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Studies of Aminosugars. XIII. The Synthesis of Paromamine*

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Paromamine has been synthesized by condensing the *N*, *N'*-dinitrophenyl derivative of deoxystreptamine with the *N*-dinitrophenyl derivative of acetobromoglucosamine in nitromethane in the presence of mercuric salts, and by then removing the protecting groups by treatment with methanolic ammonia and hydrolysis with Dowex 1X2. The synthetic sample was shown to be identical with natural paromamine by a comparison of their infrared spectra and by microbiological assay. The identity was also proved by the determination of the $\Delta[M]_{CuAm}$ values of the tri-*N*-acetate of the synthetic and natural specimens by the copper-complex methods.

Paromamine,[1] an aminoglucoside, has received wide attention in recent years in the field of chem-

* Part XXVI of "Studies on Antibiotics and Related Substances," by S. Umezawa.

1) T. H. Haskell, J. C. French and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 3480 (1959).

istry of antibiotics, since it shows antibacterial activity[2] and is a common moiety of such useful antibiotics as paromomycin I, II and kanamycin C.[3] We now know three examples of aminoglucosides, composed of two carbohydrate moieties, which exert antibiotic action on bacteria, i. e., paromamine, neamine[4] and 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-deoxystreptamine[5]; however, they have never been synthesized.

The present paper will present the details of our work on the synthesis of paromamine, work which was announced previously in a preliminary communication,[6] as well as details of some additional related studies.

The acetohalogeno derivatives of glucosamine, hitherto successfully used to synthesize α-glucosaminides of bulky alcohols, are 3, 4, 6-tri-*O*-acetyl-2-(2, 4-dinitroanilino)-2-deoxy-α-D-glucopyranosyl bromide[7] (I) and 3, 4, 6-tri-*O*-acetyl-2-(*p*-methoxybenzylidenamino)-2-deoxy-α-D-glucopyranosyl bromide[8]; however, to our knowledge, no α-glucosaminide of any kind of cyclic secondary alcohol has ever been synthesized with these acetobromo derivatives of glucosamine. Since deoxystreptamine is lacking in reactivity, and since, consequently, the conditions of glucosidation inevitably become drastic, a preliminary condensation of the more stable bromide (I) with cyclohexanol by Helferich's method[9] has been tested: Cyclohexyl 3, 4, 6-tri-*O*-acetyl-2-(2, 4-dinitroanilino)-2-deoxy-α-D-glucopyranoside (II) and its β-isomer (III) have been successfully obtained. An analogous reaction was then applied to the synthesis of paromamine.

The condensation of bis-*N*, *N'*-(2, 4-dinitrophenyl)-deoxystreptamine[10] with I in nitromethane in the presence of mercuric cyanide and bromide, gave a mixture of glucosides. The acetylation of the mixture and separation by TLC gave 4-*O*-[3, 4, 6-tri-*O*-acetyl-2-(2, 4-dinitroanilino)-2-deoxy-α-D-glucopyranosyl]-5, 6-di-*O*-acetyl-*N*, *N'*-bis-(2, 4-dinitrophenyl)-deoxystreptamine (IV).

On the other hand, paromamine was converted into tris-*N*-(2, 4-dinitrophenyl)-paromamine; when further acetylated, it gave penta-*O*-acetyl-tris-*N*-(2, 4-dinitrophenyl)-paromamine.

The infrared spectrum of IV could be completely superimposed on that of the above mentioned derivative of natural paromamine. The melting point of IV was not depressed by admixture with the derivative of natural paromamine.

The hydrolysis of IV with methanolic ammonia, followed by treatment with Dowex 1 X2 (OH^-), gave a crude base, which was then chromatographed on a column of cellulose powder. Finally, the ninhydrin-positive fraction was rechromatographed on Dowex 1 X2 (OH^-) to give a crystalline free base of 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-deoxystreptamine (V).

