STUDIES ON MACROCYCLIC LACTONE ANTIBIOTICS

VIII.[1] ABSOLUTE STRUCTURES OF RHIZOXIN AND A RELATED COMPOUND

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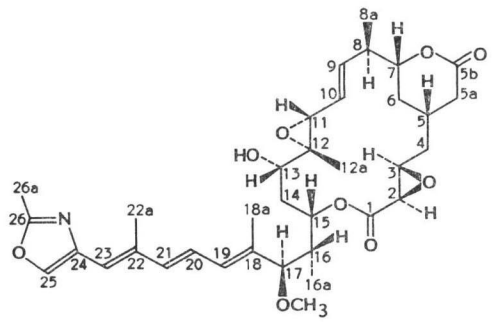
(Received for publication July 17, 1985)

The absolute structure of rhizoxin (**1**), a potent antifungal and antitumor antibiotic, was determined by interrelation with compound **2** whose structure was established by X-ray analysis. Since a ^{18}OH group was introduced at C-3 on a hydrolytic cleavage of C-2, C-3 epoxy group with alkaline $H_2{}^{18}O$, the original epoxy oxygen should be retained at C-2. The stereo-chemistry at C-2 and C-3 positions in rhizoxin was, therefore, determined as 2*R*,3*S*.

Rhizoxin (**1**) was isolated as a phytotoxin produced by *Rhizopus chinensis*,[1] the causal agent of rice seedling blight.[2] This compound exhibited potent antifungal and antitumor activity.[1,3,4] On the basis of its physico-chemical properties the skeletal structure of this compound was also assigned.[1]†

This paper deals with the absolute structures of rhizoxin (**1**) and of compound **2**.[5]

Fig. 1. Absolute structures of rhizoxin (**1**) and of compound **2**.



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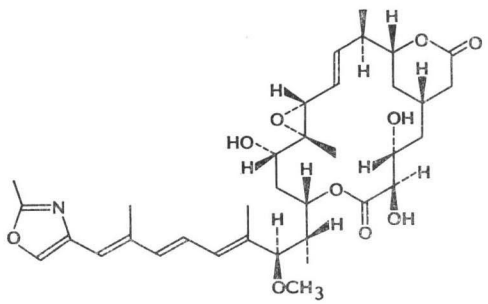

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† Some of the reported physico-chemical data of rhizoxin (**1**) in the ref 1 should be corrected as follows: $[\alpha]_D^{24} = +155.5°$ (*c* 0.8, MeOH); UV (MeOH) nm (ε) 295 (42,300), 308 (54,000), 325 (39,000); ^1H NMR H-13 3.02 ppm; ^{13}C NMR C-5 29.5 ppm, C-21 137.9 ppm.

The Structure of Compound **2**

Compound **2**, mp 127°C (from CH_3CN - H_2O), $[\alpha]_D^{25}$ +46.1° (*c* 0.7, MeOH), was isolated as an artifact formed during separation of minor metabolites of *R. chinensis*. This compound had a UV absorption maximum in methanol at 298 (ε 43,000), 310 (56,100) and 325 nm (41,400) indicating the presence of the same chromophore as the side chain of rhizoxin (**1**).

The ^1H NMR and ^{13}C NMR data of compound **2** are summarized in Tables 2 and 3 (Experimental part) and suggest the presence of the following carbon units: 7×CH_3, 4×CH_2, 3×CH, 7×HC–O, 1×–C–O, 7×=C, 2×COO.

The electron impact mass spectrum exhibited the molecular ion peak (M^+) at *m/z* 643. From the mass spectral and NMR data, the molecular formula of $C_{35}H_{49}O_{10}N$ was determined for compound **2**, indicating that it is a hydrate form of rhizoxin.

Since the NMR signals due to H-2 (δ 2.96), H-3 (3.27), C-2 (54.3) and C-3 (56.0) in the spectra of rhizoxin are shifted to δ 4.36, 3.91, 77.5 and 70.4 ppm, respectively, in the spectra of compound **2**, the compound **2** was considered to be a C-2, C-3 diol derivative. The relative and absolute stereo-chemistry of the compound **2** were established by X-ray diffraction of a crystal to be that shown in Fig. 1.

