STRUCTURE ELUCIDATION OF AN INTERMEDIATE OF 2-DEOXYSTREPTAMINE BIOSYNTHESIS

KIKUO IGARASHI, TSUNETOSHI HONMA, TAKASHI FUJIWARA and EIJI KONDO

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan

(Received for publication December 12, 1979)

The structure of a 2-deoxystreptamine (DOS) precursor named S-11-P, which was isolated by using a DOS negative mutant derived from a xylostasin-producing strain of *Bacillus circulans* B15M, has been elucidated as (1 L)-1,3,5/2,4-5-aminocyclohexanetetrol by comparison with an authentic specimen, which was synthesized from kanamycin A.

Results and Discussion

In a preceding paper, one of the authors has isolated a 2-deoxystreptamine (DOS) precursor named S-11-P by using a DOS negative mutant derived from a xylostasin-producing strain of *Bacillus circulans* B15M and inferred the structure to be that in which one of the two amino groups in DOS is replaced by a hydroxyl group. There are two possibilities for the structure of the compound, namely, (1 L)- and (1 D)-1,3,5/2,4-5-aminocyclohexanetetrols, from its ^{1}H and ^{13}C NMR spectral data. This paper deals with the preparation of the former compound and its comparison with S-11-P.

To prepare it, we have chosen 3,6′,3″-tri-N-formylkanamycin A[1] (**2**) as starting material. In the original method, **2** was prepared by partial hydrolysis of 1,3,6′,3″-tetra-N-formylkanamycin A (**1**) with 10% ammonium hydroxide for 5 days. We found that the hydrolysis was accomplished with hydrochloric acid in aq. methanol at 37°C for 20 hours and **2** was obtained in 16.4% yield after chromatography of the product on Amberlite CG-50 resin (NH_4^+ form). When **2** reacted with 1.12 molar equivalents of 3,5-di-*t*-butyl-1,2-benzoquinone[2] and the product was hydrolyzed with oxalic acid, a product (**3**), which showed a negative ninhydrin test but positive *o*-dianisidine test, was obtained in 74.4% yield. The IR spectrum of **3** showed a newly produced carbonyl band at 1730 cm^{-1}. From these results, the structure of **3** was found to be 1-deamino-3,6′,3″-tri-N-formyl-1-oxokanamycin A. Compound **3** was reduced with sodium borohydride and the removal of the formyl groups gave two products (**4** and **5**) in 39.8% and 23.5% yields, respectively. The ^{1}H NMR spectrum of a sulfate of **5** showed a multiplet centered at δ 4.97 due to the equatorial H-1. ^{13}C NMR data of the sulfates of **4** and **5** are shown in Table 1. The carbons C-1, C-2, C-5, C-6 and C-1″ of **5** are at higher field than those of **4** and the differences of the chemical shifts of these carbon atoms are very similar to those of 1-deamino-1-hydroxysisomicin[3]. From these results the configuration of the hydroxyl group of **4** appeared to be equatorial and that of **5** axial.

Hydrolysis of **4** and **5** was performed with 6 N hydrochloric acid under reflux for 20 hours and the products were chromatographed on Amberlite CG-50 resin (NH_4^+ form) to give (1 L)-1,3,5/2,4-5-aminocyclohexanetetrol (**6**) and (1 D)-1,2,4/3,5-5-aminocyclohexanetetrol (**7**) in quantitative yields, respectively. Compound **6** was found to be identical with S-11-P by comparison of their rotation values,

Chart 1.

