Communications to the editor

STRUCTURAL STUDIES ON DESTOMYCINS A AND B

Sir:

Destomycins A(**I**) and B(**II**) having anthelmintic activity were discovered in a culture filtrate of *Streptomyces rimofaciens*.[1] As reported previously,[2~4] acid hydrolysis of **I** yielded 1-*N*-methyl-2-deoxystreptamine,* D-talose and a 2,3,4,5,7-pentahydroxy-6-aminoheptanoic acid (destomic acid, **III**), and the structure of **I** except the stereochemistry of the heptosamine moiety was determined. In this communication, we will report on the absolute configuration of the heptosamine moiety in destomycin A (**I**) and the structure of destomycin B (**II**).

The formula $C_{21}H_{39}N_3O_{13}$ was assigned to **II** by the elemental analysis,[1] the carbon-13 spectrum[8] and the mass spectrum of the tri-*N*-acetyl-mono-*N*-methyl-octa-*O*-methyl derivative. **II** and its tri-*N*-acetyl derivative (mp 235~246°C, dec.) consumed 5.7 and 2.1 moles of periodate for 48 hours, respectively. The presence of two *N*-methyl groups (δ 2.83 ppm) and two pyranoside moieties having *manno*- and *gluco*-configurations (Table 1) was shown by the pmr spectrum (Varian HA 100D) of **II**. Methyl mannopyranoside was obtained by methanolysis of **II** and identified by gas chromatography[9] of its trimethylsilylated derivative.

Table 1. Pmr chemical shifts and coupling constants for two pyranoside moieties in destomycins A and B

Proton	Destomycin A		Destomycin B	
	δ(ppm)[a]	J(Hz)	δ(ppm)[a]	J(Hz)
1′	5.57	$J_{1,2}=2.4$[b]	5.72	$J_{1,2}=1.8$
2′	5.05	$J_{2,3}=6.0$[b]	5.11	$J_{2,3}=6.0$
3′	5.13	$J_{3,4}=6.0$	4.92	$J_{3,4}=6.3$
4′	~4.4		4.31	$J_{4,5}=10.3$[b]
2″	4.55	$J_{2,3}=10.0$[b]	4.39	$J_{2,3}=10.3$
3″	4.33	$J_{3,4}=3.0$[b]	4.20	$J_{3,4}=10.0$[b]
4″	4.47	$J_{4,5}=<1$		

a. Chemical shifts were measured in D_2O using TMS as the external reference.
b. These coupling constants were determined by INDOR method.

Acid hydrolysis of **II** with 6 N hydrochloric acid for 20 hours under refluxing followed by column chromatography on Dowex 1×2(OH^-) resin gave an optically inactive compound as colorless plates, mp 163°C (dec.), Anal. calcd. for $C_8H_{18}N_2O_8$: C 50.50, H 9.53, N 14.72, O 25.23. Found: C 50.46, H 9.61, N 15.46, O 25.16. 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 **II** with 1 N hydrochloric acid in a boiling water bath for 10 minutes, followed by column chromatography on Dowex 1×2 (OH^-) resin, gave a basic glycoside from the effluent, and a polyhydroxy-amino acid from the eluate with 10% aqueous acetic acid. The basic glycoside was obtained as a colorless, crystalline powder, mp 80~120°C (dec.), $[\alpha]_D^{22}$-50° (*c* 0.5, water). Anal. calcd. for $C_{14}H_{28}N_2O_8 \cdot 1/2H_2O$: C 46.53, H 8.09, N 7.75, O 37.63. Found: C 46.39, H 8.41, N 7.13, O 37.50. It showed no reducing properties and was hydrolyzed with 6 N hydrochloric acid to *N*, *N*′-dimethyl-2-deoxystreptamine and mannose. The compound was shown to be 5-*O*-(β-D-mannopyranosyl)-1,3-di-(methylamino)-1,2,3-trideoxy-*myo*-inositol by the periodate consumption of the compound (4.2 moles without formation of formaldehyde) and its di-*N*-acetyl derivative (1.8 moles), and by the application of HUDSON's rule. In the carbon-13 spectrum, the chemical shifts of the mannose moiety were in good agreement with those of methyl β-D-mannopyranoside.[10]

