STUDIES ON A NEW ANTIBIOTIC SF-2330

II. THE STRUCTURAL ELUCIDATION

JIRO ITOH, TADASHI TSUYUKI, KAYO FUJITA
and MASAJI SEZAKI

Pharmaceutical Research Laboratories,
Meiji Seika Kaisha, Ltd.,
Morooka-cho, Kohoku-ku, Yokohama 222, Japan

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The structure of antibiotic SF-2330 has been elucidated to be 11-hydroxy-5-methyl-2-(2,2′-bioxiran-2-yl)-4*H*-anthra[1,2-*b*]pyran-4,7,12-trione by spectral analyses. The olefinic side chain at C-2 of α-indomycinone is replaced by a bioxiran-2-yl group in SF-2330.

A new antibiotic SF-2330 active against Gram-positive bacteria has been isolated from the culture of *Streptomyces* sp. SF-2330[1]. In this paper, the structural elucidation by spectral analyses of the antibiotic is reported.

The molecular formula of antibiotic SF-2330 (**1**) was determined to be $C_{22}H_{14}O_7$ from the elemental analysis, FD-MS (M^+ *m/z* 390), ^1H and ^{13}C NMR spectra[1]. The UV spectrum of **1** is very similar to those of indomycinones[2] and also the pluramycin group antibiotics, *i.e.*, pluramycin[3], neopluramycin[4], hedamycin[2,5], kidamycin[2,6], griseorubin[7], largomycin[8] and PD 121,222[9], and suggested the presence of similar chromophore in the molecule of **1**. ^1H NMR spectrum of **1** indicated the presence of 14 protons containing five aromatic protons, three protons of one methyl group, four protons of two methylene groups, one proton of methine group and one proton of hydrogen-bonded hydroxyl group (Table 1).

IR spectrum of **1** showed strong absorption bands at 1675 (sh) and 1655 (quinone carbonyl and ketone CO) and 1630 cm^{-1} (hydrogen-bonded quinone CO). ^{13}C NMR spectrum of **1** showed the presence of 22 carbons containing two quinone CO, one ketone CO, 14 olefinic carbons, one methyl carbon, two methylene carbons, one methine carbon and one quaternary carbon (Table 2).

Acetylation of **1** gave a monoacetate (**2**), $C_{24}H_{16}O_8$, MS *m/z* 432 (M^+). IR spectrum of **2** showed characteristic absorption bands at 1755 (acetyl CO), 1665 and 1640 cm^{-1} (quinone CO and pyrone CO). ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 2) revealed signals due to the acetyl group (δ 2.45 s, 21.1 q, 169.5 s).

^1H NMR data of **1** are very similar to that of *O*-methylkidamycinone (**3**)[10] and, in part, to those of members of the pluramycin group antibiotics[2,11,12]. Three aromatic protons at δ 7.80, 7.67 and 7.35, observed as an ABC type spin system, was assigned to 8-H, 9-H and 10-H, respectively (Fig. 1), by ^1H-^1H decoupling experiments. The 8-H proton (δ 7.80) appeared at low field because of the peri-position of a quinone carbonyl. For the same reason, an unresolved quartet at δ 8.07 was assigned to the 6-H proton which is allylic coupled to a methyl signal at δ 2.99 (J=0.7 Hz)[2]. The remaining aromatic proton at δ 6.53 could be assigned to 3-H from the chemical shift of γ-pyrone[13]. These ^1H NMR assignments to the chromophore of **1** were confirmed by ^{13}C NMR data as follows. The signal of the quinone CO at δ 181.4 (C-7) was split into a triplet (J=4 Hz)[2,14], whereas the signal of another

Table 1. ^{1}H NMR chemical shifts of SF-2330 (**1**) and monoacetate (**2**).

Proton	δ, ppm (J Hz) in $CDCl_3$	
	1	**2**
3-H	6.53 1H, s	6.50 1H, s
6-H	8.07 1H, q (0.7)	8.02 1H, s
8-H	7.80 1H, dd (8.0, 1.1)	8.22 1H, d (7.5)
9-H	7.67 1H, dd (8.4, 8.0)	7.79 1H, t (8.0)
10-H	7.35 1H, dd (8.4, 1.1)	7.47 1H, d (6.4)
11-OH	12.73 1H, s	
13-H	2.99 3H, d (0.7)	2.89 3H, s
15-H	3.32 1H, d (6.2)	4.14 1H, d (6.2)
	3.36 1H, d (6.2)	4.15 1H, d (6.2)
16-H	4.17 1H, dd (3.7, 2.6)	4.15 1H, dd (3.6, 2.7)
17-H	2.94 1H, dd (5.5, 2.6)	2.92 1H, dd (5.6, 2.7)
	2.95 1H, dd (5.5, 3.7)	2.94 1H, dd (5.6, 3.6)
11-$OCOCH_3$		2.45 3H, s

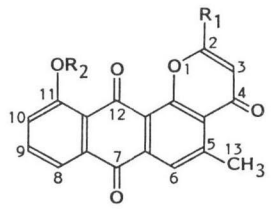
Table 2. ^{13}C NMR chemical shifts of SF-2330 (**1**) and monoacetate (**2**).

