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Studies of Antibiotics and Related Substances. XXXI. The Synthesis of Kanamycin-6′-phosphate*[1]

Sumio UMEZAWA, Kuniaki TATSUTA, Tsutomu TSUCHIYA and Eiichi YAMAMOTO

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

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The introduction of a phosphate group into the 3-amino-3-deoxy-D-glucose moiety of kanamycin, a useful antibiotic, has been described. Tetra-*N*-anisylidene kanamycin was treated with diphenyl phosphorochloridate. Hydrolysis of the product followed by hydrogenolysis afforded kanamycin-6′-phosphate in a good yield. An alternative phosphorylation of kanamycin *via* tetra-*N*-carbobenzoxykanamycin to hexa-*O*-acetyl-tetra-*N*-carbobenzoxy-6′-diphenylphosphorokanamycin was described, however, the removal of the all protecting groups except the phosphate group encountered a difficulty.

In the course of our investigation into the effects of structural changes in kanamycin on its antibacterial activity, deoxy and chlorodeoxy kanamycins were synthesized[1] and they were shown to have strong antibacterial activities essentially similar to that of kanamycin.

This paper describes the introduction of a phosphate group, that generally lowers the toxicity of drugs, into the kanamycin.

Kanamycin contains seven alcoholic hydroxyl groups, one of which is primary and the others are all secondary. The difference[2] in the reactivity of primary and secondary alcoholic group - the primary hydroxyl group can be more easily esterified than the secondary ones - was used for the phosphorylation of kanamycin. Preferential phosphorylation of the primary hydroxyl group of tetra-*N*-anisylidene kanamycin[3] with diphenyl phosphorochloridate, followed by removal of the anisylidene group by hydrolysis, gave kanamycin-6′-(diphenyl phosphate)*[2] (I) in a 56% yield.

Hydrogenolysis of the *O*-phenyl group of I with platinum oxide led to kanamycin-6′-phosphate (II) in a 97% yield. Structural evidence for II was confirmed by means of periodate oxidation. Four moles of periodate were consumed for one mole of II, indicating that the primary alcoholic group in the 3-amino-3-deoxy-D-glucose moiety of kanamycin is preferentially phosphorylated.

In a preliminary experiment, the authors attempted to synthesize II by the following reaction sequence: Tritylation of tetra-*N*-carbobenzoxykanamycin[1] (III) followed by acetylation gave hexa-*O*-acetyl-tetra-*N*-carbobenzoxy-6′-*O*-tritylkanamycin (IV), which was detritylated to hexa-*O*-acetyl-tetra-*N*-carbobenzoxykanamycin (V) and transformed to hexa-*O*-acetyl-tetra-*N*-carbobenzoxy-6′-diphenylphosphorokanamycin (VI) with diphenyl phosphorochloridate. However, the final step to remove the all protecting groups of VI except the phosphate group was unsuccessful.

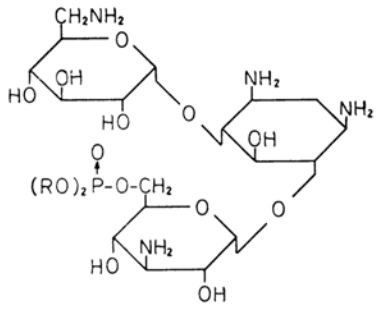
*1 Part XV of "Studies of Aminosugars," by S. Umezawa.

1) T. Tsuchiya and S. Umezawa, This Bulletin, **38**, 1181 (1965); *J. Antibiotics*, **A16**, 173 (1963).

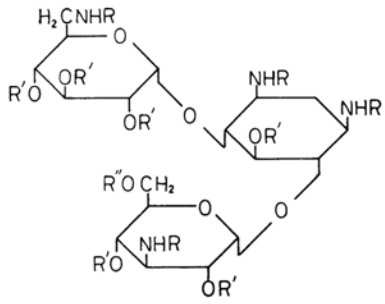
2) F. Maley and H. A. Lardy, *J. Am. Chem. Soc.*, **78**, 1393 (1956).

3) M. J. Cron, D. L. Johnson, F. M. Palermiti, Y. Perron, H. D. Taylor, D. F. Whitehead and I. R. Hooper, *J. Am. Chem. Soc.*, **80**, 752 (1958).

*2 We propose the enumeration of the system in the kanamycin structure as shown in the formulas in Chart 1; the 2-deoxystreptamine ring is first numbered according to K. L. Rinehart, Jr., M. Hichens, A. D. Argoudelis, W. S. Chilton, H. E. Carter, M. P. Georgiadis, C. P. Schaffner and R. T. Schillings, *J. Am. Chem. Soc.*, **84**, 3218 (1962), consequently followed by the 3-amino-3-deoxy-D-glucose ring and finally by 6-amino-6-deoxy-D-glucose ring.



I : R = C_6H_5
II : R = H



Kanamycin : R=R′=R″=H
III : R=$COOCH_2C_6H_5$, R′=R″=H
IV : R=$COOCH_2C_6H_5$, R′=$COCH_3$, R″=$C(C_6H_5)_3$
V : R=$COOCH_2C_6H_5$, R′=$COCH_3$, R″=H
VI : R=$COOCH_2C_6H_5$, R′=$COCH_3$, R″=$PO(OC_6H_5)_2$

Chart 1.

