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Studies on Aminosugars. XXXV. Syntheses of 3′,4′-Dideoxyneamine and 3′- and 4′-*O*-Methylneamines[1]

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3′,4′-Dideoxyneamine and 3′- and 4′-*O*-methylneamines have been prepared from neamine *via* 5,6-*O*-cyclohexylidene-tetra-*N*-methoxycarbonylneamine (**2**).

As described in previous papers, 3′-deoxykanamycin[2] and 3′,4′-dideoxykanamycin B[3] exhibited strong antibiotic activity against resistant bacteria carrying R factor and resistant *Pseudomonas aeruginosa*, whereas 3′-*O*-methylkanamycin[2,4] was inactive, suggesting the steric hindrance of binding of the antibiotic with ribosomes by 3′-*O*-methyl group. The present investigation was undertaken to prepare an active neamine derivative against resistant bacteris, and to confirm the occurrence of the same steric hindrance in neamine which is an essential moiety of kanamycin B.

In order to protect the four amino groups, neamine was treated with methyl chloroformate in aqueous acetone to give tetra-*N*-methoxycarbonylneamine (**1**). The methyl chloroformate was here selected instead of ethyl or benzyl chloroformate because *N*-methoxycarbonyl group was expected to be stable in acidic

1) A part of this paper was read by S. Umezawa at Symposium of New Natural Products Syntheses, XXIIIrd International Congress of Pure and Applied Chemistry at Boston, U.S.A., July 28, 1971; XXIIIrd ICPAC, Vol. 2, 173, Butterworth, London. Short communication: S. Umezawa, T. Tsuchiya, T. Jikihara, and H. Umezawa, *J. Antibiot.* (Tokyo), **24**, 711 (1971); **25**, 322 (1972).

2) S. Umezawa, T. Tsuchiya, R. Muto, Y. Nishimura, and H. Umezawa, *ibid.*, **24**, 274 (1971); S. Umezawa, Y. Nishimura, H. Hineno, K. Watanabe, S. Koike, T. Tsuchiya, and H. Umezawa, This Bulletin, **45**, 2847 (1972).

3) H. Umezawa, S. Umezawa, T. Tsuchiya, and Y. Okazaki, *J. Antibiot.* (Tokyo), **24**, 485 (1971); S. Umezawa, H. Umezawa, Y. Okazaki and T. Tsuchiya, This Bulletin, **45**, 3624 (1972).

4) H. Umezawa, T. Tsuchiya, R. Muto, and S. Umezawa, *ibid.*, **45**, 2842 (1972).

methanol used in a later reaction. The compound **1** was allowed to react with 1,1-dimethoxycyclohexane in dimethylformamide (DMF) in the presence of catalytic amount of *p*-toluenesulfonic acid at 50 °C under reduced pressure.[5] In the initial stage of this reaction, three derivatives, 3′,4′- and 5,6-*O*-monocyclohexylidene derivatives (**2′** and **2**, respectively) and 3′,4′;5,6-di-*O*-cyclohexylidene derivative (**2″**, minor) were formed but after standing overnight, the derivative **2′** disappeared and a mixture of **2** and **2″** was obtained. Addition of methanol converted **2″** to **2** and the conversion to the starting material (**1**) was very slight. Chromatographic separation gave **2** in a yield of 78%.

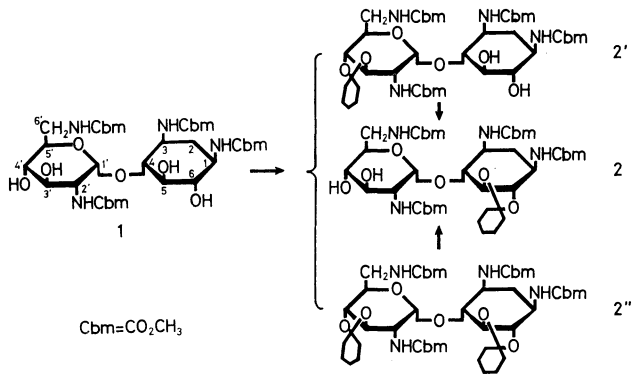

Chart 1.

