A NEW TETRACYCLINE ANTIBIOTIC WITH ANTITUMOR ACTIVITY

II. THE STRUCTURAL ELUCIDATION OF SF2575

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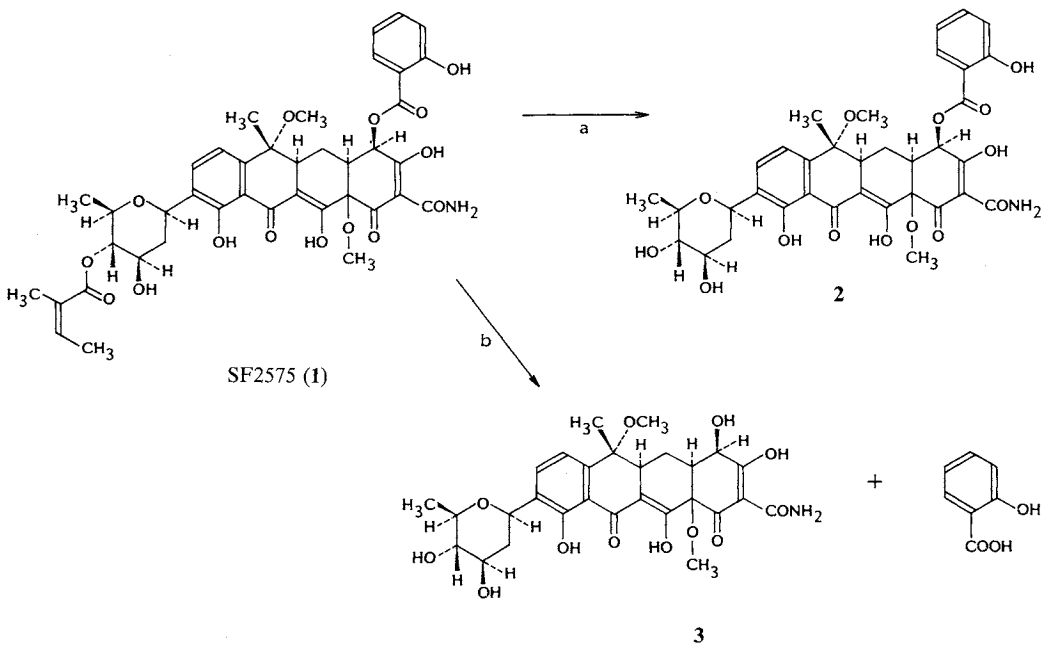
(Received for publication August 23, 1991)

The structure of a new antitumor antibiotic SF2575, has been determined by spectroscopic analyses of the antibiotic and its alkaline degradation products. The relative stereochemistry has been confirmed by X-ray crystallographic analysis. The antibiotic has a 2-naphthacenecarboxamide carbon skelton which is structurally related to the tetracycline antibiotics and it is unique by bearing *C*-glycoside, salicylic acid and angelic acid moieties.

A new antitumor antibiotic, SF2575 has been found in the culture of *Streptomyces* sp. SF2575. The antibiotic was active against Gram-positive bacteria and showed a potent antitumor activity against murine leukemia P388 *in vivo*. The producing organism, fermentation, isolation, physico-chemical and biological properties of the antibiotic were reported in the previous paper[1]. This paper describes the structural elucidation of SF2575 (**1**).

Scheme 1. The hydrolysis of SF2575.



a: 0.5 N NaOH for 2 hours at room temperature. b: 1 N NaOH for 15 hours at room temperature.

Table 1. ^{1}H NMR chemical shifts of SF2575 (**1**) and its degradation products (**2** and **3**).