Paper chromatography of the synthetic product V illustrated that its R_f value corresponds to that of paromamine. A paper chromatographic study of the synthetic product hydrolyzed by refluxing it with 3 N hydrochloric acid showed just three spots, those of D-glucosamine, deoxystreptamine and the unchanged material.

Finally, the absolute configuration of the synthetic product and its identity with the natural paromamine were confirmed by a modified method[11] using cuprammonium-glucoside complexes.[12] Tri-*N*-acetylparomamine includes two pairs of adjacent hydroxyl groups which make projected angles of about +60° and −60°, one in the glucosamine moiety and other in the deoxystreptamine moiety. Accordingly, the $\Delta[M]_{CuAm}$ of tri-*N*-acetyl paromamine may be expected to be nearly zero as a result of internal compensation. The free base V was acetylated and de-*O*-acetylated to afford 4-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyl-deoxystreptamine (VI).

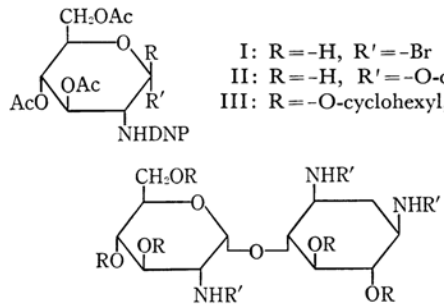

Fig. 1

2) T. Wakazawa and S. Fukatsu, *J. Antibiotics*, **A15**, 225 (1962).
3) M. Murase, ibid., **A14**, 367 (1961).
4) B. E. Leach and C. M. Teeters, *J. Am. Chem. Soc.*, **73**, 2794 (1951); H. E. Carter, J. R. Dyer, P. D. Shaw, K. L. Rinehart, Jr., and M. Hichens, ibid., **83**, 3723 (1961).
5) S. Umezawa and T. Tsuchiya, *J. Antibiotics*, **A15**, 51 (1962).
6) S. Umezawa and S. Koto, ibid., **A19**, 88 (1966).
7) P. F. Lloyd and M. Stacey, *Chem. & Ind.*, **1955**, 917.
8) E. F. Hardy, J. G. Buchanan and J. Baddiley, *J. Chem. Soc.*, **1963**, 3360.
9) B. Helferich and J. Zirner, *Chem. Ber.*, **95**, 2604 (1962).
10) S. Umezawa and S. Koto, *J. Antibiotics*, **A17**, 186 (1964).
11) S. Umezawa, T. Tsuchiya and K. Tatsuta, This Bulletin, **39**, 1235 (1966).
12) R. E. Reeves, "Advances in Carbohydrate Chemistry," Vol. VI, Academic Press, New York (1951), p. 107; M. Hichens and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **85**, 1547 (1963).

The determination of the $\Delta[M]_{CuAm}$ values of the synthetic product and the corresponding tri-*N*-acetyl derivative of natural paromamine gave values of +191 and +13 respectively. If VI were a 5-*O*- or 6-*O*-linked derivative of deoxystreptamine, instead of the 4-*O*-linked derivative mentioned above, the $\Delta[M]_{CuAm}$ values would be +2000 or +4000 respectively. The observed values for $\Delta[M]$'s of VI and tri-*N*-acetyl paromamine agree within the range of experimental error. Thus, the synthetic product, VI, was proved to be identical with the *N*-acetyl derivative of natural paromamine.

In the above-mentioned synthesis, the DNP group appears to have played an important role in the α-glucoside formation, for the dinitrophenylamino group would not be expected to exert a considerable anchimeric affect on the anomeric center. It is also conceivable that the plane of the phenyl group occupies a position almost perpendicular to the sugar body in the transition state; therefore, it is not so much of a steric hindrance, while the anchimeric effect of the 6-*O*-acetyl group[13] has a favorable influence on the formation of α-linkage in the polar solvent, nitromethane.

The synthetic product V behaved in conformity with natural paromamine in antibiotic spectra and minimal inhibitory concentrations (MIC) as is shown in Table I.

