STUDIES ON NEW ANTIBIOTIC LIVIDOMYCINS. IV
STRUCTURE OF LIVIDOMYCIN A

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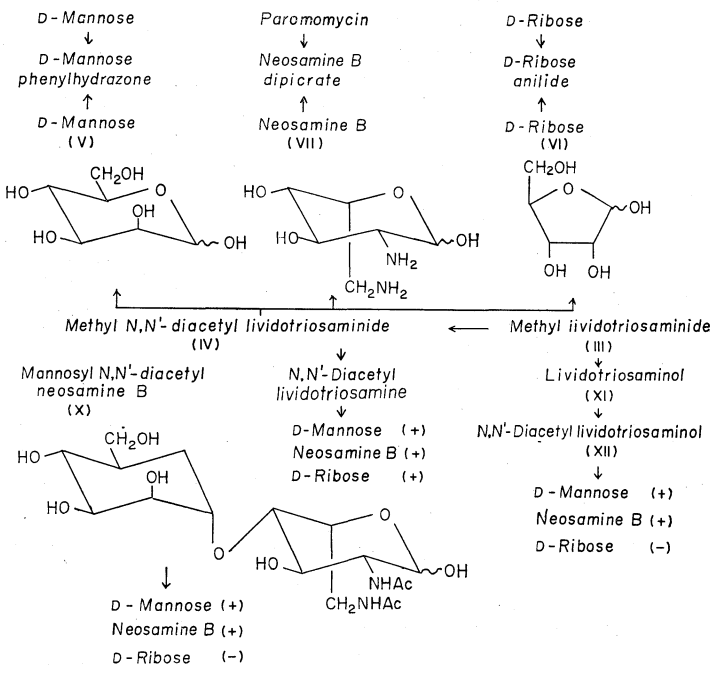
The acid hydrolysis of methyl lividotriosaminide obtained from the methanolysis of lividomycin A gave D-mannose, D-ribose and neosamine B. The sequence and the position of linkage of these three compounds were determined. 1-O-Acetyl-2,3,4,6-tetra-O-methyl-α-D-mannose and 1,3-di-O-acetyl-2,5-di-O-methyl-β-D-ribose were formed by acid hydrolysis of di-N-acetyl-deca-O-methyllividomycin A followed by acetylation and other observations. The attachment to 2-deoxystreptamine of lividotriosamine was determined by the formation of di-N-acetyl-6-O-methyl-2-deoxystreptamine. The stereochemistry of the glycosidic linkage was determined by comparison of the NMR spectra of N-acetylated derivatives of lividomycin A and paromomycin. Thus, the chemical structure of lividomycin A was concluded to be 4-O-(2-amino-2, 3-dideoxy-α-D-glucopyranosyl)-5-O-[3-O-{4-O-(α-D-mannopyranosyl)-2, 6-diamino-2, 6-dideoxy-α-L-idopyranosyl}-β-D-ribofuranosyl]-1, 3-diamino-1, 2, 3-trideoxy-*myo*-inositol or mannosyldeoxyparomomycin.

The acid methanolysis of lividomycin A (I) gave 3′-deoxy paromamine and methyl lividotriosaminide. The identification of the 3′-deoxy paromamine moiety has been reported in a previous paper[1]. This paper describes the structures of methyl lividotriosaminide and lividomycin A.

Two neutral sugars, D-mannose (**V**) and D-ribose (**VI**), and a basic sugar, neosamine B (**VII**), were obtained when methyl N, N′-diacetyllividotriosaminide (**IV**) was hydrolyzed with 1 N sulfuric

Fig. 1



acid. D-Mannose was identified as its phenylhydrazone derivative, D-ribose as the anilide and neosamine B as the dipicrate.

