THE CONFIGURATION OF DEOXYSTREPTAMINE[1]

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ABSTRACT

The nuclear magnetic resonance (n.m.r.) spectrum of 5-*O*-methyl-1,3-di-*N*-methyl-4,6-di-*O*-acetyldeoxystreptamine dihydrogenperchlorate requires the all-trans configuration since the signal for the equivalent ring hydrogens at the 4- and 6-positions is in the form of a triplet with a spacing of 10 c.p.s. This spacing requires these hydrogens to be axial and coupled with axial hydrogens at the neighboring 1-, 3-, and 5-positions.

Streptamine is obtained by hydrolysis of the antibiotic streptomycin (1) and evidence has been obtained (2, 3) that the compound is 1,3-dideoxy-1,3-diaminoscylloinositol. The substance known as deoxystreptamine, first isolated from the antibiotics neomycin A (neamine), B, and C (4), was found to be a meso form of 1,3-diamino-4,5,6-trihydroxycyclohexane (4) and its name was assigned on the basis of the assumption that it was configurationally related to streptamine. Although circumstantial evidence has accumulated (5) which rendered the correctness of this assumption highly probable, the evidence cannot be considered definitive. Deoxystreptamine is also a building unit of the kanamycins A (6) and B (7) and paromomycin (8). This widespread occurrence renders a knowledge of its configuration of particular interest and especially since Hoeksema, Argoudelis, and Wiley (9) have recently isolated the 1,3-di-*N*-methyl derivative of the 2-epimer (10) of streptamine from the antibiotic actinospectacin. The compound was termed "actinamine." It could be anticipated that a direct proof of the configuration of deoxystreptamine may be available by application of n.m.r. spectroscopy along the lines described by Lemieux, Moir, and Kullnig (11). Advantage is taken of the fact that neighboring hydrogens in axial orientation are coupled to the extent of 8 or more c.p.s. and this coupling is three to four times stronger than when the hydrogens are in gauche relationship (12).

The n.m.r. spectrum of deoxystreptamine in deuterium oxide is shown in Fig. 1. The structures of the signals for the hydrogens of the methylene group require the two amino groups to be equatorially oriented. This is evident from the 12- and 13-c.p.s. spacings found in the signal for the axial hydrogen at 1.28 p.p.m. and demonstrated through the first-order analysis shown in Fig. 1. However, the spectrum provides no simply derivable information about the configurations at the other centers because of the small chemical shift between the signals for the 4-, 5-, and 6-hydrogens in the region 3.0 p.p.m. Slomp and MacKellar (10) found the equivalent axial 4- and 6-hydrogens of actinamine to produce their signal about 0.7 p.p.m. to lower field than that for the axial 5-hydrogen at 3.46. This situation is mainly due to the deshielding effect on the 4- and 6-hydrogens by the axial and opposing hydroxyl group at the 2-position (13).

The signals for the 4-, 5-, and 6-hydrogens were also bunched together in the n.m.r. spectrum for the diperchlorate salt of tri-*O*-acetyldeoxystreptamine. However, the signals for the 1- and 3-hydrogens were well separated from those at the 4-, 5-, and 6-positions. Consequently, it was evident that a solution of the configuration of deoxystreptamine through first-order analysis of the spectrum could be obtained (14) if the acetyl group

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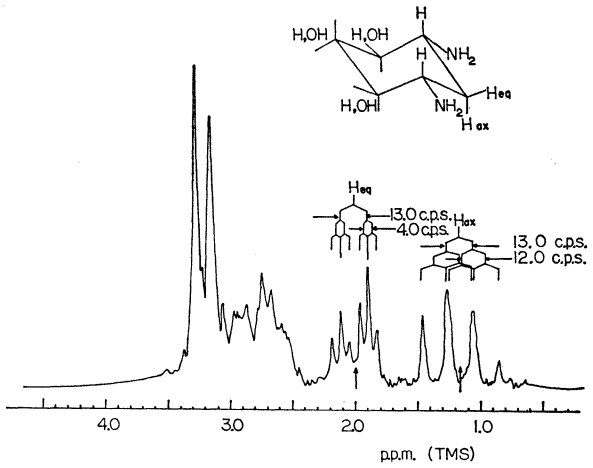

FIG. 1. The proton magnetic resonance spectrum of deoxystreptamine in deuterium oxide.

at the 5-position could be substituted in such a way as to achieve a substantial chemical shift between the signal for the 5-hydrogen and that for the neighboring 4- and 6-hydrogens. The antibiotic kanamycin A provided a convenient starting point for the preparation of such a derivative of deoxystreptamine.

