Structure and Tumor-promoting Activity of New Teleocidin-related Metabolites (Blastmycetins) from *Streptoverticillium blastmyceticum*

Nobuyuki HAGIWARA, Kazuhiro IRIE, Atsushi FUNAKI, Hideo HAYASHI,* Motoo ARAI* and Koichi KOSHIMIZU†

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan
**Department of Agricultural Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai 591, Japan*

Received August 25, 1987

Six new teleocidin-related compounds (**3**～**8**) together with (−)-N^{13}-desmethylindolactam V (**9**) were found in the culture broth of *Streptoverticillium blastmyceticum* NA34-17 producing tumor-promoting indole alkaloids. Compounds **3**～**8** proved to be a dimer of (−)-indolactam V (**3**), which bound through a methylene group at position 7, 2-oxy derivatives of (−)-indolactam V (**4**～**7**) and 14-*O*-methylteleocidin A-1 (**8**), respectively. Their tumor-promoting activities are also discussed.

Teleocidins (teleocidin A-1, A-2 and B-1～B-4)[1～5] produced by actinomycetes are strong tumor promoters[6] and peculiar indole alkaloids containing a nine-membered lactam ring and a complex monoterpenoid moiety. These characteristics attract much interest in the area of organic and biological chemistry. The last few years have seen the isolation and structural determination of a number of naturally occurring teleocidin-related compounds.[7～14]

Streptoverticillium blastmyceticum NA34-

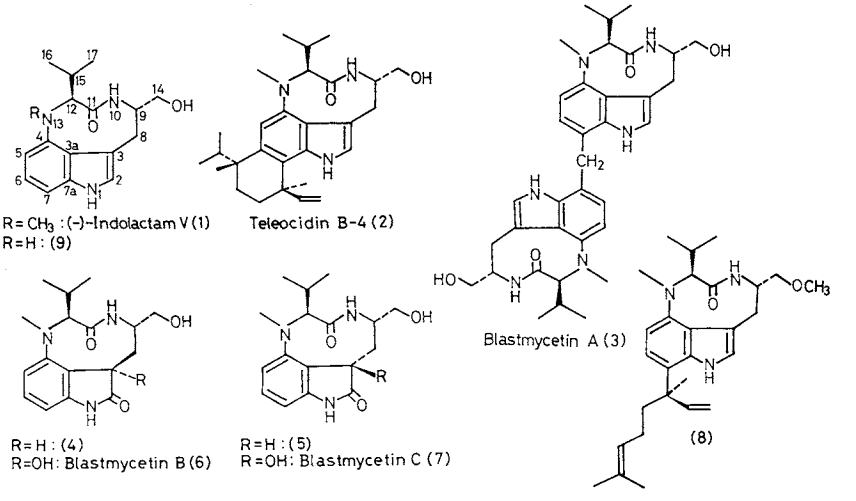

FIG. 1. Structure of (−)-Indolactam V (**1**), Teleocidin B-4 (**2**) and New Teleocidin-related Metabolites (**3**～**9**).

† To whom inquiries should be addressed.

17,[15] which was found by an Epstein-Barr virus (EBV) induction test, has a characteristic feature of producing in quantity (−)-indolactam V (**1**),[8,16] along with teleocidin B-4 (**2**)[1~3] (Fig. 1). In our preliminary publications,[11,13] we have reported the occurrence of some important teleocidin-related compounds in the culture broth of this actinomycete. This is a full report on the isolation and structural determination of these metabolites along with additional new compounds. Their biological activities are also discussed.

The purification of these metabolites was guided by Ehrlich reagent,[17] with which teleocidin-related compounds showed characteristic coloration on TLC. *S. blastmyceticum* NA34-17 was cultured by deep aerated fermentation for 48 hr or 70 hr, and the filtered broth (80 l) was extracted with ethyl acetate. The ethyl acetate extracts were chromatographed on silica gel using toluene containing increasing amounts of acetone. Each fraction was further purified by repeated column chromatography and HPLC to give five new teleocidin-related compounds (**3**~**7**) and (−)-N^{13}-desmethylindolactam V (**9**), an intermediate in the total synthesis of **1**.[16] Compounds **3**, **6** and **7** were named blastmycetin A, B and C, respectively.[13] The resultant mycelia obtained from the culture broth (48 hr cultivation time) were steeped in acetone, and then removed by filtration. The acetone extracts were redissolved in ethyl acetate, and partitioned between ethyl acetate and water. The ethyl acetate extracts gave a new teleocidin-related compound (**8**) employing a similar purification procedure as above.

Compound **3**, namely blastmycetin A, was obtained as an amorphous powder, $[\alpha]_D^{26}$ −220° (c=0.46, EtOH). Its molecular formula was established to be $C_{35}H_{46}N_6O_4$ by HR-in-beam-EIMS. The IR spectrum (KBr) showed characteristic absorption at 1650 cm^{-1} ascribable to a lactam ring of six or more members. The UV spectrum [λ_{max} (EtOH) nm (ε): 303 (16,700), 289 (15,900), 231 (49,300)] revealed the presence of an indole chromophore, and was very similar to that of **1** [λ_{max} (EtOH) nm (ε): 301 (7900), 288 (7300), 229 (28,100)].[8] The molecular extinction coefficients of **3**, however, were about twice as large as those of **1**, suggesting that there existed two indole chromophores in **3**. Compound **3** showed a quite similar ^{1}H-NMR spectrum to that of **1** in acetone-d_6, and most of the signals of **3** were doubled in a ratio of *ca.* 3:1, as was observed in **1**.*1 This is due to the existence of the two stable conformers of the nine-membered lactam ring.[16,19] Six aromatic protons of the major conformer [δ 6.39 (2H, d, J=7.6 Hz), 6.67 (2H, d, J=7.6 Hz), 7.01 (2H, s)] indicated that two indole rings of **3** were bound symmetrically at position 7. The spectrum also revealed one methylene [major conformer: δ 4.24 (2H, s)], which was deduced to be located between the two aromatic groups. To confirm this, (−)-7-benzylindolactam V (**10**) was derived from **1** by Fridel-Crafts acylation, followed by reduction with lithium aluminum hydride and aluminum chloride.[18,19] The methylene signal at the benzyl position of **10** was observed at δ 4.14 (2H, s) in acetone-d_6.