Table I. The MIC's of the synthetic (V) and natural paromamine as determined by the dilution method in bouillon

Test organisms		MIC μg./ml. V	MIC μg./ml. Paromamine
Bacillus subtilis PCI 219	(24 hr.)	125	125
Mycobacterium tuberculosis 607	(96 hr.)	>1000	>1000

In view of the established method of synthesis of deoxystreptamine by Nakajima et al.,[14] the above synthesis may be regarded as a total synthesis of paromamine.

It has been reported[15] that a kind of diastereoisomer of paromamine can be obtained from a crude product by acid reversion or, alternatively, by the reaction mentioned above, but no definite configurational assignment could be made at that time. Subsequently, the diastereoisomer was proved to be 5-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-deoxystreptamine.[16]

13) P. F. Lloyd and G. P. Roberts, *J. Chem. Soc.*, **1963**, 2962.
14) M. Nakajima, A. Hasegawa and N. Kurihara, *Tetahedron Letters*, **1964**, 967.
15) T. Tsuchiya, H. Fujita and S. Umezawa, *J. Antibiotics*, **A17**, 181 (1964).
16) S. Umezawa, T. Tsuchiya and H. Fujita, ibid., **A19**, 222 (1966).

Experimental

General Procedures.—TLC was performed on "Silica-Rider for TLC" (Dai-ich Pure Chemicals Co.) using three solvent systems, (a) benzene: MEK=4 : 1, (b) benzene : MEK=10 : 3, and (c) ethyl acetate : methanol=1 : 1, as the mobile phase. The DNP derivatives were visualized directly on an air-dried plate as yellow spots or bands, while others were detected by spraying a dried chromatogram with 50% sulfuric acid and heating it at 90—100°C. The extraction from Silica Rider was carried out with excess acetone. Paper chromatography was performed on Toyo filter paper No. 51, using the (d) solvent system (*n*-butanol : pyridine : water : acetic acid=6 : 4 : 3 : 1); ninhydrin (0.25%) in pyridine was sprayed on.

Cyclohexyl 3, 4, 6-Tri-*O*-acetyl-2-(2, 4-dinitroanilino)-2-deoxy-α-D-glucopyranoside (II).—To a solution of I (534 mg., 1 mmol.) and cyclohexanol (0.31 ml., 3 mmol.) in nitromethane (21 ml.), there was added mercury(II) cyanide (130 mg., 0.5 mmol.) and mercury(II) bromide (180 mg., 0.5 mmol.); the mixture was then stirred at 27—29°C for 6 days. The reaction mixture was evaporated, and the dark residue (1.19 g.) was treated with chloroform. The filtrate was washed with 10% aqueous potassium bromide (10 ml.×4) and distilled water (10 ml.×3), dried over sodium sulfate, and evaporated to give a reddish brown sirup (709 mg.). This was extracted with boiling ethanol (20 ml.), and then the solution was allowed to stand at room temperature overnight, thus precipitating a yellow crystalline solid (375 mg.). Two recrystallizations from ethanol gave crystals of cyclohexyl 3, 4, 6-tri-*O*-acetyl-2-(2, 4-dinitroanilino)-2-deoxy-α-D-glucopyranoside (II), m. p. 201.5°C, $[\alpha]_D^{17}$ +43° (*c* 0.81, chloroform). IR spectrum (KBr): 3320, 3100, 1620, 1590, 1530, 1332 (NH–DNP); 2910, 2840 (cyclohexyl); 1750, 1225 (O–Ac), 850 cm^{-1} (type 2a of the pyranose ring).

Found: C, 51.77; H, 5.21; N, 7.49. Calcd. for $C_{24}H_{31}N_3O_{12}$ (553): C, 52.07; H, 5.65; N, 7.59%.

The mother liquor separated from II further deposited yellow needles (200 mg.), m. p. 179—179.5°C, $[\alpha]_D^9$ −53° (*c* 1.0, chloroform). The optical rotation and the infrared spectrum of the product showed that it is cyclohexyl 3, 4, 6-tri-*O*-acetyl-2-(2, 4-dinitroanilino)-2-deoxy-β-D-glucopyranoside (III).

