STRUCTURE OF MINOSAMINOMYCIN

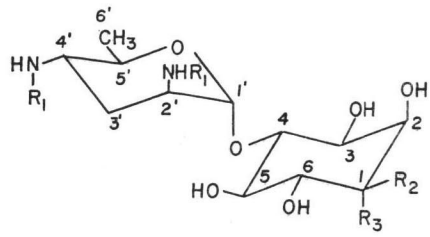
Sir:

Minosaminomycin is an antibiotic isolated from a culture filtrate of *Streptomyces* No. MA514-Al related to *Actinomyces aureomonopodiales*, and inhibits growth of mycobacteria.[1] We report on its structural elucidation and partial synthesis.*

Minosaminomycin[1] (**I**) has the formula $C_{25}H_{46}N_8O_{10}$ (derived from the elemental analysis, titration equivalent and carbon-13 spectrum**) and the following properties; mp 225~260°C (dec); $[\alpha]_D^{22}+30°$ (*c* 1.0, water); pKa′ 2.9, 6.2, 8.1 and >12; uv end absorption; ir (KBr) 3400, 2950, 1690, 1655, 1570, 1440, 1400, 1340, 1300, 1120, 1050, 1010, 970, 710 cm^{-1}; positive ninhydrin, RYDON-SMITH and pentacyanoaquoferriate; negative SAKAGUCHI, diacetyl and red tetrazolium. The pmr (D_2O, TMS as external reference) of **I** shows the presence of an isobutyl group (δ 1.38, 6H, dd and δ 1.9~2.2, 3H), a characteristic methyl group (δ 1.72, d), an anomeric proton (δ 5.45, d), two methylene protons (δ 2.35 and 2.55) and 14 other protons (δ 3.2~4.9).

A new aminocyclitol, 1D-1-amino-1-deoxy-*myo*-inositol[1] (**II**) was isolated in good yield by acid hydrolysis (6 N HCl, reflux for 5 hours) of **I**, together with a hydantoin derivative (**III**), a sugar derivative (**IV**) and a trace of leucine and a basic glycoside named minobiosamine (**V**). Alkaline hydrolysis of **I** with saturated aqueous $Ba(OH)_2$ (reflux for 4 hours) followed by column chromatography on Amberlite CG-50 (NH_4^+) resin afforded **V** in 32 % yield, mp 126~128°C (dec); $[\alpha]_D^{29}+81°$ (*c* 0.8, water); pKa′ 6.7, 7.9 and 9.0. The pmr of **V** was compared with that of kasuganobiosamine[2] (**VI**) obtained from kasugamycin and suggested the presence of the kasugamine moiety (1′-H, δ 5.38, d; 2′-H, δ 3.60; 3′-H_2, δ 2.26; 4′-H, δ 3.25; 5′-H, δ 4.45; 6′-H_3, δ 1.72, d). Acid hydrolysis (6 N HCl at 105°C for 20 hours in a sealed tube) of **V** gave **II** and **IV*****.

Benzyloxycarbonylation of **V** by the usual SCHOTTEN-BAUMANN procedure gave tri-N-benzyloxycarbonylminobiosamine (**VII**) in 85 % yield, mp 171~172°C, $[\alpha]_D^{24}+33°$ (*c* 1, dimethylformamide). Treatment of **VII** with NaH in dimethylformamide afforded a cyclic carbamate[3] of di-N-benzyloxycarbonylminobiosamine which was hydrolyzed with 5 % $Ba(OH)_2 \cdot 8H_2O$ solution in 50 % aqueous dioxane (80°C for 6 hours) to afford di-N-benzyloxycarbonylminobiosamine (**VIII**) in 49 % yield from **VII**, mp 123~126°C (dec); $[\alpha]_D^{24}+43°$ (*c* 1, dimethylformamide). Periodate oxidation of VIII in a mixture of 0.1 M sodium acetate buffer (pH 5.4) and ethanol (1:1 in volume) at room temperature for 97.5 hours gave crystalline di-N-benzyloxycarbonylkasugamine (44 % yield, mp 155~156°C $[\alpha]_D^{24}+36°$ in pyridine) which was treated with 1.3 % HCl in methanol at room temperature for 24 hours to afford an anomeric mixture of methyl di-N-benzyloxycarbonylkasugaminide. The anomeric mixture was separated into α- and β-anomers ($[\alpha]_D^{25}+21°$ and $[\alpha]_D^{25}-23°$ in chloroform, respectively) by silica gel chromatography developed with a mixture of benzene and acetone (20:1 in volume). They were identical with methyl di-N-benzyloxycarbonyl-α- and β-kasugaminide derived from di-N-benzyloxycarbonylkasuganobiosamine in all respects.



