Communications to the Editors

THE TOTAL SYNTHESIS OF KANAMYCIN B

Sir :

Previous papers[1,2] have described the total syntheses of kanamycin A and C. We now wish to report the synthesis of kanamycin B[3], another one of kanamycin congeners, from neamine[4] and 3-amino-3-deoxy-D-glucose[5]. Since we have synthesized neamine[6] (I), the combined achievements constitute the total synthesis of kanamycin B.

Neamine was converted into tetra-N-carbobenzoxy neamine (II); m. p. 259°C (decomp.), $[\alpha]_D^{18}+44°$ (c 0.67, DMF). Treatment of II with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in N,N-dimethylformamide (DMF) at 110°C gave a mixture of monoisopropylidene derivatives (IIIa and IIIb) after purification by chromatography on silica gel. Benzylation of the mixture with benzyl bromide in the presence of barium oxide and barium hydroxide in DMF followed by chromatography on silica gel afforded two products: 3′,4′-di-O-benzyl derivative (IVa), m. p. 185°C, $[\alpha]_D^{18}+57°$ (c 0.67, DMF), and 5,6-di-O-benzyl derivative (IVb), m. p. 230~231°C, $[\alpha]_D^{18}+33°$ (c 0.67, DMF). Deacetonation of IVa by treatment with aqueous acetic acid gave 4-O-(3′, 4′-di-O-benzyl-2′, 6′-dicarbobenzoxyamino-2′,6′-dideoxy-α-D-glucopyranosyl)-2-deoxystreptamine (V); m. p. 221~222°C, $[\alpha]_D^{18}+58°$ (c 0.67, DMF).

Found : C 66.74, H 6.41, N 5.58

Calcd. for $C_{58}H_{62}N_4O_{14}$: C 67.04, H 6.01, N 5.39 %

The compound V was condensed with 3-acetamido-2, 4, 6-tri-O-benzyl-3-deoxy-α-D-glucopyranosyl chloride which was previously utilized for the total synthesis[1] of kanamycin C. To a mixture of V (800 mg), mercuric cyanide (314 mg) and Drierite (3.3 g) in a mixed solvent of benzene and dioxane was added the glycosyl chloride (680 mg, 1.7 equiv.), and the mixture was stirred at 100°C for about 20 hours under anhydrous condition to give the condensation product. The product was hydrogenated in a mixture of dioxane - water - conc. hydrochloric acid (20 : 4 : 1) over palladium black with occasional addition of water to remove the O-benzyl and N-carbobenzoxy groups, and then de-N-acetylated with hot aqueous barium hydroxide. The ninhydrin-positive product was N-dinitrophenylated with 2,4-dinitrofluorobenzene in aqueous ethanol in the presence of sodium bicarbonate and then O-acetylated with acetic anhydride and anhydrous sodium acetate. The resulting product, which showed about four spots of Rf values 0.40, 0.33, 0.13 and 0.08 on a thin layer chromatogram with a solvent system A

Chart 1.

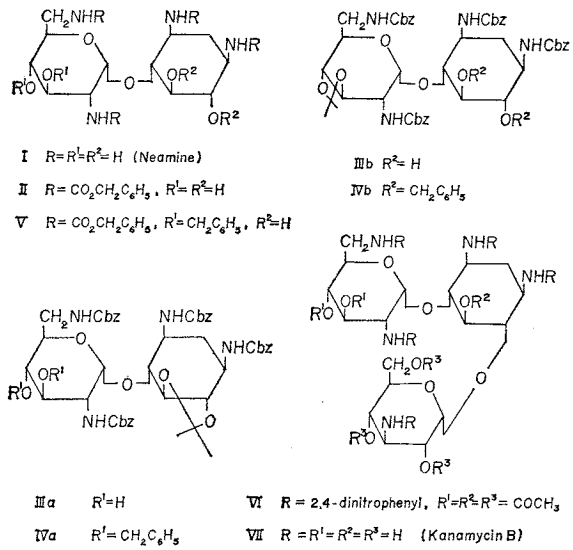

Table 1. Minimum inhibitory concentrations of synthetic and natural kanamycin B as determined by the dilution method in bouillon

Test organism	mcg/ml	
	Synthetic kanamycin B	Natural kanamycin B
Bacillus subtilis PCI 219	0.1	0.1
Staphylococcus aureus FDA 209P	0.1	0.2
Escherichia coli	7.8	7.8
Mycobacterium tuberculosis 607	0.5	1.0

(toluene – methyl ethyl ketone (MEK), 4 : 1), was chromatographed on a silica gel column with the solvent system A. The substance having an Rf value of 0.08 was isolated and recrystallized from toluene–MEK, affording yellow crystals of VI; yield 60 mg (5% over-yield from V), m. p. 212~213°C (decomp.), $[\alpha]_D^{18}+220°$ (c 0.3, acetone).

