INOSAMYCIN, A COMPLEX OF NEW AMINOGLYCOSIDE ANTIBIOTICS

II. STRUCTURE DETERMINATION

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Structures of the new aminocyclitol antibiotics, inosamycins A, B, C, D and E, have been determined by a combination of chemical degradation and spectroscopic studies. They are structurally related to neomycin, paromomycin and ribostamycin but differ in that all the inosamycin components contain 2-deoxy-*scyllo*-inosamine in place of 2-deoxystreptamine in the known aminoglycoside antibiotics.

Inosamycin is a complex of five new aminocyclitol antibiotics produced by a strain of *Streptomyces hygroscopicus* J296-21 (ATCC 39150). They exhibited *in vitro* and *in vivo* activity against Gram-positive, Gram-negative and acid-fast bacteria. In the preceding paper[1], the taxonomy of the producing strain, production, isolation and chemical and biological properties of inosamycin have been reported. This paper describes the complete structures of the inosamycin components. They are related to neomycin[2], paromomycin[3] and ribostamycin[4] in structure but contain 2-deoxy-*scyllo*-inosamine as the aglycone.

General Structural Characteristics

The molecular formula $C_{23}H_{45}N_5O_{14}$ was assigned for inosamycins A (**Ia**) and B (**Ib**), $C_{23}H_{44}N_4O_{15}$ for inosamycins C (**Ic**) and D (**Id**), and $C_{17}H_{33}N_3O_{11}$ for inosamycin E (**Ie**) from the microanalysis of their sulfates taking into consideration their ^{13}C NMR spectra. The ^1H NMR spectrum (80 MHz, D_2O) of **Ia** sulfate showed three anomeric protons at δ 6.44 (1H, d, J=4.0 Hz), 5.83 (1H, br s) and 5.71 (1H, br s) indicating the presence of three sugar moieties in the molecule. The spectrum also showed two protons on carbon not bonded to heteroatoms (δ 2.18, 1H, q, and 2.76, 1H, dt) and more than twenty protons on carbons bearing heteroatoms. The ^{13}C NMR spectrum (20 MHz, D_2O, pD $\geqq$11.0) of **Ia** exhibited twenty-three well separated carbon signals including three anomeric carbons (δ 100.0, 100.7 and 109.6 ppm) and one methylene carbon not bearing a heteroatom (δ 36.7 ppm). The spectral data of **Ib**, **Ic** and **Id** were very similar to those of **Ia**, but the ^1H and ^{13}C NMR spectra of **Ie** indicated that it contained only two sugars in the structure. Upon acetylation in anhydrous methanol, **Ia** yielded a penta-*N*-acetyl derivative (**II**).

Acid Degradation of **Ia**

Upon refluxing in 0.4 N methanolic hydrogen chloride, **Ia** was split into a biologically active fragment (**III**) and a methyl glycoside (**IV**). After purification by column chromatography, **IV** was identified as an anomeric mixture of methyl neobiosaminide B[5] by a direct comparison with an authentic