	R_1	R_2	R_3
V	H	NH_2	H
VI	H	H	OH
VII	$COOCH_2C_6H_5$	$NHCOOCH_2C_6H_5$	H
VIII	$COOCH_2C_6H_5$	NH_2	H
IX	$COCH_3$	$NHCOCH_3$	H

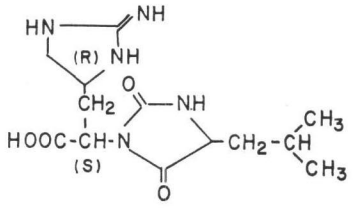
* Elemental analyses and spectroscopies gave satisfactory data on all compounds cited in the structural and synthetic studies.

** The carbon-13 FOURIER-transform nmr spectrum of **I** shows 25 carbon signals which will be described in detail elsewhere.

*** The **IV** is identical with a hydrolysis product (positive ninhydrin and red tetrazolium) of kasuganobiosamine on tlc and high-voltage paper electrophoresis.

Acetylation of V with acetic anhydride in methanol (room temperature for 19 hours) gave tri-N-acetylminobiosamine (**IX**) in 96 % yield, mp 273~274.5°C (dec); $[\alpha]_D^{26}$+49° (*c* 0.43, water), which was treated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in dimethylformamide (60°C for 2 hours) to afford tri-N-acetyl-di-O-isopropylideneminobiosamine (**X**) in 92 % yield, mp 162~166°C (dec), $[\alpha]_D^{24}$+51° (*c* 1, methanol). The pmr of **X** in deuteromethanol shows the presence of *trans*- and *cis*-O-isopropylidene groups[4] (δ 1.40, 6H in the former; δ 1.32, 3H and δ 1.50, 3H in the latter). Consequently, kasugamine must be glycosidically linked to the 4-OH or 6-OH of **II**. Mild hydrolysis of the *trans*-O-isopropylidene group in **X** with a mixture of 20 % aqueous acetic acid and methanol (2:5 in volume) at room temperature for 41 hours gave tri-N-acetyl-2,3-O-isopropylideneminobiosamine (**XI**), mp 174-176°C (dec), $[\alpha]_D^{24}$+63° (*c* 0.6, dimethylformamide). By application of the modified REEVES method (the CuAm method)[5], **IX** ($\Delta[M]_{436}$−2520°) and **XI** ($\Delta[M]_{436}$ −1290°) gave negative contribution, indicating that the structure of **V** is 1D-1-amino-1-deoxy-4-O-kasugaminyl-*myo*-inositol. Furthermore, the α-D-configuration of the glycosidic linkage of kasugamine* to **II** ($[\alpha]_D^{23}$−3.9° in water)[1] was shown by application of HUDSON's rule. Therefore, the absolute structure of minobiosamine (**V**) is 1D-1-amino-1-deoxy-4-O-(2,4-diamino-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranosyl)-*myo*-inositol.