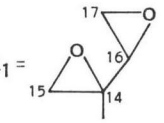
Carbon	δ, ppm in $CDCl_3$	
	1	**2**
1a	156.2 s	155.7 s
2	163.7 s	163.7 s
3	111.2 d	111.0 d
4	178.3 s	180.2 s
4a	126.5 s	126.7 s
5	150.0 s	149.9 s
6	126.1 d	125.5 d
6a	136.1 s	135.3 s
7	181.4 s	181.8 s
7a	132.1 s	134.0 s
8	119.4 d	125.5 d
9	136.6 d	134.7 d
10	125.5 d	130.5 d
11	162.6 s	148.8 s
11a	116.6 s	126.2 s
12	187.0 s	178.5 s
12a	119.6 s	121.4 s
13	24.1 q	24.0 q
14	54.5 s	54.5 s
15	53.7 t	53.7 t
16	47.6 d	47.6 d
17	45.1 t	45.0 t
11-OCO		169.5 s
11-$OCOCH_3$		21.1 q

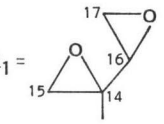
quinone CO at δ 187.0 (C-12) was observed as a sharp singlet in a proton non-decoupled ^{13}C NMR spectrum. These results indicated that the 6-H and 8-H protons were located at the peri-position to the quinone CO at δ 181.4, and the other quinone CO (δ 187.0 s) is assigned to C-12 which is hydrogen-bonded to the 11-OH (δ 12.73 s).

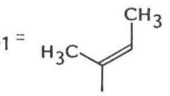
The structure of the side chain moiety comprising $C_4H_5O_2$ remained to be defined. Index of unsaturation derived from the molecular formula is sixteen. Therefore, the presence of six rings in the structure of **1** in addition to ten unsaturated bonds observed in ^{13}C NMR spectrum is required. From the spectral results described above, the chromophore moiety accounts for four rings in the 4*H*-anthra[1,2-*b*]pyran structure. Thus, it is necessary to have two-ring structures in the side chain moiety. ^{1}H NMR spectrum of the side chain moiety showed five protons containing four methylene protons (δ 2.94, 2.95, 3.32, 3.36) and one methine proton (δ 4.17). Methylene protons at δ 2.94 and 2.95 is coupled with a methine proton at δ 4.17, whereas another methylene pair at δ 3.32 and 3.36 were not coupled to other protons. ^{13}C NMR spectrum of the side chain moiety showed four carbons containing two methylene carbons at δ 45.1 (J=177 Hz) and 53.7 (J=180 Hz), one methine carbon at δ 47.6 (J=182 Hz) and one quaternary carbon at δ 54.5. The large coupling constants of two methylene carbons and one methine carbon indicated the presence of the two oxirane rings[2,11,15]. From these results the structure of the side chain moiety was deduced as bioxiran-2-yl, shown in Fig. 1.

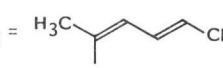
Fig. 1.



SF-2330 (**1**) R_1 =  R_2 = H

Monoacetate (**2**) R_1 =  R_2 = $COCH_3$

O-Methylkidamycinone (**3**) R_1 =  R_2 = CH_3

α-Indomycinone (**4**) R_1 =  R_2 = H

Observation of the long range couplings of a quaternary carbon (C-14 - 3-H, J=3.1 Hz and C-14 - 16-H, J=7.9 Hz) confirmed by a long range selective proton decoupling experiment indicated that the side chain moiety must be located at C-2.

Although the stereochemistry of the side chain moiety is still undetermined, the structure of SF-2330 has been shown to be 11-hydroxy-5-methyl-2-(2,2′-bioxiran-2-yl)-4*H*-anthra[1,2-*b*]pyran-4,7,12-trione.

The unique bioxiran-2-yl side chain of SF-2330 is considered to be derived by oxygenation of the (*E*)-1-methyl-1-propenyl side chain of kidamycin. It is interesting that SF-2330, having this bioxiran-2-yl side chain, showed some antibacterial activity against Gram-positive bacteria, whereas α-indomycinone, having a 1-methylpenta-1,3-dienyl side chain, was biologically inactive[16].

Experimental

Methods of Analysis

Melting points were measured using a Yamato MP-21 and are uncorrected. UV spectra were measured on a Shimadzu UV-260 spectrometer. FD-MS were carried out on a Hitachi M-80. ^1H and ^{13}C NMR spectra were recorded on a Jeol JNM-GX 400 (^1H 400 MHz and ^{13}C 100.7 MHz) spectrometer; chemical shifts are given in ppm (in δ) relative to TMS (0 ppm) as an internal standard and coupling constants (J) are recorded in Hz.

SF-2330 Monoacetate (2)

SF-2330 (20 mg) was acetylated with acetic anhydride (0.2 ml) in pyridine (1 ml) at room temp for 85 hours. The reaction mixture was poured into ice cold water and allowed to stand for 1 hour. It was extracted with $CHCl_3$ (10 ml) and the extract was washed with H_2O, dried over anhydrous Na_2SO_4

and concentrated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (Wakogel C-200, 1 g) developed with benzene - Me_2CO (10: 1) to give crude crystals (14 mg). Recrystallization from $CHCl_3$ to yield 10 mg of pale yellow needles of **2**; mp 263~266°C (dec); MS *m/z* 291 (100%), 432 (M^+, 36%). *Anal* Calcd for $C_{24}H_{16}O_8$: C 66.54, H 3.86. Found: C 66.67, H 3.73; UV $\nu_{max}^{CHCl_3}$ 372 nm (ε 10,270); IR (KBr) cm^{-1} 1755, 1665, 1640, 1630 (sh); ^1H and ^{13}C NMR were shown in Tables 1 and 2, respectively.

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