These data led us to formulate the structure of **3** as a dimer of **1**, which bound through a methylene group at position 7. To establish the absolute configuration of the nine-membered lactam ring, synthesis of **3** was carried out. Treatment of **1** with formaldehyde gave **3**, indicating that **1** and **3** had the same absolute configuration at positions 9 and 12. Compound **3** was also synthesized from (−)-7-hydroxymethylindolactam V under acidic conditions, which was obtained by reduction of (−)-7-formylindolactam V[20] with lithium aluminum hydride.

Compounds **4**~**7** were shown to have oxindole chromophores through their UV and

*1 In the ^{1}H NMR spectrum of **3**, some signals were quadrupled in a ratio of *ca.* 9:3:3:1, which is ascribable to a combination of the two conformers (conformers A and B=*ca.* 1:3)[16,19] of each (−)-indolactam V (**1**) moiety of **3**: conformers B and B, B and A, A and B, and A and A. Though four such signals are possible for every proton, these were observed only in the aromatic protons, indole NH protons and benzylmethylene.

IR spectra, and deduced to be closely related to each other. The molecular formulae of **4** and **5** were established to be $C_{17}H_{23}N_3O_3$ by HR-EIMS. The mass fragment patterns of **4** and **5** were almost the same, suggesting that they were stereoisomers. The ^{1}H-NMR spectrum of **4** in methanol-d_4 was quite similar to that of **1**. However, the signal corresponding to H-2 of **1** was not observed, and instead, a methine proton at δ 4.03 newly appeared in **4**. This signal was determined to be H-3 of the oxindole ring by spin-spin decoupling experiments between H-3 and H-8. From these observations, **4** was deduced to be 2-oxy-indolactam V. Treatment of **4** with a basic solution ($Na_2B_4O_7$–HCl buffer at pH 8.53) overnight gave a mixture of **4** and **5** (1 : 1). The same interconversion also occurred in **5**, indicating that the protons at position 3 of **4** and **5** are labile under basic conditions, and that **5** is a stereoisomer of **4** at position 3. The ^{1}H-NMR spectrum of **5** in methanol-d_4 at room temperature showed broad signals due to the interconversion of the two conformers (*ca.* 1 : 1) of the nine-membered lactam ring. Measurements in another solvents, chloroform-*d* and acetone-d_6, could not sharpen these broad signals. Measurements in methanol-d_4 at low temperature were, therefore, taken. Sharp signals were observed at 0°C. Although a complete assignment of all the signals was difficult because of overlapping of the signals and the equal signal intensity of the two conformers, protons similar to those of **4** were observed in **5**, supporting the conclusion that **5** was also 2-oxy-indolactam V. To prove these structures, transformations of **4** and **5** into **1** were carried out. Treatments of **4** and **5** with lithium aluminum hydride in ether each gave **1**, indicating that **4** and **5** had the same absolute configuration in the nine-membered lactam ring as **1**. Compounds **4** and **5** were, thus, confirmed to be (−)-2-oxy-indolactam V. The absolute configuration at position 3 is discussed later.

Compounds **6** and **7**, respectively named blastmycetin B and C, were established to have the same molecular formula of $C_{17}H_{23}N_3O_4$ by HR-EIMS. The mass fragment patterns were almost the same, suggesting that they were stereoisomers. Their mass fragment ions, *m/z* 315 ($M^+ - H_2O$) and 297 ($M^+ - 2H_2O$), revealed the existence of two hydroxyl groups in **6** and **7**, respectively. The ^{1}H-NMR spectra of **6** and **7** in methanol-d_4 showed all the signals of (−)-2-oxy-indolactam V (**4** and **5**), except for the signal ascribable to H-3 of the oxindole ring. From these results, **6** and **7** were deduced to be 3-hydroxy-2-oxy-indolactam V. This was confirmed by chemical transformations of **6** and **7** into **1**. Treatment of **6** with sodium borohydride and palladium chloride in methanol gave **4** and **5**, which led to **1** by reduction with lithium aluminum hydride in ether. Compound **7** was directly transformed into **1** with lithium aluminum hydride and aluminum chloride in ether. These results indicate that **6** and **7** are (−)-3-hydroxy-2-oxy-indolactam V and that they are stereoisomers at position 3. Compounds **6** and **7** were not interconvertible to each other in the range of pH 1.8 ~ 11.0.

The absolute configurations at position 3 of **6** and **7** were elucidated by ^{1}H-NMR chemical shift. A comparison of the ^{1}H-NMR spectra of **6** and **7** revealed large differences in the chemical shifts of H-9 (δ 3.95 and 5.61) and H-12 (δ 5.96 and 3.76), respectively. The remarkable downfield shifts of H-9 (δ 5.61) of **7** and H-12 (δ 5.96) of **6** were caused by the diaxial transannular effect of the hydroxyl group at position 3, suggesting that these protons and the hydroxyl group at position 3 were located near each other. From these findings, the absolute configurations at position 3 of **6** and **7** were determined to be 3*S* and 3*R*, respectively.

