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STRUCTURE OF BLASTMYCETIN E, A NEW TELEOCIDIN-RELATED COMPOUND, FROM STREPTOVERTICILLIUM BLASTMYCETICUM

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Abstract: A new teleocidin-related compound named blastmycetin E (1) was isolated from Streptoverticillium blastmyceticum, and the structure was elucidated by spectroscopic evidences and chemical correlation with olivoretin E (2).

Teleocidins are potent skin tumor promoters[1] produced by actinomycetes.[2] The last several years have seen the structure determination and total synthesis of teleocidin-related compounds because of their peculiar structure involving a nine-membered lactam ring and a complex monoterpenoid moiety.[3] Among the teleocidin-producing actinomycetes, Streptoverticillium blastmyceticum NA34-17,[4] which had been found to produce the Epstein-Barr virus early antigen-inducing indole alkaloids, has a characteristic feature of producing (-)-indolactam V,[5] the biosynthetic intermediate of teleocidins,[6] in quantity. This characteristic would be advantageous to obtain a wide variety of teleocidin-related compounds, especially biosynthetic intermediates of teleocidins. In our previous publication,[7] we reported the isolation of blastmycetin D, a possible precursor of teleocidins. Our continuous efforts to find new teleocidin-related compounds for elucidation of teleocidin biosynthesis have recently led to the isolation of a new metabolite named blastmycetin E (1, 45 mg) from the mycelia (8 kg, wet weight) of this actinomycete. This communication deals with the structure of blastmycetin E (1) and its significance in the biosynthesis of teleocidins.

Blastmycetin E (1), a less polar metabolite than teleocidins (B-1 - B-4,[8] A-1 and A-2[9]), 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 (observed m/z, 451.3210; calculated m/z, 451.3199), which was the same as that of teleocidin Bs. The presence of an indole ring was suggested by the UV spectrum [λ_{max}^{EtOH} nm (ε): 234 (17,200), 297 (sh., 5900), 310 (7100)]. The mass fragment pattern of 1 [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 1.

^1H NMR spectrum of 1 in chloroform-d (0.05 M, 27 °C) revealed that 1 existed as two stable conformers[5] (SOFA:TWIST = 2.4:1) and clearly showed the existence of the nine-membered lactam ring like teleocidins. Three aromatic protons [δ6.80 (1H, s), 6.95 (1H, d) and 7.18 (1H, d) for the major conformer] and lack of the signal ascribable to N-1

of the indole ring suggested that 1 was substituted at N-1 and C-7. Furthermore, the ^{1}H NMR spectrum of 1 exhibited the presence of a tert-butyl at δ0.99 (9H, s); a methyl at δ1.67 (3H, s); an alkene proton at δ4.96 (1H, m); a methylene bound to the nitrogen atom on the indole ring at δ4.46 (1H, dd) and 5.13 (1H, dd); two methylene at δ1.55 (2H, m) and 2.10 (2H, m); and a methine at δ 3.28 (1H, br.d) in the substituent at N-1 and C-7 of 1. On the basis of these data, structure of blastmycetin E was deduced to be the structure of 1 except for the stereochemistry as shown in Fig. 1. The assignments of all proton signals established by ^{1}H-^{1}H COSY are summarized in Table I. The configuration at C-20 was proved to be cis by nuclear Overhauser effect (NOE) difference spectra of 1 in chloroform-d. Saturation of the H-20 proton caused a characteristic enhancement of the H-19a and H-25 signal, and saturation of the H-24 proton resulted in a remarkable enhancement of the H-19b signal. NOE enhancements observed in the monoterpenoid moiety of 1 are summarized in Fig. 2.

To establish the stereochemistry at C-9, C-12 and C-24 of 1, chemical conversion of 1 into 2 was tried. Treatment of 1 with 1 % acetic acid in methanol and water (1:1) at 70 °C for several minutes gave quantitatively desmethylolivoretin E (3),[10] which was methylated by methyl p-toluenesulfonate in sodium and toluene[11] to give olivoretin E (2) in 20 % yield. No C-19 epimer of 3 was obtained, possibly because desmethylolivoretin E (3), whose

Table I ^{1}H NMR (400 MHz) chemical shifts of blastmycetin E (1) in $CDCl_3$[a]

