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BIOSYNTHESIS OF INDOLE ALKALOID TUMOR PROMOTERS TELEOCIDINS (I) POSSIBLE BIOSYNTHETIC PATHWAY OF THE MONOTERPENOID MOIETIES OF TELEOCIDINS

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Abstract: Possible biosynthetic pathway of the monoterpenoid moieties of teleocidins shown in Fig. 8 was proposed by feeding experiments with several D or ^{13}C-labelled precursors and isolation of new telecidin-related metabolites named blastmycetin D (6) and E (7).

Introduction

Teleocidins (teleocidin A-1, A-2, B-1 - B-4 and olivoretins)[1] are peculiar indole alkaloids which have an (-)-indolactam V (1)[2] structure and a variety of a monoterpenoid moiety at position 6 and/or 7 of the indole ring. They attract much interest in the area of organic chemistry because of their unique structures and potent tumor-promoting activity.[3] The last several years have seen intensive studies on the structural determination[1d-h,4] and total syntheses[2b,5] of teleocidins and their related metabolites. There are, however, few reports on the biosynthesis of these fascinating indole alkaloids, whose elucidation might ultimately be applicable for the efficient syntheses of a new variety of teleocidin-related compounds. In our latest preliminary publication,[6] we reported that (-)-indolactam V (1), the common biosynthetic intermediate of teleocidins, was biosynthesized from L-tryptophan, L-valine and L-methionine via N-methyl-L-valyl-L-tryptophanol as the initial step of the biosynthetic studies on teleocidins.

The monoterpenoid moieties of teleocidins are classified into two classes: teleocidin As[1c,g] with a linalyl group at position 7, and teleocidin Bs[1b,f] and olivoretins[1d,e,h] with a C_{11}-cyclic side chain at positions 6 and 7 (Fig. 1). The origin and insertion mechanism of the additional one carbon atom of the C_{11} side chain of teleocidin Bs and olivoretins are noteworthy. Olivoretin C (4) and E (5), in which the vinyl group of the cyclohexene ring is located in the 6 position side of the indole

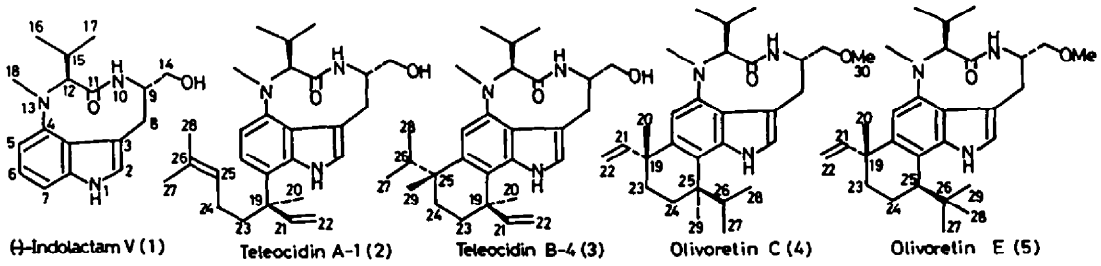


Fig. 1 Naturally occurring teleocidin-related compounds. The numbering system proposed by Sakai et al.[7] was adopted.

ring, are different in structure from teleocidin Bs, which have the vinyl group at the 7 position side, and olivoretin E (5) has a tert-butyl group at C-25 in place of an isopropyl group. Moreover, all monoterpenoid moieties of teleocidins have a 1,1-dialkylallyl structure, which is thought to be biosynthesized through some Claisen type rearrangement.

To make clear the biosynthetic pathway of these complex monoterpenoid moieties of teleocidins, we searched for biosynthetic intermediates between (-)-indolactam V (1) and teleocidins in the mycelia of Streptoverticillium blastmyceticum NA34-17,[8] which has a characteristic feature of producing 1 in quantity. We propose in this paper the possible biosynthetic pathway of the monoterpenoid moieties of the teleocidins shown in Fig. 8 on the basis of feeding experiments with several D or ^{13}C-labelled precursors and isolation of new teleocidin-related metabolites named blastmycetin D (6) and E (7), whose structures have been preliminarily mentioned.[4a,d]

Results

Feeding experiments with several D or ^{13}C-labelled precursors.

A time-course study of teleocidin B-4 (3) in a medium consisting of 2 % glucose, 1 % meat extract, 1 % polypeptone and 0.5 % NaCl (pH 7.0) showed that the production of 3 in mycelia began after 36 hr of cultivation, and that the amount of 3 reached the maximum after 70 hr of cultivation as previously reported.[6] D or ^{13}C-Labelled precursors (5 mg/100 ml) were added, therefore, to the medium at 30 hr after innoculation, and the culture fermentation broth was harvested for analysis at 70 hr after innoculation.

To elucidate the biosynthetic blocks of teleocidins, three logical precursors, DL-tryptophan-$\underline{d}_8$, L-valine-$\underline{d}_8$ and L-[CD_3]methionine, were added to the medium. DL-Tryptophan-$\underline{d}_8$ and L-valine-$\underline{d}_8$ were significantly incorporated into teleocidins: the incorporation ratio into teleocidin B-4 (3) was 2.6 % and 0.6 %, respectively. By feeding L-[CD_3]methionine, the signals ascribable to the methyl groups at C-18 (δ2.90), C-29 (δ1.35) and

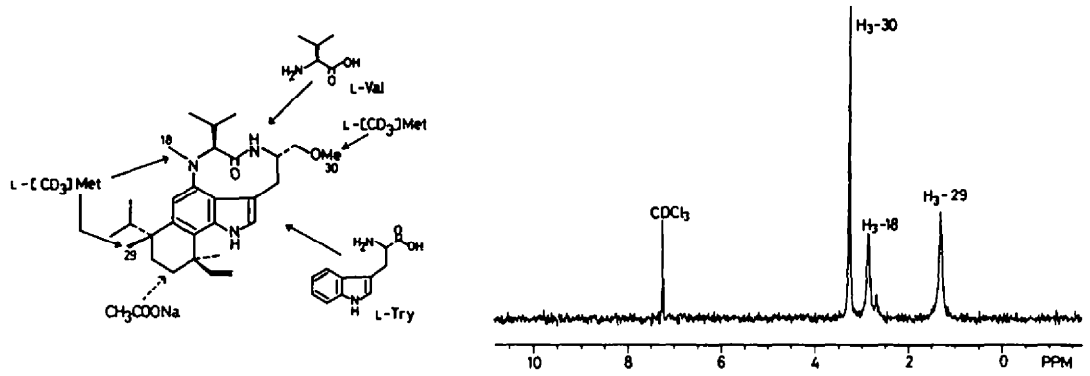


Fig. 2 D NMR of olivoretin A (8) derived from L-[CD_3]methionine and its biosynthetic blocks.

C-30 (δ3.30) of oliovretin A (14-O-methylteleocidin B-4, 8) were observed in the D NMR in chloroform (Fig. 2). In olivoretin C (4), the 7 position side methyl group (C-29) turned out to originate from L-[CD_3]methionine: the D NMR spectrum in chloroform showed three singlets at δ1.46, 2.85 and 3.33 that were ascribable to C-29, C-18 and C-30 methyl groups, respectively. The assignment of the C-29 methyl group (δ1.46) was established by the nuclear Overhauser effect (NOE) difference spectra of 4 in chloroform-d: saturation of the C-5 proton (δ6.40) caused a significant enhancement of the C-20 and C-18 methyl signals (δ1.40 and 2.85, respectively). Moreover, the L-[CD_3]methionine fed was incorporated into the tert-butyl group of olivoretin E (5). This was confirmed by the EI-MS spectrum (Fig. 3) because D NMR measurement of the deuterated 5 (less than 1 mg) was unsuccessful. Although the molecular ion [m/z (%), 465 (6.3)] of 5 significantly contained the peak of the d_9 derivative [m/z (%), 474 (0.5)], no d_9 derivative (m/z 417) was detected in the fragment ion of m/z 408 (M - tert-butyl)$^+$. On the other hand, the fragment ion of m/z 422 (M - isopropyl)$^+$ of the deuterated olivoretin C (4) clearly contained the peak of the d_9 derivative (m/z 431). These results indicate that one methyl group of the tert-butyl group of 5 originates from L-[CD_3]methionine.