The resultant absolute configurations at position 3 of **4** and **5** were deduced from a comparison of their spectral data with those of **6** and **7**. Table I and Fig. 2 show the spectral characteristics of **4** ~ **7**. These four compounds can be divided into two pairs by their spectral features, one being **4** and **6**, and the other **5** and **7**. These spectral similarities strongly suggest that the absolute configurations at position 3 of **4** and **5** are identical with those of **6** and **7**. This was confirmed by the nuclear

TABLE I. OPTICAL ROTATION AND UV SPECTRA OF COMPOUNDS 4~7

	4	5	6	7
$[\alpha]_D$ (MeOH)	−306.5°	+89.6°	−323°	+167°
UV λ_{max} nm (ε) (MeOH)	242.5 (16,200)	248 (9,700)	243 (14,300)	247 (6,300)
	309 (2,300)		325 (3,700)	285 (1,500)

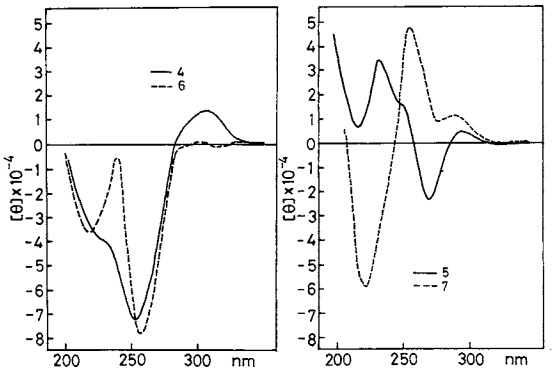

FIG. 2. The CD Spectra of Compounds 4~7.

Overhauser difference spectrum of **4**. Examination of the molecular model of **4** suggested that H-3 was located near H-12, but far from H-9. Saturation of the H-3 resulted in characteristic enhancement (>10%) of the H-12 signal, but no enhancement of the H-9 signal was observed. These results indicate that the absolute configuration at position 3 of **4** is 3*R*. Therefore, that of **5** is determined to be 3*S*. The nuclear Overhauser difference spectrum of **5** could not be measured because of the overlapping signals of the two conformers.

Compounds **4**~**7** may have been artifacts formed by autoxidation during the cultivation and/or isolation steps. However, our recent study on the metabolism of **1** by rat liver microsomes revealed that cytochrome P-450 containing mixed-function oxidases transformed **1** into **4** and **5**, which were also obtained in a small amount by autoxidation.[21] This enzyme has been known to exist in actinomycetes,[22] suggesting that a part of these 2-oxy derivatives (**4**~**7**) were enzymatically produced by this actinomycete.

Compound **8** was obtained as a gummy solid, $[\alpha]_D^{18}$ −198° (c=0.21, $CHCl_3$). Its molecular formula was established as $C_{28}H_{41}N_3O_2$ by HR-EIMS. The UV [λ_{max} (EtOH) nm (ε): 301 (9200), 286 (8900), 230 (27,000)] and IR [ν_{max} (KBr) cm^{-1}: 1650] spectra showed the presence of an indole ring and a lactam ring of six or more members. The ^{1}H-NMR spectrum in chloroform-*d* was closely similar to that of teleocidin A-1[5,23] (lyngbyatoxin A),[4] except for the presence of a methoxy group (δ 3.32) in **8**. The CD spectrum of **8** in methanol ($[\theta]_{305}$ +6300, $[\theta]_{292}$ 0, $[\theta]_{253}$ −24,000, $[\theta]_{234}$ 0, $[\theta]_{229}$ +8100, $[\theta]_{222}$ 0, $[\theta]_{217}$ −2300, $[\theta]_{212}$ 0, $c=4.42\times10^{-4}$) was almost the same as that of teleocidin A-1,[23] confirming that **8** was 14-*O*-methylteteocidin A-1. Hitherto, the same 14-*O*-methyl analogues, olivoretin A, B and C, have been isolated from *S. olivoreticuli* by Sakai *et al.*[7,9] Compound **8** is a new type of 14-*O*-methylteleocidin derivative.

Compound **9** was obtained as colorless needles, mp 199~201°C (EtOAc–*n*-hexane), $[\alpha]_D^{25}$ −81° (c=0.25, EtOH). Its spectral properties (^{1}H-NMR, UV, IR, EIMS and HR-EIMS) suggested that **9** was N^{13}-desmethylindolactam V. Treatment of **9** with methyl *p*-toluenesulfonate and sodium hydrogen carbonate in ethanol gave **1**,[16] indicating that **9** was (−)-N^{13}-desmethylindolactam V. Compound **9** was first obtained as an intermediate in the total synthesis of **1**.[16] However, this is the first report on the isolation of **9** from natural resources. Recently, the same desmethyl analogue, N^{13}-desmethylteleocidin B-4, has been isolated by Sakai *et al.*[12]

The above-mentioned metabolites contributed to the research of tumor promotion. Detailed structure–activity studies on teleocidins indicate the importance of alkyl substituents at position 7 of **1** for tumor-promoting activity.[24~26] Compound **3** induced the EBV early antigen and bound to the TPA receptor 10 times more strongly than **1**,[26] indicating the low structural requirement at position 7 of **1** for the activity. The 2-oxy derivatives of **1**

(**4**～**7**) were far less active than **1**, indicating that the double bond at position 2 of the indole ring plays an important role in tumor promotion.[26] The low activity of **9** revealed the importance of the N^{13}-methyl group.[21]