The sequence of the trisaccharide was determined as described below. Penta-N-acetyldeca-O-methyllividomycin A prepared with methyl iodide and silver oxide from penta-N-acetyllividomycin A (II), was hydrolysed with 4 N hydrochloric acid and subsequently acetylated with acetic anhydride and pyridine to yield 1-O-acetyl-2, 3, 4, 6-tetra-O-methyl-α-D-mannose (VIII), and 1,3-di-O-acetyl-2, 5-di-O-methyl-β-D-ribose (IX). Compound VIII was identical with a synthetic sample, which was synthesized from D-mannose by methylation with methyl iodide and silver oxide, followed by hydrolysis with mineral acid and subsequent acetylation. Compound IX was identical with an authentic sample prepared from paromomycin by similar treatment. From these results, D-mannose was shown to be terminal in lividotriosamine with C-1 linked to the other portion. Also, D-ribose was shown to exists in the furanose form as seen in the NMR spectrum of IX (Fig. 8). Compound IV was hydrolyzed with 1 N sulfuric acid, the resulting products were fractionated using Dowex 1×2, and fractions containing disaccharide fractions were combined and acetylated to yield mannosyl N,N'-diacetylneosamine B (X). When X was hydrolyzed with 3 N sulfuric acid, D-mannose and neosamine B were shown to be present, but D-ribose was absent. Since X was a reducing sugar, the C-1 position of neosamine B was free because D-mannose was attached to neosamine B at C-1. A reducing trisaccharide, N,N'-diacetyl lividotriosamine, which was prepared from IV, was found to be very unstable. The hydrolysis of this compound with 3 N sulfuric acid

Fig. 2

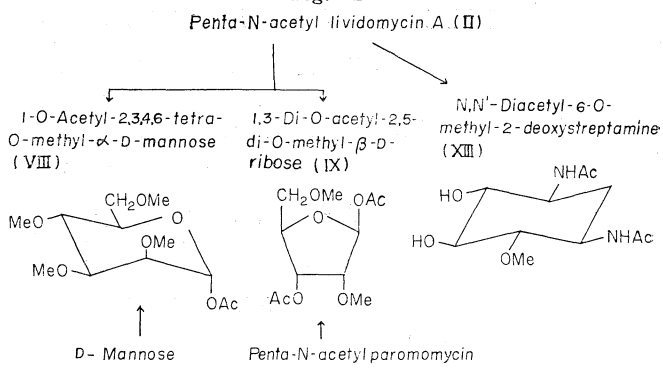


Fig. 3. Infrared absorption spectra of D-mannose phenylhydrazone (KBr tablet)
A : from lividomycin A B : from D-mannose

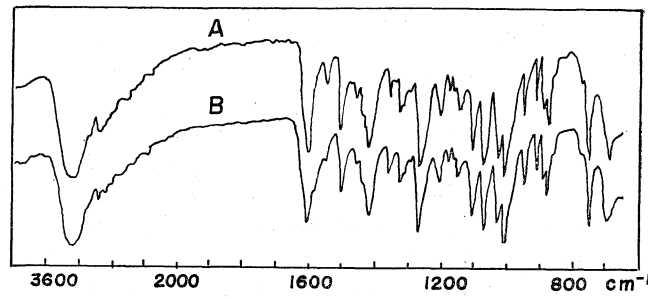
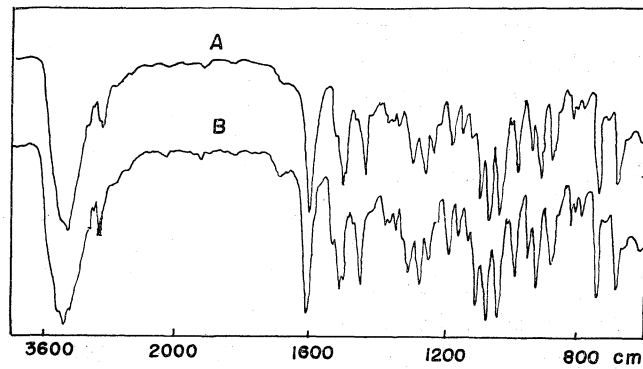


