STUDIES ON ANTIBIOTIC SF-733, A NEW ANTIBIOTIC. II

CHEMICAL STRUCTURE OF ANTIBIOTIC SF-733

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A new antibiotic SF-733[1] gave neamine[2] and methyl D-riboside on acid methanolysis and a furanoside nature of the ribose moiety was determined by periodate oxidation of tetra-N-acetyl SF-733. The site of linkage was determined by the formation of di-N-acetyl-6-O-methyl-2-deoxystreptamine after acid hydrolysis of tetra-N-acetyl-hexa-O-methyl SF-733. The NMR spectrum of tetra-N-acetyl SF-733 was compared with those of N-acetylated neomycin group of antibiotics and it was concluded that the ribofuranosyl linkage was β. Thus, the chemical structure of antibiotic SF-733 was determined to be O-β-D-ribofuranosyl-(1→5)-O-[α-2, 6-diamino-2, 6-dideoxy-D-glucopyranosyl-(1→4)]-2-deoxystreptamine.

Antibiotic SF-733 (I); $C_{17}H_{34}N_4O_{10}$, was easily solvolysed by treatment with 0.3~0.4 N HCl in methanol at room temperature and gave a methyl glycoside and an amine. The former was hydrolysed with dilute mineral acid to yield a levorotatory reducing sugar (II), which was identified to D-ribose by PPC, $[\alpha]_D$ value and by conversion to its anilide (III)[3] and phenylosazone (IV)[4].

The amine (V), which should be a bicyclic and tetraacidic base since I was a tricyclic and tetracidic base from its molecular formula, titration equivalent value and the fact that I possessed no unsaturation in its molecule, was identified to neamine

Fig. 1. Infrared absorption spectra of D-ribose anilide (KBr pellet).
A : from antibiotic SF-733. B : from commercial D-ribose.

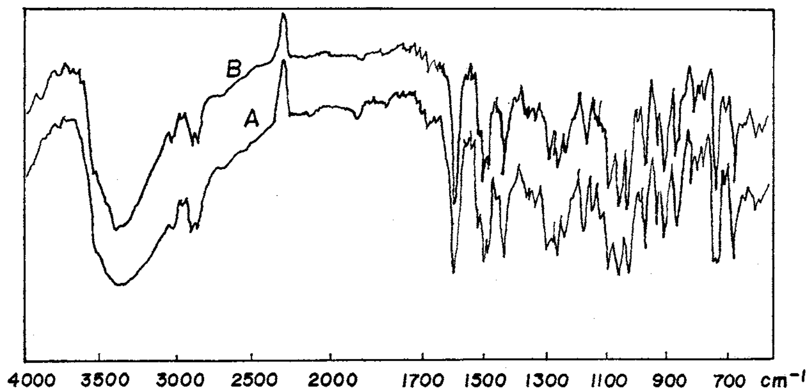


Fig. 2. Infrared absorption spectra of neamine (KBr pellet).
A : from antibiotic SF-733. B : from kanamycin B.

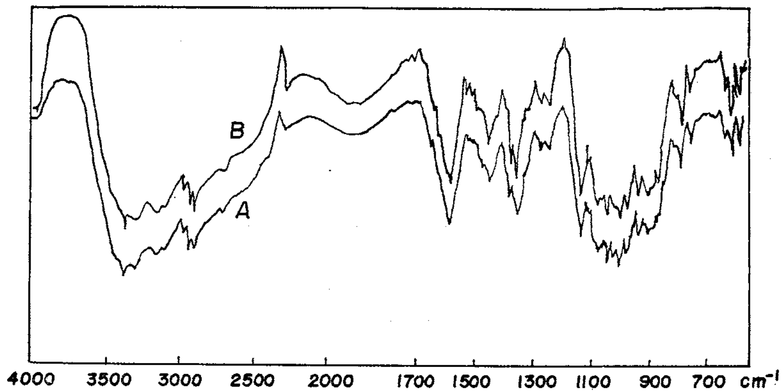


Fig. 3. Infrared absorption spectra of tetra-N-acetyl neamine (KBr pellet).
A : from antibiotic SF-733. B : from kanamycin B.

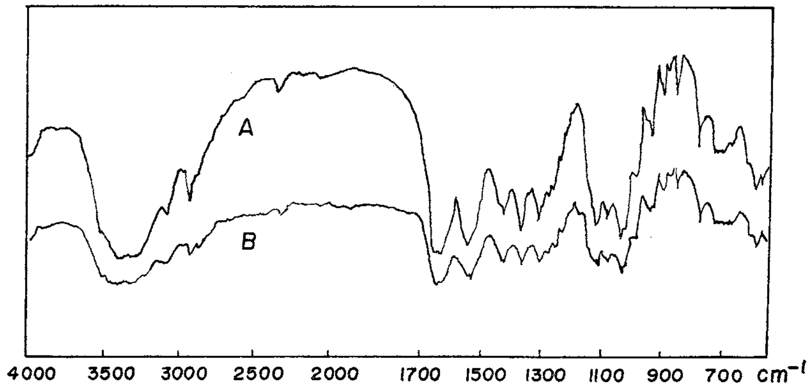


Fig. 4. Nuclear magnetic resonance spectrum of tetra-N-acetyl neamine from antibiotic SF-733.
100 MC in D_2O, TMS as external standard. δ-Values are corrected with reference to HOD signal (DSS as internal standard).