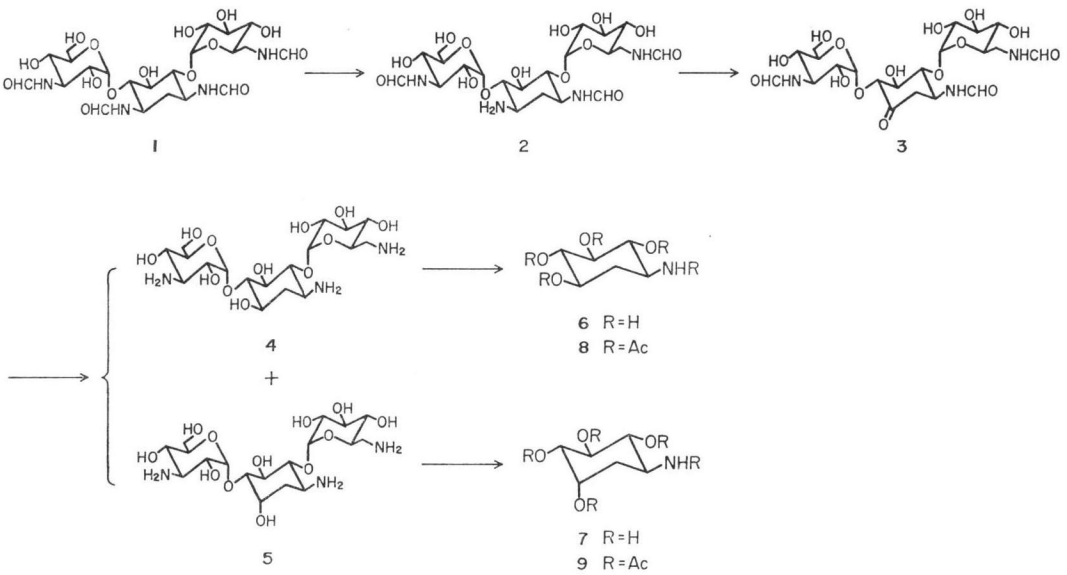
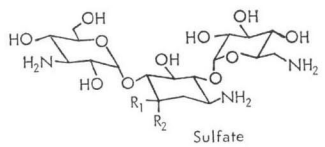
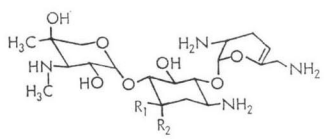


Table 1. ^{13}C NMR chemical shifts of the sulfates of 1-deamino-1-hydroxy- (**4**) and 1-deamino-1-*epi*-hydroxy- (**5**) kanamycins A compared with those of sisomicin analogs.





	R_1=OH R_2=H (**4**)	R_1=H R_2=OH (**5**)	Δ (R_1=OH)− (R_2=OH)	R_1=OH R_2=H	R_1=H R_2=OH	Δ (R_1=OH)− (R_2=OH)
C_1	69.8	64.3	5.5	71.0	65.3	5.7
C_2	33.5	31.6	1.9	36.6	34.5	2.1
C_3	48.3	47.8	0.5	49.0	47.8	1.2
C_4	79.9	79.5	0.4	84.7	85.7	−1.0
C_5	72.8	71.0	1.8	74.8	72.9	1.9
C_6	84.2	80.1	4.1	85.2	79.8	5.4
C_1'	96.8	96.8	0	100.8	101.0	−0.2
C_1''	98.8	95.5	3.3	100.5	96.8	3.7

and ^1H NMR (see Fig. 1) and IR spectra. Furthermore, the pentaacetate of **6** (**8**) and S-11-P pentaacetate were identical by comparison of their physical and spectral data and by mixture melting point determination.

Experimental

General

^1H NMR spectra were measured with a Varian T-60 NMR spectrometer using tetramethylsilane as an external standard. ^{13}C NMR spectra were measured in deuterium oxide with a Varian NV-14 FT NMR spectrometer using acetonitrile as an internal standard. Optical rotations were measured in water

with a Perkin-Elmer Model 141 polarimeter, unless otherwise stated. Sulfates were prepared, unless otherwise stated, as follows: One part of an amine was dissolved in 10 parts of water and the solution was adjusted to pH 4.5 by adding 0.1 N sulfuric acid. The solution was concentrated to one-tenth volume and ethanol was added. The resulting precipitate was collected by filtration, washed with ethanol, and dissolved in a small amount of water. The solution was treated with active carbon and lyophilized. The lyophilizate was allowed to stand in a desiccator containing 200 g of sodium bromide and 100 ml of water until the weight became constant by absorption of moisture.

3,6′,3″-Tri-N-formylkanamycin A (**2**)

To a solution of 38.6 g of 1,3,6′,3″-tetra-N-formylkanamycin A[1] (**1**) dissolved in 40 ml of water were added 5.53 ml of concentrated hydrochloric acid and 200 ml of methanol, with stirring, and the solution was kept at 37°C in a thermostated bath for 20 hours and neutralized with 95 ml of Amberlite IR-45 resin (OH^- form). The resin was filtered off and washed with 100 ml of water. The combined filtrate and washing was evaporated to dryness under reduced pressure. The residue was dissolved in 100 ml of water, adsorbed on a column of 4.0 liters of Amberlite CG-50 resin (NH_4^+ form), and eluted with water. Each fraction was 20 ml. From fractions 81~141, 12.44 g (32.2%) of **1** was recovered. From fractions 142~171, 1.48 g (4.0%) of 1,6′,3″-tri-N-formylkanamycin A was obtained as a colorless foam: $[\alpha]_D^{21.5}+118.9\pm1.5°$ (*c* 1.032) [lit.[1] $[\alpha]_D+118.3°$ (*c* 1.0)]. From fractions 186~263, 6.02 g (16.4%) of pure **2** was obtained as a colorless foam: $[\alpha]_D^{21.5}+130.5\pm1.8°$ (*c* 0.954) [lit.[1] $[\alpha]_D+123.2°$ (*c* 1.0)].