Fig. 4. Infrared absorption spectra of D-ribose anilide (KBr tablet)
A : from lividomycin A B : from D-ribose



under the same condition as described above, yielded not only D-mannose and neosamine B but D-ribose. Lividotriosaminol (**XI**) was obtained by the hydrolysis of methyl lividotriosaminide dihydrochloride (**III**) followed by reduction with sodium borohydride. When N, N'-diacetyllividotriosaminol(**XII**), prepared by N-acetylation of **XI**, was hydrolyzed with 3 N sulfuric acid, D-mannose and neosamine B were found, but D-ribose was absent. From these facts, it was clear that in lividotriosamine, the C-1 position of neosamine B is attached to the C-3 position of D-ribose and that the C-1 position of D-ribose is free. Further, methyl lividotriosaminide was found to consume 3 moles of periodate, but **IV** to consume 2 moles. Thus the chemical structure of lividotriosamine was established as 3-O-{4-O-(α-D-mannopyranosyl)-2, 6-diamino-2, 6-dideoxy-α-L-idopyranosyl}-D-ribose.

Fig. 5. Infrared absorption spectra of neosamine B dipicrate (KBr tablet)
A : from lividomycin A B : from paromomycin

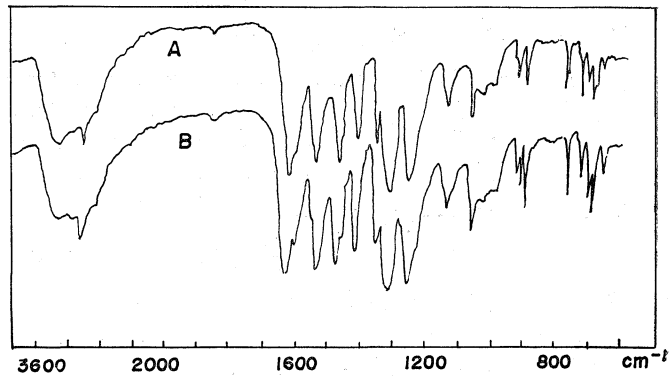


Fig. 6. Infrared absorption spectra of 1-O-acetyl-2, 3, 4, 6-tetra-O-methyl-α-D-mannose (KBr tablet)
A : from lividomycin A B : from D-mannose

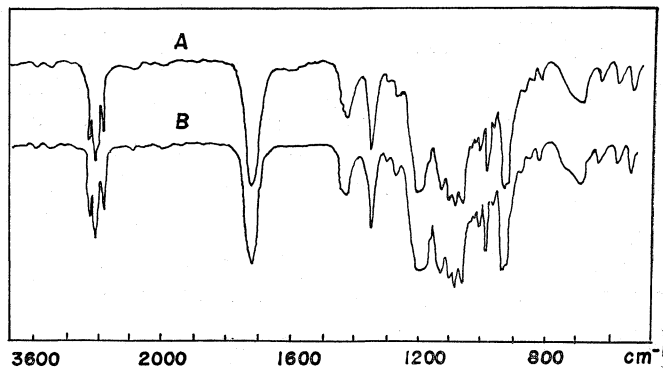
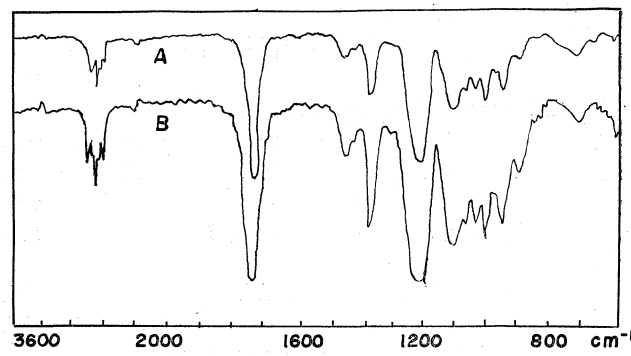