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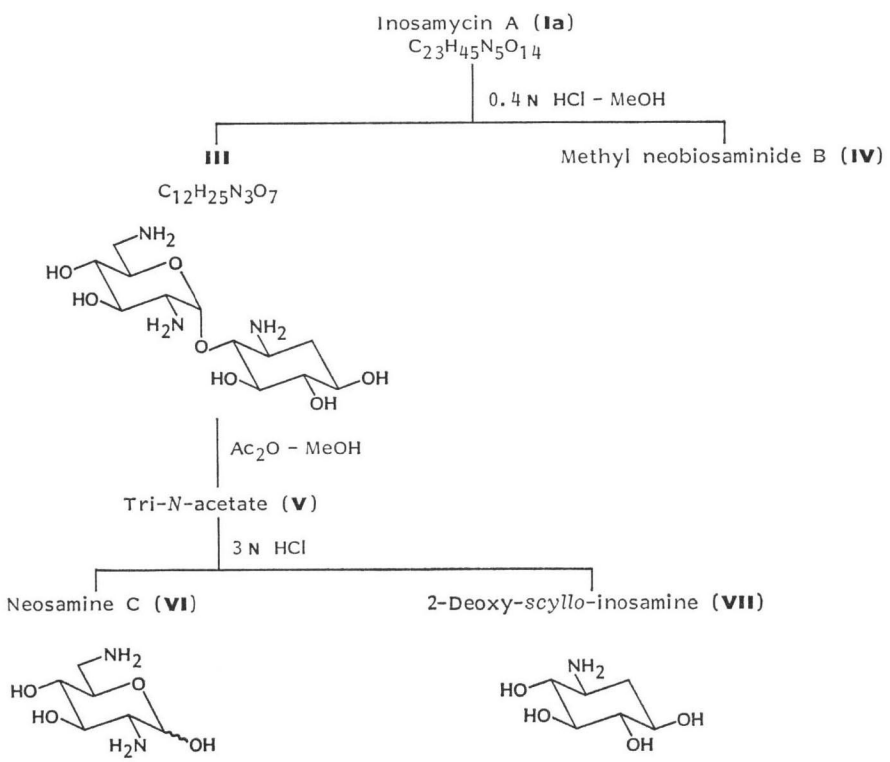
sample prepared from neomycin B. Bioactive fragment **III** was chromatographed on Amberlite CG-50 resin yielding a homogeneous white solid. MS *m/z* 324 (M+H), ^{1}H NMR (80 MHz, D_2O) δ 6.02 (1H, d, J=3.4 Hz), 3.0~4.5 (11H, m) and 1.5~2.7 (2H, m). Fragment **III** resembled neamine in physico-chemical and biological properties but they were distinguished from each other by TLC. Acetylation of **III** in anhydrous methanol afforded a crystalline tri-*N*-acetate (**V**). Elemental analysis

Table 1. ^{13}C NMR chemical shifts of **III** and neamine.

Carbon	Chemical shift, δ (ppm)					
	III			Neamine		
	pD≧11	pD≦1	β-Shift	pD≧11	pD≦1	β-Shift
1	70.2	69.1		51.1	50.6	
2	36.8	33.2	3.6	36.4	29.0	7.4
3	49.2	49.1		50.1	49.4	
4	88.1	78.9	9.2	87.9	77.7	10.2
5	76.0	75.7		76.8	76.1	
6	77.7	77.2		78.3	73.3	5.0
1′	101.9	96.4	5.5	101.6	96.1	5.5
2′	56.2	54.5		56.0	54.4	
3′	74.2	70.0	4.2	74.4	70.0	4.4
4′	72.2	71.8		72.2	71.9	
5′	73.1	69.1	4.0	73.7	69.0	4.7
6′	41.9	41.2		42.4	41.2	

Chart 1.



of V ($C_{18}H_{31}N_3O_{10}$) established a molecular formula of $C_{12}H_{25}N_3O_7$ for **III**, suggesting the presence of an additional hydroxyl group in **III** in place of an amino group in neamine.

Compound **V** was hydrolyzed in 3 N HCl for 14.5 hours. The hydrolysate was chromatographed on Dowex 50-X2 (H^+) to yield two basic substances (**VI** and **VII**). Compound **VI** was identical with 2,6-diamino-2,6-dideoxy-D-glucose (neosamine C)[6] by its IR spectrum and optical rotational value and also by a direct comparison with an authentic sample on TLC. The molecular formula of **VII** was assigned as $C_6H_{13}NO_4$ from its microanalysis and MS (M+H: *m/z* 164). In the ^1H NMR spectrum (80 MHz, D_2O), the high-field methylene signal appeared at δ 1.68 (1H, q, J=11.6 Hz) and 2.37 (1H, dt, J=3.8, 11.6 Hz). These physico-chemical and spectral data strongly suggested **VII** to be 2-deoxy-*scyllo*-inosamine (S-11-P)[7] reported by FUJIWARA *et al.* Compound **VII** was identical in all respects with an authentic sample, which was kindly provided by Dr. T. FUJIWARA.

