SYNTHESIS OF (1D)-1,3,5/2,4- AND (1L)-1,2,4/3,5-5-AMINOCYCLOHEXANETETROLS

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(Received for publication December 16, 1980)

Synthesis of (1D)-1,3,5/2,4- and (1L)-1,2,4/3,5-5-aminocyclohexanetetrols starting from kanamycin A is described.

In a previous paper[1], we reported the synthesis of (1L)-1,3,5/2,4- and (1D)-1,2,4/3,5-5-aminocyclohexanetetrols from kanamycin A. This paper deals with the synthesis of (1D)-1,3,5/2,4- and (1L)-1,2,4/3,5-5-aminocyclohexanetetrols.

Results and Discussion

Treatment of 1,6′,3″-tri-N-formylkanamycin A[1,2] (**1**) with two molar equivalents of 3,5-di-*t*-butyl-1,2-benzoquinone[3,4] and hydrolysis of the product with oxalic acid gave 3-deamino-1,6′,3″-tri-N-formyl-3-oxokanamycin A (**2**), which showed a negative ninhydrin test and a positive *o*-dianisidine test, in 84.2% yield. The IR spectrum of **2** showed a newly produced carbonyl band at 1732 cm^{-1}. Reduction of **2** with sodium borohydride and removal of the formyl groups with hydrochloric acid gave a mixture of epimeric 3-deamino-3-hydroxykanamycin A (**3**). Contrary to the case of 1-deamino-1-hydroxy derivatives[1], the mixture could not be separated by a column chromatography using Amberlite CG-50 resin (NH_4^+ form). Hydrolysis of **3** with hydrochloric acid under reflux for 24 hours and chromatography of the product gave two compounds (**4** and **5**) in 52.3 and 31.6% yields, respectively. Elemental analyses of the sulfates of both compounds agreed with $C_6H_{13}NO_4 \cdot 0.5H_2SO_4 \cdot H_2O$. Peracetylation of **4** and **5** gave their pentaacetates (**6** and **7**). The physical properties of these compounds (**4**～**7**) are summarized in Table 1. As shown, absolute rotation values of **4** sulfate and **6**, and **5** sulfate and **7** are identical with those of (1L)-1,3,5/2,4-5-amino-

Chart 1.

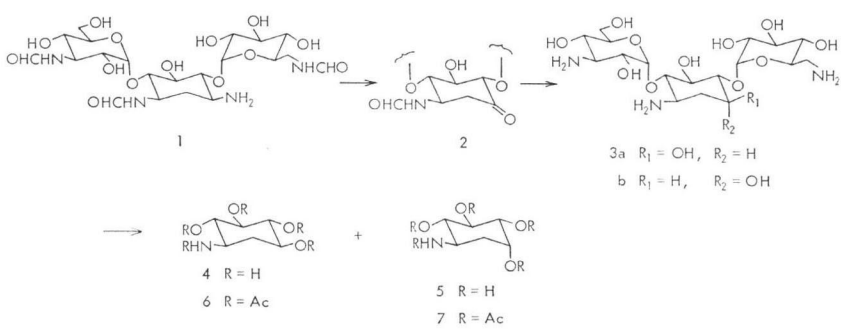
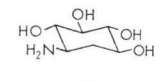
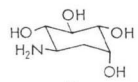
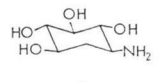
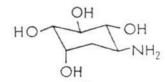
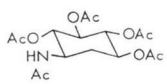
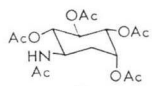
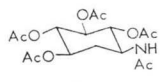
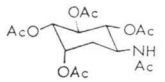


Table 1. Properties of (1D) and (1L)-1,3,5/2,4-5-aminocyclohexanetetrols, (**4**) and (**8**), (1L) and (1D)-1,2,4/3,5-5-aminocyclohexanetetrols, (**5**) and (**9**), and their respective pentaacetates (**6**, **10**, **7**, and **11**).

Sulfate				
	4	**5**	**8**	**9**
$[\alpha]_D(H_2O)$	−3.4±0.4°	−44.0±1.2°	+3.7±0.5°	+39.9±0.7°
				
	6	**7**	**10**	**11**
M.p.	191.0~192.0°	155.5~156.5°	191.5~192.0°	155.0~156.0°
$[\alpha]_D(CHCl_3)$	+11.0±0.5°	−9.7±0.5°	−11.5±0.6°	+8.9±0.5°

cyclohexanetetrol (**8**) sulfate and its pentaacetate (**10**), and (1D)-1,2,4/3,5-5-aminocyclohexanetetrol (**9**) sulfate and its pentaacetate[1] (**11**), respectively but the signs are opposite. Furthermore, ^{1}H NMR and IR spectra of the sulfates of **4** and **5** are superimposed on those of the sulfates of **8** and **9**, respectively, and IR spectra of **6** and **7** are identical with those of **10** and **11**. From these results, it was concluded that the structures of **4** and **5** are (1D)-1,3,5/2,4- and (1L)-1,2,4/3,5-5-aminocyclohexanetetrols, respectively.

Experimental

General

^{1}H NMR spectra were measured with a Varian T-60 NMR spectrometer using tetramethylsilane as an external standard. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. IR spectra were measured with a JASCO DS-403G. Sulfates were prepared by a method described previously[1].