Mesylation of **2** gave 3′,4′-di-*O*-mesyl derivative (**3**), and treatment of **3** with sodium iodide and zinc dust in DMF, similarly as described in a previous paper,[3] gave 3′-eno derivative (**4**) in a yield of 80%. Catalytic hydrogenation gave 3′,4′-dideoxy derivative (**5**), and removal of the protecting groups in a usual way gave 3′,4′-dideoxyneamine (**6**). Similar deblocking of the 3′-eno derivative (**4**) gave 3′,4′-dideoxy-3′-enoneamine (**7**).

To prepare 3′- and 4′-*O*-methylneamines, compound **2** was subjected to methylation. In preliminary experiments, methylation with methyl iodide–silver oxide and methyl iodide-barium oxide was tried, however, *O*-methylation with these reagents was accompanied by *N*-methylation, rendering the isolation of products difficult. Haworth methylation with dimethyl sulfate–aqueous sodium hydroxide was suitable in this case. Since attempts to separate 3′- and 4′-*O*-methyl derivatives (**8a**, **8b**) was unsuccessful, the mixture was acetylated and then chromatographed over silica gel to give 4′-*O*-acetyl-3′-*O*-methyl- and 3′-*O*-acetyl-4′-*O*-methyl derivatives (**9a**, **9b**) which were thereafter deblocked by the usual method to give 3′-*O*-methylneamine (**10**) and 4′-*O*-methylneamine (**11**), respectively.

The structures of **10** and **11** were confirmed by tetramine copper(II) sulfate (TACu)[6] method. The reagent forms a copper complex exclusively with vicinal amino and hydroxyl groups, which exhibits $\Delta[M]_{436\,(TACu)}$ $\pm 900°$ if the groups have $-60°$ dihedral angle. The compound **10** showed $\Delta[M]_{TACu}$ $+730°$. The high positive increment indicated that a complex is formed at 1-NH_2 and 6-OH groups. In **11**, TACu forms complexes both at 1-NH_2 and 6-OH, and at 2′-NH_2 and 3′-OH, resulting in a small increment by the intramolecular compensation of the contributions of the two complexes opposite in sign and approximately equal in magnitude.

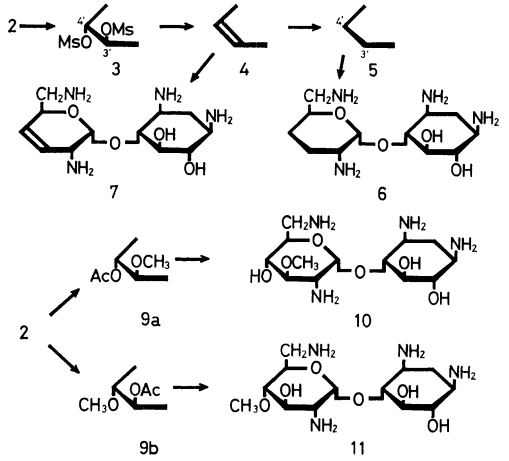

Chart 2.

The synthetic 3′,4′-dideoxyneamine showed[1] antibacterial activity against resistant *E. coli* and *Pseudomonas aeruginosa*, whereas 3′- and 4′-*O*-methylneamines showed markedly decreased activity, suggesting that, though the hydroxyl groups do not play important role in the mechanism of antibacterial action, there is a strong steric factor associated with the hydroxyl groups.

Experimental

Thin layer chromatography (tlc) was carried out on silica gel and the spots were visualized with sulfuric acid.

Tetra-N-*methoxycarbonylneamine (1)*[7]. Crude product of **1** obtained by the procedure reported[8] was desalted by dissolving it in hot dioxane and the dioxane-soluble product was reprecipitated from methanol, $[\alpha]_D^{20}$ $+67°$ (*c* 0.5, water).

Found: C, 42.50; H, 6.40; N, 9.53%. Calcd for $C_{20}H_{34}N_4O_{14}\cdot 1/2\,H_2O$: C, 42.63; H, 6.26; N, 9.94%.

NMR (dimethyl sulfoxide-d_6): τ 6.80, 6.60, 6.50, 6.43 (3H, s, each, $-COOCH_3$).

