NEW ANTIBIOTICS SF2315A AND B PRODUCED BY AN
EXCELLOSPORA SP.

II. THE STRUCTURAL ELUCIDATION

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The structures of antibiotics SF2315A and B were determined to be [4aR-(4aβ,12bβ)] or [4aS-(4aα,12bα)]-4,5,6,12b-tetrahydro-4a,8-dihydroxy-3-methylbenz[a]anthracen-1,7,12(4H)-trione and [1S-(1β,4aβ,6aβ,12β,12aβ,12bβ)] or [1R-(1α,4aα,6aα,12α,12aα,12bα)]-1,4,4a,5,6,6a,-12a,12b-octahydro-1,4a,8,12-tetrahydoxy-3-methyl-6a,12a-epoxybenz[a]anthracen-7(12H)-one, respectively from their spectroscopic analyses and single crystal X-ray diffraction analyses.

Two new antibiotics SF2315A and B which are weakly active against Gram-positive bacteria have been isolated from the culture filtrate of *Excellospora* sp. SF2315[1]. Both antibiotics have the carbon skeletons of benz[a]anthracene. In this paper we report on the structural elucidation of these antibiotics based on spectral analyses and single crystal X-ray diffraction analyses.

Structure of Antibiotic SF2315A

The molecular formula of antibiotic SF2315A (I) was determined to be $C_{19}H_{16}O_5$ from the elemental analysis, high resolution (HR)-MS (M$^+$ m/z calcd: 324.0997, found: 324.1062), ^{1}H and ^{13}C NMR spectra (Tables 1 and 2).

The UV spectrum of I in methanol exhibited maxima at 237, 269 and 419 nm which were shifted to 272 and 515 nm in basic methanol. The IR spectrum showed the absorption due to hydroxyl groups (3400 cm^{-1}), an unsaturated ketone (1690 cm^{-1}, sh), a non-chelated quinone (1660 cm^{-1}) and a chelated quinone carbonyl group (1640 cm^{-1}). The above data together with an ABX spin system of aromatic proton signals at δ 7.33 (9-H), 7.56 (11-H) and 7.74 (10-H) and a hydrogen-bonded phenolic proton at δ 11.91 (8-OH) in the ^{1}H NMR spectrum of I (Table 1) suggested the presence of a substituted 5-hydroxy-1,4-naphtoquinone (juglone)[2] moiety in I.

The ^{1}H NMR spectrum of I showed 16 proton signals (Table 1) and suggested the presence of the following partial structures: (A) CH=C(CH$_3$)CH$_2$, (B) CH$_2$CH$_2$, (C) $\overset{|}{C}$H, (D) $\overset{|}{C}$OH and (E) a substituted juglone moiety.

The ^{13}C NMR spectrum of I had 19 carbon signals, which could be attributed to two quinone carbonyls, one ketone carbonyl, ten aromatic or olefinic carbons, one quaternary carbon, one methine carbon, three methylene carbons and one methyl carbon (Table 2).

The chemical shifts in the ^{13}C NMR spectrum suggested that the ketone carbonyl carbon at δ 196.2 (C-1) was an α,β-unsaturated ketone carbonyl carbon. Therefore, the carbonyl carbon must be con-

Table 1. ¹H NMR data of SF2315A (I) and SF2315B (II).

Proton	SF2315A (I)	SF2315B (II)
1-H		4.42 m
1-OH		5.39 d (7.0)
2-H	5.78 br s	5.34 br s
3-CH₃	1.99 s	1.66 s
4-H	2.91 br d (18.4),	2.14 br d (17.5),
	2.46 d (18.4)	1.94 d (17.5)
4a-OH	5.24 s	4.36 s
5-H	1.65 m	1.56 m and 1.36 m
6-H	2.60 m	2.59 ddd (3.1, 7.8, 15.6),
		2.14 m
8-OH	11.91 s	11.33 s
9-H	7.33 br d (8.6)	6.87 br d (7.8)
10-H	7.74 dd (7.4, 8.6)	7.57 t (7.8)
11-H	7.56 br d (7.4)	7.18 br d (7.8)
12-H		5.29 d (7.0)
12-OH		5.68 d (7.0)
12b-H	3.98 br s	2.53 br d (7.0)

δ: ppm ($J=$Hz) in DMSO-d_6 at 400 MHz.

nected to the olefinic methine carbon of the partial structure (A).

