*Studies on Antibiotics and Related Substances. XIV. Synthesis of trans-2-Aminocyclohexyl-D-glucosaminide**

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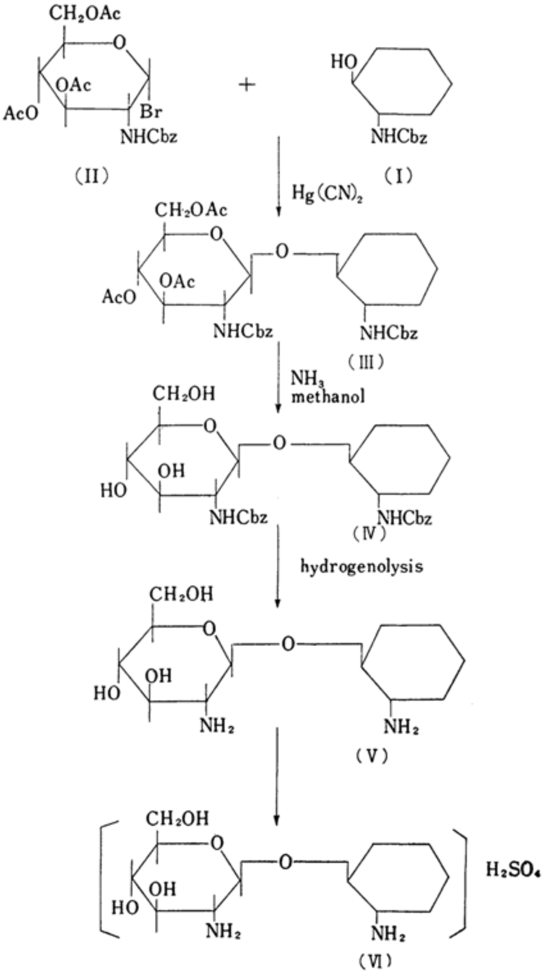
(Received July 24, 1961)

The interest in inosadiamines and inosamines and inosamines was stimulated by their occurrence in certain antibiotics. Scylloinosadiamine was isolated from streptomycin1). Another antibiotics, neomycin2) and paromomycin3) contained all-*trans*-2-deoxystreptamine. Pseudoneamine was a glucosaminide of 2-deoxystreptamine4). This occurred again in kanamycin5) in which it linked at C-4 and C-6, respectively, to kanosamine and 6-amino-6-deoxy-D-glucose. Also neosamine-2 was isolated from hygromycin A6). So it is desirable to study the relationships between chemical structures and antibiotic activities with model compounds of simpler structure. Besides our interest in this problem has been prompted by the recent development of the structural studies of kanamycin in our laboratory7).

For this purpose 2-aminocyclohexy-D-glucosaminide is selected. Both cis and trans forms of 2-aminocyclohexanol have been thoroughly characterized8). Since the stereochemical studies on streptamine indicate that natural streptamine is the all-trans meso-form9), *trans*-2-aminocyclohexanol derivatives have been adopted for the present studies.



N-Carbobenzoxy-*d*, *l*-*trans*-2-aminocyclohexanol (I) was prepared by carbobenzoxylation of *d*, *l*-*trans*-2-aminocyclohexanol10) obtained by ammonolysis of 2-chlorocyclohexanol. α-1-Bromo-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosamine (II) was prepared by the method of Irvine et al.11) and Zervas et al.12)

The condensation of I and II catalyzed by mercuric cyanide gave β-[*N*-carbobenzoxy-*d*, *l*-*trans*-2-aminocyclohexyl]-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosaminide (III) in 19.9% yield.

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* This work was reported at the 14th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1961.

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10) A. E. Osterberg and E. C. Kendall, ibid., **42**, 2616 (1920).

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The partial hydrolysis of III with absolute methanol saturated with ammonia gave β-[*N*-carbobenzoxy-*d, l-trans*-2-aminocyclohexyl]-*N*-carbobenzoxy-D-glucosaminide (IV) in 63.1% yield. By the catalytic hydrogenolysis of IV, β-[*d, l-trans*-2-aminocyclohexyl]-D-glucosaminide (V) was obtained. V was then converted into its sulfate VI by neutralization with dilute sulfuric acid.

Designation of the condensation products as β-anomers is based on their optical rotation and infrared spectra. The optical rotation of V and VI is $[\alpha]_{\mathrm{D}}^{13}$ −71.1° (*c* 1.7, water) and −44.4° (*c* 2, water) respectively. Compared with the optical rotation of kanamycin free base $[\alpha]_{\mathrm{D}}^{13}$ +140° (*c* 1, water)[13] and paromamine free base $[\alpha]_{\mathrm{D}}^{26}$ +114° (*c* 1.35, water)[14] both having α-configurations, that of V and VI are less dextrorotatory and since glucosamine is in D-series, the assignment to V and VI of β-configurations appears to be reasonable. The infrared spectra of III, IV, V and VI show absorption bands at 900, 893, 890 and 891 cm^{-1} respectively, indicating a characteristic absorption band for β-glucoside[15] while kanamycin free base shows absorption bands at 838 and 823 cm^{-1} corresponding to two α-anomeric configurations[16]. So the assignment of β-configuration to III, IV, V and VI will be reasonable.