5,6-O-Cyclohexylidene-tetra-N-*methoxycarbonylneamine (2)*. To the suspension of **1** (5.43 g) in dry DMF (57 ml), 1,1-dimethoxycyclohexane (5.76 ml) and fuse-dried *p*-toluenesulfonic acid (170 mg) were added and the mixture was heated at 55 °C under stirring and reduced pressure (20 Torr). After 20 min, the starting material (R_f 0 on tlc with chloroform–ethanol (8: 1)) disappeared and after 1.5 hr the mixture became composed from three products (R_f 0.9 (**2″**), 0.45 (**2**) and 0.40 (**2′**)). The solution was allowed to stand overnight at 25 °C, whereupon *2′* almost disappeared. Methanol (3 ml) was added and the solution was allowed to stand at 45 °C for 1.5 hr. By this treatment, **2** became major constituent

5) F. H. Bissett, M. E. Evans, and F. W. Parrish, *Carbohyd. Res.*, **5**, 184 (1967).

6) S. Umezawa, T. Tsuchiya, and K. Tatsuta, This Bulletin, **39**, 1235 (1966).

7) This experiment was performed by Yasushi Takagi and Narushi Hatsuse of our laboratory.

8) S. Umezawa, Y. Okazaki, and T. Tsuchiya, This Bulletin, **45**, 3619 (1972).

and **2″**, minor one. Saturated sodium hydrogen carbonate solution was added and the mixture was evaporated. The residue was extracted with chloroform and the solution was evaporated to give a pale brown solid (5.8 g). This was chromatographed on a column of silica gel (300 g) with chloroform–ethanol (12:1) containing 1% triethylamine. The portions containing **2** and **2″** were evaporated to give solids, 4.85 g (78%) and 0.9 g, respectively. **2**: $[\alpha]_D^{25}$ +37° (*c* 1, methanol).

Found: C, 49.44; H, 6.82; N, 8.93%. Calcd for $C_{26}H_{42}N_4O_{14}$: C, 49.20; H, 6.67; N, 8.83%.

NMR spectra of the compounds **2′** and **2″** showed the presence of mono- and di-cyclohexylidene group, respectively.

5,6-O-Cyclohexylidene-3′,4′-di-O-mesyl-tetra-N-methoxycarbonylneamine (3). To a solution of **2** (1.89 g) in dry pyridine (37 ml), mesyl chloride (1.0 g) was added and the solution was allowed to stand at 30 °C overnight and then at 50 °C for 2 hr. After addition of 0.3 ml of water, the solution was concentrated to approximately one-tenth of the original volume and the concentrate was poured into saturated sodium hydrogen carbonate solution. The precipitate was extracted with chloroform and the extract was washed with water, dried over sodium sulfate and evaporated. The residue was chromatographed on a short column of silica gel (30 g) with chloroform–ethanol (15:1) containing 1% triethylamine. The portion containing **3** was evaporated to give a solid, 2.24 g (95%), $[\alpha]_D^{25}$ +50.4° (*c* 1, methanol).

Found: C, 42.77; H, 5.85; N, 6.97; S, 7.89%. Calcd for $C_{28}H_{46}N_4O_{18}S_2$: C, 42.53; H, 5.86; N, 7.09; S, 8.11%.

NMR ($CDCl_3$): τ 6.90 and 6.73 (each 3H, s, SO_2CH_3).

5,6-O-Cyclohexylidene-3′,4′-dideoxy-3′-eno-tetra-N-methoxycarbonylneamine (4). To a solution of **3** (1.28 g) in dry DMF (30 ml), anhydrous sodium iodide (15 g) and zinc dust (7 g) were added and the mixture was heated in an oil bath (98 °C) for 2 hr under stirring. Chloroform (70 ml) was added and the resulting precipitate was removed by centrifugation and the precipitate was washed with chloroform. Mother solution and the washings combined were mixed with sodium hydrogen carbonate solution and, after filtration, the filtrate was evaporated to give a syrup, which was dissolved in chloroform. The solution was washed with sodium thiosulfate solution and with water, dried over sodium sulfate and evaporated to give amorphous powder, 0.78 g (80%), $[\alpha]_D^{25}$ −39° (*c* 1, methanol).

Found: C, 52.08; H, 6.78; N, 9.10%. Calcd for $C_{26}H_{40}N_4O_{12}$: C, 52.00; H, 6.71; N, 9.33%.

NMR ($CDCl_3$): τ 4.28 (2H, slightly broadened s, H-3′,4′).