Fig. 7. Infrared absorption spectra of 1, 3-di-O-acetyl-2, 5-di-O-methyl-β-D-ribose (KBr tablet)
A : from lividomycin A B : from paromomycin



In order to determine attachment of lividotriosamine to 2-deoxystreptamine, penta-N-acetyl-deca-O-methyl-lividomycin A was hydrolyzed with hydrochloric acid, acetylated with acetic anhydride and purified by preparative thin-layer chromatography on silica gel to yield N, N'-diacetyl-6-O-methyl-2-deoxystreptamine (**XIII**). The **XIII** was identical with an authentic sample from penta-N-acetyl-octa-O-methyl-paromomycin in IR spectrum, melting point, NMR spectrum and optical rotatory dispersion, proving that lividotriosamine is attached to C-5 position of 2-deoxystreptamine.

The stereochemistry of each glycosidic linkage in lividomycin A as determined from the NMR data of penta-N-acetyllividomycin A and penta-N-acetylparomomycin. The following results were obtained all chemical shifts measured relative to DSS.

For penta-N-acetylparomomycin :

5.51 ppm J=3.5 cps.....anomeric proton of D-glucosamine (α-linkage)
5.22 ppm J=*ca.* 1 cps.....anomeric proton of D-ribose (β-linkage)
4.98 ppm J=2 cps .....anomeric proton of neosamine B (α-linkage)

These assignments were made according to the results of RINEHART, Jr., *et al.*[2]

For penta-N-acetyllividomycin A

5.44 ppm (J=4 cps), 5.23 ppm (J=*ca.* 1 cps), 5.04 ppm (J=2 cps), 4.97 ppm (J=2 cps).

From these values, 3-deoxy-D-glucosamine has an α-glycosidic linkage, D-ribose a β-linkage and neosamine B an α-linkage, but it could not be determined from the above whether D-mannose was α- or β-linkage. For this linkage, the modified HUDSON's rule[3] was applied.

Methyl-α-D-mannoside .....$[\alpha]_D^{20}$ +79.3° M.W. 194
Methyl-β-D-mannoside .....$[\alpha]_D^{20}$ −69°
N, N′-Diacetylneosamine B .....$[\alpha]_D^{21}$ +6° M.W. 262
Mannosyl-N,N′-diacetylneosamine B .....$[\alpha]_D^{20}$ +45.4° M.W. 424

from the above data, $+79.3\times 194=+15{,}380$ $(+A+B)$
$-69\times 194=-13{,}390$ $(-A+B)$
$A=+14{,}390$, $B=+995$
$(+45.4\times 424)-(+6\times 262)=+16{,}683$

These results indicate an α-linkage for the D-mannose.

The chemical structure of lividomycin A was then concluded to be 4-O-(2-amino-2, 3-dideoxy-α-D-glucopyranosyl)-5-O-[3-O-{4-O-(α-D-mannopyranosyl)-2, 6-diamino-2, 6-dideoxy-α-L-idopyranosyl}-β-D-ribofuranosyl]-1, 3-diamino-1, 2, 3-trideoxy-*myo*-inositol or mannosyldeoxyparomomycin.

Experimental

Lividomycin A (I):

Anal. Calcd. for $C_{29}H_{55}N_5O_{18}\cdot H_2O$: C 44.67, H 7.37, N 8.98.
Found : C 44.49, H 7.08, N 8.72.

Penta-N-acetyl lividomycin A (II):

A 50 ml methanolic suspension of I (1 g) was stirred with acetic anhydride (5 ml). After the ninhydrin test became negative, the solution was evaporated to dryness, desalted and lyophilized. The lyophilized powder was purified by cellulose column chromatography with *n*-BuOH - pyridine - H_2O (6 : 4 : 3), yield 980 mg, m. p. 200~204°C (d). $[\alpha]_D^{24}$ +59.8° (*c* 0.55, H_2O).

