## Communications to the editor

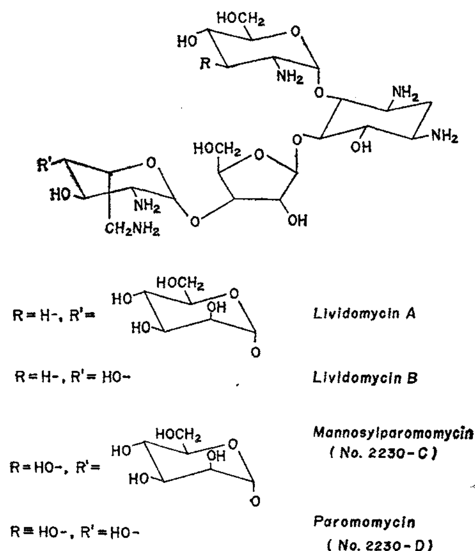
# THE STRUCTURE OF MANNOSYLPAROMOMYCIN (No. 2230-C)

Sir:

In our previous paper[1)], we reported that antibiotic No. 2230-C contains paromamine, D-ribose, neosamine B and D-mannose as shown by examination of the products of methanolysis or hydrolysis of the antibiotic, and we suggested No. 2230-C is a mannosylparomomycin. Mannosidostreptomycin[11)] and mannosidohydroxystreptomycin[12)] were already found in nature as the aminoglycosidic antibiotics contained mannose moiety. This antibiotic No. 2230-C had been isolated from the culture broth of *Streptomyces lividus* nov. sp. together with lividomycins A and B and paromomycin[1,2)], and the structures of lividomycins were reported as shown in Fig 1.[3~5)] In this comunication, we wish to summarize our structural studies on No. 2230-C.

We started with the hypothesis that when penta-N-acetyl No. 2230-C is oxidized with lead tetraacetate and followed by treatment

Fig. 1. Structures of lividomycins A and B, mannosylparomomycin (No. 2230-C) and paromomycin (No. 2230-D) isolated from culture broth of *Streptomyces lividus*.




with phenylhydrazine and acetic acid, it will be converted to paromomycin by the elimination of mannose moiety. Since *cis*-glycols were found to be more easily attacked than *trans*-glycols by lead tetraacetate in cold acetic acid[6)], the vicinal hydroxyl group of D-mannose moiety should be faster oxidized than that of D-glucosamine moiety.

Penta-N-acetyl No. 2230-C was prepared from No. 2230-C with acetic anhydride in methanol, m.p. 221~223°C (decomp.), $[\alpha]_D^{24}$ +59.4° (*c* 1.9, $H_2O$). Anal. calcd. for $C_{39}H_{65}N_5O_{24}\cdot 2H_2O$: C 45.75, H 6.79, N 6.84. Found: 45.92, H 6.51, N 6.77.

The acetate (1 g, 1.013 mmole) in 98 % aqueous acetic acid (200 ml) was oxidized with lead tetraacetate (996 mg, 2.2 mmoles) with stirring in the dark at room temperature for 17 hours. The oxidation mixture was treated with 10 % oxalic acid (6 ml), the precipitate was filtered, and filtrate was concentrated to dryness. The residue was dissolved in water and phenylhydrazine (4 ml) and acetic acid (4 ml) was added. After heating on a boiling water bath for 0.5 hour, the reaction mixture was concentrated to dryness. The residue was dissolved in 60 ml of 2 N ammonia solution, and washed with chloroform. The aqueous layer was deionized with Amberlite IR-120($H^+$) and Amberlite IRA-410($OH^-$) and lyophilized to yield 620 mg of greenish powder. The powder in 4 N sodium hydroxide (18 ml) was heated on boiling water bath for 5 hours. The reaction mixture was neutralized with 3 N sulfuric acid and adsorbed on a column of Amberlite IRC-50($NH_4^+$) (1.5×13 cm). After washing with water, the column was eluted with 0.35 N ammonia solution. The ninhydrin-positive eluate was concentrated to dryness and rechromatographed on a column of CM-Sephadex C-25($NH_4^+$) (1×76 cm) eluting with water (1,000 ml) to 0.12 N ammonia solution (1,000 ml), and 0.12 N (1,000 ml) to 0.24 N ammonia solution (1,000 ml) by linear gradient elution techniques. The unreacted No. 2230-C was eluted from the former and paromomycin from the latter. The ninhydrin-positive and biologi-

cal active eluate of the latter was lyophilized to yield 194 mg of paromomycin, m.p. 186~190°C(decomp.), $[\alpha]_D^{24}+65°$ ($c$ 1.49, $H_2O$). Anal. calcd. for $C_{23}H_{45}N_5O_{14}\cdot 3H_2O$ : C 41.25, H 7.68, N 10.46. Found: C 41.02, H 7.47, N 10.00.

