CV-1, A NEW ANTIBIOTIC PRODUCED BY A STRAIN OF *STREPTOMYCES* SP.

II. STRUCTURE DETERMINATION

Tohru Yasuzawa, Mayumi Yoshida, Michio Ichimura, Kunikatsu Shirahata and Hiroshi Sano*

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.,
3-6-6 Asahimachi, Machida-shi, Tokyo 194, Japan

(Received for publication February 3, 1987)

The structure of a new antibiotic, CV-1 was determined to be 1,2-diamino-1,2-*N,N'*-carbonyl-1,2-dideoxy-α-D-glucose hydrate by spectral and chemical studies. CV-1 possessed a unique open ring hemiaminal structure. CV-1 synthesized from *N*-carbamoyl-D-glucosamine was identical to material isolated from fermentation.

CV-1 is a new antibiotic isolated from the culture broth of *Streptomyces* sp. (CO-1), and shows antibacterial activity against Gram-negative bacteria, *Escherichia coli*, in combination with spiramycin. Spiramycin alone is not active against this organism. The fermentation, isolation and biological properties of CV-1 have been reported by Ichimura *et al.*[1] We will describe the structure determination of CV-1 in this paper.

CV-1 (**1**), mp 85~90°C, $[\alpha]_D^{28}$ +66° (*c* 0.9, H_2O), was obtained as a hygroscopic colorless powder. **1** was anisaldehyde positive and gave an Rf value 0.35 (CH_3CN - H_2O, 3:2) on HPTLC (NH_2 $F_{254}s$, Merck). The high resolution fast atom bombardment mass spectrum (HRFAB-MS) indicated that **1** had the molecular formula of $C_7H_{14}N_2O_6$. The IR spectrum of **1** contained strong absorption bands at 3350 and 1685 cm^{-1}, compatible with hydroxyl and amide groups. **1** was unstable in aqueous solution and gradually changed to a stable but biologically inactive compound **2**. This reaction was accelerated by heat or acid.

2 was also obtained by heating an aqueous solution of *N*-carbamoyl-D-glucosamine (**3**),[2] which was prepared from D-glucosamine. In the ^{13}C NMR spectra of **2** and its triacetate (**6**) chemical shifts of C-1 (δ 87.5 and 86.8, respectively) suggested that C-1 is an aminal carbon. The ^1H NMR spectrum of **6** showed the presence of couplings between H-1 and 1-NH, and between H-2 and 2-NH, and downfield shifts of the resonances assigned to H-3, H-5 and H-6a, H-6b compared with those the analogous protons of **2**. This implies that the structure of **2** is 1,2-diamino-1,2-*N,N'*-carbonyl-1,2-dideoxy-α-D-glucofuranose. **2** was identical with a degradation product of the antibiotic SF-1993 for which an incorrect pyranose structure was originally proposed.[3]

As **1** gave **2** in aqueous solution but never gave **3**, it seemed likely that **1** was an isomer of **3** and could be produced during the conversion process of **2** from **3**. This process was confirmed by the observation of the anomeric proton signals of each compound in a ^1H NMR experiment. A solution of D-glucosamine in D_2O was treated with an equivalent of potassium cyanate in an NMR tube. After 15 minutes (Fig. 1-1) the signals of D-glucosamine (a and b) were observed together with those of the newly formed **3** (c and d). Small signals of **1** (e and f) were also observed. The signal intensities of **3** and **1** increased with time (Figs. 1-2 and 1-3). After 2 hours (Fig. 1-4) the concentration of **3** was at

its maximum, and then the signals of **3** gradually diminished and were replaced by those of **1** (Fig. 1-5). After 2 days, the signal of **1** (f) became a main signal in the solution and that of **2** (g) also appeared. These results demonstrated the conversion of **3** to **1** and then to **2** (Fig. 2).

CV-1 was synthesized from D-glucosamine in a similar procedure and purified by HPLC. The sample was identical with naturally occurring material in all aspects including specific rotation.

The ^{13}C NMR spectrum of **1** showed the presence of one amide group, one aminal function, one oxygen-bearing methylene, three oxygen-bearing methine and one nitrogen-bearing methine.

The structure of **1** shown in Fig. 2 was deduced by detailed ^{1}H NMR analyses in D_2O and $(CD_3)_2SO$ solutions. The chemical shifts of all protons and their coupling constants in D_2O solution are presented in Table 1. (Decoupling experiments were difficult owing to overlap of some of these signals.) In $(CD_3)_2SO$ solution, where **1** was present as a mixture of two anomers (**1a** and **1b**), H-1 was coupled to both 1-OH and 1-NH protons, which indicated the presence of hemiaminal moiety on C-1 (Table 3). Another NH and four OH protons (4.2~4.7 ppm) were also observed. From these results the unique open-chain hexose structure having an imidazolidinone moiety was established for **1**.

