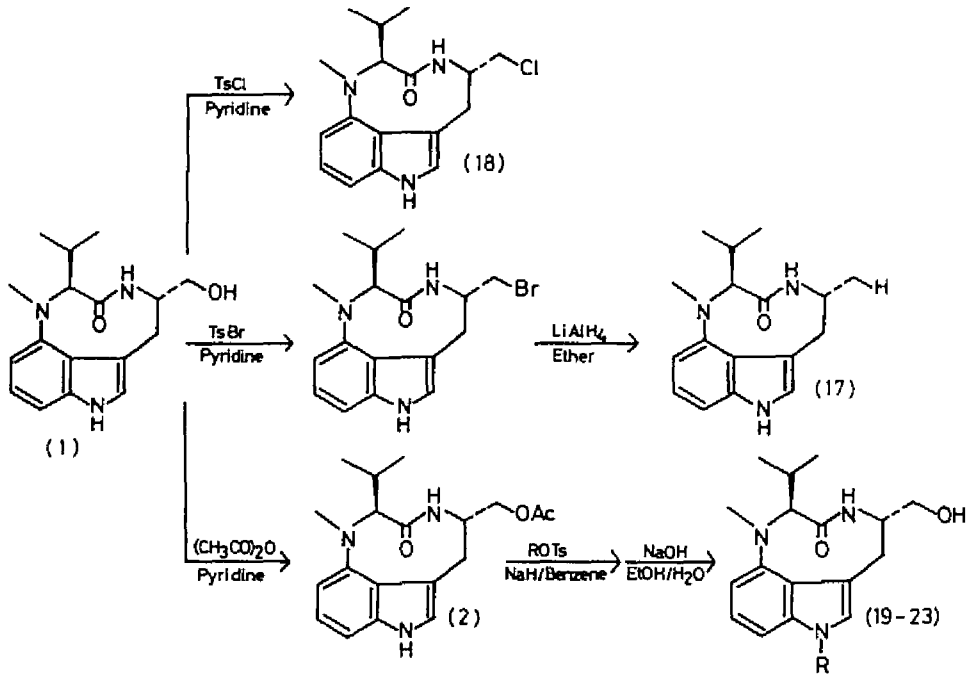


Fig. 5. Synthetic scheme of (-)-indolactam V derivatives.

examine the contribution of this moiety of **3** to activity, a series of (-)-N^1-alkylindolactam Vs (**19**~**23**) was synthesized. They were prepared from **1** by Williamson synthesis of 14-*O*-acetate of **1** with an alkyl *p*-toluenesulfonate and sodium hydride in benzene.

Next the substitution reaction on the indole ring of **1** was examined. Several methods for the introduction of alkyl or acyl substituents into position 7 of an indole ring has recently been reported.[69,77,78] However, direct introduction of substituents into position 7 of **1** is more desirable from the view point of synthesizing many derivatives for structure-activity studies. First, **1** was halogenated. Electrophilic aromatic substitution of bromine into **1** in dioxane-pyridine gave (-)-7-bromoindolactam V (**24**) and (-)-2,7-dibromoindolactam V (**25**) in 25% and 4% yield, respectively. The same reaction using iodine gave (-)-7-iodoindolactam V (**26**) in 35% yield. However, radical reaction of **1** with *N*-chlorosuccinimide in dioxane gave predominantly (-)-5-chloroindolactam V (**27**) in 40% yield. The same reactions using *N*-bromosuccinimide or *N*-iodosuccinimide gave no derivatives.

Introduction of an alkyl substituent into the indole ring of **1** was investigated. Friedel-Crafts alkylation using aluminum chloride and alkyl chloride in nitrobenzene was unsuccessful. Introduction of the first alkyl group may increase the reactivity of the ring toward further substitution and polymerization. Then Friedel-Crafts acylation was examined. Treatment of (-)-14-*O*-acetylindolactam V (**2**) with aluminum chloride and acetic anhydride in nitrobenzene, followed by hydrolysis, afforded (-)-7-acetylindolactam V (**28**) in 45% yield. (-)-2,7-Diacetyl-, (-)-5,7-diacetyl- and (-)-5-acetylindolactam V (**29**

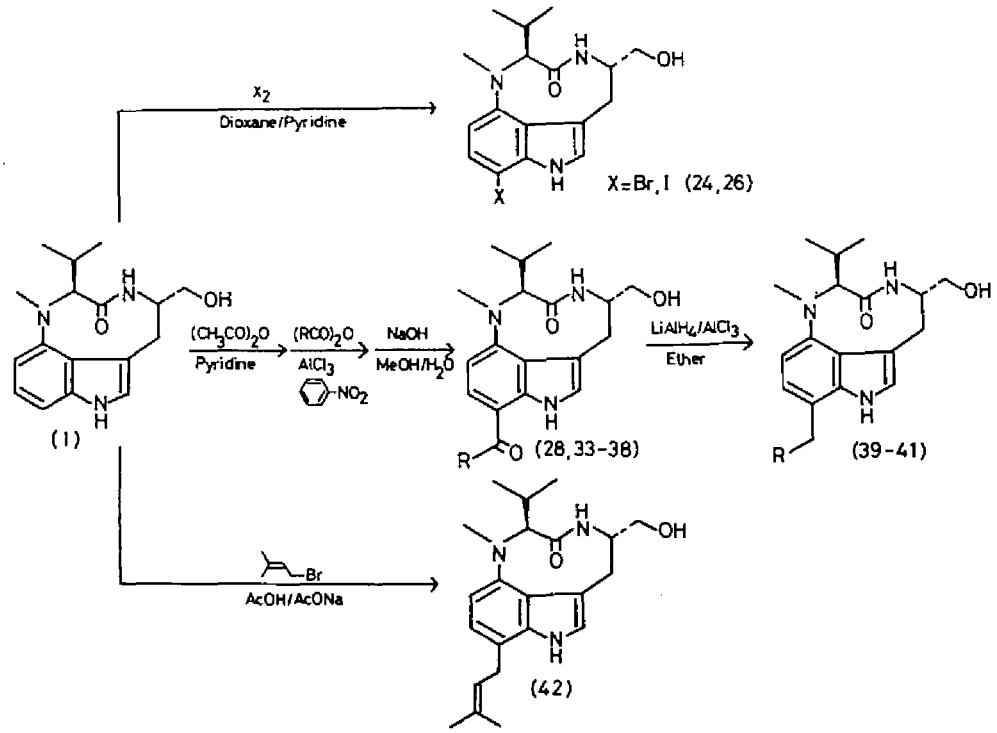


Fig. 6. Substitution reaction on the indole ring of (-)-indolactam V (**1**), the basic ring-structure of teleocidins.

~**31**) were obtained as minor products. Reduction of **28** with lithium aluminum hydride and aluminum chloride in ether gave (-)-7-ethylindolactam V (**39**) in 63% yield. A series of 7-substituted or 2, 7-disubstituted (-)-indolactam Vs (**32**~**41**) was synthesized by this method for structure-activity studies.

Direct introduction of alkyl substituents into **1** was attained under a quite mild reaction condition. For example, treatment of **1** with prenylbromide in acetic acid and sodium acetate[79] gave (-)-7-prenylindolactam V (**42**) in 10% yield. This reaction, however, was

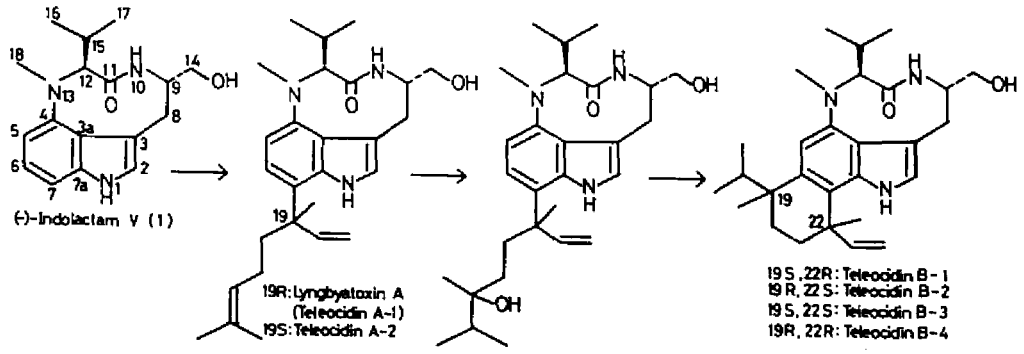


Fig. 7. Possible biosynthetic pathway of teleocidins.

not so selective as the Friedel-Crafts acylation and halogenation mentioned above, 2 or 5 substituted and disubstituted derivatives of 1 being obtained. Recently, 7-prenylindolactam V has been synthesized by Friedel-Crafts alkylation of 1-acetyl-14-*O*-TBDMS-indolactam V using prenylchloride and trifluoroacetic acid silver salt in nitromethane.[80]

The above results on the substitution reaction on the indole ring of **1** indicate that position 7 of I is most active in the electrophilic aromatic substitution, and that introduction of electron-withdrawing groups results in a good yield and selectivity since they deactivate the indole ring to prevent further reaction. (-)-Indolactam V (1) is believed to be a biosynthetic intermediate of teleocidins. High reactivity at position 7 of **1** is compatible with the biosynthetic pathway (Figure 7).[54]

As briefly described in Chapter I, teleocidins and (-)-indolactam V (**1**) exist as two stable conformers in solution at room temperature : conformer A of the SOFA type and B of the TWIST type.[68,69] Introduction of an electron-withdrawing group into position 7 will increase the resonance among the lone-pair electrons on N-13, aromatic electrons and the substituent at position 7, fixing the molecule in conformer B, in which the lone pair electrons on N-13 are more delocalizable into the indole ring. As was expected, (-)-7-acylindolactam Vs (**28**, **33**~**38**) and (-)-2,7-diacylindolactam Vs (**29** and **32**) existed only as conformer B in chloroform-d or methanol-d_4 at room temperature. This was determined by comparison of their chemical shifts with those of the two conformers of **1**, **2** and **14** (Table 7). Large differences in chemical shifts between conformer A and B are observed chiefly at H-5, 10, 12, 16, 17 and 18. The chemical shifts of these protons of **28** and **29** are summarized in Table 8, and indicate that the fixed conformer of these compounds is conformer B. The other compounds (**32**~**38**) also showed chemical shifts similar to those of conformer B (See experimental section.).

Introduction of a substituent into position 5 of **1** will hinder the resonance by a steric interaction between the substituent and the *N*-methyl group, to fix the molecule in conformer A. In the ^{1}H NMR of **27** and **31** in chloroform-d, the signals ascribable to con-

Table 7. ^{1}H NMR chemical shifts of (-)-indolactam V (1), (-)-14-*O*-acetylindolactam V (**2**) and (-)-14-*O*-methylindolactam V (**14**) in $CDCl_3$ at room temperature[a]

Conformer	1		2		14	
	A	B	A	B	A	B
H-5	NI[b]	6.50 (d, J= 7.6)	7.04 (d, J= 7.5)	6.53 (d, J= 7.6)	NI[b]	6.51 (d, J= 7.6)
H-10	4.74 (d, J=11.6)	7.37 (br. s)	4.65 (d, J=12.0)	6.01 (br. s)	NI[b]	6.14 (br. s)
H-12	NI[b]	4.40 (d, J=10.4)	2.98 (d, J=10.7)	4.36 (d, J=10.4)	NI[b]	4.36 (d, J=10.4)
H-16	0.93 (d, J= 6.4)	0.63 (d, J= 6.7)	0.94 (d, J= 6.1)	0.64 (d, J= 6.7)	0.92 (d, J= 6.4)	0.62 (d, J= 6.7)
H-17	1.25 (d, J= 6.7)	0.93 (d, J= 6.4)	1.24 (d, J= 6.7)	0.93 (d, J= 6.1)	1.24 (d, J= 7.0)	0.92 (d, J=6.4)
H-18	2.75 (s)	2.92 (s)	2.75 (s)	2.93 (s)	2.76 (s)	2.93 (s)

[a] The ratio of the two conformers were as follows; I, conformer A : B=1 : 4.4; **2**, 1 : 2.6; **14**, 1 : 7.2.
[b] The signal could not be identified because of its low intensity.

Table 8. ^{1}H NMR chemical shifts of (-)-7-acetylindolactam V (**28**), (-)-2,7-diacetylindolactam V (**29**), (-)-5-chloroindolactam V (**27**) and (-)-5-acetylindolactam V (**31**) in $CDCl_3$ at room temperature

	28	**29**	**27**[a]	**31**[a]
H-5	6.48 (d, J= 8.2)	6.46 (d, J= 8.4)	—	—
H-10	7.12 (br. s)	7.49 (br. s)	4.77 (d, J=10.7)	4.89 (d, J=10.4)
H-12	4.56 (d, J=10.4)	4.47 (d, J=10.3)	3.09 (d, J=10.4)	2.93 (d, J=10.7)
H-16	0.58 (d, J= 6.7)	0.51 (d, J= 6.6)	0.97 (d' J= 6.4)	0.84 (d, J= 6.4)
H-17	0.94 (d, J= 6.1)	0.94 (d, J= 6.2)	1.36 (d, J= 6.7)	1.03 (d, J= 7.0)
H-18	2.98 (s)	2.99 (s)	2.75 (s)	2.74 (s)

[a] The ratio of the two conformers were as follows; **27**, conformer A : B=10 : 1 ; **31**, 30 : 1.

former A were almost exclusively observed as was shown in Table 8. The ^{1}H NMR spectra of (-)-indolactam V (**1**), (-)-7-acetylindolactam V (**28**) and (-)-5-acetylindolactam V (**31**) in chloroform-d are summarized in figures 8~10.

Until now, very little has been known about conformation-activity relationship. Endo *et al.* proposed that the free energy difference between the two conformers and the free energy of activation in the conversion of the two conformers were calculated to be so small that the conformers converted easily at room temperature.[68,69] Since there exists a large difference in conformation between the two conformers, one of them is likely responsible for the biological activity. Compounds fixed as conformer A or B will give a

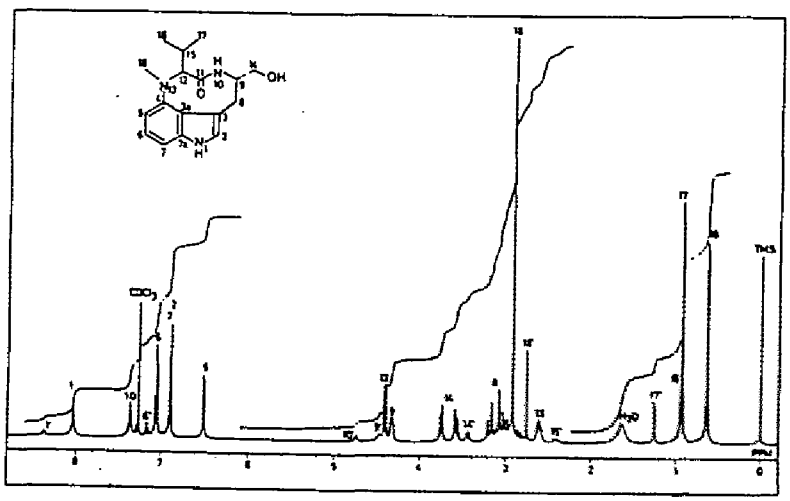


Fig. 8. ^{1}H NMR (400 MHz, $CDCl_3$) of (-)-indolactam V (**1**) at room temperature. Protons ascribable to conformer A are shown by primes.

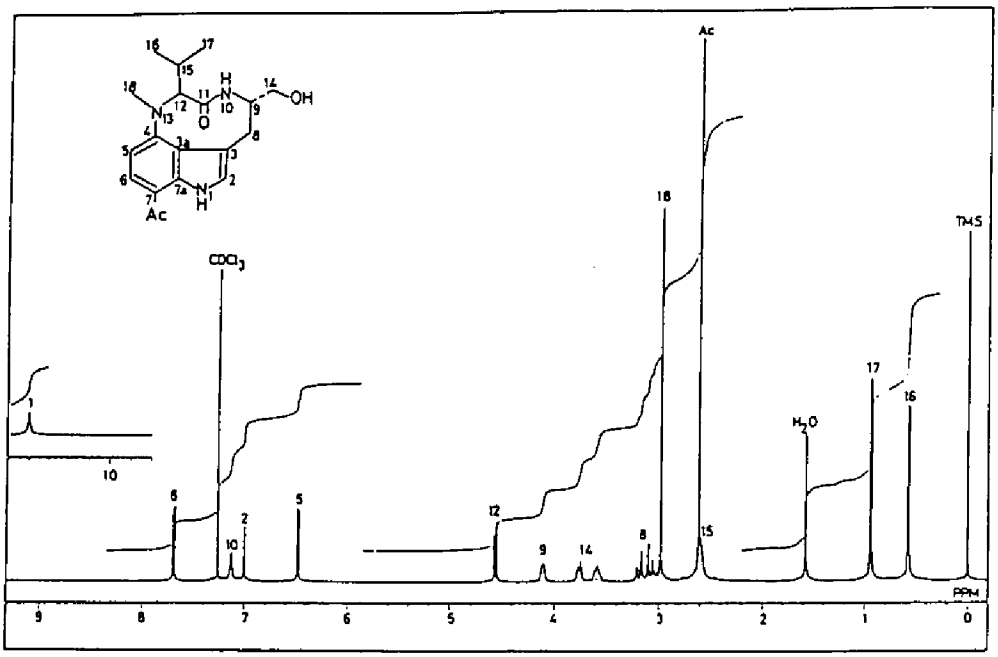


Fig. 9. ^{1}H NMR (400 MHz, $CDCl_3$) of (-)-7-acetylindolactam V (28) at room temperature.

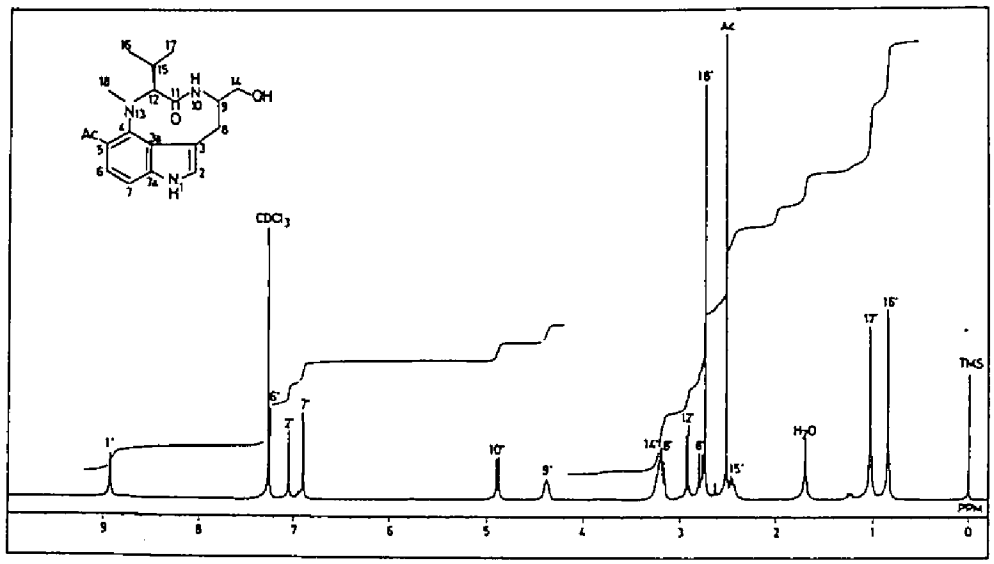


Fig. 10. ^{1}H NMR (400 MHz, $CDCl_3$) of (-)-5-acetylindolactam V (31) at room temperature.

clue to this proposition.

The biological activities of these indolactam derivatives are described in the next chapter.

Experimental

General remarks. See Chapter I.

Preparation of 14-O-acyl derivatives of (-)-indolactam V. The appropriate acid chloride (*ca.* 0.15 mmole) or acid anhydride (0.5 ml) was treated with (-)-indolactam V (1) (*ca.* 0.1 mmole) in dry pyridine (*ca.* 2 ml) at room temperature for several hours. After adding crushed ice, the reaction mixture was filtered and washed with water. The compound thus obtained was purified by column chromatography on Wako C-200 gel with 30% EtOAc in hexane, followed by HPLC on semi prep. μ-Porasil with 25% EtOAc in hexane to give the corresponding derivatives as follows.

(-)-14-O-Butanoylindolactam V (8). 89% Yield, a colorless powder, mp 76~80°C, $[\alpha]_D^{18}$-55° (c=0.199, EtOH). UV λ_{max} (EtOH) nm (ε): 297 (7200), 287 (7000), 227 (28,100). IR ν_{max} (KBr) cm^{-1}: 3350, 2970, 2890, 1735, 1660, 1502, 1180, 742. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1:2; conformer B, 0.64 (3H, d, J=7Hz), 0.95 (6H, m), 1.62 (2H, m), 2.26 (2H, m), 2.60 (1H, m), 2.95 (3H, s), 3.15 (2H, m), 3.8~4.7 (4H, m), 6.11 (1H, br.s), 6.51 (1H, d, J=8Hz), 6.8~7.3 (3H, m), 8.45 (1H, br.s); conformer A, 2.71 (s). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 371 (M^+). Anal. Calcd. for $C_{21}H_{29}N_3O_3$: C, 67.90; H, 7.87; N, 11.31. Found: C, 67.60; H, 7.88; N, 11.21.

(-)-14-O-Hexanoylindolactdm V (9). 68% Yield, a colorless powder, mp 119~124°C, $[\alpha]_D^{18}$-54° (c=0.407, EtOH). UV λ_{max} (EtOH) nm (ε): 297 (6800), 285 (6700), 227 (27,400). IR ν_{max} (KBr) cm^{-1}: 3310, 2960, 2870, 1735, 1655, 1502, 1165, 1100, 1040, 741. ^1H NMR δ ($CDCl_3$): conformer A: B=1:2.1; conformer B, 0.65 (3H, d, J=7Hz), 1.00 (6H, m), 1.30 (4H, m), 1.65 (2H, m), 2.32 (2H, m), 2.60 (1H, m), 2.96 (3H, s), 3.20 (2H, m), 3.8~4.7 (4H, m), 6.01 (1H, br.s), 6.50 (1H, d, J=8Hz), 6.8~7.2 (3H, m), 8.15 (1H, br.s); conformer A, 2.76 (s). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 399 (M^+). Anal. Calcd. for $C_{23}H_{33}N_3O_3$: C, 69.14; H, 8.33; N, 10.52. Found: C, 69.02; H, 8.50; N, 10.41.