Proton	**1**	**2**	**3**
3-OH	17.01 s	17.05 s	17.90 s
4-H	6.12 m	6.12 d (5.6)	4.60 br
4-OH	—	—	5.80 br
4a-H	3.24 m	3.21 m	2.87 m
5-H	1.63 q like (11.6),	1.65 q like (12.0),	1.26 m,
	2.21 m	2.19 m	2.12 m
5a-H	3.48 dd (11.6, 5.6)	3.48 dd	3.32 dd
6-OCH_3	3.18 s	3.18 s	3.15 s
7-H	6.98 d (7.8)	6.96 d (8.6)	6.94 d (7.8)
8-H	7.72 d (7.8)	7.67 d (8.6)	7.67 d (7.8)
10-OH	12.11 s	12.09 s	12.15 s
12-OH	14.87 s	14.80 s	14.92 s
12a-OCH_3	3.51 s	3.52 s	3.38 s
13-NH_2	9.41 br,	9.41 br,	9.12 br,
	9.46 br	9.41 br	9.22 br
14-CH_3	1.04 s	1.05 s	0.98 s
15-H	4.82 dd (11.4, 0.5)	4.72 dd	4.70 dd
16-H	1.48 q like (11.4),	1.33 q like (12.4),	1.28 m,
	2.21 m	2.12 m	2.12 m
17-H	3.80 m	3.50 m	3.50 m
17-OH	5.13 d (5.6)	4.86 d (5.5)	4.90 d (5.5)
18-H	4.59 t (9.8)	2.87 m	2.90 m
18-OH	—	4.92 d (5.5)	4.98 d (5.5)
19-H	3.61 dq (9.8, 6.4)	3.18 m	3.18 m
20-CH_3	1.12 d (6.4)	1.24 d (5.6)	1.22 d (5.8)
3′-OH	10.33 s	10.33 s	—
4′-H	7.04 d (7.2)	7.04 d (8.2)	—
5′-H	7.57 br dd (8.0, 7.2)	7.56 br dd (8.2, 7.2)	—
6′-H	7.02 br dd (8.0, 7.6)	7.02 br dd (8.0, 7.2)	—
7′-H	7.85 br d (7.6)	7.92 br d (8.0)	—
3″-H	6.11 m	—	—
4″-CH_3	1.93 br d (7.4)	—	—
5″-CH_3	1.87 br s	—	—

Measured at 400 MHz in DMSO-d_6; ppm from TMS.
Coupling constants (Hz) are in parentheses.

The molecular formula of **1** was determined to be $C_{40}H_{43}NO_{15}$ on the basis of elemental analysis, FD-MS (*m/z* 777 M^+) and ^{13}C NMR spectral data. It gave a bright yellow fluorescence on a silica gel TLC plate upon irradiation of UV light. This property and UV spectrum of **1** resemble those of tetracyclines. In the ^{1}H NMR spectrum, some characteristic signals due to the tetracycline nucleus were observed at δ 9.41 and 9.46 (2-$CONH_2$), 12.11 (10-OH), 14.87 (12-OH) and 17.01 (3-OH) (Table 2)[2]. On comparison of the ^{13}C NMR spectrum of **1** with those of the tetracycline antibiotics[3,4], it is deduced that **1** has a highly functionalized 2-naphthacenecarboxamide carbon skeleton which is similar to tetracyclines. Furthermore, the ^{1}H and ^{13}C NMR spectra of **1** indicated the presence of neutral *C*-glycoside, angelic acid and salicylic acid moieties in **1** (Tables 1 and 2).

Mild alkaline hydrolysis of **1** with 0.5 N NaOH at room temperature for 2 hours afforded **2** ($C_{35}H_{37}NO_{14}$). On comparison of the ^{13}C NMR spectra of **2** with those of **1**, the loss of five carbon signals were observed. In the heteronuclear multiple-bond correlation (HMBC) spectrum of **1**, methyl protons at δ 1.93 (4″-CH_3) were long range coupled with two olefinic carbons at δ 137.0 (C-3″) and 127.9 (C-2″). Other olefinic methyl protons at δ 1.87 (5″-CH_3) were coupled with a carbonyl carbon at δ 167.1

Table 2. ^{13}C NMR data of SF2575 (**1**) and alkaline hydrolysis products (**2** and **3**).

Carbon	1	2	3	Carbon	1	2	3
C-1	189.6 s*	189.6 s*	189.4 s*	C-13	173.2 s	173.1 s	173.2 s
C-2	95.9 s	96.0 s	95.7 s	C-14	24.7 q	24.7 q	24.4 q
C-3	189.5 s*	189.6 s*	189.2 s*	C-15	70.5 d	70.4 d	70.1 d
C-4	69.6 d	69.6 d	66.5 d	C-16	40.5 t	40.0 d	40.0 t
C-4a	36.8 d	36.7 d	38.2 d	C-17	69.3 d	72.0 d	71.7 d
C-5	19.4 t	19.4 t	18.1 t	C-18	77.7 d	77.3 d	76.0 d
C-5a	35.1 d	35.1 d	35.0 d	C-19	73.9 d	76.3 d	77.0 d
C-6	78.0 s	78.0 s	77.7 s	C-20	18.2 q	18.6 q	18.3 q
6-OCH_3	50.5 q	50.4 q	50.1 q	C-1′	167.3 s	167.3 s	—
C-6a	147.5 s	147.2 s	147.0 s	C-2′	113.5 s	113.5 s	—
C-7	114.7 d	115.0 d	114.4 d	C-3′	160.1 s	160.1 s	—
C-8	134.1 d	134.0 d	133.6 d	C-4′	117.9 d	117.9 d	—
C-9	128.9 s	129.5 s	129.2 s	C-5′	136.1 d	136.1 d	—
C-10	158.3 s	158.3 s	157.9 s	C-6′	119.7 d	119.7 d	—
C-10a	115.1 s	114.7 s	114.7 s	C-7′	130.5 d	130.5 d	—
C-11	192.6 s	192.6 s	192.2 s	C-1″	167.1 s	—	—
C-11a	108.5 s	108.5 s	108.3 s	C-2″	127.9 s	—	—
C-12	174.1 s	174.1 s	175.0 s	C-3″	137.0 d	—	—
C-12a	81.1 s	81.1 s	81.4 s	C-4″	15.7 q	—	—
12a-OCH_3	54.4 q	54.4 q	53.8 q	C-5″	20.5 q	—	—

