

F₁F₀-ATPase Inhibitor 화학 :
(-)-Citroviridin, C₂₃H₃₀O₆·CH₃OH의 결정구조에 관한 연구

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Chemistry of F₁F₀-ATPase Inhibitor :
Crystal Structure of (-)-Citroviridin, C₂₃H₃₀O₆·CH₃OH

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(-)-Citroviridin is a pyrone derivative of fungal origin that is a potent inhibitor of mitochondrial F₁F₀-ATPase activity.¹ Biological energy transduction has been hypothesized to rely on F₁F₀-ATPase (a ubiquitous membrane-bound protein), and citroviridin has been used in the investigation of the mechanism of electron transport driven phosphorylation. Previously, we reported the total synthesis of this molecule from ribonolactone.² In this total synthesis, we studied the structure of this molecule, especially the tetra-ene portion, by NMR spectroscopy. We used two different field strength (360 MHz and 500 MHz) for simulation and compared the result with the experimental ones. Previously, the crystal structure of this molecule was inaccurately reported with very high R index (R₁=0.23).³ Thus, we carried out X-ray crystallographic experiment so as to establish the clear structure of this compound (R₁=0.0339). The authentic compound was obtained by the microbial culture using *Penicillium pulvillorum*, CSIR 1406 (ATCC26219).⁴

EXPERIMENTAL

Erlenmeyer flasks (10 of 2L) containing F-14 medium (dextrose 150 g, bactopectone 50 g, NaCl 25 g, yeast extract 25 mL, beef extract 15 g, corn steep liquor 50 mL, deionized water 5 L) were inoculated with a spore suspension of *Penicillium pulvillorum*, CSIR 1406 (ATCC 26219). The mold was grown in stationary culture at 23-24 °C in the dark. After 18 days the cultures were filtered using filter paper and the mycelium was macerated with 1 L of acetone in a Waring blender. The acetone solution was evaporated to dryness. The filtrate was extracted with 700 mL of chloroform.

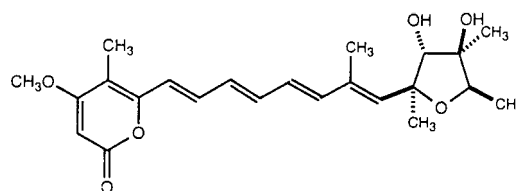


Fig. 1. (-)-Citroviridin.

The chloroform phase was concentrated, and combined with the residue of the acetone phase. This was partitioned between 1 L of *n*-hexane and 1.5 L of 90% methanol. The methanol phase was concentrated and the residue was partitioned between 1.5 L of chloroform and 500 mL of water. The chloroform solution was dried (Na₂SO₄), filtered, and evaporated to dryness. The residue was dissolved with 1 L of ethyl acetate and filtered with silica gel. Recrystallization under red light using

methanol was done to generate 1.02 g of (-)-cit-reoviridin.⁵ A yellow single crystal of size 0.53 × 0.53 × 0.4 mm was used for the X-ray crystallographic analysis. 1845 independent reflections in an asymmetric unit⁶ were collected and the intensities were corrected for L-p factors with the SDP.⁷ The extinction rule $0k0 : k = 2n$ indicated two possible space groups $P2_1$ and $P2_1/m$, but the structure was successfully elucidated with the space group $P2_1$. The structure was solved by direct methods using SHELXS86 and refined by full-matrix least

Table 1. Crystal data, data collection, and refinement of the structure for C₂₃H₃₀O₆·CH₃OH

Crystal data	
C ₂₃ H ₃₀ O ₆	MoKα radiation
Mr = 434.51	λ = 0.71073 Å
Monoclinic	Cell parameters from
$P2_1$	25 reflections
$a = 12.872(2)$ Å	$\theta = 2.33 - 23.98^\circ$
$b = 12.369(2)$ Å	$\mu = 0.088 \text{ mm}^{-1}$
$c = 7.752(1)$ Å	T = 293(2) K
$\beta = 105.990(14)^\circ$	Cube
$V = 1186.4(3)$ Å ³	0.53 × 0.53 × 0.4 mm
Z = 2	Yellow
$D_x = 1.216 \text{ Mgm}^{-3}$	
Data collection	
Enraf-Nonius CAD-4 diffractometer	$\theta_{\text{max}} = 24^\circ$
ω/θ scans	$k = 0 \rightarrow 14$,
Absorption correction: not applied	$l = -8 \rightarrow 8$
1987 measured reflections	3 standard reflections
1845 independent reflections	monitored every 300 reflections
1674 observed reflections	intensity variation:
$[F_o > 4\sigma(F_o)]$	less than 1.2%
$R_{\text{int}} = 0.0000$	
Refinement	
Refinement on F	$\Delta\rho_{\text{max}} = -0.141 \text{ eÅ}^{-3}$
$R_1 = 0.0339$	$\Delta\rho_{\text{min}} = -0.199 \text{ eÅ}^{-3}$
$R_2 = 0.1004$	Extinction correction:
$S = 0.864$	none
1674 reflections	Atomic scattering factors
415 parameter	from International
All H-atom parameters	Tables for X-ray
refined isotropically	Crystallography
$w = 1/[\sigma^2(F) + 0.000029F^2]$	(1974, Vol. IV)
$(\Delta/\sigma)_{\text{max}} = -0.217$	

