SYNTHESIS OF PAROMAMINE*

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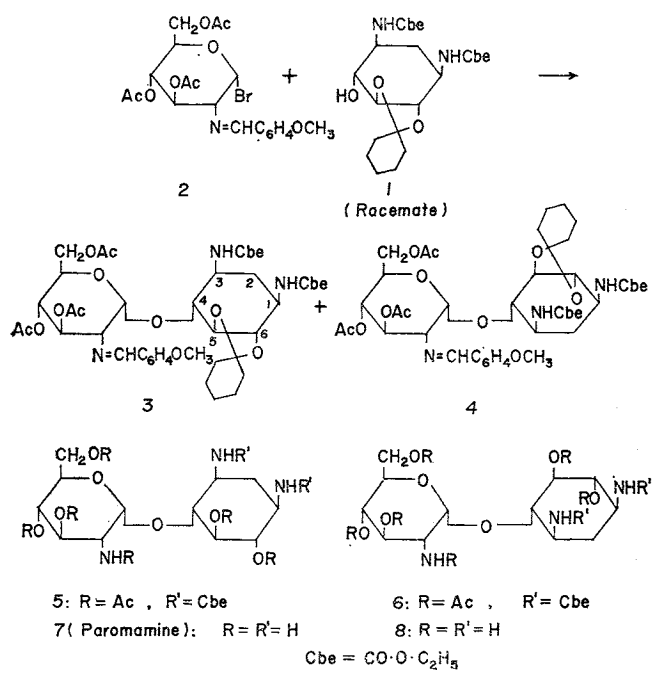
Paromamine (**7**) and its positional isomer, namely, 6-O-α-D-glucosaminyl-2-deoxystreptamine (**8**) have been synthesized in satisfactory yields by condensation of racemic mono-O-cyclohexylidene-N,N'-diethoxycarbonyl-2-deoxystreptamine (**1**) with 3,4,6-tri-O-acetyl-N-*p*-methoxybenzylidene-α-D-glucosaminyl bromide (**2**). The overall yield of paromamine from 2-deoxystreptamine is 37 %.

Paromamine[1,2] is a common and antibacterial moiety of paromomycin I and II, and kanamycin C, and was recently isolated[3] from a crude kanamycin-complex. The synthesis[4] had already been achieved in our laboratory through the condensation of bis-N,N'-(2,4-dinitrophenyl)-2-deoxystreptamine with 3,4,6-tri-O-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide. This paper describes an improved synthesis of paromamine (**7**), its position isomer (**8**) being also described.

A racemic mixture of 4,5- and 5,6-O-cyclohexylidene-N, N'-diethoxycarbonyl-2-deoxystreptamine (**1**) was prepared by the reaction of N,N'-diethoxycarbonyl-2-deoxystreptamine[5] with 1,1-dimethoxycyclohexane in DMF in the presence of *p*-toluenesulfonic acid in a yield of 83 %. Condensation of **1** with 3, 4, 6-tri-O-acetyl-N-*p*-methoxybenzylidene-α-D-glucosaminyl bromide[6] (**2**) was effected in the presence of mercuric cyanide and Drierite in dry benzene - dioxane (2 : 1) at room temperature and gave 6-O-(3,4, 6-tri-O-acetyl-N-*p*-methoxybenzylidene-α-D-glucosaminyl)-4, 5-O-cyclohexylidene-N, N'-

Fig. 1.



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diethoxycarbonyl-2-deoxystreptamine (**4**) and 4-O-(3,4,6-tri-O-acetyl-N-*p*-methoxybenzylidene-*α*-D-glucosaminyl)-5,6-O-cyclohexylidene-N, N′-diethoxycarbonyl-2-deoxystreptamine (**3**) in 36% and 49% yields, respectively (based on **1**).

Table 1. Antibacterial spectra of **7**,**8** and paromamine

Test organisms*	Minimal inhibitory concentrations (mcg/ml)		
	7	paromamine	**8**
Staphylococcus aureus FDA 209 P	>500	>500	>500
Escherichia coli NIHJ	>500	>500	>500
Bacillus subtilis PCI 219	15.6	15.6	>500

* Nutrient bouillon, 37℃, 16 hours.

It is noteworthy that the condensation is effected smoothly at room temperature, giving high yields of glycosides and that the products isolated are *α*-anomers without contamination of detectable *β*-anomers. This successful condensation may be ascribed to the presence of the nonparticipating *p*-methoxybenzylidene group at C-2 of the glycosyl halide and to the selection of pertinent solvent and condensing agent, as described by HARDY, BUCHANAN and BADDILEY[7].

