Communications to the Editor

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ELUCIDATION OF THE STRUCTURES OF OLIVORETIN B AND C

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The structures of olivoretin B and C were established by X-ray analysis as III and IV respectively. In the crystal olivoretin C was a random mixture of conformers A and B existing in the ratio of 1:1. The two conformers in the crystal are concerned with the cyclohexene ring conformation.

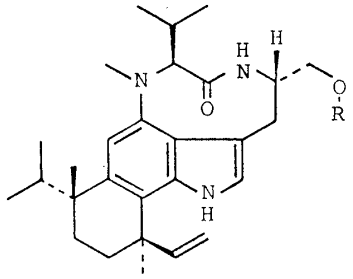
KEYWORDS —— Streptoverticillium olivoreticuli; olivoretin A; olivoretin B; olivoretin C; teleocidin B; isolation; ^{13}C-NMR; structure determination; X-ray analysis; tumor promoter

In the previous paper,[1] we have reported the isolation of olivoretin A, B, C and teleocidin B from the mycelia of Streptoverticillium olivoreticuli and described the structures of olivoretin A and teleocidin B. Teleocidin B (I) has a strong cancer-promoting activity[2] and olivoretin A is O-methylteleocidin B. In this paper, the structures of olivoretin B and olivoretin C are described.

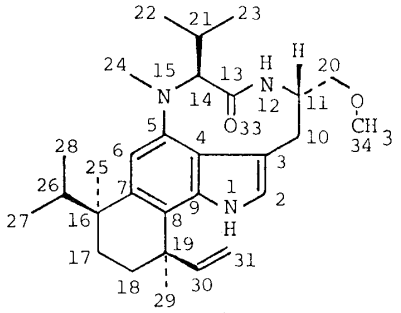
Olivoretin B was obtained as colorless needles; [$C_{29}H_{43}N_3O_2$, mp 277.5-279°C (from MeOH), $[\alpha]_D^{32}$-298.4°(c=0.35, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3400, 3380(NH), 1680(CONH), 1600, 1500($\nu_{C=C}$, arom.), 1375(δ_{CH}); MS m/z(%): 465(M^+,100), 422(41.4), 379(41.7)]. Its UV spectrum,[3] MS fragment pattern and ^{1}H-NMR were closely similar to those of olivoretin A, and so it was assumed that it was one of the diastereoisomers of olivoretin A. ^{1}H-NMR spectrum shows that olivoretin B is a mixture of the amide conformers[4] in the ratio of *ca.* 7:1 (in $CDCl_3$) which was estimated from the N_{15}-Me signal intensities.[5] The shift values shown in Table I are those of the major conformer signals. The comparison of ^{1}H-NMR spectra of olivoretin A[1] and B (Table I) shows significant differences only in chemical shifts of indolic NH and terminal methylene signals. In the ^{13}C-NMR spectrum of olivoretin B (III), C29 signal appears 4.8 ppm lower; on the contrary, C30 signal appears 4.2 ppm higher than the corresponding carbon signals of olivoretin A (II) (Table II).

From the above-mentioned observations, it was thought that olivoretin B was the C19 epimer of olivoretin A. At the same time there was another possibility -- and this was actually the case -- that they are C16 epimers and the preferential conformation of cyclohexene ring in olivoretin B is different from that of olivo-

retin A.



R = H : teleocidin B (I)
R = Me: olivoretin A (II)



olivoretin B (III)