The polyhydroxy-amino acid was obtained as colorless prisms, mp 216°C (dec.), $[\alpha]_D^{23}$

* The 1-*N*-methyl-2-deoxystreptamine was synthesized from 3-*N*-ethoxycarbonyl-2-deoxystreptamine[5] by *N*-methylation with formaldehyde and sodium borohydride followed by hydrazinolysis, and confirmed to be identical with the natural one in all respects. Therefore, the stereochemistry[2] should be revised to 1D-3-amino-1-methylamino-1, 2, 3-trideoxy-*myo*-inositol having 1*R*-configuration. This configuration was also supported by the synthesis in different routes.[6,7]

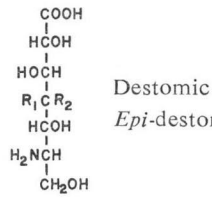
$+3.7°$ (*c* 2, water). Anal. calcd. for $C_7H_{15}NO_7$: C 37.33, H 6.71, N 6.22, O 49.74. Found: C 37.20, H 6.64, N 6.05, O 50.11. It was named *epi*-destomic acid (**IV**), because as later described the configuration at the C-4 position is different from that of **III** which was obtained from **I**.

Permethylation of tri-*N*-acetyldestomycin B by the method of HAKOMORI[11] followed by chloroform extraction afforded a colorless powder of tri-*N*-acetyl-mono-*N*-methyl-octa-*O*-methyldestomycin B (mp 122~125°C; *m/e* 793, $C_{36}H_{63}N_3O_{16}$). Mild hydrolysis of the permethylated product (750 mg) with 1 N hydrochloric acid for 10 minutes in a boiling water bath gave two compounds which were separated by treatment with Dowex 1×2 (OH^-) resin. One (the neutral compound, 380 mg) was obtained from the effluent, and its methanolysis with 3% hydrogen chloride in methanol at 90°C for 2 hours in a sealed tube, followed by column chromatography on silica gel (Wako gel C-200) eluted with a mixture of chloroform and acetone (5 : 1 in volume), gave a colorless syrup (68 mg), $[\alpha]_D^{27}$ $+79°$ (*c* 0.2, water), $\Delta[M]_{CuAm}+1030°$ at 436 nm,[12] *m/e* 191.0898 (M^+-OCH_3) (calcd. for $C_8H_{15}O_5$, 191.0918), consumption of periodate: 1.1 moles. From the spectral data and the rotatory value, it was identified as methyl 4, 6-di-*O*-methyl-α-D-mannopyranoside.[13] The other compound was eluted with 1 N hydrochloric acid from the resin and obtained as a crude powder (270 mg) by concentration of the eluate to dryness. Deacetylation of the crude powder in 2 N sodium hydroxide under reflux for 18 hours, followed by column chromatography on Dowex 50W×4 (H^+) resin, eluted with 1 N aqueous ammonia, gave the *N*-methyl-tetra-*O*-methyl derivative (128 mg) of **IV**. By reacetylation of the derivative with acetic anhydride in chloroform at room temperature for 15 hours, followed by column chromatography on silica gel eluted with a mixture of chloroform and acetone (10 : 1 in volume), a colorless syrup (35 mg) of *N*-acetyl-*N*-methyl-tetra-*O*-methyl-*epi*-destomic acid-1,5-lactone was obtained, $[\alpha]_D^{22}+55°$ (*c* 1, chloroform), *m/e* 319.1635 (calcd. for $C_{14}H_{25}NO_7$, 319.1629). By the application of HUDSON's lactone rule,[14] the D_G configuration could be assigned to the asymmetric carbon at the C-5 position of the lactone.