As for the remaining linalyl carbons, namely from C-19 to C-28, suitable precursors have not yet been found. Most plausible candidates such as DL-[2-^{13}C]mevalonolactone, [1,2-$^{13}C_2$]acetic acid and L-leucine-d_3 gave only disappointing results. It seems necessary to use radioactive precursors and to reduce the glucose content in the medium as low as possible.

Next, D-labelled (-)-indolactam V (1), teleocidin A-1 (2) and (-)-14-O-methylindolactam V (14-O-CD_3-ILV),[9] were added to the medium to investigate the biosynthetic pathway of teleocidins. As Table I shows, 1 and 2 were efficiently incorporated into teleocidins. The incorporation ratio of (-)-14-O-CD_3-ILV into olivoretin A (8) and C (4) was less than that of

(-)-indolactam V (1) and teleocidin A-1 (2). Moreover, (-)-14-O-methyl-indolactam V did not occur in the culture broth or mycelia of this actinomycete. The above results indicate that both 1 and 2 are important biosynthetic intermediates of teleocidins, and that the methylation at C-14 occurs usually after construction of the monoterpenoid moieties.

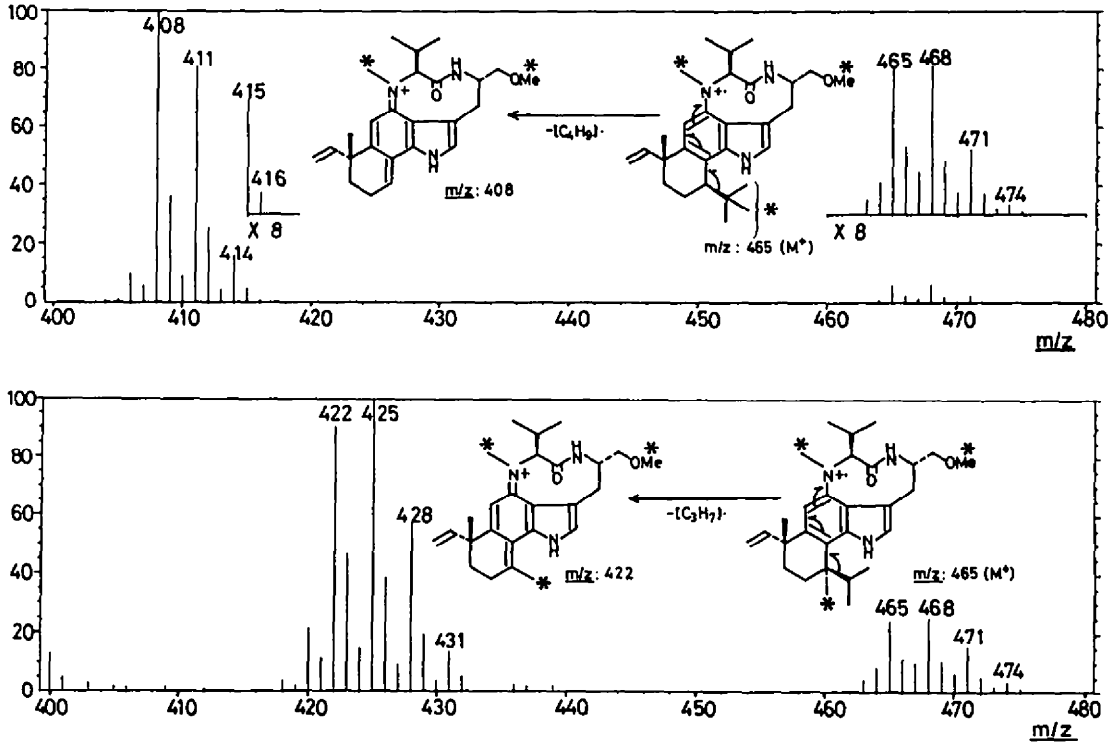


Fig. 3 Partial MS spectra of olivoretin E (top) and C (bottom) derived from L-[CD_3]-methionine. The asterisks show L-[CD_3]methionine-derived methyl groups. This fragmentation was first proposed by Sakai *et al.*[1e,h]

Table I Incorporation of D-labelled precursors into teleocidins[a]

	(-)-Indolactam V-*d*$_3$			14-O-CD_3-ILV			Teleocidin A-1-*d*$_3$		
	Prod[b]	% D[c]	% IR[d]	Prod.	% D	% IR	Prod.	% D	% IR
Teleocidin A-1 (2)	ND[e]	8.6	ND	ND	---	---	ND	---	---
Teleocidin B-4 (3)	56.4	21.0	15.9	13.9	---	---	47.9	26.4	27.9
Olivoretin A (8)	11.1	36.7	5.3	11.0	4.6	0.8	6.7	51.9	7.4
Olivoretin C (4)	10.0	28.8	3.7	5.5	7.0	0.6	5.4	45.9	5.3
Olivoretin E (5)[f]	ND	14.2	ND	ND	6.2	ND	ND	39.0	ND

[a]The results represent one simultaneous experiment using the same seed culture. Similar results were obtained in another experiment. [b]Production (mg/l). [c]Deuterium content (%). [d]Incorporation ratio (%) of a biosynthesized metabolite synthesized from a labelled precursor. [e]Not determined. [f]The quantification of olivoretin E (5) was impossible because of its quite small amount (*ca.* 0.3 mg/l).

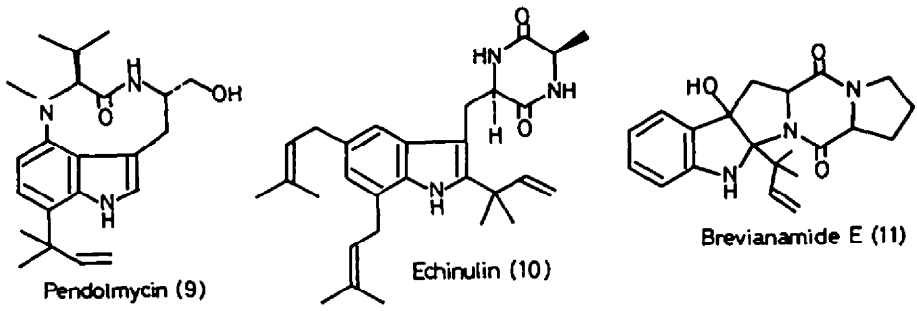


Fig. 4 Structures of several indole alkaloids with aromatic hemiterpenes.

Insertion of an isoprene unit into (-)-indolactam V (1).

The above feeding experiments indicate that insertion of a linalyl group into (-)-indolactam V (1) is the first step of teleocidin biosynthesis. To investigate the mode of introduction of the linalyl group at position 7 of teleocidin A-1 (2), the possibility of a direct S_N2' attack at position 7 of 1 was examined. Treatment of 1 with prenyl bromide in acetic acid-water medium buffered with sodium acetate,[10] simulating biological conditions, gave (-)-2, 5 or 7-prenylindolactam V in 20, 13 and 10 % yield, respectively, pendolmycin (9)[4c] with 1,1-dimethylallyl group at position 7 like teleocidin A-1 (2) being obtained only in 0.2 % yield. Similar results were obtained using geranyl bromide (data not shown). These results seem to exclude a direct S_N2' attack at position 7 of 1 by geranyldiphosphate. Some indole alkaloids like echinulin (10) and brevianamide E (11) also have 1,1-dimethylallyl groups on the indole ring.[11] In vitro rearrangement studies carried out with model compounds and several circumstantial evidences tend to support a mechanism proceeding via primary attack at N-1 with subsequent aza-Claisen rearrangement.[11] Similar aza-Claisen rearrangement was thought to be involved in the biosynthesis of teleocidins.

