SYNTHESES OF 6′-*C*-AMINOMETHYL-3′-DEOXYPAROMAMINES

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(Received for publication October 14, 1976)

The first synthesis of 6′-*C*-aminomethyl derivatives (6′-epimers) of 3′-deoxyparomamine is reported starting from 3′-deoxyparomamine by way of 6′-*O*-tritylation, *O*-acetylation, hydrolysis of the trityl group, conversion of the 6′-hydroxyl group into an aldehyde group, nitromethane condensation of the aldehyde group and catalytic reduction of the nitro group into an amino group.

Aminoglycoside antibiotics having kanamycin A, B-like structures are inactivated by resistant bacteria producing enzymes which acetylate[1,2] the 6′-amino group of the antibiotics. As an approach to counteract the inactivation, introduction of a methyl group into the 6′-amino group of 3′, 4′-dideoxykanamycin B and other related antibiotics has been found by us[3] to prevent the enzymatic 6′-*N*-acetylation to a great extent. In this paper, we describe the syntheses of 6′-*C*-aminomethyl-3′-deoxyparomamines (**6** and **6′**) starting from 3′-deoxyparomamine.[4] The syntheses were undertaken to examine the effect of the 6′-*C*-aminomethyl group on the antibacterial activity of the aminoglycoside antibiotics and on the activity against the resistant bacteria producing the kanamycin-neomycin acetyl transferase.

3′-Deoxyparomamine obtained by hydrolysis of lividomycins[5] was treated with ethyl chloroformate to give the *N*-ethoxycarbonyl derivative (**1**). Selective tritylation of **1** followed by acetylation gave the tri-*O*-acetyl-6′-*O*-trityl derivative (**2**). Acidic hydrolysis gave a derivative (**3**) which has a free hydroxyl group at C-6′. Oxidation with dimethylsulfoxide-dicyclohexylcarbodiimide-trifluoroacetic acid gave the 6′-aldehyde (**4**). Its structure was indicated by an absorption peak at δ 9.67 in the PMR spectrum assignable to an aldehyde proton. Reversal

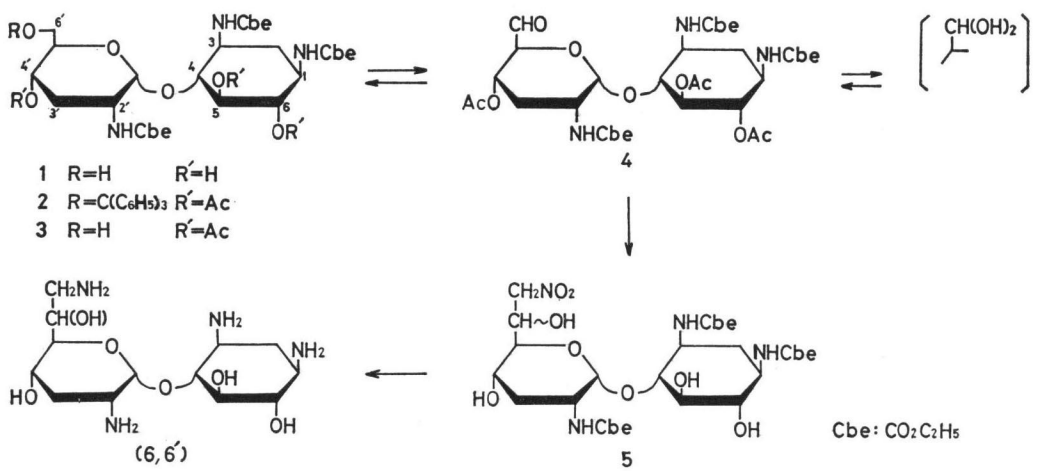

Table 1. Antibacterial spectra of **6**,6′,3′-deoxyparomamine (3′-DPA), and 3′-deoxyneamine[8] (3′-DNA).