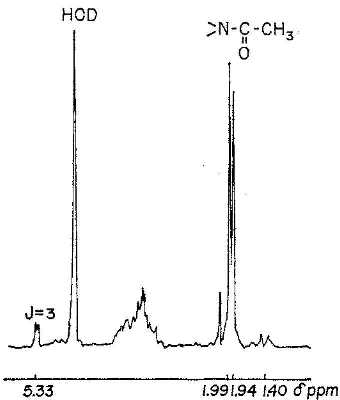


Fig. 5. Nuclear magnetic resonance spectrum of tetra-N-acetyl neamine from kanamycin B.
100 MC in D_2O, TMS as external standard. δ-Values are corrected with reference to HOD signal (DSS as internal standard).

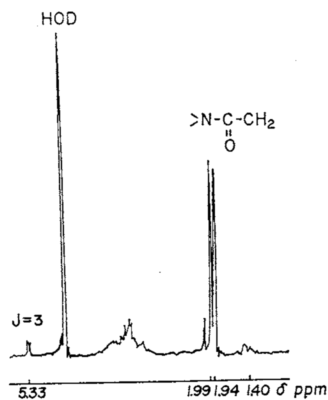


Fig. 6. Optical rotatory dispersion curves of neamine and tetra-N-acetyl neamine both from antibiotic SF-733 (solid line) and from kanamycin B (broken line).

A : neamine 0.176 % in H_2O.
B : neamine 0.181 % in H_2O.
C : tetra-N-acetyl neamine 0.087 % in H_2O.
D : tetra-N-acetyl neamine 0.084 % in H_2O.
E : tetra-N-acetyl neamine 0.087 % in CuCl/conc. NH_3 aq. (0.8 g/50 ml).
F : tetra-N-acetyl neamine 0.084 % in CuCl/conc. NH_3 aq. (0.8 g/50 ml).

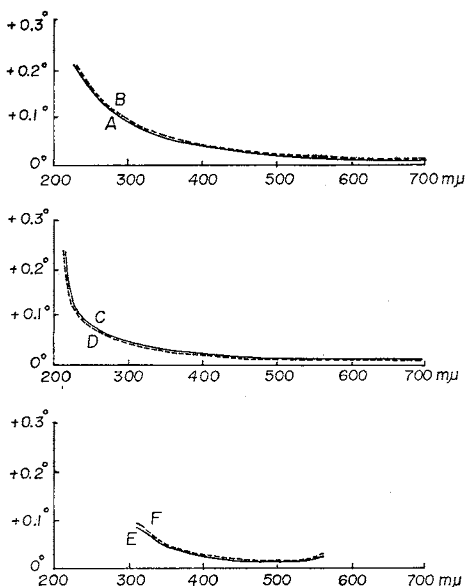
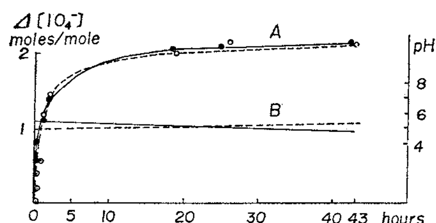


Fig. 7. Periodate oxidation of tetra-N-acetyl SF-733 (solid line) and tetra-N-acetyl neamine (broken line).

A : periodate consumption.
B : pH of the reaction mixtures.



by conversion to its tetra-N-salicylidene derivative (VI)[5,6] and its tetra-N-acetyl derivative (VII).

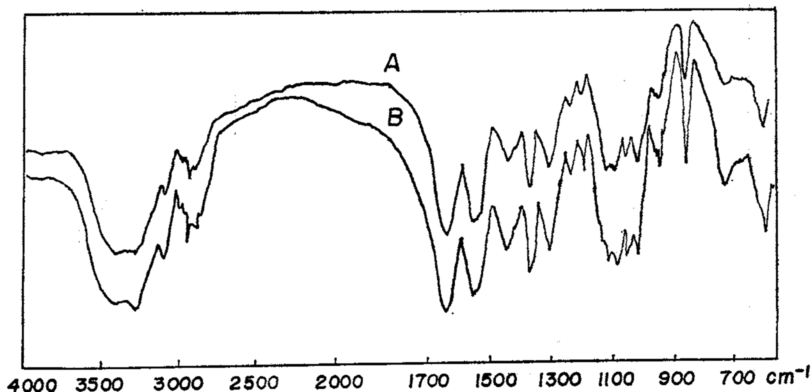
The linkage between neamine and D-ribose should be of a O-glycoside type because I did not reduce FEHLING's solution and the linkage was easily broken under a mild acidic condition.

The problem whether the ribosyl radical took a furanoside or a pyranoside structure in I, was solved by oxidizing tetra-N-acetyl SF-733 (VIII) with periodate resulting only two moles of the reagent consumption with formation of no acidic material (Fig. 7). This result clearly indicated the furanoside structure and it was also consistent with the extreme ease of acid hydrolysis of I[7].