¹H-¹³C Shift correlation spectral analysis (¹H-¹³C COSY) and long range ¹H-¹³C COSY established a benz[a]anthraquinone structure for I and permitted the assignment of all the carbon signals except for those of C-6a and C-12a (at δ 142.6 and 143.5). Carbon signals of the quinone carbonyl in the juglone moiety appear at δ 189.3 and 182.9. The signal at δ 189.3 is assigned to the hydrogen-bonded quinone carbonyl due to its relative downfield chemical shift. The carbonyl carbon signal of the non-hydrogen-bonded quinone at δ 182.9 (C-12) exhibits a long range coupling to the isolated methine proton at δ 3.98 (12b-H). Furthermore the methine proton (12b-H) was coupled to the quaternary carbon (C-4a), the α,β-unsaturated ketone carbonyl (C-1) and two aromatic carbons at δ 142.6 and 143.5 (C-6a and C-12a). One of the methylene protons at δ 2.60 (6-H) also exhibited long range coupling to C-6a and C-12a. The above results revealed structure of I to be as shown in Fig. 1.

Table 2. ¹³C NMR data of SF2315A (I) and SF2315B (II).

Carbon	SF2315A (I)	SF2315B (II)
C-1	196.2 (s)	67.2 (d)
C-2	123.2 (d)	122.8 (d)
C-3	161.2 (s)	132.8 (s)
C-4	45.9 (t)	42.9 (t)
C-4a	69.1 (s)	70.4 (s)
C-5	27.9 (t)	28.1 (t)
C-6	20.1 (t)	17.0 (t)
C-6a	*142.6 (s)	62.2 (s)
C-7	189.3 (s)	198.2 (s)
C-7a	114.7 (s)	112.3 (s)
C-8	160.5 (s)	160.3 (s)
C-9	123.9 (d)	115.6 (d)
C-10	136.8 (d)	136.4 (d)
C-11	118.9 (d)	117.7 (d)
C-11a	132.0 (s)	143.2 (s)
C-12	182.9 (s)	65.8 (d)
C-12a	*143.5 (s)	68.8 (s)
C-12b	52.0 (d)	45.4 (d)
C-13	24.0 (q)	22.7 (q)

δ: ppm ( ) multiplicity, in DMSO-d_6 at 100 MHz.

Carbon assignment marked by * may be interchangeable.

The relative configuration of I was determined by a single-crystal X-ray diffraction analysis. A computer-generated perspective drawing of the final X-ray model of I is shown in Fig. 2. The X-ray analysis defined only the relative configuration, so the enantiomer shown was arbitrarily selected.

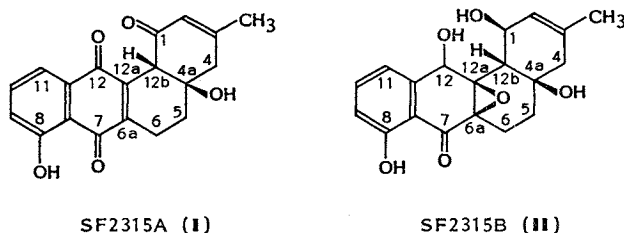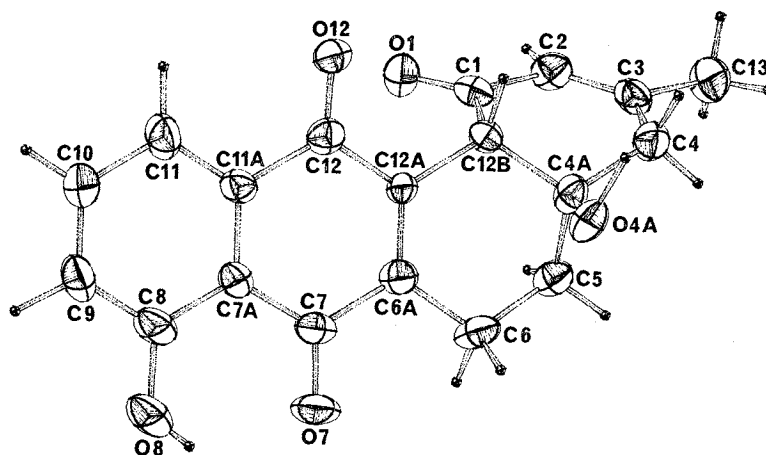Fig. 1. The structures of antibiotics SF2315A (I) and B (II).

SF2315A (I)   SF2315B (II)

Fig. 2. An ORTEP drawing of antibiotic SF2315A.

Thus, the structure of antibiotic SF2315A was determined to be either [4aR-(4aβ,12bβ)] or [4aS-(4aα,-12bα)]-4a,5,6,12b-tetrahydro-4a,8-dihydroxy-3-methylbenz[a]anthracen-1,7,12(4H)-trione.

Structure of SF2315B

The molecular formula of antibiotic SF2315B (II) was determined to be $C_{19}H_{20}O_6$ from the elemental analysis, HR-MS (M$^+$ m/z calcd: 344.1258, found: 344.1195), ^{1}H and ^{13}C NMR spectra (Tables 1 and 2).

