Fluostatins A and B, New Inhibitors of Dipeptidyl Peptidase III, Produced by *Streptomyces* sp. TA-3391

II. Structure Determination

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In the course of our screening program for new inhibitors of dipeptidyl peptidase III (EC 3.4.14.4), we discovered fluostatins A and B (**1** and **2**, Fig. 1) from the culture broth of *Streptomyces* sp. TA-3391. The taxonomy of the producing strain, fermentation, isolation, physico-chemical properties and biological properties have been described in the preceding paper[1]. Here we report on the structure determination of **1** and **2** on the basis of spectroscopic studies, chemical studies and X-ray crystallographic analysis.

The physico-chemical properties of **1** and **2** were described in the previous paper[1]. The molecular formulae of **1** ($C_{18}H_{10}O_5$) and **2** ($C_{18}H_{14}O_6$) were determined by interpretation of the HRFAB-MS, NMR spectral analyses and elemental analyses.

The ^1H and ^{13}C NMR spectra of **2** showed the presence of twelve protons and eighteen carbons. The DEPT experiment revealed that these carbons consisted of two carbonyls, twelve other sp^2 and four sp^3 carbons. The carbons bearing protons were assigned by a Hetero-nuclear Single Quantum Coherence (HSQC) experiment. These data are summarized in Table 1.

Two partial structures of **2** were established on the basis of ^1H-^1H coupling constants in the ^1H NMR spectrum and Heteronuclear Multiple Bond Correlation (HMBC) experiment (Fig. 2-b). A 4-bond coupling observed from an aromatic methine proton at δ 7.48 (H-5) to an aromatic carbon at δ 133.6 (C-6b) suggested the connectivity between 11 and 11a/6a and 6b.

To confirm the structure of **2**, it was acetylated with acetic anhydride in pyridine to give fully aromatized acetate (**3**, 41%) with an elimination of $AcOH/H_2O$. Yellow prism crystals of **3** were obtained from acetone and were suitable for the X-ray analysis. The result is shown in Fig. 3. Thus, it was confirmed that the planar structure of **2** was 1,2,3,4-tetrahydro-1,2,6,7-tetrahydroxy-3-methyl-11*H*-benzo[*a*]fluorenone-4,11-dione.

As described in the previous paper[1], **1** was not soluble in various solvents. Therefore, the sodium salt of **1** (**1**-Na) in DMSO-d_6 was used for the NMR analyses. The ^1H, and ^{13}C NMR spectral analyses, HSQC, DEPT and HMBC experiments of **1**-Na were performed. These data are summarized in Table 1. They showed that **1**-Na had one carbonyl carbon at δ 181.1 (C-1) and two aromatic carbons at δ 137.2 (C-2) and 143.7 (C-3) instead of three aliphatic carbons in the cyclohexenone moiety of **2** (C-1 through C-3). The chemical shifts of the other protons and carbons were similar between **1**-Na and **2**. These data showed that **1** had a quinone moiety instead of the cyclohexenone moiety of **2**.

Compound **3** was converted to **1**-Na to confirm the structure of **1**. Deacetylation of **3** with MeONa, followed by oxidation with manganese (IV) oxide gave **1**-Na (43%). Thus, the structure of **1** was determined to be 1,4-dihydro-6,7-dihydroxy-3-methyl-11*H*-benzo[*a*]-fluorenone-1,4,11-trione (Fig. 1-1).

Fig. 1. Structures of **1**, **2** and **3**.

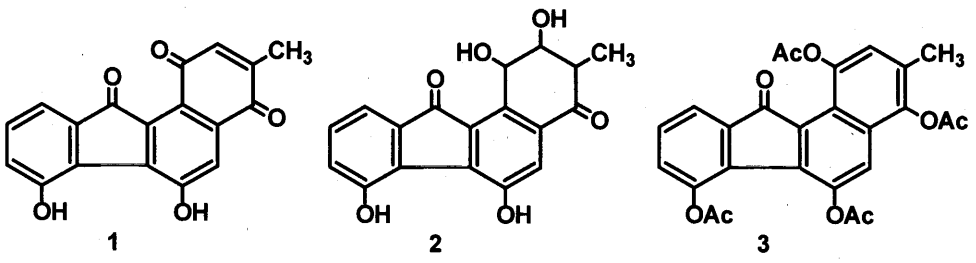
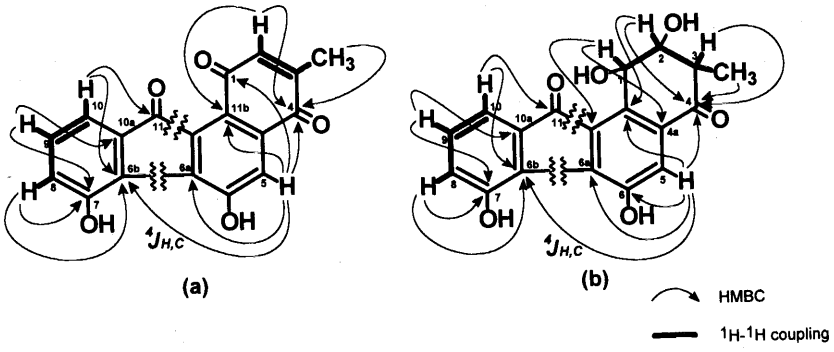