The above hydrolysis study indicated that **III** is closely related to neamine but contains 2-deoxy-*scyllo*-inosamine in place of 2-deoxystreptamine. This was supported by the MS analysis of **III** and neamine[5]. In the spectrum of **III**, the prominent fragment ions derived from 2-deoxy-*scyllo*-inosamine (*m/z* 192, 174, 164, 146 and *etc.*) were one mass unit larger than the corresponding ions of neamine, while the fragment ions from the neosamine moiety were identical for both compounds. The linkage site of **VI** to **VII** was confirmed to be C-4 by ^{13}C NMR comparison of **III** and neamine[9]. As shown in Table 1, β-protonation shifts of **III** were observed at C-2 (3.6 ppm) and C-4 (9.2 ppm), C-1′ (5.5 ppm), C-3′ (4.2 ppm) and C-5′ (4.0 ppm), the value of C-4 being close to that observed for C-4 of neamine (10.2 ppm). Thus, the structure of **III** was established as 1-deamino-1-hydroxyneamine. The de-

Table 2. ^{13}C NMR chemical shifts of inosamycin A and neomycin B.

Carbon	Chemical shift, δ (ppm)					
	Inosamycin A			Neomycin B		
	pD≧11	pD≦1	β-Shift	pD≧11	pD≦1	β-Shift
1	70.0	69.1		51.2	50.8	
2	36.7	32.7	4.0	36.5	28.8	7.7
3	50.0	49.1		51.2	49.4	
4	83.1	76.5	6.6	83.2	75.8	7.4
5	82.8	82.0		82.4	82.1	
6	77.8	77.2		78.4	73.2	5.2
1′	100.7	96.0	4.7	100.3	96.0	4.3
2′	56.4	54.5		56.4	54.5	
3′	75.0	70.2	4.8	74.1	70.3	3.8
4′	72.3	71.7		72.2	71.8	
5′	74.9	68.9	6.0	74.1	68.8	5.3
6′	42.6	41.1		42.6	41.3	
1″	109.6	110.7		109.2	110.9	
2″	74.2	74.3		74.1	74.2	
3″	77.8	75.9		77.0	75.7	
4″	84.7	85.1		85.1	85.5	
5″	62.6	61.0		62.3	61.2	
1‴	100.0	96.0	4.0	99.8	96.0	3.8
2‴	53.8	51.7		53.7	51.7	
3‴	71.9	68.0	3.9	71.6	68.1	3.5
4‴	69.7	68.5		69.4	68.5	
5‴	77.1	71.1	6.0	77.0	71.1	5.9
6‴	42.1	41.2		42.1	41.2	

Table 3. Key ^{13}C chemical shifts of inosamycins and reference samples.

Carbon	Chemical shift, δ (ppm in D_2O, pD=1.0)						
	Neomycin B	Inosamycin A (**Ia**)	Inosamycin D (**Id**)	Neomycin C	Inosamycin B (**Ib**)	Paromomycin I	Inosamycin C (**Ic**)
1	50.8	69.1	69.1	50.8	69.1	50.6	68.9
2	28.8	32.7	32.7	28.7	32.7	28.8	32.8
6	73.2	77.2	77.2	73.2	77.1	73.0	77.0
4′	71.8	71.7	71.6	71.8	71.6	69.7	69.7
5′	68.8	68.9	68.9	68.8	68.9	74.7	74.5
6′	41.3	41.1	41.1	41.2	41.1	61.2	61.0
2‴	51.7	51.7	52.1	54.5	54.5	51.7	51.7
3‴	68.1	68.0	68.5	69.8	69.8	68.1	68.1
4‴	68.5	68.5	69.8	71.6	71.6	68.4	68.4
5‴	71.1	71.1	76.2	69.6	69.6	71.0	71.0
6‴	41.2	41.2	62.1	40.9	40.9	41.3	41.3

gradation scheme and the structures of **III**, **IV**, **VI** and **VII** are depicted in Chart 1.