Crystals of **2**, crystallized from aqueous acetonitrile, belong to a monoclinic space group $P2_1$. The lattice parameters are a=10.719(4), b=17.036(6), c=10.439(4) Å and β=112.90(4)° with one molecule of **2** and two water molecules in an asymmetric unit. Intensity data were measured on a Philips PW1100 four-circle diffractometer using CuKα radiation monochromated by a graphite plate. A total of 3573, non-zero, independent reflections was collected by the θ-2θ scanning technique within a range of $3° \leq \theta \leq 78°$. No correction for absorption nor extinction were applied. The structure was solved by direct method using the program RANTAN81.[6] The structure was refined by a block-diagonal least-squares method and hydrogen atoms were located from difference maps. The final R-factor was 0.049, assuming anisotropic thermal motions for non-hydrogen atoms and isotropic motion for hydrogen atoms.

The atomic scattering factors for non-hydrogen atoms were taken from International Tables for X-ray Crystallography[7] and for hydrogen atoms were those of STEWART *et al.*[8]

To determine the absolute configuration, a small crystal with the size of 0.30×0.25×0.22 mm was chosen for the intensity measurement. The five reflections, which were of medium intensity and had a large difference between |Fc| values of *hkl* and $\bar{h}\bar{k}\bar{l}$, were used for the determination. The intensities of these reflections were carefully measured with their symmetry-equivalent reflections.

Table 1. Comparison of the calculated and observed intensity ratios for the selected Friedel-pair reflections.

h	k	l	$\lvert Fc^+\rvert$	$\frac{\lvert Fc^+\rvert^2}{\lvert Fc^-\rvert^2}$	$\sigma\left(\frac{Io^+}{Io^-}\right)$	$\frac{Io^+}{Io^-}$	$\frac{(\lvert Fo^+\rvert-\lvert Fo^-\rvert)}{\sigma(\lvert Fo^+\rvert)}$
−5	11	1	6.00	1.046	1.080	0.044	1.71
3	11	3	6.23	1.049	1.067	0.044	1.37
0	5	6	4.35	1.053	1.158	0.050	2.82
−4	1	7	5.11	1.041	1.065	0.047	1.56
−5	4	11	4.48	1.047	1.119	0.047	2.46

$|Fc^+|$, $|Fc^-|$; Calculated structure factor for *hkl* and $\bar{h}\bar{k}\bar{l}$. Io^+, Io^-; observed intensity for *hkl* and $\bar{h}\bar{k}\bar{l}$. $|Fo^+|$, $|Fo^-|$; observed structure factor for *hkl* and $\bar{h}\bar{k}\bar{l}$. $\sigma(|Fo^+|)$; estimated standard deviations for $|Fo^+|$ caluculated.

In order to estimate the absorption effect, the ψ-scan method was employed. For each reflection intensities were measured by varying the angle (azimutal angle about the reflection vector) from $-90°$ to $90°$ in step of $10°$. There were no systematic variation in intensity for different ψ angles; they agree within the limit of experimental error estimated on the basis of counting statistics. Hence, the absorption effect seemed to be negligible.

The intensities for hkl and $\bar{h}\bar{k}\bar{l}$ were obtained by averaging the two symmetry-equivalent reflections. In Table 1 the observed intensity ratios between the Friedel-pairs are compared with the ratios of $|Fc|^2$, which are calculated by using the final atomic parameters obtained from the conventional structure determination. The values of the dispersion corrections of atomic scattering factors for O, N, C atoms for CuKα radiation are $\Delta f' = 0.047$, $\Delta f'' = 0.032$; $\Delta f' = 0.029$, $\Delta f'' = 0.018$: $\Delta f' = 0.017$, $\Delta f'' = 0.009$ (International Tables for X-ray Crystallography[7]), respectively. The result cleary indicates the absolute configuration of this molecule.

The structure of this molecule is illustrated in Fig. 2 by an ORTEP drawing.[9]

Interrelation of Rhizoxin and Compound **2**

Rhizoxin (**1**) was converted to the seco-rhizoxin triacetate (**3**) as described in the preceding paper.[1] This compound was identified with the product obtained from an alkaline hydrolysis of compound **2** followed by methylation and acetylation (Fig. 3). The stereo-chemistry of rhizoxin was thus deter-

Fig. 2. ORTEP drawing of the structure of compound **2**.