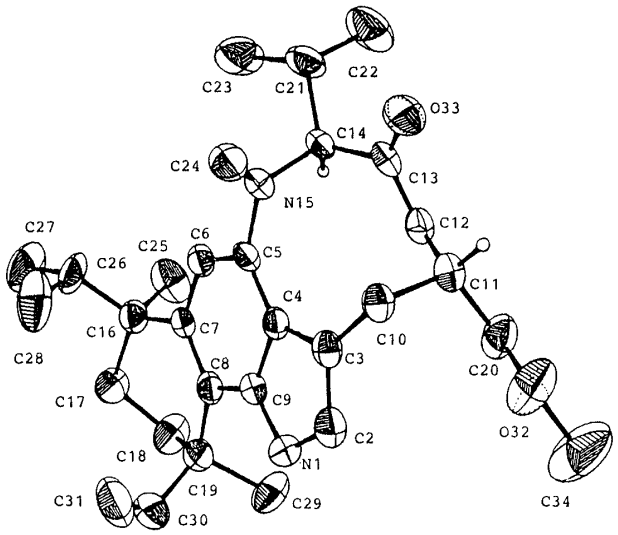


Fig. 1. ORTEP Drawing of Olivoretin B (III)

Table I. ^{1}H-NMR(270MHz) Chemical Shifts and Assignments for Olivoretin B(III)

No.	Chemical shifts[a]
1	8.14(1H,br s)
2	6.74(1H,m)
6	6.45(1H,s)
10a	3.13(1H,br d,J_{gem}=17.5Hz)
10b	2.89(1H,dd,J_{gem}=17.5,$J_{10b,11}$=3.6Hz)
11	4.43(1H,m)
12	6.13(1H,br s)
14	4.26(1H,d,$J_{14,21}$=10.2Hz)
20a	3.42(1H,dd,J_{gem}=9.6,$J_{20a,11}$=4.3Hz)
20b	3.32(1H,t,$J_{20b,20a\text{ and }11}$=9.6Hz)
21	2.59(1H,d septet,$J_{21,14}$=10.2, $J_{21,22\text{ and }23}$=6.6Hz)
22	0.60*(3H,d,$J_{22,21}$=6.6Hz)
23	0.91*(3H,d,$J_{23,21}$=6.3Hz)
24	2.90(3H,s)
25	1.30(3H,s)
26	2.12(1H,septet,$J_{26,27\text{ and }28}$=6.6Hz)
27	0.96†(3H,d,$J_{27,26}$=6.6Hz)
28	0.60†(3H,d,$J_{28,26}$=6.9Hz)
29	1.48(3H,s)
30	6.06(1H,dd,J_{trans}=17.5,J_{cis}=10.4Hz)
31a	4.96(1H,dd,J_{trans}=17.5,J_{gem}=1.7Hz)
31b	5.14(1H,dd,J_{cis}=10.4,J_{gem}=1.7Hz)
34	3.33(3H,s)

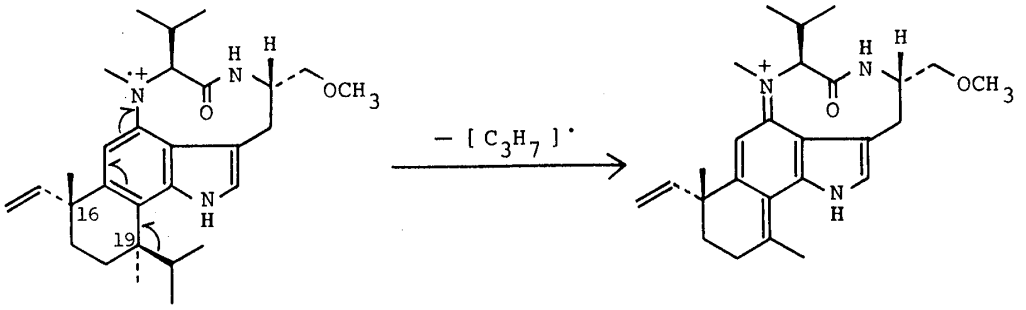
Table II. ^{13}C-NMR Chemical Shifts[a] and Assignments for Olivoretin A(II), B (III) and C(IV)