Anal. Calcd. for $C_{21}H_{36}N_4O_{14}\cdot H_2O$: C, 43.00; H, 6.53; N, 9.55.
Found: C, 43.02; H, 6.72; N, 9.45.

From fractions 298~470, 2.65 g (7.2%) of 1,3,6′-tri-N-formylkanamycin A was obtained as a colorless foam: $[\alpha]_D^{22}+113.8\pm1.5°$ (*c* 1.019) [lit.[1] $[\alpha]_D+101.8°$ (*c* 1.0)].

1-Deamino-3,6′,3″-tri-N-formyl-1-oxokanamycin A (**3**)

To a solution of 5.0 g of **2** dissolved in 70 ml of water and 70 ml of methanol was added, dropwise under nitrogen, 2.06 g of 3,5-di-*tert*-butyl-1,2-benzoquinone dissolved in 40ml of methanol and the solution was kept at room temperature overnight. Oxalic acid (890 mg) was added and the solution was stirred for 2 hours. After 300 ml of water was added the solution was extracted with dichloromethane. The water layer was treated with active carbon, concentrated to 80 ml under reduced pressure, and passed through a column of 40 ml of Amberlite MB-3 resin. The resin was washed with 200 ml of water. The eluates were treated with active carbon and concentrated to dryness under reduced pressure to give 3.827 g (74.4%) of **3** as a colorless foam. Compound **3** showed a negative ninhydrin test but a positive *o*-dianisidine test: $[\alpha]_D^{24.5}+150.2\pm1.8°$ (*c* 1.045); IR (KBr pellet) 1730 cm^{-1}.

Anal. Calcd. for $C_{21}H_{33}N_3O_{15}\cdot 2H_2O$: C, 41.79; H, 6.18; N, 6.96.
Found: C, 42.00; H, 6.48; N, 6.89.

1-Deamino-1-hydroxy- (**4**) and 1-deamino-1-*epi*-hydroxy- (**5**) kanamycins A

To a cooled solution of 3.7 g of **3** dissolved in 125 ml of water and 25 ml of methanol was added 1.32 g of sodium borohydride, with stirring. After 2.5 hours, the solution was adjusted to pH 3 by adding 10% hydrochloric acid, the solution was passed through a column of 250 ml of Amberlite MB-3 and the column was washed with 600 ml of water. The eluate was concentrated to dryness under reduced pressure. The residue was hydrolyzed with a mixture of 8 ml of water, 35 ml of methanol and 7 ml of concentrated hydrochloric acid at 36°C for 24 hours. The solution was neutralized with 150 ml of Amberlite IR-45 resin (OH^- form) and the resin was filtered off and washed with water. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in 5 ml of water, adsorbed on a column of 1.5 liters of Amberlite CG-50 resin (NH_4^+ form) and eluted with 2.5 liters of water and 2.5 liters of 1 N ammonium hydroxide by the gradient method. Each fraction was 20 ml. Since the separation of the hydroxy compounds was poor, the chromatography was repeated four times and, finally, 234 mg (38%) of the less polar compound (**4**) and 96 mg (15.6%) of the more polar compound (**5**) were obtained, together with 170 mg (27.6%) of a mixture of **4** and **5**.

Sulfate of **4**: $[\alpha]_D^{25.5}+99.6\pm1.3°$ (*c* 1.064).

Anal. Calcd. for $C_{18}H_{35}N_3O_{12}\cdot 1.5H_2SO_4\cdot 6H_2O$: C, 29.19; H, 6.81; N, 5.67; S, 6.49.
Found: C, 29.39; H, 6.76; N, 5.87; S, 6.88.

Sulfate of **5**: $[\alpha]_D^{25.5}$ +126.5±1.6° (*c* 1.034); ^{1}H NMR δ 4.97 (multiplet, H-1).

Anal. Calcd. for $C_{18}H_{35}N_3O_{12}\cdot 1.5H_2SO_4\cdot 6H_2O$: C, 29.19; H, 6.81; N, 5.67; S, 6.49.
Found: C, 29.41; H, 6.73; N, 5.70; S, 6.12.