It is indispensable for elucidating the mechanism of tumor promotion to investigate the metabolic fate of tumor promoters in a living body. Metabolic activation of some initiators is well known to be the first step of carcinogenesis. Hitherto, the metabolism of tumor promoters has been studied using 12-*O*-tetradecanoylphorbol-13-acetate. However, very little is known about that of the other tumor promoters. Our recent study has revealed that the metabolism of **1** by rat liver microsomes resulted in detoxification to produce three major metabolites, **4**, **5** and **9**, and that cytochrome P-450 containing mixed-function oxidases were involved in this metabolism.[21]

These findings on the structure–activity relationship and the metabolism could provide important information for synthesizing stable radioactive or fluorescent teleocidin derivatives. In some experiments, the position of labeling might be critical because of the metabolizing ability of tissues or cells. Recently, our group has synthesized a fluorescent teleocidin derivative, (−)-7-(2-*N*-dansylaminoethyl)indolactam V,[20] from **1** on the basis of these findings, which will be a useful tool to reveal the mechanism of tumor promotion.

EXPERIMENTAL

Melting points (mp) were uncorrected. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; ORD, Jasco Model J-5; ^{1}H-NMR, JEOL GX 400 (400 MHz) and Hitachi Model R-22 (90 MHz); IR, Shimadzu Model 435; CD, Jasco Model J-500; MS, JEOL JMS-DX 300 (70 eV, 300 μA); HPLC, 655A pump equipped with a 655A-11 variable-wavelength UV monitor (Hitachi, Ltd., Tokyo, Japan). HPLC was carried out on YMC A-211 (C_8) and A-311 (C_{18}) packed columns (Yamamura Chemical Laboratory), NOVA-PAK C_{18} and μBondapak C_{18} (Waters Associates). Wako gel (silica gel, Wako Pure Chemical Industries) and YMC I-40/64 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography.

Isolation of blastmycetin A (**3**), *B* (**6**) *and C* (**7**). *S. blastmyceticum* NA34-17 was cultured by the method reported previously,[8] and the filtered broth (70 hr cultivation time, 80 l) was concentrated to 10 l *in vacuo*, before being extracted with 30 l of EtOAc. The EtOAc layer was dried over sodium sulfate, and evaporated *in vacuo* to give a brown oily syrup. The residue (33 g) was chromatographed on silica gel using toluene containing increasing amounts of acetone to give 40% (13.2 g) and 100% (3.9 g) acetone eluates, which were found to contain compounds positive in Ehrlich reagent.

The 40% acetone eluate was chromatographed on silica gel using $CHCl_3$ containing increasing amounts of iso-PrOH. The 7% iso-PrOH eluate (57 mg) was purified by HPLC on YMC A-211 using 40% CH_3CN in water to yield **3** (8.9 mg). Compound **3**. ^{1}H-NMR δ_{Me_4Si} [$(CD_3)_2CO$]: major conformer, 0.63 (6H, d, J=6.7 Hz), 0.89 (6H, d, J=6.4 Hz), 2.56 (2H, m), 2.88 (6H, s), 3.06 (2H, dd, J=16.8, 3.4 Hz), 3.13 (2H, br.d, J=16.8 Hz), 3.47 (2H, dd, J=11.0, 9.5 Hz), 3.66 (2H, dd, J=11.0, 4.6 Hz), 4.17 (2H, m), 4.24 (2H, s), 4.41 (2H, d, J=10.1 Hz), 6.35 (2H, br.s), 6.39 (2H, d, J=7.6 Hz), 6.67 (2H, d, J=7.6 Hz), 7.01 (2H, s), 9.91 (2H, br.s). IR ν_{max} (KBr) cm^{-1}: 3350, 1650, 1510, 1040. CD $[\theta]_{314}$ 0, $[\theta]_{281}$ −28,500, $[\theta]_{276}$ −29,000 $[\theta]_{248}$ −48,500, $[\theta]_{230}$ −16,600, $[\theta]_{218}$ −40,000, $[\theta]_{209}$ 0 (c=1.53×10^{-5}, MeOH). In-beam-EIMS m/z: 614 (M^+), 571, 314, 171. HR-in-beam-EIMS m/z: 614.3603 (M^+, $C_{35}H_{46}N_6O_4$; calcd. 614.3581).