No.	A (II)	B (III)	C (IV)
2	120.8(d)	120.3(d)	120.4(d)
3	113.9(s)	113.7(s)	113.4(s)
4	116.8(s)	117.1(s)	117.6(s)
5	146.1(s)	145.4(s)	144.9(s)
6	106.2(d)	106.3(d)	109.4(d)
7	137.8(s)	137.9(s)	137.2(s)¶
8	118.0(s)	116.5(s)	119.6(s)
9	138.6(s)	139.8(s)	137.8(s)¶
10	34.1(t)	34.1(t)	34.1(t)
11	52.6(d)	52.5(d)	52.7(d)
13	173.1(s)	173.2(s)	173.3(s)
14	70.9(d)	70.8(d)	70.9(d)
16	40.1(s)	40.2(s)	39.8(s)§
17	25.0(t)	26.2(t)	33.5(t)
18	34.8(t)	35.2(t)	27.5(t)
19	39.6(s)	39.5(s)	41.5(s)§
20	74.5(t)	74.7(t)	74.6(t)
21	28.4(d)	28.4(d)	28.4(d)
22	19.6(q)*	19.2(q)*	19.4(q)*
23	21.6(q)*	21.5(q)*	21.7(q)*
24	32.8(q)	32.8(q)	33.0(q)
25	29.1(q)	27.8(q)	25.9(q)†
26	37.9(d)	37.2(d)	150.4(d)
27	17.0(q)†	17.4(q)†	110.5(t)
28	18.1(q)†	18.4(q)†	26.4(q)†
29	21.6(q)	26.4(q)	36.3(d)
30	151.9(d)	147.7(d)	18.8(q)Δ
31	111.3(t)	113.1(t)	17.5(q)Δ
34	58.4(q)	58.5(q)	58.5(q)

a) Chemical shifts in ppm downfield from TMS. Solvent $CDCl_3$.
b) Assignments bearing the same superscript on vertical column may be reversed.
c) The signals of four protons on C17 and C18 of III were observed between 1.88 and 1.22 ppm as multiplet but further assignments were unsuccessful.

In order to confirm the structure of olivoretin B, X-ray structure analysis was carried out. The crystal of olivoretin B belongs to orthorhombic space group $P2_12_12_1$, with cell constants a= 23.962(4), b= 16.755(4), c= 6.958(1)Å and z= 4. A total of 2631 unique and significant reflections (Fo > 3σ(Fo)) within the range $3° \leqq 2\theta \leqq 150°$ were measured on a 4-circle diffractometer using Cuκα radiation (λ = 1.54 Å). The structure was solved by direct method MULTAN[6] and refined by the full-matrix least-squares method[7] to R = 0.086. The ORTEP drawing[8] of the structure of olivoretin B is shown in Fig. 1. Thus the structure of olivoretin B (III) was determined as 16-epi-olivoretin A.

Olivoretin C, obtained as colorless prisms, showed the following data: [$C_{29}H_{43}N_3O_2$, mp 305-307°C(from EtOH), $[\alpha]_D^{32}$ -256.6°(c=0.07, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1675(CONH), 1500, 1480($\nu_{C=C}$, arom.), 1380(δ_{CH}); MS m/z(%): 465(M^+, 26.9), 422(100)].[9] The MS fragment pattern of olivoretin C is different from those of other olivoretins in that base peak of olivoretin C is m/z 422(M^+- isopropyl) whilst molecular ion peaks in spectra of other olivoretins are all base peaks.



olivoretin C (IV) fragment a: m/z 422

Chart 1

Table III. ^{1}H-NMR(270MHz) Chemical Shifts and Assignments for Olivoretin C (IV)