Isolation and structural determination of new teleocidin-related metabolites named blastmycetin D (6) and E (7).

On the assumption that the monoterpenoid moieties of teleocidins are introduced via aza-Claisen rearrangement from some N-1 substituted intermediate, we searched for a teleocidin-related compound with a N-1 substituent, which is expected to have a larger R_f value on a silica gel TLC than teleocidin A and Bs, and thus found two new metabolites named blastmycetin D (6) and E (7): the latter proved to have a N-1 substituent.

Purification of these metabolites was guided by Ehrlich reagent,[12] with which teleocidins showed characteristic coloration (purple - green) on TLC.

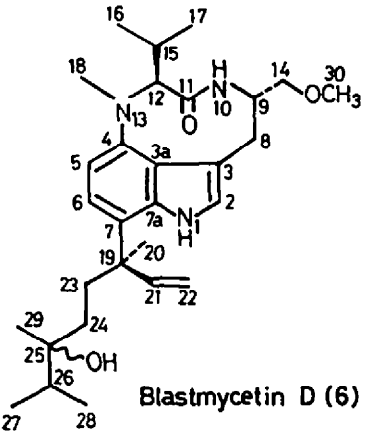
S. blastmyceticum NA34-17 was cultured by deep aerated fermentation for 45 hr, and the mycelia (6.5 kg wet weight) were steeped in acetone. The acetone extracts were concentrated in vacuo and partitioned between dichloromethane and water. The dichloromethane extracts were chromatographed on silica gel using toluene containing increasing amounts of acetone to give 15 and 20 % acetone eluates, which were further purified by repeated column chromatography and HPLC to give two new teleocidin-related metabolites, namely blastmycetin D (6, 25 mg) and E (7, 50 mg).

Blastmycetin D (6) was obtained as an amorphous powder, $[\alpha]_D^{23}$ -51° (c=0.27, MeOH). HR-EIMS established its molecular formula as $C_{29}H_{45}N_3O_3$ (obs. m/z 483.3472, calc. 483.3461). The UV spectrum [λ_{max} (MeOH) nm (ε): 298 (8400), 287 (8600), 229 (26,200)] and IR spectrum [ν_{max} (KBr) cm^{-1}: 1595, 1545, 1507] indicated the presence of an indole chromophore. Characteristic IR absorption bands were observed at 1658 and 1115 cm^{-1} that were ascribable to a lactam ring of six or more members and to an ether linkage, respectively. Though the UV and IR spectra were closely similar to those of olivoretin A (8),[1d] the MS fragment pattern of 6 [m/z (%): 483 (M^+, 22), 465 (94), 440 (7), 422 (24), 397 (7), 379 (21), 368 (100)] was different from 8 [m/z (%): 465 (M^+, 100), 422 (20), 379 (14), 321 (8)]. The fragment ion m/z 465 (M^+-18) of 6, a dehydration peak, suggests the presence of a hydroxyl group in 6.

The ^1H and ^{13}C NMR spectra of 6 in chloroform-d revealed that 6 existed as the two stable conformers[2b] (sofa:twist=1:5). The ^1H NMR spectrum (chloroform-d, 400 MHz) of 6 was similar to that of 8, except for the signals ascribable to the monoterpenoid moiety on the indole ring. Three aromatic protons for the major conformer twist were exhibited at δ6.48 (1H, d), 6.81 (1H, s) and 7.00 (1H, d), indicating that the indole ring of 6 was substituted at position 5 or 7. Substitution at position 7 is more compatible with the chemical shifts for the two vicinal aromatic protons and with the ratio of the two conformers.[13] These observations led us to assume that 6 was a compound of teleocidin A-1[1g] (lyngbyatoxin A,[1c] 2) type. A comparison between the ^1H NMR spectra of 2[14] and 6 revealed the presence of a vinyl group [δ5.28 (1H, dd), 5.30 (1H, dd), 6.15 (1H, dd)], two methyl groups [δ1.05 (3H, s), 1.45 (3H, s)], two methylenes [δ1.24 (1H, m), 1.49 (1H, m), 1.96 (2H, m)] and an isopropyl group [δ0.82 (3H, d), 0.85 (3H, d), 1.71 (1H, m)] in the substituent at position 7 of 6. This was also supported by the MS spectrum, whose fragment ion at m/z 368 was ascribable to the loss of a 2,3-dimethyl-3-pentanol group from the molecular ion. These data led us to formulate the structure of blastmycetin D as shown on the next page. The assignments of all proton and carbon signals established by ^1H-^1H and ^{13}C-^1H COSY are summarized in Table II.

Table II ^{1}H and ^{13}C NMR (400 and 100 MHz) chemical shifts and assignments for blastmycetin D (6) in chloroform-d[a]

No	^{1}H chemical shift	No	^{13}C chemical shift
1	8.54 (1H,br.s)	2	121.0 (d)
2	6.81 (1H,s)	3	113.8 (s)
5	6.48 (1H,d,J=8.2Hz)	3a	118.7 (s)
6	7.00 (1H,d,J=8.2Hz)	4	146.5 (s)
8	2.92 (1H,dd,J=17.6,3.8Hz)	5	106.4 (d)
	3.18 (1H,br.d,J=17.6Hz)	6	120.1 (d)
9	4.40 (1H,m)	7	121.6 (s)
10	6.15 (1H,br.s)	7a	137.4 (s)
12	4.30 (1H,d,J=9.9Hz)	8	34.2 (t)
14	3.32 (1H,m)	9	52.6 (d)
	3.39 (1H,dd,J=9.9,4.4Hz)	11	173.1 (s)
15	2.61 (1H,m)	12	71.1 (d)
16	0.63*(3H,d,J=7.2Hz)	14	74.5 (t)
17	0.91*(3H,d,J=6.6Hz)	15	28.5 (d)
18	2.91 (3H,s)	16	19.5*(q)
20	1.45 (3H,s)	17	21.6*(q)
21	6.15 (1H,dd,J=17.6,10.4Hz)	18	32.9 (q)
22	5.28 (1H,dd,J=10.4,1.1Hz)	19	43.0 (s)
	5.30 (1H,dd,J=17.6,1.1Hz)	20	24.2 (q)
23	1.96 (2H,m)	21	148.2 (d)
24	1.24 (1H,m)	22	112.6 (t)
	1.49 (1H,m)	23	32.0 (t)
26	1.71 (1H,m)	24	34.1 (t)
27	0.82+(3H,d,J=6.6Hz)	25	74.7 (s)
28	0.85+(3H,d,J=6.6Hz)	26	36.2 (d)
29	1.05 (3H,s)	27	16.7+(q)
30	3.32 (3H,s)	28	17.5+(q)
		29	23.2 (q)
		30	58.4 (q)



Blastmycetin D (6)

[a]Chemical shifts for twist conformer are expressed as ppm downfield from TMS. sofa:twist=1:5 (0.10 M, 27°C).
[b]Assignments bearing the same symbol may be reversed.

