# INOSAMYCIN†, A COMPLEX OF NEW AMINOGLYCOSIDE ANTIBIOTICS

## I. PRODUCTION, ISOLATION AND PROPERTIES

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A strain of *Streptomyces hygroscopicus* No. J296-21 (ATCC 39150) was found to produce a complex of new antibiotics, called inosamycins, which consisted of components A, B, C, D and E. They are novel aminocyclitol antibiotics structurally related to neomycin, paromomycin and ribostamycin. The antibiotic complex and each component of inosamycin exhibit a broad antibacterial spectrum but they are inactive against most of the aminoglycoside-resistant organisms. The antibacterial activity of inosamycin A, the major component of the complex, is comparable to that of neomycin but its acute toxicity is significantly lower (*ca.* 1/3) than that of neomycin.

In our continuing search for new antibiotics, an actinomycete strain isolated from a soil sample collected in the Philippines was shown to produce a complex of new aminocyclitol antibiotics named inosamycin[1]. The antibiotic was extracted from the broth filtrate and separated into its components by use of weakly cation exchange chromatography. Inosamycin showed inhibitory activity against Gram-positive, Gram-negative and acid-fast bacteria. This paper reports the taxonomy of the producing organism and the fermentation, isolation, physico-chemical properties and biological activity of inosamycin. The structures of the inosamycin components are reported in the following paper[2].

### Producing Organism

The inosamycin antibiotic complex was produced by an actinomycete strain No. J296-21, which was isolated from a soil sample collected in the Philippines. This organism forms aerial and substrate mycelia; the color of the aerial mycelium is white, later turning to gray. Strain J296-21 forms coiled spore-chains on monopodially branched aerial sporophores, which contain 10 to 50 arthrospores in a chain. Tightly coiled spore-chains are often formed. The spores are oval in shape, $0.6\sim0.8\times0.9\sim1.2$ μm in size, and have rugose or smooth surface. The spirals of spore-chains often coalesce as dark, moist masses.

Strain J296-21 grows well and forms aerial mycelium in both nutritionally rich organic media and chemically defined agar media except for ISP No. 6 medium. Strain J296-21 does not produce melanoid pigment in Tryptone - yeast extract broth (ISP No. 1), peptone - yeast extract - iron agar (ISP No. 6) and tyrosine agar (ISP No. 7). It grows on an agar medium containing NaCl at 6% but not at 8%. Whorl sporophore, motile spore and sporangium were not observed in any of the media

† Previously called Bu-2659 or BMY-28162[1] (inosamycin A), BMY-28163 (inosamycin B), BMY-28164 (inosamycin C) and BMY-28165 (inosamycin D).

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Table 1. Cultural characteristics of strain J296-21.

| Medium | Characteristics |
|---|---|
| CZAPEK's sucrose - nitrate agar | G: Abundant<br>R: White (263) to light gray (264)<br>A: Abundant, light gray (264) to medium gray (265)<br>D: None |
| Tryptone - yeast extract agar (ISP No. 1) | Moderately floccose, sedimented, not pigmented. |
| Yeast extract - malt extract agar (ISP No. 2) | G: Abundant<br>R: Moderate orange yellow (71) to deep yellowish brown (75)<br>A: Moderate, white (263)<br>D: None |
| Oat meal agar (ISP No. 3) | G: Abundant<br>R: White (263) to light gray (264)<br>A: Abundant, white (263) to medium gray (265), hygroscopic<br>D: None |
| Inorganic salts - starch agar (ISP No. 4) | G: Abundant<br>R: White (263) to light gray (264)<br>A: Abundant, light gray (264) to dark gray (266), hygroscopic<br>D: None |
| Glycerol - asparagine agar (ISP No. 5) | G: Abundant<br>R: Light yellow (86) to strong yellow (84)<br>A: Abundant, white (263) to light brownish gray (63), hygroscopic<br>D: Light greenish yellow (101) |
| Peptone - yeast extract - iron agar (ISP No. 6) | G: Moderate<br>R: Pale yellow (89)<br>A: None<br>D: None |
| Tyrosine agar (ISP No. 7) | G: Abundant<br>R: Strong reddish brown (40)<br>A: Abundant, white (263) to yellowish white (92)<br>D: Dark orange yellow (72) |
| BENNETT's agar | G: Abundant<br>R: Pale yellow (89) to dark yellow (88)<br>A: Abundant, white (263) to medium gray (265), hygroscopic<br>D: None |

G, Growth; R, reverse color; A, formation of aerial mycelium and aerial mass color; D, diffusible pigment.