The hydantoin derivative (**III**) was isolated from the acid hydrolysate of **I** by column chromatographies on Amberlite CG-50 (NH_4^+) resin eluted with water, and silicic acid developed with a mixture of butanol, ethanol and water (8:1:1 in volume), mp 185~190°C (dec); $[\alpha]_D^{23}$−28° (*c* 0.6, water); positive RYDON-SMITH, pentacyanoaquoferriate and red tetrazolium; negative ninhydrin. Complete acid hydrolysis (1 N HCl at 145°C for 72 hours in a sealed tube) of **III** gave two ninhydrin positive spots (leucine, Rf 0.59 and a basic amino acid (**XII**), Rf 0.09) on Silica gel G tlc (butanol-ethanol-chloroform-17 % ammonia, 4:5:2:5 in volume). Hydrazinolysis of **III** with anhydrous hydrazine at 105°C for 19.5 hours in a sealed tube did not give leucine. These results and a characteristic absorption at 1780 cm^{-1} in ir suggest that **III** has a hydantoin ring.** The basic amino acid (**XII**) was also isolated by acid hydrolysis (2 N HCl at 145°C for 72 hours) of **I** accompanied with **II**, **III** and partially racemic leucine (L-abundant mixture: $[\alpha]_D^{24}$+2.2° in acetic acid). By cellulose chromatography (microcrystalline cellulose, Avicel) developed with *n*-propanol-28% ammonia-water (7:1:2 in volume), **XII** was separated into diastereomers, **XIIa** and **XIIb** in about 3:1 of weight ratio, which were identical with enduracididine and alloenduracididine,*** respectively. All of these facts can be explained by the following structure for **III**.



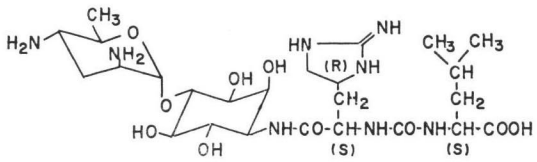
III

The chemical shifts of the 2′-H and the 4′-H in the pmr of **V** were unchanged with those of **I** (2′-H, δ 3.70; 4′-H, δ 3.35). On the other hand, the chemical shift of the 1-H on minobiosamine moiety in **I** could not be assigned, because of shifting by more than 0.6 ppm to a lower field than that in **V** (δ 3.20) and overlapping with other signals. It indicates that the 1-NH_2 of **V** is covered with a carboxyl group of the amino acid moiety in **I**. The C-terminal amino acid of **I** was determined to be leucine by hydrazinolysis. Therefore, the complete structure of minosaminomycin (**I**) is 1D-1-[{1(*S*)-carboxy-3-methylbutyl}carbamoyl-β-{(4*R*)-(2-iminoimidazolidinyl)-L-alanylamido}]-1-deoxy-4-O-(2,4-diamino-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranosyl)-*myo*-

* Methyl α-kasugaminide (methyl 2,4-diamino-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranoside) derived from kasugamycin shows $[\alpha]_D^{15}$+70° (*c* 1, water).

** Similar hydantoin derivatives have been isolated from acid hydrolysates of chymostatin[6] and elastatinal[7] having ureylene group.

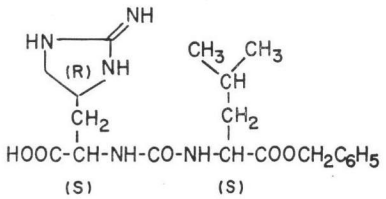
*** Authentic samples of enduracididine and alloenduracididine obtained from enduracidin[8] were kindly supplied by Dr. S. HORII of Takeda Chemical Industries, Ltd.



I

inositol.

Furthermore, the structure of **I** has been confirmed by a partial synthesis. L-Leucine benzyl ester hydrochloride was treated with trichloromethyl chloroformate in toluene (reflux for 4.5 hours) and then treated with **XIIa** in dimethylsulfoxide (room temperature for 17.5 hours), affording **XIII**. The **XIII** was coupled with **VIII** by active ester method using 1-hydroxybenzotriazole[9] and dicyclohexylcarbodiimide in dimethylformamide followed by catalytic hydrogenation with 5 % palladium-carbon in a mixture of methanol, acetic acid and water (3:1:1 in volume) to afford synthetic minosaminomycin in 7 % yield from L-leucine benzyl ester hydrochloride. The synthetic minosaminomycin was confirmed to be identical with the natural one in all respects including biological activity.



XIII

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(Received April 11, 1975)

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