No.	Chemical shifts[a]	No.	Chemical shifts
1	8.20(1H,br s)	22	0.64*(3H,d,$J_{22,21}$=6.9Hz)
2	6.82(1H,br s)	23	0.90*(3H,d,$J_{23,21}$=6.3Hz)
6	6.40(1H,s)	24	2.85(3H,s)
10a	3.16(1H,br d,J_{gem}=17.1Hz)	25	1.46§(3H,s)
10b	2.90(1H,dd,J_{gem}=17.1,$J_{10b,11}$=4.3Hz)	26	5.86(1H,dd,J_{trans}=17.5,J_{cis}=10.6Hz)
11	4.43(1H,m)	27a	4.97(1H,dd,J_{trans}=17.5,J_{gem}=1.3Hz)
12	6.11(1H,br s)	27b	5.02(1H,dd,J_{cis}=10.6,J_{gem}=1.3Hz)
14	4.24(1H,d,$J_{14,21}$=10.2Hz)	28	1.40§(3H,s)
20a	3.40(1H,dd,J_{gem}=9.6,$J_{20a,11}$=4.3Hz)	29	2.42(1H,septet,$J_{29,30\text{ and }31}$=6.7Hz)
20b	3.32(1H,t,$J_{20b,20a\text{ and }11}$=9.6Hz)	30	0.59†(3H,d,$J_{30,29}$=6.9Hz)
21	2.59(1H,d septet,$J_{21,14}$=10.2, $J_{21,22\text{ and }23}$=6.6Hz)	31	1.06†(3H,d,$J_{31,29}$=6.6Hz)
		34	3.32(3H,s)

a) Chemical shifts in ppm downfield from TMS. Solvent $CDCl_3$.
b) Assignments bearing the same superscript on vertical column may be reversed.
c) The signals of four protons on C17 and C18 of IV were observed between 2.10 and 1.40 ppm as multiplet but further assignments were unsuccessful.

This can be explained if the isopropyl group of cyclohexene ring of IV is attached not to C16 as in other olivoretins but to C19, for the fragment a (resulting from removal of isopropyl group) will be stabilized as shown in Chart 1.

The ^{1}H-NMR spectrum of olivoretin C (IV) shows that in the solution ($CDCl_3$) also IV exists as a mixture of two amide conformers in the ratio of *ca.* 2:1, which is the largest ratio of the minor amide conformer among the olivoretin congeners. The assignment of each signal was made in detail in the spin decoupling experiment. In the ^{13}C-NMR spectrum of IV, all signals are accompanied by the corresponding minor signals which arise from the other amide conformer. The shift values of ^{1}H- and ^{13}C-NMR spectra shown in Tables II and III are those of signals of the main amide conformer.

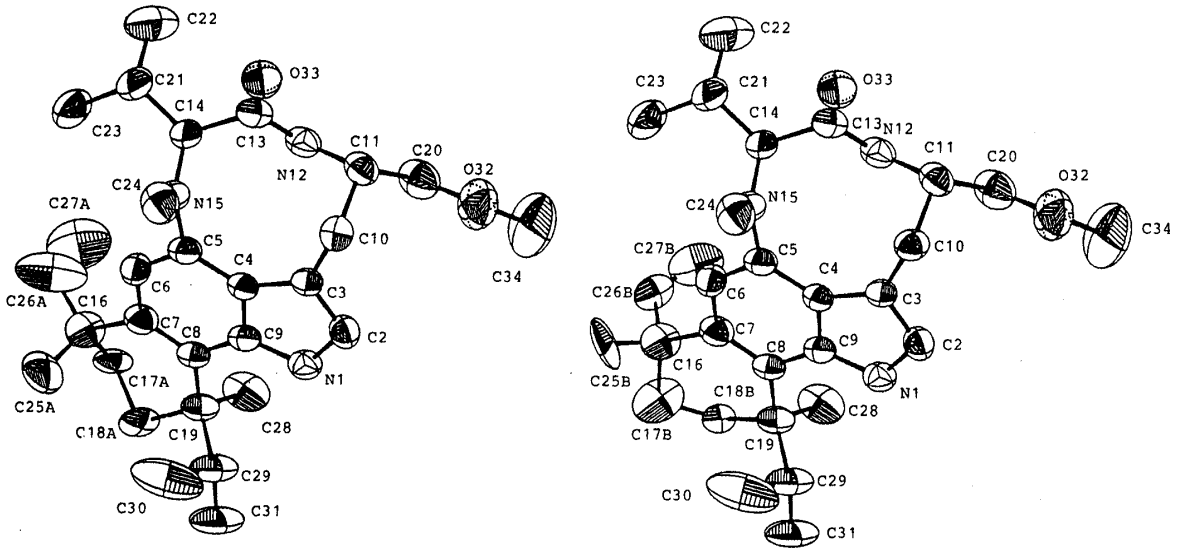
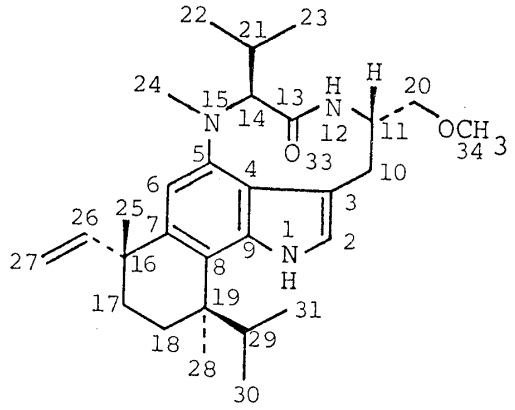