Structure of **Ia**

The ^1H NMR analysis allowed the assignment of the β-configuration for the D-ribofuranoside (δ 5.83, br s) in **Ia**. The site of attachment of the neobiosaminide B moiety (**IV**) to **III** was established by periodate oxidation and ^{13}C NMR study. Upon periodate oxidation, penta-*N*-acetyl inosamycin A (**II**) consumed 3 mol of periodate, while hexa-*N*-acetylneomycin consumed 2 mol, suggesting that in **Ia**, **IV** was attached to the C5-OH of **III**. The ^{13}C NMR determined at pD$\geqq$11 and pD$\leqq$1.0 confirmed the above assumption. As shown comparatively in Table 2, the ^{13}C NMR spectra of **Ia** and neomycin B[10] are very similar except for a distinct difference in the chemical shifts of their C-1 signals (**Ia**, δ 70.0 and neomycin B, δ 51.2 at pD$\geqq$11.0). In the spectrum of **Ia**, the C-5 signal appeared at δ 82.8, 6.8 ppm lower field than the corresponding carbon of **III** (C-5, δ 76.0) due to the deshielding effect of glycosidation. The spectrum also exhibited a 4.0 ppm β-shift at C-2 and no shift at C-6 on acidification indicating that C-1 of the antibiotic bore a hydroxyl group instead of an amino group as in neomycin B. Thus, inosamycin A (**Ia**) was determined as 1-deamino-1-hydroxyneomycin B.

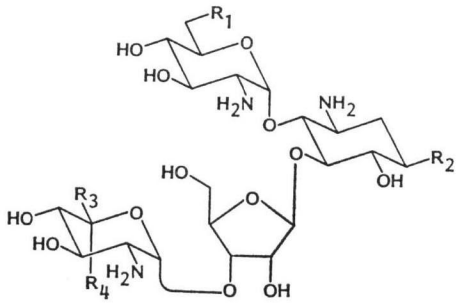
Structures of **Ib**, **Ic** and **Id**

The ^{13}C chemical shifts of key carbons of **Ia**, **Ib**, **Ic**, **Id**, neomycins B and C and paromomycin I are summarized in Table 3. The ^{13}C NMR spectrum of **Ib** closely resembled that of neomycin C but they differed in chemical shifts and β-acidification shifts at C-1, C-2 and C-6. The differences indicated that **Ib** was 1-deamino-1-hydroxyneomycin C. Methanolysis of **Ib** afforded **III** and methyl neobiosaminide C in a good yield confirming the above assignment.

The ^{13}C NMR spectra of **Ia** and **Ib** displayed two nitrogen-bearing methylenes and one oxygen-bearing methylene, while that of **Ic** showed one nitrogen-bearing methylene (δ 41.3) and two oxygen-bearing methylenes (δ 61.0 and 61.2). The chemical shifts of **Ic** were nearly consistent with the corresponding chemical shifts of parmomycin I[11] except for the three carbon signals (C-1, C-2 and C-6). The differences reflected that the amino group at C-1 of paromomycin I was replaced by a hydroxyl group in **Ic**. Thus, **Ic** was assigned as 1-deamino-1-hydroxyparomomycin I.

Id also exhibited two low-field triplet signals (δ 61.0 and 62.1) in the ^{13}C NMR spectrum showing the presence of two CH_2OH groups in the molecule. The spectrum was clearly different from those

Fig. 1. Structures of inosamycins A, B, C, D and related antibiotics.