Measured at 100 MHz in DMSO-d_6; ppm from TMS.
* May be interchangeable.

(C-1″) and two olefinic carbons at δ 127.9 (C-2″) and 137.0 (C-3″). These observation indicated the structure of C5-unit to be 2-methyl-2-butenoic acid. The large coupling constant between the carbonyl carbon C-1″ and the vicinal vinyl proton 3″-H ($^3J_{3''\text{-H,C-1''}}=14.9$ Hz) indicated that 3″-H and C-1″ are *trans* oriented[5]. This assignment was supported by the chemical shift of the β-methyl carbon (C-5″, δ 20.5), which corresponds to angelic acid (δ 20.7), but not tiglic acid (δ 11.9). From these results, the structure of the C5-unit was determined to be (*Z*)-2-methyl-2-butenoic acid (angelic acid). The high field shift of 18-H (δ 4.59 in **1**, 2.87 in **2**) and the appearance of 18-OH (δ 4.92) in **2** indicated that 18-OH was acylated with angelic acid in **1**.

Treatment of **1** with 1 N NaOH at room temperature for 15 hours followed by gel filtration with Sephadex LH-20 gave salicylic acid and **3** ($C_{28}H_{33}NO_{12}$). Salicylic acid was identified by direct comparison with an authentic sample. On comparison of the ^1H and ^{13}C NMR spectra of **3** with those of **1** (Tables 1 and 2), the loss of the signals due to salicylic acid and angelic acid were observed. The upfield chemical shift of 4-H (δ 4.60) and the apperance of 4-OH signal (δ 5.80) in **3** indicated that 4-OH was acylated with salicylic acid in **1**.

The ^1H-^1H COSY and ^{13}C-^1H COSY experiments of **1** revealed the presence of some partial structures. The sugar moiety was shown to have a pseudoanomeric carbon at δ 70.5 indicating *C*-glycoside linkage. The large coupling constants $J_{17\text{-}18}=9.8$ Hz, and $J_{18\text{-}19}=9.8$ Hz showed all *trans*-axial relation of 17-H to 19-H. Accordingly, this sugar was shown to be the *C*-glycoside of 2,6-dideoxy-*arabino*-hexopyranose. Long range couplings of 15-H with C-9 (δ 128.9) and 8-H with C-15 (δ 70.5) in the HMBC spectrum of **1** showed that the sugar moiety was located at C-9 of the tetracycline nucleus.

The carbon skelton related to the tetracyclines was elucidated by 2D NMR experiments especially analyses of the HMBC spectrum of **1** as shown in Fig. 1. The partial structure from C-4 to C-5a was elucidated easily by analysis of ^1H-^1H COSY spectrum of **1**. In the HMBC spectrum of **1**, the singlet methyl proton (14-CH_3) at δ 1.04 were coupled to three carbons at δ 35.1 (C-5a), 78.0 (C-6) and 147.5

(C-6a). This result and the chemical shift of the methyl protons indicated the methyl carbon directly attached to the sp^3 quaternary carbon (C-6, δ 78.0) which was coupled also to the methoxy protons (6-OCH_3, δ 3.18). Accordingly, the partial structure expanded from C-5a to C-6a. The doublet aromatic proton (8-H, δ 7.72) was coupled to two aromatic quaternary carbons (C-10, δ 158.3 and C-6a, δ 147.5). The hydrogen-bonded phenolic proton at δ 12.11 (10-OH) was coupled to three carbons at δ 158.3 (C-10), 128.9 (C-9) and 115.1 (C-10a). These results revealed the carbon skelton from C-6a to C-10a. The other hydrogen-bonded phenolic proton (12-OH) at δ 14.87 was coupled to three quaternary carbons at δ 174.1 (C-12), 108.5 (C-11a) and 81.1 (C-12a). The quaternary carbon C-12a was also coupled to the oxymethine proton (4-H) at δ 6.12, a methylene proton (5-H) at δ 2.21 and methoxy protons (12a-OCH_3) at δ 3.51. The results proved the position of three carbons C-11a, C-12 and C-12a. Two carbonyl carbons at δ 189.6 and 189.5 (C-1 and C-3, assignment are interchangeable) were coupled with the methine proton (4a-H) at δ 3.24. The amide protons at δ 9.41 and 9.46 were coupled to the quaternary carbon at δ 95.9 (C-2), indicating that the carboxamide was attached to C-2. The carbonyl carbon at δ 192.6 (C-11) was observed no long range CH coupling, but the location was deduced to the peri position of the hydrogen-bonded phenol (10-OH).