The CD spectrum of 6 in methanol ($[\theta]_{305}$ +7800, $[\theta]_{291}$ 0, $[\theta]_{253}$ -24,500, $[\theta]_{231}$ -8400, $[\theta]_{221}$ -20,900, $[\theta]_{211}$ 0) was very similar to that of teleocidin A-1 (2),[14] indicating that 2 and 6 had the same absolute configurations at C-9, C-12 and C-19. To prove this, chemical conversion of 6 into olivoretin A (8) was tried. Recently, Nakatsuka et al.[5c,e] have reported the total synthesis of teleocidin B-3 and B-4 (3), in the course of which they found that intramolecular cyclization of a tertiary alcohol compound like blastmycetin D (6) occurred by treatment with boron trifluoride etherate in acetic acid. Treatment of 6 with several Lewis acids, however, gave no cyclization product like olivoretin A (8). Intramolecular cyclization of 6 was achieved by treatment with phosphoric acid at room temperature for 20 hr to give 8 in 48.5 % yield, which was confirmed to be identical to the authentic sample[9] by spectral measurements (UV, ^{1}H NMR, MS and CD), melting point and co-chromatography by HPLC.

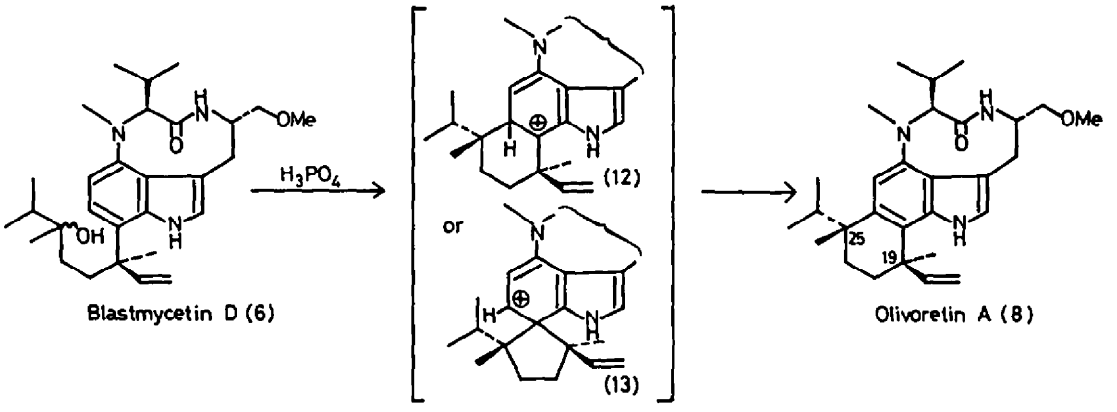


Fig. 5 Intramolecular cyclization of blastmycetin D (6) to olivoretin A (8).

The intermediate cation between 6 and 8 is deduced to be 12 or 13 (Fig. 5). However, existence of the spiro intermediate 13 could not be confirmed: olivoretin C (4), in which isopropyl and vinyl groups were reversed, was not detected at all by HPLC analysis.

In addition to olivoretin A (8), olivoretin B,[1e] a C-25 epimer of 8, was also deduced to have been formed. Although a peak corresponding to olivoretin B was found by HPLC, the substance present could not be identified because of its very small quantity. This seems reasonable because olivoretin A (8) and B occurred naturally in a ratio of ca. 15:1 in our strain.[9] This probably reflects a difference in stability between olivoretin A (8) and B. Olivoretin A (8), whose vinyl and isopropyl groups at C-19 and C-25 on the cyclohexene ring have equatorial orientations, would be more stable than olivoretin B. The above results indicate that the absolute configurations of 6 at C-9, C-12 and C-19 are S, S and R, respectively. The absolute configuration at C-25 of 6 remains to be investigated. No C-25 epimer of 6 could be found in the mycelia of our strain.

Blastmycetin E (7) was obtained as an amorphous powder, $[\alpha]_D^{22}$ -64.7° (c=0.63, EtOH). Its molecular formula was established to be $C_{28}H_{41}N_3O_2$ by HR-EIMS (obs. m/z 451.3210, calc. 451.3199), which was the same as teleocidin Bs. The presence of an indole ring in 7 was suggested by the UV spectrum [λ_{max} (EtOH) nm (ε): 310 (7100), 297 (sh., 5900), 234 (17,200)] and IR spectrum [ν_{max} (KBr) cm^{-1}: 1555, 1500]. The MS fragment pattern of 7 [m/z (%): 451 (M^+, 81), 408 (21), 394 (100), 351 (28), 307 (52)] was, however, different from that of teleocidin Bs. The fragment ion m/z 394 (M^+-57) indicated the presence of a tert-butyl group in 7.

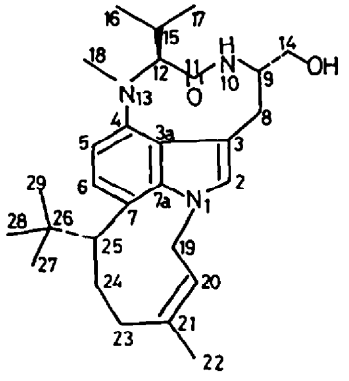
^1H NMR spectrum of 7 (chloroform-d, 400 MHz) revealed that 7 existed as

the two stable conformers[2b] (sofa:twist=2.4:1) and clearly showed the existence of the nine-membered lactam ring like teleocidins. Three aromatic protons for the major conformer sofa [δ6.80 (1H, s), 6.95 (1H, d), 7.18 (1H, d)] and lack of the signal ascribable to N-1 of the indole ring suggested that 7 was substituted at N-1 and C-7. Furthermore, the ^{1}H NMR spectrum of 7 exhibited the presence of a tert-butyl group [δ0.99 (9H, s)], a methyl group [δ1.67 (3H, s)], an alkene proton [δ4.96 (1H, m)], a methylene bound to the nitrogen atom on the indole ring [δ4.46 (1H, dd), 5.13 (1H, dd)], two methylenes [δ1.58 (1H, m), 1.95 (1H, m), 2.10 (2H, m)] and a methine [δ3.28 (1H, br.d)] in the substituent at N-1 and C-7 of 7. On the basis of these data, the structure of blastmycetin E was deduced to be the structure 7 except for the stereochemistry as shown below. The assignments of all proton signals established by ^{1}H-^{1}H and ^{13}C-^{1}H COSY are summarized in Table III. This assignment was also supported by the ^{13}C NMR spectrum (see experimental section). The configuration at C-20 was proved to be cis by the NOE

Table III ^{1}H NMR (400 MHz) chemical shifts of blastmycetin E (7) in chloroform-d[a]

No	sofa	twist
2	6.80 (1H,s)	6.64 (1H,s)
5	6.95 (1H,d,J=7.8Hz)	6.54 (1H,d,J=8.3Hz)
6	7.18 (1H,d,J=7.8Hz)	7.13 (1H,d,J=8.3Hz)
8	2.75 (1H,dd,J=14.7,1.5Hz)	2.95 (1H,dd)
	3.04 (1H,dd,J=14.7,4.4Hz)	3.13 (1H,br.d)
9	4.40 (1H,m)	4.38 (1H,m)
10	4.72 (1H,d,J=11.2Hz)	6.99 (1H,br.s)
12	3.11 (1H,d,J=10.7Hz)	4.27 (1H,d,J=9.8Hz)
14	3.48 (2H,m)	3.55 (1H,m)
		3.74 (1H,m)
15	2.38 (1H,m)	2.57 (1H,m)
16	0.95*(3H,d,J=6.4Hz)	0.62*(3H,d,J=6.8Hz)
17	1.24*(3H,d,J=6.8Hz)	0.92*(3H,d,J=6.4Hz)
18	2.71 (3H,s)	2.89 (3H,s)
19a	4.46 (1H,dd,J=16.6,5.9Hz)	ND[b]
19b	5.13 (1H,dd,J=16.6,6.8Hz)	ND
20	4.96 (1H,m)	ND
22	1.67 (3H,s)	1.68 (3H,s)
23	2.10 (2H,m)	ND
24	1.58 (1H,m)	1.50 (1H,m)
	1.95 (1H,m)	ND
25	3.28 (1H,br.d,J=9.3Hz)	3.33 (1H,br.d)
27-29	0.99 (9H,s)	1.06 (9H,s)

[a]Chemical shifts are expressed as ppm downfield from TMS. sofa:twist=2.4:1 (0.05 M, 27°C).
[b]The signals could not be identified because of their low intensity and being overlapped by the signals of the major conformer.
[c]Assignments bearing the same symbol may be reversed.