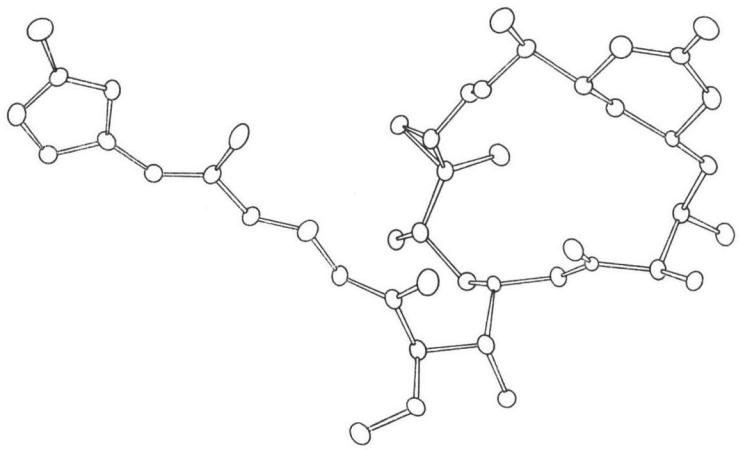


Fig. 3. Chemical interrelation of **1** and **2**.

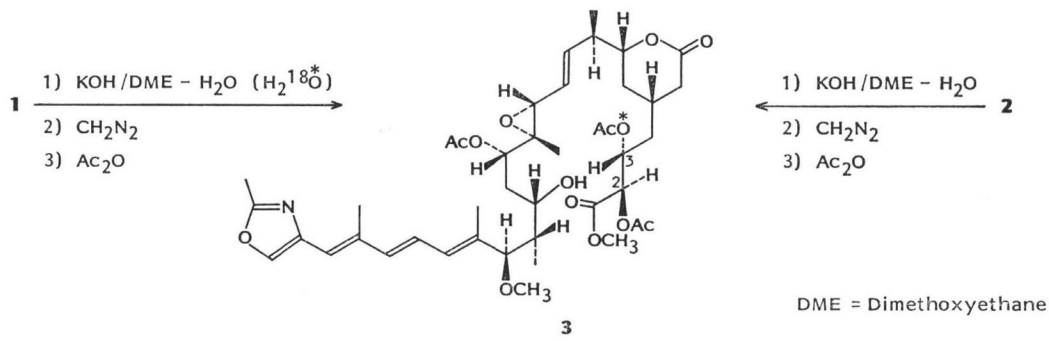
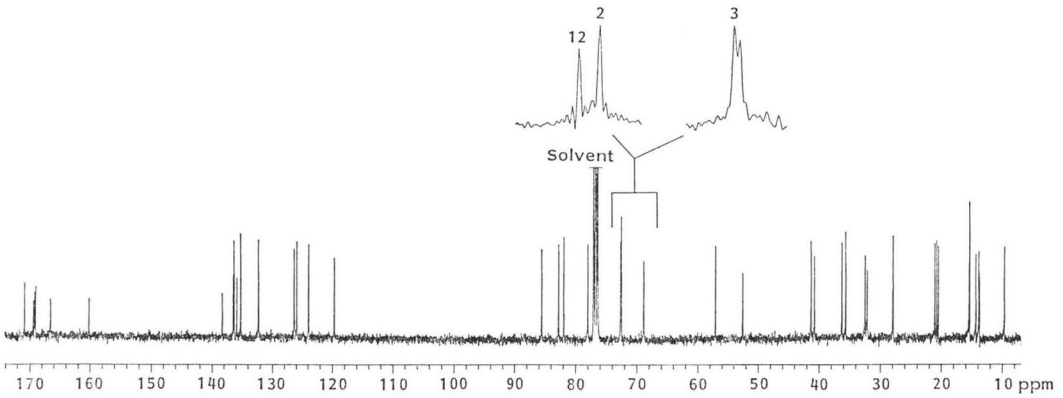


Fig. 4. ^{13}C NMR spectrum of ^{18}O-enriched seco-rhizoxin triacetate (**3**).



mined except for the configuration of the epoxy group at C-2, 3.

In order to determine the stereochemistry of the epoxide, rhizoxin was hydrolyzed using $H_2{}^{18}O$ to give ^{18}O-labeled seco-rhizoxin triacetate (**3**). The ^{13}C NMR spectrum of this copmound showed a typical oxygen-18 induced shift of the C-3 signal (δ 70.4 ppm) and no such shift of the C-2 signal (δ 77.5 ppm) (Fig. 4). These results showed that hydrolytic cleavage of the epoxide ring proceeded by attack of the ^{18}O-labeled nucleophile at C-3 and that the original epoxy oxygen atom was retained at C-2. Based on the absolute configuration at C-2 of compound **2** the stereo-chemistry of C-2, C-3 in rhizoxin was, therefore, determined as 2*R*,3*S* as shown in the Fig. 1.