The antibacterial activity and toxicity of kanamycin-6′-phosphate will be described elsewhere.

Experimental

Paper Chromatography, Cellulose and Silica-gel Chromatography and Thin-layer Chromatography. Paper chromatography was conducted by the descending technique on Toyo Roshi filter paper No. 50, and the locations of the substances on the papergrams were detected by ninhydrin spray (0.25% in pyridine). Solvent system used: *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) (Solvent A). Cellulose column was prepared by packing tightly a dry cellulose powder (Toyo Roshi Co., for chromatography). Thin-layer chromatography was conducted by the use of silica-gel (Daiichi Pure Chemicals Co.) and the prepared plates were activated at 110°C followed by storage in a desiccator. The spray reagent used was concentrated sulfuric acid. Solvent system used: toluene-methyl ethyl ketone (9 : 4) (Solvent B), benzene-ethanol (9 : 1) (Solvent C) and benzene-chloroform-ethanol (12 : 3 : 1) (Solvent D). Silica-gel column chromatography was carried out by the use of silica-gel (Kanto Chemical Co.) and activated at 110°C before use.

Kanamycin-6′-(diphenyl phosphate) (I). To a cold (−15°C) solution of tetra-*N*-anisylidene kanamycin[3] (1.9 g, 2 mmol) in dry pyridine (40 m*l*) was added diphenyl phosphorochloridate[4] (0.8 g, 3 mmol), and the mixture was kept at the same temperature for 1 hr and then at room temperature for 3 days. The resulting solution was evaporated under reduced pressure to give a yellow residue, which was dissolved in methanol (40 m*l*) containing 4 N hydrochloric acid (1.5 m*l*). The solution was refluxed for 1 min, evaporated, and the residue was treated with ether. The ether-insoluble residue virtually showed a single spot (R_f 0.25) on paper chromatography with Solvent A. The residue was dissolved in a solvent mixture of *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) and chromatographed on a cellulose column (49×230 mm) with the same solvent. The eluates were tested by paper chromatography. After 230 m*l* of eluate, a ninhydrin-positive substance (R_f 0.25) emerged in the next 180 m*l* portion, which was evaporated under reduced pressure. The aqueous solution of the residue was further purified by placing on a column (7×90 mm) of Dowex 1X2 (Cl form) resin and by developing with water. The ninhydrin-positive fractions were collected, neutralized with hydrochloric acid to pH 2, and evaporated to give a residue, which was recrystallized from 95% ethanol affording light-yellowish, crystalline tetrahydrochloride of I; yield 960 mg (56%); mp 182—184°C (decomp.), $[\alpha]_D^{18}$ +72.8° (*c* 0.5, water); IR spectrum (KBr disk): ~3380 (νOH, NH), 2900 (νC–H), 1590 ($\delta_{as}NH_3^+$), 1490 ($\delta_sNH_3^+$), 1210 (νP=O), 1185 (νC–O–P), 1050—960 (νCO), 775 (phenyl) cm^{-1}.

Found: C, 41.66; H, 6.06; N, 6.39; P, 3.9; Cl, 16.98%. Calcd for $C_{30}H_{45}O_{14}N_4P{\cdot}4HCl$: C, 41.77; H, 5.73; N, 6.50; P, 3.6; Cl, 16.44%.

Kanamycin-6′-phosphate (II). The tetrahydrochloride (600 mg) of I dissolved in 50% aqueous ethanol (60 m*l*) was hydrogenated with platinum oxide (580 mg) under 3 atm of hydrogen-pressure at room temperature for 10 hr. After removal of the catalyst, the solution was evaporated and the aqueous solution of the residue was passed through a column (7×80 mm) of Dowex 1X2 (Cl form) resin. The ninhydrin-positive eluate was neutralized with hydrochloric acid to pH 2 and followed by evaporation to dryness. Recrystallization of the residue from aqueous methanol-acetone gave crystals of tetrahydrochloride of II; yield 480 mg (97%); mp 159—161°C (decomp.), $[\alpha]_D^{18}$ +81.2° (*c* 0.5, water); IR spectrum (KBr disk): ~3400 (νOH, NH), 2960 (νC–H), 1615 ($\delta_{as}NH_3^+$), 1500 ($\delta_sNH_3^+$), 1400, 1325, 1215 (νP=O), 1145—900 (νCO) cm^{-1}.

Found: C, 30.27; H, 6.08; N, 7.60; P, 4.2; Cl, 19.68%. Calcd for $C_{18}H_{37}O_{14}N_4P{\cdot}4HCl$: C, 30.44; H, 5.82; N, 7.89; P, 4.3; Cl, 19.97%.

On paper chromatography with Solvent A, the product II showed an R_f-value of 0.18 relative to the R_f-value of kanamycin taken as 1.0.