The periodate oxidation of VIII also revealed that the ribosyl radical did not exist on the 2, 6-diamino-2, 6-dideoxy-D-glucopyranose moiety of neamine since 2-deoxystreptamine was obtained in good yield after vigorous acid hydrolysis of the periodate

Fig. 8. Infrared absorption spectra of di-N-acetyl-6-O-methyl-2-deoxystreptamine (KBr pellet).

A : from antibiotic SF-733. B : from zygomycin A_1.



oxidation product. The 2-deoxistreptamine obtained was identified to the authentic sample by converting it to its dipicrate (**IX**) and its di-N-acetate (**X**).

Though periodate oxidation of I was expected to elucidate the exact place where the ribofuranosyl radical was attached, and, actually, no 2-deoxystreptamine was recovered from the acid hydrolysate after the oxidation under either a weakly alkaline or a weakly acidic condition suggesting the substitution at C-5 of 2-deoxystreptamine moiety, there existed an uncertainty in this experiment because at an alkaline pH, the oxidation was expected to proceed with ease[8] but with a danger of overoxidation[9], and at an acidic pH, the aminoalcohol was scarecely expected to be oxidized quantitatively[9].

In order to obtain a more solid proof for the (1→5) linkage, tetra-N-acetyl-hexa-O-methyl SF-733 (**XI**) prepared by the method of KUHN and others[10], was hydrolysed with 6 N HCl and the resulting products were fractionated using Dowex 50 W×8 (H^+) by the method of HORII and others[11]. Fractions containing a substituted aminocyclitol were combined, neutralized and acetylated with acetic anhydride in methanol to give di-N-acetyl-6-O-methyl-2-deoxystreptamine (**XII**) which was identified to the authentic sample by its IR (Fig. 8), ORD (Fig. 9), NMR (Fig. 10) and melting point.

In the NMR spectrum of **XII**, the signal

Fig. 9. Optical rotatory dispersion curves of di-N-acetyl-6-O-methyl-2-deoxystreptamine.
A : from antibiotic SF-733; 0.48 % in H_2O.
B : from zygomycin A_1; 0.54 % in H_2O.
C : H_2O (blank).

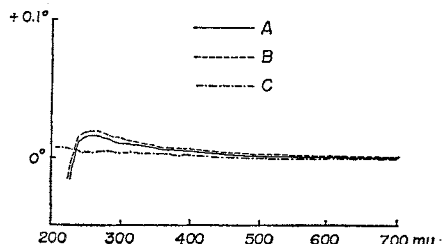


Fig. 10. Nuclear magnetic resonance spectra of di-N-acetyl-6-O-methyl-2-deoxystreptamine (100 MC in D_2O, TMS as external standard), and di-N-acetyl-5-O-methyl-2-deoxystreptamine (100 MC in D_2O, TMS as external standard).
A : di-N-acetyl-6-O-methyl-2-deoxystreptamine from antibitotic SF-733.
B : di-N-acetyl-6-O-methyl-2-deoxystreptamine from zygomycin A_1.
C : di-N-acetyl-5-O-methyl-2-deoxystreptamine from kanamycin.

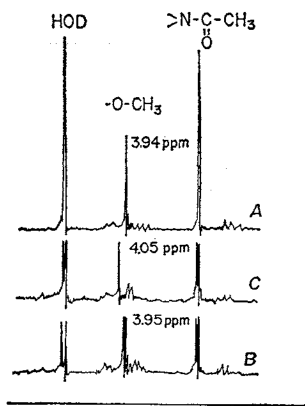
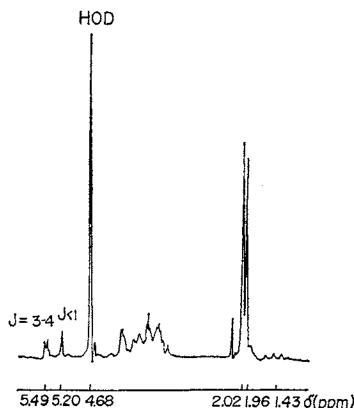


Fig. 11. Nucelar magnetic resonance spectrum of tetra-N-acetyl SF-733.
100 MC in D_2O, TMS as external standard. δ-Values are corrected with reference to HOD signal (DSS as internal standard).



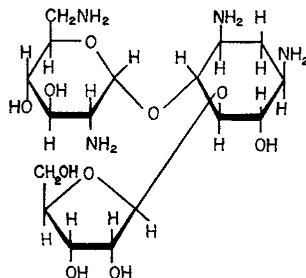
assigned to methoxyl protons appeared 3.94~3.95 ppm apart from TMS signal and was clearly distinguished from the corresponding signal that appeared 4.05 ppm apart from the standard in the spectrum of di-N-acetyl-5-O-methyl-2-deoxystreptamine (Fig. 10).

Thus, the formation of 6-O-methyl-2-deoxystreptamine from **XI** clearly indicated the (1→5) linkage in I.

The comparatively small $[\alpha]_D$ value, +42°, of I was considered to favor the β-glycoside linkage in the molecule and the further evidence for β-linkage was obtained by the examination of signals of anomeric protons in the NMR spectrum of **VIII** (Fig. 11).