Experimental

General

See reference 1.

Isolation of Compound 2

R. chinensis Rh-2 strain was cultivated in a medium composed of maltose 3%, Polypepton 1%, KH_2PO_4 0.25%, K_2HPO_4 0.75%, $MgSO_4 \cdot 7H_2O$ 0.25%, $(NH_4)_2SO_4$ 0.20% and Pharmamedia 4% at 28°C by shaking for 3 days. The ethyl acetate extract of the culture broth was separated by succesive silica gel and LH-20 column chromatographies to isolate rhizoxin and related compounds. In the course of the chromatographic separation of the metabolite mixture, compound **2** was formed as an artifact and was obtained in *ca.* 0.4 mg/liter of ultimate yield. This was further purified by crystallization from CH_3CN - H_2O (2: 1) to give colorless prisms which melted at 127°C; $[\alpha]_D^{25}$ +46.1° (*c* 0.7, MeOH); UV (MeOH) λ_{max} nm (ε) 298 (43,000), 310 (56,100), 325 (41,400); IR (KBr) ν_{max} cm^{-1} 3420, 2940, 1735, 1705, 1580, 1440, 1380, 1250, 1170, 1110, 1060, 1030, 970; ^1H NMR, see Table 2: ^{13}C NMR, see Table 3; EI-MS *m/z* 645 (M^+ 0.3), 629 (0.5), 625 (0.4), 611 (2), 595 (2), 593 (1), 577 (1.5), 549 (1), 531 (1), 232 (20), 200 (24), 153 (54), 137 (50), 111 (78), 109 (75), 95 (72), 81 (76), 55 (100).

Alkali Treatment of Rhizoxin

For the experimental procedure, and UV, IR, ^1H NMR and EI-MS spectra of compound **3** see ref 1. $[\alpha]_D^{25}$ +35.9° (*c* 0.2, MeOH); ^{13}C NMR 170.8 (1s, C-5b), 169.3, 169.1, 169.0 (3s, acetyl carbonyl), 166.5 (1s, C-1), 160.1 (1s, C-26), 138.2, 136.4, 135.8 (3s, =C), 136.2, 135.1, 132.2, 126.3, 125.9, 123.8, 119.6 (7d, =CH–), 85.5, 82.7, 80.9, 77.9, 76.3 (5d, HC–O), 72.6 (1s, C-12), 72.4 (1d, C-2), 68.8 (1d, C-3), 57.0 (1q, 17-OCH_3), 52.5 (1q, 1-OCH_3), 41.2, 40.7, 27.9 (3d, CH), 36.2, 35.6, 32.4, 32.1 (4t, CH_2), 21.0, 20.7, 20.4 (3q, acetyl methyl), 15.4, 15.4, 14.3, 13.9, 13.7, 9.6 (6q, CH_3).

Table 2. ^{1}H NMR data of compound **2** in $CDCl_3$ (400 MHz).