The UV spectrum of II in methanol exhibited maxima at 267 and 339 nm which were shifted to 232, 268 and 376 nm in basic methanol. The spectrum of II showed the absorption due to a hydroxyl group (3400 cm^{-1}) and a chelated carbonyl group (1640 cm^{-1}) but no absorption due to an unsaturated ketone and a nonchelated quinone carbonyl group which were observed in I. The above observations suggested the presence of an isosclerone[3] (3,4-dihydro-4,8-dihydroxy-1-(2H)naphthalenone) moiety in II. This postulate was also supported by the ^{1}H NMR spectrum of II (Table 1), which contained a phenolic hydroxyl proton at δ 11.33 (8-OH), a hydroxyl proton at δ 5.68 (12-OH), a methine proton at δ 5.29 (12-H) and an ABX spin system of three aromatic protons at δ 6.87, 7.18 and 7.57 (9-H, 11-H and 10-H). The other partial structures present in II were revealed as (A) $\overset{|}{C}HCH(OH)CH=C(CH_3)CH_2$, (B) CH_2CH_2 and (C) $\overset{|}{C}OH$ by spin decoupling experiments.

The ^{13}C NMR spectrum of II had 19 carbon signals, which could be attributed one ketone carbonyl, eight aromatic or olefinic carbons, three quaternary carbons, two oxymethine carbons, one methine carbon, three methylene carbons and one methyl carbon (Table 2). ^{1}H-^{13}C COSY and long range

Fig. 3.   An ORTEP drawing of antibiotic SF2315B.
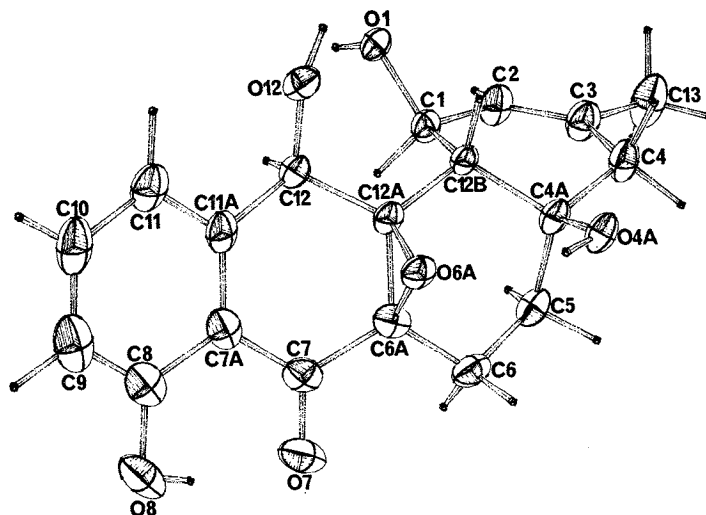
^{1}H-^{13}C COSY established the assignments of all carbon signals.

The methine proton at δ 2.53 (12b-H) was coupled to the quaternary carbon at δ 68.8 (C-12a) and the quaternary carbon at δ 70.4 (C-4a).   The methylene proton at δ 2.14 (4-H) was coupled to C-4a and the methine carbon at δ 45.4 (C-12b).   The above results indicated that the partial structures (A) and (C) formed a six membered ring which was attached to the C-12a of the isosclerone moiety.   Long range couplings were observed between C-12a and the methylene protons (6-H) which revealed the linkage of the partial structure (B) and the isosclerone moiety.

On the basis of the index of hydrogen deficiency of **II**, the remaining oxygen atom has to form an oxyran ring with the quaternary carbons of C-6a and C-12a.

By a comparison of these spectral data for **II** with those for **I**, it was concluded that the structure of **II** was closely related to that of **I**.   The α,β-unsaturated ketone carbonyl carbon (C-1) and the non-hydrogen-bonded quinone carbonyl carbon (C-12) in **I** were reduced to the oxymethine carbons in **II**.   From the above mentioned results, the structure of **II** was deduced to be that shown in Fig. 1.

The structure and the relative stereochemistry of **II** was finally determined by a single-crystal X-ray diffraction analysis.   A computer-generated perspective drawing is shown in Fig. 3.   The X-ray analysis defined only the relative configuration, so the enantiomer shown was arbitrarily selected.

Thus the structure of antibiotic SF2315B was determined to be either [1S-(1β,4aβ,6aβ,12β,12aβ,-12bβ)] or [1R-(1α,4aα,6aα,12α,12aα,12bα)]-1,4,4a,5,6,6a,12a,12b-octahydro-1,4a,8,12-tetrahydroxy-3-methyl-6a,12a-epoxybenz[a]anthracen-7(12H)-one.

Experimental

General

MP's were determined using Yamato MP-21 apparatus in glass capillary tubes.   UV spectra were recorded on a Shimadzu UV-260 spectrophotometer.   IR spectra were obtained in pressed KBr discs using a Hitachi 260-10 IR spectrophotometer.   MS spectra were measured on a Hitachi M-80B mass spectrometer.   Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JNM-GX400 spectrometer.   The chemical shifts in DMSO-d_6 refer to an internal standard of TMS (0 ppm).