On the other hand, from the mother liquor of III, a small amount of a crystalline product VII was recovered. Its elemental analysis was identical with that of III, but it showed a sharp and considerably lower melting point. VII was then treated with methanolic ammonia to yield a deacetylated compound VIII. The catalytic hydrogenolysis of VIII gave *d, l-trans*-2-aminocyclohexyl-D-glucosaminide (IX) but this material was not obtained analytically pure. The optical rotation of IX was $[\alpha]_{\mathrm{D}}^{13}$ +1.8 (*c* 3.9, water).

Considering from the fact that kanamycin and paromamine have strong dextrorotation α-anomer of *d, l-trans*-2-aminocyclohexyl-D-glucosaminide should have considerably high dextrorotation. Estimating from the molecular rotation of paromamine free base[3], the contribution of the anomeric center to the molecular rotation of *trans*-2-aminocyclohexyl-D-glucosaminide is approximately +30000, and the contribution of the remaining portion is generally about +10000. Thus assuming the β-D-configuration, the molecular rotation of *trans*-2-aminocyclohexyl-D-glucosaminide should be in the order of −20000 and that of α-D-anomer in the order of +40000. So the observed molecular rotation of V (−19600) is in good agreement with the theoretical value for β-D-configuration, but that IX is far below the theoretical value for α-D-configuration. The attempt to obtain pure α-anomer of *trans*-2-aminocyclohexyl-D-glucosaminide by different methods is under way.

Experimental

α-1-Bromo-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosamine (II).—Starting from commercially available glucosamine hydrochloride, α-1-bromo-3, 4, 6-triacetyl-D-glucosamine hydrobromide was prepared by the method of Irvine et al.[11] in a yield of 20∼30%. α-1-Bromo-3, 4, 6-triacetyl-D-glucosamine hydrobromide was introduced with the carbobenzoxyl group by the method of Zervas et al.[12] to give II melting at 97∼99°C in a yield of 30% (Found: C, 48.18; H, 4.99; N, 2.89; Br, 15.14%).

***N*-Carbobenzoxy-*d, l-trans*-2-aminocylohexanol (I).**—A 11.5 g. (0.1 mol.) portion of *d, l-trans*-2-aminocyclohexanol[9] was dissolved in 50 ml. of water and added with 8.4 g. of sodium bicarbonate. 17.1 g. (0.1 mol.) of carbobenzoxy chloride was added portionwise into the mixture with mechanical agitation. After two hours' agitation at room temperature, the reaction mixture was put in a refrigerator overnight. The precipitate was collected by filtration, washed well with water and dried in vacuo. The colorless crystalline solid weighed 20.5 g. and melted at 94∼101°C. The crude product was recrystallized from water-methanol (3 : 2) to yield 15.8 g. (63.6%) of colorless needles melting at 103∼104°C.

Found: C, 67.72; H, 7.67; N, 5.50. Calcd. for $C_{14}H_{19}NO_3$ (249.3): C, 67.44; H, 7.68; N, 5.62%.

β-[*N*-Carbobenzoxy-*d, l-trans*-2-aminocyclohexyl]-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosaminide (III).—II (2.2 g.), I (1.1 g.) and mercuric cyanide (1.9 g.) were added in 70 ml. of anhydrous benzene and the mixture was refluxed on a boiling water bath for two hours. The reaction mixture was cooled and filtered to remove an insoluble matter. The filtrate was diluted with 180 ml. of chloroform and the solution was washed twice with 100 ml. of 10% sodium chloride solution and three times with cold water. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure yielding a pale yellow residue. The residual product was washed with ether to yield 1.55 g. (52.8%) of colorless solid melting at 160∼180°C (decomp.).

The product was recrystallized repeatedly from absolute ethanol to yield 0.68 g. (23.2%) of fine needles melting at 201∼205°C. $[\alpha]_{\mathrm{D}}^{10}$ +9.6 (*c* 2.7, chloroform).

Found: C, 61.15; H, 6.53; N, 4.35. Calcd. for $C_{34}H_{42}N_2O_{16}$ (670.7): C, 60.88; H, 6.31; N, 4.18%.

β-[*N*-Carbobenzoxy-*d, l-trans*-2-aminocyclohexyl]-*N*-carbobenzoxy-D-glucosaminide (IV).—Two grams of III was suspended in 100 ml. of absolute methanol saturated with ammonia. After

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leaving overnight at room temperature, a clear solution was obtained and evaporated under reduced pressure to yield a colorless residue. The product was washed with warm ethyl acetate to remove acetamide and then recrystallized from isopropanol to yield 1.02 g. (63.0%) of colorless needles melting at 219~221°C (decomp.). A sample further recrystallized for analysis twice from isopropanol melted at 224.5~225°C (decomp.). $[\alpha]^{10}_{D}$ $-31.5°$ (c 2.9, pyridine).

Found: C, 61.94; H, 6.52; N, 4.97. Calcd. for $C_{28}H_{36}N_2O_9$ (544.6): C. 61.75; H, 6.66; N, 5.14%.