Table 1. ^{1}H and ^{13}C NMR data of **1**, **2** and **3**.

Carbon No.	**1** (sodium salt) in DMSO-d_6		**2** in DMSO-d_6		**3** in Me_2CO-d_6	
	δ_C (100MHz)	δ_H (J in Hz, 400 MHz)	δ_C (100 MHz)	δ_H (J in Hz, 400 MHz)	δ_C (100 MHz)	δ_H (J in Hz, 400 MHz)
1	181.1 (s)		63.5 (d)	5.46 (1H, d, 3.4)	145.1 (s)	
2	137.2 (d)	6.63 (1H, q, 1.9)	74.7 (d)	4.02 (1H, dd, 3.4, 2.1)	126.9 (d)	7.27 (1H, s)
3	143.7 (s)		41.5 (d)	3.17 (1H, dq, 6.9, 2.1)	130.1 (s)	
4	185.7 (s)		197.6 (s)		143.1 (s)	
4a	132.7[a] (s)		132.4 (s)		131.2[b] (s)	
5	121.3 (d)	6.96 (1H, s)	119.4 (d)	7.48 (1H, s)	123.8 (d)	7.97 (1H, s)
6	166.8 (s)		149.0 (s)		145.1 (s)	
6a	139.9 (s)		133.6 (s)		131.5[b] (s)	
6b	127.6 (s)		125.4 (s)		134.4 (s)	
7	155.7 (s)		150.6 (s)		145.7 (s)	
8	124.0 (d)	6.66 (1H, d, 8.0)	123.9 (d)	7.08 (1H, dd, 8.2, 0.9)	130.9 (d)	7.28 (1H, dd, 7.7, 0.9)
9	129.7 (d)	6.97 (1H, dd, 8.0, 6.9)	131.6 (d)	7.31 (1H, dd, 8.2, 7.1)	132.3 (d)	7.48 (1H, dd, 7.7, 7.3)
10	112.6 (d)	6.77 (1H, d, 6.9)	116.2 (d)	7.21 (1H, dd, 7.1, 0.9)	122.5 (d)	7.58 (1H, dd, 7.3, 0.9)
10a	134.0 (s)		135.3 (s)		136.3 (s)	
11	191.6 (s)		192.3 (s)		190.6 (s)	
11a	135.0[a] (s)		134.0 (s)		[b]	
11b	117.4 (s)		134.3 (s)		123.2 (s)	
12	15.2 (q)	1.98 (3H, d, 1.9)	11.3 (q)	1.17 (3H, d, 6.9)	16.2 (q)	16.2 (3H, s)
–OH		16.87 (1H, br s)		11.50 (1H, br s)		
				5.31 (1H, br s)		
–Me					20.5 (q)	2.58 (3H, s)
(4 × Ac)					21.2 (q)	2.57[c] (3H, s)
					21.3 (q)	2.57[c] (3H, s)
					21.8 (q)	2.56 (3H, s)
=CO					169.3 (s)	
(4 × Ac)					169.2 (s)	
					169.6 (s)	
					170.8 (s)	

[a,c] May be exchangable. [b] May be exchangable, however, one carbon signal was not detectable.

Fig. 2. HMBC correlations and ^{1}H-^{1}H couplings for fluostains A (a) and B (b).