The 100% acetone eluate was chromatographed on ODS using 40% MeOH in water to give two successive fractions; A (2.95 g) and B (98.9 mg). Fraction A was chromatographed on silica gel using 5% MeOH in $CHCl_3$, and was further purified by column chromatography on ODS using 30% MeOH in water to yield **7** (78.6 mg). Fraction B was purified by column chromatography on silica gel using 4% MeOH in $CHCl_3$ to yield **6** (44.3 mg). Compound **6**. $[\alpha]_D^{20}$ −323° (c=0.48, MeOH). ^{1}H-NMR δ_{Me_4Si} (CD_3OD): 0.83 (3H, d, J=6.7 Hz), 1.04 (3H, d, J=6.4 Hz), 2.05 (1H, dd, J=15.9, 1.5 Hz), 2.17 (1H, dd, J=15.9, 9.5 Hz), 2.37 (1H, m), 2.82 (3H, s), 3.3～3.4 (2H, m), 3.95 (1H, m), 5.96 (1H, d, J=9.5 Hz), 6.33 (1H, d, J=7.6 Hz), 6.47 (1H, d, J=8.5 Hz), 7.13 (1H, dd, J=8.5, 7.6 Hz). IR ν_{max} (KBr) cm^{-1}: 3300, 1710, 1640, 1615, 1575, 1500, 1450, 1060. CD $[\theta]_{258}$ −77.800, $[\theta]_{240}$ −6300, $[\theta]_{219}$ −35,400 (c=3.31×10^{-5}, MeOH). EIMS m/z: 333 (M^+), 315, 297, 290, 272, 185. HR-EIMS m/z: 333.1678 (M^+, $C_{17}H_{23}N_3O_4$; calcd. 333.1689). Compound **7**. $[\alpha]_D^{19}$ +166.5° (c=0.14, MeOH). ^{1}H-NMR δ_{Me_4Si} (CD_3OD): 1.16 (3H, d, J=6.7 Hz), 1.19 (3H, d, J=7.0 Hz), 1.46 (1H, dd, J=15.0, 9.2 Hz), 2.22 (1H, d, J=15.0 Hz), 2.40 (1H, m), 2.64 (3H, s), 3.51 (1H, dd, J=11.0, 5.8 Hz), 3.58 (1H, dd, J=11.0, 4.6 Hz), 3.76 (1H, d, J=5.5 Hz), 5.61 (1H, m), 6.75 (1H, d, J=7.6 Hz), 7.09 (1H, d, J=8.2 Hz), 7.24 (1H, dd, J=8.2, 7.6 Hz). IR ν_{max} (KBr) cm^{-1}: 3300, 1715, 1620, 1450, 1040. CD $[\theta]_{290}$ +10,300, $[\theta]_{276}$ +9400, $[\theta]_{256}$ +47,200, $[\theta]_{245}$ 0, $[\theta]_{227}$ −57,900, $[\theta]_{211}$ 0 (c=2.68×10^{-5}, MeOH). EIMS m/z: 333 (M^+), 315, 297, 290, 272, 185. HR-EIMS m/z: 333.1691 (M^+, $C_{17}H_{23}N_3O_4$; calcd.

333.1689).

Isolation of **4**, **5**, **8** *and* **9**. The other culture broth (48 hr cultivation time, 80 l) was also subjected to the same treatment as that for the first to give a 60% acetone eluate (6.9 g) containing compounds positive in Ehrlich reagent.

The 60% acetone eluate was chromatographed on ODS using 55% MeOH in water to give two successive fractions containing compounds positive in Ehrlich reagent; I (91.5 mg) and II (293 mg). Fraction I was chromatographed on silica gel using 30% acetone in toluene, and was further purified by column chromatography on silica gel using 5% iso-PrOH in $CHCl_3$, followed by HPLC on YMC A-311 using 50% MeOH in water to yield **9** (16.9 mg). Fraction II was chromatographed on silica gel using 35% acetone in toluene, and was further purified by column chromatography on silica gel using 2% MeOH in $CHCl_3$ to give **4** (37 mg) and **5** (24 mg). Compound **4**. $[\alpha]_D^{24}$ −306.5° (c = 0.21, MeOH). ^{1}H-NMR δ_{Me_4Si} (CD_3OD): 0.82 (3H, d, J = 6.6 Hz), 1.04 (3H, d, J = 6.6 Hz), 1.59 (1H, br. t, J = 12.8 Hz), 2.31 (1H, m), 2.43 (1H, m), 2.86 (3H, s), 3.46 (1H, dd, J = 11.0, 6.2 Hz), 3.51 (1H, dd, J = 11.0, 5.1 Hz), 4.03 (1H, dd, J = 11.8, 5.9 Hz), 4.14 (1H, m), 4.29 (1H, d, J = 8.8 Hz), 6.43 (1H, d, J = 7.3 Hz), 6.58 (1H, d, J = 8.1 Hz), 7.12 (1H, dd, J = 8.1, 7.3 Hz). IR ν_{max} ($CHCl_3$) cm^{-1}: 3430, 3200, 1705, 1655, 1615, 1585, 1500, 1450, 1045. CD $[\theta]_{306}$ +13,200, $[\theta]_{284}$ 0, $[\theta]_{254}$ −72,100, $[\theta]_{233}$ −40,800 ($c = 2.78 \times 10^{-5}$, MeOH). EIMS m/z: 317 (M^+), 299, 284, 274, 256, 229. HR-EIMS m/z: 317.1738 (M^+, $C_{17}H_{23}N_3O_3$; calcd. 317.1739). Compound **5**. $[\alpha]_D^{19}$ +89.6° (c = 0.17, MeOH). ^{1}H-NMR δ_{Me_4Si} (CD_3OD): 0.92 (3H, d, J = 6.4 Hz), 1.14 (3H, d, J = 6.7 Hz), 1.18 (6H, d, J = 6.7 Hz), 2.23 (1H, m), 2.56 (3H, s), 2.60 (3H, s), 3.15 (1H, d, J = 11.0 Hz), 4.06 (1H, m), 5.21 (1H, m), 6.73 (1H, d, J = 7.9 Hz), 6.75 (1H, d, J = 7.9 Hz), 6.80 (1H, d, J = 7.9 Hz), 7.07 (1H, d, J = 7.9 Hz), 7.22 (2H, t, J = 7.9 Hz), 2.00 ~ 3.90 (12H, m). The two conformers are each assumed to have one set of protons for purposes of descriptive convenience. IR ν_{max} ($CHCl_3$) cm^{-1}: 3430, 3400, 3200, 1710, 1680, 1615, 1575, 1495, 1445, 1030. CD $[\theta]_{295}$ +3800, $[\theta]_{287}$ 0, $[\theta]_{270}$ −22,900, $[\theta]_{259}$ 0, $[\theta]_{251}$ +15,500, $[\theta]_{232}$ +33,800, $[\theta]_{215}$ +6600 ($c = 3.69 \times 10^{-5}$, MeOH). EIMS m/z: 317 (M^+), 299, 284, 274, 256, 229. HR-EIMS m/z: 317.1734 (M^+, $C_{17}H_{23}N_3O_3$; calcd. 317.1739).