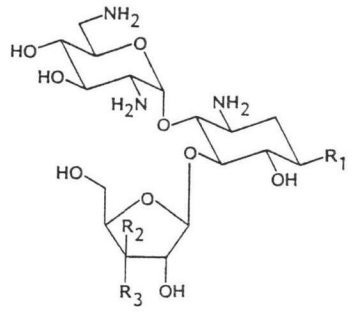


	R_1	R_2	R_3	R_4
Inosamycin A	NH_2	OH	H	CH_2NH_2
B	NH_2	OH	CH_2NH_2	H
C	OH	OH	H	CH_2NH_2
D	NH_2	OH	H	CH_2OH
Neomycin B	NH_2	NH_2	H	CH_2NH_2
C	NH_2	NH_2	CH_2NH_2	H
Paromomycin I	OH	NH_2	H	CH_2NH_2
II	OH	NH_2	CH_2NH_2	H

Table 4. ^{13}C NMR chemical shifts of inosamycin E and ribostamycin.

Carbon	Chemical shifts, δ (ppm) at pD$\leqq$1.0	
	Inosamycin E	Ribostamycin
1	69.1	50.7
2	32.7	28.7
3	49.0	49.3
4	76.7	76.1
5	85.2	85.5
6	77.2	73.3
1′	96.2	96.0
2′	54.5	54.4
3′	70.1	70.2
4′	71.6	71.7
5′	69.8	69.9
6′	41.1	41.2
1″	110.9	111.0
2″	76.2	75.8
3″	68.8	68.8
4″	83.1	83.4
5″	61.5	61.8

Fig. 2. Structures of inosamycin E, ribostamycin and S-11-A.



	R_1	R_2	R_3
Inosamycin E (Ie)	OH	H	OH
Ribostamycin	NH_2	H	OH
S-11-A	OH	OH	H

of paromomycins I and II in the C-1′~C-6′ region but quite similar to that of **Ia**. Only the C-5‴ and C-6‴ signals deviated significantly (and C-4‴ to a lesser degree) from the corresponding carbon signals of **Ia**. The observed deviations suggested that **Id** had a hydroxyl group at C-6‴ instead of the amino group of **Ia**. The L-idose structure was assigned to the 2-aminohexose of **Id** based on the fact that the specific rotation value of **Id** (+52.5° in H_2O) was consistent with those of **Ia**, **Ic**, neomycin B and paromomycin I but different from those of **Ib**, neomycin C and paromomycin II[12,13]. In addition, the chemical shifts of C-1‴ through C-6‴ carbons of **Id** coincided with the corresponding carbon signals of 6‴-deamino-6‴-

hydroxyneomycin B prepared by deamination of neomycin B[14]. Thus, **Id** is 1,6‴-dideamino-1,6‴-dihydroxyneomycin B. The structures of **Ia**, **Ib**, **Ic** and **Id** are shown in Fig. 1 with those of neomycins and paromomycins.

Structure of **Ie**

Compound **Ie** has been identified as a pseudotrisaccharide by its elemental analysis and ^{1}H and ^{13}C NMR spectra. The ^{13}C carbon signals of **Ie** compared well with the corresponding signals of ribostamycin[15] (Table 4) with exception of three carbons at C-1, C-2 and C-6 which were deshielded by 18.4, 4.0 and 3.9 ppm, respectively, in the spectrum of **Ie**. These differences corresponded to those observed between **III** and neamine. The ^{13}C NMR spectrum of **Ie** differed from that of S-11-A (1-deamino-1-hydroxyxylostasin)[16] in the chemical shifts of C-2″ and C-3″. On this basis, **Ie** was proved to be 1-deamino-1-hydroxyribostamycin. The structures of **Ie**, ribostamycin and S-11-A are shown in Fig. 2.