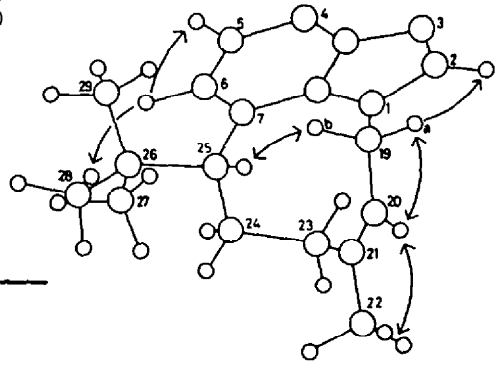


Fig. 6 The NOE enhancements observed in the monoterpenoid moiety of 7.

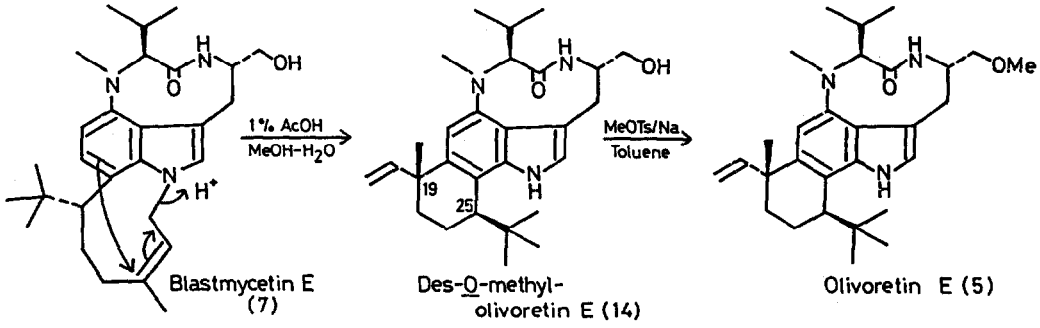


Fig. 7 Chemical conversion of blastmycetin E (7) into olivoretin E (5).

difference spectra of 7 in chloroform-d. Saturation of the H-22 protons (δ1.67) caused a remarkable enhancement (11.8 %) of the H-20 signal (δ4.96), and saturation of the H-25 (δ3.28) proton resulted in a characteristic enhancement (6.4 %) of the H-19b (δ5.13) signal. The NOE enhancements observed in the monoterpenoid moiety of 7 are summarized in Fig. 6, supporting the appropriateness of structure 7.

To establish the stereochemistry at C-9, C-12 and C-25 of 7, chemical conversion of 7 into olivoretin E (5)[1h] was investigated. After examining a number of acid catalysts, treatment of 7 with 1 % acetic acid in methanol and water (1:1) at 70 °C for several minutes proved to give quantitatively des-O-methylolivoretin E (14), whose methylation by methyl p-toluenesulfonate in sodium and toluene[13] gave olivoretin E (5) in 20 % yield (Fig. 7). No C-19 epimer of 14 was obtained, possibly because des-O-methylolivoretin E (14), whose vinyl and tert-butyl groups at C-19 and C-25 on the cyclohexene ring have equatorial orientations, would be more stable than the C-19 epimer of 14. The spectral data (UV, ^{1}H NMR, MS and CD) and melting point of the synthetic olivoretin E were identical to those of the authentic sample.[1h] The above data indicate that the absolute configurations of 7 at C-9, C-12 and C-25 are S, S and R, respectively.

Discussion

The facts that olivoretin A (8) is obtainable from blastmycetin D (6) *in vitro*, and that C-29 methyl group of 8 originated from L-methionine, indicate that the monoterpenoid moieties of teleocidins except olivoretin C (4) and E (5) are constructed by the methylation at C-25 of a teleocidin A-1 (2) type of compound, and subsequent intramolecular cyclization as shown in Fig. 8. The *in vitro* isoprenylation of (-)-indolactam V (1) by prenyl bromide and isolation of blastmycetin E (7), which is deduced to be formed through (-)-N^1-nerylindolactam V (15), suggest the involvement of aza-Claisen rearrangement in the insertion of the monoterpenoid moiety on 1. This

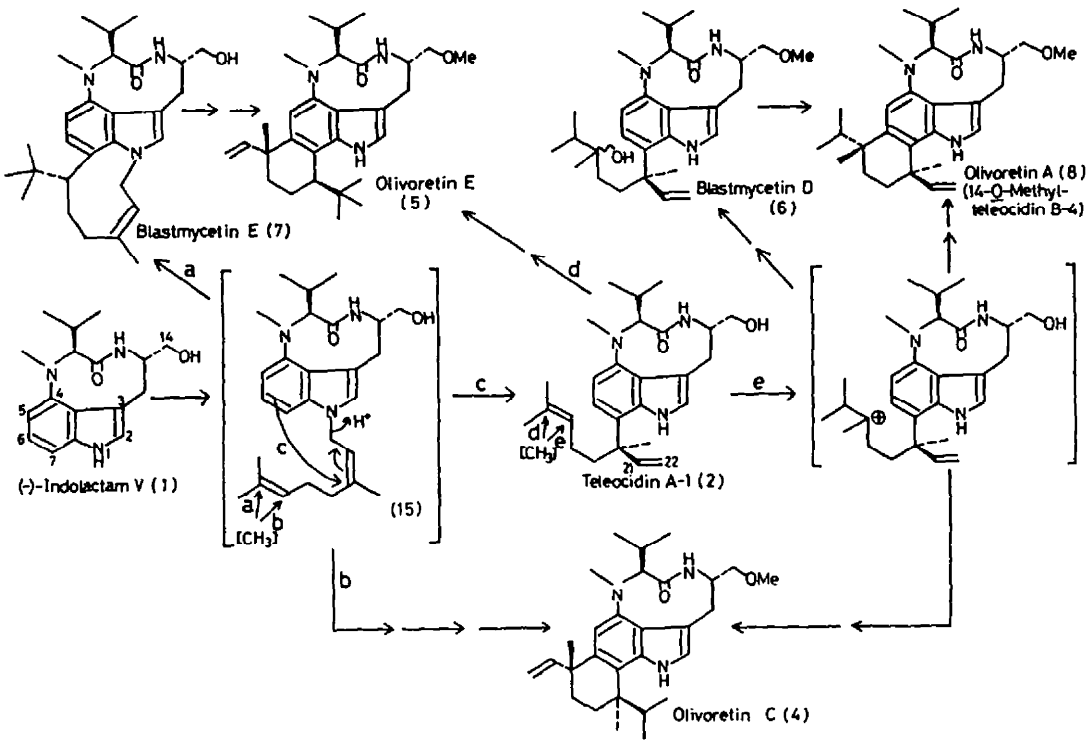


Fig. 8 Possible biosynthetic pathway of the monoterpenoid moieties of teleocidins.

possibility is strongly supported by the in vitro aza-Claisen rearrangement of blastmycetin E (7) into des-O-methylolivoretin E (14) under quite mild reaction conditions.

