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# Studies of Aminosugars. XXI. The Total Synthesis of Kanamycin C[1)]

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4-*O*-(3-*O*-Benzyl-2-carbobenzoxyamino-4,6-*O*-isopropylidene-2-deoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine has been prepared from paromamine and condensed with 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-*α*-D-glucopyranosyl chloride by a modified Koenigs-Knorr reaction. The identity of the synthetic *α*,*α*-diglycoside with a substance of the same structure derived from natural kanamycin C was shown. Removal of the masking groups gave the product identical with natural kanamycin C.

In recent years, certain strains of *Streptomyces* have been found to produce aminoglycosidic antibiotics which are entirely carbohydrate in nature, for example, streptomycins, neomycins, kanamycins and paromomycins. Interest in the chemistry of aminoglycosides has recently become widespread and has received added impetus as the recognition of the great importance of the aminoglycosidic antibiotics in medicines and biology has grown. However, none of these carbohydrate-antibiotics has never been synthesized. In this paper we describe the total synthesis of kanamycin C, a member of kanamycin congeners, syntheses of other members, kanamycin A and B, being described in the following papers.

In a previous paper[2)] we prepared the *β*,*β*-

1) Part XXXVI of "Studies on Antibiotics and Related Substances" by Sumio Umezawa. This paper was read before the 21th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1968. (See abstracts of papers of the Meeting, Vol. 3, p. 2212). A part of this work has been briefly communicated: S.Umezawa, S. Koto, K. Tatsuta and T. Tsumura *J. Antibiotics*, **21**, 162 (1968).

2) S. Koto, Y. Ito and S. Umezawa, This Bulletin, **38**, 1447 (1965).

diglycosides, which are kanamycin-analogues, from deoxystreptamine and 3-amino-3-deoxy-D-glucose or 6-amino-6-deoxy-D-glucose by way of the Koenigs-Knorr reaction and found that these compounds showed no antibiotic activity. These results underlined the fact that the approach to the synthesis of aminoglycosidic antibiotics found difficulty in the formation of *α*-glycosidic linkages. The preparation of *α*-glycopyranosides in high yields still remains the most important problem of carbohydrate chemistry, and in these circumstances we considered it advisable, as far as possible, to suitably mask 1-halogeno-sugars on one hand, and to partially mask aglycones on the other hand, considering the neighboring groups participation.

Kanamycin C was first discovered by Rothrock *et al.*,[3] and the structure (VIII) was elucidated by Murase.[4] Kanamycin C is composed of 3-amino-3-deoxy-D-glucose and paromamine[5] (I) which is a nonreducing fragment of paromomycin and is an *α*-glycoside of 2-amino-2-deoxy-D-glucose joined at C-1 to the C-4 of 2-deoxystreptamine. In this paper we described the synthesis of kanamycin C from paromamine. Since we[6] have previously synthesized paromamine, the combined achievements constitute the first synthesis of kanamycin C.

Paromamine was treated with carbobenzoxy chloride in the presence of sodium carbonate in aqueous acetone at about −10°C to give tri-*N*-carbobenzoxy-paromamine (II) in an 88% yield, which was acetonated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in *N*,*N*-dimethylformamide (DMF) at 110°C to give the diisopropylidene derivative (III), mp 243—245°C, $[\alpha]_D^{18}$ +71° (*c* 0.59, DMF), in a quantitative yield. According to the finding by Evans *et al.*[7] that the acetal exchange reaction of methyl *α*-D-glucopyranoside with 2,2-dimethoxypropane mainly gives methyl 4,6-*O*-isopropylidene-*α*-D-glucoside, the structure of III is considered to include an isopropylidene group attached to C-4 and C-6 in the glucosamine moiety.

Benzylation of III with benzyl bromide in the presence of barium oxide and barium hydroxide in DMF gave IV, mp 160—162°C, $[\alpha]_D^{18}$ +72° (*c* 0.60, DMF), in a 82% yield. Deacetonation of IV by treatment with 80% acetic acid gave quantitatively 4-*O*-(3-*O*-benzyl-2-carbobenzoxyamino-2-deoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (V), mp 270—271°C (decomp.), $[\alpha]_D^{18}$ +101° (*c* 0.52, DMF).