Proton	Chemical shift	Multiplicity and coupling constant (Hz)
H-2	4.36	d $J_{2,3}$=1 Hz
H-3	3.91	ddd $J_{3,2}$=1 Hz, $J_{3,4}$=5.1, 9.5 Hz
H-4	1.36	ddd $J_{4,4}$=12.5 Hz, $J_{4,3}$=5.1 Hz, $J_{4,5}$=*
	1.85	ddd $J_{4,4}$=12.5 Hz, $J_{4,3}$=9.5 Hz, $J_{4,5}$=*
H-5	2.38	m $J_{5,4}$=*, *, $J_{5,5a}$=5.8, 10.6 Hz, $J_{5,6}$=*, *
H-5a	2.07	dd $J_{5a,5a}$=17.6 Hz, $J_{5a,5}$=10.6 Hz
	2.62	dd $J_{5a,5a}$=17.6 Hz, $J_{5a,5}$=5.8 Hz,
H-6	1.06	ddd $J_{6,7}$=10.2 Hz, $J_{6,6}$=*, $J_{6,5}$=*
	1.82	ddd $J_{6,7}$=2.5 Hz, $J_{6,6}$=*, $J_{6,5}$=*
H-7	3.81	ddd $J_{7,6}$=2.5, 10.2 Hz, $J_{7,8}$=9.5 Hz
H-8	2.37	m $J_{8,7}$=9.5 Hz, $J_{8,8a}$=6.4 Hz, $J_{8,9}$=9.3 Hz
H-8a	1.15	d $J_{8a,8}$=6.4 Hz
H-9	5.51	dd $J_{9,8}$=9.3 Hz, $J_{9,10}$=15.6 Hz
H-10	5.41	dd $J_{10,9}$=15.6 Hz, $J_{10,11}$=8.5 Hz
H-11	3.14	d $J_{11,10}$=8.5 Hz
H-12a	1.24	s
H-13	3.18	dd $J_{13,14}$=5.6, 11.5 Hz
H-14	1.70	ddd $J_{14,14}$=15.5 Hz, $J_{14,13}$=11.5 Hz, $J_{14,15}$=*
	2.17	ddd $J_{14,14}$=15.5 Hz, $J_{14,13}$=5.6 Hz, $J_{14,15}$=*, *
H-15	4.53	ddd $J_{15,14}$=*, *, $J_{15,16}$=3.5 Hz
H-16	2.39	m $J_{16,15}$=3.5 Hz, $J_{16,16a}$=6.6 Hz, $J_{16,17}$=9.1 Hz
H-16a	1.01	d $J_{16,16a}$=6.6 Hz
H-17	3.37	d $J_{17,16}$=9.1 Hz
17-OCH_3	3.18	s
H-18a	1.89	s
H-19	6.16	d $J_{19,20}$=10.8 Hz
H-20	6.70	dd $J_{20,19}$=10.8 Hz, $J_{20,21}$=15.0 Hz
H-21	6.47	d $J_{21,20}$=15.0 Hz
H-22a	2.12	s
H-23	6.25	s
H-25	7.83	s
H-26a	2.44	s

* Spin-spin coupling was observed but its constant was not determined.

Table 3. ^{13}C NMR data of compound **2** in CD_3OD.

Carbon	Chemical shift	$^1J_{CH}$	Carbon	Chemical shift	$^1J_{CH}$
C-1	173.4	s	C-15	74.2	d
C-2	77.5	d	C-16	40.0	d
C-3	70.4	d	C-16a	9.9	q
C-4	39.5	t	C-17	88.6	d
C-5	28.0	d	17-OCH_3	56.2	q
C-5a	37.3	t	C-18	137.3	s
C-5b	173.4	s	C-18a	12.3	q
C-6	32.8	t	C-19	130.9	d
C-7	83.6	d	C-20	124.4	d
C-8	44.2	d	C-21	138.7	d
C-8a	16.8	q	C-22	137.8	s
C-9	138.2	d	C-22a	14.6	q
C-10	129.2	d	C-23	121.1	d
C-11	63.3	d	C-24	138.7	s
C-12	64.4	s	C-25	137.1	d
C-12a	11.3	q	C-26	162.2	s
C-13	76.2	d	C-26a	13.3	q
C-14	35.9	t			

Alkali Treatment of Compound **2**

Experimental procedure was according to that for rhizoxin.[1] Compound **3** was isolated in 37% yield. $[\alpha]_D^{25}$ +36.5° (*c* 0.4, MeOH). All spectral characteristics were identical with those of compound **3** obtained from rhizoxin (for the spectral data see ref 1).

Alkali Treatment of Rhizoxin using $H_2{}^{18}O$

To rhizoxin (12.5 mg, 20 μmol) in dimethoxyethane (0.9 ml) was added KOH (3.36 mg, 60 μmol) dissolved in 0.1 ml of $H_2{}^{18}O$ (97 atom %). The solution was heated to 80°C for 1 hour. After dilution of the reaction solution with 5 ml of H_2O the solution was adjusted to pH 4.0 with dil HCl. The reaction mixture was extracted with ethyl acetate and the extract was washed with H_2O and dried over sodium sulfate. The crude product was methylated with diazomethane and acetylated in benzene with each 1.5 equivalents of acetic anhydride and pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine. A silica gel column chromatography of the crude products mixture gave ^{18}O-labeled compound **3** (7.0 mg). EI-MS spectrum of this compound demonstrated that $^{18}O_1$, $^{18}O_2$- and $^{18}O_3$-enriched compounds were present in 12, 64 and 24%, respectively. In the ^{13}C NMR spectrum oxygen-18 induced shifts of the signals at 52.5 (1-OCH_3), 166.5 (C-1) and 68.8 ppm (C-3) were observed.

Acknowledgment

We are indebted to Prof. Y. SATO and Dr. Y. ODA (Miss) for the measurement of optical rotation and are grateful to the Hoansha Foundation for financial support.

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