Umezawa and co-workers (15) have reported the methylation of the tetra-*N*-acetyl derivative of kanamycin. Acid hydrolysis of the permethylated compound provided a substance, m.p. 207–209°, reported to be 5-*O*-methyldeoxystreptamine dihydrochloride. It has now been observed that methylation with methyl iodide and silver oxide in dimethylformamide (16) (instead of dimethyl sulphate in aqueous sodium hydroxide (15)) and acid hydrolysis produced a mono-*O*-methyl-di-*N*,*N*′-methyl derivative of deoxystreptamine dihydrochloride with a melting point, 207–210.5°, very close to that reported by Umezawa and his co-workers (15). It seems possible that the two compounds are identical.

The derivative of deoxystreptamine was converted to the dihydrogenperchlorate salt for *O*-acetylation (17). Although the product failed to crystallize, the n.m.r. spectrum (Fig. 2) and microanalyses require the substance to be virtually pure 5-*O*-methyl-1,3-di-*N*-methyl-4,6-di-*O*-acetyldeoxystreptamine dihydrogenperchlorate. It is seen that, as expected for this structure, sharp signals are present with the chemical shifts and intensities for two *O*-acetyl groups, two *N*-methyl groups, and one *O*-methyl group. The triplet of intensity two at 5.30 p.p.m. is in the region expected for the signal from hydrogens bonded to secondary carbons attached to acetoxy groups and must arise from the 4- and 6-hydrogens. The spacing of 10 c.p.s. requires these hydrogens to be axial and coupled with two neighboring axial hydrogens. Therefore, the spectrum comprises direct evidence for the all-trans configuration for deoxystreptamine and confirms the previous evidence for the points of attachment of the aminosugars to deoxystreptamine in kanamycin A (15, 18).

EXPERIMENTAL

Tetra-N-acetyl Kanamycin A

A solution of 100 g of kanamycin A* in 150 ml of water and 450 ml of methanol was cooled to 0–5°, and 100 ml of acetic anhydride was added dropwise. The temperature rose to 50°. After standing at room temperature for 1 hour, acetone (4 liters) was added with stirring. After 1 hour, the precipitate was collected by filtration and washed with acetone. The solid was dried *in vacuo* for 2 days. The crude material was

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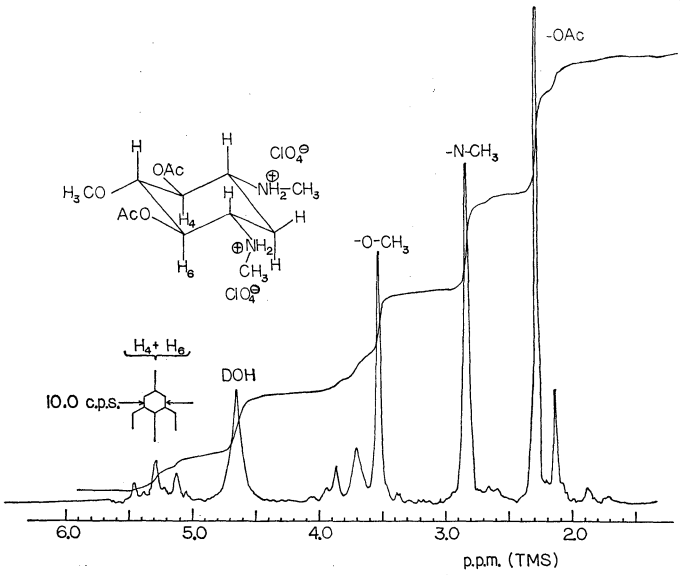

FIG. 2. The proton magnetic resonance spectrum of 4,6-di-*O*-acetyl-5-*O*-methyl-1,3-di-*N*-methyldeoxystreptamine dihydrogenperchlorate in deuterium oxide.

