Structure of Destomycin B[†]Masaru SHIMURA, Yasuharu SEKIZAWA, Katsuharu IINUMA*
Hiroshi NAGANAWA* and Shinichi KONDO*Research Laboratories, Meiji Seika Kaisha, Ltd.,
Morooka-cho, Kohoku-ku, Yokohama, Japan*Institute of Microbial Chemistry, Kamiosaki,
Shinagawa-ku, Tokyo, Japan

Received December 1, 1975

Destomycin B, a member of destomycin family antibiotics having anthelmintic activity, was shown to be 5-O-[2,3-O-(6-amino-6-deoxy-L-glycero-D-gluco-heptopyranosylidene)-β-D-mannopyranosyl]-1,3-di-(methylamino)-1,2,3-trideoxy-myo-inositol based on chemical and spectroscopic studies.

An aminoglycoside antibiotic, destomycin B was isolated from a culture filtrate of *Streptomyces rimofaciens* as a minor product.¹⁾ The properties including anthelmintic activity are similar to those of destomycin A,^{1,2)} the major product. We report herein the structural elucidation of destomycin B. Destomycin B consists of three moieties, N,N'-dimethyl-2-deoxystreptamine, D-mannose and 6-amino-6-deoxy-L-glycero-D-gluco-heptopyranose, instead of 1-N-methyl-2-deoxystreptamine, D-talose and 6-amino-6-deoxy-L-glycero-D-galacto-heptopyranose in destomycin A,^{2~5)} respectively.

Destomycin B (**I**) shows mp 181~190°C (decomp.); $[\alpha]_D^{25} + 6^\circ$ ($c=1$, H₂O); UV end absorption; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400, 2880, 1630, 1580, 1475, 1370, 1340, 1245, 1140, 1090, 1040, 905, 885, 850 and 790. The formula C₂₁H₃₉N₃O₁₃ was assigned to **I** by the elementary analysis,¹⁾ the carbon-13 spectrum⁶⁾ and the mass spectrum of the tri-N-acetyl-mono-N-methyl-octa-O-methyl derivative. **I** and its tri-N-acetyl derivative consume 5.7 and 2.1 moles of periodate for 48 hr, respectively. Methyl mannoside was obtained by methanolysis of **I** and identified by gas chromatography⁷⁾ of its trimethylsilylated derivative. In the PMR spectrum of **I**, a signal at δ 2.83 ppm assigned to two N-methyl groups was observed. As shown in Table I, coupling constants indi-

cate that the mannoside moiety is distorted from the C1 conformation and another pyranoside moiety has a *gluco*-configuration.

TABLE I. PMR CHEMICAL SHIFTS AND COUPLING CONSTANTS FOR TWO PYRANOSIDE MOIETIES IN DESTOMYCIN B

Proton	δ (ppm) ^{a)}	J (Hz)
1'	5.72	$J_{1',2'} = 1.8$
2'	5.11	$J_{2',3'} = 6.0$
3'	4.92	$J_{3',4'} = 6.3$
4'	4.31	$J_{4',5'} = 10.3^b)$
2''	4.39	$J_{2'',3''} = 10.3$
3''	4.20	$J_{3'',4''} = 10.0^b)$

a) Chemical shifts were measured in D₂O using TMS as the external reference.

b) These coupling constants were determined by INDOR method.

Acid hydrolysis of **I** with 6 N HCl for 20 hr under refluxing followed by column chromatography on Dowex 1-X2 (OH⁻) resin gave an optically inactive compound as colorless plates. The compound was identical with N, N'-dimethyl-2-deoxystreptamine which was synthesized by reduction of tri-O-acetyl-N, N'-diethoxycarbonyl-2-deoxystreptamine with lithium aluminum hydride in tetrahydrofuran.

Mild acid hydrolysis of **I** with 1 N HCl in a boiling water bath for 10 min followed by column chromatography on Dowex 1-X2 (OH⁻) resin gave a basic glycoside (**II**) from the effluent, and a polyhydroxy amino acid

[†] This work was briefly communicated, see Ref. 2).

(III) from the eluate with 10% aqueous acetic acid. The **II** was obtained as a colorless crystalline powder. It showed no reducing property and was further hydrolyzed with 6 N HCl to N,N'-dimethyl-2-deoxystreptamine and mannose which were confirmed on thin-layer chromatography by comparison with authentic samples. The basic glycoside (**II**) was suggested to be a 5-O-(mannopyranosyl)-N,N'-dimethyl-2-deoxystreptamine by the periodate consumptions of **II** (4.2 moles without formation of formaldehyde) and its di-N-acetate (1.8 moles). By the application of Hudson's rule of isorotation⁸⁾ (**II**: $[M]_D^{25} - 181^\circ$, methyl β -D-mannopyranoside: $[M]_D - 136^{(9)}$), the structure was confirmed to be 5-O-(β -D-mannopyranosyl)-1,3-di-(methylamino)-1,2,3-trideoxy-*myo*-inositol. In the carbon-13 spectrum, the chemical shifts of carbons in mannose moiety showed good agreement with those of methyl β -D-mannopyranoside,¹⁰⁾ as shown in Table II. The D-sugar was also confirmed by the identification of methyl 4,6-di-O-methyl- α -D-mannopyranoside as described later.