The aza-Claisen rearrangement of 7 into 14 also provides knowledge concerning the biosynthetic pathway of olivoretin C (4) and E (5), in which the vinyl group of the cyclohexene ring is located on the 6 position side of the indole ring: namely, the C_{11} terpenoid moiety of olivoretin E (5) is thought to be constructed by N^1-nerylation of 1 followed by the methylation at position a and intramolecular cyclization at position 7 like the case of blastmycetin D (6), and aza-Claisen rearrangement from position 1 to position 6. The biosynthesis of olivoretin C (4) can be similarly explained by the difference of the position of methylation (position b).

However, the fact that teleocidin A-1 (2) was incorporated into not only teleocidin B-4 (3) and olivoretin A (8) but also olivoretin C (4) and E (5) (Table I) suggests the existence of the biosynthetic pathway[7] proposed by Sakai et al., in which 4 and 5 arise from teleocidin A-1 (2) through some spiro intermediates by the rearrangement analogous to dienone-phenol

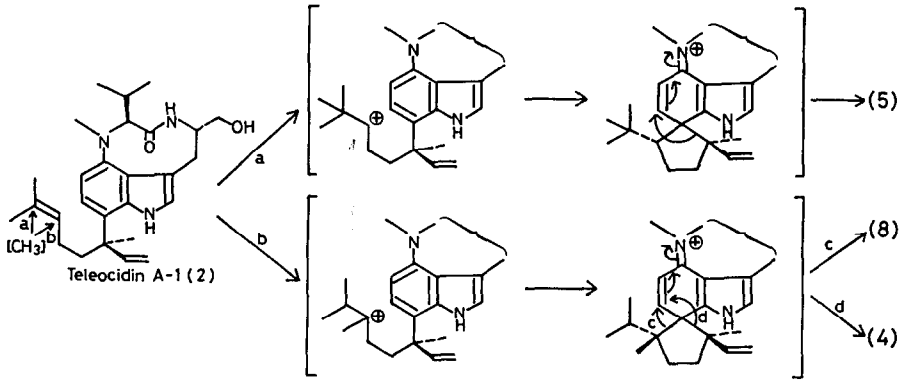


Fig. 9 Alternative biosynthetic pathway of olivoretin C (4) and E (5) from teleocidin A-1 (2) proposed by Sakai et al.[7]

rearrangement. The alternative biosynthetic pathway of **4** and **5** is shown in Fig. 9. Nakagawa et al. have recently demonstrated the participation of some spiro intermediates in the Pictet-Spengler reaction of N_b-hydroxytryptamines and cysteinals,[15] supporting the appropriateness of this pathway. On the basis of these considerations, the possible biosynthetic pathway of the monoterpenoid moieties of teleocidins was proposed as shown in Fig. 8.

We could not obtain, however, direct evidence of the involvement of some spiro intermediates in the biosynthesis of **4** and **5**: the in vitro intramolecular cyclization of blastmycetin D (**6**) did not give olivoretin C (**4**) at all, and no structural isomers of des-O-methylolivoretin E (**14**), in which tert-butyl and vinyl groups were reversed, were obtained in the aza-Claisen rearrangement of **7** into **14**. Moreover, the feeding experiment with 21,22-dihydroteleocidin A-1 (**16**) gave only dissapointing results: though ca. 5 % of the **16** fed was transformed into 21,22-dihydroolivoretin A (**17**) by S. blastmyceticum NA34-17, the amount of 21,22-dihydroolivoretin C (**18**) was below detection limits. Since olivoretin A (**8**) and C (**4**) occurred naturally in a ratio of ca. 5:4 in our strain (Table I), this seems to indicate the possibility that the **2** fed was transformed into **4** and **5** via (-)-N^1-neryl-indolactam V (**15**) by reverse aza-Claisen rearrangement. Further studies are necessary to demonstrate the biosynthetic pathway through some spiro intermediates.

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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 and ^{13}C NMR, JEOL GX400 (400 and 100 MHz, ref. TMS) and Brucker AC250 (250 and 62.9 MHz, ref. TMS); IR, Shimadzu Model 435; HPLC, Waters Model 600 E with a UV detector; CD, Jasco Model J-500; MS, JEOL JMS-DX300 (70 eV, 300 μA).

HPLC was carried out on YMC packed S343-10 (ODS), AM322 (ODS), ALL313 (ODS) and A023 (silica gel) column (Yamamura Chemical Laboratory), and NOVA-PAK C_{18} (ODS) column (Waters Associates). Wako C-100 and C-200 gel (silica gel, Wako Pure Chemical Industries) and YMC I-40/64 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography.

D or ^{13}C-Labelled precursors.

DL-Tryptophan-$\underline{d}_8$, L-valine-$\underline{d}_8$, L-[CD_3]methionine, DL-[2-^{13}C]mevalonolactone, [1,2-$^{13}C_2$]acetic acid and L-leucine-$\underline{d}_3$ were purchased from MSD ISOTOPES. Deuterated (-)-indolactam V (1) was synthesized from (-)-$\underline{N}^{13}$-desmethylindolactam V[2b,4b] and iodomethane-$\underline{d}_3$ in ethanol.[2b] Treatment of 1 with iodomethane-$\underline{d}_3$ in toluene and Na at 100 °C for 30 min gave (-)-14-$\underline{O}$-CD_3-ILV.[9] Deuterated teleocidin A-1 (2) was obtained by feeding experiments with L-[CD_3]methionine. The deuterium content was 35.9 %.

Feeding experiments with D or ^{13}C-labelled precursors.

S. blastmyceticum NA34-17 kept on Waksman's medium was transferred to a 500 ml shaking flask containing 100 ml of a medium consisting of 1 % glucose, 1 % polypeptone (Daigo Eiyo Kagaku), 1 % meat extract (Wako Pure Chemical Industries) and 0.5 % NaCl (pH 7.0), and the flask was shaken at 30 °C for 70 hr. Two milliliters of the seed culture thus obtained was transferred to a 500 ml shaking flask containing 100 ml of a medium (2 % glucose, 1 % polypeptone, 1 % meat extract, 0.5 % NaCl). After shaking at 30 °C for 30 hr, a D or ^{13}C-labelled precursor (ca. 5 mg) dissolved in DMSO (0.5 ml) was added to the medium and shaked. The culture fermentation broth was harvested for analysis at 70 hr after innoculation. Several flasks were incubated for each sample.

The mycelia were filtered and steeped in acetone. An aliquot of this extract was subjected to HPLC analysis to quantify the amount of teleocidin B-4 (3), olivoretin A (8) and C (4). The conditions were respectively as follows (column; column size; solvent; flow rate; t_R): YMC A311; 6 mm i.d. x 100 mm; 80 % CH_3CN; 1.0 ml/min; 8.8 min for 3, 11.2 min for 4 and 14.6 min for 8.

The remaining acetone extracts were purified by column chromatography on Wako C-100 gel, eluting with toluene containing increasing amounts of acetone. The eluates with 10 and 15 %, and 20 and 25 % acetone, were combined, respectively. The former was purified by HPLC on YMC AM322 column with 85 % MeOH, followed with 80 % CH_3CN to give olivoretin A (8), C (4) and E (5). The latter was purified by HPLC on YMC AM322 column with 80 % CH_3CN to give teleocidin A-1 (2) and B-4 (3). These were subjected to MS and NMR measurements.

Insertion of an isoprene unit into (-)-indolactam V (1).