Color and number in parenthesis follow the color standard described by K. L. KELLY and D. B. JUDD: ISCC-NBS color-name charts illustrated with centroid colors. U.S. Dept. of Comm. Circ. 553, Washington, D.C., Nov., 1975.

examined. The cultural and physiological characteristics of strain J296-21 are shown in Tables 1 and 2, respectively. The pattern of carbohydrate utilization is shown in Table 3.

The above-mentioned characteristics of strain J296-21 indicate that it belongs to the genus *Streptomyces*. According to the descriptions in BERGEY's Manual[3], strain J296-21 resembles the species group, *spirales*, gray series, non-chromogenic, and smooth spore surface, which includes 65 species and 7 subspecies. Hygroscopic change of the aerial mycelium (blackening and moistening) is an additional important property of strain J296-21. DIETZ[4] classified hygroscopic *Streptomyces* strain into two species, *Streptomyces hygroscopicus* and *S. neohygroscopicus*. Based on the descriptions in the BERGEY's Manual and the studies of DIETZ, strain J296-21 was concluded to belong to the species,

Table 2. Physiological reactions.

| Test | Strain No. J296-21 | *Streptomyces hygroscopicus* NRRLB-1340 | Methods and Materials |
|---|---|---|---|
| Nitrite from nitrate | Positive | Negative | Inorganic medium: CZAPEK's glucose nitrate broth. |
| | Positive | Negative | Organic medium: Yeast extract 0.5%, glucose 1%, $KNO_3$ 0.5%, $CaCO_3$ 0.1%. |
| Sodium chloride tolerance | Moderate growth at 0.5% NaCl. Restricted growth at 1.0~6.0% NaCl. No growth at 8% NaCl. | Moderate growth at 1.5% NaCl. Restricted growth at 8.0% NaCl. | Basal medium: Yeast extract 1%, soluble starch 2%, agar 1.5%. |
| Casein hydrolysis in agar medium | Weakly positive (1~2 mm hydrolyzed band after 7 days). | Positive (3~5 mm hydrolyzed band after 7 days). | LUEDEMANN's agar medium[a] |
| Reactions in skimmed milk solution | Not coagulated and completely peptonized. | Not coagulated and completely peptonized. | |
| Gelatin stab | Liquefied | Liquefied | |
| Formation of melanoid | Negative | Negative | Tyrosine agar and peptone - yeast - iron agar and tryptone - yeast extract broth. |
| Effect of temperature | Maximal growth at 28~37°C. Moderate growth at 20°C and 43°C. No growth at 5°C and 45°C. | Maximal growth at 28~37°C. Moderate growth at 20°C. No growth at 5°C and 43°C. | Yeast extract - malt extract agar. |

[a] LUEDEMANN, G. M.: Int. J. Syst. Bacteriol. 21: 240~247, 1971

*Streptomyces hygroscopicus*.

## Antibiotic Production

A loopful of strain No. J296-21 on an agar slant was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium composed of malt extract 2%, fish meal 2%, peptone 0.2%, NaCl 0.5%, $CaCO_3$ 0.4% and $MgSO_4 \cdot 7H_2O$ 0.05%. The pH was adjusted to 7.2 before sterilization. The seed culture was incubated at 28°C for 96 hours on a rotary shaker (250 rpm), and 3 ml of the growth was transferred to 500-ml Erlenmeyer flasks containing 100 ml of production medium having the same composition as the seed medium. Upon shaking fermentation at 28°C, the broth pH gradually rose to 8.0~8.4 after 4~7 days and the antibiotic production reached a maximum of 300~350 μg/ml. The antibiotic activity in the fermentation broth was determined by the paper disc - agar diffusion method using *Bacillus subtilis* PCI 219 as the test organism.