The resultant mycelia obtained from this culture broth (48 hr cultivation time) were steeped in 15 l of acetone, and then removed by filtration. The acetone extracts (85.3 g) were redissolved in EtOAc, and partitioned between EtOAc and water. The EtOAc layer (10 l) was dried over sodium sulfate, and evaporated *in vacuo* to give a brown oily syrup (59.3 g). The residue was chromatographed on silica gel using toluene containing increasing amounts of acetone. An Ehrlich positive compound was eluted with a 20% acetone eluate (1.84 g).

The 20% acetone eluate was chromatographed on ODS using 93% MeOH in water. A major impurity (olivoretin C)[9] was removed at this point by crystallization from EtOH. The filtrate was purified by HPLC on μBondapak C_{18} using 80% CH_3CN in water to give **8** (4.5 mg). Compound **8**. ^{1}H-NMR δ_{Me_4Si} ($CDCl_3$): conformer A : B = 1 : 4; conformer B, 0.64 (3H, d, J = 6.7 Hz), 0.91 (3H, d, J = 6.1 Hz), 1.47 (3H, s), 1.50 (3H, br. s), 1.65 (3H, br. s), 1.70 ~ 1.97 (4H, m), 2.61 (1H, m), 2.91 (3H, s), 2.92 (1H, dd, J = 17.4, 3.7 Hz), 3.18 (1H, br. d, J = 17.4 Hz), 3.32 (3H, s), *ca.* 3.32 (1H, m), 3.39 (1H, dd, J = 9.8, 4.0 Hz), 4.31 (1H, d, J = 10.1 Hz), 4.40 (1H, m), 5.08 (1H, m), 5.28 (1H, dd, J = 10.7, 1.2 Hz), 5.30 (1H, dd, J = 17.7, 1.2 Hz), 6.17 (1H, dd, J = 17.7, 10.7 Hz), 6.17 (1H, br. s), 6.48 (1H, d, J = 7.9 Hz), 6.80 (1H, br. s), 6.97 (1H, d, J = 7.9 Hz), 8.50 (1H, br. s); conformer A, 1.24 (d, J = 6.7 Hz), 1.45 (s), 1.63 (s), 2.38 (m), 2.74 (s), 5.36 (d, J = 10.7 Hz), 6.24 (dd, J = 17.7, 10.7 Hz), 7.08 (d, J = 7.9 Hz), 8.74 (br. s). The other peaks had weak intensities and overlapped those of the major conformer. IR ν_{max} ($CHCl_3$) cm^{-1}: 3440, 3380, 1650, 1595, 1505, 1450, 1115. EIMS m/z: 451 (M^+), 408, 365, 340, 307. HR-EIMS m/z: 451.3192 (M^+, $C_{28}H_{41}N_3O_2$; calcd. 451.3199).

^{1}*H-NMR spectrum of* **1**. δ_{Me_4Si} [$(CD_3)_2CO$]: conformer A : B = 1 : 4; conformer B, 0.60 (3H, d, J = 6.7 Hz), 0.88 (3H, d, J = 6.4 Hz), 2.56 (1H, m), 2.87 (3H, s), 3.06 (1H, dd, J = 17.4, 3.7 Hz), 3.12 (br. d, J = 17.4 Hz), 3.46 (1H, ddd, J = 11.0, 9.2, 6.7 Hz), 3.66 (1H, ddd, J = 11.0, 4.6, 1.2 Hz), 4.14 (1H, m), 4.43 (1H, d, J = 10.4 Hz), 6.34 (1H, br. s), 6.45 (1H, dd, J = 7.6, 1.2 Hz), 6.90 (1H, dd, J = 7.9, 1.2 Hz), 6.95 (1H, dd, J = 7.9, 7.6 Hz), 7.03 (1H, d, J = 0.9 Hz), 10.03 (1H, br. s); conformer A, 0.88 (d, J = 6.4 Hz), 1.22 (d, J = 6.7 Hz), 2.33 (m), 2.71 (s), 3.03 (d, J = 10.7 Hz), 3.28 (m), 4.30 (br. s), 5.24 (d, J = 9.5 Hz), 7.20 (s), 7.31 (d, J = 7.9 Hz), 10.32 (br. s). The other peaks had weak intensities and overlapped those of the major conformer.

Synthesis of **3**. Compound **1** (41 mg) was treated with 37% formaldehyde (15 μl) in 10% MeOH–water at 90°C for 13 hr. The reaction mixture was partitioned between EtOAc and water. The EtOAc extract was chromatographed on silica gel using acetone–toluene to give **3** (18.9 mg, 45.2% yield). Synthetic **3** was found to be identical to the authentic sample by spectral measurements ($[\alpha]_D$, ^{1}H-NMR, IR, UV, CD and EIMS) and co-chromatography by HPLC.