(-)-Indolactam V (1, 300 mg) was dissolved in 13 N 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 were 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 (-)-2, 5 or 7-prenylindolactam V and pendolmycin (9), in 20, 13, 10 and 0.2 % yield, respectively. (-)-2-Prenylindolactam V: amorphous powder, $[\alpha]_D^{24.5}$ -116.4° (c=0.79, EtOH). UV λ_{max} (EtOH) nm (ε): 298 (9000), 234 (29,600). ^1H NMR δ ($CDCl_3$, 0.013 M, 27 °C) ppm: sofa:twist=1:3; twist, 0.63 (3H, d, J=7.0Hz), 0.94 (3H, d, J=6.4Hz), 1.75 (3H, s), 1.79 (3H, s), 2.59 (1H, m), 2.91 (3H, s), 3.01 (2H, m), 3.39 (2H, m), 3.51 (1H, m), 3.72 (1H, m), 4.34 (1H, m), 4.37 (1H, d, J=10.4Hz), 5.27 (1H, m), 6.49 (1H, d, J=7.3Hz), 6.84 (1H, d, J=7.3Hz), 6.85 (1H, br.s), 6.99 (1H, t, J=7.6Hz), 7.84 (1H, br.s); sofa, 0.94 (d, J=6.7Hz), 1.25 (d, J=6.7Hz), 1.77 (s), 1.82 (s), 2.37 (m), 2.74 (s), 2.83 (d, J=15.0Hz), 7.09 (t, J=7.6Hz), 7.20 (d, J=7.6Hz), 8.09 (br.s). Other peaks of the sofa conformer had weak intensities and/or overlapped the peaks of the twist conformer. EIMS m/z (%): 369 (M^+, 100), 351 (44), 326 (28), 300 (89), 283 (51), 239 (87). HR-EIMS m/z: 369.2429 (M^+, calc. for $C_{22}H_{31}N_3O_2$, 369.2416). (-)-5-Prenylindolactam V: amorphous powder, $[\alpha]_D^{24.5}$ +18.3° (c=0.88, EtOH). UV λ_{max} (EtOH) nm (ε): 300 (6200), 293 (6400), 231 (26,500). ^1H NMR δ ($CDCl_3$, 0.014 M, 27 °C) ppm: only sofa conformer; 0.97 (3H, d, J=6.4Hz), 1.29 (3H, d, J=7.0Hz), 1.74 (3H, s), 1.76 (3H, d, J=1.2Hz), 2.67 (1H, m), 2.75 (3H, s), 2.79 (1H, dd, J=14.3, 1.5Hz), 3.03 (1H, d, J=10.4Hz), 3.23 (1H, dd, J=14.3, 4.3Hz), 3.35-3.44 (2H, m), 3.51 (1H, dd, J=15.6, 7.0Hz), 3.71 (1H, dd, J=15.6, 6.4Hz), 4.45 (1H, m), 4.94 (1H, d, J=10.7Hz), 5.27 (1H, m), 7.02 (1H, d, J=2.5Hz), 7.09 (1H, d, J=8.2Hz), 7.22 (1H, d, J=8.2Hz), 8.22 (1H, br.s). EIMS m/z (%): 369 (M^+, 78), 351 (57), 326 (62), 283 (52), 239 (100). HR-EIMS m/z; 369.2434 (M^+, calc. for $C_{22}H_{31}N_3O_2$, 369.2416). The spectral data of (-)-7-prenylindolactam V and pendolmycin coincided with those reported previously.[17,4c]

Isolation of blastmycetin D (6) and E (7).

The above mentioned seed culture was transferred to a 30-liter jar fermentor (Marubishi type MSJ-02) containing 20 liters of 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; cultivation time, 45 hr.

The mycelia (6.5 kg wet weight, 260 liters of culture broth) were steeped in 20 liters of acetone, and then removed by filtration. The acetone extracts were partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer (40 liters) was dried over sodium sulfate (10 kg), and evaporated *in vacuo* to give a brown oily syrup (197 g). The residue was chromatographed on Wako C-100 gel (2.5 kg) using toluene containing increasing amounts of acetone to give 15 and 20 % acetone eluates (6.9 g) containing two compounds positive in Ehrlich reagent, named blastmycetin D (6) and E (7), both of which had larger R_f value on a silica gel TLC (acetone-toluene) than teleocidin A and Bs.

The above acetone eluates (6.9 g) were chromatographed again on Wako C-100 gel (250 g) using toluene containing increasing amounts of acetone to give 15 and 20 % acetone eluates (3.2 g), which were purified by MPLC on Wako C-200 gel (150 g) using toluene containing increasing amounts of acetone. Blastmycetin D (6) and E (7) were found in 6, 8 and 10 % acetone eluates. These eluates were purified by HPLC on YMC S343-10 using 75 % CH_3CN, followed on YMC A023 using 87 % n-hexane, 10 % $CHCl_3$ and 3 % i-PrOH to give blastmycetin D (6, 25 mg) and E (7, 50 mg). Blastmycetin D (6): IR ν_{max} (KBr) cm^{-1}: 3450, 1658, 1595, 1545, 1507, 1115, 1040. ^1H NMR δ ($CDCl_3$, 0.10 M, 27 °C) ppm: sofa:twist=1:5; sofa, 0.78 (d, J=6.6Hz), 1.01 (s), 1.24 (d, J=6.6Hz), 1.49 (s), 2.39 (m), 2.73 (s), 2.81 (d, J=14.3Hz), 4.55 (br.s), 5.36 (d, J=17.6Hz), 6.23 (dd, J=17.6, 10.4Hz), 6.93 (d, J=2.2Hz), 6.99 (d, J=7.7Hz), 7.10 (d, J=7.7Hz), 8.79 (br.s). Other peaks of the sofa conformer had weak intensities and/or overlapped the peaks of the twist conformer. Blastmycetin E (7): IR ν_{max} (KBr) cm^{-1}: 3370, 2970, 1655, 1555, 1500. CD $[\theta]_{320}$ 0, $[\theta]_{310}$ -6200, $[\theta]_{304}$ -3400, $[\theta]_{289}$ -13,100, $[\theta]_{282}$ -8600, $[\theta]_{259}$ -28,500, $[\theta]_{250}$ 0, $[\theta]_{236}$ +110,000, $[\theta]_{220}$ +36,100, $[\theta]_{210}$ 0 (c=0.005, MeOH, 22.5°C). ^{13}C NMR δ ($CDCl_3$, 0.13 M, 27 °C, 62.9 MHz) ppm: sofa, 19.7 (q, C-16 and 17), 24.5 (d, C-15), 26.0 (q, C-22), 28.4 (t, C-8), 29.1 (q, C-27, 28, 29), 31.7 (t, C-24), 32.8 (t, C-23), 35.2 (s, C-26), 35.7 (q, C-18), 48.3 (d, C-25), 50.9 (t, C-19), 55.4 (d, C-9), 63.4 (t, C-14), 76.9 (d, C-12), 108.3 (s, C-3), 118.9 (d, C-20), 121.2 (d, C-6), 122.7 (d, C-5), 123.7 (s, C-3a), 130.1 (s, C-7), 134.0 (d, C-2), 140.4 (s, C-7a or 21), 140.7 (s, C-7a or 21), 143.0 (s, C-4), 172.6 (s, C-11); twist, 19.8 (q, C-16 or 17), 21.7 (q, C-16 or 17), 26.3 (q, C-22), 28.4 (d, C-15), 29.6 (q, C-27, 28, 29), 30.5 (t, C-24), 33.5 (q, C-18), 33.6 (t, C-23), 33.7 (t, C-8), 34.4 (s, C-26), 46.7 (d, C-25), 51.3

(t, C-19), 55.6 (d, C-9), 65.1 (t, C-14), 71.2 (d, C-12), 108.7 (d, C-5), 112.6 (s, C-3), 119.5 (d, C-20), 120.5 (s, C-3a), 120.9 (d, C-6), 130.0 (s, C-7), 130.7 (d, C-2), 139.9 (s, C-7a or 21), 140.9 (s, C-7a or 21), 145.2 (s, C-4), 174.6 (s, C-11).

Intramolecular cyclization of blastmycetin D (6) to olivoretin A (8).