In contrast to this, the condensation[5,8] of 2-O-benzylglycosyl halides with N-protected 2-deoxystreptamine derivatives required a higher reaction temperature and longer period, yields being not necessarily satisfactory.

Romoval of the *p*-methoxybenzylidene and cyclohexylidene groups **3** and **4** by treatment with aqueous acetic acid resulted in the partial formation of N-acetylated products, probably by O→N acetyl migration. Therefore, the products were acetylated respectively to give the corresponding hexaacetyl derivatives, **5** and **6**. Removal of the protecting groups from **5** and **6** by hydrazinolysis gave 4-O-*α*-D-glucosaminyl-2-deoxystreptamine (**7**) (paromamine) and 6-O-*α*-D-glucosaminyl-2-deoxystreptamine (**8**), respectively. An alternative procedure, which did not involve the isolation of the totally acetylated product, gave a high yield (96%) of **7**.

The synthetic glycoside **7** showed IR and NMR spectra and optical rotation identical to those of natural paromamine: however, the IR spectra of **7** and **8** were almost indistinguishable and their NMR spectra also were quite similar except for the shift-values of their anomeric protons.

The determination of their $\Delta[M]_{TACu}$ values[9] served for elucidating the structures of **7** and **8**. In the case of paromamine, tetraminecopper (II) sulfate (TACu) may form copper complexes at the 1-NH_2 and 6-OH, and at the 2′-NH_2 and 3′-OH groups, to give a small $\Delta[M]_{TACu}$ value by the intramolecular compensation of $\Delta[M]$ contributions of the two complexes opposite in sign and approximately equal in magnitude. In the case of **8**, the two pairs, 3-NH_2 and 4-OH and 2′-NH_2 and 3′-OH, may form complexes, however, since $\Delta[M]$ contributions of the two complexes are both negative in sign, the $\Delta[M]_{TACu}$ of **8** is expected to be a large negative value.

The $\Delta[M]_{TACu}$ value of the synthetic paromamine (**7**) is in accord with that of natural paromamine. The position isomer (**8**) gave a predicted $\Delta[M]_{TACu}$ value and proved to be 6-O-*α*-D-glucosaminyl-2-deoxystreptamine.

The result of antibacterial tests of **7**, **8** and natural paromamine is shown in Table 1. Antibacterial activity of synthetic paromamine (**7**) agreed with that of natural paromamine. The 6-O-isomer (**8**) showed no activity against the organisms tested.

This observation is compatible with the previous observation[8] that 6-O-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine has no antibacterial activity.

Experimental

The NMR spectra were measured with a Varian A-60 D spectrometer. TMS was used as internal standard except that DSS was used in D_2O solution. Thin-layer chromatography (TLC) was carried out on microscope slides coated with silica gel. Paper chromatography was performed on Toyo Roshi No. 50 paper with 1-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) and the spots were visualized with 0.5 % ninhydrin in pyridine.

Racemic Mono-O-cyclohexylidene-N,N'-diethoxycarbonyl-2-deoxystreptamine (1).

To a solution of N,N'-diethoxycarbonyl-2-deoxystreptamine[5] (4.62 g) in dry DMF (dried over CaH_2, 56 ml), anhydrous *p*-toluenesulfonic acid (dehydrated *in vacuo* at 90°C for 3 hours, 520 mg) and dry 1,1-dimethoxycyclohexane (10.8 ml) were added and the solution was stirred at 50°C (bath temperature) *in vacuo* (35 Torr) for 1.5 hour. Evaporation of the solution followed by azeotropic evaporation with toluene gave a solid. Saturated barium hydroxide solution (50 ml) and ethyl acetate were added and, after vigorous agitation, the organic layer separated was washed with water, dried over anhydrous sodium sulfate and evaporated to give a residue, which was triturated with petroleum ether to give a colorless solid, 4.82 g (83 %), mp 128~130°C. TLC: Rf 0.73 on TLC with benzene - ethanol (5 : 1). NMR (in pyridine-d_5 containing a small amount of D_2O): τ 8.88 (6H t, J=7 Hz, $NHCOOCH_2C\underline{H}_3$), 8.15~8.65 (10 H, cyclohexylidene protons).

Calcd for $C_{18}H_{30}N_2O_7$: C 55.94, H 7.83, N 7.25 %.
Found: C 55.93, H 8.04, N 6.92 %.

