Communications to the Editor

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ELUCIDATION OF THE STRUCTURE OF OLIVORETIN A AND D (TELEOCIDIN B)

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The known metabolites, indole-3-aldehyde, indole-3-carboxylic acid, cyclo-Leu-Val, cyclo-Pro-Leu and phenyl acetamide were isolated from Streptoverticillium olivoreticuli together with pimprinin derivatives. Four more complex natural products having strong vesicatory toxicity, olivoretin A, B, C and D, were also isolated and based on the chemical correlation with D, A was proved to be O-methyl olivoretin D.

By X-ray analysis the structure of olivoretin D, having strong epigenetic activities, was found to have exactly the same structure as teleocidin B.

KEYWORDS ——— Streptoverticillium olivoreticuli; olivoretin A; olivoretin B; olivoretin C; teleocidin B; isolation; ^{13}C-NMR; X-ray analysis

One (Y. K.) of the present authors earlier reported isolation, characterization and synthesis of several indolic bases, pimprinin derivatives, found as metabolites of Streptoverticillium olivoreticuli.[1] We further identified several other known metabolites, indole-3-aldehyde, indole-3-carboxylic acid and cyclo-Leu-Val, cyclo-Pro-Leu and phenyl acetamide isolated from the culture broth.

In addition to above metabolites, four more complex metabolites were isolated from the mycelia by repeated column chromatography and HPLC (Radial Pak.-C_{18}, Waters, mobile phase, 20% H_2O-MeOH). Three of the metabolites were named olivoretin A, B and C whose molecular formulae are the same and represented by $C_{29}H_{43}N_3O_2$ as shown by high resolution mass spectrometry.

Olivoretin A was colorless plates, mp 251-253°C (from MeOH), $[\alpha]_D$ -314.9° (in $CHCl_3$), High-resolution MS, *m/z*: 465.3331 (M^+, Calcd for $C_{29}H_{43}N_3O_2$, *m/z*: 465.3355). Olivoretin B was obtaind as colorless needles, mp 277.5-279°C (from MeOH), $[\alpha]_D$ -298.4° (in $CHCl_3$), M^+; *m/z*: 465.3341 (Found) and olivoretin C was recrystallized from EtOH as prisms, mp 305-307°C, $[\alpha]_D$ -256.6° (in $CHCl_3$), M^+; *m/z*: 465.3400 (Found). The more polar compound, olivoretin D, was recrystallized from diisopropyl

ether as colorless prisms, mp 228-229°C, $[\alpha]_D$-141.5°(in MeOH). High-resolution mass spectrometry and elemental analysis established the molecular formula of olivoretin D as $C_{28}H_{41}N_3O_2$ (M^+, Calcd: *m/z* 451.3199. Found: *m/z* 451.3165) having one methylene less than olivoretins A, B and C. The ^{13}C nuclear magnetic resonance (NMR) and ^1H-NMR spectra (Table I and II)[2] suggested that olivoretin D had the same structure as teleocidin B.[3] We could not identify both compounds directly, because the latter compound was not in a crystalline state and the authentic material is not available any more.

Recently, dihydroteleocidin B and its congeners were shown to have strong epigenetic activities. Promotion (not initiation) of malignant cell growth by these chemicals is recognized.[4] Olivoretin A, B, C and D also cause strong inflammation and vesication to human skin. All of these prompted us to further study the structures of olivoretins.

The ^{13}C-NMR spectrum of olivoretin A (I) shows the presence of a methoxy group (δ: 58.5,q), but other signals were not distinguishable from those of olivoretin D except for carbon atoms around the methoxy group (C_{11} and C_{20}) as shown in Table I. The UV spectrum[5] and CD curves of both compounds were very similar. From these facts we concluded that olivoretin A was O-methylolivoretin D (I). To prove this, olivoretin A was demethylated with BBr_3 in CH_2Cl_2, at r.t. It gave rise to olivoretin D in 62% yield after purification by HPLC.