(-)-14-O-Ocatanoylindolactam V (10). 16% Yield, a colorless powder, mp 101~105°C, $[\alpha]_D^{18}$-47° (c=0.201, EtOH). UV λ_{max} (EtOH) nm (ε): 295 (8000), 285 (8000), 227 (29,800). IR ν_{max} (KBr) cm^{-1}: 3300, 2920, 2860, 1735, 1658, 1502, 1165, 1039, 741. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1:2.3; conformer B, 0.65 (3H, d, J=7Hz), 0.90 (6H, m), 1.29 (8H, m), 1.60 (2H, m), 2.31 (2H, m), 2.60 (1H, m), 2.93 (3H, s), 3.15 (2H, m), 3.8~4.7 (4H, m), 6.06 (1H, br.s), 6.51 (1H d, J=8Hz), 6.8~7.2 (3H, m), 8.12 (1H, br.s); conformer A, 2.76 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 427.2820 (M^+, calcd. for $C_{25}H_{37}N_3O_3$, 427.2835).

(-)-14-O-Decanoylindolactam V (11). 13.5% Yield, a colorless powder, mp 90~92°C, $[\alpha]_D^{15}$-47° (c=0.149, EtOH). UV λ_{max} (EtOH) nm (ε): 297 (7400), 286 (7400), 227.5 (29,200). IR ν_{max} (KBr) cm^{-1}: 3300, 2920, 2850, 1735, 1655, 1502, 1165, 1040, 743. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1:2.1; conformer B, 0.67 (3H, d, J=7Hz), 0.95 (6H, m), 1.30 (12H, br.s), 1.60 (2H, m), 2.31 (2H, m), 2.60 (1H, m), 2.95 (3H, s), 3.15 (2H, m), 3.8~4.7 (4H, m), 6.09 (1H, br.s), 6.54 (1H, d, J=7Hz), 6.8~7.3 (3H, m), 8.25 (1H, br.s); conformer A, 2.78 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 455.3148 (M^+, calcd. for $C_{27}H_{41}N_3O_3$, 455.3148).

(-)-14-O-Trimethylacetylindolactam V (12). 68% Yield, a colorless powder, mp 98~102°C, $[\alpha]_D^{17}$-58° (c=0.206, EtOH). UV λ_{max} (EtOH) nm (ε): 296.5 (7300), 285 (7200), 227 (28,200). IR ν_{max} (KBr) cm^{-1}: 3350, 2960, 2870, 1730, 1655, 1502, 1280, 1155, 1035, 745. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1:1.8; conformer B, 0.65 (3H, d, J=7.0Hz), 0.93 (3H, d, J=6.4Hz), 1.20 (9H, s), 2.62 (1H, m), 2.93 (3H, s), 3.11 (1H, dd, J=17.4Hz, 4.0Hz), 3.22 (1H, br. d, J=17.4Hz), 4.03 (1H, dd, J=11.6Hz, 8.2Hz), 4.16 (1H, dd, J=11.6Hz, 3.8Hz), 4.35 (1H, d, J=10.4Hz), 4.54 (1H, br.m), 6.01 (1H, br.s), 6.53 (1H, d, J=7.6Hz), 6.90 (1H, s), 6.91 (1H, d, J=7.6Hz), 7.07 (1H, t, J=7.6Hz), 8.13 (1H, br.s); conformer A, 0.93 (d, J=6.4Hz), 1.19 (s), 1.24 (d, J=6.7Hz), 2.41 (m), 2.75 (s), 2.78 (d, J=14.7Hz), 2.96 (d, J=11.0Hz), 4.63 (m), 6.95 (d, J=2.4Hz), 7.04 (d,

J=7.6Hz), 7.16 (t, J=7.6Hz), 7.28 (d, J=7.6Hz), 8.41 (br. s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 385.2383 (M^+, calcd. for $C_{22}H_{31}N_3O_3$, 385.2365).

(-)-14-O-Benzoylindolactam V (*13*). 43% Yield, a colorless powder, mp 108~117°C, $[\alpha]_D^{17}$-51° (c=0.118, EtOH). UV λ_{max} (EtOH) nm (ε): 297 (7700), 283.5 (8100), 277 (sh., 7300), 228.5 (42,100). IR ν_{max} (KBr) cm^{-1}: 3350, 2950, 2870, 1720, 1660, 1605, 1500, 1450, 1270, 1110, 1025, 715. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 1.5; conformer B, 0.65 (3H, d, J=6.7Hz), 0.94 (3H, d, J=6.4Hz), 2.64 (1H, m), 2.95 (3H, s), 3.23 (1H, dd, J=16.8Hz, 4.0Hz), 3.30 (1H, br. d, J=16.8Hz), 4.30 (1H, dd, J=11.6Hz, 7.9Hz), 4.40 (1H, d, J=9.5Hz), 4.41 (1H, dd, J=11.6Hz, 3.7Hz), 4.68 (1H, m), 6.21 (1H, br. s), 6.53 (1H, d, J=7.6Hz), 6.92 (1H, d, J=7.6Hz), 6.93 (1H, s), 7.07 (1H, t, J=7.6Hz), 7.39 (1H, d, J=7.6Hz), 7.45 (2H, t, J=7.6Hz), 8.03 (2H, d, J=7.6Hz), 8.15 (1H, br. s); conformer A, 0.94 (d, J=6.4Hz), 1.25 (d, J=6.7Hz), 2.41 (m), 2.77 (s), 2.89 (d, J=14.6 Hz), 3.02 (d, J=11.0Hz), 3.19 (br. d, J=14.6Hz), 4.11 (m), 4.79 (m), 6.98 (d, J=2.1Hz), 7.17 (t, J=7.9Hz), 7.26 (d, J=7.9Hz), 7.54 (d, J=7.6Hz), 7.58 (t, J=7.6Hz), 7.88 (d, J=7.6Hz), 8.35 (br. s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 405.2047 (M^+, calcd. for $C_{24}H_{27}N_3O_3$, 405.2052).

Preparation of 14-O-alkyl derivatives of (-)-indolactam V.

(-)-14-O-Methylindolactam V (*14*). (-)-Indolactam V (1) (67.8 mg) was added to stirred toluene-Na (from 5 ml of toluene and 100 mg of Na) at room temperature. After stirring for 30 min at 100 °C, methyl *p*-toluenesulfonate (25 μl) was added to the mixture. The mixture was refluxed for 30 min. After cooling, EtOH was added to the mixture. It was then neutralized with AcOH, and evaporated *in vacuo* to dryness. The residue was extracted with EtOAc. The EtOAc layer was washed with water, dried over sodium sulfate, and evaporated *in vacuo* to dryness. The EtOAc extract was purified by column chromatography on Wako C-200 gel (5 g) with acetone-toluene (25: 75), followed by HPLC on NOVA-PAK C_{18} with 60% MeOH in water. Compound **14** was obtained as colorless plates by recrystallization from EtOH-H_2O (9.3 mg, 13% yield, mp 246~247°C). $[\alpha]_D^{17}$-103° (c=0.467, EtOH). UV λ_{max} (EtOH) nm (ε): 300 (7900), 286 (7400), 228 (28,400). IR ν_{max} (KBr) cm^{-1}: 3370, 2940, 1640, 1502, 1112, 1041, 740. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 7.2; conformer B, 0.62 (3H, d, J=6.7Hz), 0.92 (3H, d, J=6.4Hz), 2.62 (1H, m), 2.93 (3H, s), 2.95 (1H, dd, J=17.7Hz, 3.7Hz), 3.20 (1H, br. d, J=17.7Hz), 3.32 (3H, s), 3.33~3.41 (2H, m), 4.36 (1H, d, J=10.4Hz), 4.38 (1H, br. s), 6.14 (1H, br. s), 6.51 (1H, d, J=7.6Hz), 6.89 (1H, s), 6.90 (1H, d, J=7.6Hz), 7.06 (1H, t, J=7.6Hz), 8.02 (1H, br. s); conformer A, 0.92 (d, J=6.4Hz), 1.24 (d, J=7.0Hz), 2.76 (s). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 315 (M^+). Anal. Calcd. for $C_{18}H_{25}N_3O_2I/4H_2O$: C, 67.57; H, 8.03; N, 13.13. Found: C, 67.40; H, 8.08; N, 13.04.

(-)-14-O-Butylindolactam V (*15*). A mixture of **1** (56.7 mg), *n*-butyl *p*-toluenesulfonate (25 μl) and NaH (60% dispersion in oil, washed with dry benzene, 190 mg) in dry benzene (3 ml) was refluxed for 6 hr. The reaction mixture was worked up in the same way as above to give 3.3 mg (4.9% yield) of **15**, which was recrystallized from EtOAc-hexane to yield colorless rods, mp 98~100 °C, $[\alpha]_D^{23}$-115° (c=0.161, EtOH). UV λ_{max} (EtOH) nm (ε): 299.5 (7500), 286 (7100), 228.5 (27,300). IR ν_{max} (KBr) cm^{-1}: 3480, 3290, 2950, 2860, 1645, 1605, 1565, 1500, 1440, 1375, 1105, 1039, 780, 740. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 7.2; conformer B, 0.61 (3H, d, J=7.0Hz), 0.91 (6H, m), 1.34 (2H, m), 1.52 (2H, m), 2.61 (1H, m), 2.92 (3H, s), 2.93 (1H, dd, J=17.4Hz, 3.7Hz), 3.19 (1H, br. d, J=17.4Hz), 3.31~3.44 (4H, m), 4.36 (1H, d, J=10.1Hz), *ca.* 4.36 (1H, br. s), 6.17 (1H, br. s), 6.50 (1H, d, J=7.6Hz), 6.87 (1H, br. s), 6.88 (1H, d, J=7.6Hz), 7.05 (1H, t, J=7.6Hz), 8.01 (1H, br. s); conformer A, 2.75 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 357.2422 (M^+, calcd. for $C_{21}H_{31}N_3O_2$, 357.2416).

(-)-14-O-Hexylindolactam V (*16*). A mixture of **1** (68.4 mg), *n*-hexyl *p*-toluenesulfonate (40 μl) and NaH (180 mg) in benzene (2 ml) was refluxed for 10 hr and stirred at room temperature overnight. The reaction mixture was worked up in the same way as above to give 24.4 mg (27.9% yield) of **16**, which was recrystallized from EtOAc-hexane to yield colorless needles, mp 108~110°C.

$[\alpha]_D^{27}$-118° (c=0.161, EOH). UV λ_{max} (EtOH) nm (ε): 300 (8300), 285 (7900), 228.5 (30,500). IR ν_{max} (KBr) cm^{-1}: 3380, 3290, 2920, 2850, 1645, 1605, 1500, 1460, 1385, 1105, 1038, 780, 740. ^1H NMR δ [$(CD_3)_2CO$] ppm: conformer A: B=1: 3.8; conformer B, 0.63 (3H, d, J=7Hz), 0.90 (6H, m), 1.1~1.6 (8H, m), 2.60 (1H, m), 2.90 (3H, s), 3.10 (2H, m), 3.3~3.5 (4H, m), 4.30 (1H, br.s), 4.45 (1H, d, J=11Hz), 6.23 (1H, br.s), 6.50 (1H, d, J=8Hz), 7.00 (3H, m), 8.05 (1H, br.s); conformer A, 2.71 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 385.2724 (M^+, calcd. for $C_{23}H_{35}N_3O_2$, 385.2729).

Preparation of (-)-*14-dehydroxyindolactam V* (*17*). Lithium aluminum hydride (40 mg) was dissolved in dry ether (5 ml) and CH_2Cl_2 (5 ml). To the solution, (-)-14-bromoindolactam V (57 mg) prepared from **1** and *p*-toluenesulfonyl bromide was added, and the reaction mixture was stirred at room temperature for 3 hr. After adding crushed ice carefully, the mixture was extracted with EtOAc. The EtOAc extracts were purified on Wako C-100 gel with 8% acetone in toluene, followed by HPLC on NOVA-PAK C_{18} with 50% CH_3CN in water to give **17** (12 mg, 27% yield) as amorphous powder. $[\alpha]_D^{20}$-11° (c=0.910, EtOH). UV λ_{max} (EtOH) nm (ε): 297 (5750), 284 (5650), 227 (22,700). IR ν_{max} (KBr) cm^{-1}: 3350, 3270, 2950, 2870, 1650, 1605, 1500, 1445, 1362, 1270, 1210, 1095, 1038, 778, 740. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 1; conformer B, 0.62 (3H, d, J=6.7Hz), 0.91 (3H, d, J=6.4Hz), 1.24 (3H, d, J=6.7Hz), 2.60 (1H, m), 2.91 (3H, s), 3.02 (1H, dd, J=17.1Hz, 3.7 Hz), 3.32 (1H, br.d, J=17.1Hz), 4.30 (1H, m), 4.36 (1H, d, J=10.1Hz), 5.61 (1H, br.s), 6.49 (1H, d, J=7.9Hz), 6.87 (1H, s), 6.88 (1H, d, J=7.9Hz), 7.05 (1H, t, J=7.9Hz), 8.00 (1H, br.s); conformer A, 0.87 (d, J=6.7Hz), 0.92 (d, J=6.4Hz), 1.22 (d, J=6.1Hz), 2.38 (m), 2.51 (d, J=14.4Hz), 2.74 (s), 2.89 (d, J=10.7Hz), 3.18 (br.d, J=14.4Hz), 4.51 (br.s), 6.99 (d, J=2.4Hz), 7.03 (dd, J=7.6Hz, 1.2Hz), 7.14 (t, J=7.6Hz), 7.28 (dd, J=7.6Hz, 1.2Hz), 8.29 (br.s). HR-EIMS m/z: 285.1840 (M^+, calcd. for $C_{17}H_{23}N_3O$, 285.1841).

Preparation of (-)-*14-chloroindolactam V* (*18*). *p*-Toluenesulfonyl chloride (43 mg) was treated with **1** (50.8 mg) in dry pyridine (2 ml) at room temperature over night. The reaction mixture was evaporated to dryness, and partitioned between EtOAc and water. The EtOAc extracts were purified by column chromatography on Wako C-200 gel with 10% acetone in toluene, followed by HPLC on semi prep. μ-Porasil with 20% EtOAc in hexane to give **18** (12.0 mg, 22% yield) as amorphous powder. $[\alpha]_D^{20}$-26° (c=0.252, EtOH). UV λ_{max} (EtOH) nm (ε): 287 (7200), 226 (27,800). IR ν_{max} (KBr) cm^{-1}: 3360, 3290, 2960, 2870, 1659, 1608, 1501, 1438, 1039, 745. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 1.4; conformer B, 0.64 (3H, d, J=6.4Hz), 0.93 (3H, d, J=6.4Hz), 2.61 (1H, m), 2.93 (3H, s), 3.23 (1H, dd, J=17.1Hz, 3.9Hz), 3.30 (1H, br.d, J=17.1Hz), 3.56 (1H, dd, J=11.7 Hz, 6.8Hz), 3.68 (1H, dd, J=11.7Hz, 3.9Hz), 4.32 (1H, d, J=10.3Hz), 4.63 (1H m), 6.01 (1H, br.s), 6.54 (1H, d, J=7.8Hz), 6.92 (1H, d, J=7.8Hz), 6.93 (1H, s), 7.08 (1H, t, J=7.8Hz), 8.04 (1H, br.s); conformer A, 0.93 (d, J=6.4Hz), 1.25 (d, J=6.3Hz), 2.39 (m), 2.75 (s), *ca.* 3.1 (m), 4.57 (br.s), 7.05 (d, J=7.3Hz), 7.13 (d, J=2.4Hz), 7.17 (t, J=7.3Hz), 7.29 (d, J=7.3Hz), 8.32 (br. s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 319.1437 (M^+, calcd. for $C_{17}H_{22}N_3OCl$, 319.1451).

Preparation of (-)-N^1-*alkylindolactam* V. A mixture of (-)-14-*O*-acetylindolactam V (**2**, *ca.* 0.1 mmole), alkyl *p*-toluene sulfonate (*ca.* 0.15 mmole) and NaH (60% dispersion in oil, washed with dry benzene, 100 mg) in dry benzene (3 ml) was refluxed for 10 hr. To the reaction mixture, a small amount of *n*-BuOH was added and the solution was neutralized with acetic acid. This reaction mixture was evaporated *in vacuo* to dryness and the residue was partitioned between EtOAc and water. The concentrates of the EtOAc layer were purified by column chromatography on Wako C-100 gel with 25% acetone in toluene, followed by HPLC on NOVA-PAK C_{18} with 70~80% MeOH in water to give the corresponding derivatives as follows.

(-)-N^1-*Methylindolactam V* (*19*). 35% Yield, colorless needles from MeOH, mp 260~262°C, $[\alpha]_D^{22}$ -241° (c=0.199, $CHCl_3$). UV λ_{max} (EtOH) nm (ε): 314 (9100), 307.5 (9300), 287 (6800), 230.5 (30,200). IR ν_{max} (KBr) cm^{-1}: 3360, 3260, 2920, 2880, 1635, 1552, 1495, 1282, 1072, 998, 728. ^1H NMR δ ($CDCl_3$) ppm; conformer A: B=1: 4.5, conformer B, 0.62 (3H, d, J=6.7Hz), 0.92 (3H, d, J=6.1Hz), 2.59 (1H, m), 2.91 (3H, s), 3.02 (1H, dd, J=17.7Hz, 3.5Hz), 3.16 (1H, br.d, J=

17.7Hz), 3.56 (1H, m), 3.69 (3H, s), 3.72 (1H, m), 4.29 (1H, br.s), 4.41 (1H, d, J=10.1Hz), 6.50 (1H, d, J=7.6Hz), 6.75 (1H, s), 6.83 (1H, d, J=7.6Hz), 7.10 (1H, t, J=7.6Hz), 7.31 (1H, br.s); conformer A, 0.94 (d, J=6.7Hz), 1.25 (d, J=6.4Hz), 2.74 (s), 3.76 (s). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 315 (M^+). Anal. Calcd. for $C_{18}H_{25}N_3O_2$: C, 68.54; H, 7.99; N, 13.32. Found: C, 68.71; H, 8.00; N, 13.19.

(-)-*N*^{1}-*Ethylindolactam V* (*20*). 55% Yield, amorphous powder, $[\alpha]_D^{18}$-145° (c=0.759, EtOH). UV λ_{max} (EtOH) nm (ε): 314 (7100), 308.5 (7200), 288 (5100), 231 (22,200). IR ν_{max} (KBr) cm^{-1}: 3350, 2920, 2860, 1650, 1599, 1560, 1492, 1279, 1070, 778, 730. ^{1}H NMR δ ($CDCl_3$) ppm: conformer A: B =1: 5.4; conformer B, 0.63 (3H, d, J=6.7Hz), 0.92 (3H, d, J=6.1Hz), 1.44 (3H, t, J=7.3Hz), 2.58 (1H, m), 2.91 (3H, s), 3.04 (1H, dd, J=17.4Hz, 3.7Hz), 3.16 (1H, br.d, J=17.4Hz), 3.57 (1H, m), 3.73 (1H, m), 4.07 (2H, q, J=7.3Hz), 4.31 (1H, m), 4.41 (1H, d, J=10.1Hz), 6.49 (1H, d, J=7.6Hz), 6.80 (1H, d, J=0.6Hz), 6.85 (1H, d, J=7.6Hz), 7.07 (1H, t, J=7.6Hz), 7.64 (1H, br.s); conformer A, 0.93 (d, J=6.4Hz), 1.24 (m), 2.40 (m), 2.74 (s), 3.42 (m), 4.14 (q, J=7.3 Hz), 6.95 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 329.2106 (M^+, calcd. for $C_{19}H_{27}N_3O_2$, 329.2103).

(-)-*N*^{1}-*Butylindolactam V* (*21*). 32% Yield, amorphous powder, $[\alpha]_D^{18}$-134°, c=0.539, EtOH). UV λ_{max} (EtOH) nm (ε): 313 (7400), 308.5 (7600), 289 (5200), 231.5 (22,900). IR ν_{max} (KBr) cm^{-1}: 3350, 2920, 2860, 1650, 1598, 1560, 1495, 1278, 1070, 778, 730. ^{1}H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 5.5; conformer B, 0.67 (3H, d, J=6.5Hz), 1.00 (6H, m), 1.33 (2H, m), 1.84 (2H, m), 2.60 (1H, m), 2.93 (3H, s), 3.11 (2H, m), 3.5~3.8 (2H, m), 4.00 (2H, t, J=7Hz), 4.30 (1H, m), 4.41 (1H, d, J=10Hz), 6.48 (1H, d, J=8Hz), 6.77 (1H, s), 6.81 (1H, d, J=8Hz), 7.06 (1H, t, J=8Hz), 7.68 (1H, br.s); conformer A, 2.76 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 357.2424 (M^+, calcd. for $C_{21}H_{31}N_3O_2$, 357.2416).

(-)-*N*^{1}-*Hexylindolactam V* (*22*). 34% Yield, amorphous powder, $[\alpha]_D^{25}$-134° (c=0.447, EtOH). UV λ_{max} (EtOH) nm (ε): 314 (8300), 308 (8500), 288.5 (5900), 231.5 (25,400). IR ν_{max} (KBr) cm^{-1}: 3320, 2920, 2860, 1655, 1598, 1560, 1492, 1278, 1070, 778, 728. ^{1}H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 5.3; conformer B, 0.64 (3H, d, J=6.5Hz), 0.94 (6H, m), 1.33 (6H, m), 1.80 (2H, m), 2.60 (1H, m), 2.91 (3H, s), 3.09 (2H, m), 3.5~3.8 (2H, m), 3.99 (2H, t, J=7Hz), 4.29 (1H, m), 4.40 (1H, d, J=10Hz), 6.49 (1H, d, J=8Hz), 6.78 (1H, s), 6.82 (1H, d, J=8Hz), 7.07 (1H, t, J=8Hz), 7.81 (1H, br.s); conformer A, 2.73 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 385.2733 (M^+, calcd. for $C_{23}H_{35}N_3O_2$, 385.2729).