The positions of attachment of the salicylic acid and angelic acid moieties were also confirmed by the observation of the long range couplings of 4-H proton to C-1′ carbonyl carbon at δ 167.3 and 18-H proton to C-1″ carbonyl carbon at δ 167.1, respectively. From the above-mentioned results, the gross structure of SF2575 was deduced to be **1**.

The relative stereochemistry of **1** was determined by X-ray crystallographic analysis using a transparent prismatic crystal. The structure was solved successfully only by the MAGEX[6] program with inputting the common structure for tetracycline. In the final refinement, the nonhydrogen atoms were refined isotropically by full-matrix least-squares. The hydrogen atoms were not included in calculated positions. A PLUTO[7] drawing of the molecule is shown in Fig. 2.

Thus, **1** was different from the known tetracycline antibiotics by the presence of the *C*-glycoside acylated with an angelic acid at C-9 and the salicyloyl group at C-4. Further study on the absolute stereochemistry is progress.

Fig. 1. The summary of HMBC experiment of **1**.

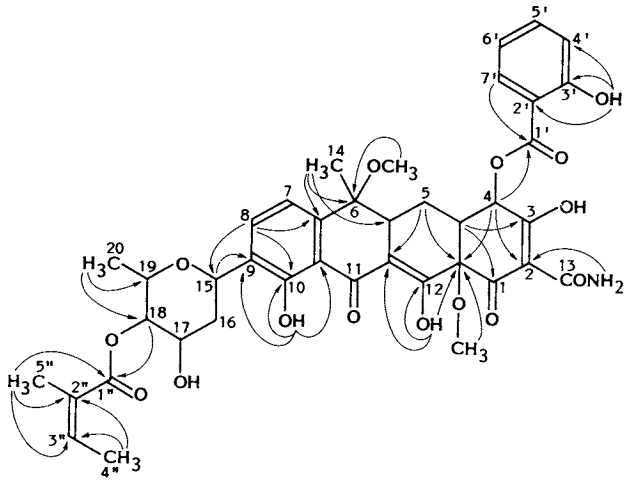
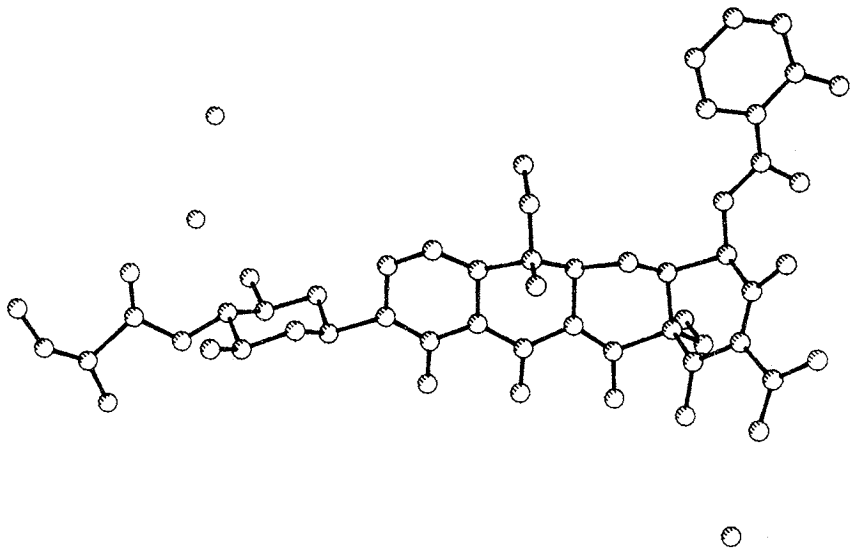


Fig. 2. A PLUTO drawing of **1**.