Fig. 2. ORTEP Drawings of Two Conformers of Olivoretin C (IV)

The structure of IV was finally elucidated by X-ray analysis. The crystal of IV also belongs to orthorhombic space group $P2_12_12_1$, with cell constants a= 23.359(6), b= 17.106(3), c= 6.844(2)Å and z=4. A total of 2299 reflections (Fo > 3σ(Fo)) within the range $3° \leqq 2\theta \leqq 150°$ were measured on the diffractometer using Cu$K\alpha$ radiation. The solution was obtained straightforwardly also using the MULTAN program, but the thermal ellipsoids of C17, C18, C25, C26 and C27 were abnormally elongated, and therefore accurate molecular structure concerning these five atoms could not be deduced whilst that of the remaining part was definitely determined. This phenomenon can be explained on the assumption that the two different conformational isomers regarding the cyclohexene ring conformation were mixed in the crystal at random. Each of the five carbon atoms was divided into two atoms along the largest principal axis of the thermal ellipsoid, based on the geo-



Olivoretin C (IV)

metrical and stereochemical considerations, in order to construct the two conformer models A and B. The validity of the two conformer models was confirmed by comparing the coordinates of the divided atoms with the electron-density maps as well as difference electron-density maps generated by eliminating the atoms concerned. Successive full-matrix least-squares refinement including the two conformers was carried out varying the atomic and population parameters for the five atoms of one of the conformers, while those of the other one were fixed, and *vice versa*. The population ratio of the two conformers A and B converged to 1:1 and the final *R*-value was 0.100. The ORTEP drawings of the two conformers of olivoretin C are shown in Fig. 2.

The CD spectra (in MeOH) of olivoretin B[10] and C[11] show a similar pattern to those of olivoretin A and teleocidin B.[1] The absolute configurations of the latter two have been already decided as 11S and 14S.[12] Therefore it was assumed that III and IV have the same absolute configurations, namely 11S and 14S.

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3) UV spectrum of III, λ_{max}^{MeOH} nm(logε): 232(4.65), 286.5(4.11), 298sh(4.03), 310sh (3.84).
4) Detailed analyses will be published.
5) The numbering system adopted here is tentative.
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8) C.K. Johnson, ORTEP-ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A., 1965.
9) UV spectrum of IV, λ_{max}^{MeOH} nm(logε): 233(4.56), 289(4.00), 298sh(3.98).
10) CD spectrum of III, CD(c=0.0003, MeOH) [θ] (nm): +4200(311.5), 0(301.5), -29600(257), -22700(244), -23300(242), -12700(235), -39000(223), 0(209).
11) CD spectrum of IV, CD(c=0.0003, MeOH) [θ] (nm): +5400(308), 0(296), -41900 (257), -2100(236), -31100(224), 0(214.5).
12) Yasuyuki Endo, Koichi Shudo, Kimio Furuhata, Haruo Ogura, Shin-ichiro Sakai, Norio Aimi, Yukio Hitotsuyanagi, and Yasumasa Koyama, Chem. Pharm. Bull., 32, 358(1984).

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