(-)-*N*^{1}-*Octylindolactam V* (*23*). 34% Yield, amorphous powder, $[\alpha]_D^{21}$-132° (c=0.683, EtOH). UV λ_{max} (EtOH) nm (ε): 314 (8500), 308 (8700), 288.5 (6000), 231 (25,200). IR ν_{max} (KBr) cm^{-1}: 3330, 2920, 2860, 1655, 1599, 1560, 1492, 1279, 1070, 778, 725. ^{1}H NMR δ ($CDCl_3$) ppm: conformer A: B =1: 6; conformer B, 0.68 (3H, d, J=6.5Hz), 0.96 (6H, m), 1.32 (10H, br.s), 1.84 (2H, m), 2.60 (1H, m), 2.93 (3H, s), 3.12 (2H, m), 3.5~3.8 (2H, m), 4.00 (2H, t, J=7Hz), 4.31 (1H, m), 4.42 (1H, d, J=10Hz), 6.48 (1H, d, J=8Hz), 6.77 (1H, s), 6.82 (1H, d, J=8Hz), 7.06 (1H, t, J=8Hz), 7.71 (1H, br.s); conformer A, 2.77 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 413.3044 (M^+, calcd. for $C_{25}H_{39}N_3O_2$, 413.3042).

Halogenation of (-)-*indolactam V* (*1*).

Bromination of 1. (-)-Indolactam V (1) (79.4 mg) was dissolved in dioxane (2 ml) and pyridine (0.1 ml). To the solution, Br_2 (8 μl) was added, and stirred at room temperature for 2 hr. The reaction mixture was partitioned between EtOAc and water. The EtOAc extracts were purified by column chromatography on Wako C-200 gel (5 g), eluting with toluene containing increasing amounts of acetone. The eluates with 20, 25 and 30% acetone were combined and chromatographed on YMC I-40/64 gel (10 g) with 65% MeOH in water to give (-)-7-bromoindolactam V (**24**) (24.8 mg, 25% yield) and (-)-2,7-dibromoindolactam V (**25**) (5.0 mg, 4% yield). Compound **24**. Colorless rods from EtOH-water, mp 198 °C dec., $[\alpha]_D^{22}$-210° (c=0.110, EtOH). UV λ_{max} (EtOH) nm (ε): 314 (9400), 310 (9700), 287.5 (7600), 230.5 (28,000). IR ν_{max} (KBr) cm^{-1}: 3370, 3300, 2940, 2870, 1655, 1622, 1607, 1500, 1445, 1038, 865, 792. ^{1}H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 6.3; confor-

mer B, 0.62 (3H, d, J=6.7Hz), 0.93 (3H, d, J=6.1Hz), 2.59 (1H, m), 2.89 (3H, s), 3.03 (1H, dd, J=17.7Hz, 3.7Hz), 3.17 (1H, br.d, J=17.7Hz), 3.56 (1H, dd, J=11.0Hz, 8.6Hz), 3.74 (1H, dd, J=11.0 Hz, 4.0Hz), 4.24 (1H, br.s), 4.33 (1H, d, J=10.1Hz), 6.40 (1H, d, J=8.2Hz), 6.95 (1H, s), 7.03 (1H, br.s), 7.18 (1H, d, J=8.2Hz), 8.19 (1H, br.s); conformer A, 0.94 (d, J=6.4Hz), 1.23 (d, J=6.7Hz), 2.40 (m), 2.73 (s). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 379 (M^+). Anal. Calcd. for $C_{17}H_{22}N_3O_2Br$: C, 53.69; H, 5.83; N, 11.05. Found: C, 53.75; H, 5.61; N, 10.94. Compound **25**. Colorless rods from acetone, mp 184~185°C, $[\alpha]_D^{18}$-154° (c=0.163, EtOH). UV λ_{max} (EtOH) nm (ε): 314 (8500), 290 (7600), 235 (23,100). IR ν_{max} (KBr) cm^{-1}: 3320, 3140, 2940, 1655, 1638, 1600, 1500, 1442, 1047, 795. ^1H NMR δ (CD_3OD) ppm: conformer A: B=1: 2.3; conformer B, 0.61 (3H, d, J=6.7Hz), 0.90 (3H, d, J=6.1Hz), 2.53 (1H, m), 2.86 (3H, s), 2.93 (1H, br.d, J=17.9Hz), 3.20 (1H, dd, J=17.9Hz, 3.8Hz), 3.49 (1H, dd, J=11.0Hz, 8.5Hz), 3.57 (1H, dd, J=11.0Hz, 4.7Hz), 4.18 (1H, m), 4.31 (1H, d, J=10.4Hz), 6.45 (1H, d, J=8.2Hz), 7.11 (1H, d, J=8.2Hz); conformer A, 0.90 (d, J=6.1Hz), 1.22 (d, J=6.7Hz), 2.30 (m), 2.69 (s), 6.90 (d, J=7.9Hz), 7.26 (d, J=7.9Hz). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 457.0008 (M^+, calcd. for $C_{17}H_{21}N_3O_2Br_2$, 457.0001).

(-)-7-Iodoindolactam V (*26*). (-)-Indolactam V (I) (96.5 mg) was dissolved in dioxane (2 ml) and pyridine (0.1 ml). To the solution, I_2 (46.2 mg) was added and stirred at room temperature for one day. The reaction mixture was worked up in the same way as above to give 48.5 mg (35.4% yield) of **26**, which was recrystallized from MeOH-water to yield yellow platelets, mp 202°C dec., $[\alpha]_D^{29}$-240° (c=0.129, EtOH). UV λ_{max} (EtOH) nm (ε): 315.5 (10,800), 311.5 (11,200), 288 (8700), 233 (30,800). IR ν_{max} (KBr) cm^{-1}: 3420, 3320, 3200, 2900, 2870, 1630, 1595, 1535, 1492, 1440, 1342, 1060, 1038, 800, 790, 685. ^1H NMR δ (CD_3OD) ppm: conformer A: B=1: 2.2; conformer B, 0.61 (3H, d, J=6.7Hz), 0.89 (3H, d, J=6.4Hz), 2.54 (1H, m), 2.87 (3H, s), 3.08 (2H, m), 3.46 (1H, dd, J=11.0Hz, 8.9Hz), 3.61 (1H, dd, J=11.0Hz, 4.6Hz), 4.16 (1H, m), 4.43 (1H, d, J=10.4 Hz), 6.32 (1H, d, J=8.2Hz), 7.03 (1H, s), 7.30 (1H, d, J=8.2Hz); conformer A, 0.89 (d, J=6.4Hz), 1.22 (d, J=6.7Hz), 2.30 (m), 2.71 (s), 4.26 (m), 6.76 (d, J=7.9Hz), 7.20 (s), 7.46 (d, J=7.9Hz). Other peaks had weak intensities and overlapped those of the major conformer. EIMS m/z: 427 (M^+). Anal. Calcd. for $C_{17}H_{22}N_3O_2I$: C, 47.79; H, 5.19; N, 9.83. Found: C, 47.78; H, 5.18; N, 9.75.

(-)-5-Chloroindolactam V (*27*). A mixture of 1 (106 mg) and *N*-chlorosuccinimide (66 mg) in dioxane (6 ml) was stirred at room temperature for 10 min. After adding water and EtOH, this reaction mixture was evaporated to dryness and partitioned between EtOAc and water. The EtOAc extract was purified by column chromatography on Wako C-200 gel (20 g) with 22.5% acetone in toluene, followed by on YMC I-40/64 gel (10 g) with 65% MeOH in water to give **27** as amorphous powder (47 mg, 40% yield). $[\alpha]_D^{29}$+46° (c=0.153, EtOH). UV λ_{max} (EtOH) nm (ε): 298 (5900), 233 (26,000). IR ν_{max} (KBr) cm^{-1}: 3290, 2960, 2930, 1642, 1490, 1462, 1040, 775. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=10: 1; conformer A, 0.97 (3H, d, J=6.4Hz), 1.36 (3H, d, J=6.7Hz), 2.62 (1H, m), 2.75 (3H, s), 2.79 (1H, dd, J=14.7Hz, 1.8Hz), 3.09 (1H, d, J=10.4Hz), 3.18 (1H, dd, J=14.7Hz, 4.3Hz), 3.35 (2H, m), 4.46 (1H, m), 4.77 (1H, d, J=10.7Hz), 7.06 (1H, d, J=2.1 Hz), 7.18 (1H, d, J=8.6Hz), 7.27 (1H, d, J=8.6Hz), 8.38 (1H, br.s); conformer B, 1.07 (d, J=6.7Hz), 1.27 (d, J=6.4Hz). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 335.1418 (M^+, calcd. for $C_{17}H_{22}N_3O_2Cl$, 335.1401).

Friedel-Crafts acylation of 1. The appropriate acid chloride or acid anhydride (*ca.* 0.15 mmole) was treated with (-)-14-*O*-acetylindolactam V (**2**) (0.1 mmole) and $AlCl_3$ (0.2 mmole) in nitrobenzene (1 ml) at room temperature for one day. After adding water and MeOH, the products were hydrolyzed by NaOH (*ca.* 5 mmole) at room temperature for 30 min. The reaction mixture was partitioned between EtOAc and water. The EtOAc extracts were purified by column chromatography on Wako C-200 gel (10 g) with 2-PrOH-$CHCl_3$, followed by purification on YMC I-40/64 gel (10 g) with MeOH-water to give the corresponding derivatives as follows.

(-)-7-Acetylindolactam V (*28*). 45% Yield, pale yellow rods from EtOH-water, mp 219~222°C,

$[\alpha]_D^{24}$-670° (c=0.18, EtOH). CD $[\theta]_{364}$−37,000, $[\theta]_{292}$−6400, $[\theta]_{274}$−13,900, $[\theta]_{262}$−10,100, $[\theta]_{231}$−34,300, $[\theta]_{210}$−63,400 ($c=1.04\times10^{-4}$, MeOH). UV λ_{max} (EtOH) nm (ε): 370 (20,900) 258(15,500). IR ν_{max} (KBr) cm^{-1}: 3390, 2950, 1660, 1645, 1575, 1502, 1281, 1165, 1048, 795. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.58 (3H, d, J=6.7Hz), 0.94 (3H, d, J=6.1Hz), 2.60 (1H, m), 2.61 (3H, s), 2.98 (3H, s), 3.07 (1H, dd, J=17.4Hz, 3.4Hz), 3.18 (1H, br.d, J=17.4Hz), 3.59 (1H, m), 3.75 (1H, m), 4.10 (1H, m), 4.56 (1H, d, J=10.4Hz), 6.48 (1H, d, J=8.2Hz), 7.00 (1H, s), 7.12 (1H, br.s), 7.70 (1H, d, J=8.2Hz), 10.80 (1H, br.s). EIMS m/z: 343 (M^+). Anal. Calcd. for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.46; H, 7.35; N, 12.16.

(-)-*2,7-Diacetylindolactam V* (*29*). Amorphous powder, $[\alpha]_D^{23}$-655° (c=0.740, EtOH). UV λ_{max} (EtOH) nm (ε): 398 (22,200), 319 (15,900), 258 (16,200). IR ν_{max} (KBr) cm^{-1}: 3370, 2920, 1665, 1650, 1575, 1515, 1505, 1280. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.51 (3H, d, J=6.6Hz), 0.94 (3H, d, J=6.2Hz), 2.55 (1H, m), 2.63 (3H, s), 2.65 (3H, s), 2.99 (3H, s), 3.05 (1H, dd, J=19.1Hz, 3.3Hz), 3.64 (2H, m), 4.10 (1H, br.s), 4.29 (1H, dd, J=19.1Hz, 3.7Hz), 4.47 (1H, d, J=10.3Hz), 6.46 (1H, d, J=8.4Hz), 7.49 (1H, br.s), 7.72 (1H, d, J=8.4Hz), 11.46 (1H, br.s). HR-EIMS m/z: 385.2000 (M^+, calcd. for $C_{21}H_{27}N_3O_4$, 385.2002).

(-)-*5,7-Diacetylindolactam V* (*30*). Amorphous powder, $[\alpha]_D^{22}$+135° (c=0.565, EtOH). UV λ_{max} (EtOH) nm (ε): 354 (6800), 247.5 (14,600), 227 (13,600). IR ν_{max} (KBr) cm^{-1}: 3380, 3290, 2960, 2920, 2880, 1678, 1653, 1585, 1570, 1458, 1232, 1038. ^1H NMR δ (CD_3OD) ppm: conformer A: B=5.5:1; conformer A; 0.87 (3H, d, J=6.7Hz), 1.04 (3H, d, J=7.0Hz), 2.43 (1H, m), 2.57 (3H, s) 2.67 (3H, s), 2.75 (3H, s), 2.92 (1H, dd, J=14.7Hz, 1.5Hz), 3.09∼3.20 (4H, m), 4.27 (1H, m), 7.33 (1H, s), 7.68 (1H, s); conformer B, 0.97 (d, J=6.4Hz), 1.05 (d, J=7.9Hz), 2.66 (s), 2.72 (s), 2.87 (s), 7.19 (s), 8.06 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 385.2026 (M^+, calcd. for $C_{21}H_{27}N_3O_4$, 385.2002).

(-)-*5-Acetylindolactam V* (*31*). Amorphous powder, $[\alpha]_D^{19}$+57° (c=0.40, EtOH). CD $[\theta]_{289}$−7900, $[\theta]_{264}$ 0, $[\theta]_{257}$+4400, $[\theta]_{248}$ 0, $[\theta]_{229.5}$+76,100, $[\theta]_{214}$+21,200 ($c=1.04\times10^{-4}$, MeOH). UV λ_{max} (EtOH) nm (ε): 290 (4800), 248 (sh., 9000), 227 (18,000). IR ν_{max} (KBr) cm^{-1}: 3370, 3290, 2960, 2930, 2880, 1675, 1655, 1605, 1500, 1465, 1350, 1140, 782. ^1H NMR δ ($CDCl_3$): conformer A: B=30:1; conformer A, 0.84 (3H, d, J=6.4Hz), 1.03 (3H, d, J=7.0Hz), 2.45 (1H, m), 2.52 (3H, s), 2.74 (3H, s), 2.78 (1H, d, J=14.4Hz), 2.93 (1H, d, J=10.7Hz), 3.16 ∼3.27 (3H, m), 4.38 (1H, br.m), 4.89 (1H, d, J=10.4Hz), 6.90 (1H, d, J=8.2Hz), 7.05 (1H, d, J=2.1Hz), 7.25 (1H, d, J=8.2Hz), 8.93 (1H, br.s). HR-EIMS m/z: 343.1890 (M^+, calcd. for $C_{19}H_{25}N_3O_3$, 343.1896).

(-)-*2,7-Dibutanoylindolactam V* (*32*). Amorphous powder, $[\alpha]_D^{18}$-527° (c=0.61, EtOH). UV λ_{max} (EtOH) nm (ε): 398 (20,300), 320 (14,300), 258.5 (14,900). IR ν_{max} (KBr) cm^{-1}: 3370, 2950, 2870, 1665, 1573, 1513, 1265, 1170. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.52 (3H, d, J=7.0Hz), 0.94 (3H, d, J=6.2Hz), 1.05 (6H, m), 1.82 (4H, m), 2.56 (1H, m), 2.99 (3H, s), 2.92∼3.07 (5H, m), 3.63 (2H, m), 4.09 (1H, m), 4.34 (1H, dd, J=19.2Hz, 3.5Hz), 4.48 (1H, d, J=10.3Hz), 6.46 (1H, d, J=8.6Hz), 7.35 (1H, br.s), 7.86 (1H, d, J=8.6Hz), 11.59 (1H, br.s). HR-EIMS m/z: 441.2621 (M^+, calcd. for $C_{25}H_{35}N_3O_4$, 441.2628).

(-)-*7-Butanoylindolactam V* (*33*). 29% Yield, pale yellow leaflets from MeOH, mp 207∼209°C, $[\alpha]_D^{20}$-546° (c=0.90, EtOH). UV λ_{max} (EtOH) nm (ε): 370 (19,300), 258.5 (14,300). IR ν_{max} (KBr) cm^{-1}: 3460, 3280, 2960, 1660, 1630, 1572, 1502, 1157. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.57 (3H, d, J=6.7Hz), 0.93 (3H, d, J=6.4Hz), 1.02 (3H, t, J=7.6Hz), 1.80 (2H, sextet, J=7.6Hz), 2.55 (1H, m), 2.95 (3H, s), 2.96 (2H, m), 3.10 (1H, dd, J=17.7Hz, 3.4Hz), 3.15 (1H, br.d, J=17.7Hz), 3.60 (1H, m), 3.73 (1H, m), 3.90 (1H, br.s, OH), 4.12 (1H, m), 4.56 (1H, d, J=10.1Hz), 6.45 (1H, d, J=8.5Hz), 6.99 (1H, s), 7.72 (1H, d, J=8.5Hz), 7.86 (1H, br.s), 10.87 (1H, br.s). EIMS m/z: 371 (M^+). Anal. Calcd. for $C_{21}H_{29}N_3O_3$: C, 67.90; H, 7.87; N, 11.31. Found: C, 68.06; H, 7.94; N, 11.40.

(-)-*7-Hexanoylindolactam V* (*34*). 38% Yield, amorphous powder, $[\alpha]_D^{19}$-509° (c=1.59, EtOH). UV λ_{max} (EtOH) nm (ε): 370 (19,800), 259 (14,800). IR ν_{max} (KBr) cm^{-1}: 3380, 2910, 2870, 1655, 1640, 1572, 1501, 1295, 1150. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.58 (3H, d, J=6.7Hz), 0.92

(3H, t, J=7.0Hz), 0.94 (3H, d, J=6.4Hz), 1.38 (4H, m), 1.76 (2H, m), 2.57 (1H, m), 2.96 (3H, s), 2.96 (2H, m), 3.09 (1H, dd, J=17.7Hz, 3.7Hz), 3.17 (1H, br.d, J=17.7Hz), 3.36 (1H, br.s, OH), 3.60 (1H, m), 3.74 (1H, m), 4.12 (1H, br.s), 4.56 (1H, d, J=10.1Hz), 6.46 (1H, d, J=8.5 Hz), 7.00 (1H, s), 7.66 (1H, br.s), 7.72 (1H, d, J=8.5Hz), 10.87 (1H, br.s). HR-EIMS m/z : 399.2515 (M^+, calcd. for $C_{23}H_{33}N_3O_3$, 399.2522).

(-)-*7-Octanoylindolactam V* (*35*). 56% Yield, amorphous, $[\alpha]_D^{15}$-516° (c=1.29, EtOH). UV λ_{max} (EtOH) nm (ε) : 370 (21,100), 258.5 (15,800). IR ν_{max} (KBr) cm^{-1} : 3400, 2920, 1658, 1635, 1575, 1502, 1150. ^1H NMR δ ($CDCl_3$) ppm : conformer B only, 0.58 (3H, d, J=6.5Hz), 0.95 (6H, m), 1.35 (8H, m), 1.82 (2H, m), *ca.* 2.6 (1H, m), 2.97 (3H, s), 2.85~3.25 (4H, m), 3.45~3.85 (2H, m), 4.12 (1H, br.s), 4.55 (1H, d, J=10Hz), 6.45 (1H, d, J=8.5Hz), 6.97 (1H, s), 7.67 (1H, br.s), 7.69 (1H, d, J=8.5Hz), 10.92 (1H, br.s). HR-EIMS m/z : 427.2836 (M^+, calcd. for $C_{25}H_{37}N_3O_3$, 427.2835).

(-)-*7-Dodecanoylindolactam V* (*36*). 41% Yield, amorphous, $[\alpha]_D^{18}$-477° (c=1.26, EtOH). UV λ_{max} (EtOH) nm (ε) : 370.5 (21,800), 258.5 (16,500). IR ν_{max} (KBr) cm^{-1} : 3390, 2910, 1655, 1635, 1575, 1501, 1145. ^1H NMR δ ($CDCl_3$) ppm : conformer B only, 0.56 (3H, d, J=7Hz), 0.90 (6H, m), 1.30 (16H, m), 1.72 (2H, m), 2.60 (1H, m), 2.96 (3H, s), 2.85~3.25 (4H, m), 3.5~3.8 (2H, m), 4.10 (1H, br.s), 4.57 (1H, d, J=10Hz), 6.47 (1H, d, J=8.5Hz), 6.98 (1H, s), 7.62 (1H, br.s), 7.71 (1H, d, J=8.5Hz), 10.86 (1H, br.s). HR-EIMS m/z : 483.3461 (M^+, calcd. for $C_{29}H_{45}N_3O_3$, 483.3461).

(-)-*7-Benzoylindolactam V* (*37*). 22% Yield, amorphous, $[\alpha]_D^{30}$-611° (c=0.74, EtOH). UV λ_{max} (EtOH) nm (ε) : 389 (18,900), 264 (10,300), 225 (sh., 14,700). IR ν_{max} (KBr) cm^{-1} : 3370, 2960, 2920, 1660, 1615, 1580, 1565, 1502, 1282, 1119. ^1H NMR δ ($CDCl_3$) ppm : conformer B only, 0.63 (3H, d, J=6.5Hz), 0.98 (3H, d, J=6.5Hz), 2.60 (1H, m), 2.98 (3H, s), 3.17 (2H, m), 3.48 (1H, m), 3.70 (1H, m), 4.12 (1H, m), 4.62 (1H, d, J=10Hz), 6.45 (1H, d, J=8Hz), 7.07 (1H, br.s), 7.4~7.8 (7H, m), 10.88 (1H, br.s). HR-EIMS m/z : 405.2056 (M^+, calcd. for $C_{24}H_{27}N_3O_3$, 405.2052).