When V was caused to react with 2,2-dimethoxypropane at room temperature, partial acetonation of V was successful to give monoisopropylidene derivative (VI), mp 239—241°C, $[\alpha]_D^{18}$ +75° (*c* 0.63, DMF), in a 55% yield. It is worth mentioning that the hydroxyl groups of C-4 and C-6 in the glucosamine moiety were acetonated in preference to the hydroxyl groups in deoxystreptamine.

3-Acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-*α*-D-glucopyranosyl chloride[8] was condensed with VI in the presence of mercuric cyanide and Drierite in benzene-dioxane at 100°C. The product was treated with acetic acid to remove the isopropylidene group, hydrogenated over palladium black in a mixture of dioxane-water-concentrated hydrochloric acid to remove carbobenzoxy and benzyl groups, and de-*N*-acetylated with barium hydroxide to give a ninhydrin-positive product. This was dinitrophenylated with 2,4-dinitrofluorobenzene in the presence of sodium bicarbonate in aqueous ethanol and then *O*-acetylated with acetic anhydride and anhydrous sodium acetate. The product, which showed about four spots with $R_f$-values of 0.56, 0.45, 0.35 and 0.27 on a thin-layer chromatogram (TLC) with a solvent system: toluene-MEK (2 : 1), was chromatographed on a silica-gel column with the same solvent system. The substance of $R_f$-value 0.35 was isolated to give synthetic hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl)-kanamycin C (VII), mp 208—211°C (decomp.), $[\alpha]_D^{18}$ +285° (*c* 0.75, acetone), in a 15% overall yield from VI.

On the other hand, natural kanamycin C was dinitrophenylated and acetylated to give hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl) derivative of kanamycin C, mp 208—211°C (decomp.), $[\alpha]_D^{18}$ +299° (*c* 0.64, acetone).

The identity of the synthetic VII with the above mentioned derivative of natural kanamycin C was established by elemental analyses, specific rotations, by their failure to depress the mixed melting point and identical mobilities on TLC and infrared spectra.

Hydrolysis of VII with methanolic ammonia followed by treatment with Dowex 1×2 ($OH^-$) resin gave a crystalline free base of synthetic kanamycin C (VIII), $[\alpha]_D^{18}$ +139° (*c* 0.50, water), which was identical with an authentic sample of the natural kanamycin C. Optical rotations were essentially equal and infrared spectra were superimposable. On descending paper chromatography the $R_f$-value of the synthetic VIII agreed with

3) J. W. Rothrock, R. T. Goegelman and F. J. Wolf, *Antibiotics Annual*, **1958/59**, 796.

4) M. Murase, *J. Antibiotics*, **A14**, 367 (1961).

5) T. H. Haskell, J. C. French and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 3480 (1959).

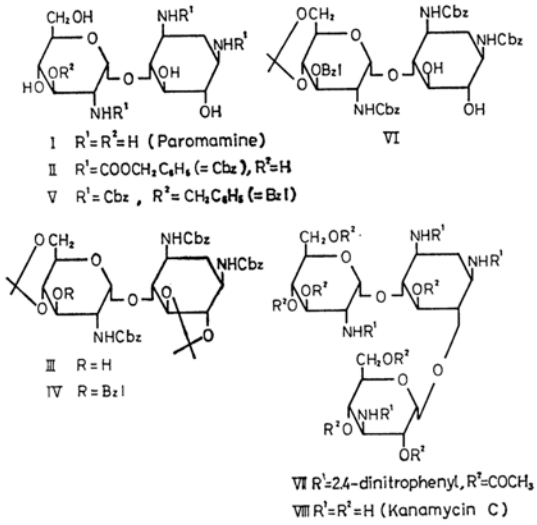
6) S. Umezawa and S. Koto, This Bulletin, **39**, 2014 (1966).

