Structure of Fusariocin C, A Cytotoxic Metabolite from *Fusarium Moniliforme*

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The structure of fusariocin C, $C_{27}H_{28}O_6$ has been established by X-ray diffraction, PMR and IR studies. It is a pseudo dimer structure containing the tropolone skeleton.

In the previous paper,¹⁾ one of the authors (T.I.) reported the isolation of a new cytotoxic metabolite, fusariocin C, $C_{27}H_{28}O_6$, $[\alpha]_D^{20} = -20.6^\circ$ ($c=0.25$, $CHCl_3$), from the culture filtrate of *Fusarium Moniliforme* strain A-130. Because of the difficulties in elucidating molecular structure by chemical and spectroscopic method, X-ray crystal analysis was applied.

METHODS

Prismatic crystals, mp $242\sim 244^\circ C$, were grown from a pyridine solution. Preliminary photographic measurement revealed that there are two molecules in the asymmetric unit. In order to obtain the crystals more suitable for X-ray analysis, many different solvents for recrystallization were tried in vain. Only thiophene solution gave very small crystals, but they were isomorphous with those from the pyridine solution and not enough for accurate intensity measurement.

As the crystal obtained from the pyridine solution decomposed easily in air, it was sealed in a glass-capillary. The crystal is monoclinic with $a=14.443(1)$, $b=30.998(5)$, $c=8.0443(7)$ Å, $\beta=91.804(9)^\circ$ and $V=3599.3(7)$ Å³. The density measured by flotation is 1.29 g/cm³, whereas the calculated density is 1.27 g/cm³ if the crystal is assumed to have four fusariocin C and twelve pyridine molecules in the unit cell. Systematic absence of reflections is $0k0$ for $k=2n+1$, the space group being $P2_1$. The molecular arrangement, however, seemed to have an approximate $P2_1/a$ symmetry because $h0l$ reflections are relatively weak for $h=2n+1$. Intensity data up to $2\theta \leq 115$ were collected

on a Rigaku automated four-circle diffractometer with Ni-filtered Cu $K\alpha$ radiation, using a crystal of $0.2 \times 0.3 \times 0.5$ mm³. A total of 3750 independent reflections, ($|F_o| \geq 3\sigma(|F_o|)$), were obtained.

At the first approximation with the assumed $P2_1/a$ symmetry, the structure was solved by the direct method using the program MULTAN.²⁾ The atomic positions of fusariocin C and one pyridine were obtained from the E -map and the successive electron density map. After several cycles of the block-diagonal least-squares refinement, the R -value became 0.25. The peaks for the atoms of seven-membered rings and their substituents are relatively definite on the electron density map and the temperature factors of these atoms are not so large. This suggests that in the two crystallographically independent molecules the seven-membered rings are related approximately by a center of symmetry. A trial structure based on $P2_1$ was constructed, in which the atomic parameters of the seven-membered rings, their substituents and two pyridine molecules were taken from the refinement based on $P2_1/a$, those of the other atoms being obtained by resolving the average structure. Further refinement was carried out by the block-diagonal least-squares. Other three pyridine molecules were found on the difference map. The position of remaining one could not be assigned because only a broad peak appeared on the difference map. The anisotropic temperature factors were taken account only for the atoms of fusariocin C. The final R -value was 0.17. The atomic parameters are listed in Table I with their standard deviations.*

* The observed and calculated structure factor table and the temperature factor table may be obtained from the author (Y.O.) on request.

TABLE I. FINAL ATOMIC COORDINATES WITH THEIR STANDARD DEVIATIONS, MULTIPLIED BY 10^4 FOR THE ATOMS OF FUSARIOCIN C AND BY 10^3 FOR THOSE OF PYRIDINE