(-)-*7-(3-Methylcarboxy-propanoyl) indolactam V* (*38*). 6% Yield, amorphous, $[\alpha]_D^{24}$-513° (c=0.133, EtOH). UV λ_{max} (EtOH) nm (ε) : 370 (20,400), 258.5 (15,300). ^1H NMR ($CDCl_3$) ppm : conformer B only, 0.58 (3H, d, J=6.7Hz), 0.94 (3H, d, J=6.4Hz), 2.59 (1H, m), 2.77 (2H, t, J=6.7Hz), 2.98 (3H, s), 3.05 (1H, dd, J=17.7Hz, 3.4Hz), 3.17 (1H, br.d, J=17.7Hz), 3.38 (2H, t, J=6.7Hz), 3.56 (1H. m), 3.72 (3H, s), 3.73 (1H, m), 4.08 (1H, br.s), 4.55 (1H, d, J=10.1Hz), 6.48 (1H, d, J=8.5Hz), 6.93 (1H, br.s), 6.97 (1H, s), 7.76 (1H, d, J=8.5Hz), 10.75 (1H, br.s). HR-EIMS m/z : 415.2108 (M^+, calcd. for $C_{22}H_{29}N_3O_5$, 415.2107).

Reduction of (-)-*7-Acylindolactam V*. $LiAlH_4$ (*ca.* 0.1 mmole) was added to $AlCl_3$ (0.2 mmole) in ether (2 ml). After the bubbling ceased, the mixture was added to the solution of (-)-7-acylindolactam V (*ca.* 0.05 mmole) in ether (*ca.* 10 ml). The reaction mixture was stirred at room temperature for 1 hr. Excess of the reagent was destroyed by adding EtOAc, followed by addition of 10% H_2SO_4. After stirring for 30 min, the mixture was partitioned between EtOAc and water. The EtOAc extracts were purified by column chlomatography to give (-)-7-alkylindolactam V as follows.

(-)-*7-Ethylindolactam V* (*39*). 63% Yield, amorphous powder, $[\alpha]_D^{18}$-110.5° (c=0.973, EtOH). UV λ_{max} (EtOH) nm (ε) : 295 (sh., 6900), 287 (7100), 228.5 (23,800). IR ν_{max} (KBr) cm^{-1} : 3350, 2960, 2870, 1650, 1605, 1505, 1447, 1370, 1275, 1038, 805. ^1H NMR δ [$(CD_3)_2CO$] ppm : conformer A : B = 1 : 2.1 ; conformer B, 0.61 (3H, d, J=7.0Hz), 0.88 (3H, d, J=6.4Hz), 1.27 (3H, t, J=7.6Hz), 2.56 (1H, m), 2.85 (3H, s), 2.86 (2H, m), 3.05 (1H, dd, J=17.4Hz, 3.7Hz), 3.10 (1H, br.d, J=17.4Hz), 3.46 (1H, dd, J=11.0Hz, 9.2Hz), 3.65 (1H, dd, J=11.0Hz, 4.6Hz), 4.16 (1H, m), 4.29 (1H, br.s, OH), 4.38 (1H, d, J=10.1Hz), 6.37 (1H, br.s), 6.42 (1H, d, J=7.6Hz), 6.81 (1H d, J=7.6Hz), 7.02 (1H, s), 9.97 (1H, br.s) ; conformer A, 0.89 (d, J=6.4Hz), 1.22 (d, J=6.7Hz), 1.31 (t, J=7.6Hz), 2.33 (m), 2.70 (s), 2.79 (q, J=7.3Hz), 3.02 (d, J=11.0Hz), 4.22 (m), 6.89 (d, J=7.6Hz), 6.90 (d, J=7.6Hz), 7.18 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z : 329.2096 (M^+, calcd. for $C_{19}H_{27}N_3O_2$, 329.2103).

(-)-*7-Octylindolactam V* (*40*). 90% Yield, amorphous powder, $[\alpha]_D^{17}$-114° (c=0.487, EtOH). UV λ_{max} (EtOH) nm (ε) : 299 (8000), 286.5 (8000), 228.5 (27,700). IR ν_{max} (KBr) cm^{-1} : 3320, 2920,

2870, 1650, 1602, 1507, 1450, 1362, 1275, 1035, 798. ^{1}H NMR δ [$(CD_3)_2CO$] ppm: conformer A: B= 1: 2; conformer B, 0.59 (3H, d, J=7Hz), 0.86 (6H, m), 1.27 (10H, m), 1.7 (2H, m), 2.55 (1H, m), 2.81 (3H, s), 2.7~3.1 (4H, m), 3.2~3.7 (2H, m), 4.1 (2H, m), 4.34 (1H, d, J=10Hz), 6.28 (1H, br.s), 6.37 (1H, d, J=8Hz), 6.78 (1H, d, J=8Hz), 6.96 (1H, br.s); conformer A, 2.66 (s), 7.11 (d, J=2Hz). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 413.3040 (M^+, calcd. for $C_{25}H_{39}N_3O_2$, 413.3042).

(-)-7-Benzylindolactam V (41). 88% Yield, amorphous powder, $[\alpha]_D^{29}$-132° (c=0.51, EtOH). UV λ_{max}(EtOH) nm (ε): 300 (7300), 289 (7500), 230 (22,900). IR ν_{max} (KBr) cm^{-1}: 3360, 2960, 2880, 1650, 1602, 1505, 1450, 1370, 1042. ^{1}H NMR δ [$(CD_3)_2CO$] ppm: conformer A: B=1: 4; conformer B, 0.61 (3H, d, J=6.7Hz), 0.88 (3H, d, J=6.4Hz), 2.56 (1H, m), 2.86 (3H, s), 3.05 (1H, dd, J=16.8 Hz, 3.7Hz), 3.10 (1H, br.d, J=16.8Hz), 3.45 (1H, dd, J=11.0Hz, 9.2Hz), 3.65 (1H, dd, J=11.0Hz, 4.6Hz), 4.14 (2H, s), 4.14 (1H, m), 4.39 (1H, d, J=10.1Hz), 6.34 (1H, br.s), 6.44 (1H, d, J=7.9 Hz), 6.77 (1H, d, J=7.9Hz), 7.00 (1H, s), 7.25 (5H, m), 9.78 (1H, br.s); conformer A, 1.21 (d, J=6.7Hz), 2.32 (m), 2.70 (s), 5.23 (d, J=11.0Hz), 6.84 (d, J=7.6Hz), 6.89 (d, J=7.6Hz). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 391.2260 (M^+, calcd. for $C_{24}H_{29}N_3O_2$, 391.2260).

Preparation of (-)-7-prenylindolactam V (42). (-)-Indolactam V (1, 300 mg) was dissolved in 13N acetic acid (2 ml) and sodium acetate (200 mg). To the solution, prenyl bromide (150 μl) was added and stirred at room temperature under N_2 for 1 hr. The reaction mixture was neutralized with $NaHCO_3$ and extracted with EtOAc. The EtOAc extracts were chromatographed on Wako C-200 gel (40 g) using 0.5 % MeOH in $CHCl_3$ and was further purified by column chromatography on YMC I-40/64 gel (15 g) using 75% MeOH, followed by HPLC on NOVA-PAK C_{18} using 55% CH_3CN to give **42** (37 mg) as amorphous powder. 10% Yield, $[\alpha]_D^{25}$-143° (c=0.35, EtOH). EIMS m/z: 369 (M^+).

Preparation of (-)-7-thiomethylindolactam V (43). Compound **43** was obtained by treatment of **1** (72 mg) with conc. hydrochloric acid (50 μl) in dimethylsulfoxide (0.5 ml) at 70°C for 30 min. 19% Yield, amorphous powder, $[\alpha]_D^{18}$-274° (c=0.285, EtOH). UV λ_{max} (EtOH) nm (ε): 317 (12,300), 295 (sh., 8900), 234 (23,300). IR ν_{max} (KBr) cm^{-1}: 3350, 2920, 2870, 1650, 1595, 1500, 1342, 1040, 798. ^{1}H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 9.8; conformer B, 0.63 (3H, d, J=6.5Hz), 0.94 (3H, d, J=6.5Hz), 2.38 (3H, s), 2.60 (1H, m), 2.89 (3H, s), 3.10 (2H, m), 3.3~3.8 (2H, m), 4.22 (1H, m), 4.33 (1H, d, J=10Hz), 6.40 (1H, d, J=8Hz), 6.89 (1H, br.s), 7.15 (1H, d, J=8Hz), 7.53 (1H, br.s), 8.50 (1H, br.s); conformer A, 1.22 (d, J=6Hz), 2.48 (s), 2.72 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 347.1676 (M^+, calcd. for $C_{18}H_{25}N_3O_2S$, 347.1668).

IV. Structure-Activity Studies of Indole Alkaloid Tumor Promoter Teleocidin

Introduction

Structure-activity studies using a variety of teleocidin derivatives are especially important since they contribute to the elucidation of the structural requirements for tumor-promoting activity and provide a basis for designing a TPA antagonist. Furthermore, the structural similarity of the several potent tumor promoters, phorbol esters, teleocidins and aplysiatoxins, will be more obvious. As mentioned in Chapter III, the author synthesized a wide range of indolactam derivatives for structure-activity studies. This section comprises the EBV-EA inducing activity of these derivatives and discusses in detail the structure-activity relationship of teleocidins. The binding ability of these compounds to the TPA receptor in a mouse epidermal particulate fraction was also measured to evaluate their

possible tumor-promoting activity *in vivo* and analyze quantitatively the effects of the substituents on the activity.

Materials and Methods

Chemicals.

See Chapter III. The syntheses of (-)-7-formylindolactam V (**44**) and (-)-7-nitrovinylindolactam V (**45**) are briefly described in Chapter V.

EBV-EA induction test.

EBV genome-carrying human lymphoblastoid Raji cells were grown in RPMI 1640 medium supplemented with 8% fetal calf serum, 200 U/ml penicillin and 250 μg/ml streptomycin. The spontaneous induction rate of our subline Raji cells was <0.1% at any given time. The cells were grown to a density of 1×10^6 cells/ml, pelleted by centrifugation and resuspended in a culture medium (RPMI 1640 supplemented with 4% fetal calf serum, 200 U/ml penicillin and 250 μg/ml streptomycin) containing sodium *n*-butyrate (4 mM) and a test compound. After cultivation at 37°C for 48 hr, smears were made from the cell suspension, and the EBV-expressing cells were stained by a conventional indirect immunofluorescence technique with high-titer EBV-positive sera from NPC patients, in the manner already described.[19,81] In each assay, at least 500 cells were counted and the proportion of EA-positive cells was recorded.

The EBV-EA inducing activity is expressed as the percentage of EA-positive cells, and evaluated in terms of this percentage and of the effective concentration, EC_{50}, defined as the concentration that induces half of the percentage of EA-positive cells induced at the optimum concentration. EC_{50} values were calculated by a computer program (SAS) with a probit procedure.[82]

*Effect of S,S,S-tributyl phosphorotrithioate (DEF) on the EBV-EA inducing activity of teleocidin B-4 (**3**), (-)-indolactam V (**1**) and (-)-14-O-hexanoylindolactam V (**9**).*

Raji cells were adjusted to a density of 1×10^6 cells/ml, pelleted by centrifugation and resuspended in the culture medium described above. The cells were incubated with sodium *n*-butyrate (4 mM), a test compound (0.1 μM) and DEF at a concentration of 1, 10 or 100 μM for 48 hr at 37°C, then the proportion of EA-positive cells was similarly determined.

Inhibition of the specific binding of [^{3}H]TPA to a mouse epidermal particulate fraction.

Inhibition of specific [^{3}H]TPA binding was assayed by the cold-acetone filter method.[83,84] An epidermal particulate fraction was prepared from the dorsal epidermis of female SENCAR mice by the method reported previously. One hundred micrograms of protein from the particulate fraction were incubated at 0°C for 3 hr with [^{3}H]TPA (4 nM) and a test compound in 1 ml of 20 mM Tris-HCl buffer (pH 7.4) containing 2-mercaptoethanol (2 mM). Non-specific binding was determined by measuring the binding of the [^{3}H]TPA to the particulate fraction in the presence of a 500-fold excess

of unlabeled TPA.

The binding affinity was evaluated by the concentration required to cause 50% inhibition, ID_{50}. Since there were subtle variations in ID_{50} values, depending on the particulate fraction prepared, all compounds were tested simultaneously using the same particulate fraction.

Table 9. EBV-EA inducing activity of indolactam derivatives[a]

Compound number	Percentage of EA-positive cells at the indicated concentration (log 1/M)											EC_{50}(M)	$\log 1/EC_{50} \pm SEM$		n
	10.00	9.52	9.00	8.52	8.00	7.52	7.00	6.52	6.00	5.52	5.00				
1	0.1	0.2	1.1	5.4	12.6	24.0	29.9	32.2	27.0	22.1		1.26×10^{-8}	7.90	0.04	8
2					5.1	10.5	22.8	25.3	29.1	19.7		3.47×10^{-8}	7.46		2
3	1.1	3.4	6.1	11.8	20.4	28.9	32.8	29.5	21.0			4.47×10^{-9}	8.35	0.04	8
8				7.3	9.1	15.5	26.7	30.3	27.6	20.7		1.82×10^{-8}	7.74		2
9				4.9	9.1	10.5	23.9	32.4	27.3	21.4		3.10×10^{-8}	7.51		2
10				4.2	5.5	15.5	27.8	31.8	30.5	23.4		2.57×10^{-8}	7.59		2
11				3.5	4.1	7.7	17.0	31.8	26.5	22.6		5.50×10^{-8}	7.26		2
12				6.0	8.4	13.1	19.1	19.1	24.6	15.0		2.29×10^{-8}	7.64		2
13				3.1	5.3	9.2	19.1	23.0	24.1	20.6		3.16×10^{-8}	7.50		2
14				0.1	0.1	0.1	0.1	0.1	0.1	1.6		not active			2
15				0.1	0.1	0.1	0.1	0.1	0.1	0.1		not active			2
16				0.1	0.1	0.1	0.1	0.1	0.1	0.1		not active			2
17						7.2	10.2	13.7	18.9	21.4	15.7	9.55×10^{-8}	7.02	0.21	2
18					4.3	5.2	9.2	14.2	20.9	29.4	22.3	2.04×10^{-7}	6.69	0.15	2
19			2.8	4.0	9.7	11.8	16.1	20.5	24.9	31.0	29.4	6.03×10^{-8}	7.22	0.05	2
20			1.4	3.8	10.7	12.2	14.9	22.4	24.7	29.6	27.1	5.13×10^{-8}	7.29	0.01	2
21			3.1	5.3	9.5	12.3	15.2	22.2	30.5	22.2	19.2	4.27×10^{-8}	7.37	0.08	2
22						2.1	4.5	10.0	15.1	27.5	12.7	4.57×10^{-7}	6.34	0.02	2
23						4.7	8.4	12.5	15.5	27.4	18.6	2.95×10^{-7}	6.53	0.04	2
24	1.0	2.1	5.8	10.4	21.0	28.4	32.7	28.9	22.2	15.6		4.68×10^{-9}	8.33	0.06	2
25			0.1	8.3	16.4	27.3	32.8	25.7	22.9	11.6		8.91×10^{-9}	8.05	0.03	2
26	0.1	1.1	3.7	7.4	21.2	25.2	28.7	31.8	24.1	20.9		7.41×10^{-9}	8.13	0.03	2
27			0.1	2.8	13.3	18.5	25.4	30.5	24.4	18.1		1.82×10^{-8}	7.74	0.03	2
28	0.1	0.3	2.8	7.2	17.2	26.3	30.3	26.1	17.2	8.6		6.92×10^{-9}	8.16	0.03	5
29				0.1	0.1	0.1	0.1	0.1	3.0	5.7		not active			2
30				0.1	0.1	0.1	0.1	0.1	1.0	4.4		not active			2
31				0.1	0.1	0.1	0.1	0.1	0.1	0.6		not active			2
32				0.1	0.1	0.6	2.0	2.4	5.1	5.9		not active			2
33	1.9	6.6	7.9	11.7	19.7	25.0	30.6	26.9	15.5	7.2		3.47×10^{-9}	8.46	0.02	2
34	1.6	2.5	7.7	10.6	17.0	26.8	29.4	21.9	13.7	7.4		3.98×10^{-9}	8.40	0.00	2
35	1.1	2.9	7.6	9.8	17.3	25.9	28.3	21.6	12.6	8.4		3.80×10^{-9}	8.42	0.05	3
36	1.1	2.1	3.8	12.2	15.8	21.4	26.7	19.4	12.0	5.9		4.79×10^{-9}	8.32	0.05	2
37	2.2	5.2	13.4	16.1	20.6	25.7	30.3	24.3	19.8	17.8	11.7	2.29×10^{-9}	8.64	0.07	2
38		0.1	4.6	9.0	11.8	20.7	26.2	28.1	30.0	29.0	25.7	9.77×10^{-9}	8.01	0.01	2
39	0.1	0.1	6.4	7.9	18.0	24.0	29.3	26.6	18.7	8.0		5.89×10^{-9}	8.23	0.04	2
40	1.2	7.1	8.7	13.0	20.5	27.7	28.9	24.5	12.7	8.0		2.34×10^{-9}	8.63	0.01	2
41	2.1	7.1	14.2	16.9	23.0	27.0	31.1	24.9	22.4	19.2	11.8	1.91×10^{-9}	8.72	0.07	2
42			6.1	10.5	21.6	28.2	33.0	16.6	15.6			5.13×10^{-9}	8.29		1
43			0.1	1.8	12.7	25.1	30.7	27.8	24.4	21.0		1.23×10^{-8}	7.91	0.06	2
44			1.2	4.5	10.1	15.8	23.8	31.1	31.0	27.1	21.3	2.19×10^{-8}	7.66		1
45			0.1	2.5	10.3	16.7	25.8	27.0	20.5			1.62×10^{-8}	7.79		1
46	0.1	2.2	5.5	11.0	17.9	25.2	28.4	22.4	11.3			4.37×10^{-9}	8.36	0.05	2

[a] Sodium *n*-butyrate was added to all samples to enhance the sensitivity of Raji cells. Only 0.1% EA-induction was demonstrated at 4 mM. The viability of the cells exceeded 60% in each experiment.

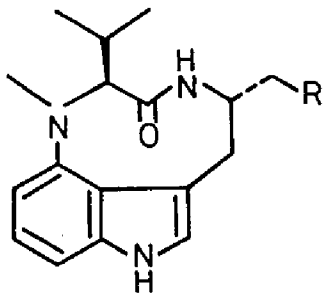
Results

EBV-EA inducing activity of indolactam derivatives.

The EBV-EA inducing activity of indolactam derivatives is summarized in Table 9. All 14-*O*-alkyl derivatives (**14**～**16**) were found to be far less active than (-)-indolactam V (**1**). The series of 14-*O*-acyl derivatives (**2**, **8**～**13**), however, showed a high EBV-EA inducing activity. Of these compounds, the *n*-acyl derivatives (**8**～**11**) induced about 30% of EA-positive cells at an optimum concentration of 0.3 μM, which was comparable with that of **1**. The activity of the trimethylacetyl derivative (**12**) and the benzoyl derivative (**13**) was lower than that of the *n*-acyl derivatives (**2**, **8**～**11**). (-)-14-Dehydroxyindolactam V (**17**) and (-)-14-chloroindolactam V (**18**), which have no hydroxyl group at position 14, showed a significant EBV-EA induction compared to the series of (-)-14-*O*-alkylindolactam Vs (**14**～**16**).

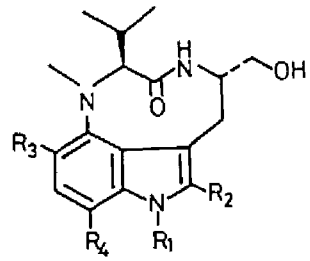
The activity of (-)-N^1-methyl-, (-)-N^1-ethyl- and (-)-N^1-butylindolactam V (**19**～**21**) was about 5 times weaker than that of **1**, and (-)-N^1-hexyl- and (-)-N^1-octylindolactam V (**22** and **23**) were even less active. (-)-2,7-Dibromoindolactam V (**25**) had lower activity than (-)-7-bromoindolactam V (**24**), and (-)-2,7-diacetylindolactam V (**29**) and (-)-2,7-dibutanoylindolactam V (**32**) were inactive. (-)-5-Chloroindolactam V (**27**) had lower activity than **1**, and (-)-5-acetylindolactam V (**31**) and (-)-5,7-diacetylindolactam V (**30**) were inactive.

On the other hand, all 7-substituted (-)-indolactam Vs (**24**, **26**, **28**, **33**～**45**) showed a high EBV-EA inducing activity. The activity of a series of (-)-7-acylindolactam Vs (**28**,



R		R	
$OCO(CH_2)_2CH_3$	(8)	OCH_3	(14)
$OCO(CH_2)_4CH_3$	(9)	$O(CH_2)_3CH_3$	(15)
$OCO(CH_2)_6CH_3$	(10)	$O(CH_2)_5CH_3$	(16)
$OCO(CH_2)_8CH_3$	(11)	H	(17)
$OCOC(CH_3)_3$	(12)	Cl	(18)
$OCOC_6H_5$	(13)		

Fig. 11. 14-Derivatives of (-)-indolactam V (**1**).



R_1	R_2	R_3	R_4	
CH_3	H	H	H	(19)
CH_2CH_3	H	H	H	(20)
$CH_2(CH_2)_2CH_3$	H	H	H	(21)
$CH_2(CH_2)_4CH_3$	H	H	H	(22)
$CH_2(CH_2)_6CH_3$	H	H	H	(23)
H	H	H	Br	(24)
H	Br	H	Br	(25)
H	H	H	I	(26)
H	H	Cl	H	(27)
H	H	H	$COCH_3$	(28)
H	$COCH_3$	H	$COCH_3$	(29)
H	H	$COCH_3$	$COCH_3$	(30)
H	H	$COCH_3$	H	(31)
H	$CO(CH_2)_2CH_3$	H	$CO(CH_2)_2CH_3$	(32)
H	H	H	$CO(CH_2)_2CH_3$	(33)
H	H	H	$CO(CH_2)_4CH_3$	(34)
H	H	H	$CO(CH_2)_6CH_3$	(35)
H	H	H	$CO(CH_2)_{10}CH_3$	(36)
H	H	H	COC_6H_5	(37)
H	H	H	$CO(CH_2)_2CO_2CH_3$	(38)
H	H	H	CH_2CH_3	(39)
H	H	H	$CH_2(CH_2)_6CH_3$	(40)
H	H	H	$CH_2C_6H_5$	(41)
H	H	H	$CH_2CH=C(CH_3)_2$	(42)
H	H	H	SCH_3	(43)
H	H	H	CHO	(44)
H	H	H	$CH=CHNO_2$	(45)

Fig. 12. 1, 2, 5 or 7-Substituted (-)-indolactam Vs.