Accoording to RINEHART Jr. and others[12], the anomeric proton of tetra-N-acetyl neamine aforded a doublet (J=3.1) at 0.66 ppm lower than the signal of water and in case of hexa-N-acetyl neomycin B and C, the corresponding protons gave doublets (J=3.5 and 3.6) at 0.80 ppm and 0.79 ppm lower than that of water.

In the present case, **VIII** gave two anomeric proton signals both 0.81 ppm (J=3~4) and 0.52 ppm (J<1) lower than the signal of water. As the former signal was assigned to the anomeric proton of 2, 6-diamino-2, 6-dideoxy-D-glucopyranose moiety, the latter should be assigned to that of ribofuranose moiety. The very small coupling constant together with the chemical shift value of this signal clearly indicated the β-linkage when compared with the report[12] in which the substituted β-D-ribofuranosyl anomeric proton of either acetylated neomycin B or C was described to give a signal at field 0.50 ppm (J<1) lower than that of water.



In conclusion, the chemical structure of antibiotic SF-733 (I) was determined to be O-β-D-ribofuranosyl-(1→5)-O-[α-2, 6-diamino-2, 6-dideoxy-D-glucopyranosyl-(1→4)]-2-deoxystreptamine.

Experimental

NMR spectra were taken by using JEOL JNM-4H-100, IR spectra were taken by using JASCO DS-401G, ORD curves were obtained by using JASCO ORD/UV-5 and vapor pressure osmometric determination of molecular weight was done by using Hitachi-Perkin-Elmer 115 M.W. apparatus.

Antibiotic SF-733 (I):

Crystals or the material of equivalent purity was submitted to the structural study.

Anal. Calcd. for $C_{17}H_{34}N_4O_{10}$: C 44.93, H 7.54, N 12.33, O 35.20.

Found: C 44.16, 44.19, H 7.55, 7.39, N 11.92, 11.60, O 36.21.

Tetra-N-acetyl SF-733 (VIII):

A 65 ml methanolic suspension of 1 g of I was added with 5 ml of acetic anhydride under stirring until dissolved. After standing at room temperature for 30 minutes the solution was further stood for 21 hours at 5°C, added with 400 ml of ethyl ether and the resulting precipitate was collected; 1247.7 mg, colorless powder, decomposed at 205°C (sinter at 180°C), $[\alpha]_D^{23}$ +40° (*c* 1, H_2O), NMR: δ=5.49 (J=3~4), δ=5.20 (J<1), each 1 proton; δ=1.96~2.02, 12 protons (100 MC in D_2O, TMS external standard, δ-values were corrected with reference to HOD signal (DSS as internal standard)).

Anal. Calcd. for $C_{25}H_{42}N_4O_{14}\cdot H_2O$: C 46.90, H 6.89, N 8.75, O 37.5.

Found: C 45.01, H 6.94, N 8.59, Molecular weight (calcd.): 640, Molecular weight (found): 630.

Decaacetyl SF-733:

A 5 ml solution of 200 mg of **VIII** in a mixed reagent consisted of pyridine and acetic anhydride (2:1) was stood at 5°C overnight, dried in vacuum, chromatographed on a 10 g column of sillicic acid and each 10 g fraction was taken with elution by $CHCl_3$-MeOH (20:1). Fractions Nos. 23~44 were combined and dried to give a 195 mg of solid material which was recrystallized from $CHCl_3$-ethyl ether to yield a 109.7 mg of crystalline precipitate; m. p. 156°C (145°C sinter), decomposed at 258°C with remelting; pure on TLC: $CHCl_3$-MeOH (5:1), acetone-MeOH (10:1), $CHCl_3$-MeOH-H_2O-ethyl acetate (100:40:7:40) Silicagel G, $[\alpha]_D$ +48.3° (*c* 0.48, MeOH), NMR: δ=5.51 (doublet), δ=5.39 (doublet), each 1 proton; δ=2.13, 2.08, 2.05, 2.03, 1.97, 1.93, 1.91 (30 protons) in $CDCl_3$, TMS as internal standard, 100 MC.

Anal. Calcd. for $C_{37}H_{54}N_4O_{20}$: C 50.80, H 6.22, N 6.40, O 36.58.
Found: C 49.48, H 6.39, N 6.19, O 36.86.

Tetra-N-salicylidene-SF-733:

A 1.28 ml aqueous solution of 95.0 mg of **I** was added with a 1.28 ml portion of 800 mg of salicylaldehyde in 10 ml of EtOH. After 10 minutes, 12 ml of H_2O was added to the reaction mixture and resulting precipitate was collected; 135.5 mg, crystalline powder m.p. 175°C (152°C sinter), pure on TLC: $CHCl_3$-MeOH (5:1), benzene-EtOH (5:1) Silicagel G.

Anal. Calcd. for $C_{45}H_{50}N_4O_{14}\cdot H_2O$: C 60.80, H 5.90, N 6.30, O 27.00.
Found: C 60.60, H 6.10, N 6.03.