II

SCHEME 1.

The polyhydroxy amino acid (**III**) was obtained as colorless prisms from the eluate of Dowex 1-X2 resin by the mild acid hydrolysis of **I**. The properties were similar to those of destomic acid⁴⁾ which was obtained from destomycin A. As shown in Table I, the PMR spectrum of **I** shows that the heptosamine moiety has a *gluco*-configuration. Consequently, **III** was suggested to be 4-*epi*-destomic acid. The configuration at C-5 and C-6 position of **III** was determined by the following

derivations.

Periodate-permanganate oxidation⁴⁾ of the 2, 4-dinitrophenyl derivative of **III** followed by extraction with ethyl acetate at acid afforded

TABLE II. CARBON-13 CHEMICAL SHIFTS OF THE BASIC GLYCOSIDE (**II**)

Carbon	II (δ)	Methyl β -D-mannopyranoside ¹⁰⁾ (δ^a)
1	58.4	
2	28.7	
3	58.4	
4	73.8	
5	86.2	
6	75.4	
7	32.0	
8	32.0	
1'	102.0	102.0
2'	71.4	71.5
3'	74.3	74.2
4'	67.4	68.0
5'	77.3	77.5
6'	61.7	62.3

^{a)} δ : ppm from TMS using dioxane ($\delta = 67.4$ ppm) as the internal reference.

the 2, 4-dinitrophenyl derivative of the oxidized product which was identical with authentic 2,4-dinitrophenyl-L-serine¹¹⁾ in all respects. Therefore, the stereochemistry at the C-6 position of **III** was determined to be the *S*-configuration.

Permethylation of tri-N-acetyldestomycin B by the method of Hakomori,¹²⁾ followed by chloroform extraction afforded a colorless powder of tri-N-acetyl-mono-N-methyl-octa-O-methyldestomycin B (*m/e* 793). After mild hydrolysis of the permethylated product with 1 N HCl for 10 min in a boiling water bath, neutral and acidic compounds were obtained by the treatment of Dowex 1-X2 (OH⁻) resin (Chart I). The eluate with 1 N HCl from the resin was concentrated to dryness yielding the crude powder of the acidic compound. Deacetylation of the crude compound in 2 N NaOH under refluxing for 18 hr followed by column chromatography on Dowex 50W-X4 (H⁺) resin eluting with 1 N aqueous ammonia gave the N-methyl-tetra-O-methyl derivative of

III. By the reacetylation of the derivative with acetic anhydride in chloroform at room temperature for 15 hr, followed by column chromatography of silica gel eluting with a mixture of chloroform and acetone (10:1 in volume), a colorless syrup of N-acetyl-N-methyl-tetra-O-methyl-4-*epi*-destomic acid-1,5-lactone was obtained, $[\alpha]_D^{25} + 55^\circ$ ($c=1$, chloroform). The application of Hudson's lactone rule^{13,14} (the value by addition of one equivalent KOH in methanol: $[\alpha]_D^{25} + 12^\circ$) suggested assignment of the D_G-configuration to the asymmetric carbon at C-5 position of the lactone. From these results, the structure of 4-*epi*-destomic acid (**III**) was determined to be 6-amino-6-deoxy-L-*glycero*-D-*gluco*-heptonic acid.

SCHEME 2.

Methanolysis of the neutral compound described above, with 3% methanolic hydrogen chloride at 90°C for 2 hr in a sealed tube gave a derivative of N,N'-dimethyl-2-deoxystreptamine which was crystallized from chloroform, and a derivative of mannose as chloroform-soluble product (Chart I). Deacetylation of the derivative of N,N'-dimethyl-2-deoxystreptamine with 6 N HCl for 8 hr under refluxing followed by column chromatography on Amberlite CG 50 (NH₄⁺) resin afforded colorless needles of N,N'-dimethyl-4,6-di-O-methyl-2-deoxystreptamine. The derivative of mannose was chromatographed on a column of silica gel eluted with a mixture of chloroform and acetone (5:1 in volume) to

give a colorless syrup, $[\alpha]_D^{27} + 79^\circ$ ($c=0.2$, H₂O). It consumes 1.1 moles of periodate for 48 hr. By the application of the modified Reeves' method¹⁵) ($\Delta[M]_{\text{C}_{10}\text{H}_{16}\text{O}_m} + 1030^\circ$ at 436 nm), it was confirmed to be D-sugar. From the spectral data and rotatory value, it was identified as methyl 4,6-di-O-methyl- α -D-mannopyranoside.¹⁶)

The carbon-13 spectrum of **I** showed the presence of an orthoester carbon at 121.7 ppm (Table III). Therefore, the absolute structure