Periodate-permanganate oxidation[3] of the 2, 4-dinitrophenyl derivative (mp 177~179°C) of **IV**, followed by extraction with ethyl acetate at acid afforded the 2, 4-dinitrophenyl derivative of the oxidized product which was identical with authentic 2, 4-dinitrophenyl-L-serine.[15] Thus, the stereochemistry at the C-6 position of **IV** was confirmed to be the *S*-configuration and the structure of **IV** was determined to be 6-amino-6-deoxy-L-*glycero*-D-*gluco*-heptonic acid.

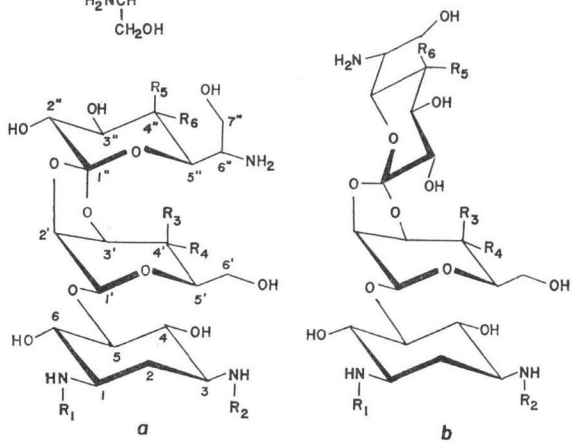
As described in a subsequent paper,[7] the carbon-13 spectrum of **I** or **II** showed the presence of an orthoester carbon at δ 121.2 or 121.7 ppm, respectively. Therefore, the absolute structure of destomycin B (**II**) except the configuration of the orthoester carbon of the heptosamine moiety, was confirmed 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 (**IIa** or **IIb**).

Coupling constants in the pmr spectrum of



Destomic acid: **III**; R_1=OH, R_2=H

Epi-destomic acid: **IV**; R_1=H, R_2=OH



a *b*

Destomycin A: **Ia** or **Ib**;
R_1=CH_3, R_3, R_5=OH, R_2, R_4, R_6=H

Destomycin B: **IIa** or **IIb**;
R_1, R_2=CH_3, R_4, R_6=OH, R_3, R_5=H

Hygromycin B: **Va** or **Vb**;
R_2=CH_3, R_3, R_5=OH, R_1, R_4, R_6=H

A-396-I: **VIa** or **VIb**;
R_3, R_5=OH, R_1, R_2, R_4, R_6=H

I (Table 1) indicated that the D-talose moiety was distorted from the *C*1 conformation[16] and the heptosamine moiety had a *galacto* configuration. The configurations at C-5 and C-6 positions of the heptosamine moiety in **I** were determined by the chemical methods described in the structural determination of **II**.

From the hydrolyzate of tri-*N*-acetyl-di-*N*-methyl-octa-*O*-methyldestomycin A (mp 121~123°C; *m/e* 793, $C_{36}H_{63}N_3O_{16}$), *N*-acetyl-*N*-methyl-tetra-*O*-methyl-destomic acid-1, 5-lactone was obtained as colorless syrup, $[\alpha]_D^{25}$ +69° (*c* 1, chloroform), *m/e* 319.1599 (calcd. for $C_{14}H_{25}NO_7$, 319.1628). The D_G configuration could also be assigned to the asymmetric carbon of the C-5 position of the lactone by the application of HUDSON's lactone rule. By periodate-permanganate oxidation of the 2, 4-dinitrophenyl derivative[3] of **III**, 2, 4-dinitrophenyl-L-serine was also isolated. From these results, the structure of **III** was determined to be 6-amino-6-deoxy-L-*glycero*-D-*galacto*-heptonic acid.

From the data described above, the absolute stereochemistry of destomycin A (**I**) except the configuration of the orthoester carbon was confirmed to be 5-*O*-[2, 3-*O*-(6-amino-6-deoxy-L-*glycero*-D-*galacto*-heptopyranosylidene)-β-D-talopyranosyl]-1D-3-amino-1-methylamino-1,2, 3-trideoxy-*myo*-inositol (**Ia** or **Ib**). Hygromycin B[17] and A-396-I[18] can be also presented by structure **Va** or **Vb** and **VIa** or **VIb**, respectively, from the published data of their structural studies.

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