Synthesis of **10**. The 14-*O*-acetate of **1** (45 mg) was treated with benzoic anhydride (100 mg) and $AlCl_3$ (40 mg) in nitrobenzene at room temperature for one day. After adding water and MeOH, the reaction mixture was treated with NaOH at room temperature for 30 min. The mixture was partitioned between EtOAc and water, and the EtOAc extract was purified by column chromatography on silica gel using iso-PrOH–$CHCl_3$, followed by purification on ODS using CH_3CN–water to give (−)-7-benzoylindolactam V (11.7 mg, 20% yield). $[\alpha]_D^{30}$ −611° (c = 0.74, EtOH). ^{1}H-NMR δ_{Me_4Si} ($CDCl_3$): conformer B

only, 0.63 (3H, d, J=6.5 Hz), 0.98 (3H, d, J=6.5 Hz), *ca.* 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=10.0 Hz), 6.45 (1H, d, J=8.0 Hz), 7.07 (1H, br. s), 7.4 ~ 7.8 (7H, m), 10.88 (1H, br. s). UV λ_{max} (EtOH) nm (ε): 389 (18,900), 264 (10,300), 225 sh (14,700). IR ν_{max} (KBr) cm^{-1}: 3370, 1660, 1615, 1580, 1565, 1502, 1282, 1119. EIMS *m/z*: 405 (M^+), 387, 372, 362, 344, 319, 275, 105. HR-EIMS *m/z*: 405.2056 (M^+, $C_{24}H_{27}N_3O_3$; calcd. 405.2052).

$LiAlH_4$ (20 mg) was added to $AlCl_3$ (10 mg) in Et_2O. After the bubbling had ceased, the mixture was added to a solution of (−)-7-benzoylindolactam V (11.7 mg) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 10 min. The excess reagent was destroyed by adding EtOAc, followed by adding 10% H_2SO_4 in water. After stirring for 30 min, the mixture was partitioned between EtOAc and water. The EtOAc extract was chromatographed on ODS using CH_3CN–water to give **10** (9.9 mg, 87.6% yield). $[\alpha]_D^{29}$ −132° (c=0.51, EtOH). ^{1}H-NMR δ_{Me_4Si} [$(CD_3)_2CO$]: conformer A : B = 1 : 4; conformer B, 0.61 (3H, d, J=6.7 Hz), 0.88 (3H, d, J=6.4 Hz), 2.56 (1H, m), 2.86 (3H, s), 3.05 (1H, dd, J=16.8, 3.7 Hz), 3.10 (1H, br. d, J=16.8 Hz), 3.45 (1H, dd, J=11.0, 9.2 Hz), 3.65 (1H, dd, J=11.0, 4.6 Hz), 4.14 (2H, s), 4.14 (1H, m), 4.39 (1H, d, J=10.1 Hz), 6.34 (1H, br. s), 6.44 (1H, d, J=7.9 Hz), 6.77 (1H, d, J=7.9 Hz), 7.00 (1H, s), 7.25 (5H, m), 9.78 (1H, br. s); conformer A, 1.21 (d, J=6.7 Hz), 2.32 (m), 2.70 (s), 5.23 (d, J=11.0 Hz), 6.84 (d, J=7.6 Hz), 6.89 (d, J=7.6 Hz). The other peaks had weak intensities and overlapped those of the major conformer. UV λ_{max} (EtOH) nm (ε): 300 (7300), 289 (7500), 230 (22,900). IR ν_{max} (KBr) cm^{-1}: 3360, 1650, 1602, 1505, 1450, 1042. EIMS *m/z*: 391 (M^+), 373, 358, 348, 330, 305, 261, 91. HR-EIMS *m/z*: 391.2260 (M^+, $C_{24}H_{29}N_3O_2$; calcd. 391.2260).

Reduction of **4** *and* **5**. Compound **4** (2 mg) was dissolved in CH_2Cl_2–Et_2O, and $LiAlH_4$ (5 mg) was added to the solution. After ultrasonication for 20 hr, 10% H_2SO_4 in water was added to the reaction mixture, which was then neutralized with NaOH. The mixture was partitioned between EtOAc and water, and the EtOAc extract was treated with SEP-PAK (silica gel, Waters Associates), eluting with 50% acetone in toluene. This eluate was purified by HPLC on NOVA-PAK C_{18} using CH_3CN–water to give **1** (100 μg, 5.3% yield). Synthetic **1** was found to be identical to the authentic sample by spectral measurements (UV, EIMS and CD) and co-chromatography by HPLC.

Compound **5** (2.5 mg) was also treated under the same conditions as those just mentioned to give **1** (148 μg, 6.2% yield). Synthetic **1** was found to be identical to the authentic sample by spectral measurements (UV, EIMS and CD) and co-chromatography by HPLC.

Reduction of **6**. Compound **6** (4.5 mg) was treated with $NaBH_4$ (5.1 mg) and $PdCl_2$ (4.8 mg) at room temperature for 1.5 hr. The reaction mixture was filtered, and then partitioned between EtOAc and water. The EtOAc layer was dried over sodium sulfate, and evaporated *in vacuo* to give **4** and **5**. The EtOAc extract (3.7 mg) was dissolved in CH_2Cl_2–Et_2O, and $LiAlH_4$ (5 mg) was added to the solution. After ultrasonication for 5 hr, 10% H_2SO_4 in water was added to the reaction mixture, which was then neutralized with NaOH. The mixture was partitioned between EtOAc and water, and the EtOAc extract was purified by column chromatography on silica gel using acetone–toluene, followed by HPLC on YMC A-311 using CH_3CN–water to give **1** (150 μg, 3.7% yield from **6**). Synthetic **1** was found to be identical to the authentic sample by spectral measurements (UV, EIMS and CD) and co-chromatography by HPLC.