Anal. Calcd. for $C_{39}H_{65}N_5O_{23}\cdot 3H_2O$: C 45.66, H 6.98, N 6.83.
Found : C 45.57, H 6.63, N 6.78.

Methyl N, N′-diacetyl lividotriosaminide (IV):

Methyl lividotriosaminide dihydrochloride (III)[1] (230 mg) was dissolved in water and adsorbed on a column of CM-Sephadex C-25 (NH_4^+) (30×1 cm). The ninhydrin-positive effluent eluted with 0.5 N NH_4OH, was collected and concentrated to dryness. The residue was dissolved in MeOH (10 ml), and added to acetic anhydride (5 ml). The solution was stirred at room temperature overnight. After the ninhydrin test of this solution became negative, the solvent was evaporated, and the residue was dissolved in water, desalted and

lyophilized. The powder obtained was purified by cellulose column chromatography with *n*-BuOH – pyridine – H_2O (6 : 4 : 3) to yield 240 mg of white powder (**IV**). m.p. 160~162°C (d).

Anal. Calcd. for $C_{22}H_{38}N_2O_{15}\cdot 2H_2O$:
C 43.63, H 6.99, N 4.63.
Found :
C 43.93, H 6.94, N 4.73.

Isolation of D-mannose (**V**) and D-ribose (**VI**) from **IV**:

A solution of **IV** (1 g) in 20 ml of 1 N H_2SO_4 was heated on a boiling water bath for 3 hours. The pH was adjusted to 7 with $Ba(OH)_2$ and the solution was filtered. The filtrate was desalted and adsorbed on a column of Dowex 1×2 (borate form) (50×2 cm). **VI** and **V** were eluted successively with 0.015 mole $K_2B_4O_7$ solution. Each fraction was collected, passed through a Dowex 50×2 (H^+) and concentrated to dryness. The residue was repeatedly dissolved in MeOH and evaporated to remove boric acid completely.

D-Ribose was converted to its anilide and recrystallized from EtOH[4], yield 13 mg, m. p. 122~123°C (d). $[\alpha]_D^{25}$ +63°→+45° (*c* 0.16, pyridine).

Anal. Calcd. for $C_{11}H_{15}NO_4\cdot{}^1/_2H_2O$:
C 56.40, H 6.89, N 5.98.
Found :
C 57.26, H 7.00, N 6.16.

D-Ribose anilide was reported[4] to have m.p. 125~127°C and $[\alpha]_D^{25}$ +63°→+49° (*c* 1, pyridine).

Fig. 8. Nuclear magnetic resonance spectrum of 1, 3-di-O-acetyl-2, 5-di-O-methyl-β-D-ribose
(60 MHz in $CDCl_3$, TMS internal standard)

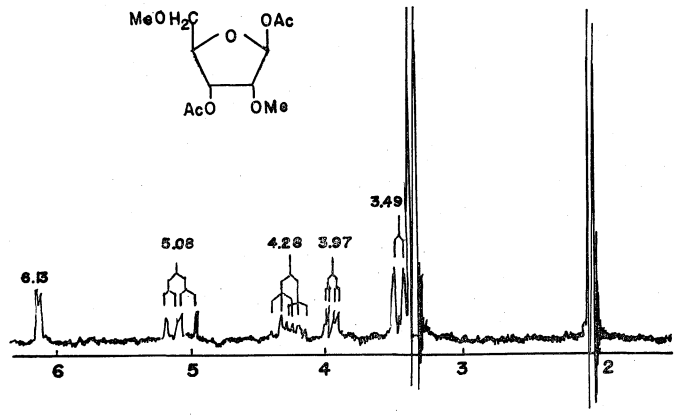


Fig. 9. Infrared absorption spectra of N, N′-diacetyl-6-O-methyl-2-deoxystreptamine (KBr tablet)
A : from lividomycin A B : from paromomycin

