Studies on Antibiotics and Related Substances. XIX. The Synthesis of cis-2-Aminocyclohexyl D-Glucosaminide

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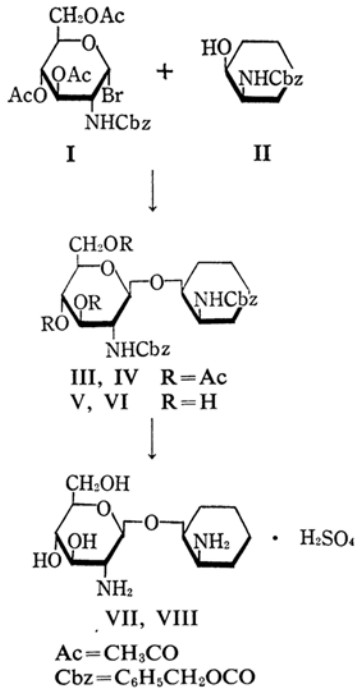
Previous studies of the synthesis of *trans*-2-aminocyclohexyl D-glucosaminide[1] have stimulated our interest in the synthesis of *cis*-2-aminocyclohexyl D-glucosaminide. In the present paper, the synthesis of two diastereomers of *cis*-2-aminocyclohexyl D-glucosaminide will be described.

The starting compound, *dl-cis*-2-aminocyclohexanol, was prepared by the following two method: the hydrogenation of *o*-acetamidophenol,[2] and the inversion reaction of *dl-trans*-2-aminocyclohexanol.[3] An attempt to resolve the racemic *cis*-2-aminocyclohexanol with tartaric acid or di-*O*-benzoyl tartaric acid did not yield a satisfactory result. Therefore, *dl-cis*-2-aminocyclohexanol was treated with carbobenzyloxy chloride to yield *N*-carbobenzyloxy-*dl-cis*-2-aminocyclohexanol (II) in 86% yield. The condensation of II with α-1-bromo-3, 4, 6-tri-*O*-acetyl-*N*-carbobenzyloxy-D-glucosamine (I)[4] in the presence of mercuric cyanide gave a mixture of glucosaminides (III and IV), which was then separated by fractional recrystallization to III, m. p. 155°C, $[\alpha]_D^{12}$ +25.2° and IV, m. p. 138°C, $[\alpha]_D^{28}$ −36.5°. Considering their optical rotations and elemental analyses, III and IV might be identified as *N*-carbobenzyloxy-*d-cis*-2-aminocyclohexyl 3, 4, 6-tri-*O*-acetyl-*N*-carbobenzyloxy-β-D-glucosaminide and *N*-carbobenzyloxy-*l-cis*-2-aminocyclohexyl 3, 4, 6-tri-*O*-acetyl-*N*-carbobenzyloxy-β-D-glucosaminide respectively. The deacetylation of

1) T. Suami, S. Ogawa and S. Umezawa, This Bulletin, **36**, 459 (1963).
2) G. E. McCasland, R. K. Clark, Jr., and H. E. Carter, *J. Am. Chem. Soc.*, **71**, 637 (1949).
3) A. C. Cope, E. Ciganek and J. Lazar, ibid., **82**, 2591 (1962).
4) J. C. Jruine, D. McNicoll and A. Hynd, *J. Chem. Soc.*, **1911**, 250; L. Zervas and S. Konstas, *Chem. Ber.*, **93**, 435 (1960).



III and IV with methanol saturated with ammonia gave V, $[\alpha]_D^{20}$ +20.6° and VI, $[\alpha]_D^{25}$ −59.2° respectively. The catalytic hydrogenolysis of V and VI, followed by neutralization with sulfuric acid, yielded crystalline sulfate, VII $[\alpha]_D^{15}$ +10.2° and VIII, $[\alpha]_D^{15}$ −50.8° respectively. The designation of VII and VIII as β-anomers is based on their optical rotations, which are less dextrorotatory than those of α-anomers in an analogous compound,[5] and on their infrared spectra.[6]

Experimental

All melting points are corrected. The infrared spectra were recorded in potassium bromide pellets.

dl-cis-2-Aminocyclohexanol Hydrochloride.—a) *dl-cis*-2-Aminocyclohexanol hydrochloride was prepared from *o*-acetamidophenol by the method of Carter et al.[2] in 14% yield.

b) *dl-cis*-Isomer was obtained from *dl-trans*-2-aminocyclohexanol by the method of Cope et al.[3] in 50% yield.

dl-cis-2-Acetamidocyclohexanol Acetate.—A two gram portion of *dl-cis*-2-aminocyclohexanol was treated with a large excess of acetic anhydride for 48 hr. at room temperature. The mixture was then evaporated under reduced pressure, and the residue was distilled in vacuo to yield 2.5 g. (72%) of a viscous oil, b. p. 140~155°C/4 mmHg. After it had stood for three months at room temperature, it crystallized completely. The product was recrystallized twice from benzene and petroleum ether to give the melting point of 71~73°C.

Found: C, 60.58; H, 8.76; N, 7.11. Calcd. for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.66; N, 7.03%.

The Kunz hydrolysis[7] of the product indicated that one acetyl group (1.05) was bound in an ester and another one in an amide form.

***N*-Carbobenzyloxy-*dl-cis*-2-aminocyclohexanol (II).** — *dl-cis*-2-Aminocyclohexanol hydrochloride was treated with carbobenzyloxy chloride in the presence of an excess of sodium bicarbonate to give II, m. p. 72~73°C in 85.5% yield (lit.[8] m. p. 72~73°C).