Reduction of **7**. $LiAlH_4$ (0.46 mg) was added to $AlCl_3$ (3.2 mg) in Et_2O. After the bubbling had ceased, the mixture was added to a solution of **7** (1.2 mg) in CH_2Cl_2–Et_2O. After stirring at room temperature for 1 hr, 10% H_2SO_4 in water was added to the reaction mixture, whic was then neutralized with NaOH. The mixture was partitioned between EtOAc and water, and the EtOAc extract was purified by HPLC on YMC A-311 using CH_3CN–water to give **1** (100 μg, 11.1% yield). Synthetic **1** was found to be identical to the authentic sample by spectral measurements (UV, EIMS and CD) and co-chromatography by HPLC.

Acknowledgments. The authors express their thanks to Dr. S. Sakai of the Faculty of Pharmaceutical Sciences at Chiba University for supplying the spectral data of teleocidin A-1 and A-2, to Dr. N. Takeda of the Faculty of Pharmacy at Meijo University for the MS measurements, and to Mr. R. Imamura of the Faculty of Science at Kyoto University and Mr. A. Okada at Sumitomo Chemical Industries Co., Ltd. for the FT-^{1}H NMR measurements. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1) M. Takashima and H. Sakai, *Bull. Agric. Chem. Soc. Jpn.*, **24**, 647 (1960).
2) H. Nakata, H. Harada and Y. Hirata, *Tetrahedron Lett.*, **23**, 2515 (1966).
3) Y. Hitotsuyanagi, H. Fujiki, M. Suganuma, N. Aimi, S. Sakai, Y. Endo, K. Shudo and T. Sugimura, *Chem. Pharm. Bull.*, **32**, 4233 (1984).
4) J. H. Cardellina II, F.-J. Marner and R. E. Moore, *Science*, **204**, 193 (1979).
5) S. Sakai, Y. Hitotsuyanagi, N. Aimi, H. Fujiki, M. Suganuma, T. Sugimura, Y. Endo and K. Shudo, *Tetrahedron Lett.*, **27**, 5219 (1986).
6) H. Fujiki, M. Mori, M. Nakayasu, M. Terada, T. Sugimura and R. E. Moore, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 3872 (1981).

7) S. Sakai, N. Aimi, K. Yamaguchi, Y. Hitotsuyanagi, C. Watanabe, K. Yokose, Y. Koyama, K. Shudo and A. Itai, *Chem. Pharm. Bull.*, **32**, 354 (1984).
8) K. Irie, M. Hirota, N. Hagiwara, K. Koshimizu, H. Hayashi, S. Murao, H. Tokuda and Y. Ito, *Agric. Biol. Chem.*, **48**, 1269 (1984).
9) Y. Hitotsuyanagi, K. Yamaguchi, K. Ogata, N. Aimi, S. Sakai, Y. Koyama, Y. Endo, K. Shudo, A. Itai and Y. Iitaka, *Chem. Pharm. Bull.*, **32**, 3774 (1984).
10) K. Irie, N. Hagiwara, K. Koshimizu, H. Hayashi, S. Murao, H. Tokuda and Y. Ito, *Agric. Biol. Chem.*, **49**, 221 (1985).
11) N. Hagiwara, K. Irie, K. Koshimizu, H. Hayashi, S. Murao, H. Tokuda and Y. Ito, *Agric. Biol. Chem.*, **49**, 2529 (1985).
12) S. Sakai, Y. Hitotsuyanagi, K. Yamaguchi, N. Aimi, K. Ogata, T. Kuramochi, H. Seki, R. Hara, H. Fujiki, M. Suganuma, T. Sugimura, Y. Endo, K. Shudo and Y. Koyama, *Chem. Pharm. Bull.*, **34**, 4883 (1986).
13) K. Irie, N. Hagiwara, T. Kurome, H. Hayashi, M. Arai and K. Koshimizu, *Agric. Biol. Chem.*, **51**, 285 (1987).
14) K. Irie, N. Hagiwara, A. Funaki, H. Hayashi, M. Arai and K. Koshimizu, *Agric. Biol. Chem.*, **51**, 1733 (1987).
15) K. Irie, N. Hagiwara, K. Koshimizu, H. Hayashi, S. Murao and Y. Ito, *Agric. Biol. Chem.*, **49**, 845 (1985).
16) Y. Endo, K. Shudo, A. Itai, M. Hasegawa and S. Sakai, *Tetrahedron*, **42**, 5905 (1986).
17) R. A. Heacock and M. E. Mahon, *J. Chromatogr.*, **17**, 338 (1965).
18) S. Nakatsuka, T. Masuda, O. Asano, T. Teramae and T. Goto, *Tetrahedron Lett.*, **27**, 4327 (1986).
19) K. Irie, H. Hayashi, M. Arai and K. Koshimizu, *Agric. Biol. Chem.*, **50**, 2679 (1986).
20) K. Irie, N. Hagiwara and K. Koshimizu, *Tetrahedron*, **43**, 5251 (1987).
21) N. Hagiwara, K. Irie, H. Tokuda and K. Koshimizu, *Carcinogenesis*, **8**, 963 (1987).
22) F. S. Sariasian and D. A. Kunz, *Biochem. Biophys. Res. Commun.*, **141**, 405 (1986).
23) S. Sakai, personal communication (1987).
24) H. Fujiki, M. Suganuma, H. Hakii, M. Nakayasu, Y. Endo, K. Shudo, K. Irie, K. Koshimizu and T. Sugimura, *Proc. Jpn. Acad. Ser. B*, **61**, 45 (1985).
25) K. Irie, H. Tokuda, N. Hagiwara, K. Koshimizu, H. Hayashi, S. Murao and Y. Ito, *Int. J. Cancer*, **36**, 485 (1985).
26) K. Irie, N. Hagiwara, H. Tokuda and K. Koshimizu, *Carcinogenesis*, **8**, 547 (1987).