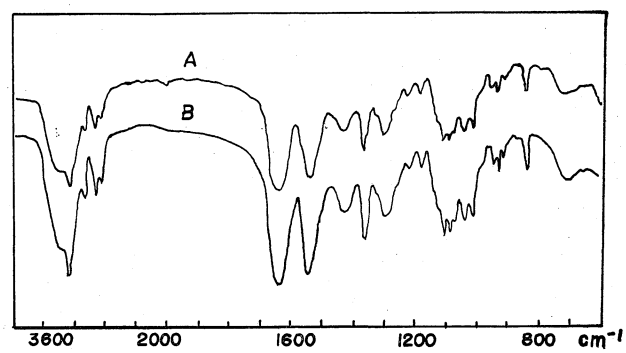
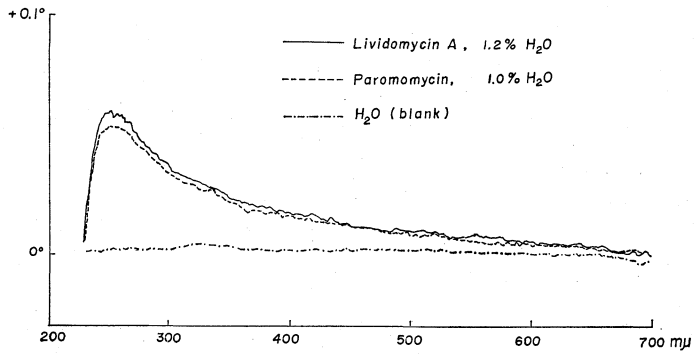


Fig. 10. Optical rotatory dispersion curves of N, N′-diacetyl-6-methyl-2-deoxystreptamine



D-Mannose was converted to its phenylhydrazone[5] and recrystallized from water and EtOH, yield 40 mg, m. p. 198~199°C (d). $[\alpha]_D^{25}$ +25°→+33° (*c* 0.35, pyridine).

Anal. Calcd. for $C_{12}H_{18}N_2O_5$: C 53.32, H 6.71, N 10.37.
Found : C 53.57, H 7.05, N 10.05.

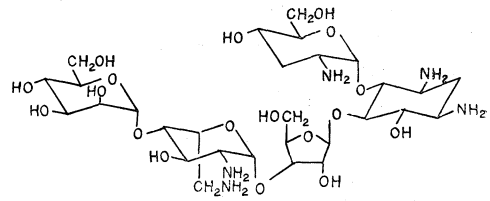
D-Mannose phenylhydrazone was reported[6] to have m. p. 199~200°C and $[\alpha]_D^{20}$ +26°→

+34° (pyridine).

Isolation of neosamine B (VII) from IV :

A solution of IV (1 g) in 40 ml of 6 N HCl was heated on a boiling water bath for one hour and neutralized with Dowex 3 (OH^-). The solution was adsorbed on a column of Dowex 50×2 (H^+) (50×2 cm) and eluted with 0.4 N HCl. The ninhydrin- and TTC*[7]-positive effluent was concentrated to dryness and reprecipitated from MeOH - acetone to yield 300 mg of hygroscopic neosamine B hydrochloride.

Fig. 11. Structure of lividomycin A



A portion of the above material in water with saturated aqueous picric acid yielded a crystalline picrate which after recrystallization from water melted at 126~128°C (d). $[\alpha]_D^{26}$ +8° (*c* 1, H_2O).

Anal. Calcd. for $C_6H_{14}N_2O_4 \cdot 2C_6H_3N_3O_7$: C 33.97, H 3.17, N 17.61.
Found : C 33.84, H 3.40, N 17.33.

Neosamine B dipicrate was reported[8] to have m. p. 125~126.5°C and $[\alpha]_D$ +13° (*c* 0.94, H_2O).