A small quantity of tetrahydrochloride of II was hydrolyzed in 3 N hydrochloric acid with heating in a boiling water bath for 2 hr, and the hydrolyzate was paper chromatographed with Solvent A. Detection by ninhydrin showed the spots corresponding to deoxystreptamine and 6-amino-6-deoxy-D-glucose, but not to 3-amino-3-deoxy-D-glucose.

4) A. B. Foster, W. G. Overend and M. Stacey, *J. Chem. Soc.*, **1951**, 980.

The Periodate Oxidation of II and Kanamycin. An accurately weighed sample (25.0 mg) of tetrahydrochloride of II or kanamycin tetrahydrochloride was dissolved in a mixture of 0.1 M sodium metaperiodate (3.00 m*l*) and 0.1 N sodium acetate buffer solution (pH 4.7, 10 m*l*), and the oxidation was carried out at 5°C in the dark. Iodometric titrations with sodium arsenite[5] showed that both II and kanamycin consumed about 4 mol of periodate per mole of substances in 24 hr (Table 1).

Hexa-*O*-acetyl-tetra-*N*-carbobenzoxy-6'-*O*-tritylkanamycin (IV). A solution of tetra-*N*-carbobenzoxykanamycin[1] (III) (3.05 g, 3.0 mmol) in dry pyridine (50 m*l*) was refluxed with triphenylmethyl chloride (2.07 g, 7.4 mmol) at 110°C for 4 hr. After cooling, acetic anhydride (30 m*l*) was added to the solution and the mixture was allowed to stand at room temperature for 2 days. The resulting brownish solution was poured into a large volume of ice and water. The precipitated mass was washed with water and treated with eight 50-m*l* portions of hot isopropyl ether to remove triphenylcarbinol. The insoluble residue, which showed a single spot (R_f 0.5) on thin-layer chromatography with Solvent B, was recrystallized from 80% ethanol; yield 3.69 g (81.8%); mp 130—133°C.

Table 1. Periodate oxidation of tetrahydrochlorides of II and kanamycin

Reaction time hr	Moles of periodate per mole of	
	II·4HCl	Kanamycin·4HCl
0.5	2.52	2.68
5.0	3.54	3.65
23.0	4.01	4.19
31.0	4.10	4.25
46.0	4.20	4.35

Found: C, 64.45; H, 5.80; N, 3.64%. Calcd for $C_{81}H_{86}O_{25}N_4$: C, 64.19; H, 5.72; N, 3.70%.

Hexa-*O*-acetyl-tetra-*N*-carbobenzoxykanamycin (V). To the compound IV (3.0 g, 2 mmol) dissolved in chloroform (30 m*l*) was added acetic acid (27.2 m*l*) and the solution was concentrated to a volume of about 24 m*l*. After cooling to 5°C, to the solution was added a cooled (5°C) mixture of acetyl bromide (0.163 m*l*), water (0.04 m*l*) and acetic acid (1.43 m*l*), whereupon trityl bromide immediately began to precipitate. The precipitate was removed and the solution was poured into a large volume of ice and water, and extracted with chloroform several times. Evaporation of the organic layer gave a solid, which was washed with two 120-m*l* portions of isopropyl ether to remove triphenylcarbinol. Recrystallization from acetone-petroleum ether (boiling point range, 30—60°C); yield 1.60 g (63.5%); mp 251—255°C (decomp.).

Found: C, 58.42; H, 5.52; N, 4.28%. Calcd for $C_{62}H_{72}O_{25}N_4$: C, 58.48; H, 5.70; N, 4.40%.

On thin-layer chromatography with Solvent C, the product showed a single spot having an R_f 0.45.

Hexa-*O*-acetyl-tetra-*N*-carbobenzoxy-6'-diphenylphosphorokanamycin (VI). To a cold (0°C) solution of V (500 mg, 0.393 mmol) in dry pyridine (11 m*l*) was added diphenyl phosphorochloridate (405 mg, 1.50 mmol), and the mixture was allowed to stand overnight at room temperature. The solution was poured into a large volume of ice and water. The resulting precipitate was collected and washed with water, yielding 600 mg of a crude product. On thin layer chromatography with Solvent D, the crude product showed three spots having R_f 0.16 (minor), 0.21 (minor) and 0.27 (main). The crude product was dissolved in a solvent mixture of benzene-chloroform-ethanol (12 : 3 : 1) and chromatographed on a silica-gel column (35×320 mm) with the same solvent. After about 150 m*l* of eluate, fractions (6 g each) were tested by thin-layer chromatography. The substance having R_f 0.27 was eluted in the fractions of tube Nos. 2—7. The fractions were combined and evaporated. Recrystallization of the residue from 70% ethanol; yield 315 mg (53.4%); mp 102—104°C (decomp.).

Found: C, 59.45; H, 5.38; N, 3.72; P, 1.9%. Calcd for $C_{74}H_{81}O_{28}N_4P$: C, 59.04; H, 5.42; N, 3.72; P, 2.0%.

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5) J. M. Bobbitt, "Advances in Carbohydrate Chemistry," Vol. 11, Academic Press, New York (1956), pp. 1—14.