4-O-(3, 4, 6-Tri-O-acetyl-N-*p*-methoxybenzylidene-α-D-glucosaminyl)-5, 6-O-cyclohexylidene-N,N'-diethoxycarbonyl-2-deoxystreptamine (3) and 6-O-(3,4,6-Tri-O-acetyl-N-*p*-methoxybenzylidene-α-D-glucosaminyl)-4, 5-O-cyclohexylidene-N, N'-diethyoxycarbonyl-2-deoxystreptamine (4).

To a solution of **1** (209 mg, 0.54 mmol) in dry benzene - dioxane* (2 : 1, 3.1 ml), freshly prepared Drierite (420 mg), mercuric cyanide (dried *in vacuo* at 110°C, 410 mg) and 3, 4, 6-tri-O-acetyl-N-*p*-methoxybenzylidene-α-D-glucosaminyl bromide[7] (423 mg, 0.87 mmol) were added and the mixture was stirred at room temperature for 5 hours. Evaporation gave a syrup, which was dissolved in chloroform and the solution was washed with sodium hydrogen carbonate solution and with water, dried over anhydrous sodium sulfate and evoporated to give a pale-yellow syrup. On TLC with benzene - ethanol (25 : 1), it showed two spots, Rf 0.5 and 0.4. Column chromatography with silica gel (110 g) and ether - chloroform (4 : 1) separated the products. Purification was effected by reprecipitation with coloroform - petroleuum ether.

Compound **3** (212 mg, 49 %) had Rf 0.4 with benzene - ethanol (25 : 1), Rf 0.22 with ether - chloroform (4 : 1), mp 121~123°C, $[\alpha]_D^{23}$ +68° (*c* 1, chloroform), NMR (in $CDCl_3$) τ 8.75 (6 H, t, J=7 Hz, $NHCOOCH_2C\underline{H}_3$), 8.2~8.7 (10 H, a broadened signal, cyclohexylidene protons), 7.98, 7.95 and 7.92 (3 H, each, OAc), 6.15 (3 H, s each, OCH_3), 2.45~3.2 (4 H m, typical for *p*-disubstituted benzene ring), mass spectral peaks at *m/e* 791 (M^+), 746 and 731.

Calcd for $C_{38}H_{53}N_3O_{15}$: C 57.64, H 6.74, N 5.31 %.
Found: C 57.35, H 6.52, N 5.51 %.

Compound **4** (153 mg, 36 %) had Rf 0.5 with benzene-ethanol (25 : 1), Rf 0.36 with ether - chloroform (4 : 1), mp 116~117°C, $[\alpha]_D^{23}$ +70° (*c* 1, chloroform), NMR (in $CDCl_3$) τ 8.83 and 8.75 (3 H, t each, J=7 Hz, $NHCOOCH_2C\underline{H}_3$), 8.2~8.7 (10 H, a broadened signal, cyclohexylidene protons), 7.98, 7.95 and 7.92 (3 H, s each, OAc), 6.18 (3 H, s, OCH_3), 2.45 ~3.2 (4 H, m), mass spectral peaks at *m/e* 791 (M^+), 746 and 731.

* Benzene was dried over $LiAlH_4$ and dioxane was heated at reflux in the presence of sodium metal.

Calcd for $C_{38}H_{53}N_3O_{15}$: C 57.64, H 6.74, N 5.31 %.
Found: C 57.78, H 6.71, N 5.53 %.

4-O-(3, 4, 6-Tri-O-acetyl-N-acetyl-α-D-glucosaminyl)-5, 6-O-acetyl-N, N'-diethoxycarbonyl-2-deoxystreptamine (5).

To a solution of **3** (500 mg) in methanol (1.0 ml), 50 % acetic acid (2.5 ml) was added and the solution was heated in a boiling water bath for 1 hour. The solution was evaporated to give a syrup, which was dissolved in ethanol containing a small amount of hydrochloric acid. To the solution was added a large amount of ether, and the resulting precipitate was washed with ether - ethyl acetate (1 : 1) to give a solid (323 mg). On TLC with benzene - ethanol (2 : 1), it gave a spot of Rf 0.50 accompanied by another of Rf 0.55. The solid (300 mg) was allowed to react with acetic anhydride in pyridine for 30 hours. Evaporation gave a syrup, which was dissolved in chloroform. The solution was washed successively with potassium hydrogen sulfate solution, sodium hydrogen carbonate solution and water, dried over anhydrous sodium sulfate and evaporated to give a syrup. Trituration with ether gave a colorless solid, 345 mg (83 % based on **3**), mp 133~136°C, $[\alpha]_D^{25}$+67° (*c* 1, chloroform). TLC: Rf 0.56 (benzene-ethanol 5 : 1).