Tetra-N-acetyl-hexa-O-methyl SF-733 (XI):

A 8 ml solution of 966.25 mg of **VIII** in DMF was added with 2.8 ml of methyl iodide and BaO-$Ba(OH)_2$ mixture (BaO 2.8 g, $Ba(OH)_2\cdot 8H_2O$ 101 mg) under cooling with water. After standing at room temperature for about 1.5 hours the exothermic reaction occurred and when the evolution of heat ceased after additional 30 minutes, the reaction vessel was mounted on a reciprocal shaker and shaking was continued for 24 hours at 28°C. Then the reaction mixture was added with 12.5 ml of MeOH and 25 ml of $CHCl_3$, and chromatographed on a 100 g column of Al_2O_3 (Sumitomo activated alumina C-1). Elution was executed with $CHCl_3$-MeOH (2:1) and the first 166 ml fraction was dried, re-dissolved into 10 ml of $CHCl_3$-MeOH (10:1) and re-chromatographed on a 100 g column of Al_2O_3. Elution was done with $CHCl_3$-MeOH (10:1). The first 150 ml was dried to yield 1,179 mg of the methylated material which was dissolved into 10 ml of acetone and precipitated with addition of 120 ml of ethyl ether; 898 mg, white powder, recrystallized from acetone-ethyl ether; colorless needles or small rods, m. p. 206°C, $[\alpha]_D$ +59.2° (*c* 0.71, MeOH); NMR: δ=5.17 (2 protons), 3.53 (12 protons), 3.42 (3 protons), 3.33 (3 protons), 2.09, 2.02, 1.98, 1.95 (12 protons) in $CDCl_3$, TMS as internal standard, 100 MC. Pure on TLC: $CHCl_3$-MeOH (5:1), acetone-MeOH (10:1), $CHCl_3$-MeOH-H_2O-ethyl acetate (100:40:7:40) Silicagel G.

Anal. Calcd. for $C_{31}H_{54}N_4O_{14}$: C 52.68, H 7.70, N 7.93, O 31.69.
Found: C 51.46, H 7.90, N 7.74, O 30.81.

Methanolysis of antibiotic SF-733:

A 250 ml solution of 976.6 mg of **I** in 0.4 N HCl/MeOH was kept at 28°C for 6 days and 260 ml of ethyl ether was added to the reaction mixture. The resulting precipitate was removed by filtration and the filtrate was neutralized with MeOH washed anion exchanger and dried to give a 269.2 mg of solid material; yield 76.3 % as methyl D-riboside, pure on TLC: *n*-BuOH-AcOH-H_2O (2:1:1), *t*-BuOH-AcOH-H_2O (2:1:1) Silicagel G.

The removed precipitate was ninhydrin positive; 859.0 mg yield 85.2 % as neamine tetrahydrochloride, pure on TLC: the same solvent system, Silicagel G.

D-Ribose (II) from antibiotic SF-733:

A 22.54 mg of the methyl glycoside fraction obtained by the methanolysis of **I** was

dissolved into 2.5 ml of 0.1 N HCl in a glass ampule, sealed and hydrolysed at 120°C for 2 hours. The resulting reaction mixture was neutralized with Amberlite IRA400 (OH^-) and condensed to a syrupy solid; 8.55 mg, $[\alpha]_D$ −17.5° (*c* 0.285, H_2O), chromatographically pure: $R_{glucose}$ 1.43, $R_{galactose}$ 1.46, $R_{fructose}$ 1.21, $R_{arabinose}$ 1.21, R_{lyxose} 1.16, R_{xylose} 1.11, R_{ribose} 1.00 (PPC: *n*-BuOH-AcOH-H_2O (4:1:5, upper layer), ascend. 20 hours, detected by the method of TREVELYAN-PETRONICI[13]), $R_{gluc.}$ 1.28, $R_{galac.}$ 1.29, $R_{fruc.}$ 1.23, $R_{arab.}$ 1.14, $R_{xyl.}$ 1.02, $R_{rib.}$ 1.00 (PPC: *n*-BuOH-EtOH-H_2O (40:11:19), ascend. 20 hours, detected by the same method as above), $R_{lyx.}$ 1.46, $R_{xyl.}$ 1.00, $R_{rib.}$ 1.00 (PPC: ethyl acetate-pyridine-H_2O (8:2:1), ascend. 2.5 hours, detected by the same method as above), $R_{gluc.}$ 2.50, $R_{galac.}$ 1.80, $R_{fruc.}$ 1.12, $R_{arab.}$ 1.21, $R_{xyl.}$ 0.76, $R_{rib.}$ 1.00 (TLC: ethyl acetate-(iso-PrOH-H_2O (2:1)) (65:35), ascend. detected with anilin hydrogen phthalate).

D-Ribose anilide (III) from antibiotic SF-733:

A 269 mg portion of the methyl glycoside obtained by the methanolysis was hydrolysed with 10 ml of 0.5 N H_2SO_4 at 90~95°C for 1 hour and 40 minutes, after cooling, added with 1.56 ml of satd. aqueous $Ba(OH)_2$ solution to pH 6 and freeze-dried to give a 250 mg of faintly brown solid. A 124.3 mg portion of this solid was converted to its anilide by the method described in the text[3]; 41.17 mg, crystals, m. p. 92~95°C (microblock), recrystallized from EtOH-H_2O (1:2); 22.42 mg, colorless needles, m. p. 121°C (109°C sinter), $[\alpha]_D$ +62.5° (*c* 0.3, pyridine), pure on TLC: benzene-EtOH (5:1), $CHCl_3$-MeOH (5:1) Silicagel G, Rf values on TLC were identical with those of the authentic sample, IR spectrum was identical with that of the authentic sample and it did not depress its melting point on admixture with the authentic sample.