3-Deamino-1,6′,3″-tri-N-formyl-3-oxokanamycin A (2)

To a solution of 1.258 g of 1,6′,3″-tri-N-formylkanamycin A[1,2] dissolved in 20 ml of water was added, dropwise under nitrogen, 540 mg of 3,5-di-*t*-butyl-1,2-benzoquinone dissolved in 10 ml of methanol for 20 minutes and the solution was kept at room temperature overnight. Oxalic acid (340 mg) was added and the solution was stirred for 2 hours. The solution was diluted with 300 ml of water and extracted with dichloromethane. The water layer was treated with active carbon, concentrated to 20 ml, and passed through a column of 25 ml of Amberlite MB-3 resin. The resin was washed with 50 ml of water. The eluates were treated with active carbon and concentrated to dryness under reduced pressure to give 1.057 g (84.2%) of **2** as a colorless foam. Compound **2** showed a negative ninhydrin test but a positive *o*-dianisidine test: $[\alpha]_D^{26}$ +103.1±1.4° (*c* 1.005, H_2O); IR (KBr pellet) 1732 cm^{-1}.

Anal. Calcd. for $C_{21}H_{33}N_3O_{15}\cdot 3H_2O$: C, 40.58; H, 6.32; N, 6.76.
Found: C, 40.89; H, 6.45; N, 6.77.

Epimeric mixture of 3-deamino-3-hydroxy kanamycin A (3)

To an ice-cooled solution of 908 mg of **2** dissolved in 32 ml of water and 6.3 ml methanol was added 343 mg of sodium borohydride, with stirring. After 2.5 hours, the solution was adjusted to pH 3 by adding 10% hydrochloric acid and passed through a column of 60 ml of Amberlite MB-3 resin. The resin was washed with 180 ml of water. The eluates were concentrated to dryness under reduced

pressure and the residue was hydrolyzed with a mixture of 2 ml of water, 8.6 ml of methanol and 1.7 ml of concentrated hydrochloric acid at 36°C for 24 hours. The solution was neutralized with 50 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 1.5 ml of water and adsorbed on a column of 300 ml of Amberlite CG-50 resin (NH_4^+ form). The column was eluted with 2 liters of water and 2 liters of 0.5 N ammonium hydroxide by the gradient method. Each fraction was 12 ml. Fractions 230~269 were combined, treated with active carbon, and evaporated to dryness under reduced pressure to give 508 mg (71.7%) of **3** as a colorless foam.

(1D)-1,3,5/2,4- (**4**) and (1L)-1,2,4/3,5-5-aminocyclohexanetetrols (**5**), and their pentaacetates (**6** and **7**)

A solution of 463 mg of **3** dissolved in 5 ml of water and 5 ml of concentrated hydrochloric acid was refluxed for 24 hours. After cooling, the solution was treated with active carbon and evaporated to dryness under reduced pressure. The residue was dissolved in a small amount of water, adsorbed on a column of 200 ml of Amberlite CG-50 resin (NH_4^+ form) and eluted with 700 ml of water and 700 ml of 0.4 N ammonium hydroxide by the gradient method. Each fraction was 12 ml. Fractions 68~73 were combined, treated with active carbon and evaporated to dryness to give 81 mg (52.3%) of **4** as a colorless foam. The sulfate of **4** showed $[\alpha]_D^{25.5}$ $-3.4\pm0.4°$ (*c* 0.993, H_2O) and its ^1H NMR (D_2O) and IR (KBr pellet) spectra were identical with those of (1L)-1,3,5/2,4-5-aminocyclohexanetetrol sulfate[1].

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, 31.39; H, 7.27; N, 6.31; S, 6.83.

To a solution of 51 mg of **4** 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 mixture 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 toluene was evaporated. The residue was recrystallized from ether to give 80 mg (68.6%) of **6**, mp 191~192°C, $[\alpha]_D^{25}$ $+11.0\pm0.5°$ (*c* 1.030, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{23}NO_9$: C, 51.47; H, 6.21; N, 3.75.
Found: C, 51.26; H, 6.31; N, 3.69.

The IR spectrum (KBr pellet) and melting point of **6** were identical with those of (1L)-1,3,5/2,4-5-aminocyclohexanetetrol pentaacetate[1].

Fractions 75~85 of the above chromatography were combined and treated as described above to give 49 mg (31.6%) of **5** as a colorless foam. The sulfate of **5** showed $[\alpha]_D^{25.5}$ $-44.0\pm1.2°$ (*c* 0.697, H_2O) and its ^1H NMR (D_2O) and IR (KBr pellet) spectra were identical with those of (1D)-1,2,4/3,5-5-aminocyclohexanetetrol sulfate[1].

Anal. Calcd. for $C_6H_{13}NO_4 \cdot 0.5H_2SO_4 \cdot 0.5H_2O$: C, 32.57; H, 6.83; N, 6.33; S, 7.25.
Found: C, 32.48; H, 6.81; N, 6.24; S, 7.06.

Compound **5** was acetylated as described above and the acetate (**7**) was recrystallized from ether: mp 155.5~156.5°C: $[\alpha]_D^{25.5}$ $-9.7\pm0.5°$ (*c* 1.024, $CHCl_3$).

Anal. Calcd. for $C_{16}H_{23}NO_9$: C, 51.47; H, 6.21; N, 3.75.
Found: C, 51.29; H, 6.22; N, 3.65.

The IR spectrum (KBr pellet) and melting point of **7** were identical with those of (1D)-1,2,4/3,5-5-aminocyclohexanetetrol pentaacetate[1].

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