Fig. 1. ^{1}H NMR spectra of the reaction mixture of D-glucosamine with potassium cyanate in D_2O (400 MHz).

Additional proof was obtained: Treatment of **1** with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine gave two trimethylsilylates of dehydrates (**4** and **5**) (Fig. 3). The structures of **4** and **5** were confirmed by ^{1}H NMR decoupling studies. The ^{1}H NMR spectrum of **4** showed the presence of four TMS and two amide protons, and allyl coupling between H-1 and H-3 were observed, thereby confirming the sequence between the C-1 methine and the C-6 methylene. These observations substantiate the structure of **1**.

Fig. 2. Conversion process from **3** to **2**.

Table 1. ^{1}H NMR data for **1**, **2**, **4**, **5** and **6**.[a,b]

Proton	**1**	**2**	**4**
1	5.21 (d, *J*=2.4)	5.74 (d, *J*=6.3)	6.11 (br s)
2	3.75 (dd, *J*=5.9, 2.4)	4.22 (d, *J*=6.3)	—
3	3.85 (dd, *J*=5.9, 1.8)	4.20 (br s)	4.51 (dd, *J*=6.8, 0.8)
4	3.62 (dd, *J*=8.6, 1.8)	*ca.* 3.8 (m)	3.66 (dd, *J*=6.8, 2.1)
5	*ca.* 3.75 (m)		3.74 (ddd, *J*=6.5, 6.1, 2.1)
6	3.65 (dd, *J*=11.7, 6.1)	3.75 (dd, *J*=12.0, 2.3)	3.62 (dd, *J*=9.8, 6.5)
	3.83 (dd, *J*=11.7, 2.8)	3.59 (dd, *J*=12.0, 5.3)	3.33 (dd, *J*=9.8, 6.1)
N*H*CON*H*	—	—	9.81 (br s)
	—	—	8.02 (br s)
			0.12, 0.11, 0.08, 0.07 (TMS, all s)

Proton	**5**	**6**
1	5.60 (d, *J*=6.0)	5.79 (dd, *J*=6.2, 1.1)
2	3.93 (d, *J*=6.0)	4.19 (dd, *J*=6.2, 1.7)
3	4.13 (d, *J*=1.9)	5.02 (d, *J*=2.9)
4	3.59 (dd, *J*=8.8, 1.9)	4.33 (dd, *J*=9.1, 2.9)
5	3.98 (ddd, *J*=8.8, 7.2, 2.1)	5.25 (ddd, *J*=9.1, 5.6, 2.4)
6	3.51 (dd, *J*=10.7, 7.2)	4.56 (dd, *J*=12.3, 2.4)
	3.82 (dd, *J*=10.7, 2.1)	4.11 (dd, *J*=12.3, 5.6)
N*H*CON*H*	—	—
	—	—
	0.32, 0.28, 0.20, 0.08, 0.07 (TMS, all s)	

[a] **1** and **2** were measured in D_2O with DSS as an internal standard, and **4**, **5** and **6** were in $CDCl_3$ with TMS as an internal standard.
[b] 400 MHz; chemical shifts in ppm, coupling constants in Hz.

Table 2. ^{13}C NMR data for **1**, **2**, **4**, **5** and **6**.[a,b]

Carbon	**1**	**2**	**4**	**5**	**6**
1	80.0	87.5	106.4	90.1	86.8
2	64.4	64.6	123.3	67.3	62.1
3	70.4[c]	75.2	80.3	77.0	75.4
4	71.2[c]	79.2	68.8	78.9	75.3
5	71.6[c]	69.4	72.8	71.3	67.5
6	63.7	64.4	62.8	65.3	63.0
NHCONH	164.0	164.5	155.3	166.2	161.9
			(TMS) 0.6 (3)	(TMS) 1.3 (3)	(CH_3CO) 20.4 (3)
			0.3 (3)	1.0 (3)	170.4
			0.0 (3)	0.0 (3)	169.6
			−0.6 (3)	−0.6 (3)	169.4
				−0.7 (3)	

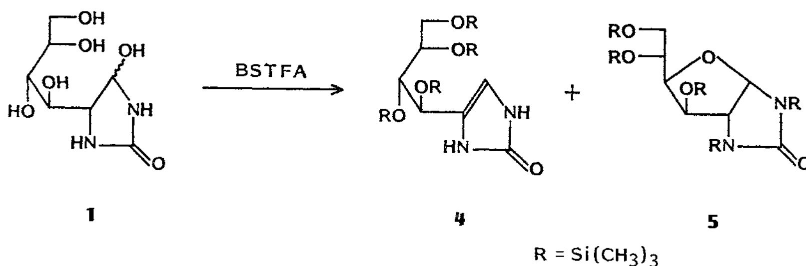
[a] 100 MHz; chemical shifts in ppm.
[b] **1** and **2** were measured in D_2O with DSS as an internal standard, and **4**, **5** and **6** were in $CDCl_3$ with TMS as an internal standard.
[c] These assignments may be interchangeable.