Test organisms*	Minimal inhibitory concentration (mcg/ml)			
	6	**6′**	3′-DPA	3′-DNA
Staphylococcus aureus FAD 209 P	200	100	200	25
Sarcina lutea PCI 1001	>200	>200	>200	>200
Bacillus subtilis NRRL B-558	25	12.5	25	3.12
Salmonella typhi T-63	100	50	100	12.5
Escherichia coli NIHJ	>200	>200	>200	50
〃 K-12	200	200	>200	25
〃 〃 R-5***	>200	>200	>200	>200
〃 〃 ML 1629	>200	200	>200	50
〃 〃 ML 1410	>200	>200	200	50
Psedomonas aeruginosa A3	>200	>200	200	12.5
Mycobacterium smegmatis ATCC 607**	200	100	100	100

* Agar dilution streak method (nutrient agar, 37°C, 18 hours)
** 48 hours.
*** A strain producing 6′-*N*-acetyl transferase.

of **4** to **3** by reduction with zinc borohydride further supported the presence of the aldehyde group.

Hydrolysis of **4** with sodium methoxide in methanol followed by treatment with nitromethane gave a pair of 6′-epimers (**5**). The structure of **5** was determined by the microanalysis, IR and PMR[6] spectroscopy.

Catalytic reduction of the nitro compounds followed by hydrolysis of the ethoxycarbonyl groups with barium hydroxide and separation by Sephadex column chromatography gave the epimeric 6′-*C*-aminomethyl derivatives (**6** and **6′**), although the configuration at C-6′ remained undetermined.

The antibacterial activities of **6** and **6′** are shown in Table 1 with other reference antibiotics. The result shows that the introduction of the 6′-aminomethyl group into 3′-deoxyparomamine scarcely changes the antibacterial spectrum.

Experimental

PMR spectra were recorded at 60 and 100 MHz with Varian A-60D and HA-100 spectrometers, respectively. Thin-layer chromatography (TLC) was carried out on Wakogel B-5 with sulfuric acid spray for detection unless otherwise stated. For column chromatography, silica gel (Wakogel C-200) was used. Paper chromatography (ppc) was carried out on Toyo Roshi No. 50 with 1-butanol - pyridine - water - acetic acid (6:4:3:1) as the developer, descending for 18 hours, and colorized with 0.5% ninhydrin in pyridine. Paper electrophoresis was conducted in a Savant LT 20A apparatus (Savant Instrument Inc.) on Toyo Roshi No. 51 in formic acid - acetic acid - water (1:3:36) at 3,500 V for 15 minutes and after drying the paper in a hood, it was colorized as stated above.

3′-Deoxy-1, 3, 2′-tri-*N*-ethoxycarbonylparomamine (**1**)

To a mixture of 3′-deoxyparomamine trihydrochloride (3.24 g) and anhydrous sodium carbonate (3.7 g) in aqueous acetone (1:1, 70 ml), ethyl chloroformate (2.65 ml) was added and the mixture was vigorously stirred at room temperature for 2 hours. The methanol extract of the reaction mixture showed, on TLC with ethyl acetate - methanol (5:1), a single spot at Rf

0.58. Evaporation of the solvent of the slurry followed by extraction of the residue with methanol and concentration of the solution gave a colorless solid (4.93 g) which was used without purification to the next step. A portion (32.0 mg) of the solid was recrystallized from hot aqueous methanol (1:1) to give needles of **1**, 18.9 mg (69%), mp 290°C (dec.), $[\alpha]_D^{12}$ + 61° (*c* 1, pyridine).

Found: C 46.74, H 6.82, N 7.86%.
Calcd for $C_{21}H_{37}N_3O_{12}\cdot H_2O$: C 46.57, H 7.26, N 7.76%.