## Isolation and Purification

The fermentation broth (32 liters, 300 μg/ml) was filtered with filter aid and the filtrate was adjusted to pH 7.0 with 6 N HCl. The antibacterial activity in the filtrate was adsorbed on a column of Amberlite IRC-50 ($NH_4^+$, 1.6 liters). The column was washed with water (5 liters) and then developed with 0.5 N $NH_4OH$ solution (4 liters). Active eluates were combined and concentrated *in vacuo* to a small volume. The concentrate was chromatographed using a column of Amberlite CG-50 ($NH_4^+$,

Table 3. Carbohydrate utilization.

| | Strain J296-21 | *Streptomyces hygroscopicus* NRRLB-1340 | | Strain J296-21 | *Streptomyces hygroscopicus* NRRLB-1340 |
|---|---|---|---|---|---|
| Glycerol | + | + | Cellobiose | + | + |
| D(−)-Arabinose | + | − | Melibiose | + | − |
| L(+)-Arabinose | + | + | Trehalose | + | + |
| D-Xylose | + | + | Raffinose | + | − |
| D-Ribose | + | + | D(+)-Melezitose | − | − |
| L-Rhamnose | + | − | Soluble starch | + | + |
| D-Glucose | + | + | Cellulose | + | − |
| D-Galactose | + | + | Dulcitol | − | − |
| D-Fructose | + | + | Inositol | + | − |
| D-Mannose | + | + | D-Mannitol | + | + |
| L(−)-Sorbose | − | − | D-Sorbitol | + | − |
| Sucrose | − | − | Salicin | + | + |
| Lactose | + | − | | | |

Basal medium: PRIDHAM-GOTTLIEB's inorganic medium.

Table 4. TLC of inosamycins A, B, C, D and E.

| System[a] | Rf value (by ninhydrin reagent) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Inosamycin | | | | | Neo-mycin B | Paromo-mycin I | Ribosta-mycin |
| | A | B | C | D | E | | | |
| S-110 | 0.31 | 0.35 | 0.39 | 0.43 | 0.43 | 0.23 | 0.33 | 0.33 |
| S-115S | 0.47 | 0.54 | 0.54 | 0.63 | 0.60 | 0.40 | 0.51 | 0.52 |
| S-115A | — | — | — | 0.57 | 0.06 | — | — | 0.11 |

[a] S-110: $SiO_2$ plate, $CHCl_3$ - MeOH - conc $NH_4OH$ - $H_2O$ (1:4:2:1).
S-115S: $SiO_2$ plate, $CHCl_3$ - MeOH - 17% $NH_4OH$ (2:1:1, upper layer).
S-115A: $Al_2O_3$ plate, solvent system same as S-115S.

1.0 liter). The column was washed with water (2 liters) and then developed with increasing concentrations of aqueous ammonia. A mixture of components B, C, D and E was eluted first with 0.21 N $NH_4OH$ (3 liters) followed by component A which was eluted with 0.43 N $NH_4OH$ (4 liters). The mixture of components B, C, D and E was separated by chromatography on an Amberlite CG-50 ($NH_4^+$, 220 ml) column. A mixture of components D and E was eluted first with 0.1 N $NH_4OH$ (0.5 liter), then components C and B were eluted successively with 0.17 N (0.7 liter) and 0.21 N (1.0 liter) $NH_4OH$ solutions, respectively. The mixture of components D and E was further separated into each component by silica gel column chromatography (Wakogel C-200, 40 g) using a solvent system of MeOH - conc $NH_4OH$ - $H_2O$ (9:1:1). Yields for components A, B, C, D and E in the above experiment were 7,100 mg, 370 mg, 350 mg, 40 mg and 20 mg, respectively.

The free base of inosamycin A (1,150 mg) was dissolved in a small amount of water and the pH adjusted to 4.5 with diluted $H_2SO_4$. Addition of methanol to the solution yielded a white precipitate which was collected by filtration and dried *in vacuo* to give the sulfate of inosamycin A (1,280 mg). The sulfates of other components were prepared by the same manner.

## Physico-chemical Properties

The sulfates of inosamycin components are freely soluble in water, slightly soluble in methanol

Table 5. Physico-chemical properties of inosamycin components (sulfates).