Table 2. Fractional atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{Å}^2 \times 10^3$) for C₂₃H₃₀O₆·CH₃OH

	$U_{ij} = (1/3) \sum \sum U_j \rho_i^* a_i^* a_r a_s$			U(eq)
	x	y	z	
O(1)	7565(2)	8018	644(3)	41(1)
O(2)	5909(2)	8428(3)	-841(4)	56(1)
O(3)	7128(2)	4811(3)	-315(4)	55(1)
O(4)	11929(2)	16188(3)	3407(4)	54(1)
O(5)	14461(2)	15886(3)	5238(4)	56(1)
O(6)	12746(2)	15237(3)	763(3)	47(1)
O(7)	4170(3)	8036(4)	-3703(6)	93(1)
C(1)	6575(3)	7697(3)	-361(5)	41(1)
C(2)	6409(3)	6589(3)	-721(5)	41(1)
C(3)	7220(3)	5882(3)	-31(5)	38(1)
C(4)	8252(3)	6230(3)	1071(4)	36(1)
C(5)	8396(3)	7311(3)	1325(4)	35(1)
C(6)	6101(5)	4421(4)	-1418(10)	72(2)
C(7)	9121(4)	5422(4)	1793(7)	53(1)
C(8)	9377(3)	7877(4)	2277(5)	41(1)
C(9)	9498(3)	8946(4)	2160(5)	40(1)
C(10)	10451(3)	9551(4)	3047(5)	43(1)
C(11)	10518(3)	10620(4)	2841(5)	44(1)
C(12)	11401(3)	11331(4)	3643(5)	42(1)
C(13)	11359(3)	12401(4)	3332(5)	44(1)
C(14)	12171(3)	13214(3)	4104(5)	40(1)
C(15)	11922(3)	14252(4)	3668(5)	43(1)
C(16)	13218(3)	12830(4)	5361(6)	50(1)
C(17)	12544(3)	15277(4)	4364(5)	43(1)
C(18)	13640(3)	15350(4)	3927(4)	38(1)
C(19)	13346(2)	15939(3)	2124(4)	36(1)
C(20)	12536(3)	16762(3)	2411(5)	38(1)
C(21)	12625(6)	15441(5)	6351(6)	70(1)
C(22)	14301(4)	16415(4)	1607(7)	55(1)
C(23)	11790(4)	17243(4)	751(7)	56(1)
C(24)	3359(7)	8727(11)	-3669(14)	127(4)

squares using SHELX93.⁹ All H-atoms were found from difference Fourier map and refined isotropically. Details of the data collection together with structure refinement are summarized in the crystallographic data.

DISCUSSION

Atomic position parameters and equivalent isotropic thermal parameters are provided in *Table*

Table 3. Selected geometric parameters (Å, °)