Table I. ^{13}C-NMR Chemical Shifts[a] and Assignments for A (I) and D(II)

No	A (I)	D (II)
2	120.8(d)	120.8(d)
3	113.9(s)	114.1(s)
4	116.8(s)	116.9(s)
5	146.1(s)	146.0(s)
6	106.2(d)	106.3(d)
7	137.8(s)	137.9(s)
8	118.0(s)	118.1(s)
9	138.6(s)	138.6(s)
10	34.1(t)	33.8(t)
11	52.6(d)	56.0(d)
13	173.1(s)	174.7(s)
14	70.9(d)	70.8(d)
16	40.1(s)	40.1(s)
17	25.0(t)	25.0(t)
18	34.8(t)	34.8(t)
19	39.6(s)	39.6(s)
20	74.5(t)	65.0(t)
21	28.4(d)	28.5(d)
22	21.6(q)*	21.6(q)*
23	19.6(q)*	19.6(q)*
24	32.8(q)	33.0(q)
25	29.1(q)	29.1(q)
26	37.9(d)	37.9(d)
27	17.0(q)†	17.0(q)†
28	18.1(q)†	18.0(q)†
29	21.6(q)	21.7(q)
30	151.9(d)	151.9(d)
31	111.3(t)	111.3(t)
34	58.4(q)	

Table II. ^1H-NMR(270MHz) Chemical Shifts and Assignments for Olivoretin A (I)

No	Chemical Shifts[a]
1	8.80(1H,br s)
2	6.76(1H,m)
6	6.51(1H,s)
10a	3.14(1H,br d,J_{gem}=17.4Hz)
10b	2.87(1H,dd,J_{gem}=17.4, $J_{10b,11}$=3.6Hz)
11	4.41(1H,m)
12	6.12(1H,br s)
14	4.28(1H,d,$J_{14,21}$=10.2Hz)
20	3.38-3.28(2H,m)
21	2.63(1H,d septet,$J_{21,14}$=10.2, $J_{21,22\text{ and }23}$=6.6Hz)
22	0.91*(3H,d,$J_{22,21}$=6.6Hz)
23	0.68*(3H,d,$J_{23,21}$=6.6Hz)
24	2.91(3H,s)
25	1.35(3H,s)
26	2.25(1H,septet,$J_{26,27\text{ and }28}$=6.7Hz)
27	0.54†(3H,d,$J_{27,26}$=6.7Hz)
28	1.01†(3H,d,$J_{28,26}$=6.7Hz)
29	1.51(3H,s)
30	6.17(1H,dd,J_{trans}=17.8, J_{cis}=10.6Hz)
31a	5.41(1H,dd,J_{trans}=17.8, J_{gem}= 1.2Hz)
31b	5.24(1H,dd,J_{cis}=10.6, J_{gem}=1.2Hz)
34	3.30(3H,s)

a) Chemical shifts in ppm downfield from TMS. Solvent $CDCl_3$.
b) Assignments bearing the same superscript on vertical column may be interchanged.
c) Abbreviations: s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet, gem= geminal.

The structure of olivoretin D (II) was determined by X-ray analysis. Crystals of (II) belong to orthorhombic space group $P2_12_12$, with cell constants of a= 13.357 (2)Å, b= 18.424(3)Å, c= 10.805(2)Å. A total of 2549 unique and significant reflections (Fo > 3σ(Fo)) within the range 3° ≤ 2θ ≤ 55° were measured on a 4-circle diffractometer using Mo Kα radiation (λ= 0.71Å). The structure was solved by the direct method MULTAN and refined anisotropically (isotropically for H) by the least squares method to R= 0.074. The ORTEP drawing of the structure of olivoretin D (II) is shown in Fig. 1. The arbitrarily chosen enantiomeric form is believed to show the correct absolute configuration of the natural product as is discussed below, and in a forthcoming paper.[6] The elucidated structure of olivoletin D (II) is exactly the same as teleocidin B as expected from dihydroteleocidin B monobromoacetate which was resolved by X-ray analysis.[7]

