STRUCTURE OF MINOSAMINOMYCIN

Sir:
Minosaminomycin is an antibiotic isolated from a culture filtrate of Streptomyces No. MA514-Al related to Actinomycetes aureomonomodales, and inhibits growth of mycobacteria.1) We report on its structural elucidation and partial synthesis.*

Minosaminomycin1) (I) has the formula C<sub>25</sub>H<sub>46</sub>N<sub>8</sub>O<sub>10</sub> (derived from the elemental analysis, titration equivalent and carbon-13 spectrum**) and the following properties; mp 225--260°C (dec); [α]<sub>22</sub>D<sup>+</sup>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<sup>-1</sup>; positive ninhydrin, RYDON-SMITH and pentacyanoaquoferriate; negative SAKAGUCHI, diacetyl and red tetrazolium. The pmr (D<sub>2</sub>O, 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-myoinositol 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)<sub>2</sub> (reflux for 4 hours) followed by column chromatography on Amberlite CG-50 (NH<sub>4</sub><sup>+ </sup>) resin afforded V in 32 % yield, mp 126--128°C (dec); [α]<sub>D</sub><sup>+</sup>81° (c 0.8, water); pKa' 6.7, 7.9 and 9.0. The pmr of V was compared with that of kasuganobiosamine2) (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<sub>2</sub>, δ 2.26; 4'-H, δ 3.25; 5'-H<sub>2</sub>, δ 4.45; 6'-H<sub>3</sub>, 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***.

Benzyloxy carbonylation of V by the usual SCHOTTEN-BAUMANN procedure gave tri-N-benzyloxy carbamylminobiosamine (VII) in 85 % yield, mp 171--172°C, [α]<sub>D</sub><sup>+</sup>+33° (c 1, dimethylformamide). Treatment of VII with NaH in dimethylformamide afforded a cyclic carbonate3) of di-N-benzyloxy carbamylminobiosamine which was hydrolyzed with 5 % Ba(OH)<sub>2</sub>·8H<sub>2</sub>O solution in 50 % aqueous dioxane (80°C for 6 hours) to afford di-N-benzyloxy carbamylminobiosamine (VIII) in 49 % yield from VII, mp 123--126°C (dec); [α]<sub>D</sub><sup>+</sup>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-benzyloxy carbamylkasugamine (44 % yield, mp 155--156°C [α]<sub>D</sub><sup>+</sup>+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-benzyloxy carbamylkasugaminide. The anomeric mixture was separated into α- and β-anomers ([α]<sub>D</sub>+21° and [α]<sub>D</sub>+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-benzyloxy carbonyl-α- and β-kasugaminide derived from di-N-benzyloxy carbamylkasugamidinose in all respects.

* 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); [a]_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), [a]_D^26 +51° (c 1, methanol). The pmr of X in deuteromethanol shows the presence of trans- and cis-O-isopropylidene groups (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-isopropylideminobiosamine (XI), mp 174-176°C (dec), [a]_D^24 +63° (c 0.6, dimethylformamide). By application of the modified Reeves method (the CuAm method), IX (δ[M]_436 -2520°) and XI (δ[M]_436 -1290°) gave negative contribution, indicating that the structure of V is 1β-1-amino-1-deoxy-4-O-kasugaminyl-myo-inositol. Furthermore, the αD-configuration of the glycosidic linkage of kasugamine* to II ([α]_D^15 +3.9° in water) was shown by application of Hudson’s rule. Therefore, the absolute structure of minobiosamine (V) is 1β-1-amino-1-deoxy-4-O-kasugaminyl-myo-inositol.

The hydantoin derivative (III) was isolated from the acid hydrolysate of I by column chromatographies on Amberlite CG-50 (NH₄⁺) 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); [α]_D^23 -28° (c 0.6, water); positive RYDON-SMITH, pentacyanoaquoferriate and red tetrazolium; negative ninhydrin. Complete acid hydrolysis (1N 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 (2N HCl at 145°C for 72 hours) of I accompanied with II, III and partially racemic leucine (L-abundant mixture: [α]_D^23 +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.

![Chemical Structure](image)

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-NH₂ 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 minosaminycin (I) is 1β-1-{[(S)-carboxy-3-methylbutyl]-carbamoyl-β-{[(4R)-(2-iminoimidazolidinyl)-L-alanylamido]}-1-deoxy-4-O-(2,4-diamino-2,3,4,6-tetrahydroxy-α-D-arabino-hexopyranosyl)-myo-inositol.

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* Methyl α-kasugaminide (methyl 2,4-diamino-2,3,4,6-tetrahydroxy-α-D-arabino-hexopyranoside) derived from kasugamycin shows [α]_D^23 +70° (c 1, water).
** Similar hydantoin derivatives have been isolated from acid hydrolysates of chymostatin and elastatinal having ureylene group.
*** Authentic samples of enduracididine and alloenduracididine obtained from enduracidin were kindly supplied by Dr. S. Hori of Takeda Chemical Industries, Ltd.
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 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.

References

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