Atom	x	y	z	Atom	x	y	z
O(1A)	2210(11)	3724(6)	2008(22)	C(17B)	4919(73)	1132(17)	9686(57)
C(2A)	1373(18)	3587(6)	1101(33)	O(1'B)	3142(11)	3519(5)	7349(22)
C(3A)	734(21)	3904(12)	705(53)	C(2'B)	3955(14)	3412(7)	8167(28)
C(4A)	998(13)	4383(7)	727(27)	C(3'B)	4747(25)	3680(10)	8045(47)
C(5A)	1939(18)	4465(9)	1471(26)	C(4'B)	4463(15)	4214(7)	8260(26)
C(6A)	2177(13)	4949(6)	1615(23)	C(5'B)	3589(17)	4276(7)	7661(27)
C(7A)	2964(14)	5093(6)	2131(28)	C(6'B)	3288(15)	4729(8)	7560(27)
C(8A)	3724(14)	4921(7)	3087(26)	C(7'B)	2456(15)	4919(6)	6916(29)
C(9A)	3901(12)	4531(6)	3285(21)	C(8'B)	1628(13)	4850(7)	6140(25)
C(10A)	3382(13)	4189(6)	2813(23)	C(9'B)	1496(12)	4330(6)	5953(22)
C(11A)	2532(12)	4181(6)	2188(22)	C(10'B)	2009(13)	4065(6)	6526(28)
C(12A)	1172(19)	3227(9)	1676(34)	C(11'B)	2786(14)	3997(6)	7127(22)
C(13A)	1308(20)	2853(11)	973(36)	C(12'B)	3962(20)	3059(12)	8301(54)
C(14A)	1458(13)	5263(9)	921(29)	C(13'B)	3915(18)	2583(11)	8304(33)
O(15A)	4336(10)	5230(5)	3659(21)	C(14'B)	4227(18)	5055(8)	8143(29)
O(16A)	4722(9)	4427(5)	4087(19)	O(15'B)	1094(10)	5099(4)	5475(18)
C(17A)	-232(22)	3813(10)	-35(38)	O(16'B)	635(9)	4287(4)	5171(19)
O(1'A)	1981(10)	1423(5)	2610(20)	-----			
C(2'A)	1082(20)	1537(8)	1655(35)	N(1C)	345(2)	267(1)	337(4)
C(3'A)	496(19)	1234(9)	1196(53)	C(2C)	353(2)	232(1)	355(3)
C(4'A)	628(12)	864(7)	1249(26)	C(3C)	431(2)	216(1)	327(4)
C(5'A)	1581(12)	681(8)	2181(26)	C(4C)	497(2)	234(1)	279(4)
C(6'A)	1707(12)	235(6)	2415(24)	C(5C)	527(2)	262(1)	262(4)
C(7'A)	2475(13)	6(7)	3133(25)	C(6C)	435(3)	306(1)	280(5)
C(8'A)	3361(13)	242(7)	3860(27)	N(1D)	54(2)	205(1)	678(4)
C(9'A)	3638(15)	647(8)	4031(28)	C(2D)	135(2)	216(1)	638(3)
C(10'A)	3094(13)	1054(7)	3634(26)	C(3D)	144(2)	255(1)	640(3)
C(11'A)	2052(14)	1036(8)	2717(28)	C(4D)	73(2)	291(1)	657(4)
C(12'A)	1043(22)	2114(10)	1687(55)	C(5D)	-15(2)	267(1)	639(4)
C(13'A)	1126(17)	2389(8)	1456(32)	C(6D)	5(2)	223(1)	669(4)
C(14'A)	979(12)	-73(6)	1685(29)	N(1E) ^a	655(4)	135(2)	523(7)
O(15'A)	3978(10)	-34(6)	4102(21)	C(2E) ^a	706(4)	116(2)	479(8)
O(16'A)	4419(9)	727(5)	4734(18)	C(3E) ^a	787(5)	119(3)	534(9)
O(1B)	2814(11)	1239(5)	7844(19)	C(4E) ^a	837(4)	141(2)	488(8)
C(2B)	3803(19)	1326(8)	8426(32)	C(5E) ^a	814(5)	155(3)	355(9)
C(3B)	4175(18)	1065(9)	9250(37)	C(6E) ^a	718(6)	153(3)	377(10)
C(4B)	4095(15)	663(8)	9236(31)	N(1F) ^b	846(4)	362(2)	417(6)
C(5B)	3124(13)	513(6)	8311(23)	C(2F) ^b	822(3)	388(1)	499(5)
C(6B)	2949(15)	166(9)	8250(26)	C(3F) ^b	688(4)	382(2)	451(7)
C(7B)	2124(14)	-147(8)	7612(27)	C(4F) ^b	732(4)	390(2)	530(7)
C(8B)	1248(17)	5(7)	6962(32)	C(5F) ^b	717(5)	351(2)	356(8)
C(9B)	1121(16)	502(8)	6453(26)	C(6F) ^b	818(5)	329(2)	350(9)
C(10B)	1696(15)	863(8)	6774(29)	N(1G) ^c	717(7)	243(4)	-41(13)
C(11B)	2658(15)	876(7)	7746(26)	C(2G) ^c	746(10)	216(4)	-27(16)
C(12B)	3677(17)	1880(10)	8770(39)	C(3G) ^c	791(7)	232(4)	30(13)
C(13B)	3971(19)	2188(8)	8095(33)	C(4G) ^c	807(6)	298(3)	148(11)
C(14B)	3724(17)	-219(7)	9043(33)	C(5G) ^c	780(6)	321(3)	56(12)
O(15B)	665(11)	-227(5)	6554(22)	C(6G) ^c	718(6)	305(3)	-148(11)
O(16B)	279(9)	562(4)	5752(16)				