TABLE III. CARBON-13 CHEMICAL SHIFTS OF DESTOMYCIN FAMILY ANTIBIOTICS

Carbon	Destomy- cin A (δ) ^{a)}	Destomy- cin B (δ)	Destomy- cin C (δ)	A-396-I (δ)
1	58.7 d ^{b)}	58.6 d	58.6 d	51.0 d
2	32.2 t	28.2 t	28.6 t	35.3 t
3	50.8 d	58.6 d	58.6 d	51.0 d
4	76.6 d	73.7 d	73.9 d	76.3 d
5	86.7 d	87.0 d	86.9 d	86.3 d
6	75.6 d	75.1 d	75.4 d	77.8 d
7	32.0 q	31.8 q	32.0 q	
8		31.8 q	32.0 q	
1'	100.5 d	100.4 d	100.5 d	100.4 d
6'	62.0 t	61.5 t	62.0 t	62.0 t
1''	121.2 s	121.7 s	121.2 s	121.2 s
6''	52.3 d	52.5 d	52.3 d	52.4 d
7''	62.7 t	62.7 t	62.7 t	62.5 t
2'~5'	76.1 d	81.0 d	76.1 d	76.1 d
	75.6 d	76.2 d	75.5 d	75.3 d
	74.7 d	75.1 d	74.7 d	74.7 d
	73.0 d	74.5 d	73.0 d	73.0 d
	72.4 d	73.0 d	72.4 d	72.5 d
2''~5''	69.9 d	70.0 d	69.9 d	69.9 d
	69.9 d	69.0 d	69.9 d	69.9 d
	64.4 d	68.8 d	64.4 d	64.4 d

^{a)} δ : ppm from TMS using dioxane ($\delta=67.4$ ppm) as the internal reference.

^{b)} s, d, t, q: multiplicity on off-resonance experiment; singlet, doublet, triplet and quartet, respectively.

of destomycin B (**I**) except the configuration of the orthoester carbon of the heptosamine moiety was determined to be 5-O-[2,3-O-(6-amino-6-deoxy-L-*glycero*-D-*gluco*-heptopyranosylidene)- β -D-mannopyranosyl]-1,3-di-(methylamino)-1, 2, 3-trideoxy-*myo*-inositol (**Ia** or **Ib**).

As shown in Table III and Fig. 1, **I** shows reasonable spectral data of the carbon-13 spectrum and ORD in comparison with other destomycin family antibiotics, hygromycin B,¹⁷⁾ A-396-I,¹⁸⁾ destomycins A³⁾ and C⁸⁾ which can be presented by the following structures.

FIG. 1. Optical Rotatory Dispersion Curves of Destomycin Family Antibiotics.

Samples (free base) were measured in aqueous solution ($c=2$).

a, destomycin A; b, destomycin B; c, destomycin C; d, hygromycin B; e, A-396-I.

EXPERIMENTAL

Melting points were determined in capillary tubes and uncorrected. For infrared absorption spectrum, a Hitachi 215 recording spectrometer was used. UV absorption was measured by a Hitachi EPS-3T recording spectrometer. For optical measurement, a Jasco DIP-SL automatic polarimeter or a Jasco Model ORD/UV-5 automatic optical rotatory dispersion

Destomycin A: $R_1=CH_3$, R_3 , $R_5=OH$, R_2 , R_4 , $R_6=H$.

Destomycin B (Ia or Ib): R_1 , $R_2=CH_3$, R_4 , $R_6=OH$, R_3 , $R_5=H$.

Destomycin C: R_1 , $R_2=CH_3$, R_3 , $R_5=OH$, R_4 , $R_6=H$.

Hygromycin B: $R_2=CH_3$, R_3 , $R_6=OH$, R_1 , R_4 , $R_5=H$.

A-396-I: R_3 , $R_5=OH$, R_1 , R_2 , R_4 , $R_6=H$.

SCHEME 3.

recorder was used. Proton magnetic resonance spectrum was measured by a Varian HA-100D spectrometer. The spectrometer modified by an INDOR kit (Varian) was employed for the INDOR method. Carbon-13 Fourier-transform spectrum was measured by a Varian

XL-100 spectrometer. A Hitachi RMU-6M and a Hitachi RMU-7M with a Hitachi 002 datalyzer system were used for mass spectrometry and high-resolution mass spectrometry, respectively. Through all the experiments, silica gel plate (Art 5714, Merck, West Germany) was used for thin-layer chromatographic analysis. Ninhydrin (sprayed with 0.2% solution in acetone and pyridine (20:1 in volume) and heated) and orcinol (sprayed with 2% solution in ethanol, then with 50% H_2SO_4 and heated) reagents were used.

Destomycin B free base (I)

Destomycin B free base was prepared by the method previously reported.¹³ The homogeneity was confirmed by TLC. The spot was visualized by ninhydrin reagent (pinkish purple) or by orcinol reagent (dark blue). Pure I showed a single spot at R_f 0.24, 0.17 and 0.15 by using solvent mixture of butanol-28% ammonia-ethanol (4:3:1 in volume), butanol-ethanol-chloroform-17% ammonia (4:5:2:5 in volume) and chloroform-methanol-4% ammonia (2:1:1 in volume, upper layer), respectively. I was obtained as a white amorphous powder; mp 181~190°C (decomp.); $[\alpha]_D^{21} + 6^\circ$ ($c=1$, H_2O). *Anal.* Calcd. for $\text{C}_{21}\text{H}_{30}\text{N}_3\text{O}_{13} \cdot \text{H}_2\text{O}$: C, 45.07; H, 7.39; N, 7.51; O, 40.03; amino N 2.50. Found: C, 45.34; H, 7.37; N, 7.69; O, 39.40; amino N 2.57%.