Anal. Calcd. for $C_{11}H_{15}NO_4 \cdot 1/2H_2O$: C 56.40, H 6.89, N 5.98, O 30.74.
Found: C 56.83, H 6.60, N 6.50.

D-Ribose anilide (authentic) from D-ribose:

The authentic sample of D-ribose anilide was prepared from commercial D-ribose according to the text; 57.72 mg from 144.8 mg of marketed D-ribose, colorless needles m. p. 116°C (109°C sinter), $[\alpha]_D$ +63.8° (*c* 0.3, pyridine).

Anal. Calcd. for $C_{11}H_{15}NO_4 \cdot 1/2H_2O$: C 56.40, H 6.89, N 5.98, O 30.74.
Found: C 57.96, H 7.22, N 6.17.

D-Ribose phenylosazone (IV) from antibiotic SF-733:

A 63 mg of the crude hydrolysate of the methyl glycoside obtained was dissolved into 1.2 ml of H_2O and added with 123.56 mg of phenylhydrazine hydrochloride and 182.40 mg of NaOAc. The resulting faintly yellow or yellowish brown colored reaction mixture was filtered and the filtrate was warmed at 60°C bath for 30 minutes. After cooling the precipitate formed was collected; 6.76 mg moss-like yellow needles, m.p. 156°C, $[\alpha]_D$ −44° (*c* 0.34, pyridine-EtOH (4:6)), a second crop from the mother liquor; 24.89 mg.

Neamine (V) from antibiotic SF-733:

A 85 ml methanolic solution of 859 mg of the ninhydrin-positive precipitate obtained on addition of ethyl ether to the methanolysate of I, was passed through a 60 ml column of Amberlite IRA400 (OH^-) resin (prewashed with MeOH). The effluent and washed solution of the column were combined and the first 170 ml, from which crystals were separated spontaneously, was added with 200 ml of ethyl ether to give crystalline precipitate; 252.23 mg, decomposed at 234~241°C, $[\alpha]_D$ +112° (*c* 0.3, H_2O), IR spectrum was identical with that of the authentic sample, ORD was also identical and it did not depress its decomposing point on admixture with the authentic sample.

Anal. Calcd. for $C_{12}H_{26}N_4O_6$: C 44.71, H 8.13, N 17.38, O 29.78.
Found: C 44.73, H 8.31, N 16.43, O 30.56.

The mother liquor and the second fraction of the column gave the second crop; 217.5 mg, $[\alpha]_D$ +108° (*c* 3.4, H_2O); total yield of **V** amounted 66.9 %.

Neamine (authentic):

Crystals, decomposed at 244~247°C, $[\alpha]_D$ +126° (*c* 0.3, H_2O).

Anal. Calcd. for $C_{12}H_{26}N_4O_6$: C 44.71, H 8.13, N 17.38, O 29.78.
Found: C 44.66, H 8.27, N 17.05.

Tetra-N-salicylidene neamine (VI) from **V**:

Tetra-N-salicylidene neamine was prepared by the method described in the literature[5]; yellow crystals, pure on TLC: $CHCl_3$-MeOH (5:1), benzene-EtOH (5:1) Silicagel G, showed an identical Rf values on TLC with the authentic sample and gave the same pattern of ORD curve with the authentic sample: peaks at 288, 308, 400 mμ, a trough at 346 mμ[6].

Anal. Calcd. for $C_{40}H_{42}N_4O_{10}\cdot 2H_2O$: C 62.01, H 5.98, N 7.23, O 24.78.
Found: C 62.08, H 6.22, N 7.17.

Tetra-N-salicylidene neamine (authentic) from neamine:

Tetra-N-salicylidene neamine was prepared by the method of literature[5]; yellow crystals, ORD peaks at 288, 308, 400 mμ, a trough at 346 mμ.

Tetra-N-acetyl neamine (VII) from **V**:

A 29 ml suspension of 141.5 mg of **V** in MeOH was added with 2.9 ml of acetic anhydride, the resulting solution was kept at room temperature for 38 hours and added with 170 ml of ethyl ether. The 210.87 mg of precipitate formed was collected by filtration, extracted with 35 ml of warm MeOH and the trace of insoluble material was filtered off. The filtrate was condensed to yield crystals which were redissolved at 70°C and recrystallized on cooling; 116.29 mg, colorless needles, decomposed at 310°C, $[\alpha]_D$ +92°±10° (*c* 0.3, H_2O), IR spectrum and ORD curves were identical with those of the authentic sample (copper complex method[14] was applied to one of the observations of ORD curves), NMR spectrum was also identical with the authentic sample: δ=5.33 (J=3) 1 proton, 1.99, 1.94 (12 protons), 1.40 (quart.) 2 protons in D_2O, TMS as external standard, δ values were corrected with reference to HOD signal (DSS as internal standard), 100 MC. No depression of decomposing point on admixture with the authentic sample.