(1 L)-1,3,5/2,4-5-Aminocyclohexanetetrol (**6**) and its pentaacetate (**8**)

A solution of 1.05 g of **4** dissolved in 20 ml of 6 N hydrochloric acid was refluxed for 23 hours. After cooling, the solution was treated with activated carbon and evaporated to dryness under reduced pressure. The residue was dissolved in a small amount of water, adsorbed on a column of 250 ml of Amberlite CG-50 resin (NH_4^+ form) and eluted with 2 liters of water and 2 liters of 0.1 N ammonium hydroxide by the gradient method. Each fraction was 18 ml. From fractions 165~184, 349 mg (98.8%) of **6**

Fig. 1. ^{1}H NMR spectra of the sulfates of S-11-P and (1 L)-1,3,5/2,4-5-amino- (**6**) and (1 D)-1,2,4/3,5-5-aminocyclohexanetetrols (**7**) (in D_2O)

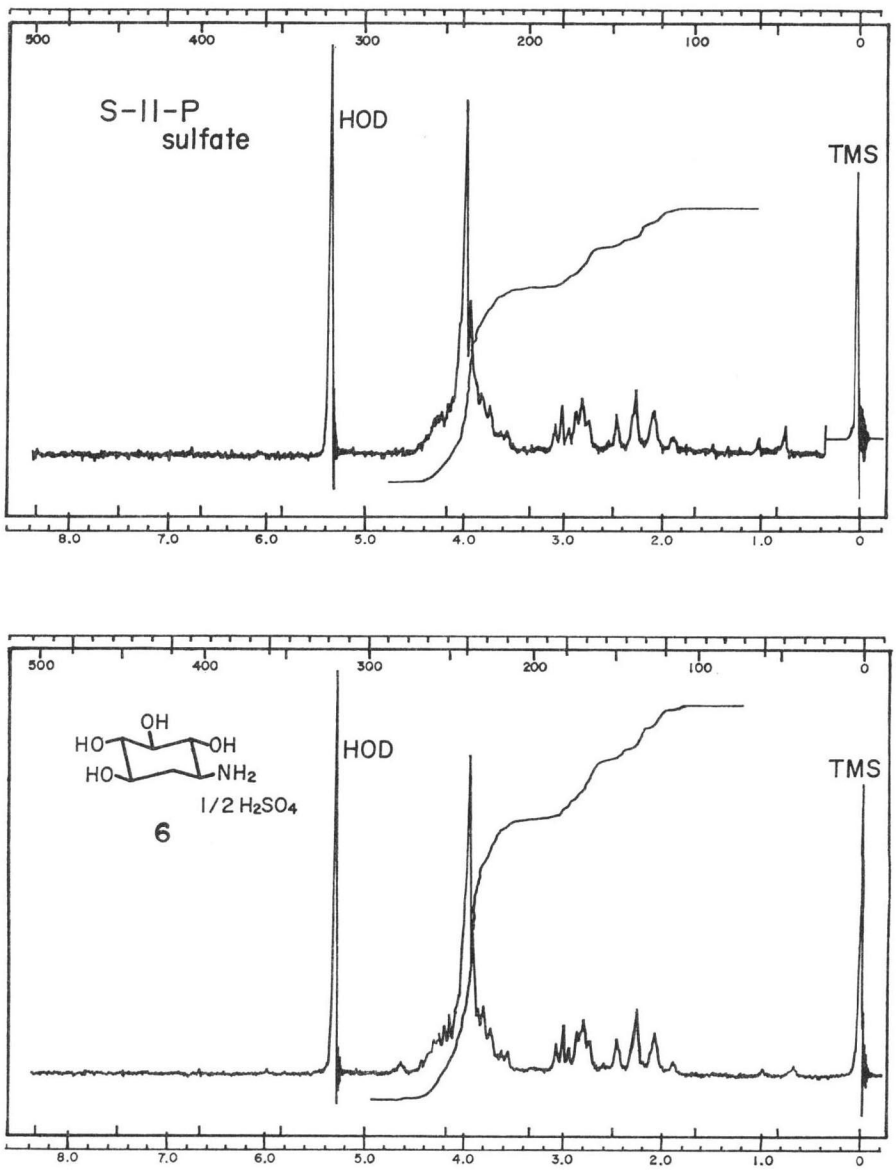
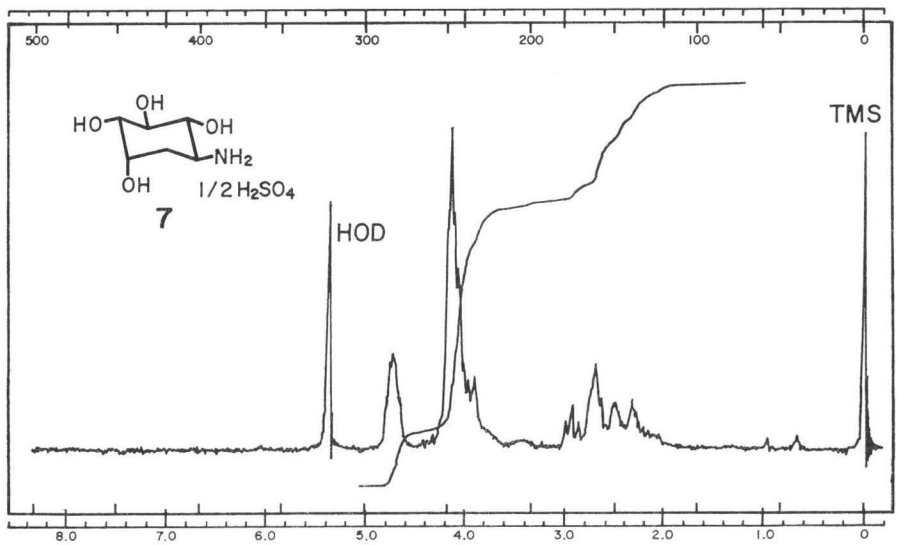