However, absolute configuration of teleocidin B (= olivoletin D) has not yet been determined. The CD spectra[8] of olivoretin A (I) and D (II) were found to be very similar to dihydroteleocidin B and also to tetrahydrolyngbyatoxin A.[9] Therefore, all the above metabolites of Streptomyces and Streptoverticillium have the same absolute configuration. When it is assumed that the composing natural amino acid has the S-configuration on the chiral carbon atom (C_{14}), teleocidin B (= olivoretin D) and olivoretin A are represented by formulae (II) and (I) as shown in Fig. 2.[10]

The structures of other metabolites of Streptoverticillium are now under investigation.

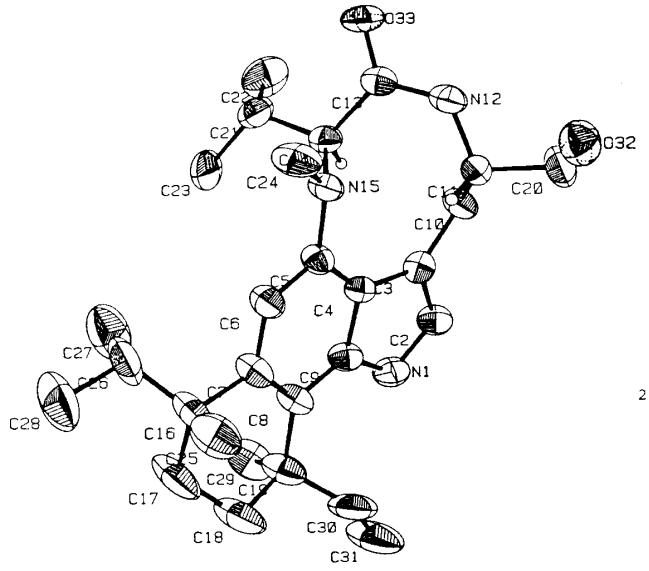

Fig. 1. The ORTEP drawing of Olivertin D (II)

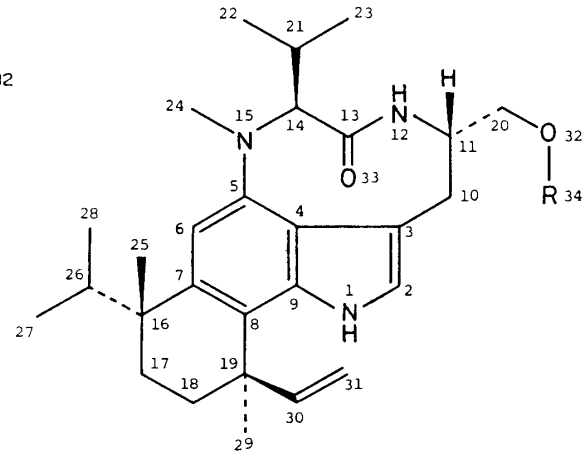

Fig. 2. R= Me: Olivoretin A (I)
R= H : Teleocidin B (Olivoretin D) (II)

REFERENCES AND NOTES

1) Yasumasa Koyama, Kazuteru Yokose and Lloyd J. Dolby, Agric. Biol. Chem., 45, 1285 (1981).

2) ^{1}H- and ^{13}C-NMR spectra show that olivoretins A, B, C and D are in equilibrium between two stable conformers respectively, presumably originating from the amide rings. In A and D, the ratios are 12 : 1 and 7 : 1 respectively, based on the signal area of the N-methyl group in the ^{1}H-NMR spectrum. The ^{13}C- and ^{1}H-NMR data in Table I, II and ref. 3) are for the major signals.