The occupancy factors for a) are 0.5, for b) 0.5 and for c) 0.3.

RESULTS

The structures of the two crystallographically independent molecules are essentially identical to each other. Figure 1 shows one of them projected along the *c* axis. The structural formula is given in Fig. 2, in which the averaged bond distances between the two molecules are also shown. Because of the pseudo symmetry described above and the low resolution of the solvent molecules, the estimated standard deviations of the bond distances are rather large, 0.03~0.05 Å. Moreover, the maximum difference between the corresponding bond distances of the two crystallographically independent molecules reaches 0.35 Å. This brought about considerable doubt as to whether an atom is carbon or oxygen and whether a bond is single or double.

In the light of this structure, the PMR and IR spectroscopic data and additional spin decoupling study are unequivocally rationalized. The PMR spectrum is illustrated in Fig. 3. The signal at δ 2.35 (6H, S) was assigned to the methyl protons of C (14) and C (14'). The signal at δ 7.00 (2H, S) and 7.12 (2H, S) were done to the H (7) and H (7') protons and H (10) and H (10'), respectively, or *vice versa*. On irradiation at 1.90 ppm, (A) in Fig. 3, doublet at δ 1.06 became to singlet, double doublet at δ 4.15 to doublet, and multiplet at δ 2.65 suffered considerable change. From such spin decoupling, the signal for H (3) (H, m), which is mixed with that for the methylene protons of H (3'1) and H (3'2) (2H, m) at *ca.* δ 2.00, has a center at δ 1.90. The signal at δ 1.06 (3H, d, $J=7$) was assigned to the methyl protons of the C (17) methyl group, the δ 4.15 (H, d, d) to H (2), and δ 2.65 (4H, m) to the four me-

FIG. 1. Molecular Structure of Fusariocin C Viewed along the *c* Axis. Oxygen atoms are shadowed.

FIG. 2. The Average Bond Distances between Two Crystallographically Independent Molecules.

FIG. 3. PMR Spectrum of Fusariocin C in CDCl_3 (100 MHz), Measured on a JEOL JNM PFT 100.

Chemical shifts are indicated as δ values from TMS and J values are recorded as Hz. Inserts show spin-spin decoupling spectra resulting from irradiation at (A) 1.90 and (B) 5.85 ppm.

thylene protons of H (41), H (42), H (4'1) and H (4'2). When the signal at δ 5.85 was irradiated, (B) in Fig. 3, double doublet at δ 4.15 was decoupled to doublet, multiplet at δ 4.60 to double doublet and multiplet at δ 6.38 to double doublet. Thus the signal at δ 5.85 (2H, m) was assigned to H (12) and H (12') and δ 4.15 (H, d, d) signal to H (2) as stated above. The δ 4.60 (H, m) signal was assigned to H (2') and δ 6.38 (2H, d, d) to H (13) and H (13').

In the IR spectrum,¹⁾ the absorption bands at 1625 and 1590 cm^{-1} were assigned to the C=O and C=C double bonds of tropolone, respectively, because the two intense absorptions in the range of 1500~1650 cm^{-1} are characteristic to tropolone derivatives.³⁾

All the above results indicate that fusariocin C has a pseudo dimer structure drawn in Fig. 1 and is represented by the structural formula shown in Fig. 2.

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