Fig. 1. (continued)



was obtained as a colorless foam. The sulfate of **6** was so hygroscopic that it was analyzed immediately after lyophilization: $[\alpha]_D^{24.5}+3.7\pm0.5°$ (*c* 1.023).

Anal. Calcd. for $C_6H_{13}NO_4 \cdot 0.5H_2SO_4 \cdot H_2O$: C, 31.30; H, 7.01; N, 6.08; S, 6.96.
Found: C, 31.20; H, 7.34; N, 5.98; S, 6.84.

This was identical with S-11-P sulfate, $[\alpha]_D^{24.5}+3.6\pm0.5°$ (*c* 1.015), by comparison of their thin-layer mobilities and IR and ^1H NMR spectra.

To a solution of 55 mg of **6** dissolved in 0.2 ml of water and 0.6 ml of methanol was added 0.06 ml of acetic anhydride and the solution was left at room temperature for 3 hours. The solution was evaporated to dryness under reduced pressure, the residue was dissolved in 1 ml of pyridine and 0.5 ml of acetic anhydride, and the solution was kept at room temperature overnight. The solution was evaporated to dryness under reduced pressure, the residue was dissolved in 10 ml of toluene and the solution was evaporated. The residue was recrystallized from ethanol to give 81 mg of **8**, mp 191.5~192°C, $[\alpha]_D^{24.3}-11.5\pm0.6°$ (*c* 1.039, $CHCl_3$), as colorless leaflets.

Anal. Calcd. for $C_{16}H_{23}NO_9$: C, 51.47; H, 6.21; N, 3.75.
Found: C, 51.23; H, 6.38; N, 3.72.

This was identical with S-11-P pentaacetate, mp 192°C, $[\alpha]_D^{22.3}-11.4\pm0.5°$ (*c* 1.040, $CHCl_3$), by comparison of their IR and ^1H NMR spectra and mixture melting point determination.

(1 D)-1,2,4/3,5-5-Aminocyclohexanetetrol (**7**) and its pentaacetate (**9**)

Compound **5** was also hydrolyzed and treated as described above to give **7** in quantitative yield. The sulfate was also very hygroscopic and was analyzed immediately after lyophilization: $[\alpha]_D^{25}+39.9\pm0.7°$ (*c* 1.093).

Anal. Calcd. for $C_6H_{13}NO_4 \cdot 0.5H_2SO_4 \cdot 1.5H_2O$: C, 30.12; H, 7.16; N, 5.85; S, 6.70.
Found: C, 30.51; H, 7.51; N, 5.76; S, 7.16.

Compound **7** was acetylated as described above and the acetate was recrystallized from ethanol: mp 155~156°C: $[\alpha]_D^{25}+8.9\pm0.5°$ (*c* 1.042, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{23}NO_9$: C, 51.47; H, 6.21; N, 3.75.
Found: C, 51.17; H, 6.27; N, 3.80.

References

1) Horii, S.; H. Fukase, Y. Kameda & N. Mizokami: A new method for selective N-acylation of aminoglycoside antibiotics. Carbohyd. Res. 60: 275~288, 1978

2) COREY, E. J. & K. ACHIWA: A new method for the oxidation of primary amines to ketones. J. Am. Chem. Soc. 91: 1429~1432, 1969

3) MALLAMS, A. K.; D. H. DAVIES, D. L. BOXLER, H. F. VERNARY & P. REICHERT: The synthesis and antibacterial activity of 1-*epi*-derivatives of netilmicin, sisomicin and gentamicin. Abstr. Paper No. 251, 17th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, N.Y., Oct. 1977