5, 6, 4′-Tri-*O*-acetyl-3′-deoxy-1, 3, 2′-tri-*N*-ethoxycarbonyl-6′-*O*-tritylparomamine (**2**)

To a solution of crude **1** (4.90 g) in pyridine (150 ml), trityl chloride (13.0 g) was added and the solution was kept at 25°C for 16 hours. The solution showed, on TLC with benzene-methanol (5:1), a spot at Rf 0.52. Acetic anhydride (16 ml) was added and the solution was heated at 50°C for 13 hours. The solution showed, on TLC with benzene-ethyl acetate (1:2), a major spot at Rf 0.55. Water (5 ml) was added and the solution was concentrated. The resulting brown solid was chromatographed over silica gel with benzene (to elute tritylcarbinol), then with benzene-ethyl acetate (2:3) to give a colorless solid of **2**, 4.29 g, mp 126.5~127.5°C, $[\alpha]_D^{13}$+ 63° (*c*. 0.5, methanol). PMR ($CDCl_3$) δ: 1.05, 1.24, and 1.27 (each 3H t, J=7 Hz, $CO_2CH_2C\underline{H}_3$); 1.77 (3H s, Ac at 4′-O (?)), 2.05 (6H s, Ac).

Found: C 61.95, H 6.43, N 4.53%.
Calcd for $C_{46}H_{57}N_3O_5$: C 61.94, H 6.44, N 4.71%.

5, 6, 4′-Tri-*O*-acetyl-3′-deoxy-1, 3, 2′-tri-*N*-ethoxycarbonylparomamine (**3**)

A solution of **2** (1.42 g) in 70% acetic acid (34 ml) was heated at 60°C for 1.5 hours and allowed to stand at room temperature for 1 hour. The mixture was filtered to remove precipitated tritylcarbinol and the filtrate was concentrated to give a solid. To a chloroform solution of the solid, diisopropyl ether was added to cause crystallization of **3**, 944 mg (92%), mp 185~186.5°C, $[\alpha]_D^{13}$ + 55° (*c* 0.5, methanol). PMR ($CDCl_3$-D_2O) δ: 1.24 (3H) and 1.28 (6H) (each t, J=7 Hz, $CO_2CH_2C\underline{H}_3$); 2.02, 2.04, and 2.07 (each 3H s, Ac).

Found: C 49.62, H 6.58, N 6.25%.
Calcd for $C_{27}H_{43}N_3O_{15}$: C 49.92, H 6.67, N 6.47%.

5, 6-Di-*O*-acetyl-4-*O*-(4-*O*-acetyl-2, 3-dideoxy-2-ethoxycarbonylamido-D-*ribo*-hexodialdo-1, 5-pyranosyl)-2-deoxy-1,3-di-*N*-ethoxycarbonylstreptamine (**4**)

To a solution of **3** (2.13 g) in a mixture of dry benzene-dry dimethylsulfoxide (dried over CaH_2)-dry pyridine-trifluoroacetic acid (100:10:2:1) (32 ml), dicyclohexylcarbodiimide (3.37 g) was added and the solution was stirred at room temperature for 1 hour. The solution showed, on TLC with water-saturated chloroform-ethanol (15:1) and triphenyltetrazolium chloride (TTC), a single spot at Rf 0.66 (*cf* **3**: Rf 0.44, TTC negative). The reaction mixture was filtered and the filtrate was concentrated. A chloroform solution (200 ml) of the residue was washed with water, dried (Na_2SO_4), and concentrated to its half-volume. Addition of petroleum ether gave a pale-yellow solid. The solid was chromatographed over silica gel with water-saturated chloroform-ethanol (20:1) to give a colorless solid, 1.11 g (~52%). Since the product appeared to be a hydrated form, a sample of the solid was dried at 130°C *in vacuo* until it gave constant weight, mp 226.5°C (dec.), $[\alpha]_D^{16}$ + 47° (*c* 1, chloroform). PMR ($CDCl_3$) δ: 1.22, 1.25, and 1.27 (each 3H t, $CO_2CH_2C\underline{H}_3$), 2.04 (9H s, Ac), 9.67 (1H slightly broadend s, the strength being decreased on deuteration, CHO).

Found: C 50.22, H 6.39, N 6.33%.
Calcd for $C_{27}H_{41}N_3O_{15}$: C 50.07, H 6.38, N 6.49%.