Tri-N-acetyldestomycin B

Two g of I was suspended in 20 ml of methanol to which 1 ml of acetic anhydride was added gradually under stirring and the reaction mixture was stood at 5°C overnight. Addition of 20 ml of ethyl ether to the solution gave a white precipitate which was separated by filtration and washed with 200 ml of ethyl ether. The precipitate was crystallized with a mixture of methanol and ethyl ether to give a colorless crystalline powder (1.8 g) of tri-N-acetyldestomycin B, mp 235~246°C (decomp.). *Anal.* Calcd. for $\text{C}_{21}\text{H}_{30}\text{N}_3\text{O}_{13} \cdot 3(\text{COCH}_3)$: C, 46.35; H, 7.03; N, 6.24; O, 40.38. Found: C, 46.50; H, 7.47; N, 5.89; O, 40.42%. TLC: butanol-ethanol-chloroform-17% ammonia (4:5:2:5 in volume); R_f 0.28, *tert*-butanol-acetic acid-water (2:1:1 in volume); R_f 0.37.

Determination of methyl mannoside by gas chromatography

A carbohydrate in the methanolysate of I was determined by the method of Yamakawa *et al.*¹⁷ A solution of I (5 mg) in 5 ml of 3% methanolic hydrogen chloride was heated in a sealed tube at 105°C for 3.5 hr. The solution was neutralized with Amberlite IR 45 (OH^-) resin and the resin was filtered off. The filtrate was concentrated to dryness under reduced pressure. To the pyridine solution (1 ml) of the residue, 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchloro-

silane were added. The solution was warmed at 50°C for 2 min and allowed to stand at room temperature overnight. After addition of 3 ml of chloroform, the solution was washed with water (6 ml $\times$ 3). The chloroform layer was dried with anhydrous sodium sulfate and concentrated to dryness yielding a yellow syrup. The syrup was dissolved in chloroform and subjected to gas chromatography. Gas chromatographic conditions were as follows; instrument: a Hitachi 063 with a stainless steel column (0.3 $\times$ 200 cm), liquid phase: 5% Ucon LB 550 X, support: Shimalite W (60~80-mesh), column temperature: 190°C, carrier gas: nitrogen 1.2 kg/cm³. The retention time of the trimethylsilylated derivative of the methanolysate corresponded with that of authentic methyl D-mannopyranoside (8.7 min).

Isolation of N,N'-dimethyl-2-deoxystreptamine

A solution of I (2 g) in 20 ml of 6 N HCl was heated under refluxing for 20 hr and concentrated to dryness. An aqueous solution (10 ml) of the residue was subjected to column chromatography on Dowex 1-X2 (OH^- , 100~200 mesh, 3.5 $\times$ 30 cm) resin and the column was developed with water. The effluent was cut into 10-ml fractions. The fractions (Nos. 16~20) which showed negative orcinol test and positive ninhydrin test were combined and concentrated to dryness. Crystallization with a mixture of methanol (5 ml) and acetone (50 ml) gave 275 mg of colorless plates, mp 173~176°C (decomp.), $[\alpha]_D^{25} 0$ ($c=2$, H_2O). *Anal.* Calcd. for $\text{C}_8\text{H}_{18}\text{N}_2\text{O}_3$: C, 50.50; H, 9.54; N, 14.73; O, 25.23. Found: C, 50.46; H, 9.61; N, 15.46; O, 25.16%. PMR $\delta_{\text{Me}_4\text{Si}}^{\text{D}_2\text{O}}$ (external): 1.38 (1H, quartet, $J=12$ Hz, $\text{H}_{\text{ax}}-2$), 2.60~3.05 (3H, multiplet, $\text{H}_{\text{eq}}-2$, H-1,3, overlapped with methyl signal), 2.78 (6H, singlet, $\text{N}-\text{CH}_3 \times 2$), 3.5~3.9 (3H, multiplet, H-4,5,6). TLC: butanol-ethanol-chloroform-17% ammonia (4:5:2:5 in volume); R_f 0.38, *tert*-butanol-acetic acid-water (2:1:1 in volume); R_f 0.22. Dihydrochloride of this compound was crystallized with a mixture of 6 N HCl and ethanol, colorless needles, mp 283~286°C (decomp.), $[\alpha]_D^{30} 0$ ($c=0.9$, H_2O). This compound was identical with synthetic N,N'-dimethyl-2-deoxystreptamine in all respects.