| | Inosamycin A | | Inosamycin B | | Inosamycin C | | Inosamycin D | | Inosamycin E | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nature | White amorphous solid | | White amorphous solid | | White amorphous solid | | White amorphous solid | | White amorphous solid | |
| MP (°C, dec) | 210~215 | | 200~206 | | 195~201 | | 193~204 | | 195~218 | |
| $[\alpha]_D^{25}$ ($H_2O$) | +52° (*c* 0.6) | | +73° (*c* 0.6) | | +55° (*c* 0.6) | | +52.5° (*c* 0.6) | | +47° (*c* 0.37) | |
| Molecular formula | $C_{23}H_{45}N_5O_{14}$ | | $C_{23}H_{45}N_5O_{14}$ | | $C_{23}H_{44}N_4O_{15}$ | | $C_{23}H_{44}N_4O_{15}$ | | $C_{17}H_{33}N_3O_{11}$ | |
| *Anal* | $C_{23}H_{45}N_5O_{14}\cdot 2.5H_2SO_4\cdot 2H_2O$ | | $C_{23}H_{45}N_5O_{14}\cdot 2.5H_2SO_4\cdot 2.5H_2O$ | | $C_{23}H_{44}N_4O_{15}\cdot 2H_2SO_4\cdot 3H_2O$ | | $C_{23}H_{44}N_4O_{15}\cdot 2H_2SO_4\cdot 3H_2O$ | | $C_{17}H_{33}N_3O_{11}\cdot 1.5H_2SO_4\cdot 2.5H_2O$ | |
| | Calcd | Found | Calcd | Found | Calcd | Found | Calcd | Found | Calcd | Found |
| C | 30.81 | 30.94 | 30.50 | 30.57 | 31.87 | 31.89 | 31.87 | 31.50 | 31.53 | 31.24 |
| H | 6.07 | 6.62 | 6.12 | 6.35 | 6.28 | 6.29 | 6.28 | 6.15 | 6.38 | 6.34 |
| N | 7.81 | 8.01 | 7.73 | 7.52 | 6.46 | 6.27 | 6.46 | 6.83 | 6.49 | 6.60 |
| S | 8.92 | 9.23 | 8.83 | 8.68 | 7.40 | 7.46 | 7.40 | 7.79 | 7.43 | 7.65 |
| NMR spectrum (δ, ppm) | 2.18 (1H, q, *J*=11.6), 2.76 (1H, dt, *J*=11.6, 4.0), 3.5~5.5 (22H, m), 5.71 (1H, br s), 5.83 (1H, br s), 6.44 (1H, d, *J*=4.0) | | 2.18 (1H, q, *J*=11.6), 2.77 (1H, dt, *J*=11.6, 4.0), 3.5~5.5 (22H, m), 5.86 (2H, br s), 6.44 (1H, d, *J*=4.0) | | 2.10 (1H, q, *J*=11.6), 2.76 (1H, dt, *J*=11.6, 4.0), 3.7~5.5 (22H, m), 5.71 (1H, br s), 5.78 (1H, br s), 6.17 (1H, d, *J*=4.0) | | 2.18 (1H, q, *J*=11.6), 2.78 (1H, dt, *J*=11.6, 4.0), 3.5~5.5 (22H, m), 5.67 (1H, br s), 5.83 (1H, br s), 6.45 (1H, d, *J*=4.0) | | 2.16 (1H, q, *J*=11.6), 2.76 (1H, dt, *J*=11.6, 4.0), 3.5~4.7 (16H, m), 5.76 (1H, s), 6.44 (1H, d, *J*=4.0) | |

Fig. 1. IR spectrum of inosamycin A (KBr).