Conversion of **4** into **3**

To a solution of **4** (77 mg) in dioxane-ether (1:1, 3 ml), zinc borohydride in ether was added and the solution was kept at room temperature for 15 minutes. After addition of acetone, the mixture was filtered and the filtrate was concentrated. A chloroform solution of the residue was successively washed with 0.1 M aqueous hydrochloric acid, 0.2 M aqueous

sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated to give a solid (61.5 mg). It was identical with **3** in all respects.

Treatment of **4** with nitromethane

To a solution of **4** (352 mg) in dry methanol (9 ml), sodium methoxide (44.4 mg) in methanol (0.6 ml) was added and the solution was kept at room temperature for 20 minutes. The solution showed, on TLC with benzene-ethanol (4:1), a single spot at Rf 0.5 (*cf* **4**: Rf 0.7). Nitromethane (330 mg) was added and the solution was again kept at room temperature for 16 hours. The solution showed, on TLC, a single spot at Rf 0.55. After addition of acetic acid (0.1 ml), the solution was concentrated and the residue was washed with benzene and water to give a solid of **5**, 269 mg (85%), mp 248°C (dec.), $[\alpha]_D^{12}$ +50° (*c* 1, pyridine). PMR (pyridine-d_5) δ: 5.15~5.4[4] (3H, H-1′, 7′).

Found: C 44.63, H 6.43, N 9.14%.
Calcd for $C_{22}H_{38}N_4O_4 \cdot \frac{1}{2}H_2O$: C 44.67, H 6.64, N 9.47%.

6′-*C*-Aminomethyl derivatives (**6** and **6′**) of 3′-deoxyparomamine

To a solution of **5** (239 mg) in aqueous dioxane (1:2, 13 ml), acetic acid (0.5 ml) was added and the solution was hydrogenated in the presence of platinum oxide under hydrogen (50 lb/in^2) at room temperature overnight. The solution showed, on TLC with chloroform-methanol-28% ammonia (6:3:1), two ninhydrin-positive spots of Rf 0.44 (minor) and 0.47 (major). Filtration followed by concentration gave a solid, 239 mg (95%). The solid (206 mg) in 0.5 M barium hydroxide (7.5 ml) was refluxed for 16 hours. The solution showed, on electrophoresis, two ninhydrin-positive spots of $Rf_{3'\text{-deoxyparomamine}}$ 0.8 (minor, a cyclic 1, 3-ureylene derivative[7] showing an absorption at 1640 cm^{-1} in its IR spectrum) and 1.3 (major). Introduction of carbon dioxide followed by filtration and concentration of the filtrate gave a pale-yellow solid (~200 mg). The solid was chromatographed over CM-Sephadex C-25 column (16 ml) with ammonia (0.01~0.2 M, gradually increased). After elution of the minor product (70~75 ml), two products were separately eluted. From the earlier (110~120 ml) and the later fractions (135~150 ml), solids of 25.1 mg (**6**, 22%) and 30.9 mg (**6′**, 27%) were obtained, respectively. They both had the same $Rf_{3'\text{-deoxyparomamine}}$ 0.27 on ppc.

6: $[\alpha]_D^{12}$ +85° (*c* 0.4, water). Found: C 42.26, H 7.45, N 14.04%. Calcd for $C_{13}H_{28}N_4O_6 \cdot H_2CO_3$: C 42.21, H 7.59, N 14.06%.

6′: $[\alpha]_D^{12}$ +81° (*c* 1, water). Found: C, 40.73, H 7.38, N 13.50%. Calcd for $C_{13}H_{28}N_4O_6 \cdot H_2CO_3 \cdot H_2O$: C 40.38, H 7.75, N 13.45%.

Acknowledgement

The authors are grateful to Prof. HAMAO UMEZAWA, Director of Institute of Microbial Chemistry, for his support and discussions, and to Dr. E. AKITA, Central Research Laboratories of Meiji Seika Company for his kind supply of a sample of 3′-deoxyneamine.

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