Found : C 46.16, H 4.23, N 12.96
Calcd. for $C_{60}H_{59}N_{15}O_{36}$: C 46.01, H 3.83, N 13.42 %

On the other hand, kanamycin B[8] was dinitrophenylated and acetylated to give hexa-O-acetyl-penta-N-(2, 4-dinitrophenyl) kanamycin B; m. p. 217~218° (decomp.), $[\alpha]_D^{18}+240°$ (c 0.4, acetone).

Found : C 46.31, H 4.21, N 13.38
Calcd. for $C_{60}H_{59}N_{15}O_{36}$: C 46.01, H 3.83, N 13.42 %

On thin-layer chromatography with the solvent system A, the synthetic product VI and the above-mentioned derivative of natural kanamycin B showed identical mobilities. Their infrared spectra were superimposable. The melting point of a mixture of the synthetic VI and the derivative of natural kanamycin B was 217~218°C (decomp.).

Ammonolysis of the synthetic VI and the above-mentioned derivative of kanamycin B with methanolic ammonia followed by hydrolysis with an excess of Dowex 1×2 (OH^-) resin gave free bases, respectively, each of which was purified by chromatography on a column of Dowex 1×2 (OH^-) resin using water and recrystallized from water-ethanol to give a free base. On descending paper chromatography by ninhydrin coloration using a solvent system of *n*-butanol – pyridine – water – acetic acid (6 : 4 : 3 : 1), the Rf values of the above-mentioned free bases agreed with that of natural kanamycin B. The antibiotic spectra and minimal inhibitory concentrations of the synthetic kanamycin B against test organisms were in agreement with those of the natural kanamycin B as shown in Table 1.

The details of the present work will be published in Bull. Chem. Soc. Japan.

Acknowledgement

We wish to express our thanks to Kawasaki Factory of Meiji Seika Co., Ltd. for providing us with kanamycin B. This research was supported in part by a grant from the Microbial Chemistry Foundation, to which the authors' thanks are due.

Sumio Umezawa
Shinkiti Koto
Kuniaki Tatsuta
Hirokuni Hineno
Yoshio Nishimura
Takayuki Tsumura

Department of Applied Chemistry,
Faculty of Engineering,
Keio University,
Koganei-shi, Tokyo

(Received April 25, 1968)

References

1) Umezawa, S.; S. Koto, K. Tatsuta & T. Tsumura : The total synthesis of kanamycin C. J. Antibiotics 21 : 162~163, 1968
2) Umezawa, S.; K. Tatsuta & S. Koto : The total synthesis of kanamycin A. J. Antibiotics 21 : 367~368, 1968
3) Schmitz, H.; O. B. Fardig, F. A. O'Herron, M. A. Rousche & I. R. Hooper : Kanamycin. III. Kanamycin B. J. Am. Chem. Soc. 80 : 2911~2912, 1958
Wakazawa, T.; Y. Sugano, M. Abe, S. Fukatsu & S. Kawaji : Studies on kanamycin B. I. Isolation of kanamycin B and its chemical and biological properties. J. Antibiotics, Ser. A 14 : 180~186, 1961.
Ito, T.; M. Nishio & H. Ogawa : The structure of kanamycin B. J. Antibiotics, Ser. A : 189~193, 1964
4) Peck, R.L.; C.E. Hoffhine, Jr., P.H. Gale & K. Folkers : Streptomyces antibiotics. XXV. Isolation of neomycin A. J. Am. Chem. Soc. 75 : 1018~1021, 1953
5) Cron, M. J.; D. L. Evans, F. M. Palermiti, D. F. Whitehead, I. R. Hooper, P. Chu & R. U. Lemieux : Kanamycin. V. The structure of kanosamine. J. Am. Chem. Soc. 80 : 4741~4742, 1958
Baer, H. H. : Synthetic kanosamine. J. Am. Chem. Soc. 83 : 1882~1885, 1961.
6) Tatsuta, K.; E. Kitazawa & S. Umezawa : Studies of antibiotics and related substances. XXXII. Synthesis of neamine and its analogue. Bull. Chem. Soc. Japan 40 : 2371~2375, 1967