O(1)-C(1)	1.357(4)	O(1)-C(5)	1.371(4)
O(2)-C(1)	1.231(4)	O(3)-C(3)	1.342(4)
O(3)-C(6)	1.445(6)	O(4)-C(20)	1.429(4)
O(4)-C(17)	1.457(5)	O(5)-C(18)	1.413(4)
O(6)-C(19)	1.419(4)	O(7)-C(24)	1.355(8)
C(1)-C(2)	1.403(5)	C(2)-C(3)	1.354(5)
C(3)-C(4)	1.434(5)	C(4)-C(5)	1.356(5)
C(4)-C(7)	1.490(5)	C(5)-C(8)	1.453(5)
C(8)-C(9)	1.337(5)	C(9)-C(10)	1.439(5)
C(10)-C(11)	1.338(6)	C(11)-C(12)	1.436(5)
C(12)-C(13)	1.343(6)	C(13)-C(14)	1.455(5)
C(14)-C(15)	1.344(5)	C(14)-C(16)	1.505(5)
C(15)-C(17)	1.516(5)	C(17)-C(21)	1.529(6)
C(17)-C(18)	1.541(5)	C(18)-C(19)	1.529(5)
C(19)-C(22)	1.512(5)	C(19)-C(20)	1.517(5)
C(20)-C(23)	1.501(6)		
C(1)-O(1)-C(5)	123.0(3)	C(3)-O(3)-C(6)	116.9(3)
C(20)-O(4)-C(17)	110.9(2)	O(2)-C(1)-O(1)	115.1(3)
O(2)-C(1)-C(2)	127.0(3)	O(1)-C(1)-C(2)	117.8(3)
C(3)-C(2)-C(1)	119.6(3)	O(3)-C(3)-C(2)	123.3(3)
O(3)-C(3)-C(4)	114.8(3)	C(2)-C(3)-C(4)	121.9(3)
C(5)-C(4)-C(3)	116.8(3)	C(5)-C(4)-C(7)	123.2(3)
C(3)-C(4)-C(7)	119.9(3)	C(4)-C(5)-O(1)	120.8(3)
C(4)-C(5)-C(8)	127.8(3)	O(1)-C(5)-C(8)	111.4(3)
C(9)-C(8)-C(5)	122.9(4)	C(8)-C(9)-C(10)	125.6(4)
C(11)-C(10)-C(9)	122.1(4)	C(10)-C(11)-C(12)	128.5(4)
C(13)-C(12)-C(11)	122.5(4)	C(12)-C(13)-C(14)	128.2(4)
C(15)-C(14)-C(13)	117.5(3)	C(15)-C(14)-C(16)	124.9(4)
C(13)-C(14)-C(16)	117.5(3)	C(14)-C(15)-C(17)	130.0(4)
O(4)-C(17)-C(15)	107.8(3)	O(4)-C(17)-C(21)	106.6(4)
C(15)-C(17)-C(21)	110.6(3)	O(4)-C(17)-C(18)	104.2(3)
C(15)-C(17)-C(18)	113.4(3)	C(21)-C(17)-C(18)	113.6(3)
O(5)-C(18)-C(17)	112.4(3)	O(5)-C(18)-C(19)	114.7(3)
C(19)-C(18)-C(17)	102.6(3)	O(6)-C(19)-C(22)	110.9(3)
O(6)-C(19)-C(20)	105.3(3)	C(22)-C(19)-C(20)	114.8(3)
O(6)-C(19)-C(18)	109.6(3)	C(22)-C(19)-C(18)	114.4(3)
C(20)-C(19)-C(18)	101.1(3)	O(4)-C(20)-C(23)	110.1(3)
O(4)-C(20)-C(19)	104.3(3)	C(23)-C(20)-C(19)	116.3(3)

Table 2 lists interatomic distances and angles. *Table 3* shows hydrogen-bonding geometry. *Fig. 2* drawn by ORTEP¹⁰ illustrates the molecular conformation with atomic labelling. O(4), C(17), C(18) and C(20) atoms are coplanar within 0.01 Å and C(19) atom deviates by 0.6 Å out of plane so that furanose five-membered ring has an envelope form. The pyrone ring including O(2), O(3), C(6), C(7) and C(8) atoms are in a plane with maximum deviation 0.065 Å. O(1), O(4), C(5), C(8), C(9), C(10), C(11), C(12), C(13), C(14), C(15), C(16), C(17) atoms are also coplanar within 0.1 Å. All of the olefines of the tetra-ene portion have trans structure. As shown in *Table 4*, the HO6 hydrogen atom participates in a bifurcated hydrogen bonding with O(1) and O(2) atoms in other molecule, and O(7) atom of the solvent methanol is hydrogen bonded

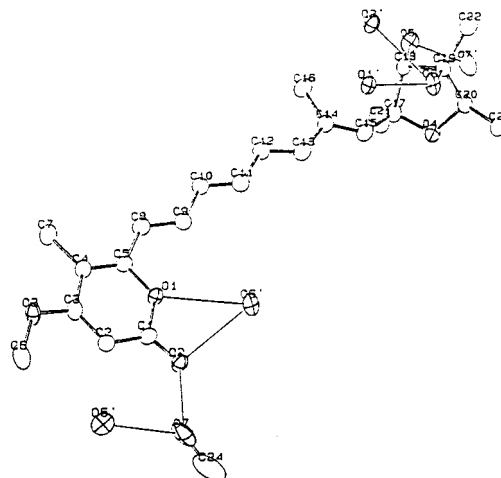


Fig. 2. ORTEP10 drawing of the molecule with atom-labelling scheme. Hydrogen bonds are indicated by thin lines. Displacement ellipsoids are plotted at the 50% probability level.