Experimental

General

UV spectra were measured on a Shimadzu UV-260 spectrometer. IR spectra in KBr discs were recorded on a Hitachi 260-10 infrared spectrometer. MS spectra were measured on a Hitachi M-80B mass spectrometer. Optical rotations were recorded with a Perkin Elmer model 141 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on a Jeol JNM-GX400 or Jeol JNM-GSX400 spectrometer. The chemical shifts are expressed in ppm with TMS as an internal standard. For TLC, silica gel plate (E. Merck, Art. No. 5715) was used.

Mild Alkaline Hydrolysis of **1**

A solution of **1** (100 mg) in 0.5 N NaOH (10 ml) was stirred for 2 hours at room temperature. The reaction solution was acidified to pH 2.0 with 1 N HCl and extracted with EtOAc (10 ml × 2). The solvent layer was evaporated to give an orange powder. The powder was dissolved in MeOH and purified by a column of Sephadex LH-20 using MeOH as a developing solvent to yield **2** (35 mg) as a pale yellow powder; mp 212~214°C; $[\alpha]_D^{24}$ +158.8° (*c* 0.1, MeOH); FD-MS *m/z* 695 $(M)^+$; UV λ_{max}^{MeOH} nm (ε) 228 (25,600), 243 (28,300), 320 (13,900), 359 (18,800), $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 242 (28,300), 386 (21,700); IR (KBr) cm^{-1} 3370, 1720 (sh), 1650, 1610; soluble in MeOH, DMSO and Me_2CO, hardly soluble in $CHCl_3$, insoluble in H_2O and hexane; TLC Rf 0.57 ($CHCl_3$-MeOH-H_2O, 65:25:4); ^{1}H NMR and ^{13}C NMR spectra are shown in Tables 1 and 2, respectively.

Alkaline Hydrolysis of **1**

A solution of **1** (300 mg) in 1 N NaOH (10 ml) was stirred for 15 hours at room temperature. The reaction solution was acidified to pH 2.0 with 2 N HCl and extracted with EtOAc (10 ml × 2). The solvent layer was evaporated to give a yellow powder (151 mg), which was chromatographed on Sephadex LH-20 (250 ml) using MeOH as a developing solvent. Salicylic acid (10 mg) was obtained from the first eluate (fraction Nos. 39~42) (5 g/fraction). It was identified with an authentic sample by TLC and ^{1}H NMR spectrum. From the subsequent eluate (fraction Nos. 44~50), a yellow powder of **3** (210 mg) was obtained, mp 203~205°C; $[\alpha]_D^{24}$ +209.8° (*c* 0.1, MeOH); FD-MS *m/z* 575 (M^+); UV λ_{max}^{MeOH} nm (ε) 226 (15,800), 256 (15,200), 358 (14,500), $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 242 (16,600), 260 (sh, 14,000), 386 (16,100); IR (KBr) cm^{-1} 3400, 1650 (sh), 1610; soluble in MeOH, DMSO and Me_2CO, hardly soluble in $CHCl_3$, insoluble in H_2O and hexane; TLC Rf 0.34 ($CHCl_3$-MeOH-H_2O, 65:25:4); ^{1}H NMR and ^{13}C NMR spectra are shown

in Tables 1 and 2, respectively.

X-Ray Crystallographic Analysis of **1**

Purified preparation of **1** was recrystallized from $CHCl_3$ as transparent prismatic crystals. A crystal of approximate dimensions $0.2 \times 0.2 \times 0.3$ mm was mounted on a Phillips PW-1100 X-ray diffractometer. All X-ray measurements were made using graphite monochromated CuK_α radiation. The lattice constants were derived from setting angles of 15 higher angle ($\theta = 15° \sim 24°$) reflections. Crystal data are as follows; $C_{40}H_{43}NO_{15} \cdot 3H_2O$, MW 831, orthorhombic, space group $P2_12_12_1$, $a = 18.136(9)$, $b = 24.355(12)$, $c = 9.268(2)$Å, $U = 4{,}094.2$Å^3, $Z = 4$, $D_{calc} = 1.26$ g cm^{-3}, μ for CuK_α radiation $= 9.03$ cm^{-1}. Intensities were measured by a $2\theta-\omega$ scan method with the scan speed 0.1°/second in ω. Background was measured at each end of the scan for half the total scan time. For the weak reflections whose intensities were less than 3,000 counts during the single scan, the scans were repeated. A total of 3,483 reflections ($R_{sym}(F) = 0.17$) in the 2θ range $6° \sim 120°$ were measured. The structure was refined to a R value of 0.130. The calculation were done on a micro-Vax computer, using the program MITHRIL[8] in the TEXSAN package. Atomic coordinates have been deposited with the Cambridge Crystallographic Data-base.

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