Discussion

The above structural studies demonstrated that inosamycins A, B, C, D and E are novel aminocyclitol antibiotics structurally related to neomycin, paromomycin and ribostamycin containing 2-deoxy-*scyllo*-inosamine (1-deamino-1-hydroxy-2-deoxystreptamine; HDOS) as the aglycone. 2-Deoxystreptamine (DOS) is the common aglycone of the classical aminocyclitol antibiotics and 1,4-diaminocyclitols have been found as the constituents of newer aminoglycosides. There have been several examples of HDOS-containing aminocyclitol antibiotics, *e.g.* S-11-A[7], SU-1, 2 and 3[17] and K-144 e and g[18], but they were produced only by mutants (DOS-idiotrophs) of known aminoglycoside-producing strains. Inosamycin represents the first HDOS-containing aminocyclitol antibiotics produced by a naturally occurring microorganism.

The biosynthesis of the aminocyclitol antibiotics was extensively studied by RINEHART, and STROSHANE[19]. FUJIWARA *et al.*[20] recently reported HDOS to be the direct precursor of 2-deoxystreptamine. Production of the HDOS-containing analogues of neomycin, paromomycin and ribostamycin gives further evidence for the biosynthetic route proposed by them. More recently, AUTISSIER *et al.* isolated 6‴-deamino-6‴-hydroxy analogues of neomycin and paromomycin from a fermentation broth and suggested that 6‴-amination took place at the final step of the biosynthesis of neomycin and paromomycin[12]. Inosamycin D is an another example of a 6‴-deamino-6‴-hydroxy aminocyclitol antibiotic from the natural origin.

As described in the preceding paper, inosamycin A (1-deamino-1-hydroxyneomycin B) exhibited antibacterial activity comparable to neomycin with acute toxicity significantly lower than neomycin. It is interesting that inosamycin E (1-deamino-1-hydroxyribostamycin) and compound **III** (1-deamino-1-hydroxyneamine) showed much lower antibacterial activity than the corresponding 1-amino analogues. This might be explained by the fact that at least four basic functions are essential to render substantial activity to this group of aminocyclitol antibiotics, as exemplified by the very weak activity of paromamine relative to neamine.

Experimental

Thin-layer chromatography (TLC) was performed on silica gel (Kieselgel 60 F_{254}, Merck) or cellulose plates (DC Fertigplatten Cellulose F, Merck) using the solvent systems shown below:

System No.	Plate	Solvent system
S-108	Silica gel	Acetone - AcOH - H_2O (20: 6: 74)
S-110	Silica gel	$CHCl_3$ - MeOH - 28% NH_4OH - H_2O (1: 4: 2: 1)
S-123	Silica gel	10% $AcONH_4$ - MeOH - 10% NH_4OH (9: 10: 1)
S-12	Cellulose	BuOH - Pyridine - AcOH - H_2O (6: 4: 1: 3)
S-101	Silica gel	PrOH - Pyridine - AcOH - H_2O (15: 10: 3: 10)
SG-6	Silica gel	*tert*-BuOH - AcOH - H_2O (2: 2: 1)

IR spectra were determined in KBr pellet using a Jasco IRA-1 spectrometer and ^1H NMR spectra and ^{13}C NMR spectra were recorded on a Varian FT80A apparatus operated in Fourier transform mode. Mass spectra were measured on a Hitachi RMU-6MG mass spectrometer modified with an in-beam/direct inlet system.

Penta-*N*-acetylinosamycin A (**II**)

A solution of **Ia** (100 mg) in 10 ml of anhydrous methanol and 2 ml of acetic anhydride was stirred at room temp overnight. The reaction mixture was evaporated and poured into acetone with stirring. The white crystalline precipitate (**II**) obtained was collected by filtration, washed with acetone and dried *in vacuo*, yield 95.3 mg. MP 166~174°C (dec), $[\alpha]_D^{26.5}$ +41° (*c* 0.6, MeOH).

Anal Calcd for $C_{33}H_{55}N_5O_{16}\cdot 4H_2O$: C 44.15, H 7.02, N 7.80.
Found: C 44.64, H 6.68, N 7.43.