33~**36**) increased as the acyl chain extended and reached the maximum at (-)-7-butanoylindolactam V (**33**), whose activity was comparable to that of teleocidin B-4 (**3**). Further elongation of the acyl chain resulted in a slight decrease in the activity. The derivatives having an electron-withdrawing group (**28**, **35** and **37**), and those having an electron-donating group (**39**~**41**) showed a similar activity. The activity of (-)-7-benzoyl- and (-)-7-benzylindolactam V (**37** and **41**) with a bulky substituent was almost equal to that of (-)-7-acyl- and alkylindolactam Vs, and even blastmycetin A (**46**),[53,55] which is a dimer of (-)-indolactam V (**1**), showed potent EBV-EA inducing activity.

It is nearly impossible to determine what physicochemical parameters of the substituents at position 7, for example, steric, hydrophobic and electronic, are significant for EBV-EA induction since the difference in the activity between the weakest derivative (**44**) and the strongest one (**41**) is very small (less than 10-fold).

Effect of DEF on the EBV-EA inducing activity of teleocidin B-4 (***3***), (-)-*indolactam V* (***1***)

Table 10. Effect of DEF on the EBV-EA inducing activity of teleocidin B-4 (**3**), (-)-indolactam V (**1**) and (-)-14-*O*-hexanoylindolactam V (**9**)[a]

Compound	Percentage of EA-positive cells when incubated with DEF at the indicated concentration (μM)			
	0	1	10	100
Teleocidin B-4	31.6	30.3	30.1	31.7
		(4.1)[b]	(4.7)	(0.0)
(-)-Indolactam V	30.1	30.0	29.6	27.1
		(0.0)	(1.7)	(10.0)
(-)-14-*O*-Hexanoylindolactam V	23.4	22.1	12.6	9.6
		(5.6)	(46.2)	(59.0)

[a] Raji cells were incubated with sodium *n*-butyrate (4 mM), test compound (0.1 μM) and DEF at a concentration of 1, 10 or 100 μM. The values represent the average of 2 experiments. DEF showed only 0.1% EA-positive cells at all concentrations used. Cell viability exceeded 60% in each experiment.

[b] Values in parentheses are percentage of inhibition.

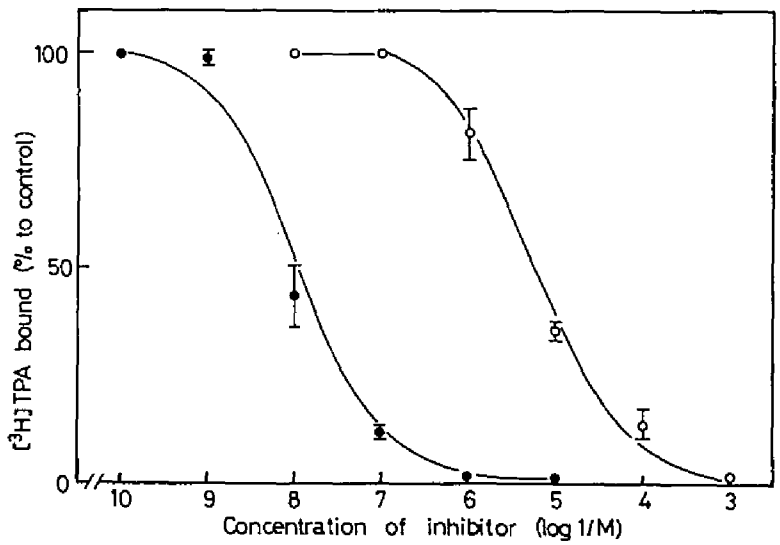


Fig. 13. Dose-response curves of inhibition of the specific binding of [^{3}H]TPA to an epidermal particulate fraction, which was prepared from the dorsal epidermis of female SENCAR mice. Each point is the average ± SD. ● Teleocidin B-4 (3) (n=5); ○ (-)-indolactam V (1) (n=3).

and (-)-14-O-hexanoylindolactam V (**9**).

It is likely that the activity of the acyl derivatives (**2**, **8**～**13**) is due to **1** formed through hydrolysis of the ester groups. To examine this, Raji cells were incubated with (-)-14-*O*-hexanoylindolactam V (**9**) and an esterase inhibitor, DEF, which was a potent potentiator of the insecticide, [(dimethoxyphosphinothioyl)thio]butanedioic acid diethyl ester (malathion).[85,86] DEF was selected because of its low toxicity. The other esterase inhibitors, tri-*o*-cresyl phosphate and 2-phenoxy-4H-1, 3, 2-benzodioxaphosphoran-2-one,[87] caused marked lowering of cell viability. As shown in Table 10, the activity of (-)-14-*O*-hexanoylindolactam V (**9**) fell drastically with increasing inhibitor concentration, in contrast to those of (-)-indolactam V (**1**) and teleocidin B-4 (**3**).

Table 11. Inhibition of the specific binding of [^{3}H]TPA

Compound	ID_{50}(M)	log $1/ID_{50}$ ± SEM		n
1	6.76×10^{-6}	5.17	0.06	3
3	1.29×10^{-8}	7.89	0.05	5
14	$>10^{-4}$	<4		1
17	$>10^{-4}$	<4		1
19	7.41×10^{-6}	5.13	0.03	2
20	5.75×10^{-6}	5.24	0.01	2
21	1.82×10^{-6}	5.74	0.06	2
23	5.50×10^{-8}	7.26	0.01	2
24	3.63×10^{-7}	6.44	0.11	2
25	*ca.* 3×10^{-5}	4.5		2
26	2.88×10^{-7}	6.54	0.04	2
27	*ca.* 3×10^{-5}	4.5		2
28	2.88×10^{-6}	5.54	0.05	2
29	$>10^{-4}$	<4		1
30	$>10^{-4}$	<4		1
31	$>10^{-4}$	<4		1
32	$>10^{-4}$	<4		1
33	2.04×10^{-7}	6.69	0.01	2
34	1.26×10^{-7}	6.90	0.03	2
35	8.13×10^{-8}	7.09	0.02	2
36	2.57×10^{-7}	6.59	0.00	2
37	1.15×10^{-7}	6.94	0.09	2
38	1.10×10^{-5}	4.96	0.02	2
39	3.55×10^{-7}	6.45	0.03	2
40	2.95×10^{-8}	7.53	0.14	2
41	1.05×10^{-7}	6.98	0.07	2
42	4.07×10^{-8}	7.39		1
43	1.66×10^{-7}	6.78	0.02	2
44	6.31×10^{-6}	5.20		1
45	7.41×10^{-7}	6.13		1
46	1.10×10^{-6}	5.96	0.07	2

Inhibition of the specific binding of [^{3}H]TPA to a mouse epidermal particulate fraction.

Dose-response curves of (-)-indolactam V (**1**) and teleocidin B-4 (**3**) for inhibition of the specific binding of [^{3}H]TPA to a mouse epidermal particulate fraction are shown in Figure 13. The ID_{50} values are summarized in Table 11. The ID_{50} values of **3** and **1** were 1.29×10^{-8} M and 6.76×10^{-6} M, respectively. (-)-14-*O*-Methylindolactam V (**14**) and (-)-14-dehydroxyindolactam V (**17**) did not bind to the TPA receptor in the range $10^{-4}\sim10^{-7}$ M.

The substituents at position 2 or 5 conspicuously lowered the binding affinity : for example, halogen derivatives (**25** and **27**) exhibited weaker inhibition of the specific binding of [^{3}H]TPA than (-)-indolactam V (**1**), and acyl derivatives (**29**~**32**) were at least 10^4 times weaker than teleocidin B-4 (**3**). (-)-N^1-Methyl-, (-)-N^1-ethyl and (-)-N^1-butylindolactam V (**19**~**21**), however, bound to the receptor with almost the same potency as (-)-indolactam V (**1**). All 7-substituted (-)-indolactam Vs (**24**, **26**, **28**, **33**~**45**) remarkably inhibited the specific binding.

The effects of the substituents at position 7 on inhibition of the specific binding have been scrutinized. Since the difference in ID_{50} values between the strongest inhibitor (**3**) and the weakest one (**38**) is large (10^3-fold), it is possible to clarify what physicochemical parameters play a critical role in the receptor binding. Compounds with hydrophobic substituents are potent inhibitors, while hydrophilic substituents lower the binding affinity (**28**, **38** and **44**), indicating that hydrophobicity of the substituents plays an important role

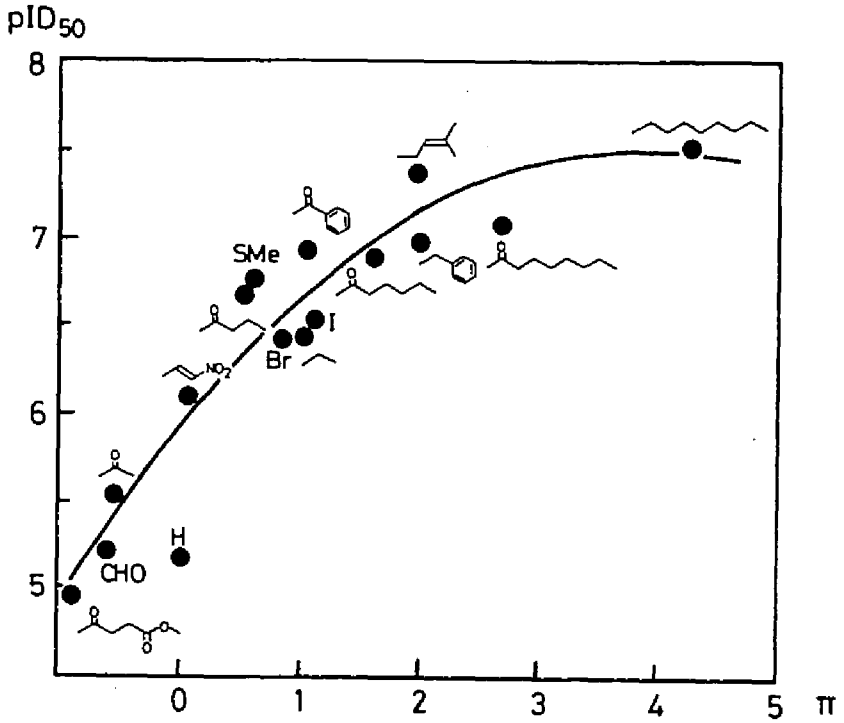


Fig. 14. Relationship between the hydrophobic parameter π and pID_{50}.

Table 12. Physicochemical parameters and calculated pID_{50} values

Compound number	π	pID_{50} (log 1/M)		
		Observed	Calculated	Deviation
1	0.00	5.17	5.87	−0.70
24	0.86	6.44	6.54	−0.10
26	1.12	6.54	6.70	−0.16
28	−0.55	5.54	5.36	0.18
33	0.53	6.69	6.30	0.39
34	1.61	6.90	6.97	−0.07
35	2.69	7.09	7.36	−0.27
36[a]	4.85	6.58	7.31	−0.73
37	1.05	6.94	6.66	0.28
38	−0.89	4.96	5.00	−0.04
39	1.02	6.45	6.64	−0.19
40	4.26	7.53	7.43	0.10
41	2.01	6.98	7.15	−0.17
42	2.00	7.39	7.15	0.24
43	0.61	6.78	6.36	0.42
44	−0.65	5.20	5.26	−0.06
45	0.11	6.13	5.97	0.16

[a] This compound was excluded from the regression analysis.

in the receptor binding. The similar activity of acyl derivatives (**35** and **37**) and alkyl derivatives (**40** and **41**) indicates that the electronic factor is not so important. The high activity of **37**, **41** and **46** with bulky substituents suggests that a steric factor is not so significant. On the basis of these impressions, the hydrophobic parameter π[88] of these derivatives (**1**, **24**, **26**, **28**, **33**～**35**, **37**～**45**) was plotted against log $1/ID_{50}$ (Figure 14). Good correlation was observed between π and pID_{50}. Furthermore, this was analyzed quantitatively by regression analysis. pID_{50} was expressed by the following equation :

$$pID_{50} = 0.87(\pm 0.25)\pi - 0.12(\pm 0.07)\pi^2 + 5.87(\pm 0.21)$$
$$(n = 16,\ s = 0.31,\ r = 0.94)$$

where n is the number of compounds, s is the standard deviation and r is the correlation coefficient. Figures in parentheses are 95% confidence limits. The physicochemical parameters and calculated pID_{50} values are summarized in Table 12. Since (-)-7-dodecanoylindolactam V (**36**) may interact non-specifically with the cell membrane because of its high lypophilicity and long chain, this compound was excluded from the regression analysis.

Discussion

Two biological activities, EBV-EA inducing activity and affinity to the TPA receptor, correlated very well for each derivative. This indicates that the EBV-EA induction test is highly effective for searching for TPA-type tumor promoters as Ito *et al.* proposed

previously.[19] However, there is a slight difference between these two activities. The difference in the EBV-EA inducing activity between (-)-indolactam V (**1**) and teleocidin B-4 (**3**) (only 2.8 times) was far less than that in the binding affinity to the TPA receptor (500 times). Possibly the monoterpenoid moiety in **3** contributes to the receptor binding more significantly than to EBV-EA induction. This means that the structure of **1** already has a requisite condition for EBV-EA induction.

The series of (-)-14-*O*-alkylindolactam Vs (**14**~**16**) did not induce EBV-EA, and (-)-14-*O*-methylindolactam V (**14**) did not bind to the TPA receptor, indicating that the free hydroxyl group at position 14 played an important role in the activities. The series of acyl derivatives (**2**, **8**~**13**), however, showed a high EBV-EA inducing activity. Since ester groups are hydrolyzed more easily than ether groups, the activity of the acyl derivatives might be ascribable to **1** formed through hydrolysis of the ester groups. The assumption is supported by the fact that the percentage of EA-positive cells at the optimum concentration of the trimethylacetyl derivative (**12**) and the benzoyl derivative (**13**), which resist hydrolysis because of the bulkiness of the substituents, are lower than that of the other acyl derivatives. The large difference in activity between (-)-14-*O*-hexanoylindolactam V (**9**) and (-)-14-*O*-hexylindolactam V (**16**) can be explained well by this mechanism. To prove this mechanism, Raji cells were incubated with (-)-14-*O*-hexanoylindolactam V (**9**) and the esterase inhibitor, DEF. As shown in Table 10, the activity of **9** fell drastically with increasing inhibitor concentration, in contrast to those of (-)-indolactam V (**1**) and teleocidin B-4 (**3**). In addition, the existence of an esterase in Raji cells was confirmed by a conventional biochemical method. These findings strongly suggest that some part of the activity of 14-*O*-acyl derivatives arises through hydrolysis of the ester groups.

It is noteworthy that (-)-14-dehydroxyindolactam V (**17**) and (-)-14-chloroindolactam V (**18**) are far more potent EBV-EA inducers than (-)-14-*O*-alkylindolactam Vs (**14**~**16**), though their activity is about 10 times weaker than that of (-)-indolactam V (**1**). This indicates that the hydroxyl group at position 14 is not indispensable, at least for EBV-EA induction.

The present results, based on a wide range of 14-derivatives of **1**, strongly suggest the importance of this hydroxyl group at position 14. Horiuchi *et al.* have recently proposed, after comparing the activities of 14-*O*-methyl derivatives of teleocidins (olivoretins) with those of teleocidins, that this hydroxyl group is necessary for induction of ODC, adhesion of HL-60 cells and binding to the TPA receptor.[89] The author has also tested the EBV-EA inducing activity of olivoretins which were isolated from *Streptoverticillium blastmyceticum* NA34-17. As shown in Figure 15, olivoretins induced only about 20% of EA-positive cells at a 10 times higher concentration compared to the optimum concentration for teleocidin B-4 (**3**).

Hecker *et al.* reported that the primary hydroxyl group at position 20 of TPA must be free for expression of the irritating and tumor-promoting activity.[20] Teleocidins have the same effects in cell culture systems as TPA,[33] and it has recently been proposed that teleocidins and TPA bind to the same receptor,[62~64] which is deduced to be PKC.[5] It can be postulated, therefore, that the hydroxyl group at position 14 of teleocidins plays

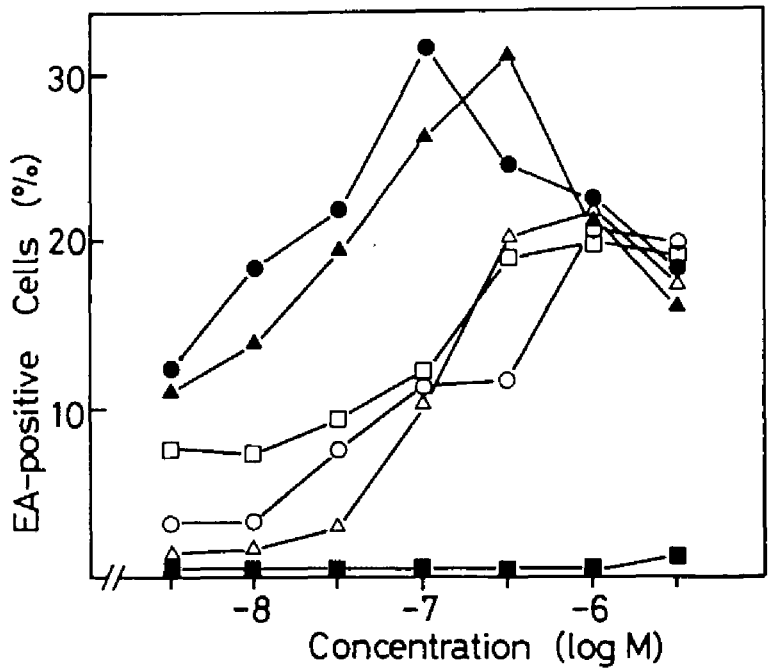


Fig. 15. The EBV-EA inducing activity of olivoretins. The results represent the average of duplicated experiments when all compounds were tested simultaneously. Sodium *n*-butyrate was added to all samples to enhance the sensitivity of Raji cells. It showed only 0.1% EA induction at 4 mM. The viability percentage of the cells was higher than 60% in each sample. ○ Olivoretin A; △ olivoretin B; □ olivoretin C; ▲ teleocidin B-4; ● (-)-indolactam V; ■ (-)-14-*O*-methylindolactam V.

a similar role to that of TPA at position 20 on the receptor site. The computer-assisted molecular modeling of TPA and teleocidins supports this structural similarity.[65,66]

It is obvious that the monoterpenoid moiety of teleocidins acts to enhance the activity. Large substituents at position 2 or 5 of the indole ring of **1**, however, remarkably lowered the EBV-EA inducing activity and the binding affinity to the TPA receptor. Hydrophobic substituents at position 7 of **1** enhanced the activities. Although alkyl substituents at N-1 did not cause the disappearence of the activities, comparison of the activities of (-)-N^1-ethylindolactam V (**20**) with those of (-)-7-ethylindolactam V (**39**) suggests that introduction of substituents into position 7 is more desirable. Recently, Fujiki *et al.* examined (±)-N^1-prenyl- and (±)-N^1-geranylindolactam V for such activities as induction of ODC, adhesion of HL-60 cells and binding to the TPA receptor, and proposed that the imino group at *N*-1 of **1** must be free for full expression of tumor-promoting activity.[61] To enhance the activity of (-)-indolactam V (**1**), it proved necessary to introduce a suitable substituent at position 6 or 7. This agrees with the high tumor-promoting activity of teleocidin B-4 (**3**) and lyngbyatoxin A[46] (teleocidin A-1, **5**),[44] which carries a monoterpenoid moiety at position 7.

The quantitative structure-activity study on the 7-substituted derivatives suggests that

hydrophobicity of the substituents plays a critical role in the receptor binding, and the high activity of blastmycetin A (**46**), which is a dimer of **1**, suggests the less structural requirement at position 7 of **1** for its varied activity. It is proposed that the long fatty acid chain of TPA at position 12 participates in interacting with phospholipids in the cell membrane in a non-specific manner.[90] The present results strongly suggest that the monoterpenoid moiety of teleocidins is similarly involved in the non-specific hydrophobic interaction with the phospholipids. The appropriateness of the regression analysis equation is confirmed by expectation of pID_{50} value of lyngbyatoxin A (**5**). The π value of this monoterpenoid chain is calculated to be 3.80 and the expected pID_{50} value from the equation is 7.48. Fujiki *et al.*[60] reported that teleocidin B-4 (**3**) and lyngbyatoxin A (**5**) had the same receptor binding potency, indicating that the high binding affinity of lyngbyatoxin A (**5**) is explained well by the equation.

It is known that teleocidins and (-)-indolactam V (**1**) exist as two stable conformers in solution at room temperature : conformer A of the SOFA type and B of the TWIST type.[68,69] Since there is a large difference in conformation between the two conformers, one of them might be responsible for the biological activity. Until now, very little has been known about the relationship between conformation and activity. As shown in Chapter III and V, (-)-7-acylindolactam Vs (**28**, **33**~**38**, **44**) and (-)-7-nitrovinylindolactam V (**45**) existed only as conformer B in chloroform-d or methanol-d_4 at room temperature, while 5-substituted derivatives (**27** and **31**) existed almost exclusively as conformer A. As shown in Tables 9 and 11, compound **28**, **33**~**38** and **44** had high activity, but **27** and **31** were far less active, indicating that conformer B might play a significant role in tumor-promoting activity.

There is a strict structural requirement for activity at position 2. To strictly discuss the effect of the substituents at position 2, 2-substituted derivatives must be assayed. However, direct introduction of substituents into only position 2 is very difficult, so 2, 7-disubstituted derivatives were prepared. It seems reasonable to discuss the effect at position 2 by use of 2, 7-disubstituted derivatives since introduction of the substituents into position 7 increases activity. Recently, Shudo *et al.* have reported that isopropyl group at position 12 played similar role to the monoterpenoid moiety at positions 6 and 7.[91] These results indicate that the region from position 2 to position 11 participates in the specific binding.