3) ^{1}H-NMR spectrum of olivoretin D: H_1, 8.67(1H, br s); H_2, 6.78(1H, br s); H_6, 6.50(1H, s); H_{10a}, 3.10(1H, d, J=16.9Hz); H_{10b}, 3.02(1H, dd, J_1=16.9, J_2=3.4Hz); H_{11}, 4.34(1H, m); H_{12}, 7.70(1H, br s); H_{14}, 4.32(1H, d, J=10.2Hz); H_{20a}, 3.71 (1H, dd, J_1=12.1Hz, J_2=3.4Hz); H_{20b}, 3.55(1H, dd, J_1=12.1Hz, J_2=7.9Hz); H_{21}, 2.60(1H, d of sep., J_1=10.2Hz, J_2=6.4Hz); H_{22}, 0.91(3H, d, J=6.4Hz); H_{23}, 0.69 (3H, d, J=6.4Hz); H_{24}, 2.90(3H, s); H_{25}, 1.35(3H, s); H_{26}, 2.25(1H, sep., J=6.8 Hz); H_{27}, 0.53(3H, d, J=6.8Hz); H_{28}, 1.01(3H, d, J=6.8Hz); H_{29}, 1.51(3H, s); H_{30}, 6.16(1H, dd, J_1=17.5Hz, J_2=10.6Hz); H_{31a}, 5.40(1H, d, J=17.5Hz); H_{31b}, 5.24 (1H, d, J=10.6Hz). (δ_{ppm} in $CDCl_3$, 270MHz).
a) Hisao Nakada, Hirokichi Harada and Yoshimasa Hirata, Tetrahedron Lett., 23 2515(1966); b) *Idem,* Nippon Kagaku Zasshi, 87, 86 (1966).

4) Hirota Fujiki, Masami Mori, Michie Nakayasu, Masaaki Terada, Takashi Sugimura and Richard E. Moore, Proc. Natl. Acad. Sci. USA, 78, 3872(1981).

5) UV spectrum of olivoretin A : λ_{max}^{MeOH} nm(log ε): 232(4.55), 286(4.01), 297sh(3.95) ; olivoretin D : λ_{Max}^{EtOH} nm(log ε): 234(4.56), 287(4.03), 298sh(3.98).

6) A study of the absolute configuration of olivoretins will be reported in a forthcoming paper.

7) a) N. Sakabe, H. Harada, Y. Hirata, Y. Tomiie and I. Nitta, Tetrahedron Lett., 23, 2523(1966); b) Hirokichi Harada, Noriyoshi Sakabe , Yoshimasa Hirata , Yujiro Tomiie and Isamu Nitta, Bull. Chem. Soc. Jpn., 39, 1773(1966).

8) CD of olivoretin A: (MeOH) [θ] (nm): +3,600(312), 0(302.5), -28,800(255), -26,900(248), -36,900(240), -25,400(234.5), -46.700(224), 0(208.5). olivoretin D: (MeOH), +4,400(311), 0(302), -26,900(256), -25,200(248), -41,800(240), -27,900(234), -49,500(224), 0(210).

9) John H. Cardellina II, Franz-Josef Marner and Richard E. Moore, Science, 204, 193(1979).

10) It may be essential to point out here a mispresentation of the structural formulae of teleocidin derivatives in references 3b, 4, 7a and 9. Evidently the correct structure of dihydroteleocidin B mono bromoacetate was obtained from the X-ray analysis as shown in Fig. 1 in ref. 7a and in Fig. 1 and Tables in ref. 7b. However, the structural formula is erroneously printed in Fig. 2 in ref. 7a, regarding the relative stereochemistry of the four chiral centers. In the structure, the isopropyl group and the bromoacetyl group-carrying hydroxymethyl group in the amide part are shown as β- and α-oriented, respectively. In that case, the stereochemistry of both of the two other chiral centers in the alicyclic part should be inversed.
The correct relative stereochemistry is shown for olivoretin D (teleocidin B) in Fig. 2 in our present paper. This comment is made to prevent future misquotation of the structure and to avoid the useless confusion about the structures of teleocidin B and related compounds. For the same reason we use the name of teleocidin B instead of olivoretin D hereafter.

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