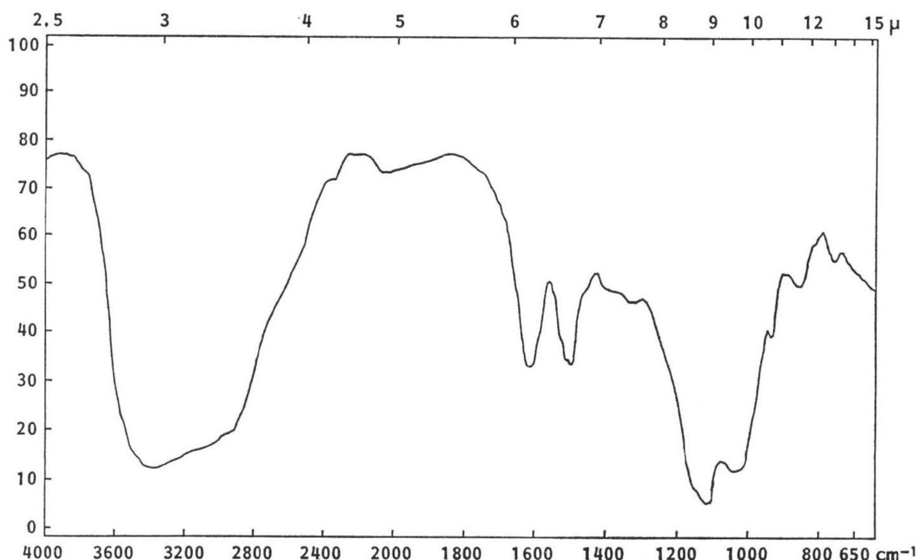
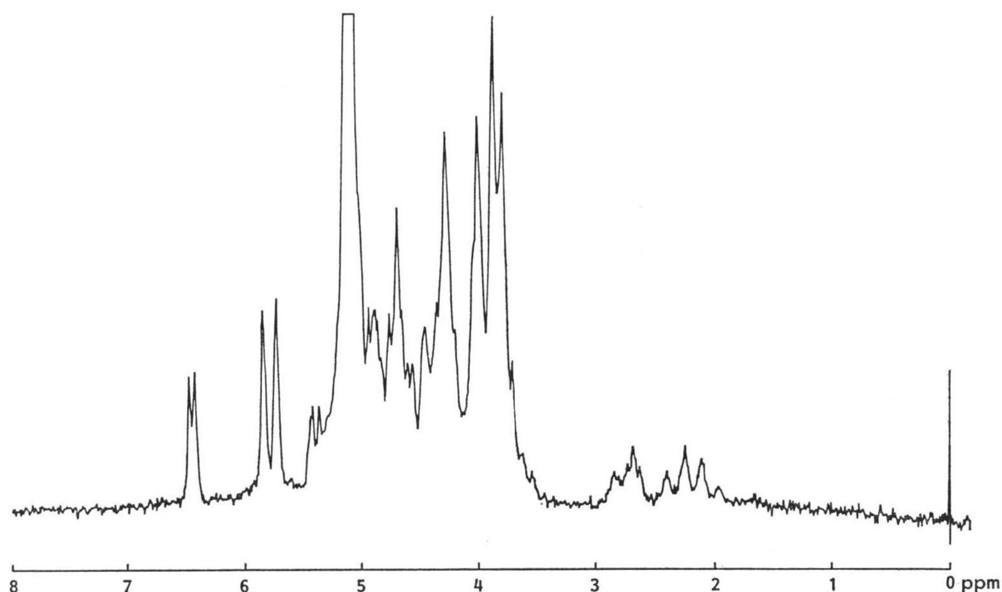


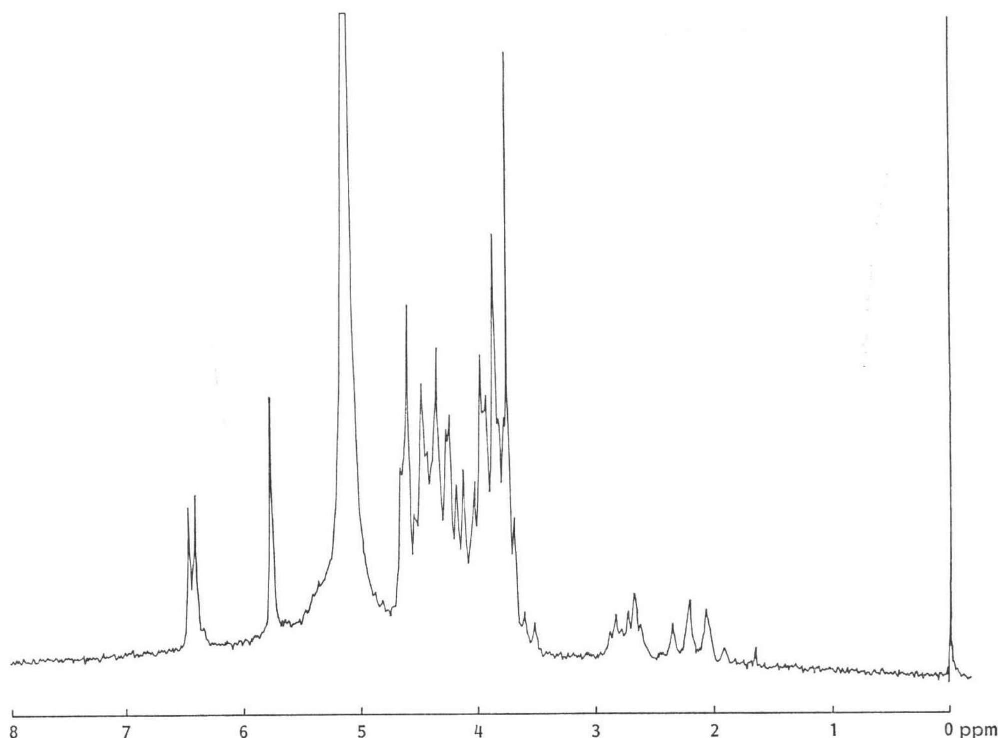

Fig. 2. $^1$H NMR spectrum of inosamycin A in $D_2O$ (80 MHz, pD=4.6).