No	δ (SOFA)	δ (TWIST)
2	6.80 (s)	6.64 (s)
5	6.95 (d,J=7.8Hz)	6.54 (d,J=8.3Hz)
6	7.18 (d,J=7.8Hz)	7.13 (d,J=8.3Hz)
8a	2.75 (dd,J=14.7,1.5Hz)	2.95 (dd)
8b	3.04 (dd,J=14.7,4.4Hz)	3.13 (br.d)
9	4.40 (m)	4.38 (m)
10	4.72 (d,J=11.2Hz)	6.99 (br.s)
12	3.11 (d,J=10.7Hz)	4.27 (d,J=9.8Hz)
14a	3.48 (m)	3.55 (m)
14b	3.48 (m)	3.74 (m)
15	2.38 (m)	2.57 (m)
16	0.95 (d,J=6.4Hz)	0.62 (d,J=6.8Hz)
17	1.24 (d,J=6.8Hz)	0.92 (d,J=6.4Hz)
18	2.71 (s)	2.89 (s)
19a	4.46 (dd,J=16.6,5.9Hz)	ND[b]
19b	5.13 (dd,J=16.6,6.8Hz)	ND
20	4.96 (m)	5.07 (m)
22	2.10 (m)	1.95 (m)
23	1.55 (m)	1.50 (m)
24	3.28 (br.d,J=9.3Hz)	3.33 (br.d)
25	1.67 (s)	1.68 (s)
27-29	0.99 (s)	1.06 (s)

a) Chemical shifts are expressed as ppm downfield from TMS.
b) The signal could not be identified because of its low intensity and being overlapped by the signals of the major conformer.

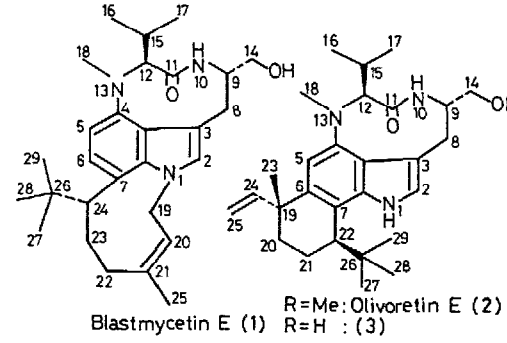

Fig. 1 Teleocidin-related compounds.

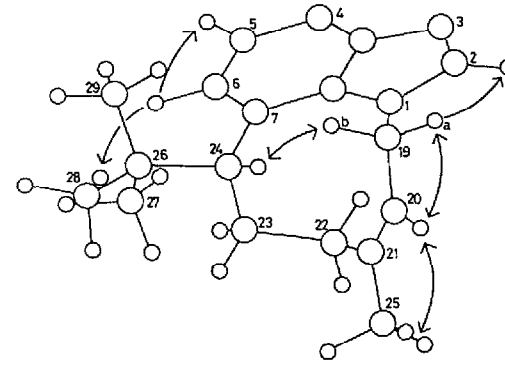

Fig. 2 NOE enhancements observed in 1.

vinyl and tert-butyl group at C-19 and C-22 on the cyclohexene ring have equatorial orientations, would be more stable than the C-19 epimer of 3. 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.[12] The above data indicate that the absolute configurations of 1 at C-9, C-12 and C-24 are S, S and R, respectively.

The fact that quite mild acid treatment of 1 resulted in the recyclization to give 3 strongly indicates that 1 is a possible biosynthetic precursor of olivoretin E (2). Olivoretin E (2) and C, in which the vinyl group of the cyclohexene ring is attached to C-19, are different in structure of the monoterpenoid moiety from teleocidin Bs, which have the vinyl group at C-22. Very little has been known about the biosynthesis of olivoretin E (2) and C type compounds. The present results along with the previous results on blastmycetin D[7] suggest that the C_{11} terpenoid moiety of olivoretin E is constructed by N^1-nerylation followed by the methylation at C-25 and oxidation at C-24, subsequent intramolecular cyclization at C-7 like blastmycetin D,[7] and the Claisen type rearrangement from N-1 to C-6 as shown in Fig. 3. The intermediate cation between 1 and 3 is deduced to be 4 because no structural isomers of 3, in which tert-butyl and vinyl groups were reversed, were obtained by the above mentioned in vitro rearrangement. The biosynthesis of olivoretin C can be similarly explained. Moreover, teleocidin As might be biosynthesized from (-)-N^1-nerylindolactam V by Claisen type rearrangement from N-1 to C-7.