Isolation of 1-O-acetyl-2, 3, 4, 6-tetra-O-methyl-α-D-mannose (VIII) and 1,3-di-O-acetyl-2,5-di-O-methyl-β-D-ribose (IX) from II :

To a stirred solution of II (9 g) in dimethylformamide (100 ml), MeI (50 g) and Ag_2O (30 g) were added. After 40-hour stirring at room temperature, the silver salts were collected by filtration and washed with dimethylformamide. The combined filtrate and wash were evaporated to dryness, dissolved in 100 ml of 4 N HCl and heated on a boiling water bath for one hour, and the solution was evaporated to a syrup, which was dissolved in 50 ml of water and extracted with $CHCl_3$

(1) The $CHCl_3$ solution was evaporated and was acetylated with pyridine (25 ml) and acetic anhydride (50 ml). After 12 hours, the reaction mixture was evaporated, and the residue was dissolved in MeOH and again evaporated and was added to the top of a column of silica gel (30×1.5 cm). The anisaldehyde[9]-positive effluent eluted by $CHCl_3$ - MeOH (100 : 1) was collected and concentrated to give 160 mg of syrup (VIII). The syrup was purified by vacuum distillation. b. p. 96°C/0.04 mmHg.

Anal. Calcd. for $C_{12}H_{22}O_7$: C 51.79, H 7.97.
Found : C 51.50, H 7.79.

This sample was identical with 1-O-acetyl-2, 3, 4, 6-tetra-O-methyl-α-D-mannose prepared from D-mannose in IR and NMR spectrum.

(2) The aqueous layer after chloroform extraction was passed through Dowex 3 (OH^-) and Dewex 50×2 (H^+). The effluent was evaporated and acetylated with pyridine (25 ml) and acetic anhydride (50 ml). After solvent was evaporated, the acetylated product was purified by preparative thin-layer chromatography on silica gel with $CHCl_3$ - MeOH (200 : 1). The portion having Rf 0.75 detected by an anisaldehyde test[9] was collected and yielded 20 mg of syrup (IX).

Anal. Calcd. for $C_{11}H_{18}O_7$: C 50.38, H 6.92.
Found : C 50.02, H 6.77.

This sample was identical in IR and NMR spectrum to 1, 3-di-O-acetyl-2, 5-di-O-methyl-β-D-ribose prepared from paromomycin.

Mannosyl N, N'-diacetyl neosamine B (X) :

A solution of IV (650 mg) in 15 ml of 1 N H_2SO_4 was heated on a boiling water bath for one hour. The mixture was neutralized with Dowex 3 (OH^-) and evaporated to a syrup. The syrup was dissolved in MeOH, and Dowex 1×2 (carbonate form) (15 g) and acetic anhydride (2 ml) were added to the solution. The mixture was stirred in an ice

* TTC : Triphenyltetrazolium chloride (200 mg)/0.5 N methanolic NaOH (10 ml).

bath for 2 hours, then the resin was removed from the mixture. The filtrate was evaporated to yield 160 mg of colorless syrup. This syrup was gradient eluted from a column of Dowex 1×2 (borate form) (30×1 cm) using 0.001 to 0.015 mole $K_2B_4O_7$ solution. The effluent giving a positive anthrone - H_2SO_4 test and a negative phloroglucinol - HCl test was collected, passed through Dowex 50×2 (H^+) and evaporated to dryness. The residue was repeatedly dissolved in MeOH and evaporated to remove boric acid, yield 174 mg of white powder (**X**), m. p. 170~173°C (d). $[\alpha]_D^{20}$ +45.4° (*c* 1, H_2O) final.

Anal. Calcd. for $C_{16}H_{28}N_2O_{11}$: C 45.28, H 6.65, N 6.60.
Found : C 45.09, H 6.30, N 6.24.