This compound was identical with an authentic sample of paromomycin in all respects including biological activity.

To a solution of penta-N-acetyl No. 2230-C (3 g) in dimethylformamide (100 ml), methyl iodide (20 ml) and silver oxide (25 g) was added. After overnight stirring at room temperature in dark, the precipitate was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in 3 N hydrochloric acid (100 ml) and heated on a boiling water bath for 3 hours. The reaction mixture was neutralized with 2 N sodium hydroxide and passed through a column of CM-Sephadex C-25 ($NH_4^+$) (2×33 cm). The effluent was extracted with chloroform and extract was evaporated to dryness. The residue was acetylated with acetic anhydride (20 ml) in pyridine (10 ml), and after evaporation of the reaction mixture, the residue was extracted with chloroform and the extract was washed with water. After removal of solvent, the residue was distilled at b.p. 105~106°C/0.04 mmHg to obtain 320 mg of 1-O-acetyl-2,3,4,6-tetra-O-methyl-$\alpha$-D-mannose, $[\alpha]_D^{24}+54.8°$ ($c$ 1, pyridine). Anal. calcd. for $C_{12}H_{22}O_7$ : C 51.79, H 7.97. Found: C 51.68, H 7.62.

This compound was identical with an authentic sample synthetized from D-mannose or obtained from lividomycin A by the similar treatments[4] in optical rotation, IR and NMR.

Methyl N,N′-diacetylglycoside (2 g), which was prepared with acetic anhydride in methanol from methyl glycoside isolated from methanolysis products of No. 2230-C[1], was hydrolyzed with 1 N sulfuric acid (46 ml) on a boiling water bath for an hour. The reaction mixture was neutralized with Dowex 3($OH^-$) and evaporated to a brown syrup. The syrup was acetylated with acetic anhydride (10 ml) and Dowex 1×2 (carbonate form, 20 g) in methanol (100 ml) according to the method of ROSEMAN and LUDOWIG[7]. The reaction mixture was purified with a column of Dowex 1×2 (borate form) (1.4×68 cm) and yielded 90 mg of white power, m.p. 169~172°C(decomp.), $[\alpha]_D^{24}+45.6°$ ($c$ 1.26, $H_2O$, final). Anal. calcd. for $C_{16}H_{18}N_2O_{11}\cdot H_2O$ : C 43.44, H 6.83, N 6.33. Found: C 43.91, H 6.59, N 6.33.

When the compound was hydrolyzed with 3 N sulfuric acid, D-mannose and neosamine B were obtained by the detection of anisaldehyde- or ninhydrin-reagent using thin-layer chromatography on Kieselguhr G (Merck) respectively[1], but D-ribose was absent. And then, the compound was a reducing sugar and was identical with mannosyl N,N'-diacetylneosamine B obtained from lividomycin A by the similar treatments[4] in IR, optical rotation and melting point.

The compound consumed 1.9 mole of periodate by the usual method[8]. Therefore, it was clear that D-mannose was glycosidically linked to paromomycin at C-4 position of neosamine B moiety.

The stereochemistry of glycosidic linkage of D-mannose is determined by the application of the modified HUDSON's rule[9] as follows:

$\alpha$-Methyl mannoside $[\alpha]_D+79.3°$ M.W.194
$\beta$-Methyl mannoside $[\alpha]_D-69°$
N,N'-Diacetyl neosamine B $[\alpha]_D+6°$[10] M.W. 262
Mannosyl N,N′-diacetyl neosamine B $[\alpha]_D+45.6°$ M.W.424

$$+79.3\times194=+154\times10^2\ (+A+B)$$
$$-69\ \times194=-134\times10^2\ (-A+B)$$
$$A=+144\times10^2,\ B=+10\times10^2$$
$$(+45.6\times424)-(+6\times262)-(+10\times10^2)=+168\times10^2$$

This result indicates an $\alpha$-linkage for D-mannose moiety in No. 2230-C. Therefore, the structure of No. 2230-C was concluded to be 4-O-(2-amino-2-deoxy-$\alpha$-D-glucopyranosyl)-5-O-[3-O-{4-O-($\alpha$-D-mannopyranosyl)-2, 6-diamino-2, 6-dideoxy-$\alpha$-L-idopyranosyl}-$\beta$-D-ribofuranosyl]-1,3-diamino-1, 2, 3-trideoxy-*myo*-inositol or mannosylparomomycin as shown in Fig. 1.

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TOSHITO MORI
YOSHINORI KYŌTANI
ISAMU WATANABE
TAKESHI ODA

Kowa Co., Ltd., Tokyo Research Laboratories
Noguchi-cho, Higashimurayama, Tokyo, Japan

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