Communications to the Editor

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ISOLATION AND STRUCTURE ELUCIDATION OF TELEOCIDIN B-1, B-2, B-3, AND B-4

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From the mycelia of Streptomyces mediocidicus four metabolites teleocidin B-1, B-2, B-3 and B-4 were isolated in crystalline states and their structures were determined by comparison with the known des-O-methylolivoretin B, and olivoretin D (teleocidin B of Hirata), and by studying the ^{13}C-nuclear magnetic resonance (NMR) spectra.

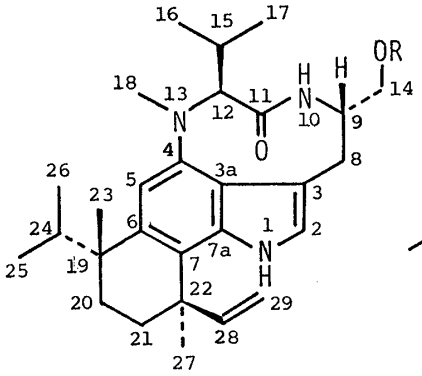
KEYWORDS —— Streptomyces mediocidicus; Streptoverticillium olivoreticuli; teleocidin B-1; teleocidin B-2; teleocidin B-3; teleocidin B-4(olivoretin D = teleocidin B of Hirata); olivoretin B; des-O-methylolivoretin C; ^{13}C-NMR; tumor promoter

"Teleocidin" was first isolated from the myceria of Streptomyces mediocidicus as a mixture of highly toxic compounds by Takashima and Sakai.[1] The structure of teleocidin B, one of these metabolites, was presented as shown by formula I[2] by Hirata et al.[3] After "teleocidin" was revealed to be a potent tumor promoter in mouse skin, we found that "teleocidin" consists of two teleocidin A isomers (A-1, A-2; MW 437) and four teleocidin B isomers (B-1, B-2, B-3, B-4; MW 451) which could be separated by high perfomance liquid chromatography (HPLC) in that elution order.[4,5]

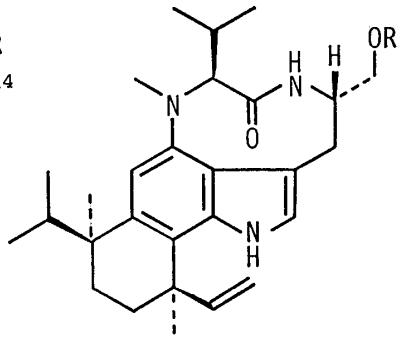
The structures of olivoretin A,B,C and D isolated from Streptoverticillium olivoreticuli were determined by X-ray analysis and by chemical comparison of A and D as shown by the formula II, III, IV, and I.[2,6]

In this communication, we describe the isolation and the complete characterization of teleocidin B-1~B-4 in crystalline states. Also we report that teleocidin B-1 is des-O-methylolivoretin B (V) and teleocidin B-4 is identical with olivoretin D (above teleocidin B of Hirata). From the ^{13}C-NMR spectra the structures of teleocidin B-2 and B-3 were elucidated as formulas VI and VII.

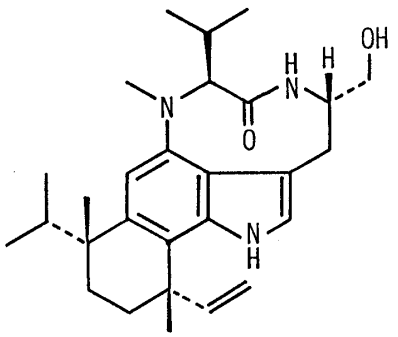
Thus ca. 10 g of extract from the mycelia of S. mediocidicus was purified by using flash column chromatography (SiO_2, 230-400 mesh) with 3% MeOH-$CHCl_3$, medium pressure liquid chromatography (MPLC, Lobar column SiO_2, 230-400 mesh) with 2% MeOH-$CHCl_3$ and ODS column (30-50μ, Wako RG-1) with 20% H_2O-MeOH. The Ehrlich positive compounds (230 mg) were obtained as "teleocidin" which showed single spots



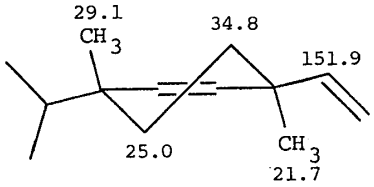
teleocidin B-4:R = H (I)
(olivoretin D = teleocidin B of Hirata)
olivoretin A :R = CH_3 (II)