β-[*d, l*-*trans*-2-Aminocyclohexyl]-D-glucosaminide (V).—IV (800 mg.) was suspended in 45 ml. of dioxane-water mixture (1 : 1) and added to a prehydrogenated suspension of 200 mg. of palladium black in 10 ml. of dioxane-water mixture (1 : 1). The reaction mixture was hydrogenated under mechanical shaking at 36~38°C. After four hours, 50 ml. of hydrogen had been absorbed. The reaction mixture was filtered to remove palladium black and the filtrate was evaporated under reduced pressure to yield a colorless syrup. Th product was dissolved in absolute ethanol and added with an hydrous petroleum ether to yield 100 mg. (24.7%) of fine needles melting at 160~161°C (decomp.). $[\alpha]^{13}_{D}$ $-71.1°$ (c 1.7, water).

Found: C, 51.76; H, 8.56; N, 9.99. Calcd. for $C_{12}H_{24}N_2O_5$ (276.3): C, 52.16; H, 8.75; N, 10.14%.

β-[*d, l-trans*-2-Aminocyclohexyl]-D-glucosaminide Sulfate (VI).—The crude free base (470 mg.) was neutralized with 0.1 N sulfuric acid to pH 7 and the solution was filtered to remove a small amount of insoluble matter. Then the filtrate was evaporated under reduced pressure to yield 1 ml. of a residual solution. Addition of absolute ethanol to the residue resulted in precipitation of colorless crystals. After standing for two hours in a refrigerator, the crystals were collected by filtration and washed with absolute ethanol to yield 300 mg. (47.5%) of fine crystals melting at 270~271°C (decomp.). $[\alpha]^{13}_{D}$ $-44.4°$ (c 2, water).

Found: C, 38.07; H, 6.79; N, 7.30. Calcd. for $C_{12}H_{24}N_2O_5 \cdot H_2SO_4$ (374.4): C, 38.49; H, 7.00; N, 7.48%.

***d, l-trans*-2-Aminocyclohexyl-D-glucosaminide (IX).**—After standing the mother liquor of III for five days at room temperature, crystals appeared again and were collected by filtration. The product was recrystallized twice from absolute ethanol to yield 0.15 g. (5.1%) of crystals of *N*-carbobenzoxy-*d, l-trans*-2-aminocyclohexyl-3, 4, 6-triacetyl-*N*-carbobenzoxy-D-glucosaminide (VII) melting at 186~189°C. $[\alpha]^{10}_{D}$ $+16°$ (c 2.6, chloroform). Further recrystallization from absolute ethanol did not raise its optical rotation.

Found: C, 61.01; H, 6.17; N, 4.33. Calcd. for $C_{34}H_{42}N_2O_{12}$ (670.7): C, 60.88; H, 6.31; N, 4.18%.

VII (477 mg.) was suspended in 25 ml. of absolute methanol saturated with ammonia. After standing overnight at room temperature, the solution was evaporated under reduced pressure to dryness. The product was washed with warm ethyl acetate and recystallized from isopropanol to yield 262 mg. (67.5%) of crystals of *N*-carbobenzoxy-*d, l-trans*-2-aminocyclohexyl-*N*-carbobenzoxy-D-glucosaminide (VIII) melting at 198~199°C. The recrystallization of the product from isopropanol raised the m. p. to 199~200°C. $[\alpha]^{15}_{D}$ $+6.3°$ (c 1.9, pyridine).

Found: C, 61.52; H, 6.55; N, 5.35. Calcd. for $C_{28}H_{36}N_2O_9$ (544.6): C, 61.75; H, 6.66; N, 5.14%.

VIII (189 mg.) was suspended in 10 ml. of dioxane-water mixture (1 : 1) and added to a prehydrogenated suspension of 50 mg. of palladium black in 3 ml. of dioxane-water mixture (1 : 1). After four hours' hydrogenation at 37~39°C, the reaction mixture was filtered to remove palladium black and the filtrate was evaporated under reduced pressure to yield 92 mg. (90.2%) of amorphous solid of IX melting at 190~193°C (decomp.). $[\alpha]^{13}_{D}$ $+1.8°$ (c 3.9, water). The attempt to obtain the product in a crystalline form failed.

Found: C, 49.52; H, 9.01; N, 9.29. Calcd. for $C_{12}H_{24}N_2O_5 \cdot H_2O$ (294.3): C, 48.96; H, 8.90; N, 9.52%.

Chromatography. — No satisfactory chromatographic separation of α- and β-anomer could be accomplished by ascending paper chromatography. Glucosamine hydrochloride, V and IX showed R_f values 0.12, 0.28 and 0.30 respectively in 1-butanol-ethanol-water system (5 : 1 : 4) and 0.12, 0.28 and 0.26 in 1-butanol-acetic acid-water system (4 : 1 : 5). These spots were developed by ninhydrin in pyridine,

Bioassays.—V and IX did not show antimicrobial activity against *E. coli* in a dilution ratio 1 : 1000 by a dilution assay method.

The authors were indebted to Mr. S. Nakada for microanalysis and to Miss S. Fukuyama for bioassays.

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