Synthesis of N,N'-dimethyl-2-deoxystreptamine¹⁹⁾

Di-N-ethoxycarbonyl-2-deoxystreptamine²⁰⁾ (501 mg) prepared from 2-deoxystreptamine was dissolved in 2 ml of dry pyridine and the 2 ml of acetic anhydride was added under stirring and cooling with an ice bath. After stirring overnight, the solution was transferred into 30 ml of ice-water to yield colorless crystals (278-mg) of the tri-O-acetate, mp 220~221°C. To a solution of the acetate (215 mg) in 5 ml of dry tetrahydrofuran, a suspension of LiAlH_4 (307 mg) in 5 ml of the same solvent was added and the mixture was refluxed at 85°C for 24 hr. The reaction mixture was made up

to 50 ml with water and ethanol. The solution, after removing white precipitate, was neutralized with 6 N HCl and passed through a column of Amberlite CG-50 (NH_4^+ , 15 ml) resin. After washing the column with 75 ml of water, elution was done with 180 ml of 0.1 N NH_4OH . The eluate was cut into 2-ml fractions. Ninhydrin-positive fractions (Nos. 46~75) were concentrated to dryness yielding a colorless hygroscopic powder. Crystallization with a mixture of 6 N HCl and ethanol gave colorless needles (81 mg) of N,N'-dimethyl-2-deoxystreptamine dihydrochloride, mp $283 \sim 286^\circ\text{C}$ (decomp.), $[\alpha]_{\text{D}}^{30} 0$ ($c=0.87$, H_2O). Free base of the compound was obtained as colorless plates by treatment of Dowex 1-X2 (OH^-) resin, mp $173 \sim 176^\circ\text{C}$ (decomp.) Anal. Calcd. for $\text{C}_8\text{H}_{18}\text{N}_2\text{O}_3 \cdot 1/2\text{H}_2\text{O}$: C, 48.23; H, 9.61; N, 14.06; O, 28.10. Found: C, 49.10; H, 9.38; N, 13.96; O, 27.31%.

Isolation of the basic glycoside (II)

A solution of **I** (2.7 g) in 20 ml of 1 N HCl was heated in a boiling water bath for 10 min and then concentrated to dryness. The residue was dissolved in 20 ml of water and charged to a column (3×82 cm) of Dowex 1-X2 (OH^- , 100~200 mesh, 190 ml). The column was developed with water (480 ml) and the effluent was cut into 20-ml fractions. The ninhydrin and orcinol-positive fractions (Nos. 9~16) were combined and concentrated to dryness yielding a colorless crystalline powder (1.2 g) of **II**, mp $80 \sim 120^\circ\text{C}$ (decomp.), $[\alpha]_{\text{D}}^{22} -50^\circ$ ($c=0.5$, H_2O), $[\text{M}]_{\text{D}}^{22} -181^\circ$. Anal. Calcd. for $\text{C}_{14}\text{H}_{23}\text{N}_3\text{O}_5 \cdot 1/2\text{H}_2\text{O}$: C, 46.53; H, 8.09; N, 7.75; O, 37.63. Found: C, 46.39; H, 8.41; N, 7.13; O, 36.50%. PMR $\delta_{\text{Me}_4\text{Si}}^{\text{D}_2\text{O}}$ (external): 1.39 (1H, quartet, $J=12$ Hz, $\text{H}_{\text{ax}}-2$), 2.6~3.1 (3H, multiplet, $\text{H}_{\text{eq}}-2$, $\text{H}-1,3$, overlapped with methyl signal), 2.80 (6H, singlet, $\text{N}-\text{CH}_3 \times 2$), 3.75~4.56 (9H, multiplet), 5.33 (1H, $J < 1$ Hz, $\text{H}-1'$). TLC: butanol-ethanol-chloroform-17% ammonia (4:5:2:5 in volume); $R_f 0.28$, *tert*-butanol-acetic acid-water (2:1:1 in volume); $R_f 0.14$. It was hydrolyzed with 1 N H_2SO_4 at 100°C for 1 hr. On TLC developed with *tert*-butanol-acetic acid-water (2:1:1 in volume) or butanol-28% ammonia-ethanol (4:3:1 in volume), the hydrolysate showed a ninhydrin-positive spot at $R_f 0.16$ or $R_f 0.47$ and an orcinol-positive spot at $R_f 0.49$ or $R_f 0.21$, respectively. The former spot corresponded with that of N,N'-dimethyl-2-deoxystreptamine and the latter one with that of D-mannose. The di-N-acetate of **II** was prepared by the similar method for tri-N-acetate of **I** using acetic anhydride in methanol, a colorless crystalline powder, mp $184 \sim 190^\circ\text{C}$ (decomp.).

Isolation of 4-*epi*-destomic acid (III)

The Dowex 1-X2 column described in the preceding section was eluted with 260 ml of 10% acetic acid. The fractions showing positive ninhydrin were collected

and concentrated to dryness yielding a colorless crystalline powder. Recrystallization with a mixture of hot water and methanol afforded colorless prisms (818 mg) of **III**, mp 216°C (decomp.), $[\alpha]_{\text{D}}^{23} +3.7^\circ$ ($c=2$, H_2O). Anal. Calcd. for $\text{C}_7\text{H}_{15}\text{NO}_7$: C, 37.33; H, 6.71; N, 6.22; O, 49.73. Found: C, 37.20; H, 6.64; N, 6.05; O, 50.11%. TLC: butanol-28% ammonia-ethanol (4:3:1 in volume); $R_f 0.30$, *tert*-butanol-acetic acid-water (2:1:1 in volume); $R_f 0.34$.