recrystallized from a minimum amount of water to which equal volumes of methanol and ethanol were added until crystals began to appear. The product was powdered and dried for 6 hours in a vacuum oven at 80°. The yield was 112.5 g of a product which melted at 265–266° (literature (6) m.p. 250–255° (with decomposition)), gave a negative test with ninhydrin, showed absorption at 1640 cm^{-1} characteristic of the *N*-acetyl group, and had $[\alpha]_D$ +88.3° (*c*, 5 in water). Calculated for $C_{26}H_{44}N_4O_{15}$: CH_3CO, 26.35%. Found: CH_3CO, 24.15%.

Methylation of Tetra-N-acetyl Kanamycin A

A suspension of 25.8 g of tetra-*N*-acetyl kanamycin A (39.5 mmoles) in dimethylformamide (800 ml) and methyl iodide (142 ml) was reacted with 280 g of freshly prepared silver oxide. The silver oxide was added to the rapidly stirred mixture in four 70-g portions at intervals of 20 minutes. After stirring at room temperature for 12 hours, a further 70 g of silver oxide and 50 ml of methyl iodide were added. Stirring was continued for a total of 48 hours. The silver salts were collected by filtration and washed with dimethylformamide (2×100 ml). The combined filtrate and washings were evaporated. The salts were extracted with several portions of methanol and evaporated to dryness. The amber oil was dissolved in chloroform and the solution was washed once with water. The chloroform was removed and several small portions of benzene were distilled from the residue to leave an orange amorphous solid (13.0 g, 41%); m.p. 123–125°; $[\alpha]_D$ +94.5 (*c*, 4 in chloroform). The infrared absorption indicated that the substance was free of hydroxyl groups. The product, formed on hydrolysis in refluxing 4 *N* hydrochloric acid for 20 minutes, when chromatographed on paper using 1-butanol – pyridine – acetic acid – water (6:4:3:1) showed three ninhydrin-positive (19) spots, R_f = 0.16 (brown), 0.34 (orange), 0.55 (violet), and no trace of deoxystreptamine, R_f = 0.05.

5-O-Methyl-1,3-di-N-methyldeoxystreptamine Dihydrochloride

Fully methylated kanamycin A tetra-*N*-acetate (4.80 g) was dissolved in 4 *N* hydrochloric acid (150 ml) and the solution refluxed for 1.5 hours. The solution was evaporated *in vacuo* at 50°. The solid residue was dissolved in water (20 ml) and decolorized with charcoal (0.6 g). Evaporation left 4.02 g of a solid which was dissolved in methanol (6 ml), and ether (29 ml) was then added to precipitate the 5-*O*-methyl-1,3-di-*N*-methyldeoxystreptamine dihydrochloride (2.26 g). A further similar treatment yielded 1.0 g of material which was dissolved in the developing phase (8 ml) for chromatograghy on a column of cellulose (18 cm×1.5 cm) using 1-butanol – ethanol – water (4:1:5). Three-milliliter fractions were collected and fractions 11 to 13 contained 560 mg of 5-*O*-methyl-1,3-di-*N*-methyldeoxystreptamine dihydrochloride, which was recrystallized by solution in a minimum amount of methanol and the addition of ethanol to turbidity. The yield was 510 mg of a crystalline substance; m.p. 207–210.5°. Chromatography on paper using the above solvent system showed only one spot (R_f = 0.16) using either ninhydrin (19) or periodate–permanganate (20) spray reagents. Calculated for $C_9H_{22}Cl_2N_2O_3 \cdot H_2O$: C, 36.95; H, 8.19; N, 9.49; *O*-CH_3, 10.51%. Found: C, 36.71, 37.01; H, 7.80, 8.03; N, 9.80; *O*-CH_3, 13.16%.