and ethanol but practically insoluble in butanol, acetone and other organic solvents. They give positive reactions with ninhydrin and anthrone reagents, but are negative in Tollens, Fehling and Sakaguchi reactions. The thin-layer chromatogram (TLC) Rf values of inosamycin components are shown in Table 4 comparatively with those of neomycin, paromomycin and ribostamycin.

The physico-chemical properties of the sulfates of inosamycins A, B, C, D and E are summarized in Table 5. Inosamycin components exhibit only end absorption in the UV spectra. The IR spectrum of inosamycin A (Fig. 1) is very similar to that of components B, C, D and E, showing charac-

Fig. 3. $^1H$ NMR spectrum of inosamycin E in $D_2O$ (80 MHz, pD=4.6).




teristics of the spectra of aminoglycoside antibiotics such as neomycin, paromomycin and ribostamycin. In the NMR spectra of inosamycins A, B, C and D, the presence of three anomeric protons was indicated while two anomeric protons were observed in the spectrum of component E (Table 5 and Figs. 2 and 3).

## Biological Activity

### Antibacterial Spectrum

The minimum inhibitory concentrations (MIC) of inosamycins A, B, C, D and E were determined for a variety of Gram-positive and Gram-negative bacteria by the serial two-fold agar dilution method using the Steer's multi-inoculating apparatus. The inoculum was standardized as a $10^4$ dilution of an overnight culture of the test organisms in Heart Infusion Broth (Difco). Mueller-Hinton agar medium (Difco) was generally used for the MIC determination for most test organisms, GC medium (Eiken) for fastidious bacteria such as streptococci, *Neisseria* and *Haemophilus* species, and No. 1001 agar medium* for mycobacteria. Neomycins B and C and its commercial product**, paromomycin*** and ribostamycin were used as reference antibiotics.

The *in vitro* antibacterial spectra of inosamycin components are shown in Table 6. Inosamycin

* Glycerol 3%, sodium L-glutamate 0.3%, peptone 0.2%, $Na_2HPO_4 \cdot 12H_2O$ 0.31%, $KH_2PO_4$ 0.1%, ammonium citrate 0.005%, $MgSO_4 \cdot 7H_2O$ 0.001%, agar 1.5%.

** In the following description, "neomycin" means the commercial product containing neomycins B and C at an approximate ratio of 7: 3.

*** Commercial product containing 90% of paromomycin I and 10% of paromomycin II.

Table 6. Antibacterial activities of inosamycin components.