7) M. E. Evans, F. W. Parrish and L. Long, Jr., *Carbohydrate Research*, **3**, 453 (1967).

8) This compound was reported by S. Umezawa *et al.* at the 20th Annual Meeting of the Chemical Society of Japan, Tokyo, March 31, 1967. (See abstracts of the papers of the Meeting, III, p. 596.) S. Koto, T. Tsumura, Y. Kato and S. Umezawa, This Bulletin, **41**, 2765 (1968).

9) S. Umezawa, S. Koto, K. Tatsuta and T. Tsumura, *J. Antibiotics*, **21**, 163 (1968).

that of natural kanamycin C. The antibiotic spectra[9] and minimal inhibitory concentrations of the synthetic VIII against test organisms were in agreement with those of natural kanamycin C.




## Experimental

**General Procedures.** Thin layer chromatography (TLC) was performed on "Silica-Rider for TLC" (Daiichi Pure Chemicals Co.) using the following solvent systems: (a) benzene - methyl ethyl ketone (MEK) (4 : 1), (b) benzene - MEK (1 : 1), and (c) toluene - MEK (2 : 1). The 2,4-dinitrophenyl (DNP) derivatives were visualized directly on an air-dried plate as yellow spots, while others were detected by spraying a dried chromatogram with 50% sulfuric acid and heating it at 110°C. Paper chromatography was performed on Toyo filter paper No. 51, using a solvent system: (d) *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1); ninhydrin in pyridine (0.3%) was sprayed on. Infrared spectra were determined in potassium bromide pellets.

**Tri-*N*-carbobenzoxyparomamine (II).** To a mixture of paromamine (I) (5 g) and sodium carbonate (15 g) in water (50 m*l*) and acetone (150 m*l*) was added slowly carbobenzoxychloride in toluene (30%, 28 g) under stirring at −10—−5°C. After having been stirred for about 4 hr, the reaction mixture was set aside in a refrigerator overnight. The resulting white solid was shaken with a mixture of water (400 m*l*) and ether (20 m*l*), collected by filtration and pressed between filter papers to remove moisture and oily matters. The waxy product was triturated with 1 N hydrochloric acid (300 m*l*), filtered, washed with water and dried *in vacuo*. Recrystallization from dioxane (400 m*l*) gave a colorless solid, 6.7 g, mp 258°C (decomp.), $[\alpha]_D^{18}$ +64.5° (*c* 0.67, DMF). The mother liquor deposited a further crop, 3.1 g. Total yield 88%. IR spectrum: 3330, 1697 and 1542 cm$^{-1}$ (NHCbz).

Found: C, 59.74; H, 6.28; N, 5.55%. Calcd for $C_{36}H_{43}N_3O_{13}$: C, 59.58; H, 5.97; N, 5.79%.

**4-*O*-(2-Carbobenzoxyamino-4,6-*O*-isopropylidene-2-deoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-5,6-*O*-isopropylidene-2-deoxystreptamine (III).** To a solution of II (4.7 g) in anhydrous DMF (30 m*l*) was added *p*-toluenesulfonic acid monohydrate (0.06 g) and 2,2-dimethoxypropane (6.2 m*l*), and the solution was heated at 110°C for 4 hr with occasional stirring. The mixture was neutralized with Amberlite IRA-400 (OH type) washed with methanol before use and filtered. Concentration gave a residue, which solidified when triturated with water. Recrystallization from ethanol; colorless crystals, 5.2 g (98%), mp 243—245°C, $[\alpha]_D^{18}$ +71° (*c* 0.59, DMF), IR spectrum: 3340, 1698 and 1533 (NHCbz), 1167, 1137 and 1047 cm$^{-1}$ (ketal).

Found: C, 62.63; H, 6.50; N, 5.39%. Calcd for $C_{42}H_{51}N_3O_{13}$: C, 62.60; H, 6.38; N, 5.21%.