Anal. Calcd. for $C_{20}H_{34}N_4O_{10}$: C 48.97, H 6.99, N 11.42, O 32.62.
Found: C 49.03, H 7.45, N 11.61, O 32.55.

Tetra-N-acetyl neamine (authentic) from neamine:

Prepared by the method described above; colorless needles, decomposed at 310°C, $[\alpha]_D$ +83°±10° (*c* 0.3, H_2O), NMR: δ=5.33 (J=3) 1 proton, 1.99, 1.94 (12 protons), 1.40 (quart.) 2 protons in D_2O, TMS as external standard, δ values were corrected with reference to HOD signal (DSS as internal standard), 100 MC.

Anal. Calcd. for $C_{20}H_{34}N_4O_{10}$: C 48.97, H 6.99, N 11.42, O 32.62.
Found: C 48.96, H 7.04, N 11.95, O 33.14.

Periodate oxidation of tetra-N-acetyl SF-733 (VIII) and tetra-N-acetyl neamine (authentic):

The measurement was executed according to the method described by RAMMLER and RABINOWITZ[15], but the reaction itself was done under the condition without buffer in order to detect the formation of formic acid by pH change of the reaction mixture: Either a 1.15 ml portion of solution A or solution B, which was placed in the dark at room temperature, was added with 1.85 ml of buffer solution and the absorbancy at 300 mμ was measured spectrophotometrically against blank solution; observations were made after 1, 5, 10, 15, 60, 113 minutes, 18.5, 25 and 42.5 hours for solution A, 8, 20, 30, 63, 120 minutes, 19, 26 and 43 hours for solution B.

Solution A: Prepared by adding an 80 ml portion of 100 ml aqueous solution of 536.83 mg (2.51 mM) of $NaIO_4$ to a 49 ml portion of 50 ml aqueous solution of 310.66 mg (0.5 mM) of **VIII**.

Solution B: Prepared by adding an 8 ml portion of 100 ml aqueous solution of 536.83 mg (2.51 mM) of $NaIO_4$ to a 4 ml portion of 5 ml aqueous solution of 24.89 mg (0.05 mM) of the authentic tetra-N-acetyl neamine.

Buffer solution : 13.6 g of NaOAc was added with 13.3 ml of AcOH and H_2O to make 110 ml (WALPOLE buffer ; pH 4.3).

Blank solution A : A 1.1 ml portion of 50 ml aqueous solution of 310.66 mg (0.5 mM) or **VIII** was added with 4.625 ml of buffer solution and 1.775 ml of H_2O.

Blank solution B : A 1.1 ml portion of 5 ml aqueous solution of 24.89 mg (0.05 mM) of the authentic tetra-N-acetyl neamine was added with 4.625 ml of buffer solution and 1.775 ml of H_2O.

Isolation of 2-deoxystreptamine from the acid hydrolysate of the periodate oxidation product of **VIII** :

A 122 ml aliquot of the periodate oxidation reaction mixture (solution A, 42.5 hours ; cf. preceding item) was added with 0.2 ml of propylene glycol in order to terminate the oxidation reaction, and then, the mixture was further added with 130 ml of conc. HCl, refluxed for 13 hours, condensed to dryness and redissolved into about 20 ml of H_2O. This was neutralized with Amberlite IRA400(OH^-) to pH 7~8 and condensed to a syrup.

2-Deoxystreptamine dipicrate **IX** from acid hydrolysate of the periodate oxidation product of **VIII** :

The syrup above obtained was dissolved into 0.4 ml of hot water and a 2 ml solution of 109 mg of picric acid. The resulting brown colored solution gave yellow crystals on standing for a while. The crystals were collected by filtration after 16 hours at room temperature ; 142.03 mg, yield 49.5 % from **VIII**, yellow rods, decomposed at 215~260°C.

Anal. Calcd. for $C_{18}H_{20}N_8O_{17}$: C 34.85, H 3.25, N 18.06, O 43.84.
Found : C 35.13, H 3.32, N 17.90.

Di-N-acetyl-2-deoxystreptamine (**X**) form **IX** :

A 60 ml solution of 98.42 mg of **IX** in MeOH - acetone (1 : 2) was added with 0.6 ml of conc. HCl. The white precipitate formed was collected by filtration to obtain a 27.83 mg of dihydrochloride. This was dissolved into 1 ml of H_2O and passed through a column of 2.3 ml of Amberlite IRA400(OH^-). The effluent and the wash were combined and evaporated to give 13.29 mg of free base which was dissolved into 0.64 ml of MeOH, added with 0.064 ml of acetic anhydride and kept at room temperature for 2 days.

The resulting reaction mixture separated crystals ; 14.85 mg, decomposed at 292°C, no depression of melting point on admixture with the authentic sample, NMR : δ=1.98 (6 protons), 1.39 (quart.) 2 protons, in D_2O, TMS as external standard, δ values were corrected with reference to HOD signal (DSS as internal standard), 100 MC.

Anal. Calcd. for $C_{10}H_{18}N_2O_5$: C 48.77, H 7.37, N 11.38, O 32.48.
Found : C 48.92, H 7.31, N 11.16.