Found: C, 52.10; H, 5.68; N, 7.49. Calcd. for $C_{24}H_{31}N_3O_{12}$ (553): C, 52.07; H, 5.65; N, 7.59%.

4-*O*-[3, 4, 6-Tri-*O*-acetyl-2-(2, 4-dinitroanilino)-2-deoxy-α-D-glucopyranosyl]-5, 6-di-*O*-acetyl-*N*, *N'*-bis-(2, 4-dinitrophenyl)-deoxystreptamine (IV).—A suspension of I (5.0 g., 9.4 mmol.), bis-*N*, *N'*-(2, 4-dinitrophenyl)-deoxystreptamine[12] (3.1 g., 6.3 mmol.), mercury(II) cyanide (1.2 g., 4.8 mmol.) and mercury-(II) bromide (1.7 g., 4.8 mmol.) in nitromethane (15 ml.) was heated at about 95°C for 50 hr. while occasionally being stirred. The reaction mixture was then diluted with chloroform (100 ml.), and the dark brown filtrate was dried up in vacuo to give a residue (8.0 g.) which left a hard gummy substance (fraction A) (3.1 g.) when extracted with hot chloroform (50 ml.). The evaporation of the chloroform solution gave a dark residue (fraction B) (4.0 g.). Fraction B was chromatographed on silicon dioxide (170 g., 3.8×24 cm.) with benzene-ethanol (10 : 1, v/v) to give a yellow solid

(fraction B') (0.91 g.). Fraction A was again dissolved in ethyl acetate, filtered and evaporated to afford a brown residue (fraction A') (2.9 g.). Fractions A' and B' were combined and chromatographed on thin layer plates (20×20×0.05 cm; 160 sheets) with the a a solvent system; the distance of development was 10 cm. Three fractions, C, D and E (0.93 g., 0.83 g., and 1.58 g.), were obtained; the rate of flow of C was the largest. The fraction C was then further separated into two nearly equal fractions by preparative TLC using the b solventsystem. A yellow homogeneous substance (270 mg.) was obtained from the fraction with the smaller R_f value. The product was then acetylated with acetic anhydride (4 ml.) and pyridine (4 ml.) overnight at room temperature. The crude acetate was subjected to preparative TLC using the b solvent system; the distance of development was 17 cm. Extraction with acetone from the main band gave a yellow solid. This solid was taken up in acetone and filtered, and an excess of ethanol was added to afford fine yellow needles of the title compound (IV), m. p. 275—280°C (decomp.; sintered at about 200°C). $[\alpha]_D^{24}$ +250° (*c* 0.9, acetone). IR spectrum (KBr): 3345, 3100, 1628, 1593, 1525, 1336, 745 (NH–DNP); 1758, 1220 cm^{-1} (O–Ac).

Found: C, 46.47; H, 4.21; N, 12.37. Calcd. for $C_{40}H_{41}N_9O_{24}$ (1031): C, 46.56; H, 4.01; N, 12.22%.

4-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-deoxystreptamine (V).—A sample (30 mg.) of IV was dissolved by gently stirring into methanolic ammonia (40 g.) saturated at 0°C and then kept standing at room temperature overnight. Evaporation in vacuo was followed by letting the mixture stand under reduced pressure for several hours in order to remove the acetamide. The red residue was then dissolved in an acetone-water mixture (1 : 1), and an excess of Dowex 1X2 (OH^-) was added to the yellow solution. The mixture was shaken at room temperature overnight, and filtered, and the colorless filtrate was evaporated in vacuo. The product (10 mg.) was mixed with cellulose powder (200 mg.), placed on a column of cellulose powder (4.0 g.; 18×1.05 cm.), and eluted with the d solvent mixture, the fractions being cut into 20-ml. portions. The appearance of the product was examined by means of ninhydrin coloration. After the evaporation of the solvent at about 25°C, the fractions of tube Nos. 3—4 afforded a colorless sirup (8 mg.); this sirup was deionized with Dowex 1X2 (OH^-) and distilled water. The evaporation of drop Nos. 15—45 gave the pure free base (V), 7.0 mg. (74% from IV), $[\alpha]_D^{24}$ +111° (*c* 0.75, water).