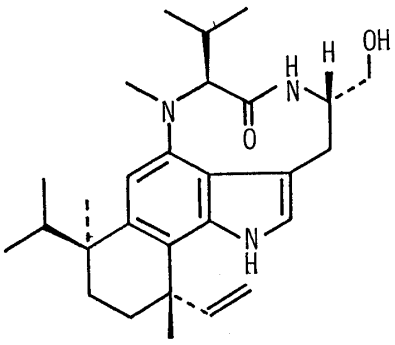
teleocidin B-1:R = H (V)
olivoretin B :R = CH_3 (III)



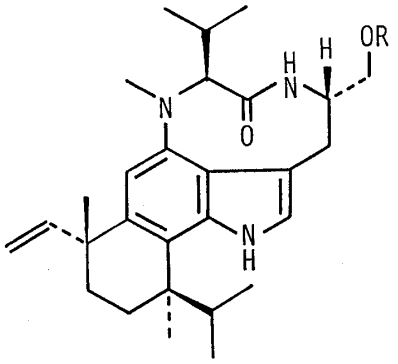
teleocidin B-2 (VI)



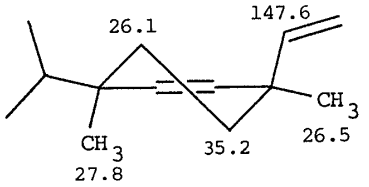
teleocidin B-4 (I)



teleocidin B-3 (VII)



olivoretin C :R = CH_3 (IV)
des-O-methylolivoretin C : R = H (VIII)



teleocidin B-1 (V)

Fig. 1. Stable Conformation and ^{13}C-NMR Chemical Shifts of I and V

Table 1. ^{13}C-NMR Chemical Shifts of Teleocidin B-1 ~ B-4 around the Cyclohexene Ring of Molecules

No.	B_1	B_2	Δ(B-1 - B-2)	B_3	B_4	Δ(B-3 - B-4)
6	137.9	137.6	(+0.3)	137.3	137.9	(-0.6)
7	116.6	117.4	(-0.8)	118.1	118.1	(0)
7a	139.8	139.7	(+0.1)	138.3	138.6	(-0.3)
19	40.2*	40.1*	(+0.1)	39.6*	40.1	(-0.5)
20	26.1	26.7	(-0.6)	24.9	25.0	(-0.1)
21	35.2	35.6	(-0.4)	34.8	34.8	(0)
22	39.5*	39.6*	(-0.1)	39.4*	39.6	(-0.2)
23	27.8	27.9	(-0.1)	29.8	29.1	(+0.7)
24	37.2	38.2	(-1.0)	37.0	37.9	(-0.9)
25	17.4	17.5	(-0.1)	17.0	17.0	(0)
26	18.4	18.6	(-0.2)	18.3	18.0	(+0.3)
27	26.5	25.4	(+1.1)	21.6	21.7	(-0.1)
28	147.6	148.3	(-0.7)	151.6	151.9	(-0.3)
29	113.2	112.8	(+0.4)	111.1	111.3	(-0.2)

Chemical shifts in ppm downfield from TMS. Solvent $CDCl_3$. Assignments bearing the same superscript on vertical column may be reversed.

on SiO_2 thin layer chromatograms. They were purified finally by using repeated HPLC (ODS, 5μ, YMC Pack A-324, 10×300 mm and TSKgel ODS-120A and 120T, 4.6×250 mm) with MeOH-H_2O-$CHCl_3$ (78 : 20 : 2). The purified teleocidin B-1, B-2, B-3 and B-4 were obtained together with a teleocidin A-1 and A-2 mixture in a total amount of 150 mg and their weight ratio was 2.5 : 24 : 22 : 38 and 31 of the mixture of A-1 and A-2.

Teleocidin B-1: mp 153-155°C(capillary tube), colorless prisms (from isopropyl ether), high-resolution MS *m/z*: 451.3212 (M^+, Calcd for $C_{28}H_{41}N_3O_2$, *m/z*: 451.3199). Teleocidin B-2: mp 203-204°C (capillary tube), colorless prisms (from acetone), high-resolution MS *m/z*: 451.3178 (M^+, Calcd for $C_{28}H_{41}N_3O_2$, *m/z*: 451.3199). Teleocidin B-3: mp 160-162°C (capillary tube), colorless prisms (from isopropyl ether), high-resolution MS *m/z*: 451.3206 (M^+, Calcd for $C_{28}H_{41}N_3O_2$, *m/z*: 451.3199). Teleocidin B-4: mp 230-232.5°C (capillary tube), colorless prisms (from isopropyl ether), MS *m/z*(%): 452(32, M^++1), 451(100, M^+), 450(45), 408(40), 365(71), 321(31).

The ultraviolet (UV) spectra and CD curves of all four teleocidin B group compounds were almost the same.[7] Therefore, these four compounds were assumed to have the same aromatic chromophore and the same absolute configuration of the nine-membered lactam moiety.