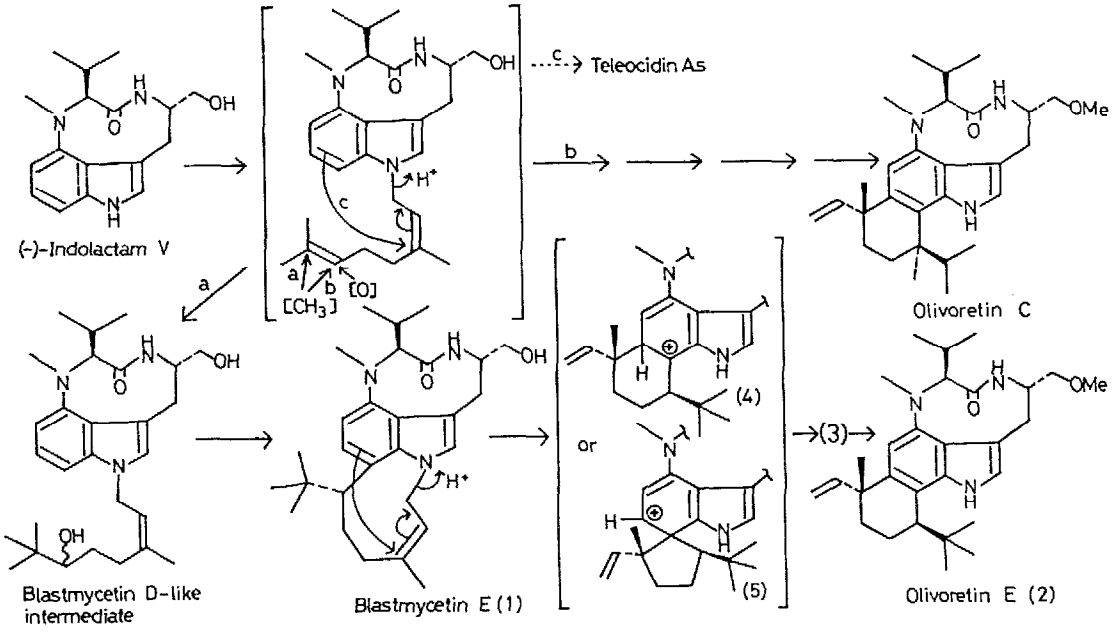

Fig. 3 Possible biosynthetic pathway of olivoretin E and C from (-)-indolactam V.

However, the possibility that olivoretin C and E are biosynthesized from teleocidin A-1 via the intermediate cation like 5[13], and that a direct S_N2' attack at C-7 of (-)-indolactam V by the geranylpyrophosphate gives teleocidin As, cannot be excluded. Further investigation on these biosynthetic pathways is in progress.

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10) Spectral data of 3: Colorless needles from MeOH, mp 276 - 279 °C, $[\alpha]_D^{24}$ -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): 235 (33,300), 291 (9600), 298 (sh., 9200). ^{1}H 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), 0.90 (3H, d, J=6.1Hz, H_3-17), 1.03 (9H, s, H_3-27,28,29), 1.51 (3H, s, H_3-23), 1.75 (2H, m, H_2-21), 2.10 (2H, m, H_2-20), 2.57 (1H, m, H-15), 2.75 (1H, m, H-22), 2.89 (3H, s, H_3-18), 3.00 (1H, dd, J=17.7, 4.3Hz, Ha-8), 3.15 (1H, br. d, J=17.7Hz, Hb-8), 3.58 (1H, dd, J=11.6, 8.6Hz, Ha-14), 3.77 (1H, dd, J=11.6, 3.7Hz, Hb-14), 4.30 (1H, d, J=9.8Hz, H-12), 4.36 (1H, dd, J=17.1, 1.5Hz, Ha-25), 4.40 (1H, m, H-9), 4.76 (1H, dd, J=10.4, 1.5Hz, Hb-25), 5.83 (1H, dd, J=17.1, 10.4Hz, H-24), 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). HR-EIMS m/z: 451.3221 (M^+, calcd. for $C_{28}H_{41}N_3O_2$, 451.3199).

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