5,6-O-Cyclohexylidene-3′,4′-dideoxy-tetra-N-methoxycarbonylneamine (5). A solution of **4** (368 mg) in methanol (7 ml) containing water (0.2 ml) was hydrogenated with platinum oxide and hydrogen (50 psi) at room temperature for 3 hr. The reaction mixture was filtered and evaporated to give amorphous powder, 339 mg (92%), $[\alpha]_D^{23}$ +34° (*c* 0.6, methanol).

Found: C, 51.58; H, 6.95; N, 9.20%. Calcd for $C_{26}H_{42}N_4O_{12}$: C, 51.82; H, 7.03; N, 9.30%.

NMR ($CDCl_3$): τ 4.92 (1H d, J=3.5 Hz, H-1′), 6.30, 6.32 and 6.34 (6H, 3H, 3H, s, respectively, $NHCO_2C\underline{H}_3$), 8.1—8.8 (~15H broadened signal, cyclohexylidene protons, H-3′,3′,4′,4′ and H-2_{ax}).

3′,4′-Dideoxyneamine (6). To a solution of **5** (299 mg) in methanol (5.3 ml), 0.5 M barium hydroxide solution (17 ml) was added and the mixture was heated at 105 °C for 11 hr. Carbon dioxide was introduced at 100 °C and the precipitate was removed by centrifuging and the precipitate was washed with hot water. Mother solution and the washings combined were evaporated to give a residue, which was again dissolved in water, filtered, and evaporated. The solid residue was dissolved in 1 M hydrochloric acid (5 ml) and the solution was refluxed for 1 hr. After concentration with occasional addition of water, the concentrate was neutralized with Amberlite IRA-400 (OH form) to pH 4 and, after filtration, the solution was charged on a column of Amberlite CG 50 (NH_4^+ form) and developed with 0—0.3 M ammonia with gradual increase in concentration. 2-Deoxystreptamine and by-products were eluted in an early stage (at about 0.2 M ammonia) and **6** followed (at about 0.3 M ammonia). The portion containing **6** was evaporated to give a solid, 115 mg (74%), $[\alpha]_D^{20}$ +102° (*c* 1, water).

Found: C, 48.56; H, 9.12; N, 18.42%. Calcd for $C_{12}H_{26}N_4O_4\cdot 1/2H_2O$: C, 48.14; H, 9.09; N, 18.72%.

NMR (D_2O): τ 9.1—7.8 (6H m, H-2,3′,4′), 4.82 (1H d, J=3.5 Hz, H-1′). The pattern at τ 9.1—7.8 was quite similar with that of 3′,4′-dideoxykanamycin B base[3].

3′,4′-Dideoxy-3′-enoneamine (7). Compound **4** (1.9 g) was treated similarly as described for the preparation of **6** from **5** and the product obtained by treatment of Amberlite CG 50 column chromatography was neutralized with sulfuric acid solution. Addition of methanol gave a solid, which was further purified by passing a short column of active carbon, 802 mg (80%), $[\alpha]_D^{20}$ −19.4° (*c* 1, water).

Found: C, 27.85; H, 6.40; N, 10.28; S, 12.28%. Calcd for $C_{12}H_{24}N_4O_4\cdot 2H_2SO_4\cdot 2H_2O$: C, 27.69; H, 6.20; N, 10.76; S, 12.32%.

NMR (D_2O): τ 8.3—7.2 (2H m, H-2), 4.30 (1H d, J=3 Hz, H-1′), 4.1—3.5 (2H m, H-3′,4′). The pattern at τ 4.1—3.5 was similar with that of methyl 2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hex-3-enopyranoside sulfate.[8]

A Mixture of 5,6-O-Cyclohexylidene-tetra-N-methoxycarbonyl-3′-O-methylneamine (8a) and 5,6-O-Cyclohexylidene-tetra-N-methoxycarbonyl-4′-O-methylneamine (8b). To a solution of **2** (2.90 g) in acetone (58 ml), 30% sodium hydroxide solution (4 ml) and dimethyl sulfate (1 ml) were added and the mixture was stirred at room temperature for 1 hr. On tlc with chloroform–2-propanol (15:1) the solution showed three spots of R_f 0.26 (**8a**+**8b**), 0.35 (mono-*O*-methyl-mono-*N*-methyl derivatives) and 0.5 (di-*O*-methyl derivative). Ammonia solution was added and, after stirring for a while, the solution was evaporated to give a residue, which was dissolved in chloroform and the solution was washed with sodium hydrogen carbonate solution and with water, dried over sodium sulfate and evaporated. A pale yellow residue (2.8 g) was chromatographed on a column of silica gel (120 g) with chloroform–2-propanol (25:1). The portion containing **8a** and **8b** was evaporated to give a solid, 1.40 g (47%).