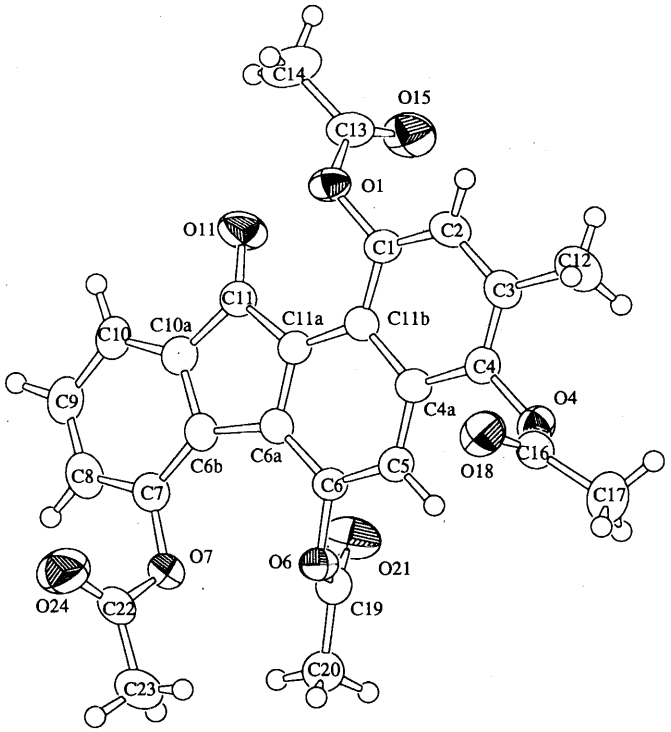


Experimental

General

Chemicals employed were as follows: Acetic anhydride from Wako Pure Chemicals Industries, Ltd., Osaka, Japan; Manganese(IV) oxide from Aldrich Chemical Co.,Inc., Milwaukee, USA; Diaion HP-20 from Nippon Rensui Co., Japan; All other chemicals were of analytical grade. NMR spectra were recorded on a Jeol JNM-GX400 NMR spectrometer. MS spectra were obtained

Fig. 3. Molecular structure of **3**.



The thermal ellipsoids are given at 50% probability.

on a HITACHI M-80H mass spectrometer.

Preparation of **3**

To compound **2** (118.4 mg) in pyridine (10 ml) was added acetic anhydride (0.628 ml) at room temperature. The mixture was stirred for 26 hours, then acetylation was stopped by adding 3 drops of MeOH. The reaction mixture was diluted with water (50 ml) and extracted with EtOAc (50 ml). The organic layer was dried over Na_2SO_4 and evaporated. The residue was dissolved in Me_2CO-hexane and crystallized to give **3** as yellow prisms (70.9 mg, 41%): Rf 0.16 (Toluene - EtOAc, 4 : 1); EI-MS *m/z* 476 (M^+).

X-Ray Crystallographic Analysis of **3**

A yellow prism crystal of **3** having approximate dimensions of $0.60 \times 0.40 \times 0.30$ mm was mounted in a quartz capillary. All measurements were made on a Rigaku AFC5R diffractometer using Ni-filtered Cu-Kα radiation. The crystal data are as follows: Empirical formula; $C_{26}H_{20}O_9$. F. W.; 476.44. Crystal system; orthorhombic. Lattice parameters; a = 13.560(2) Å, b = 19.075(1) Å, c = 17.291(1) Å, V = 4472.5(6) Å^3. Space group; Pbca. Z value; 8. Dcalc; 1.415 g/cm^3. μ (CuKα); 9.12 cm^{-1}. The intensity data were collected by using the ω–2θ scan technique to a maximum 2θ value of 124°. Of the 3959 reflections which were collected, 2293 were unique. The structure was solved by direct methods and refined by the method of full-matrix least-squares with anisotropic thermal parameters for all non-hydrogen atoms. The final *R* value was 0.061.

Chemical Conversion from **3** to **1**-Na

Compound **3** (57.7 mg) in MeOH (100 ml) was treated with 28% MeONa - MeOH (0.245 ml) for 1 minute at 4°C. Manganese (IV) oxide (10 mg) was added and the solution was stirred for another 68 hours at 4°C. Undisolved material was filtered off. The filtrate was neutralized by AcOH and evaporated under reduced pressure. The residue (34.7 mg) was dissolved in 0.005 N aq. $NaHCO_3$ (100 ml), and was adsorbed on Diaion HP-20 column (100 ml) at room temperature. After washing with 10% aq. Me_2CO, the column was eluted with 30% aq. Me_2CO to give **1**-Na as purple needles (17.6 mg, 43%). ^{1}H NMR spectrum of the product was essentially the same as that of natural **1**-Na.

Reference

1) AKIYAMA, T.; S. HARADA, F. KOJIMA, Y. TAKAHASHI, C. IMADA, Y. OKAMI, Y. MURAOKA, T. AOYAGI & T. TAKEUCHI: Fluostatins A and B, new inhibitors of dipeptidyl peptidase III, produced by *Streptomyces* sp. TA-3391. I. Taxonomy, production and biological activities. J. Antibiotics 51: 553～559, 1998