The EBV-EA inducing activity and affinity to the TPA receptor correlate well with the tumor-promoting activity *in vivo*. There are, however, some exceptions : compounds which bind to the TPA receptor do not always act as tumor promoters. Two-stage carcinogenesis experiments with these compounds is indispensable to extrapolate these results to tumor-promoting activity.

The structural similarity of the three potent tumor promoters, TPA, teleocidin B-4 and aplysiatoxin is being investigated by the use of computer graphics.[65,66] The present results will contribute to the elucidation of this structural similarity and to the development of new probes for receptor analysis of tumor promoters, which is described in the subsequent chapter.

V. New Probes for Receptor Analysis of Tumor Promoters : Synthesis of Fluorescent and Photolabile Derivatives of (-)-Indolactam V

As shown in Chapter IV, the structure-activity studies on a wide range of indolactam derivatives have elucidated the structural requirements for tumor-promoting activity and the structural similarity of the two potent tumor promoters, phorbol esters and teleocidins. In addition to this indirect approach, however, direct identification of the putative receptor site of tumor promoters using fluorescent or photolabile probes is indispensable to reveal the mechanism of tumor promotion. Fluorescent probes are especially useful to investigate macromolecule-ligand interactions since their spectroscopic characteristics often change drastically by binding to proteins. In this section, the synthesis of fluorescent and photolabile indolactam derivatives is described.

The structure-activity study in Chapter IV indicated that large substituents at position 2 or 5 of the indole ring of (-)-indolactam V (**1**) conspicuously lowered the activity, and that hydrophobic substituents at position 7 enhanced the activity. A low structural requirement at position 7 was also revealed. These results suggest that position 7 of **1** is most suitable for introducing a fluorescent group without decreasing activity. The results of the substitution reaction on the indole ring of **1** have revealed that the position 7 of **1** was most active in the electrophilic aromatic substitution, and that introduction of electron-withdrawing groups resulted in a good yield and selectivity.

On the basis of these considerations, position 7 of (-)-14-*O*-acetylindolactam V (**2**) was formylated. Treatment of **2** with titanium tetrachloride and dichloromethyl methyl ether in dry methylene chloride afforded (-)-7-formyl-14-*O*-acetylindolactam V (**47**) in 25% yield along with unreacted **2** (30%). Compound **47** was hydrolized by alkaline treatment to (-)-7-formylindolactam V (**44**). Condensation of **44** with nitromethane was achieved in ammonium acetate and acetic acid to give (-)-7-nitrovinylindolactam V (**45**) in 76% yield. In this reaction, a large excess of nitromethane was necessary to obtain sufficient yield. Reduction of **45** was accomplished by use of lithium aluminum hydride and aluminum chloride in tetrahydrofuran to yield the primary amine (**48**) in 56% yield. Treatment of **48** with dansyl chloride in triethylamine and methylene chloride gave (-)-7-(2-*N*-dansylaminoethyl)indolactam V (**49**) in 89% yield. The overall yield of **49** was 10%. This synthetic method is also available for photolabile and other fluorescent indolactam derivatives using **48** as an intermediate. Compound **48** can also be bound to agarose gel for affinity chromatography. Using this method, another fluorescent indolactam derivative, (-)-7-[2-*N*-(2,6-mansyl)aminoethyl]indolactam V (**50**) and a photolabile indolactam derivative, (-)-7-[2-*N*-(4-azidobenzoyl)aminoethyl]indolactam V (**51**), were synthesized. A tritiated compound of **51** was similarly obtained using tritiated succinimidyl 4-azidobenzoate.

An inactive derivative which has a quite similar structure to the active one is necessary as a reference in the experiment employing fluorescent or photolabile probes. Since the hydroxyl group at position 14 of the teleocidins must be free for the appearence of tumor-promoting activity (Chapter IV), the 14-*O*-methyl derivative of **49** was synthesized.

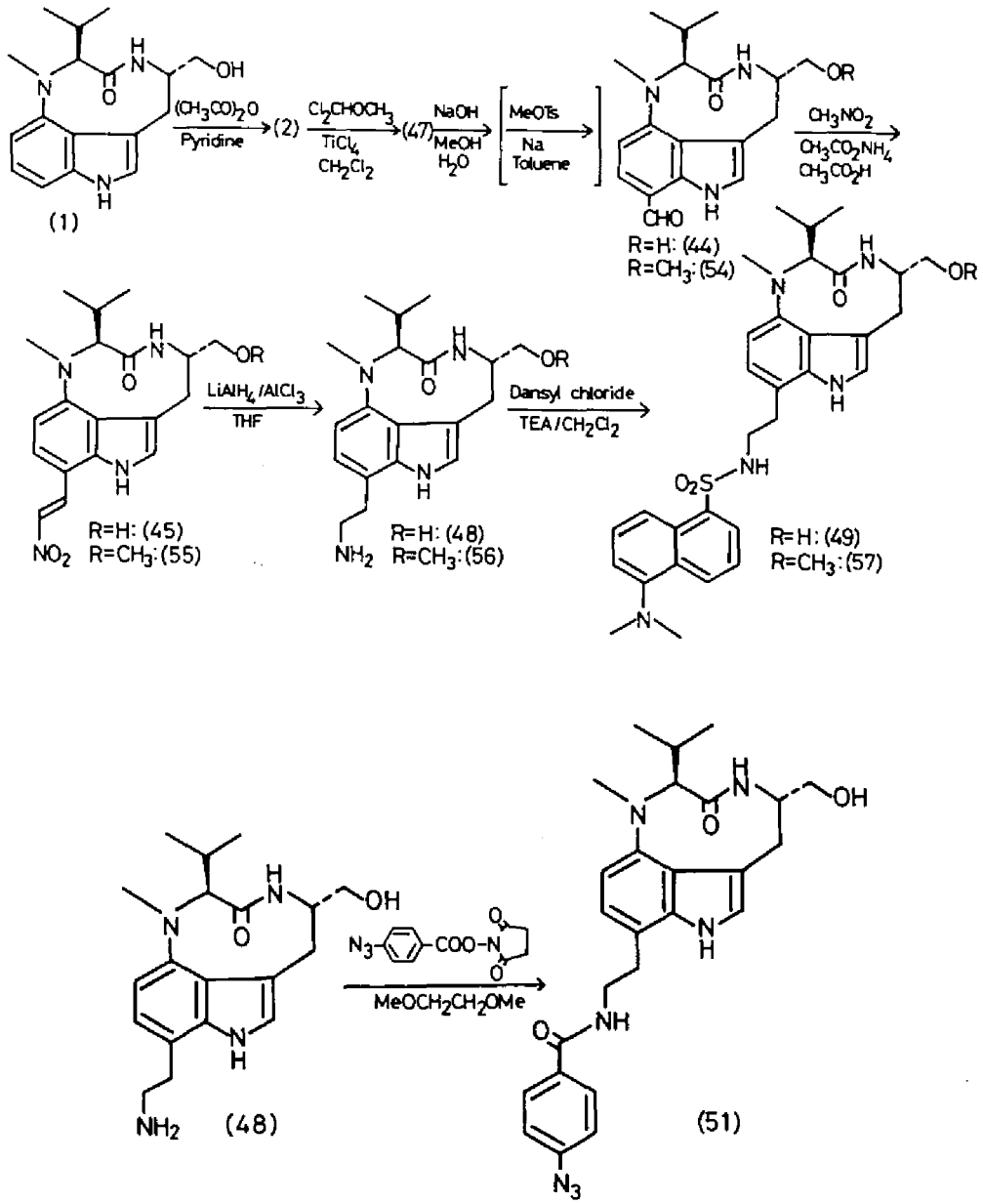


Fig. 16. Synthetic scheme of fluorescent and photolabile indolactam derivatives.

As there are three positions (imino group at N-1, amido group at N-10 and hydroxyl group at C-14) which can be methylated by Williamson ether synthesis in **1**, the selective methylation at position 14 was investigated. Methyl *p*-toluenesulfonate (MeOTs) was used as a methylating reagent. Treatment of **1** with MeOTs in sodium hydride and tetrahydrofuran did not show any selectivity and gave mainly (-)-1, 14-*O*-dimethylin-

dolactam V (**52**). When a less polar solvent, benzene, was used instead of tetrahydrofuran, a little selectivity was observed and (-)-14-*O*-methylindolactam V (**14**) was obtained in 9% yield. Further, treatment of **1** with MeOTs in sodium and toluene gave **14** in 13% yield. The reactivity at position 10 was very low and this position was methylated only when **1** was heated with MeOTs in sodium hydride and tetrahydrofuran. Next, the same reaction in sodium and toluene was carried out with (-)-7-formylindolactam V (**44**), whose imino group at N-1 was deduced to be less reactive than **1** because of the intramolecular interaction between the formyl group and the imino group, to give **54** in 29% yield. (-)-7-(2-*N*-dansylaminoethyl)-14-*O*-methylindolactam V (**57**) was synthesized from **54** by the same method as was used in the synthesis of **49**. The overall yield of **57** was 3%.

(-)-Indolactam V (**1**) has the advantage of possessing an indole ring which can fluoresce by such chemical modifications as the introduction of various substituents into this moiety. This advantage is peculiar only to teleocidins. Substitution reaction on the indole ring of **1** revealed that (-)-2, 7-diacylindolactam Vs fluoresced strongly in ethanol. However, these were completely inactive (Chapter IV). Since a large substituent at position 2 of **1** was expected to result in a drastic decrease in activity, the smallest acyl substituent was introduced into position 2 of **1**. Based on this concept, (-)-2-formyl-7-decanoylindolactam V (**58**) was synthesized by the Friedel-Crafts acylation followed by formylation. (-)-2, 7-Didecanoylindolactam V (**59**) was also prepared from **1** as an inactive control.

The biological activities of these fluorescent indolactam derivatives and their cellular uptake are mentioned in the next chapter.

Experimental

General remarks. See Chapter I.

(-)-*7-Formyl-14-O-acetylindolactam V* (*47*). (-)-14-*O*-Acetylindolactam V (**2**) (340 mg) was dissolved in dry CH_2Cl_2 (2 ml) and cooled by acetone-ice. To the reaction mixture, $TiCl_4$ (100 μl) and Cl_2CHOCH_3 (150 μl) was added and stirred at room temperature for 20 hr. After partitioning between CH_2Cl_2 and water, the CH_2Cl_2 layer was chromatographed on Wako C-100 gel eluting with toluene containing increasing amounts of acetone. The eluates with 10, 15 and 20% acetone were combined and chromatographed on YMC I-40/64 gel with 30% CH_3CN in water to give **47** as amorphous powder (91.6 mg, 25% yield). $[\alpha]_D^{21}$-447° (c=2.15, EtOH). UV λ_{max} (EtOH) nm (ε): 371 (17,400), 261.5 (11,100). IR ν_{max} (KBr) cm^{-1}: 3320, 2960, 1740, 1665, 1650, 1587, 1500, 1240, 1142, 1042. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.59 (3H, d, J=6.7Hz), 0.95 (3H, d, J=6.1Hz), 2.10 (3H, s), 2.62 (1H, m), 3.01 (3H, s), 3.17 (1H, dd, J=17.7Hz, 3.7Hz), 3.23 (1H, br.d, J=17.7Hz), 4.00 (1H, dd, J=12.2Hz, 9.2Hz), 4.22 (1H, dd, J=12.2Hz, 3.7Hz), 4.24 (1H, m), 4.54 (1H, d, J=10.4Hz), 6.11 (1H, br.s), 6.58 (1H, d, J=8.2Hz), 7.03 (1H, s), 7.51 (1H, d, J=8.2Hz), 9.85 (1H, s), 10.42 (1H, br.s). HR-EIMS m/z: 371.1835 (M^+, calcd. for $C_{20}H_{25}N_3O_4$, 371.1845).

(-)-*7-Formylindolactam V* (*44*). Treatment of **47** with MeOH and water (pH 11) gave **44** quantitatively, which was recrystallized from EtOH. Pale yellow rods, mp 246~248°C. $[\alpha]_D^{21}$-558° (c=0.25, EtOH). UV λ_{max} (EtOH) nm (ε): 372 (18,600), 262 (11,900). IR ν_{max} (KBr) cm^{-1}: 3480, 3390, 2960, 1660, 1650, 1585, 1498, 1365, 1295, 1242, 1142, 1043. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.58 (3H, d, J=6.7Hz), 0.95 (3H, d, J=6.1Hz), 2.60 (1H, m), 3.00 (3H, s), 3.08 (1H, dd, J=17.7Hz, 3.7Hz), 3.18 (1H, br.d, J=17.7Hz), 3.58 (1H, m), 3.76 (1H, m), 4.07 (1H, m), 4.58 (1H, d, J=10.1Hz), 6.56 (1H, d, J=8.2Hz), 6.98 (1H, br.s), 7.02 (1H, s), 7.51 (1H, d, J=8.2Hz), 9.83

(1H, s), 10.41 (1H, br.s). EIMS *m/z*: 329 (M^+). Anal. Calcd. for $C_{16}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.48; H, 6.91; N, 12.64.

(-)-*7-Nitrovinylindolactam V* (***45***). Compound **44** (35 mg) was dissolved in AcOH (0.1 ml) and CH_3NO_2 (0.9 ml). $AcONH_4$ (90 mg) was added to the solution and refluxed for 30 min. The reaction mixture was evaporated to dryness with toluene and partitioned between EtOAc and water. The EtOAc extracts were chromatographed on Wako C-200 gel with 0.75% MeOH in $CHCl_3$ to give **45** (30 mg, 76% yield). Amorphous powder, $[\alpha]_{650}^{26}$-1085° (c=0.21, EtOH). UV λ_{max} (EtOH) nm (ε): 476 (20,500), 285 (10,000), 233 (19,200). IR ν_{max} (KBr) cm^{-1}: 3360, 2950, 1655, 1615, 1567, 1512, 1430, 1260, 1212, 1150. ^1H NMR δ (CD_3OD) ppm: conformer B only, 0.57 (3H, d, J=7.0Hz), 0.91 (3H, d, J=6.4Hz), 2.55 (1H, m), 2.95 (3H, s), 3.10 (1H, br.d, J=17.7Hz), 3.17 (1H, dd, J=17.7Hz, 3.4Hz), 3.48 (1H, dd, J=11.3Hz, 8.9Hz), 3.61 (1H, dd, J=11.3Hz, 4.6Hz), 4.08 (1H, m), 4.61 (1H, d, J=10.4Hz), 6.60 (1H, d, J=8.5Hz), 7.08 (1H, s), 7.47 (1H, d, J=8.5Hz), 7.88 (1H, d, J=13.4Hz), 8.47 (1H, d, J=13.4Hz). HR-EIMS *m/z*: 372.1781 (M^+, calcd. for $C_{19}H_{24}N_4O_4$, 372.1798).

(-)-*7-(2-Aminoethyl)indolactam V* (***48***). Compound **45** (21.5 mg) was dissolved in THF (0.5 ml). $AlCl_3$ (18 mg) and $LiAlH_4$ (8 mg) in THF (1 ml) was added to the solution and refluxed for 40 min. The reaction mixture was extracted with water (pH 1, HCl), followed by partitioning between EtOAc and water (pH 14, NaOH), to give the primary amine (**48**), which gave a characteristic coloration with ninhydrin. This crude amine was purified by column chromatography on YMC I-40/64 gel (20 g) using 50% MeOH and 0.4% NH_4OH in water to give **48** (11.1 mg, 56% yield). A viscous liquid, $[\alpha]_D^{22}$-148° (c=0.29, EtOH). UV λ_{max} (EtOH) nm (ε): 303 (7000), 287 (6700), 230 (22,200). ^1H NMR δ (CD_3OD) ppm: conformer A: B=1:1; conformer B, 0.62 (3H, d, J=6.7 Hz), 0.89 (3H, d, J=6.4Hz), 2.54 (1H, m), 2.88 (3H, s), 2.9~3.2 (6H, m), 3.46 (1H, dd, J=11.3 Hz, 9.2Hz), 3.61 (1H, dd, J=11.3Hz, 4.6Hz), 4.21 (1H, m), 4.43 (1H, d, J=10.1Hz), 6.44 (1H, d, J=7.9Hz), 6.81 (1H, d, J=7.9Hz), 6.99 (1H, s); conformer A, 0.89 (d, J=6.4Hz), 1.24 (d, J=6.7Hz), 2.31 (m), 2.70 (s), 3.06 (d, J=11.0Hz), 4.24 (m), 6.92 (s), 7.14 (s). Other peaks had weak intensities and overlapped those of conformer B. HR-EIMS *m/z*: 344.2199 (M^+, calcd. for $C_{19}H_{28}N_4O_2$, 344.2212).

(-)-*7-(2-N-Dansylaminoethyl)indolactam V* (***49***). Compound **48** (16 mg) was treated with dansyl chloride (46 mg) in CH_2Cl_2 (2 ml) and $N(CH_2CH_3)_3$ (1 ml) for 40 min at room temperature. After partitioning between EtOAc and water, the EtOAc extracts were chromatographed on Wako C-200 gel with 1% MeOH in $CHCl_3$ to give **49** as amorphous powder (24 mg, 89% yield). $[\alpha]_D^{22}$-80° (c=0.26, MeOH). UV λ_{max} (EtOH) nm (ε): 335 (4700), 305.5 (11,200), 288.5 (10,200), 250 (17.500), 218.5 (62,000). IR ν_{max} (KBr) cm^{-1}: 3370, 2490, 2880, 1650, 1588, 1507, 1315, 1141, 1075. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1:3.5; conformer B, 0.61 (3H, d, J=6.4Hz), 0.92 (3H, d, J=6.4Hz), 2.58 (1H, m), 2.86 (3H, s), 2.88 (6H, s), 2.90~3.25 (6H, m), 3.54 (1H, m), 3.73 (1H, m), 4.24 (1H, br.s), 4.34 (1H, d, J=10.3Hz), 5.01 (1H, br.s), 6.36 (1H, d, J=8.1 Hz), 6.68 (1H, d, J=8.1Hz), 6.92 (1H, s), 7.04 (1H, br.s), 7.18 (1H, d, J=7.7Hz), 7.51 (2H, m), 8.20 (1H, d, J=8.6Hz), 8.24 (1H, d, J=7.9Hz), 8.54 (1H, d, J=8.5Hz), 8.68 (1H, br.s); conformer A, 1.22 (d, J=6.8Hz), 2.37 (m), 2.71 (s), 4.70 (d, J=11.1Hz), 9.04 (br.s). Other peaks had weak intensities and overlapped those of the major conformer. HR-in-beam-EIMS *m/z*: 577.2740 (M^+, calcd. for $C_{31}H_{39}N_5O_4S$, 577.2723).

(-)-*7-[2-N-(2,6-Mansyl)aminoethyl]indolactam V* (***50***). Compound **50** was prepared by treatment of **48** with 2,6-mansyl chloride[92] in CH_2Cl_2 and $N(CH_2CH_3)_3$. 57% Yield, amorphous powder, $[\alpha]_D^{26}$ -75° (c=0.259, EtOH). UV λ_{max} (EtOH) nm (ε): 360 (sh., 7300), 318 (20,800), 280 (sh., 16,700), 256.5 (25,200), 228 (51,400). ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1:3; conformer B, 0.62 (3H, d, J=6.7Hz), 0.93 (3H, d, J=6.4Hz), 2.59 (1H, m), 2.88 (3H, s), 2.9~3.3 (6H, m), 3.44 (3H, s), 3.54 (1H, m), 3.73 (1H, m), 4.27 (1H, m), 4.34 (1H, d, J=10.1Hz), 4.74 (1H, t, J=6.1 Hz), 6.40 (1H, d, J=7.9Hz), 6.75 (1H, d, J=7.9Hz), 6.80 (1H, br.s), 6.92 (1H, s), 7.1~7.7 (10H m), 8.23 (1H, s), 8.68 (1H, br.s); conformer A, 1.22 (d, J=6.4Hz), 2.39 (m), 2.72 (s), 4.45 (m), 6.86 (d, J=7.6Hz), 6.93 (d, J=7.6Hz), 7.06 (d, J=2.5Hz), 9.05 (br.s). Other peaks had weak

intensities and overlapped those of the major conformer. EIMS m/z : 639 (M^+).

(-)-*7-[2-N-(4-Azidobenzoyl)aminoethyl]indolactam V* (*51*). Compound **48** (3.3 mg) was treated with succinimidyl 4-azidobenzoate (4.5 mg) in 1,2-dimethoxyethane (0.2 ml) at room temperature for 1 hr. After partitioning between EtOAc and water, the EtOAc extracts were chromatographed on Wako C-100 gel with toluene containing increasing amounts of acetone. The 30 and 40% acetone eluates were purified by HPLC on YMC A-311 using 75% MeOH in water to give **51** (2.5 mg, 53% yield) as amorphous powder. UV λ_{max} (EtOH) nm (ε) : 300 (sh., 11,500), 291 (sh., 16,300), 283 (sh., 20,700), 271.5 (24,300), 229 (28,300). IR ν_{max} (KBr) cm^{-1} : 3300, 2150 ($-N_3$), 1640, 1600, 1540, 1500, 1445, 1280, 1040. ^1H NMR δ ($CDCl_3$) ppm : conformer A : B=1 : 1.9 ; conformer B, 0.65 (3H, d, J=6.7 Hz), 0.94 (3H, d, J=6.4Hz), 2.61 (1H, m), 2.92 (3H, s), 3.01~3.23 (4H, m), 3.45~3.77 (4H, m), 4.32 (1H, m), 4.40 (1H, d, J=10.1Hz), 6.41 (1H, d, J=7.6Hz), 6.63 (1H, br.t, J=6.8Hz), 6.67 (1H, br.s), 6.83 (1H, d, J=7.6Hz), 7.04 (1H, s), 7.10 (2H, d, J=8.6Hz), 7.80 (2H, d, J=8.6Hz), 10.27 (1H, br.s) ; conformer A, 0.94 (d, J=6.4Hz), 1.24 (d, J=7.0Hz), 2.40 (m), 2.75 (s), 2.86 (d, J=14.4Hz), 2.98 (d, J=11.0Hz), 4.45 (m), 4.81 (d, J=11.0Hz), 6.93 (d, J=7.3Hz), 6.96 (d, J=7.3Hz), 7.18 (d, J=2.5Hz), 10.73 (br.s). Other peaks had weak intensities and overlapped those of the major conformer. HR-in-beam-EIMS m/z : 489.2512 (M^+, calcd. for $C_{26}H_{31}N_7O_3$, 489.2488).