Isolation of **III** and Methyl Neobiosaminide B (**IV**) from **Ia**

A solution of **Ia** (1.56 g) in 455 ml of 0.4 N methanolic hydrogen chloride was refluxed for 4 hours. The solution was concd *in vacuo* and the residue was dissolved in H_2O, neutralized with 6 N NaOH and applied to a column of Amberlite CG-50 (NH_4^+, 360 ml). The column was washed with H_2O and then developed with 0.1 N and 0.2 N NH_4OH. The eluates were collected in fractions which were examined by bioassay with *Bacillus subtilis* PCI 219 and ninhydrin reagent. The bioactive, ninhydrin-positive fractions which were eluted with 0.2 N NH_4OH were pooled, concd *in vacuo* and lyophilized to give 657 mg of **III**.

^1H NMR (D_2O), δ 6.02 (1H, d, J=3.4 Hz), 3.0~4.5 (11H, m), 1.5~2.7 (2H, m). MS (in beam) m/z 324 (M+H). TLC Rf 0.47 (S-108), 0.55 (S-110), 0.48 (S-123). The hydrochloride was prepared by dissolving **III** in dil HCl, followed by precipitation with addition of acetone. MP 229~235°C (dec), $[\alpha]_D^{22}$ +89° (*c* 0.36, H_2O).

Anal Calcd for $C_{12}H_{25}N_3O_7\cdot 3HCl\cdot \frac{1}{2}H_2O$: C 32.63, H 6.62, N 9.51, Cl 24.08.
Found: C 32.53, H 6.79, N 9.45, Cl 23.92.

The bio-inactive, ninhydrin-positive fractions eluted with 0.1 N NH_4OH were pooled and concd *in vacuo*. The residue was dissolved in H_2O and adsorbed on a column of CG-50 (NH_4^+, 60 ml). The column was washed with H_2O and eluted with 0.05 N, 0.08 N and 0.1 N NH_4OH, successively. Ninhydrin-positive fractions were combined and lyophilized to afford 180 mg of white powder (**IV**). ^1H NMR (D_2O), δ 5.37 (1H, d, J=1.7 Hz), 4.75 (β-H, d, J=4.2 Hz) and 4.58 (α-H, d, J=2.1 Hz), 3.52 (β-OCH_3, s) and 3.48 (α-OCH_3, s). MS (in beam) m/z 325 (M+H). TLC:

Methyl neobiosaminide (mixture of α- and β-anomers)	Rf		
	S-12	S-101	S-110
From inosamycin A	0.14, 0.24	0.46, 0.49	0.57, 0.64
〃 neomycin B	0.14, 0.24	0.46, 0.49	0.57, 0.64
〃 neomycin C	0.13, 0.21	0.43, 0.46	0.60, 0.68

The hydrochloride of **IV** was prepared by the usual manner for analysis. MP 184~189°C (dec), $[\alpha]_D^{22}$ +15° (*c* 0.5, H_2O).

Anal Calcd for $C_{12}H_{24}N_2O_5\cdot 2HCl$: C 36.28, H 6.60, N 7.05, Cl 17.85.
Found: C 36.12, H 6.58, N 6.87, Cl 17.72.

Compound **IV** was identical with methyl neobiosaminide B obtained from neomycin B in specific rotation, IR spectrum and TLC.

Tri-*N*-acetate of **III** (**V**)

A solution of **III** (350 mg) and acetic anhydride (7 ml) in 35 ml of anhydrous methanol was stirred overnight at room temp. White crystals which deposited were collected by filtration and recrystallized from MeOH - acetone (1 : 1). Yield 277 mg. MP 210~212°C, $[\alpha]_D^{23}$ +73° (*c* 0.6, H_2O). ^1H NMR (D_2O), δ 5.33 (1H, d, J=3 Hz), 2.07 (6H, s), 2.02 (3H, s). MS (in beam) m/z 449 (M).