Calcd for $C_{30}H_{45}N_3O_{17}$: C 50.07, H 6.30, N 5.84 %.
Found: C 50.07, H 6.28, N 5.69 %.

6-O-(3,4,6-Tri-O-acetyl-N-acetyl-α-D-glucosaminyl)-4,5-di-O-acetyl-N,N'-diethoxycarbonyl-2-deoxystreptamine (6).

Compound **4** was treated as in the preceding section to give **6** in a yield of 77 %; mp 264~266°C (decomp.), $[\alpha]_D^{25}$+60° (*c* 1, chloroform), TLC Rf 0.49 (benzene - ethanol 5 : 1).

Calcd for $C_{30}H_{45}N_3O_{17}$: C 50.07, H 6.30, N 5.84 %.
Found: C 49.76, H 6.09, N 5.77 %.

4-O-α-D-Glucosaminyl-2-deoxystreptamine (Paromamine) (7).

A) From **5**. A solution of **5** (167 mg) in 80 % hydrazine hydrate (25 ml) was heated in a sealed tube at 160°C for 30 hours. Evaporation of the solution followed by azeotropic evaporation with water gave a solid, which was dissolved in water. After removal of insoluble matter, the solution was evaporated to give a syrup. Addition of acetone gave a solid (54 mg). An aqueous solution of the solid was charged on a column of CM-Sephadex C-25 (NH_4^+ form) and, after washing with water (100 ml) to remove accompanying hydrazine, elution was first effected with 0.03 N ammonia to give a partially de acetylated substance (paper chromatography $Rf_{Paromamine}$ 2.32, 23 mg). Further elution with 0.06 N ammonia gave paromamine, 37.0 mg (50 %). Repeated reaction of the partially deacetylated substance (Rf 2.32) with hydrazine gave another crop of paromamine (18.3 mg); total yield 75 %, $[\alpha]_D^{20}$ +103° (*c* 1, water)**, $[\alpha]_{436}^{20}$+182° (*c* 1, water), $\Delta[M]_{TACu}$−203° (natural paromamine, $\Delta[M]_{TACu}$ −150°), NMR (in D_2O) τ 8.74 (1 H, q, J~12 Hz, H-2_{ax}), 8.00 (1 H doublet of triplets, J~12, ~3.5 and ~3.5 Hz, H-2_{eq}), 4.74 (1 H, d, J=3.5 Hz, anomeric proton).

Calcd for $C_{12}H_{25}N_3O_7$: C 44.58, H 7.79, N 13.00 %.
Found: C 44.36, H 7.82, N 12.85 %.

B) From **3**. To a solution of **3** (662 mg) in methanol (1 ml), 50 % acetic acid (3.3 ml) was added and the solution was heated in a boiling water bath for 1 hour. Evaporation of the solution gave a syrup, which was dissolved in ethanol. To the solution, ether was added and the resulting precipitate was heated with 80 % hydrazine hydrate (70 ml) in a sealed tube at 160°C for 30 hours. Subsequent treatment followed as in A) to give paromamine, 260 mg (96 %), $[\alpha]_D^{20}$+103° (*c* 1, water).

6-O-α-D-Glucosaminyl-2-deoxystreptamine (8).

Compound **6** was treated like in A) above to give **8** in a yield of 70 %; $[\alpha]_D^{20}$+102° (*c*

** Although the value is a little smaller than that reported[1] (+114°), a specimen of purified natural paromamine also showed the same value as the synthetic specimen.

1, water), $[\alpha]_{436}^{20}+183°$ (*c* 1, water), $\Delta[M]_{TACu}-1590°$, NMR (in D_2O) τ 8.73 (1 H, q, $J\sim12$ Hz, H-2_{ax}), 8.00 (1 H doublet of triplets, $J\sim12$, ~3.5 and ~3.5 Hz, H-2_{eq}), 4.87 (1 H, d, $J=3.5$ Hz, anomeric proton).

Calcd for $C_{12}H_{25}N_3O_7$: C 44.58, H 7.79, N 13.00 %.
Found: C 44.40, H 7.54, N 12.71 %.

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