Teleocidin B-4 was proved to be identical with olivoretin D (I) (teleocidin B of Hirata) by the mixed mp, ^{1}H-NMR (270 MHz), IR, CD and retention time in HPLC. Olivoretin B (III) was demethylated with BBr_3 in CH_2Cl_2 at r.t. It gave rise to des-O-methylolivoretin B (V), mp 153-155°C, high-resolution MS *m/z*: 451.3196 (M^+, Calcd for $C_{28}H_{41}N_3O_2$, *m/z*: 451.3199), which was established to be identical with teleocidin B-1 by comparison of the mixed mp and ^{1}H-NMR, CD curves and retention time in HPLC.

The des-O-methylolivoretin C (VIII)[8] obtained from olivoretin C (IV) was recrystallized from benzene as colorless plates, mp 268-270°C, high-resolution MS *m/z*(%): 451.3219(M^+, 32, Calcd for $C_{28}H_{41}N_3O_2$, *m/z*: 451.3199), 408(100). The UV spectrum of VIII showed the same absorption curve as teleocidin B-2 and B-3, but the CD curve of VIII was similar but different. The appearance of the base peak (M^+- $CH(CH_3)_2$, *m/z*: 408) in the MS of VIII showed the presence of the isopropyl group at C22.[5] In the mass spectra of teleocidins B-2 and B-3 no such strong (M^+- $CH(CH_3)_2$) peak was observed. Therefore, teleocidins B-2 and B-3 were found to be the stereoisomers of teleocidins B-1 and B-4, not the olivoretin C type regioisomers.

The difference in the ^{13}C-NMR chemical shift value of each carbon between teleocidin B-1 (V) and B-2 (VI) appeared under 1.0 ppm except in C27 (1.1 ppm) as shown in Table 1. Similarly the Δ(B-3 - B-4) values were also revealed to be under 1.0 ppm. However, a difference in chemical shift between the B-1, B-2 group and the B-3, B-4 group sometimes occured over 1.0 ppm; particularly large differences (3.3 - 4.9 ppm) were observed for C27 and C28. These large differences were interpreted to be ascribable to a γ-gauche effect between C20 and axially substituted methyl (C27 at B-3, B-4) and vinyl (C28 at B-1, B-2) groups as shown in Fig. 1. It is quite reasonable to presume that the preferred conformations of tereocidin B-1 ~ B-4 have the equatorial orientation of big isopropyl group on cyclohexene ring, and this was strongly supported by the previous X-ray crystallographical works on the structures of olivoretin D (teleocidin B of Hirata)[2] and olivo-

retin B.[5] All the ^{13}C-NMR chemical shifts of the carbons not shown in Table 1 are completely identical through B-1 to B-4, thus proving the same structure of a nine-membered lactam ring part. These observations indicate that the two pairs B-1, B-2 and B-3, B-4 seem to have the same conformational and configurational structures respectively. The results were satisfactorily explained when the substituted cyclohexene ring part of teleocidin B-1 and B-2 (and also those of teleocidin B-3 and B-4) are regarded as having the partial mirror image relationship. Thus, the structure forms VI and VII have been given for teleocidin B-2 and B-3 respectively.

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7) UV spectra(in MeOH): λ_{max}^{MeOH}nm(log ε): B-1; 233(4.54), 286(3.99), 298sh(3.91). B-2; 233(4.51), 287(3.98), 298sh(3.93). B-3; 232(4.54), 286(4.00), 298sh(3.92). B-4; 232(4.54), 287(4.01), 298sh(3.94). CD curves (in MeOH): θ(nm): B-1; +1,000 (340), +5,500(311.5), 0(300), -23,800(257), -19,800(245.5), -22,200(240), -17,600(235), -38,600(225), 0(210). B-2; +100(345), +7,900(308.5), 0(295), -24,500(262), -23,600(250), -41,400(240), -34,900(235), -45,700(228), 0(214.5), B-3; 0(335), +7,800(309.5), 0(294), -23,400(255), -22,100(248.5), -29,400(240), -20,700(234.5), 0(213). B-4; 0(335), +3,700(312), 0(303), -25,700(255), -24,400 (248.5), -38,600(239.5), -30,000(234), -45,200(225), 0(208.5).
8) Des-O-methylolivoretin C (VIII) was also isolated as a minor metabolite (mp 269 -270°C) from Streptoverticillium olivoreticuli and found by comparison to be identical to the demethylated compound of IV using the mixed mp, ^{1}H-, ^{13}C-NMR, IR and retention time in HPLC. UV spectrum of VIII: λ_{max}^{MeOH} nm (log ε): 233(4.52), 289(3.96), 298sh(3.94). CD curve of VIII (in MeOH): θ(nm): +5,400(310), 0 (297.5), -32,900(257), +1,200(236), -26,700(225), 0(216.5).

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