Blastmycetin D (6, 4.2 mg) dissolved in 200 µl of MeOH was treated with 85 % phosphoric acid (2 ml) at room temperature for 20 hr. After neutralization with 1 N NaOH, the reaction mixture was partitioned between $CHCl_3$ and water. The $CHCl_3$ extract was purified by HPLC on YMC ALL313 using 75 % CH_3CN, followed on YMC A023 using 70 % $CHCl_3$ in n-hexane to give olivoretin A (2.0 mg, 48.5 % yield).

Aza-Claisen rearrangement of blastmycetin E (7) to des-O-methylolivoretin E (14).

Blastmycetin E (7, 3.7 mg) was dissolved in methanol and water (1:1) containing 1 % AcOH, and heated at 70 °C for several minutes. After adding toluene, the reaction mixture was evaporated to dryness. The residue was subjected to HPLC analysis, indicating that the reaction proceeded quantitatively. The product was recrystallized from MeOH to give **14** as colorless needles (mp. 276 - 279 °C). Des-O-methylolivoretin E (14): $[\alpha]_D^{24}$ -112° (c=0.25, EtOH). CD $[\theta]_{332}$ 0, $[\theta]_{313}$ +2600, $[\theta]_{304}$ 0, $[\theta]_{265}$ -63,300, $[\theta]_{243}$ 0, $[\theta]_{236}$ +45,200, $[\theta]_{227}$ +11,600, $[\theta]_{210}$ +105,000 (c=0.005, MeOH, 22.5 °C). UV λ_{max} (EtOH) nm (ε): 298 (sh., 9200), 291 (9600), 235 (33,300). IR ν_{max} (KBr) cm^{-1}: 3450, 3370, 3300, 2950, 2870, 1675, 1602, 1498, 1038. ^1H NMR δ ($CDCl_3$, 0.11 M, 27 °C) ppm: sofa:twist= 1:1.5; twist, 0.64 (3H, d, J=6.7Hz, H_3-16 or 17), 0.90 (3H, d, J=6.1Hz, H_3-16 or 17), 1.03 (9H, s, H_3-27, 28, 29), 1.51 (3H, s, H_3-20), 1.75 (2H, m, H_2-24), 2.10 (2H, m, H_2-23), 2.57 (1H, m, H-15), 2.80 (1H, m, H-25), 2.89 (3H, s, H_3-18), 2.97 (1H, dd, J=17.7, 4.0Hz, H_2-8), 3.17 (1H, br.d, J=17.7Hz, H_2-8), 3.58 (1H, dd, J=11.6, 8.6Hz, H_2-14), 3.77 (1H, dd, J=11.6, 3.7Hz, H_2-14), 4.30 (1H, d, J=9.8Hz, H-12), 4.36 (1H, dd, J=17.1, 1.5Hz, H_2-22), 4.40 (1H, m, H-9), 4.76 (1H, dd, J=10.4, 1.5Hz, H_2-22), 5.83 (1H, dd, J=17.1, 10.4Hz, H-21), 6.41 (1H, s, H-5), 6.85 (1H, s, H-2), 7.47 (1H, br.s, H-10), 7.92 (1H, br.s, H-1); sofa, 0.92 (d, J=6.1Hz), 1.04 (s), 1.21 (d, J=6.7Hz), 1.55 (s), 2.35 (m), 2.73 (s), 3.40 (dd, J=11.0, 7.0Hz), 3.48 (dd, J=11.0, 6.7Hz), 4.53 (dd, J=17.1, 1.5Hz), 4.69 (br.d, J=11.3Hz), 4.78 (dd, J=10.4, 1.5Hz), 5.88 (dd, J=17.1, 10.4Hz), 6.92 (s), 6.97 (d, J=2.1Hz), 8.27 (br.s). Other peaks of the sofa conformer had weak intensities and/or overlapped the peaks of the twist conformer. EIMS m/z (%): 451 (M^+, 8), 433 (2), 394 (100), 376 (52). HR-EIMS m/z: 451.3221 (M^+, calc. for $C_{28}H_{41}N_3O_2$, 451.3199).

Synthesis of 21,22-dihydroolivoretin A (17) and C (18).

21,22-Dihydroolivoretin A (17) and C (18) were obtained, respectively, from olivoretin A (8) and C (4) by reduction using Wilkinson catalyst [tris(triphenylphophine)rhodium (1) chloride] and hydrogen.[16] Compound 17: amorphous powder, $[\alpha]_D^{23}$ -141° (c=0.16, $CHCl_3$). UV λ_{max} (EtOH): 287 (8600), 232 (30,400). ^1H NMR δ [$(CD_3)_2CO$, 0.011 M, 27 °C] ppm: sofa:twist= 1:4; twist, 0.50 (3H, t, J=7.3Hz), 0.51 (3H, d, J=6.7Hz), 0.70 (3H, d, J=7.0Hz), 0.88 (3H, d, J=6.4Hz), 1.01 (3H, d, J=7.0Hz), 1.34 (3H, s), 1.42 (3H, s), 1.62 (1H, m), 1.35-2.35 (6H, m), 2.59 (1H, m), 2.87 (3H, s), 3.00 (1H, dd, J=17.4, 4.0Hz), 3.11 (1H, br.d, J=17.4Hz), 3.31 (3H, s), 3.35 (1H, d, J=9.5Hz), 3.42 (1H, dd, J=9.5, 4.3Hz), 4.30 (1H, m), 4.32 (1H, d, J=10.1Hz), 6.17 (1H, br.s), 6.55 (1H, s), 6.95 (1H, s), 9.53 (1H, br.s); sofa, 0.99 (d, J=7.0Hz), 1.24 (d, J=6.4Hz), 1.46 (s), 2.69 (s), 3.26 (s), 6.99 (s), 7.00 (s), 9.79 (br.s). Other peaks of the sofa conformer had weak intensities and/or overlapped the peaks of the twist conformer. EIMS m/z (%): 467 (M^+, 100), 438 (50), 424 (38), 410 (32), 396 (20), 381 (28), 323 (17). HR-EIMS m/z: 467.3524 (M^+, calc. for $C_{29}H_{45}N_3O_2$, 467.3511). Compound 18: colorless rods from $CHCl_3$-MeOH (mp. >300 °C). $[\alpha]_D^{25}$ -108° (c=0.05, $CHCl_3$). UV λ_{max} (EtOH) nm (ε): 286 (6500), 232 (23,600). ^1H NMR δ [$(CD_3)_2CO$, 0.004 M, 27 °C] ppm: sofa:twist=1:2; twist, 0.38 (3H, d, J=6.7Hz), 0.61 (3H, t, J=7.3Hz), 0.71 (3H, d, J=6.7Hz), 0.88 (3H, d, J=6.4Hz), 1.01 (3H, d, J=6.1Hz), 1.29 (3H, s), 1.50 (3H, s), 1.30-2.00 (7H, m), 2.59 (1H, m), 2.87 (3H, s), 2.98-3.13 (2H, m), 3.31 (3H, s), 3.34 (1H, d, J=9.5Hz), 3.42 (1H, dd, J=9.5, 4.3Hz), 4.29 (1H, m), 4.31 (1H, d, J=9.8Hz), 6.17 (1H, br.s), 6.50 (1H, s), 6.95 (1H, s), 9.59 (1H, br.s); sofa, 1.25 (d, J=6.7Hz), 1.30 (s), 1.55 (s), 2.69 (s), 3.26 (s), 6.99 (d, J=2.1Hz). Other peaks of the sofa conformer had weak intensities and/or overlapped the peaks of the twist conformer. EIMS m/z (%): 467 (M^+, 23), 424 (100), 396 (17), 381 (12), 323 (7). HR-EIMS m/z: 467.3514 (M^+, calc. for $C_{29}H_{45}N_3O_2$, 467.3511).

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