**4-*O*-(3-*O*-Benzyl-2-carbobenzoxyamino-4,6-*O*-isopropylidene-2-deoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-5,6-*O*-isopropylidene-2-deoxystreptamine (IV).** To a solution of III (5.1 g) in anhydrous DMF (80 m*l*) was added pulverized barium oxide (5.2 g) and barium hydroxide octahydrate (6.2 g) with stirring and the mixture was cooled to −10°C. Benzyl bromide (3.7 m*l*) was added drop by drop to the mixture under vigorous agitation. The reaction temperature was allowed to rise gradually to 0°C during 4 hr and then to room temperature during an additional 4 hr. The slightly colored mixture was further stirred continuously for 20 hr at room temperature. Chloroform (150 m*l*) was added and the mixture was filtered through a layer of Celite (thickness 20 mm). The filtrate was evaporated to give a sirup, which was chromatographed on a silica-gel column (400 g, 51 × 510 mm) with a solvent mixture (a). An eluate between 1400—2300 m*l* was evaporated to dryness. The residue was recrystallized from ethanol to give IV; yield 4.7 g (82%), mp 160—162°C, $[\alpha]_D^{18}$ +72.4° (*c* 0.61, DMF), IR spectrum: 3335, 1730—1700 and 1540—1520 cm$^{-1}$ (NHCbz), 1172, 1132 and 1040 cm$^{-1}$ (ketal).

Found: C, 65.47; H, 6.67; N, 4.80%. Calcd for $C_{49}H_{57}N_3O_{13}$: C, 65.68; H, 6.41; N, 4.69%.

**4-*O*-(3-*O*-Benzyl-2-carbobenzoxyamino-2-deoxy-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (V).** A suspension of IV (3.0 g) in aqueous acetic acid (80%; 100 m*l*) was stirred at 50°C until the solution became clear and then kept at room temperature overnight. The resulting precipitate was collected, washed with water and dried *in vacuo*. Recrystallization from ethanol gave colorless needles of V; yield 2.7 g (98%), mp 270—271°C (decomp.), $[\alpha]_D^{18}$ +101° (*c* 0.52, DMF), IR spectrum: 3340, 1699 and 1538 (NHCbz) 1026 cm$^{-1}$ (*O*-benzyl).

Found: C, 63.08; H, 6.13; N, 5.24%. Calcd for $C_{43}H_{49}N_3O_{13}$: C, 63.30; H, 6.05; N, 5.15%.

**4-*O*-(3-*O*-Benzyl-2-carbobenzoxyamino-2-deoxy-4,6-*O*-isopropylidene-*α*-D-glucopyranosyl)-*N*,*N*′-dicarbobenzoxy-2-deoxystreptamine (VI).** To a solution of V (2.2 g) in DMF (13 m*l*) was added *p*-toluenesulfonic acid (0.03 g) and 2,2-dimethoxypropane (0.8 m*l*), and the mixture was allowed to stand at room temperature overnight. After neutralization with Amberlite IRA-400 (OH type) washed with methanol before use, the solution was evaporated to give a residue, which showed two spots of $R_f$ values 0.86 and 0.53 on TLC with the solvent system (b). The residue was chromatographed on a silica-gel column (200 g, 50 × 295 mm) with the solvent system (b), the effluent being cut into fractions of 15 m*l* each. The substance of

$R_f$ 0.53 appeared in the fractions of tube Nos. 30—53, which afforded 1.3 g of VI (55%). Recrystallization from ethanol; mp 239—241°C, $[\alpha]_D^{18}$ +75.2° (*c* 0.63, DMF), IR spectrum: 3335, 1697 and 1535 (NHCbz), 1170, 1130 and 1035 (ketal), 1026 $cm^{-1}$ (*O*-benzyl).

Found: C, 64.29; H, 6.11; N, 4.78%. Calcd for $C_{46}H_{53}N_3O_{13}$: C, 64.55; H, 6.24; N, 4.91%.

The diisopropylidene derivative IV ($R_f$ 0.86) was also obtained from the fractions between tube Nos. 31—33 and weighed 0.5 g.