Table 3. ^{1}H NMR data of **1** (**1a** and **1b**) (400 MHz, DMSO-d_6).

Proton	**1a**	**1b**
H-1	4.86 (br d, *J*=*ca.* 7 Hz)	4.96 (br t, *J*=*ca.* 6 Hz)
1-OH	5.83 (d, *J*=7.2 Hz)	5.56 (d, *J*=7.2 Hz)
1-NH	6.98 (br s)	6.84 (br s)
2-NH	5.87 (br s)	5.83 (br s)

Couplings between H-1 and H-2, between H-1 and 1-OH and between H-1 and 1-NH were confirmed by decoupling experiments.

Fig. 3. Trimethylsilylation of 1.



BSTFA : *N,O*-Bis(trimethylsilyl)trifluoroacetamide

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer with TMS (0 ppm), 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt hydrate (DSS, 0 ppm) and dioxane (67.4 ppm) as the internal standards. IR spectra were obtained using a Shimadzu IR-27G spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Secondary ion mass spectra (SI-MS) and high resolution electron impact mass spectra (HREI-MS) were measured on Hitachi M-80B mass spectrometer. Melting points were taken with a Yanagimoto melting point apparatus and were not corrected. Thin-layer chromatography was performed on pre-coated plates, Merck HPTLC NH_2 F_{254}S and compounds were detected with anisaldehyde.

Synthesis of 2

To a solution of D-glucosamine hydrochloride (2.0 g) in H_2O (5 ml), potassium cyanate (380 mg) was added and stirred for 30 minutes at room temp. The reaction mixture was passed through a column of Sephadex G-10 and the eluent containing *N*-carbamoyl-D-glucosamine was heated on a water bath (50°C) for 2 hours. The solution was lyophilized to give **2** (1.8 g, 95%) as a colorless powder. Rf 0.49 (CH_3CN - H_2O, 7:3); $[\alpha]_D^{28}$ −47.0° (*c* 1.0, H_2O); IR (KBr) cm^{-1} 3350, 1690.

Acetylation of 2

To a solution of **2** (50 mg) in pyridine (1 ml), acetic anhydride (0.5 ml) was added and stirred for 2 hours at room temp. The reaction mixture was poured into water and extracted with EtOAc. The extract was washed with dil HCl followed by satd $NaHCO_3$ and brine, dried and evaporated to afford a colorless powder, which was subjected to silica gel column chromatography eluted with $CHCl_3$ - MeOH (10:1) to give **6** (75 mg, 91%) as colorless needles (recrystallization from MeOH). MP 183~184°C; $[\alpha]_D^{28}$ +19.6° (*c* 0.62, $CHCl_3$); EI-MS *m/z* 331 $(M+H)^+$, 271, 228, 211, 168, 151, 127; HR-MS calcd for $C_{13}H_{19}N_2O_8$: 331.1140, found: 331.1118.

Synthesis and Purification of 1, 2 and 3

To a solution of D-glucosamine hydrochloride (100 mg) in H_2O (5 ml), potassium cyanate (45 mg) was added and stirred for 40 hours at room temp. The solution was purified by HPLC [Shimadzu LC-3A with DuPont ZORBAX NH_2 (4.6 mm×25 cm)] eluting with CH_3CN - H_2O (4:1) to afford **3** (25 mg, 25%), **2** (26 mg, 28%) and **1** (46 mg, 44%) in the order of elution.

Trimethylsilylation of CV-1

To a solution of CV-1 (**1**, 40 mg) in pyridine (1 ml), BSTFA (0.5 ml) was added and stirred for 30 minutes at room temp. The reaction mixture was evaporated and chromatographed on silica gel column with hexane - EtOAc (30:1) to give tetrasilylate **4** (36 mg, 41%) and pentasilylate **5** (19 mg, 16%) in the order of elution.

Acknowledgment

We wish to thank Mr. A. NAKAMURA for NMR spectroscopy and Miss Y. ISHIHARA for mass spectroscopy.

References

1) ICHIMURA, M.; T. KOGUCHI, T. YASUZAWA & F. TOMITA: CV-1, a new antibiotic produced by a strain of *Streptomyces* sp. I. Fermentation, isolation and biological properties of the antibiotic. J. Antibiotics 40: 723~726, 1987
2) MICHEEL, F. & W. LENGSFELD: Über die Reaktionen des D-Glucosamines, II. Mitteil.; Verbindungen von D-Glucosamin mit Aminosäuren. Chem. Ber. 89: 1246~1253, 1956
3) OMOTO, S.; T. SHOMURA, H. SUZUKI & S. INOUYE: Studies on actinomycetales producing antibiotics only on agar culture. II. Isolation, structure and biological properties of N-carbamoyl-D-glucosamine (substance SF-1993). J. Antibiotics 32: 436~441, 1979