(-)-*1,14-O-Dimethylindolactam V* (*52*). (-)-Indolactam V (**1**) (24.5 mg) was dissolved in THF (1 ml). To the solution, NaH (20 mg) in THF (1 ml) was added and stirred at 0°C for 20 min. MeOTs (39 mg) was added to the above reaction mixture and stirred at room temperature for 30 min. This reaction mixture was treated with *n*-BuOH and partitioned between EtOAc and water. The EtOAc layer was chromatographed on Wako C-100 gel with toluene containing increasing amounts of acetone. The eluates with 10 and 15% acetone was collected and recrystallized from MeOH to give **52** as colorless needles (16.5 mg, 62% yield). mp 203~204°C, $[\alpha]_D^{25}$-171° (c=0.14, EtOH). UV λ_{max} (EtOH) nm (ε) : 315 (8900), 307 (9100), 286 (6700), 230 (29,800). IR ν_{max} (KBr) cm^{-1} : 3425, 3225, 2925, 1670, 1600, 1560, 1495, 1120. ^1H NMR δ ($CDCl_3$) ppm : conformer A : B=1 : 8.5 ; conformer B, 0.62 (3H, d, J=6.7Hz), 0.91 (3H, d, J=6.4Hz), 2.61 (1H, m), 2.91 (1H, dd, J=17.4Hz, 3.7Hz), 2.92 (3H, s), 3.18 (1H, br.d, J=17.4Hz), 3.31 (3H, s), 3.33 (1H, m), 3.38 (1H, dd, J=9.8Hz, 4.3Hz), 3.69 (3H, s), 4.37 (1H, m), 4.38 (1H, d, J=10.1Hz), 6.13 (1H, br.s), 6.52 (1H, d, J=7.9Hz), 6.73 (1H, d, J=1.2Hz), 6.83 (1H, d, J=7.9Hz), 7.10 (1H, t, J=7.9Hz) ; conformer A, 1.24 (d, J=6.7Hz), 2.74 (s). EIMS m/z : 329 (M^+). Anal. Calcd. for $C_{19}H_{27}N_3O_2$: C, 69.27 ; H, 8.26 ; N, 12.75. Found : C, 69.01 ; H, 8.43 ; N, 12.69.

(-)-*1,10,14-O-Trimethylindolactam V* (*53*). The reaction mixture of **1** (25 mg), NaH (20 mg) and MeOTs (77.5 mg) in THF (2 ml) was refluxed for 4 hr and stood at room temperature for 12 hr. This was worked up by the same method as above and chromatographed on Wako C-200 gel with 2 % acetone in toluene, followed by HPLC using NOVA-PAK C_{18} with 55% CH_3CN in water to give **53** as a viscous oil (7.2 mg, 26% yield). $[\alpha]_D^{25}$-93° (c=0.35, EtOH). UV λ_{max} (EtOH) nm (ε) : 316 (7800), 307 (8400), 289 (6500), 231 (29,200). IR ν_{max} (KBr) cm^{-1} : 3425, 2950, 1625, 1600, 1570, 1495 1325, 1280, 1120, 1110, 975. ^1H NMR δ ($CDCl_3$) ppm : major conformer, 0.49 (3H, d, J=6.7Hz), 0.66 (3H, d, J=6.7Hz), 2.59 (1H, m), 2.9~4.0 (17H, m), 4.06 (1H, d, J=10.4Hz), 6.57 (1H, d, J=7.6Hz), 6.76 (1H, s), 6.81 (1H, d, J=7.6Hz), 7.06 (1H, t, J=7.6Hz). HR-EIMS m/z : 343.2244 (M^+, calcd. for $C_{20}H_{29}N_3O_2$, 343.2260).

(-)-*7-Formyl-14-O-methylindolactam V* (*54*). (-)-7-Formylindolactam V (**44**) (30 mg) was refluxed with MeOTs (40 μl) and Na (100 mg) in toluene (2 ml) for 30 min. After filtration, the reaction mixture was partitioned between EtOAc and water. The EtOAc extracts were chromatographed on Wako C-200 gel with toluene containing increasing amounts of acetone. The 15 and 20% acetone eluates were collected and chromatographed on YMC I-40/64 gel with 57% MeOH in water to give **54** (9 mg, 29% yield). Pale yellow rods from MeOH, mp 271~273°C, $[\alpha]_D^{26}$-719° (c=0.44, $CHCl_3$). UV λ_{max} (EtOH) nm (ε) : 372 (24,100), 262 (15,300). IR ν_{max} (KBr) cm^{-1} : 3400, 3250, 2900, 1670, 1638, 1585, 1500, 1297, 1247, 1143, 1112, 1040. ^1H NMR δ ($CDCl_3$) ppm : conformer B only, 0.57 (3H, d, J=6.7Hz), 0.94 (3H, d, J=6.4Hz), 2.61 (1H, m), 3.00 (3H, s), 3.01 (1H, dd, J=17.4Hz,

3.4Hz), 3.19 (1H, br. d, J=17.4Hz), 3.33 (3H, s), 3.33 (1H, m), 3.41 (1H, dd, J=9.5Hz, 4.3Hz), 4.13 (1H, m), 4.55 (1H, d, J=10.1Hz), 6.21 (1H, br. s), 6.56 (1H, d, J=8.2Hz), 7.00 (1H, s), 7.50 (1H d, J=8.2Hz), 9.83 (1H, s), 10.41 (1H, br. s). EIMS m/z: 343 (M^+). Anal. Calcd. for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.49; H, 7.41; N, 12.09.

(-)-*7-Nitrovinyl-14-O-methylindolactam V* (*55*). Compound **54** (10 mg) in AcOH (0.1 ml) and CH_3NO_2 (1 ml) was mixed with $AcONH_4$ (50 mg). After reflux for 1 hr, the reaction mixture was evaporated *in vacuo* with toluene to dryness. The residue was partitioned between EtOAc and water, and the EtOAc layer was chromatographed on Wako C-200 gel with $CHCl_3$, followed by HPLC on NOVA-PAK C_{18} with 67% MeOH in water to give **55** (7.2 mg, 65% yield). Dark red rods from EtOH, mp 229°C dec., $[\alpha]_{610}^{27}$-1068° (c=0.029, $CHCl_3$). UV λ_{max} (EtOH) nm (ε): 474.5 (19,700), 282 (9700), 232.5 (18,100). IR ν_{max} (KBr) cm^{-1}: 3260, 2920, 1660, 1610, 1565, 1507, 1330, 1268, 1200, 1148, 1115. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.57 (3H, d, J=6.7Hz), 0.94 (3H, d, J=6.4Hz), 2.61 (1H, m), 2.98 (3H, s), 3.02 (1H, dd, J=17.7Hz, 3.4Hz), 3.20 (1H, br. d, J=17.7Hz), 3.35 (3H, s), 3.35 (1H, m), 3.42 (1H, dd, J=9.5Hz, 4.3Hz), 4.17 (1H, m), 4.48 (1H, d, J=10.4 Hz), 6.23 (1H, br. s), 6.57 (1H, d, J=8.2Hz), 7.01 (1H, s), 7.38 (1H, d, J=8.2Hz), 7.64 (1H, d, J=13.4Hz), 8.33 (1H, d, J=13.4Hz), 8.70 (1H, br. s). EIMS m/z: 384 (M^+). Anal. Calcd. for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78; N, 14.50. Found: C, 62.02; H, 6.73; N, 14.41.

(-)-*7-(2-N-Dansylaminoethyl)-14-O-methylindolactam V* (*57*). Compound **57** was obtained from **55** (20 mg) by the same method as was used in the synthesis of **49**. Amorphous powder (9.1 mg, 30% yield), $[\alpha]_D^{23}$-66° (c=0.46, EtOH). UV λ_{max} (EtOH) nm: 335 (4000), 304.5 (9200), 289 (8400), 250 (14,500), 218.5 (51,700). IR ν_{max} (KBr) cm^{-1}: 3370, 2930, 2870, 1648, 1588, 1575, 1507, 1452, 1315, 1141. ^1H NMR δ ($CDCl_3$) ppm: conformer A: B=1: 5; conformer B, 0.61 (3H, d, J=6.7Hz), 0.92 (3H, d, J=6.4Hz), 2.60 (1H, m), 2.89 (9H, s), 2.92~3.22 (6H, m), 3.33 (3H, s), 3.33 (1H, m), 3.42 (1H, dd, J=9.8Hz, 4.3Hz), 4.32 (1H, d, J=10.4Hz), 4.36 (1H, m), 4.77 (1H, t, J=6.4Hz), 6.14 (1H, br. s), 6.38 (1H, d, J=7.9Hz), 6.69 (1H, d, J=7.9Hz), 6.94 (1H, s), 7.19 (1H, d, J=7.6Hz), 7.53 (2H, m), 8.19 (1H, d, J=8.6Hz), 8.26 (1H, d, J=7.3Hz), 8.56 (1H, d, J=8.6Hz), 8.64 (1H, br. s); conformer A, 1.21 (d, J=6.7Hz), 2.73 (s). Other peaks had weak intensities and overlapped those of the major conformer. HR-EIMS m/z: 591.2856 (M^+, calcd. for $C_{32}H_{41}N_5O_4S$, 591.2879).

(-)-*2-Formyl-7-decanoylindolactam V* (*58*). Friedel-Crafts formylation of (-)-7-decanoyl-14-*O*-acetylindolactam V (20 mg) using $TiCl_4$ (25 μl) and Cl_2CHOCH_3 (20 μl) in dry CH_2Cl_2 (2 ml) at room temperature for 24 hr, followed by alkaline treatment (pH 14) and purification, afforded **58** (9.5 mg) as amorphous powder. 50% Yield, $[\alpha]_D^{23}$-527° (c=0.474, EtOH). UV λ_{max} (EtOH) nm (ε): 401 (20,500), 322 (20,000), 318 (sh., 19,400), 260 (14,900). IR ν_{max} (KBr) cm^{-1}: 3390, 2920, 2850, 1650, 1575, 1507, 1165, 1145. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.57 (3H, d, J=6.8Hz), 0.88 (3H, t, J=6.8Hz), 0.96 (3H, d, J=6.3Hz), *ca.* 1.3 (12H, m), 1.76 (2H, quintet, J=7.3Hz), 2.60 (1H, m), 2.96 (2H, m), 3.00 (3H, s), 3.23 (1H, dd, J=18.1Hz, 2.4Hz), 3.67 (1H, m), 3.78 (1H, m), 3.88 (1H, dd, J=18.1Hz, 3.4Hz), 4.17 (1H, br. s), 4.41 (1H, d, J=10.3Hz), 6.47 (1H, d, J=8.3Hz), 7.40 (1H, br. s), 7.85 (1H, d, J=8.3Hz), 10.05 (1H, s), 11.39 (1H, br. s). HR-EIMS m/z: 483.3090 (M^+, calcd. for $C_{28}H_{41}N_3O_4$, 483.3097).

(-)-*2,7-Didecanoylindolactam V* (*59*). Friedel-Crafts reaction of (-)-14-*O*-acetylindolactam V (**2**, 78 mg) using $AlCl_3$ (61 mg) and decanoic anhydride (100 μl) in nitrobenzene (1.5 ml) at 60°C for 10 hr gave 14-*O*-acetate of **59** as a minor product, which was hydrolized by alkaline treatment to **59** (13 mg, 9% yield). Viscous liquid, $[\alpha]_D^{23}$-444° (c=0.619, EtOH). UV λ_{max} (EtOH) nm (ε): 398 (23,800), 320 (17,800), 258.5 (18,200). IR ν_{max} (KBr) cm^{-1}: 3370, 2920, 2850, 1665, 1640, 1575, 1515, 1162. ^1H NMR δ ($CDCl_3$) ppm: conformer B only, 0.52 (3H, d, J=6.5Hz), 0.90 (9H, m), 1.3 (24H, m), 1.7 (4H, m), 2.6 (1H, m), 2.98 (3H, s), 2.85~3.20 (5H, m), 3.65 (2H, m), 4.10 (1H, m), 4.25 (1H, m), 4.48 (1H, d, J=10Hz), 6.48 (1H, d, J=8Hz), 7.37 (1H, br. s), 7.88 (1H, d, J=8Hz), 11.51 (1H, br. s). HR-EIMS m/z: 609.4492 (M^+, calcd. for $C_{37}H_{59}N_3O_4$, 609.4506).

VI. Cellular Uptake Studies of Fluorescent Indolactam Derivatives

Introduction

As was described in Chapter V, a variety of fluorescent indolactam derivatives (Figure 17) was synthesized. This section describes the fluorescence spectra, the EBV-EA inducing activity and the binding ability to the TPA receptor of these derivatives. To investigate the interactions of tumor promoters with their cellular targets and to screen out more effective fluorescent probes for receptor analysis of tumor promoters, the cellular uptake of these derivatives by HeLa cells and C3H 10T1/2 cells was also examined.

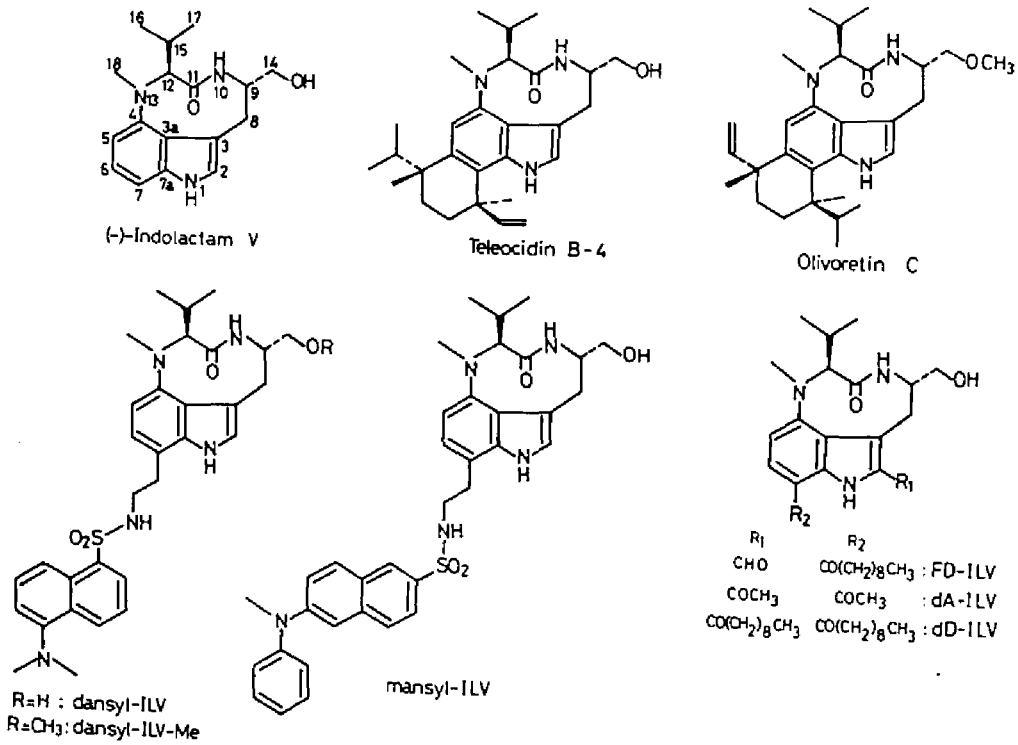


Fig. 17. Naturally occurring teleocidin-related compounds and fluorescent indolactam derivatives.

Materials and Methods

Chemicals.

Syntheses of the fluorescent indolactam derivatives, (-)-7-(2-*N*-dansylaminoethyl) indolactam V (**49**, dansyl-ILV), (-)-7-(2-*N*-dansylaminoethyl)-14-*O*-methylindolactam V (**57**, dansyl-ILV-Me), (-)-7-[2-*N*-(2,6-mansyl) aminoethyl] indolactam V (**50**, mansyl-ILV), (-)-2-formyl-7-decanoylindolactam V (**58**, FD-ILV), (-)-2,7-diacetylindolactam

V (**29**, dA-ILV) and (-)-2,7-didecanoylindolactam V (**59**, dD-ILV), were shown in Chapters III and V. The purity of all compounds was >99%, determined by HPLC and ^{1}H NMR. In particular the purity of FD-ILV, which was obtained from (-)-7-decanoylindolactam V, was checked strictly because 7-acyl derivatives of (-)-indolactam V were potent agonists, and found to be >99.7% pure. All other chemicals and reagents were purchased from chemical companies and were special grade.

Spectra.

Ultraviolet spectra were measured on a Shimadzu UV-200 spectrophotometer and fluorescence spectra were recorded on a Shimadzu RF-503A spectrofluorimeter at 25°C.

EBV-EA induction test.

See Chapter IV.

Inhibition of the specific binding of [^{3}H]TPA to a mouse epidermal particulate fraction.

See Chapter IV. Female ICR mice were used.

Fluorescence microscopy.

HeLa cells (*ca.* 10^5 cells/ml) were cultured on a 22 mm glass coverslip, which was placed in a 3.5 cm petri dish, for 2~3 days in Eagle's minimum essential medium (EMEM) plus 8% fetal bovine serum. C3H 10T1/2 cells (*ca.* 10^5 cells/ml) were cultured in Dulbecco's modified EMEM supplemented with 8% fetal bovine serum. These cells were cultured in monolayers at 37°C in a humidified CO_2 incubator. For microscopy, the cells were incubated in a serum free EMEM with a fluorescent indolactam derivative for 30 min at 37°C, and washed 3 times with phosphate-buffered saline (PBS). The coverslips were removed and mounted in 20% glycerol on a microscopy slide. Fluorescence photographs of dansyl-ILV and dansyl-ILV-Me were taken by a fluorescence microscope (Olympus BHF). An excitation filter passed wavelengths below 400 nm and an emission filter, those over 510 nm. Photographs were taken using Fuji-color 1600 film.

The intensity of the cellular fluorescence of each HeLa cell was measured by a fluorescence cytophotometer (Olympus MMSP-FR-II) equipped with an incident illumination system.[93] An excitation filter passing wavelengths below 400 nm was used and an emission filter passing wavelengths over 425 nm was employed to detect almost all cellular fluorescence. A pinhole of suitable size was selected in order to cut off interference by surrounding fluorescence. The size of this pinhole was not changed during any one set of the experiment. Globular HeLa cells were employed to reduce any aberration due to the round pinhole being used to measure a single cell's fluorescence. The background fluorescence was subtracted from the total fluorescence. At least 20 cells were measured. The cellular fluorescence intensity did not change significantly within several hours after mounting.

Cell extraction.

HeLa cells were cultured in EMEM supplemented with 8% fetal bovine serum for

2~3 days. After treatment with trypsin, the cells were harvested and suspended in serum free EMEM. The cell number was counted and the suspension was diluted to *ca.* 10^6 cells/ml with serum free EMEM. Three milliliters of this cell suspension in a chlorotrimethylsilane-treated 13×100 mm glass test tube were incubated with a fluorescent indolactam derivative (0.25 μM) for 30 min at 37°C. After washing 3 times with PBS, the cells were resuspended in 2 ml of PBS and extracted 2 times with 2 ml of chloroform-methanol (2 : 1). No fluorescence was detectable at the interface, in the upperphase or in cells. The chloroform layer was dried over sodium sulfate. After evaporation, the extracts were submitted to HPLC analysis.

Results

Fluorescence spectra.

The fluorescence data of the six indolactam derivatives are summarized in Table 13. The 2,7-diacyl derivatives (FD-ILV, dA-ILV and dD-ILV) all had higher fluorescence

Table 13. Effects of solvents on the fluorescent indolactam derivatives[a]

Compound	Solvent	λ_{max} (Excitation)	λ_{max} (Emission)	Peak wt[b]
dansyl-ILV	20% EtOH	345 nm	535 nm	0.13
	50% EtOH	345	525	0.32
	EtOH	345	515	1.00
	Octanol	345	510	0.71
	$CHCl_3$	355	510	0.83
dansyl-ILV-Me	20% EtOH	345	535	0.09
	50% EtOH	345	525	0.26
	EtOH	345	515	0.91
	Octanol	345	515	1.09
	$CHCl_3$	355	505	0.58
mansyl-ILV	10% EtOH	330	460	0.29
	50% EtOH	330	515	0.24
	EtOH	330	485	1.62
FD-ILV	10% EtOH	400	540	0.35
	50% EtOH	400	575	0.65
	EtOH	400	555	2.41
	Octanol	400	535	6.52
	$CHCl_3$	405	490	30.64
dA-ILV	10% EtOH	400	565	0.66
	EtOH	400	520	7.13
dD-ILV	10% EtOH	400	505	2.44
	EtOH	400	520	10.13

[a] Measurements were carried out at 25°C in the concentration 10^{-6}~5×10^{-6} M. Each sample was excited at the wavelength of its excitation maximum.
[b] Dansyl-ILV in ethanol was chosen as the standard of the relative fluorescence quantum yield.

intensity than either dansy-ILV, dansyl-ILV-Me or mansyl-ILV in ethanol. Since all compounds had excitation maxima at <400 nm and emission maxima at >460 nm, excitation (<400 nm) and emission (>425 nm) filters were selected for fluorescence microscopy. It is worthy of note that the fluorescence intensity of these six fluorescent derivatives increased with decreasing solvent polarity, suggesting that they would fluoresce strongly in such a hydrophobic environment as the interior region of a protein or membrane. This tendency was especially obvious in 2, 7-diacyl probes. This characteristic is very similar to those of 1-anilinonaphthalene-8-sulfonate (ANS),[94] *N*-methyl-2-anilinonaphthalene-6-sulfonate (MANS)[92] and 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS)[95] which have often been used as hydrophobic probes.

Possible tumor-promoting activity of the fluorescent indolactam derivatives.