**Proof for the Structure of VI.** Monoisopropylidene derivative VI (13 mg) in DMF (1 m*l*) was benzylated in the above-mentioned manner with barium oxide (24 mg), barium hydroxide octahydrate (29 mg) and benzyl bromide (18 m*l*). The TLC of the oily product showed that VI completely vanished and a new spot appeared. The oily product was dissolved in hot aqueous acetic acid (80%) and allowed to stand at room temperature for 5 hr. The colorless solid separated was collected, yield 8 mg. The deacetonated product was hydrolyzed in a mixture of glacial acetic acid, methyl cellosolve, water and concentrated hydrochloric acid (10 : 5 : 3 : 2) by heating at 100°C for 10 hr under reflux to remove carbobenzoxy groups and to cleave the glycosidic linkage. On the other hand, a control experiment was carried out with II. The paper chromatogram of the hydrolyzates using the solvent system (d) and ninhydrin-coloration showed that the fully benzylated VI gave neither 2-amino-2-deoxy-D-glucose nor 2-deoxystreptamine, while II gave both of them, indicating that the isopropylidene and benzyl groups are present in the glucosamine moiety of VI.

**Synthetic Hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl)-kanamycin C (VII).** A mixture of VI (1.13 g), dried powdery mercuric cyanide (0.46 g) and freshly activated Drierite (3.66 g) in a mixture of dioxane (5.5 m*l*) and benzene (11 m*l*) was heated to reflux for a few minutes under agitation. To the mixture, which was somewhat cooled, was added well dried 3-acetamido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride[8] (1.3 g, 1.7 eq.) and the reaction mixture was stirred vigorously under reflux for 9 hr under anhydrous conditions. During the course of reaction the mixture turned gradually dark and somewhat pasty. The resulting mixture was filtered and thoroughly washed with dioxane and ethyl acetate. The filtrate combined with washings was evaporated to give a sirup, which was extracted with ethyl acetate (150 m*l*). The extract was washed with three 100 m*l* portions of 20% aqueous sodium chloride, dried over anhydrous sodium sulfate and evaporated to give an oily product (1.8 g). Aqueous acetic acid (80%; 20 m*l*) was added and the mixture was warmed to 50°C under stirring until the solution became clear. The solution was allowed to stand for 1 hr and evaporated to give the deacetonated product, which was then hydrogenated over palladium black (0.3 g) in a mixture of dioxane (21 m*l*), water (3.5 m*l*), ethanol (0.5 m*l*) and concentrated hydrochloric acid (2 m*l*), under 3 atm of hydrogen pressure at about 40°C with occasional addition of portions of water whose total volume was 35 m*l*. The hydrogenolysis took 48 hr for completion. After removal of the catalyst, the solution was evaporated at a temperature below 30°C to give a sirup (0.74 g). The ninhydrin-positive sirup was dissolved in 1 N aqueous barium hydroxide (40 m*l*) and heated over a boiling water bath for 2 hr. The mixture was acidified with 3 N sulfuric acid to pH 2 and the mixture was centrifuged to remove barium sulfate. The supernatant was neutralized with Dowex 1×2 (OH type) and evaporated to give a colorless sirup (0.6 g) of free base. Paper chromatography and bioautography of the product showed that the product mainly contained the substance whose $R_f$-value coincided with that of natural kanamycin C. The product was dinitrophenylated with 2,4-dinitrofluorobenzene (0.84 g) in the presence of sodium bicarbonate (0.4 g) in 50% aqueous ethanol (60 m*l*) at room temperature under stirring overnight. Precipitation of a yellow gummy substance was observed. The resulting mixture was neutralized with 0.1 N hydrochloric acid and followed by evaporation to give a sirup. This was acetylated with acetic anhydride (20 m*l*) and anhydrous sodium acetate (1 g) with stirring at 100°C. After evaporation, the residue was extracted with acetone and the extract was evaporated to give a yellow glass (1.8 g), which showed about four spots with $R_f$ value of 0.56, 0.45, 0.35 and 0.27 on TLC with the solvent system (c). The product was chromatographed on a silica-gel column (150 g, 49×310 mm) with the same solvent system, being cut into fractions of 8 g each. The main product of $R_f$ value 0.35 appeared in fractions of tube Nos. 34—48, which was evaporated and followed by recrystallization from the solvent system (c) to give yellow needles of VII (overall yield from VI, 15%); mp 208—211°C (decomp.), $[\alpha]_D^{18}$ +285° (*c* 0.75, acetone), IR spectrum: 3320, 1620, 1595, 1550, 1525, 1335, 835 and 745 (NHDNP), 1750, 1365 and 1220 $cm^{-1}$ (OAc).