Lividotriosaminol (**XI**) :

A solution of **III** (90 mg) in 10 ml of 0.5 N HCl was heated at 90~95°C for 10 hours. The mixture was neutralized with 2 N NaOH and stirred with excess $NaBH_4$ at room temperature. Afterward, the excess $NaBH_4$ in the reaction mixture wes decomposed by adding 2 N HCl. Then, the solution was neutralized with 2 N NaOH and chromatographed on CM-Sephadex C-25 (NH_4^+) (40×1 cm) with 0.025 N NH_4OH. The effluent with negative TTC test and positive ninhydrin test, was collected, concentrated and lyophilized to yield 50.5 mg of white powder (**XI**), m.p. 153°C (d). $[\alpha]_D^{21}$ +54° (*c* 0.24, H_2O). NMR $\delta_{DSS}^{D_2O}$ 4.97 ppm (1H, d, J=2 cps), 5.03 ppm (1H, d, J=2 cps).

Anal. Calcd. for $C_{17}H_{34}N_2O_{13}\cdot H_2O$: C 41.46, H 7.37, N 5.69.
Found : C 41.62, H 6.88, N 5.76.

Di-N-acetyl lividotriosaminol (**XII**) :

A solution of **XI** (335 mg) in MeOH (10 ml) and acetic anhydride (1 ml) was stirred at room temperature for 2 hours. The solution was evaporated to dryness, dissolved in water, desalted and concentrated to dryness yielding 333 mg of white powder (**XII**), m. p. 236~240°C (d). $[\alpha]_D^{21}$ +40° (*c* 1.25, H_2O). NMR $\delta_{DSS}^{D_2O}$ 2.01 ppm (3H, s), 2.04 ppm (3H, s), 4.98 ppm (1H, d, J=1.5 cps), 5.05 ppm (1H, d, J=2 cps).

Anal. Calcd. for $C_{21}H_{38}N_2O_{15}$: C 43.90, H 6.67, N 4.88.
Found : C 44.00, H 6.76, N 5.05.

Periodate oxidation of methyl lividotriosaminide and methyl N,N'-diacetyl lividotriosaminide :

A solution of sample (0.014 m mole) in 17 ml of $NaIO_4$ (0.01 m mole/ml) was stirred at room temperature in the dark. The amount of periodate consumed was determined by the usual method[10].

Sample	Periodate consumed per mole		
	3 hours	6 hours	8 hours
Methyl lividotriosaminide	3.1 moles	3.2 moles	3.2 moles
Methyl N, N'-diacetyl lividotriosaminide	1,7 moles	2.0 moles	2.1 moles

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

To a solution of **II** (3 g) in dimethylformamide (100 ml), MeI (60 ml) was added, and Ag_2O (4 g) was added twice at 20 minutes intervals with stirring. The stirring was continued for 4 hours. The reaction mixture was filtered, concentrated and dissolved in 100 ml of 3 N HCl. This solution was heated on a boiling water bath for 3 hours and evaporated to dryness after treatment with carbon. The residue was dissolved in water and chromatographed on a column of CM-Sephadex C-25 (NH_4^+) (40×1 cm), washed with water and 0.035 N NH_4OH and finally gradient eluted with 0.035 N to 0.15 N NH_4OH. The effluent was evaporated and acetylated with acetic anhydride in MeOH, and the N-acetylated material was separated with preparative thin-layer chromatography on silica gel with $CHCl_3$ - MeOH (3 : 1). Compound **XIII** was extracted from the plate and recrystallized from absolute EtOH, yield 200 mg, m. p. 278~281°C (d). $[\alpha]_D^{24}$ +4° (*c* 1.23, H_2O). NMR $\delta_{DSS}^{D_2O}$ 3.52 ppm (3H, s).

Anal. Calcd. for $C_{11}H_{20}N_2O_5$: C 50.75, H 7.75, N 10.76.
Found : C 50.30, H 7.74, N 10.56.

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