2,4-Dinitrophenyl-4-*epi*-destomic acid

A solution of 1-fluoro-2,4-dinitrobenzene (752 mg) in 1 ml of ethanol was added to an aqueous solution (10.5 ml) of **III** (497 mg) and sodium bicarbonate (719 mg). The mixture was stirred for 90 min at room temperature in the dark and thereafter concentrated to dryness. An aqueous solution (50 ml) of the residue was washed with ethyl ether (50 ml $\times$ 3) and the aqueous layer was extracted with ethyl acetate (50 ml $\times$ 6) at pH 2.0 adjusted with 1 N HCl. The extract was dried with anhydrous sodium sulfate and concentrated to about 50 ml which was stood at room temperature overnight to yield yellow needles (604 mg) of 2,4-dinitrophenyl-4-*epi*-destomic acid, mp $177 \sim 179^\circ\text{C}$. Anal. Calcd. for $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}_{11}$: C, 39.90; H, 4.38; N, 10.73; O, 44.98. Found: C, 40.11; H, 3.96; N, 10.76; O, 44.84%.

Isolation of 2,4-dinitrophenyl-L-serine

A solution of 2,4-dinitrophenyl-4-*epi*-destomic acid (528 mg), K_2CO_3 (760 mg), NaIO_4 (406 mg) and KMnO_4 (19 mg) in 360 ml of water was allowed to stand in the dark at room temperature for 3 hr. After removing a blackish brown precipitate, the solution was acidified to pH 2~3 with 10% H_2SO_4 and extracted with ethyl acetate (60 ml $\times$ 2). The extract was dried with anhydrous sodium sulfate and concentrated to dryness yielding 390 mg of an yellow gummy solid. Crystallization with a mixture of ethyl acetate and petroleum ether gave 175 mg of yellow prisms, mp $175 \sim 177^\circ\text{C}$, $[\alpha]_{\text{D}}^{22} +110^\circ$ ($c=0.5$, 4% NaHCO_3 in H_2O). Anal. Calcd. for $\text{C}_9\text{H}_9\text{N}_3\text{O}_7$: C, 39.85; H, 3.34; N, 15.49; O, 41.30. Found: C, 39.81; H, 3.25; N, 15.10; O, 40.97%. TLC: butanol-acetic acid-water (4:1:1 in volume); $R_f 0.56$. This compound was identical with authentic 2,4-dinitrophenyl-L-serine (Tokyo Kasei Kogyo Co., Ltd.) in all respects.

Permethylation⁽¹²⁾ of tri-N-acetyldestomycin B

A solution of sodium hydride in 250 ml of dimethylsulfoxide was stirred for 30 min and then 1.1 g of tri-N-acetyldestomycin B was added. After stirring for 30 min, 4 ml of methyl iodide was added gradually to the solution which was stirred for further 60 min. After addition of 500 ml of chloroform and 250 ml of water

under vigorous shaking, chloroform layer was separated and washed with water (250 ml $\times$ 2). The chloroform extract was dehydrated with anhydrous sodium sulfate and concentrated to dryness yielding a yellow syrup. The syrup was dissolved in a small amount of chloroform and then added 100 ml of *n*-hexane. The solution was kept in refrigerator overnight to give a colorless powder (1.2 g) of tri-*N*-acetyl-mono-*N*-methyl-octa-*O*-methyldestomycin B, mp 122~125°C. MS *m/e*: 793 (M^+) ($C_{36}H_{33}N_3O_{16}$). IR $\nu_{\max}^{film}$ cm^{-1} : 3000, 2950, 2840, 1635, 1550, 1450, 1410, 1375, 1300, 1240, 1200, 1140, 1100, 1070, 1020, 980, 850, 750. TLC: chloroform-methanol (100: 8 in volume); *R*_f 0.25.

Isolation of N-acetyl-N-methyl-tetra-O-methyl-4-epi-destomic acid-1,5-lactone

A solution of tri-*N*-acetyl-mono-*N*-methyl-octa-*O*-methyldestomycin B (750 mg) in 20 ml of 1 *N* HCl was heated in a boiling water bath for 10 min and the solution was concentrated to dryness. The residual solid was dissolved in 20 ml of water which was adjusted to pH 8~9 by addition of Dowex 1-X2 (OH^- , 40 ml) resin. The resin separated was eluted with 80 ml of 1 *N* HCl to afford 270 mg of a solid which showed a main spot at *R*_f 0.16 on TLC using a solvent mixture of chloroform-acetone (2: 1 in volume). A solution of this solid in 4 ml of 2 *N* NaOH was heated under refluxing for 18 hr. The solution was diluted with 150 ml of water and passed through a column of Dowex 50W-X4 (H^+ , 50 ml) resin. After washing with 600 ml of water, the column was eluted with 1 *N* NH_4OH . The eluate was cut into 5-ml fractions. Each fraction was detected by spraying with 10% H_2SO_4 on silica gel plate. Fractions Nos. 18~20 were combined and concentrated to dryness yielding 128 mg of a solid. To a solution of this solid in 4 ml of chloroform, 0.4 ml of acetic anhydride was added and the solution was allowed to stand at room temperature overnight. The solution was concentrated to dryness and the residue was dissolved in 3 ml of chloroform. The solution was poured on a column of silica gel (Wakogel C-200, 7 g). The column was washed with 50 ml of chloroform and then eluted with a mixture of chloroform-acetone (10: 1 in volume). The eluate was cut into 5-ml fractions and each fraction was detected by TLC. Fractions (Nos. 20~40) showing a single spot were concentrated to dryness yielding a colorless syrup (35 mg) of *N*-acetyl-*N*-methyl-tetra-*O*-methyl-4-epi-destomic acid-1,5-lactone, $[\alpha]_D^{22} + 55^\circ$ ($c=1.0$, $CHCl_3$), $[\alpha]_D^{22} + 12^\circ$ (the value by addition of one equivalent KOH in methanol). PMR $\delta_{Me_4Si}^{CDCl_3}$: 2.1 (3H, singlet, $CO-CH_3$), 3.05 (3H, singlet, $N-CH_3$), 3.34, 3.53, 3.58, 3.68 (3H $\times$ 4, singlet, $O-CH_3$). MS *m/e*: 319.1635 (M^+), (calcd. for $C_{14}H_{25}NO_8$, 319.1629). TLC: chloroform-acetone (2: 1 in volume); *R*_f 0.33.