Table 4. Hydrogen-bonding geometry (Å, °)

D-H...A	D-H	H...A	D...A	D-H...A
O5-HO5...O7 ^a	0.90(7)	1.97(7)	2.839(6)	163(5)
O6-HO6...O1 ^b	0.74(6)	2.48(6)	2.940(4)	122(5)
O6-HO6...O2 ^b	0.74(6)	2.09(6)	2.820(4)	168(7)
O7-HO7...O2	0.97(6)	1.76(6)	2.726(4)	169(7)
O1 ^b ...HO6...O2 ^b				56(2)

Symmetry code: ^a1+x, 1+y, 1+z; ^b1-x, 0.5+y, 1-z.

to O(2) in a molecule and O(5) of another molecule. Therefore all the molecules in the crystal are held together by a strong hydrogen bonding scheme.

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Supplementary material available. Detailed description of X-ray works, listings of anisotropic thermal parameters, hydrogen positional parameters, and observed and calculated structure factors are available from one of the authors (H. Suh) upon request.

REFERENCES

- Hirata, Y.; Goto, T.; Sakabe, N. *Tetrahedron Lett.* **1964**, 1825.
- Suh, H.; Wilcox, C. S. *J. Am. Chem. Soc.* **1988**, *110*, 470.
- Furusake, A.; Watanabe, T.; Sakate, T.; Hirata, Y. 22nd Annual Meeting of the Chemical Society of Japan, Tokyo, Program of Abstracts # 1626, 1969.
- Nagel, D. W.; Steyn, P. S.; Ferriera, N. P. *Phytochemistry* **1972**, *11*, 3215.
- Mp 106 °C; R_f 0.25(SiO₂, 70% ethyl acetate-CH₂-Cl₂); [α]_D²³-77.0°(c 0.10, CHCl₃); UV(MeOH) λ_{max} 385, 294, 286, 230, 204 nm; IR(CHCl₃) 3615, 3300, 2912, 2857, 1695, 1624, 1603, 1588, 1562, 1535, 1453, 1406, 1292, 1246, 1145, 1094, 1051, 999, 810 cm⁻¹; ¹H NMR (500 MHz, CD₂Cl₂)(CH₂Cl₂, internal standard) δ 7.15(dd, 1H, J_{8,9}=15.0 Hz, J'_{9,10}=11.2 Hz, H9), 6.57(1H, J_{10,11}=14.7 Hz, J'_{11,12}=9.7 Hz, H11), 6.43(1H, J_{9,10}=11.2 Hz, J'_{10,11}=14.7 Hz, H10), 6.39(1H, J_{3,9}=15.0 Hz, H8), 6.36(1H, J_{12,13}=15.6 Hz, H13), 6.34(1H, J_{12,13}=15.6 Hz, J'_{11,12}=9.7 Hz, H12), 5.86(1H, J_{15,16}=1.0 Hz, H15), 5.45(s, 1H, H2), 3.92(d, 1H, J=4.9 Hz H18), 3.82(s, 3H, H6), 3.77(q, 1H, J_{20,23}=6.3 Hz, H20), 3.41(d, 3H, J=4.5 Hz, H24), 1.96(s, 3H, H7), 1.93(d, 3H, J_{15,16}=1.0 Hz, H16), 1.33(s, 3H, H21), 1.16(s, 3H, H22), 1.12(d, 3H, J_{20,23}=6.3 Hz, H23); ¹³C NMR(90 MHz, CD₂Cl₂) δ 171.5, 163.6, 154.9, 142.3, 141.4, 139.1, 136.2, 134.2, 131.3, 127.3, 118.9, 108.5, 88.7, 86.1, 84.5, 81.1, 78.1, 56.7, 50.1, 21.44, 18.2, 13.5, 12.6, 9.0; MS, m/e calcd. for C₂₃H₃₀O₆, 402.20422, measured 402.20542.
- (a) Suh, I. H.; Suh, J. M.; Ko, T. S.; Aoki, K.; Yamazaki, H. *J. Appl. Cryst.* **1988**, *21*, 521. (b) Suh, I. H.; Suh, J. M.; Ko, T. S.; Aoki, K.; Yamazaki, H. *J. Appl. Cryst.* **1989**, *22*, 183.
- Enraf-Nonius. Structure Determination Package, Enraf-Nonius, Delft, The Netherlands, 1985.
- Sheldrick, G. M. SHELX86. Program for the Solution of Crystal Structure, Univ. of Goettingen, Germany, 1985.
- Sheldrick, G. M. SHELX93. Program for the Solution of Crystal Structure, Univ. of Goettingen, Germany, 1993.
- Johnson, C. K. ORTEPII. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA, 1976.