Di-N-acetyl-2-deoxystreptamine (authentic) :

2-Deoxystreptamine dihydrochloride was converted to di-N-acetate by the same method described ; crystals, decomposed at 292°C.

Anal. Calcd. for $C_{10}H_{18}N_2O_5$: C 48.77, H 7.37, N 11.38, O 32.48.
Found : C 49.26, H 7.27, N 11.28.

Periodate oxidation of **I** :

Each 1.54 ml aliquot of 3.08 ml aqueous solution of 17.51 mg of **I** was added with 3.08 ml WALPOLE buffer (pH 4.3, described already) and 3.08 ml of 1 M $NaHCO_3$ solution (pH 9). The each mixture was diluted with 0.02 M $NaIO_4$ aqueous solution to 9.2 ml ; 0.4 ml of sample was collected from each reaction mixture after 0, 45 minutes, 2, 5, 19, 43 and 94 hours at room temperature in the dark and the reaction was halted by addition of each one drop of 10 % aqueous propylene glycol solution. The sample collected was added with 0.2 ml of conc. HCl and hydrolysed at 90°C for 2 hours. The each hydrolysate was neutralized with Amberlite IR45(OH^-) and chromatographed on paper. No ninhydrin-positive spot was given except with 0-time sample.

Periodate oxidaiion of kanamycin A (control) :

The same procedure was run with kanamycin A, and the PPC revealed the formation of ninhydrin-positive product which was considered to be 2-deoxystreptamine; Rf 0.74 (n-PrOH - AcOH - H_2O (10 : 1 : 9)), 18 hours, ascend.

Di-N-acetyl-6-O-methyl-2-deoxystreptamine (**XII**) from **XI**:

A 19.8 ml solution of 804 mg of **XI** in 6 N HCl was sealed in a glass ampule, hydrolysed at 110°C for 2 hours and the dark brown reaction mixture was added with H_2O to make 25 ml (pH<1). This solution was chromatographed on a column of 108 ml of Dowex 50 W×8 (H^+) and each fraction (20 ml) was collected. Development was exercised by eluting with 1 N HCl from tube Nos. 1~28 and with 1.5 N HCl from No. 29 to the end. Tubes Nos. 3~8 gave reactions of a neutral sugar, Nos. 42~64 gave those of an aminocyclitol and Nos. 74~96 gave those of an aminosugar. The content of the tubes Nos. 42~64 was combined and the solvent was evaporated to give a 314 mg of solid material which was redissolved into 10 ml of H_2O, applied on a 28 ml column of Amberlite IRA400(OH^-) and the column was washed with H_2O. The wash gave on evaporation of water a 177.45 mg of pale yellow solid. A 5 ml methanolic solution of this solid was added with 0.3 ml of acetic anhydride, trace of insoluble material being removed by filtration and the filtrate was added with 100 ml of ethyl ether. The resulting 143.14 mg of precipitate was reprecipitated by the same procedure, dissolved into 3 ml of n-BuOH - pyridine - H_2O (2 : 1 : 1) and chromatographed on a 160 ml column of Sephadex LH-20 packed with the same solvent mixture. Development was done by the same solvent system and each 5 ml fraction was collected. The content of tubes Nos. 27~33 was combined, dried and a 109.53 mg portion of the 141.90 mg of crude crystals obtained was recrystallized from hot EtOH to yield crystals of **XII**; 25.69 mg, yield 11.3 %, decomposed at 264~271°C, colorless short needles, NMR: δ=3.94 (3 protons), 2.44 (6 protons), 1.86 (quart.) 2 protons, in D_2O, TMS as external standard, δ values were uncorrected, 100 MC.

IR spectrum, NMR spectrum and ORD curves were identical with those of the authentic sample, and it did not depress its decomposing point on admixture with the authentic sample.

Anal. Calcd. for $C_{11}H_{20}N_2O_5$: C 50.76, H 7.74, N 10.76, O 30.73.
Found: C 51.00, H 8.00, N 10.97.

Di-N-acetyl-6-O-methyl-2-deoxystreptamine (authentic):

Colorless short needles, decomposed at 279°C, NMR: δ=3.95 (3 protons), 2.45 (6 protons), 1.86 (quart.) 2 protons, in D_2O, TMS as external standard, δ values were uncorrected, 100 MC.

Di-N-acetyl-5-O-methyl-2-deoxystreptamine (authentic):

A 1 ml aqueous solution of 8 mg of 5-O-methyl-2-deoxystreptamine dihydrochloride from kanamycin was applied on a 0.4 ml column of Amberlite IRA400 (OH^-), the column being washed with water and 10 ml of the wash was dried to give a 4.65 mg of solid. Without isolation, this was dissolved into 1 ml of MeOH and acetylated with acetic anhydride, 0.04 ml, for 16 hours at room temperature; white crystalline powder, 7.94 mg, obtained by adding 20 ml of ethyl ether to the reaction mixture was subjected to NMR: δ=4.05 (3 protons), 2.43 (6 protons), 1.81 (quart.) 2 protons, in D_2O, TMS as external standard, δ values were uncorrected, 100 MC.

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