Found: C, 46.68; H, 4.34; N, 11.84%. Calcd for $C_{56}H_{58}N_{12}O_{34}$: C, 46.61; H, 4.05; N, 11.65%.

**Synthetic Kanamycin C (VIII).** A sample (47 mg) of VII was dissolved in 20 m*l* of methanol saturated with ammonia at 0°C and allowed to stand at room temperature for 4 hr to remove acetyl groups. Evaporation of the reaction mixture *in vacuo* gave a yellow glass (39 mg), which was treated with 0.5 m*l* of Dowex 1×2 (OH type) in moistened acetone (3 m*l*) at room temperature for 4 hr to remove dinitrophenyl groups. The resin was filtered off, and the filtrate was evaporated. The residue was passed through a small column of Dowex 1×2 (OH type) with water to give a colorless solution of pure free base. Evaporation followed by recrystallization from aqueous methanol containing small amount of ethanol gave VIII; $[\alpha]_D^{18}$ +139° (*c* 0.50, water).

Found: C, 44.40; H, 7.28; N, 11.80%. Calcd for $C_{18}H_{36}N_4O_{11}$: C, 44.62; H, 7.49; N, 11.56%.

The natural kanamycin A showed $[\alpha]_D^{18}$ +145° (*c* 0.58, water) [lit.[4] $[\alpha]_D^{18}$ +126° (*c* 1, water)]. On descending paper chromatography by ninhydrin-coloration using the solvent system (d), the $R_f$-value of the synthetic product (VIII) agreed with that of the natural kanamycin C. Infrared spectra of VIII and the natural specimen were superimposable. The antibiotic spectra[9] and minimal inhibitory concentrations of the synthetic product VIII against test organisms coincided with that of natural kanamycin C.

**Natural Hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl)-kanamycin C.** To a solution of natural kanamycin C (50 mg) in water (0.5 m*l*) was added sodium bicarbonate (26 mg) and 2,4-dinitrofluorobenzene (58 mg) in ethanol (0.6 m*l*) and the mixture was vigorously stirred at room temperature for 3 hr. Water (1 m*l*) was

added and agitated overnight to precipitate a yellow solid, which was collected and washed thoroughly with water; yield 95 mg (82%). The *N*-dinitrophenyl derivative (95 mg) was acetylated with acetic anhydride (5 m*l*) and anhydrous sodium acetate (0.5 g) by heating at 110°C under stirring for 2 hr. The resulting mixture was concentrated under reduced pressure and followed by dilution with acetone and filtration. The filtrate was again evaporated and made free from acetic anhydride by co-evaporating with toluene three times to give a yellow glass (140 mg), which was purified by chromatography on a silica-gel column (10 g, 23×100 mm) with the solvent system (c) being cut into 3 m*l* each. The fraction Nos. 8—20 were collected and evaporated to give a yellow solid; 130 mg (89%). Crystallization from the solvent system (c) afforded yellow needles of hepta-*O*-acetyl-tetra-*N*-(2,4-dinitrophenyl)-kanamycin C; mp 208—211°C (decomp.), $[\alpha]_D^{18}$ +299° (*c* 0.64, acetone), IR spectrum: 3320, 1620, 1595, 1550, 1525, 1335, 835 and 745 (NHDNP), 1750, 1365 and 1220 $cm^{-1}$ (OAc).

Found: C, 46.65; H, 4.24; N, 11.78%. Calcd for $C_{56}H_{58}N_{12}O_{34}$: C, 46.61; H, 4.05; N, 11.65%.

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