Found: C, 50.21; H, 6.95; N 8.32%. Calcd for $C_{27}H_{44}N_4O_{14}$: C, 49.99; H, 6.84; N, 8.64%.

4′-O-Acetyl-5,6-O-cyclohexylidene-tetra-N-methoxycarbonyl-3′-O-methylneamine (9a) and 3′-O-Acetyl-5,6-O-cyclohexylidene-tetra-N-methoxycarbonyl-4′-O-methylneamine (9b). The mixture of **8a** and **8b** described above was treated with acetic anhydride and pyridine in a usual manner to give a mixture of monoacetyl derivatives quantitatively. On tlc with chloroform–2-propanol, the mixture showed a spot of R_f 0.5, while on tlc with benzene–ethyl acetate (1:4), it showed two spots of R_f 0.26 (**9a**) and 0.39 (**9b**), although **8a** and **8b** could not be separated by column chromatography with this system. The mixture (1.4 g) was chromatographed on a column of silica gel (95 g) with benzene–ethyl acetate (2:5) and the portions containing **9a** (640—1200 ml portion) and **9b** (300—570 ml portion) were evaporated to give solids, 0.58 g (42%) and 0.72 g (52%), respectively. **9a**: mp 129—131 °C, $[\alpha]_D^{20}$ +46° (*c* 1, methanol).

Found: C, 50.28; H, 6.54; N, 8.04%. Calcd for $C_{29}H_{46}N_4O_{15}$: C, 50.43; H, 6.71; N, 8.11%.

NMR ($CDCl_3$): τ 8.8—8.2 (11H, cyclohexyl and H-2_{ax}), 7.88 (3H s, OAc), 6.55 (3H s, OCH_3), 6.31 (12H s, $NHCO_2CH_3$), 5.12 (1H t, J=9.5 Hz, H-4′), 4.81 (1H d, J=3.5 Hz, H-1′). **9b**: mp 123—127 °C, $[\alpha]_D^{20}$ +44° (*c* 1, methanol).

Found: C, 50.56; H, 6.85; N, 8.00%. Calcd for $C_{29}H_{46}N_4O_{15}$: C, 50.43; H, 6.71; N, 8.11%.

NMR ($CDCl_3$): τ 8.8—8.2 (11H), 7.92 (3H s, OAc), 6.53 (3H s, OCH_3), 6.53 (3H), 6.32 (6H) and 6.28 (3H) (each s, $NHCO_2CH_3$), 4.83 (1H t, J=10 Hz, H-3′), 4.80 (1H d, J=3.5 Hz, H-1′).

3′-O-Methylneamine (10). A sample of **9a** was treated likewise as described in the preparation of **6** from **5**, yield 74%, $[\alpha]^{20}$ +122° (*c* 1, water), $\Delta[M]^{20}_{436(TACu)}$ +730°.

Found: C, 43.86; H, 8.59; N, 16.06%. Calcd for $C_{13}H_{28}N_4O_6\cdot H_2O$: C, 44.05; H, 8.53; N, 15.81%.

NMR (D_2O): τ 8.80 (1H q, J=12 Hz, H-2_{ax}), 8.02 (1H double t, J=4, 4 and 13 Hz, H-2_{eq}), 6.40 (3H s, OCH_3), 4.71 (1H d, J=3.5 Hz, H-1′).

4′-O-Methylneamine (11). A sample of **9b** was treated as above, yield 78%, $[\alpha]_D^{20}$ +126° (*c* 1, water), $\Delta[M]^{20}_{436(TACu)}$ −200°.

Found: C, 43.93; H, 8.56; N, 16.00%. Calcd for $C_{13}H_{28}N_4O_6\cdot H_2O$: C, 44.05; H, 8.53; N, 15.81%.

NMR (D_2O): τ 8.80 (1H q, J=12 Hz, H-2_{ax}), 8.02 (1H double q, J=4, 4 and 13 Hz, H-2_{eq}), 6.44 (3H s, OCH_3), 4.72 (1H d, J=3.5 Hz, H-1′).