4,6-Di-O-acetyl-5-O-methyl-1,3-di-N-methyldeoxystreptamine Dihydrogenperchlorate

5-*O*-Methyl-1,3-di-*N*-methyldeoxystreptamine dihydrochloride (104.0 mg, 0.375 mmole) was added to 0.194 ml of 3.87 *M* perchloric acid (0.75 mmole) in acetic acid. The mixture was cooled to 0–5° and acetic anhydride (0.4 ml) was added dropwise. Solution was completed after 1 hour. After 4 hours, the reaction was complete, as indicated by a constant n.m.r. spectrum. The solution was freeze-dried to remove excess acetic anhydride and acetic acid. The residue was dissolved in water and the solution freeze-dried to leave a white amorphous solid. The substance was triturated with boiling chloroform and, after drying, dissolved in water and freeze-dried. Calculated for $C_{13}H_{26}Cl_2N_2O_{13}\cdot 2H_2O$: C, 29.74; H, 5.75; N, 5.33; *O*-CH_3, 5.90; *N*-CH_3, 5.72; CH_3CO, 16.36%. Found: C, 29.14, 29.36; H, 5.27, 5.33; N, 5.51; *O*-CH_3, 6.25; *N*-CH_3, 4.65; CH_3CO, 14.00, 15.76%.

After freeze-drying twice from deuterium oxide (2×1 ml), the material (145 mg, 88%) gave the n.m.r. spectrum shown in Fig. 2. The ratio of the intensity of the signal of the *N*-methyl groups to that of the *O*-acetyl groups was only 0.9 to 1. A further indication that the compound was not pure was the presence of the low-intensity triplet to slightly higher field in the region 5.30 p.p.m. for the 4- and 6-hydrogens.

When a paper chromatogram, using the above-mentioned conditions, was run for 60 hours, two components were separated. It seems most probable that the substance was about a 9:1 mixture of the di-*N,N'*-methyl and mono-*N*-methyl derivatives of 4,6-di-*O*-acetyl-5-*O*-methyldeoxystreptamine dihydrogenperchlorate.

Deoxystreptamine

Kanamycin A (25 g) in 4 *N* hydrochloric acid (250 ml) was refluxed for 20 minutes. The cooled solution was diluted with water (200 ml) and treated with decolorizing charcoal (40 g). The filtered solution was concentrated *in vacuo* to 50 ml and methanol (50 ml) was added. When crystals began to appear, 400 ml of ethanol was added with stirring. After 24 hours at room temperature, the crude deoxystreptamine dihydrochloride (14.4 g) was collected and dissolved in a minimum amount of boiling methanol. Ethanol was added to turbidity. The precipitate was examined by paper chromatography and found to contain traces of 6-deoxy-6-amino-D-glucose hydrochloride. The precipitate was therefore added to a Dowex 50 (H^+) ion exchange resin contained in a column (32 cm×2.5 cm) which had been washed with 4 liters of 0.7 *N* hydrochloric acid. The column was washed free of the aminosugar with 0.7 *N* hydrochloric acid. Stronger hydrochloric acid (2.5 *N*) was required to elute the deoxystreptamine dihydrochloride (21). The free base was prepared by passing the hydrochloride through an Amberlite IR-120 (OH^-) column and eluting with water.

4,5,6-Tri-O-acetyldeoxystreptamine Dihydrogenperchlorate

A solution of 25 ml of acetic acid, 2 ml of 70% perchloric acid, and 15 ml of acetic anhydride was added to 500 mg of deoxystreptamine dihydrochloride. The mixture was shaken overnight, and the hydrochloride slowly dissolved. The white gelatinous product was collected by filtration, washed with acetic acid, and dried *in vacuo*. The yield was 500 mg (67%). After two recrystallizations from water, the nicely crystalline compound possessed the following physical properties: the infrared spectrum showed a strong band at 1726 cm^{-1} characteristic of the carbonyl group of *O*-acetates but no signal in the region (about 1640 cm^{-1}) characteristic of *N*-acetates, the n.m.r. spectrum showed two bands for the *O*-acetyl groups, one at 2.22 p.p.m. which was half the intensity of that at 2.28 p.p.m. Calculated for $C_{12}H_{22}Cl_2N_2O_{14}$: C, 29.47; H, 4.53; N, 5.73; CH_3CO, 26.32%. Found: C, 29.51; H, 4.33; N, 5.79; CH_3CO, 24.80, 25.70%.

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