***N*-Carbobenzyloxy-*cis*-2-aminocyclohexyl 3, 4, 6-Tri-*O*-acetyl-*N*-carbobenzyloxy-β-D-glucosaminide (III and IV).** — A mixture of 8.0 g. of I and 8.0 g. of II in 100 ml. of anhydrous benzene was heated under reflux while being mechanically stirred for 7 hr. with 8.0 g. of mercuric cyanide. The mixture was then filtered to remove any insoluble substance. The filtrate was diluted with 230 ml. of chloroform and washed with a 10% sodium chloride solution and cold water. The solvent was removed under reduced pressure to give a semicrystalline residue. The residue was dissolved in 23 ml. of absolute ethanol and kept in a refrigerator to yield 1.4 g. of the product, m. p. 150~165°C. The crude product was repeatedly recrystallized from methanol-water (2 : 1) to yield 0.87 g. of needles (III), m. p. 154~155°C. $[\alpha]_D^{12}$ +25.2° (*c* 1.63, chloroform).

Found: C, 61.04; H, 6.25; N, 4.11. Calcd. for $C_{34}H_{42}N_2O_{16}$: C, 60.88; H, 6.31; N, 4.18%.

The alcoholic mother liquor was added to ether to give 3.0 g. of the product, m. p. 66~68°C. The crude product was fractionally recrystallized from methanol-water (2 : 1) to yield 1.2 g. of needles (IV), m. p. 136~138°C. $[\alpha]_D^{28}$ −36.5° (*c* 1.07, chloroform).

Found: C, 61.07; H, 6.48; N, 4.25. Calcd. for $C_{34}H_{42}N_2O_{16}$: C, 60.88; H, 6.31; N, 4.18%.

Further recrystallizations of III and IV did not raise their melting points or optical rotations.

***N*-Carbobenzyloxy-*d-cis*-2-aminocyclohexyl *N*-Carbobenzyloxy-β-D-glucosaminide (V).** — III (0.7 g.) was suspended in 30 ml. of methanol saturated with ammonia at 5°C. After it had been kept overnight in a refrigerator, the solution was evaporated under reduced pressure to yield a colorless syrup. The product was washed with a small amount of water and then repeatedly recrystallized from acetone-ether to yield 0.35 g. of the amorphous product (61.6%), m. p. 114~116.5°C, $[\alpha]_D^{20}$ +20.6° (*c* 1.92, methanol).

Found: C, 61.60; H, 6.59; N, 5.07. Calcd. for $C_{28}H_{36}N_2O_9$: C, 61.75; H, 6.66; N, 5.14%.

***N*-Carbobenzyloxy-*l-cis*-2-aminocyclohexyl *N*-Carbobenzyloxy-β-D-glucosaminide (VI).**—IV (0.6 g.) was treated by being placed in a refrigerator overnight with 30 ml. of methanol saturated with ammonia. The solution was treated similarly with III to yield 0.4 g. of crystals (89%), m. p. 160.5~162°C, $[\alpha]_D^{25}$ −59.2° (*c* 0.92, methanol).

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6) S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *Chem. & Ind.*, **1953**, 196; *J. Chem. Soc.*, **1954**, 171.

7) A. Kunz and C. S. Hudson, *J. Am. Chem. Soc.*, **48** 1982 (1926).

8) D. Ben-Ishai, ibid., **78**, 4962 (1956).

Found: C, 61.91; H, 6.46; N, 5.05. Calcd. for $C_{28}H_{36}N_2O_9$: C, 61.75; H, 6.66; N, 5.14%.

***d-cis*-2-Aminocyclohexyl β-D-Glucosaminide Sulfate (VII).**—A solution of V (1.09 g.) in 25 ml. of absolute methanol was hydrogenated for 40 hr. at room temperature in a hydrogen stream over palladium black (400 mg.). The mixture was then filtered to remove the catalyst, and the filtrate was evaporated under reduced pressure to dryness. The crude product (0.57 g.) was obtained after drying it over phosphorus pentoxide. $[\alpha]_D^{20}$ −12.6° (*c* 2.85, methanol). The crude free base (0.55 g.) was neutralized with 0.1 N sulfuric acid to pH 7, and the solution was filtered to remove a small amount of insoluble substance. Then the filtrate was evaporated under reduced pressure to yield 1 ml. of a residual solution. The addition of absolute ethanol to the solution gave 253 mg. of colorless needles (34%) melting at 275°C (decomp.). $[\alpha]_D^{15}$ +10.2° (*c* 2.64, water).

Found: C, 38.76; H, 6.82; N, 7.56; S, 8.23. Calcd. for $C_{12}H_{24}N_2O_5 \cdot H_2SO_4$: C, 38.49; H, 7.00; N, 7.48; S, 8.56%.

IR: 883 cm^{-1} (type 2b of β-D-glucopyranoside).

***l-cis*-2-Aminocyclohexyl β-D-glucosaminide Sulfate (VIII).**—A 0.33 g. portion of VI was treated in a manner similar to that described above for V to give 140 mg. of crystals (31.4%) melting at 270°C (decomp.). $[\alpha]_D^{15}$ −50.8° (*c* 3.27, water).

Found: C, 38.34; H, 7.11; N, 7.37; S, 8.27. Calcd. for $C_{12}H_{24}N_2O_5 \cdot H_2SO_4$: C, 38.49; H, 7.00; N, 7.48; S, 8.56%.

IR: 887 cm^{-1} (type 2b of β-D-glucopyranoside).

Paper Chromatography.—VII and VIII showed a single spot of R_f 0.38 and 0.26 respectively, in an ascending development at 22.5°C in an ethyl acetate-pyridine-acetic acid-water (5 : 5 : 1 : 3) system[9] (R_f of D-glucosamine hydrochloride: 0.29) on Toyo filter paper No. 50. The spots were detected by means of a spray of ninhydrin in pyridine.

Bioassays.—VII and VIII showed almost no antimicrobial activity against E. coli when examined with a paper-disk method.

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