Found: C, 44.15; H, 7.64; N, 12.67. Calcd. for $C_{12}H_{25}N_3O_7$ (323.5): C, 44.57; H, 7.79; N, 13.00%.

Descending paper chromatography of the synthetic product illustrated that its R_f value corresponded well with that of paromamine as ascertained by ninhydrin coloration.

4-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyl-deoxystreptamine (VI).—A sample (11 mg.) of V was acetylated with acetic anhydride (1 ml.) and pyridine (1 ml.) at about 30°C for one week; then, after the evaporation of the excess reagent, the crude product was de-*O*-acetylated with methanolic sodium methylate (4 ml., 0.02%) at room temperature overnight. Neutralization with Amberlite IR 120 (H^+), followed by evaporation, gave a colorless residue which was then deionised by the use of Dowex 1X2 (0.8×8 cm.) and Dowex 50WX8 (0.3×4 cm.) successively, and developed with water. The elute was evaporated to yield a crystalline solid (4.7 mg.); $[\alpha]_D^{15}$ +110° (*c* 0.34, water), $[\alpha]_{436}^{15}$ +200° (*c* 0.34, water), $[\alpha]_{436}^{15}$ +243° (*c* 0.33, CuAm), $\Delta[M]_{CuAm}$ +191. The R_f value of the product, as ascertained using the c solvent system, corresponded well with that of tri-*N*-acetyl paromamine obtained by the same procedure of acetylation and de-*O*-acetylation.

Found: C, 47.87; H, 6.92; N, 9.41. Calcd. for $C_{18}H_{31}N_3O_{10}$ (449.5): C, 48.10; H, 6.95; N, 9.35%.

Penta-*O*-acetyl-tris-*N*-(2, 4-dinitrophenyl)-paromamine.—A suspension of paromomycin sulfate (Sankyo Co.) (1.0 g.) in methanol (25 ml.) containing concentrated hydrochloric acid (1 ml.) was refluxed gently for 8 hr. The resulting colorless crystals were then collected and washed with methanol; yield, 356 mg. The crude product (340 mg.) was dissolved in distilled water (1.0 ml.), put on the Dowex 1×2 (OH^-) column (1×13 cm.), and developed with distilled water, the fractions being cut into 5-ml. portions. The evaporation of the fraction of tube Nos. 2—4, which was detected by ninhydrin coloration, followed by crystallization from methanol, gave a sample of chromatographically-pure paromamine (190 mg.).

Found: C, 44.36; H, 7.59; N, 12.86. Calcd. for $C_{12}H_{25}N_3O_7$ (323.5): C, 44.57; H, 7.79; N, 13.00%.

A mixture of the paromamine (163 mg.) and sodium bicarbonate (193 mg.) in water (6 ml.) was stirred for a while. To the solution there were then added 2, 4-dinitrofluorobenzene (308 mg.) and ethanol (3.5 ml.), after which the mixture was stirred for about 6 hr. at 29—30°C, until the yellow gummy product initially formed became powdery; yield, 350 mg. (85%). The crude product of tris-*N*-(2, 4-dinitrophenyl)-paromamine was acetylated with acetic anhydride (5 ml.) and pypyridine (5 ml.). The resulting yellow glass was crystallized from acetone as fine needles by the gradual addition of ethanol. Two recrystallizations from an acetone-ethanol mixture gave a chromatographically-pure product, m. p. 275—280°C (decomp.; sintered at about 200°C); $[\alpha]_D^{24}$ +262° (*c* 0.9, acetone).

Found: C, 46.23; H, 3.98; N, 12.24. Calcd. for $C_{40}H_{41}N_9O_{24}$ (1031): C, 46.56; H, 4.01; N, 12.22%.

The infrared spectra of the product and of IV were identical.

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