Possible tumor-promoting activity of the six fluorescent indolactam derivatives was estimated by EBV-EA inducing activity and inhibition of the specific binding of [^{3}H]TPA to a mouse epidermal particulate fraction. The EBV-EA induction test is highly effective for estimating tumor-promoting activity because many potent tumor promoters efficiently induce the viral cycle in latently infected EBV genome-carrying cells.[18,70,96] The EBV-EA inducing activity of the six fluorescent indolactam derivatives is summarized in Table 14. Dansyl-ILV and mansy-ILV with a fluorescent group at position 7 of the indole ring proved potent EBV-EA inducers comparable to (-)-indolactam V. Dansyl-ILV-Me was nearly inactive as expected. The EBV-EA inducing activity of dD-ILV with a large substituent at position 2 was weak, and dA-ILV was inactive as mentioned in Chapter IV. It is noteworthy, however, that FD-ILV induced EBV-EA of the similar potency as (-)-indolactam V. Possibly due to the smaller size of the formyl group this compound induces EBV-EA.

Dose-response curves of the fluorescent indolactam derivatives for inhibition of the

Table 14. EBV-EA inducing activity of fluorescent indolactam derivatives[a]

Compound	Percentage of EA-positive cells at the indicated concentration (log 1/M)								EC_{50}(M)	log 1/EC_{50} ±SEM		n
	9.00	8.52	8.00	7.52	7.00	6.52	6.00	5.52				
dansyl-ILV	2.0	7.3	13.5	20.4	25.6	31.0	15.2	12.1	1.32×10^{-8}	7.88	0.06	2
dansyl-ILV-Me	0.1	0.1	0.1	1.5	6.2	10.1	5.6	2.1	not active			2
mansyl-ILV		1.2	5.5	11.8	18.1	23.7	19.6		2.82×10^{-8}	7.55	0.09	2
FD-ILV		5.7	10.4	12.8	24.3	31.1	27.7	22.6	2.24×10^{-8}	7.65	0.07	2
dA-ILV[b]		0.1	0.1	0.1	0.1	0.1	3.0	5.7	not active			2
dD-ILV		0.1	1.8	4.3	12.2	21.9	30.9	20.3	1.20×10^{-7}	6.92	0.01	2
(-)-indolactam V[b]	1.1	5.4	12.6	24.0	29.9	32.2	27.0	22.1	1.26×10^{-8}	7.90	0.04	8
teleocidin B-4[c]	5.9	12.4	20.6	29.0	35.1	19.6			5.62×10^{-9}	8.25	0.02	5

[a] Sodium *n*-butyrate was added to all samples to enhance the sensitivity of Raji cells. It showed only 0.1% EA-induction at 4 mM. The viability of the cells exceeded 60% in each experiment. At least two experiments were carried out and mean values are given.

[b] The activity of these compounds was shown in Table 9.

[c] This compound was elected a positive control.

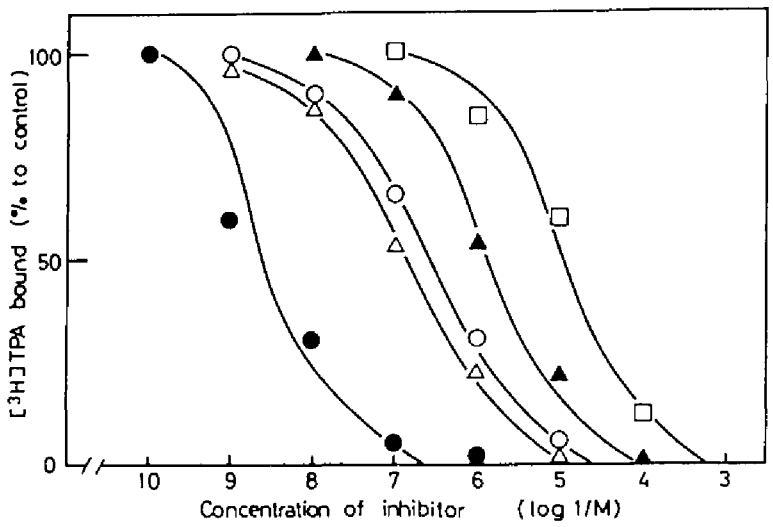


Fig. 18. Dose-response curves of inhibition of the specific binding of [³H]TPA to an epidermal particulate fraction, which was prepared from the dorsal epidermis of female ICR mice. Each point is the average of at least duplicate experiments. ● Teleocidin B-4 ; △ mansyl-ILV ; ○ dansyl-ILV ; ▲ (-)-indolactam V ; □ FD-ILV.

Table 15. Inhibition of the specific binding of [³H]TPA[a]

Compound	ID_{50} (M)	log $1/ID_{50}$	±SEM	n
dansyl-ILV	2.63×10^{-7}	6.58	0.14	2
dansyl-ILV-Me	$>10^{-4}$	<4		2
mansyl-ILV	1.91×10^{-7}	6.72	0.25	2
FD-ILV	1.23×10^{-5}	4.91	0.04	3
dA-ILV	$>10^{-4}$	<4		2
dD-ILV	$>10^{-4}$	<4		3
(-)-indolactam V[b]	1.62×10^{-6}	5.79	0.08	2
teleocidin B-4[b]	2.88×10^{-9}	8.54	0.04	2

[a] Dorsal epidermis from ICR mice was used.
[b] The activity of these compounds was reported previously by Fujiki *et al.*[60] Since there were subtle variations in the activity, depending on the particulate fraction prepared, the activity of these compounds were measured simultaneously with the fluorescent indolactam derivatives to evaluate their relative activities.

specific binding of [³H]TPA to a mouse epidermal particulate fraction are shown in Figure 18. ID_{50} values are summarized in Table 15. Dansyl-ILV and mansyl-ILV bound to the TPA receptor about 10 times more strongly than (-)-indolactam V. FD-ILV exhibited lower binding ability compared to these probes. However, this binding affinity is of significance. This is the first biologically active fluorescent compound without a fluorescent group such as a dansyl group. Dansyl-ILV-Me, dA-ILV and dD-ILV did not inhibit the specific binding of [³H]TPA in the range $10^{-4}\sim10^{-7}$ M.

Thus, two biological activities, EBV-EA inducing activity and affinity to the TPA receptor, correlated very well for each derivative.

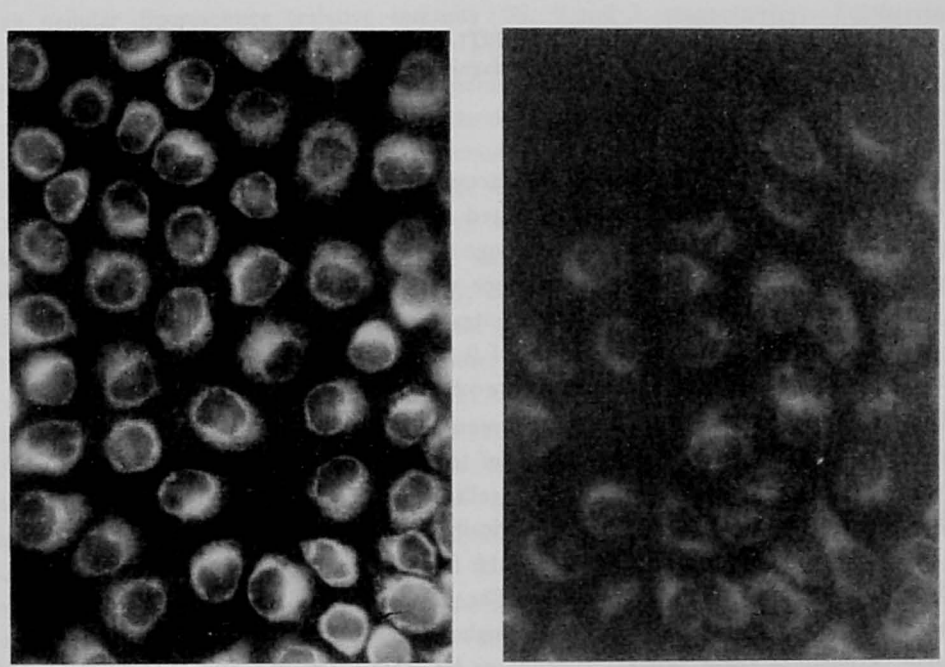
Table 16. The relative fluorescence intensity of HeLa cells after treatment of fluorescent indolactam derivatives[a]

Compound		Relative intensity[b]	±SEM	n
dansyl-ILV[c]		**100**	5	4
	+teleocidin B-4 (100-fold)	30	6	2
	+olivoretin C (100-fold)	88	6	2
dansyl-ILV-Me[c]		36	14	3
mansyl-ILV		54	0	2
FD-ILV		428	38	3
	+teleocidin B-4 (100-fold)	46	0	2
	+olivoretin C (100-fold)	374	42	2
dA-ILV		0	0	2
dD-ILV		7	1	2

[a] The concentration of all samples was 0.25 μM.

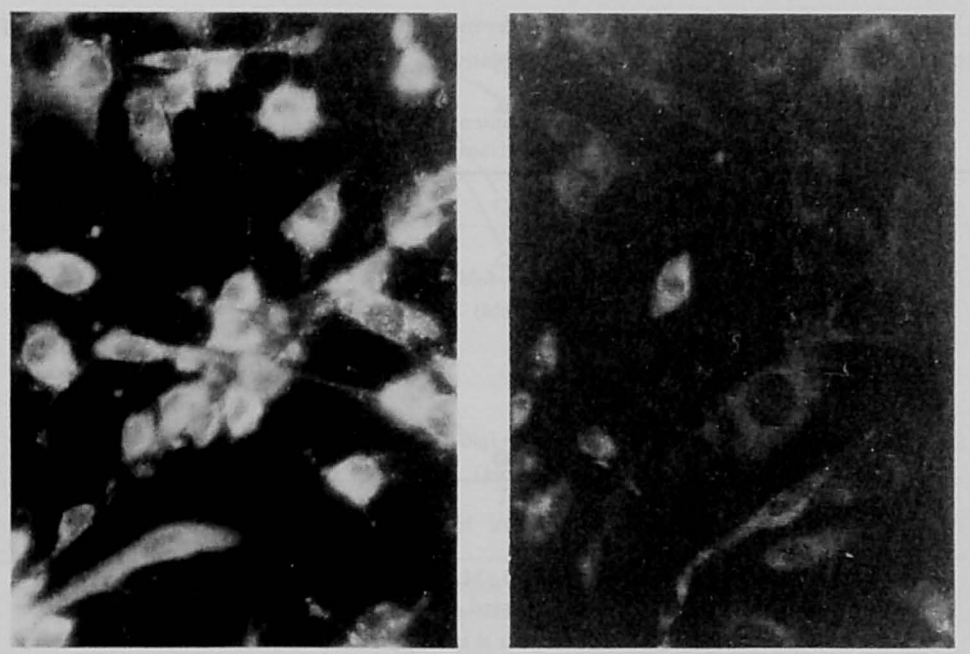
[b] The fluorescence of dansyl-ILV was elected the standard. The standard deviation of each petri dish ranged from 10 to 20%.

[c] The fluorescence photographs of these compounds were shown in Figures 19 and 20.



(a) (b)

Fig. 19. Cellular fluorescence of HeLa cells after treatment of dansyl-ILV (a) or dansyl-ILV-Me (b). Magnification × 132.



(a) (b)

Fig. 20. Cellular fluorescence of C3H 10T1/2 cells after treatment of dansyl-ILV (a) or dansyl-ILV-Me (b). Magnification × 132.

Cellular uptake study.

The uptake of the six fluorescent indolactam derivatives by HeLa cells was investigated in detail. Time course studies suggested that this uptake occurred within several minutes and that the staining did not change significantly within several hours. Dose-response studies revealed that this fluorescence was easily detected with as little as 0.025 μM dansyl-ILV. Since the maximal concentration for dansyl-ILV to induce EBV-EA was 0.3 μM (Table 15), a concentration of 0.25 μM was used in the following experiments. No cytotoxic effects on the cells were observed at this concentration.

To strictly discuss the relationship between the cellular fluorescence and biological activity, the relative fluorescence intensity of each cell was measured. Table 16 shows the relative fluorescence intensity of HeLa cells after being treated respectively with the six fluorescent indolactam derivatives. Biologically active derivatives (dansyl-ILV, mansyl-ILV and FD-ILV) showed remarkable cellular fluorescence. The fluorescence photograph of dansyl-ILV is shown in Figure 19-a. The most intense fluorescence was observed mainly in the area outside the nucleus, throughout the entire cytoplasm. FD-ILV fluoresced most strongly of these probes. Inactive or less active ones (dansyl-ILV-Me, dA-ILV and dD-ILV) showed only weak fluorescence (Figure 19-b). In addition, the intense fluorescence of dansyl-ILV and FD-ILV was suppressed drastically by 100-fold teleocidin B-4, a potent agonist. Olivoretin C,[51] an inactive teleocidin-related compound, did not

change the cellular fluorescence significantly.

Quite similar observations were made in the experiment with C3H 10T1/2 cells (Figure 20-a and b). The cellular fluorescence measurement of C3H 10T1/2 cells was difficult since these cells were not globular. Most of the fluorescent compounds uptaken by HeLa cells was extracted with chloroform-methanol, and no metabolite was detected in the extracted compounds by HPLC analysis.

Discussion

As shown in Tables 14~16, biologically active probes (dansyl-ILV, mansyl-ILV and FD-ILV) had an inclination to give intense cellular fluorescence (relative intensity : 100, 54 and 428, respectively). Since all these probes had characteristics similar to the well-known hydrophobic probes (ANS, MANS and TNS),[92,94,95] increase in the fluorescence intensity can be attributed to the receptor binding and/or hydrophobic interactions with lipids. The relative intensity of the three active probes was in the order FD-ILV≫ dansyl-ILV>mansyl-ILV. This would principally be due to their respective fluorescence peculiarities (Table 13) and the environment of the fluorescent groups on the occasion of the receptor binding, that is, the position to which the fluorescent groups were attached.

Inactive or less active ones (dansyl-ILV-Me, dA-ILV and dD-ILV) showed only weak cellular fluorescence (relative intensity : 36, 0 and 7, respectively). Furthermore, the intense fluorescence of dansyl-ILV or FD-ILV was suppressed by a potent tumor promoter, teleocidin B-4. These results present strong evidence that most of the cellular fluorescence caused by the fluorescent indolactam derivatives reveals the specific binding sites of these probes. In addition, more accurate correlation was observed between the cellular fluorescence intensity and the affinity to the TPA receptor, suggesting that some part of the intense cellular fluorescence of dansyl-ILV, mansyl-ILV and FD-ILV reflects the TPA receptor binding. Inactive dansyl-ILV-Me showed, however, significant cellular fluorescence (relative intensity : 36), albeit weak (Table 16 and Figures 19 and 20). This is presumably because only a small quantity of dansyl-ILV-Me might bind to the TPA receptor. Of course, there is another possibility that there are specific binding sites of dansyl-ILV-Me in addition to the TPA receptor.

Present structure-activity study using a number of indolactam derivatives (Chapter IV) have revealed that the nine-membered lactam ring and the pyrrole moiety of the indole ring played an important role in the receptor binding, and that the side chain at position 7 of the indole ring was involved in the non-specific hydrophobic interaction with phospholipids in the cell membrane. It is also proposed that the long fatty acid chain of TPA at position 12 participates in the interaction with the phospholipids in a non-specific manner.[90] These results of the structure-activity studies indicate that the cellular fluorescence of FD-ILV, which results from the indole ring itself, might reflect the interactions with specific binding sites, maybe proteins, while that of dansyl-ILV and mansyl-ILV, the non-specific hydrophobic interaction with the phospholipids. Hitherto, several phorbol ester derivatives for receptor analysis of tumor promoters have been

synthesized. These include electron spin labeled,[97] photoaffinity labeled[90] and fluorescence labeled[98,99] phorbol esters. Almost all of these probes were labeled at position 12 of TPA since introduction of these labels at this position did not decrease biological activity. Dansyl-ILV and mansyl-ILV were prepared based on the same concept. All these compounds are essentially the same from the view point of probing the non-specific hydrophobic interaction with the phospholipids. FD-ILV is, however, a new type of fluorescent probe, in which the specific binding region interacting with the putative receptors is labeled. FD-ILV might be effective for investigating the specific binding sites of tumor promoters. It would be also of practical use for searching for TPA-type tumor promoters because of its drastic change of fluorescence intensity (10-fold) caused by teleocidin B-4 and its high fluorescence quantum yield in hydrophobic solvents (Table 13). The only drawback of FD-ILV is its low biological activity. The synthesis of more active FD-ILV type fluorescent probes remains to be investigated.

The present results strongly suggest that the cellular fluorescence caused by dansyl-ILV and FD-ILV reflects the specific binding sites of the tumor promoters. It is not known, however, to what extent these binding sites participate in the pleiotropic effects on intact cells, and whether all these sites reflect PKC in the cell membrane and the cytoplasm. Recently, cellular uptake and localization of the fluorescent derivatives of phorbol ester-type tumor promoters, 12-*O*-(3-*N*-dansylaminotetradecanoyl)phorbol-13-acetate[98] and 12-*O*-(12-*N*-dansylaminododecanoyl)phorbol-13-acetate,[99] have been reported. These results indicated that these probes rapidly entered cells and bound to specific sites distributed throughout the cytoplasm. Hitherto, it has been proposed that the target site of TPA was a cell membrane-associated PKC.[5] The results of the fluorescent indolactams and phorbol esters suggest, however, that putative receptors of tumor promoters exist also in a cytoplasm. The variety of the fluorescent indolactam derivatives might give a clue to identify the cellular receptors of tumor promoters in combination with these fluorescent phorbol esters. Especially, dansyl-ILV and FD-ILV would become effective fluorescent probes because their intense cellular fluorescence was found to reflect the specific binding sites of tumor promoters.

Summary and Conclusion

Carcinogenic promoters produced by microorganisms were sought using the EBV-EA induction test,[18,19] a short-term promoter test system, and one strain, *Streptoverticillium blastmyceticum* NA34-17, was found to induce potent EBV-EA. Three biologically-active indole alkaloids, **1**, **2** and **3**, were isolated from the culture broth, and **1** was shown to be (-)-indolactam V, the basic ring-structure of teleocidins. Compound **2** was identified as (-)-14-*O*-acetylindolactam V and **3** as teleocidin B-4,[41,45] which has been found to be a potent tumor promoter by Fujiki *et al.*[49]

(-)-Indolactam V is a key compound for structure-activity studies of teleocidin-related compounds because its structure is fundamental to teleocidins and it has various activities *in vivo* and *in vitro*.[61,71] Hitherto, structural requirement for tumor-promoting activity has been examined only in TPA and its analogues.[20] Since teleocidins were first potent tumor

promoters whose structure was quite different from TPA, structure-activity studies using a wide range of indolactam derivatives will reveal further structural requirement for tumor-promoting activity and provide a basis for designing TPA antagonists and new probes for receptor analysis of tumor promoters.

The author synthesized about 50 indolactam derivatives, and examined the EBV-EA inducing activity and the binding ability to the TPA receptor. The results clarified the structural factors involved in the activity. The hydroxyl group at position 14 of (-)-indolactam V was shown to play an important role in the activity. Large substituents at position 2 or 5 of the indole ring conspicuously lowered the activity, and hydrophobic substituents at position 7 enhanced the activity. The effects of the substituents at position 7 on binding ability to the TPA receptor were analyzed quantitatively using physicochemical substituent parameters and regression analysis. Hydrophobicity of the substituents was found to play a critical role for receptor binding, suggesting that the monoterpenoid moiety of teleocidins was involved in the non-specific hydrophobic interaction with phospholipids in the cell membrane to stabilize the receptor-ligand binding. It was further indicated that conformer B of the TWIST type might play a significant role in tumor-promoting activity of teleocidins. The structural similarity of three potent tumor promoters, TPA, teleocidins and aplysiatoxins is being investigated by the use of computer graphics.[65,66] The present results will also contribute to the elucidation of this structural similarity.

In addition to these structure-activity studies, direct identification of the putative receptor sites of tumor promoters using photolabile or fluorescent derivatives is indispensable to reveal the mechanism of tumor promotion. Fluorescent probes are especially useful to investigate macromolecule-ligand interactions since their spectroscopic characteristics often change drastically by binding to proteins. The author synthesized a variety of fluorescent indolactam derivatives on the basis of the present structure-activity studies. To screen out more effective fluorescent probes for receptor analysis, the cellular uptake of these fluorescent indolactam derivatives by HeLa cells or C3H 10T1/2 cells was examined. The results indicated that (-)-7-(2-*N*-dansylaminoethyl)indolactam V (dansyl-ILV) and (-)-2-formyl-7-decanoylindolactam V (FD-ILV) would be of practical use for investigating the cellular receptors of tumor promoters in combination with their inactive analogues. The synthetic method of the fluorescent indolactam derivatives is also applicable to photolabile indolactam derivatives using (-)-7-(2-aminoethyl)indolactam V (**48**) as an intermediate. A biologically active ^{3}H-labeled photolabile probe, (-)-7-[2-*N*-(4-azido[3,5-^{3}H]benzoyl)aminoethyl]indolactam V, was synthesized from **48** by this method. Compound **48** can also be bound to agalose gel for affinity chromatography. A variety of the approach to identify the putative receptor of tumor promoters is possible employing the new probes derived from (-)-indolactam V.

Teleocidins attracted much interest in the area of organic chemistry because of their peculiar structures of a nine-membered lactam ring and a complex monoterpenoid moiety. A wide range of studies on teleocidins have been done actively by several groups in the 1980's. These include structure determination of teleocidin-related compounds,[50-55] total synthesis of (-)-indolactam V[36,56,57] and teleocidins,[58,59] structure-activity relationship[61,71,89]

(Chapter IV) and synthesis of new probes for receptor analysis of tumor promoters (Chapter V). These not only contributed largely to the elucidation of the mechanism of tumor promotion but also developed a new field in cancer research. From now on, an organic chemical approach as well as a biological one will play a more important role in the research on chemical carcinogenesis.

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List of Publications

Original articles

1) Irie, K., Hirota, M., Hagiwara, N., Koshimizu, K., Hayashi, H., Murao, S., Tokuda, H. and Ito, Y. (1984) The Epstein-Barr virus early antigen-inducing indole alkaloids, (-)-indolactam V and its related compounds, produced by Actinomycetes. *Agric. Biol. Chem.*, **48**, 1269~1274.
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