| Test organism | MIC (μg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Inosamycin | | | | | Neomycin | | | Paromomycin | Ribostamycin |
| | A | B | C | D | E | * | B | C | | |
| *Staphylococcus aureus* 209P | 0.8 | 3.1 | 3.1 | 6.3 | 25 | 0.4 | 0.4 | 0.8 | 0.8 | 1.6 |
| 〃 Smith | 1.6 | 3.1 | 6.3 | 25 | 50 | 0.8 | 0.4 | 1.6 | 1.6 | 3.1 |
| 〃 D136 | 0.8 | 6.3 | 6.3 | 25 | 50 | 0.8 | 0.4 | 1.6 | 0.8 | 3.1 |
| *Micrococcus luteus* PCI 1001 | 3.1 | >100 | >100 | 100 | >100 | 0.8 | 0.8 | 50 | 6.3 | 6.3 |
| *M. flavus* D12 | 6.3 | >100 | >100 | 100 | >100 | 3.1 | 1.6 | 100 | 12.5 | 12.5 |
| *Bacillus subtilis* PCI 219 | 0.1 | 0.4 | 0.8 | 1.6 | 3.1 | <0.05 | <0.05 | <0.05 | 0.2 | 0.4 |
| *Escherichia coli* NIHJ | 1.6 | 12.5 | 6.3 | 12.5 | 50 | 1.6 | 1.6 | 3.1 | 3.1 | 3.1 |
| 〃 Juhl | 3.1 | 50 | 25 | 50 | >100 | 3.1 | 3.1 | 12.5 | 12.5 | 6.3 |
| *Klebsiella pneumoniae* D-11 | 0.2 | 1.6 | 1.6 | 1.6 | 12.5 | 0.4 | 0.2 | 0.4 | 0.4 | 0.4 |
| *Proteus vulgaris* A9436 | 0.4 | 3.1 | 1.6 | 3.1 | 12.5 | 0.8 | 0.8 | 1.6 | 0.8 | 0.8 |
| *P. mirabilis* A9554 | 0.8 | 6.3 | 3.1 | 6.3 | 12.5 | 0.8 | 0.8 | 3.1 | 0.8 | 1.6 |
| 〃 A9906 | 0.8 | 6.3 | 3.1 | 6.3 | 25 | 0.8 | 0.8 | 1.6 | 0.8 | 1.6 |
| *P. morganii* A9553 | 1.6 | 12.5 | 6.3 | 12.5 | 25 | 3.1 | 3.1 | 3.1 | 1.6 | 1.6 |
| *P. rettgeri* A15167 | 0.8 | 3.1 | 3.1 | 3.1 | 12.5 | 1.6 | 1.6 | 1.6 | 1.6 | 0.8 |
| *Pseudomonas aeruginosa* D15 | 3.1 | >100 | 25 | >100 | >100 | 3.1 | 3.1 | >100 | 50 | >100 |
| 〃 A9930 | 1.6 | >100 | 12.5 | 50 | >100 | 3.1 | 3.1 | 50 | 12.5 | 100 |
| *Serratia marcescens* A20019 | 3.1 | 100 | 6.3 | 100 | >100 | 1.6 | 1.6 | 6.3 | 1.6 | 25 |

* Commercial product (neomycin B: C=*ca.* 7: 3).

Table 7. Antibacterial activities of inosamycin A.

| Test organisms | Media | MIC (μg/ml) | |
|---|---|---|---|
| | | Inosamycin A | Neomycin |
| *Streptococcus pyogenes* A20201 | GC | 25 | 12.5 |
| *S. pneumoniae* Type I | 〃 | 25 | 25 |
| *Neisseria gonorrhoeae* A20143 | 〃 | 50 | >400 |
| *N. meningitidis* A21496 | 〃 | 50 | 400 |
| *Haemophilus influenzae* A9729 | 〃 | 50 | 400 |
| *Mycobacterium smegmatis* 607 | No. 1001 | 0.4 | 0.4 |
| *M. phlei* | 〃 | 0.1 | 0.2 |
| *M. ranae* | 〃 | 0.4 | 0.4 |

Table 8. Activities of inosamycin A and reference antibiotics against aminoglycoside-resistant organisms.

| Test organism | Inactivating[b] enzyme | MIC[a] (μg/ml) | | | |
|---|---|---|---|---|---|
| | | Inosa-mycin A | Neomycin | Paromo-mycin | Ribosta-mycin |
| *Staphylococcus aureus* A20239 | APH(3′)-I, II | 12.5 | 25 | >100 | >100 |
| *Bacillus brevis* IFO 12334 | ANT(4′) | >100 | 25 | >100 | >100 |
| *Escherichia coli* ML 1630 | APH(3′)-I | >100 | >100 | >100 | >100 |
| 〃 A20107 | APH(3′)-II | 25 | 100 | >100 | >100 |
| 〃 JR66/W677 | APH(3′)-II<br>ANT(2″) | >100 | >100 | >100 | >100 |
| 〃 JR88 | AAC(3)-I | 1.6 | 1.6 | 1.6 | 1.6 |
| 〃 JR35/C600 | APH(3′)-I | 25 | >100 | >100 | >100 |
| 〃 A20732 | ANT(2″) | 0.8 | 0.8 | 1.6 | 1.6 |
| 〃 NR79/W677 | AAC(6′)-I | 50 | 50 | >100 | >100 |
| *Enterobacter cloacae* A20364 | APH(3′)-I | 50 | >100 | >100 | >100 |
| *Pseudomonas aeruginosa* A20601 | AAC(3)-I<br>APH(3′)-II | 6.3 | 12.5 | >100 | >100 |
| 〃 A20896 | AAC(3)-II | >100 | >100 | >100 | >100 |

[a] Mueller-Hinton agar (Difco).
[b] APH, Aminoglycoside phosphotransferase; ANT, aminoglycoside nucleotidyltransferase; AAC, aminoglycoside acetyltransferase.