Single-crystal X-Ray Diffraction Analysis of SF2315A

A crystal of approximate dimensions $0.15 \times 0.15 \times 0.4$ mm was mounted on a Philips PW-1100 X-ray diffractometer. All X-ray measurements were made using graphite monochromated CuKα radiation. The lattice constants were derived from setting angles of 24 higher angle ($\theta = 21.1° \sim 34.3°$) reflections. Crystal data: $C_{19}H_{16}O_5$ Orthorhombic, $P2_12_12_1$, $a=10.958(6)$, $b=15.887(8)$, $c=8.906(5)$Å, $U=1550.4$ Å^3, $Z=4$, $D_{calc}=1.389$ gcm^{-3}, μ for CuKα radiation$=7.93$ cm^{-1}. Intensities were measured by a $2\theta - \omega$ scan method with a scan speed 0.1°/sec in ω. Backgrounds were measured at each end of the scan for half the total scan time. For weak reflections whose intensities were less than 3000 counts during the single scan, the scans were repeated once. A total of 1177 reflections in the 2θ range $6° \sim 156°$ was measured.

The phases of 124 strong reflections with $|E| > 1.47$ were determined by direct method using MULTAN[4]. The E-map for the best solution yielded positions for all the non-hydrogen atoms.

In the final refinement, the non-hydrogen atoms were refined anisotropically by block-diagonal least-squares. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined.

The final R value was 0.083 for 1177 observed reflections. In the present structure determination, no attempt has been made to assign the absolute configuration. An ORTEP[5] drawing of the molecule is shown in Fig. 2.

Single-crystal X-Ray Diffraction Analysis of SF2315B

A purified preparation of antibiotic SF2315B was recrystallized from ethanol as transparent prismatic crystals. A crystal of approximate dimensions $0.15 \times 0.15 \times 0.3$ mm was mounted on a Philips PW-1100 X-ray diffractometer. All X-ray measurements were made using graphite monochromated CuKα radiation. The lattice constants were derived from setting angles of 16 higher angle ($\theta = 15.1° \sim 25.7°$) reflections.

Crystal data: $C_{19}H_{20}O_6$, Orthorhombic, space group $P2_12_12_1$, $a=14.287(7)$, $b=20.780(10)$, $c=5.645(3)$ Å, $U=1675.9$ Å^3, $Z=4$, $D_{calc}=1.365$ gcm^{-3}, μ for CuKα radiation$=8.04$ cm^{-1}.

Intensities were measured by a $2\theta - \omega$ scan method with the scan speed 0.1°/sec in ω. Backgrounds were measured at each end of the scan for half the total scan time. For the weak reflections whose intensities were less than 3000 counts during the single scan, the scans were repeated once. A total of 1079 reflections in the 2θ range $6° \sim 156°$ was measured. The phases of 200 strong reflections with $|E| > 1.25$ were determined by direct methods using MULTAN[4]. The E-map for the best solution yielded positions for all the non-hydrogen atoms.

In the final refinement, the non-hydrogen atoms were refined anisotropically by block-diagonal least-squares. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined.

The final R value was 0.052 for 1079 observed reflections. In the present structure determination, no attempt has been made to assign the absolute configuration. All the crystallograhic calculations were done on a IBM 4381 computer at the Meiji information system center, using the UNICS III program[6]. An ORTEP[5] drawing of the molecule is shown in Fig. 3.

References

1) SASAKI, T.; J. YOSHIDA, M. ITOH, S. GOMI, T. SHOMURA & M. SEZAKI: New antibiotics SF2315A and B produced by an *Excellospora* sp. I. Taxonomy of the strain, isolation and characterization of antibiotics. J. Antibiotics 41: 835~842, 1988

2) MORTON, R. A. & W. T. EARLAM: Absorption spectra in relation to quinones: 1,4-naphthoquinone, anthraquinone and their derivatives. J. Chem. Soc. Chem. Commun. 1941: 159~169, 1941

3) MORITA, T. & H. AOKI: Isosclerone, a new metabolite of *Sclerotinia sclerotiorum* (LIB.) DE BARY. Agric. Biol. Chem. 38: 1501~1505, 1974

4) GERMAIN, G.; P. MAIN & M. M. WOOLFSON: The application of phase relationships to complex struc-
    tures. III. The optimum use of phase relationships. Acta Crystallogr. Sec. A 27: 368~376, 1971
5) JOHNSON, G. K.: ORTEP. A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations.
    Report ORNL-3794. Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A., 1965
6) SAKURAI, T. & K. KOBAYASHI: On the universal crystallographic computation program system (5). UNICS
    III system. Rep. Inst. Phys. Chem. Res. 55: 69~77, 1979