Isolation of N,N'-dimethyl-4,6-di-O-methyl-2-deoxy-streptamine

The filtrate from the Dowex 1-X2 resin described in the preceding section was concentrated to dryness yielding a neutral compound (380 mg). A solution of the neutral compound in 5 ml of 3% methanolic hydrogen chloride was heated in a sealed tube at 90°C for 2 hr. The solution was concentrated to dryness which was crystallized from chloroform to give colorless crystals (53 mg) of *N,N'*-diacetyl-*N,N'*-dimethyl-4,6-di-*O*-methyl-2-deoxystreptamine, mp 133~135°C. The crystals were hydrolyzed with 6 *N* HCl for 8 hr under refluxing to give 20 mg of a colorless powder (positive ninhydrin). An aqueous solution (2 ml) of the powder was poured on a column of Amberlite CG50 (NH_4^+ , 10 ml) resin. After washing with 20 ml of water, the column was eluted with 200 ml of 0.1% NH_4OH . The eluate was cut into 3.3-ml fractions. Ninhydrin-positive fractions (Nos. 20~22) were concentrated to dryness yielding colorless needles (6.2 mg) of *N,N'*-dimethyl-4,6-di-*O*-methyl-2-deoxystreptamine, $[\alpha]_D^{23} 0$ ($c=0.2$, H_2O). MS *m/e*: 218 (M^+) ($C_{10}H_{22}N_2O_9$). PMR $\delta_{Me_4Si}^{D_2O}$ (external): 1.4 (1H, quartet, $J=12$ Hz, H_{ax-2}), 2.65~3.10 (3 H, multiplet, H_{eq-2} , $H-1,3$, overlapped with *N*-methyl signal), 2.81 (6H, singlet, $N-CH_3 \times 2$), 3.53 (2H, triplet, $J=10$ Hz, $H-4,6$), 3.95~4.15 (1H, $H-5$, overlapped with *O*-methyl signal), 4.03 (6H, singlet, $O-CH_3 \times 2$). TLC: butanol-ethanol-chloroform-17% ammonia (4: 5: 2: 5 in volume); *R*_f 0.59.

Isolation of methyl 4,6-di-O-methyl-α-D-mannopyranoside

The mother liquor (chloroform solution) of the crystallization of *N,N'*-diacetyl-*N,N'*-dimethyl-4,6-di-*O*-methyl-2-deoxystreptamine described in the preceding section was concentrated to dryness yielding 330 mg of a syrup. An aqueous solution of this syrup was passed through a column of Dowex 50W-X8 (H^+ , 50 ml) resin to give 210 mg of a colorless syrup. It was chromatographed on a column of silica gel (Wakogel C-200, 12 g) using a mixture of chloroform and acetone (5: 1 in volume). The effluent was cut into 5-ml fractions. Orcinol-positive fractions (Nos. 28~40) were concentrated to dryness yielding a colorless syrup (68 mg) of methyl 4,6-di-*O*-methyl-α-*D*-mannopyranoside, $[\alpha]_D^{27} + 79^\circ$ ($c=0.2$, H_2O), (lit.¹⁰) $[\alpha]_{5780}^{20} + 80.5^\circ$ ($c=1.2$, H_2O), $[\lambda]_{CuAm}^{20} + 1030^\circ$ at 436 nm.¹⁰ MS *m/e*: 191.0898 ($M^+ - OCH_3$), (calcd. for $C_8H_{16}O_6$, 191.0918). PMR $\delta_{Me_4Si}^{CDCl_3}$: 2.98 (2H, singlet, $-OH \times 2$), 3.36, 3.44, 3.53 (3H $\times$ 3, singlet, $O-CH_3$), 4.73 (1H, broad singlet, $J < 1$ Hz, $H-1$). TLC: chloroform-acetone (2: 1 in volume); *R*_f 0.23.