Anal Calcd for $C_{18}H_{31}N_3O_{10}\cdot H_2O$: C 46.25, H 7.12, N 8.99.
Found: C 46.39, H 6.96, N 8.85.

Isolation of 2,6-Diamino-2,6-dideoxy-D-glucose (Neosamine C) (**VI**) and 2-Deoxy-*scyllo*-inosamine (**VII**) from **V**

A solution of **V** (540 mg) in 3 N HCl (50 ml) was refluxed for 14.5 hours and then evaporated *in vacuo*. The residue was dissolved in H_2O and applied to a column of Dowex 50-X2 (H^+, 30 ml). The column was washed with H_2O and eluted with 0.25 N, 0.5 N and 2 N HCl, successively. The elution was followed by ninhydrin test on silica gel plate. Compound **VII** was eluted first with 0.5 N HCl. The fractions containing **VII** were collected, concd *in vacuo* and lyophilized to give 206 mg of **VII** hydrochloride. Subsequent ninhydrin-positive fractions containing **VI** were eluted with 2 N HCl. The relevant fractions were concd and poured into acetone to yield 153 mg of hygroscopic **VI** hydrochloride.

Compound **VI** hydrochloride. MP 145~150°C (dec), $[\alpha]_D^{24}$ +55° (*c* 0.5, H_2O). ^1H NMR (D_2O), δ 5.52 (β-H, d, J=3.5 Hz) and 5.02 (α-H, d, J=7.8 Hz), 2.9~4.4 (6H, m). MS (in beam) *m/z* 179 (M+H). TLC: Rf 0.20 (SG-6), 0.23 (S-101).

Anal Calcd for $C_6H_{14}N_2O_4 \cdot 2HCl \cdot \frac{1}{2}H_2O$: C 27.71, H 6.59, N 10.77, Cl 27.30.
Found: C 28.05, H 6.71, N 10.77, Cl 27.12.

Compound **VI** was identical with an authentic sample of neosamine C obtained from *N*-acetylneamine in specific rotation, melting point, IR spectrum and TLC.

Compound **VII** hydrochloride (207 mg) was dissolved in 10 ml of H_2O and passed through a column of Dowex 1-X2 (OH^-, 15 ml) with H_2O elution. The ninhydrin-positive eluates were evaporated *in vacuo* to afford white hygroscopic powder (**VII** free base, 103 mg). MS (in beam) *m/z* 164 (M+H). Compound **VII** sulfate was prepared by the usual method for analysis. MP 141~143°C, $[\alpha]_D^{22}$ +5° (*c* 1.0, H_2O).

Anal Calcd for $C_6H_{13}NO_4 \cdot \frac{1}{2}H_2SO_4 \cdot \frac{1}{2}H_2O$: C 32.58, H 6.83, N 6.33, S 7.25.
Found: C 32.59, H 7.19, N 6.17, S 7.25.

^1H NMR (D_2O), δ 3.43 (5H, m), 2.37 (1H, dt, J=3.8, 11.6 Hz), 1.68 (1H, q, J=11.6 Hz). TLC: Rf 0.38 (S-101) and 0.18 (S-12). Compound **VII** was consistent in all respects with 2-deoxy-*scyllo*-inosamine (S-11-P).

Periodate Oxidation of *N*-Acetylinosamycin A (**II**) and *N*-Acetylneomycin

Periodate oxidation experiments were carried out on *N*-acetylinosamycin A and *N*-acetylneomycin by essentially the same procedure as published by OGAWA *et al.*[21]. The results are shown below:

	Periodate consumption*				Theoretical
	2 hours	8 hours	10 hours	24 hours	
N-Acetylinosamycin A	1.67	3.03	2.97	2.91	3 mol
N-Acetylneomycin	0.78	1.75	2.09	1.94	2 mol

* Mol of periodate/mol of compound.

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