A showed the highest activity among the five inosamycin components and its activity was almost comparable to that of neomycin and about 2 times more active than ribostamycin. Inosamycins B and C were approximately 1/4 as active as inosamycin A, while inosamycins D and E were still less active than component B.

The antibacterial activity of inosamycin A against strains of *Streptococcus*, *Neisseria*, *Haemophilus* and *Mycobacterium* is shown in Table 7. Inosamycin A and neomycin showed equivalent activity against species of *Streptococcus* and *Mycobacterium*, while inosamycin A was more active than neomycin against *Neisseria* and *Haemophilus* species.

A variety of aminoglycoside-resistant organisms which have been shown to produce aminoglycoside-modifying enzymes were examined for their susceptibility toward inosamycin A and reference antibiotics. The results are shown in Table 8. Inosamycin A was more active than neomycin, paromomycin and ribostamycin against several resistant strains which produce APH(3′) or AAC(3).

*In Vivo* Activity

The *in vivo* activity of inosamycin A was assessed against experimental systemic infections in

Table 9. *In vivo* activity of inosamycin A.

| Test organisms | $PD_{50}$ (mg/kg, im) | |
|---|---|---|
| | Inosamycin A | Neomycin |
| *Escherichia coli* Juhl | 3.1 | 3.1 |
| *Klebsiella pneumoniae* D-11 | 1.1 | 0.9 |
| *Proteus mirabilis* A9906 | 2.5 | 1.8 |
| *Staphylococcus aureus* Smith | 0.63 | 0.31 |
| *Streptococcus pyogenes* A20201 | 50 | 50 |

Table 10. Acute toxicities of inosamycin A.

| | $LD_{50}$ (mg/kg) | | |
|---|---|---|---|
| | iv | ip | sc |
| Inosamycin A | 110 | 460 | 720 |
| Neomycin | 34 | 180 | 280 |

mice. The pathogenic bacteria used in the *in vivo* tests were *Escherichia coli* Juhl, *Klebsiella pneumoniae* D-11, *Proteus mirabilis* A9906, *Staphylococcus aureus* Smith and *Streptococcus pyogenes* A20201. Mice were challenged intraperitoneally with approximately $100 \times LD_{50}$ of the pathogens in a 5% suspension of hog gastric mucin (American Laboratories, Omaha, Neb.). A single intramuscular treatment with the antibiotic was given immediately after the bacterial challenge. Group of 5 mice was used for each dosage level and the animals were observed for 4 days to determine the median protective dose ($PD_{50}$). Neomycin was tested comparatively as a reference compound. As shown in Table 9, inosamycin A afforded excellent protection in mice against the five experimental infections tested. Inosamycin A showed *in vivo* activity comparable to that of neomycin against infections with three Gram-negative bacteria and *S. pyogenes*, but was about one-half as active as neomycin against the *S. aureus* strain tested.

### Acute Toxicity

The acute toxicity of inosamycin A was determined in mice by intravenous, intraperitoneal and subcutaneous routes. Neomycin was tested comparatively. As shown in Table 10, inosamycin A was significantly less toxic than neomycin by any of the three routes of administration. The toxicity of inosamycin A was approximately 1/3 that of neomycin in terms of $LD_{50}$ values.

## Discussion

Inosamycin is a complex of new aminocyclitol antibiotics produced by a strain of *S. hygroscopicus*. Five components of the complex, inosamycins A, B, C, D and E are active against a variety of Gram-positive and Gram-negative bacteria including acid-fast bacteria *in vitro* and *in vivo*. The structure study reported in the accompanying paper[2] showed that inosamycin components are structurally related to neomycin, paromomycin and ribostamycin but differ in having 2-deoxy-*scyllo*-inosamine in place of the 2-deoxystreptamine group of the latter antibiotics. It is interesting to note that inosamycin A, 1-deamino-1-hydroxyneomycin B, has the same level of antibacterial activity as neomycin B, in spite of the fact that the 1-deamino-1-hydroxyl analogs of aminocyclitol antibiotics reported so far[5~8] are less active than the parent antibiotics containing 2-deoxystreptamine. Furthermore, inosamycin A is significantly less acutely toxic (*ca.* 1/3) than neomycin, which might present a clinical advantage of the antibiotic.

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