Determination of periodate oxidation

Periodate consumptions and formaldehyde formations of I, tri-*N*-acetyl-I, II, di-*N*-acetyl-II and

TABLE IV. PERIODATE CONSUMPTION AND FORMALDEHYDE FORMATION

Sample	Periodate consumption (moles)			Formaldehyde formation (moles)	
	Theoretical	Found		Theoretical	Found
		24 hr	48 hr		
Destomycin B (I)	5	5.0	5.7	1	1.01
Tri-N-acetate of I	2	1.4	2.1		
Basic glycoside (II)	4	3.6	4.2	0	0.21
Di-N-acetate of II	2	2.1	1.8		
Methyl 4,6-di-O-methyl- α -D-mannopyranoside	1	0.91	1.1		

methyl 4,6-di-O-methyl- α -D-mannopyranoside were quantitatively determined, and these results were shown in Table IV. Sample (ca. 0.02 mmole) was dissolved in 8 ml of water to which 2 ml of 0.1 M sodium periodate was added and the solution was kept in the dark at room temperature. Two-ml aliquots were taken to which was added immediately 0.5 ml of 8% solution of sodium hydrogen carbonate, 2 ml of 0.1M sodium arsenite solution, and 2 ml of 20% solution of potassium iodide and titrated with 0.1 N iodine solution according to the method described by Dyer.²¹⁾

Formaldehyde formed by periodate oxidation was determined by the method of Odea and Gibbons.²²⁾ To 1 ml of the sample solution (0.5 μ mole/ml) in water, 0.5 ml of 1 N NaHCO₃ and 5 ml of 0.015 N NaIO₄ in 0.045 N H₂SO₄ were added. The reaction mixture was stood at room temperature for 48 hr in the dark. One ml of the reaction mixture was taken and centrifuged after addition of 1 ml of 10% lead acetate aqueous solution. To 1 ml of the supernatant, 9 ml of chromotropic acid reagent consisting of 100 ml of 1% chromotropic acid aqueous solution and 400 ml of 66% sulfuric acid was added. After kept at room temperature for 30 min, the reaction mixture was recentrifuged. The supernatant was heated in a boiling water bath for 30 min and after cooling its absorbance at 570 nm was measured.

Acknowledgement. The authors wish to express their deep gratitude to Prof. H. Umezawa, Director of Institute of Microbial Chemistry for his guidance and encouragement.

REFERENCES

- 1) S. Kondo, M. Sezaki, M. Koike, M. Shimura, E. Akita, K. Satoh and T. Hara, *J. Antibiot.*, Ser. A, **18**, 38 (1965).
- 2) S. Kondo, K. Iinuma, H. Naganawa, M. Shimura and Y. Sekizawa, *J. Antibiot.*, **28**, 79 (1975).
- 3) S. Kondo, M. Sezaki, M. Koike and E. Akita, *J. Antibiot.*, Ser. A, **18**, 192 (1965).
- 4) S. Kondo, E. Akita and M. Sezaki, *ibid.*, **19**, 192 (1966).
- 5) S. Kondo, E. Akita and M. Koike, *ibid.*, **19**, 137 (1966).
- 6) M. Shimura, Y. Sekizawa, K. Iinuma, H. Naganawa and S. Kondo, *J. Antibiot.*, **28**, 83 (1975).
- 7) T. Yamakawa, N. Ueta and I. Ishizuka, *Japan. J. Exp. Med.*, **34**, 231 (1964).
- 8) C. S. Hudson, *J. Amer. Chem. Soc.*, **31**, 66 (1909).
- 9) J. Stanek, M. Cerny, J. Kocourek and J. Pacak, "The Monosaccharide," Academic Press, Inc., New York, 1963, pp. 53~54.
- 10) A. S. Perlin, B. Casu and J. Koch, *Can. J. Chem.*, **48**, 2596 (1970).
- 11) J. P. Greenstein and M. Winitz, "Chemistry of the amino acids," Vol. II, John Wiley & Sons, New York, 1961, p. 1564.
- 12) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
- 13) C. S. Hudson, *J. Amer. Chem. Soc.*, **32**, 338 (1910).
- 14) B. Witkop, *Experientia*, **12**, 372 (1956).
- 15) S. Umezawa, T. Tsuchiya and K. Tatsuta, *Bull. Chem. Soc. Japan.*, **39**, 1235 (1966).
- 16) R. G. Ault, W. N. Hawworth and E. L. Hirst, *J. Chem. Soc.*, **1935**, 1012 (1935).
- 17) N. K. Neuss, K. F. Koch, B. B. Molloy, W. Day, L. L. Huckstep, D. F. Dorman and J. D. Roberts, *Helv. Chim. Acta*, **53**, 2314 (1970).
- 18) J. Shoji and Y. Nakagawa, *J. Antibiot.*, **23**, 569 (1970).
- 19) W. T. Shier, S. Ogawa, M. Hichens and K. L. Rinehart, Jr., *ibid.*, **26**, 551 (1973).
- 20) Y. Nishimura, T. Tsuchiya and S. Umezawa, *Bull. Chem. Soc. Japan.*, **43**, 2960 (1970).
- 21) J. R. Dyer, "Methods of Biochemical Analysis," Vol. III, ed. D. Glick, Interscience Pub., 1954, p. 111.
- 22) J. F. Odea and R. A. Gibbons, *Biochem. J.*, **55**, 580 (1953).