Apramycin, a Unique Aminocyclitol Antibiotic[1]

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Apramycin, $C_{21}H_{41}N_5O_{11}$, a broad-spectrum aminocyclitol antibiotic, is produced by a strain of *Streptomyces tenebrarius*. It does not fall into the tobramycin, kanamycin, gentamicin group and a detailed examination of its structure involving degradative, synthetic, and spectroscopic analysis leads to the assignment of an unusual structure, the main features of which are a 4-amino-4-deoxy-D-glucose moiety, a 1–1′ sugar linkage, and an octadiose which exists as a rigid bicyclic system. The proposed structure is confirmed by an x-ray diffraction study.

Nebramycin,[3] an aminocyclitol antibiotic complex produced by *Streptomyces tenebrarius*, was first reported in 1967. The structure of tobramycin, derived from a component of the complex, has been elucidated by Koch and Rhoades.[4] It was shown to be a member of the kanamycin–gentamicin group in that it is an O^4,O^6-disubstituted deoxystreptamine. We report here, the structure of apramycin, formerly called nebramycin factor 2.[5]

Studies on apramycin were initiated with the expectation that its structure would prove to be similar to that of tobramycin. We find, however, that apramycin is markedly different from previously reported antibiotics of this class.

Isolation and Characterization. Crude apramycin[5] was purified on IRC-50 resin and the amorphous base was crystallized from aqueous ethanol as the monohydrate. Table I lists some properties of the crystalline material. Microanalysis (see Experimental Section) in combination with the x-ray derived molecular weight yielded two possible molecular formulas for the anhydrous base, viz., $C_{21}H_{41}N_5O_{11}$ and $C_{21}H_{43}N_5O_{11}$.

To differentiate between these two possibilities, the compound was N-acetylated and the resulting penta-*N*-acetylapramycin was permethylated with sodium hydride/methyl iodide in dimethylformamide. The product gave a molecular ion at *m/e* 889.4897. The theoretical value for $C_{41}H_{71}N_5O_{16}$ [$C_{21}H_{41}N_5O_{11} + 5(CH_2{=}C{=}O) + 10(CH_2)$] is 889.48955. The molecular formula is, therefore, $C_{21}H_{41}N_5O_{11}$ and the absence of any evidence of multiple bonds in the molecule (ir, chemical reduction, catalytic reduction) requires that apramycin contain *four rings*. Van Slyke nitrogen determination on apramycin revealed the presence of four primary amino groups. The ^1H NMR spectrum of the antibiotic in D_2O (Figure 1) contains a sharp three-proton singlet at 2.37 ppm assigned to an *N*-methyl group. Only four of the five nitrogens in penta-*N*-acetylapramycin could, therefore, have accepted a methyl group on reaction with NaH–CH_3I–DMF leaving six methyl groups to be accounted for. The absence of any signal ascribable to a *C*-methyl group in the ^1H NMR of the methylation product requires that the remaining methyl groups be on oxygen. Apramycin, therefore, contains six hydroxyl groups. Periodate oxidation of apramycin led to overoxidation. Penta-*N*-acetylapramycin, however, consumed 2 mol of oxidant.

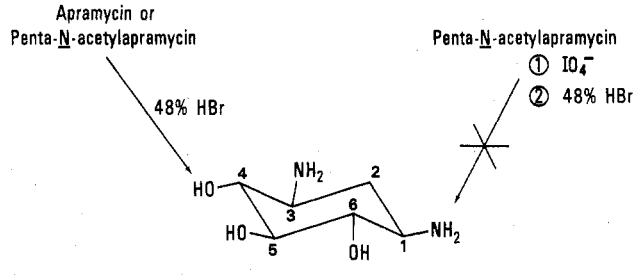
The 220-MHz spectrum of apramycin in D_2O (Figure 1) in the presence of Dowex 1 × 2 (OH^-) resin (to remove carbon dioxide), contains the following features.

(a) Four protons between 2.18 and 1.00 ppm. The chemical shifts and coupling patterns are reminiscent of the methylene hydrogens in 2-deoxystreptamine.[6]
(b) A three-proton singlet at 2.37 ppm, an *N*-methyl group.
(c) Three one-proton triplets in the region 3.054–3.7 ppm typical of the protons on the oxygen-bearing carbons in an O^4- or O^6-monosubstituted 2-deoxystreptamine ring.
(d) A sharp triplet, J = 3 Hz, at 4.246 ppm.
(e) Three doublets below 4.8 ppm, J = 8.5, 3.5, and 3.0 Hz, respectively. These are taken to be anomeric protons.

Commencing with the signal at lowest field, the anomeric protons in apramycin were irradiated (at 100 MHz) resulting in decoupling at 3.595, 2.96, and 2.67 ppm, respectively, suggesting that the anomeric at lowest field was flanked by a carbon bearing an oxygen while the remaining anomeric positions were each flanked by a carbon bearing a nitrogen.

Degradative Studies. Refluxing either apramycin or its penta-*N*-acetate overnight with 48% HBr yielded 2-deoxystreptamine in 90% yield (based on one deoxystreptamine moiety per molecule). When the penta-*N*-acetate was first treated with periodate and then subjected to the above degradation, no deoxystreptamine was obtained (Scheme I).

Scheme I



Deoxystreptamine is, therefore, monosubstituted at either C_4 or C_6, as suggested by ^1H NMR, and the consumption of 1 mol of periodate by penta-*N*-acetylapramycin is accounted for.

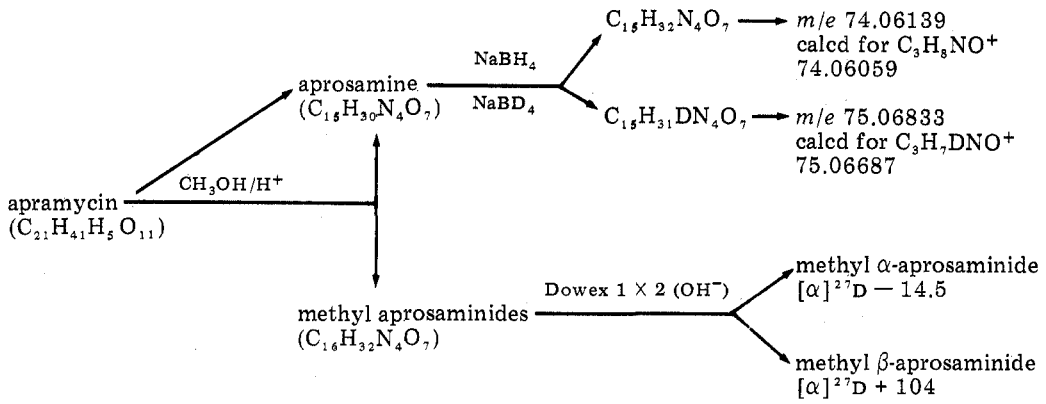
Methanolysis of apramycin using a sulfonic acid resin as catalyst resulted in extensive cleavage of the molecule. Three fractions were obtained by eluting the resin with increasing concentrations of aqueous hydrochloric acid.

Fraction I, obtained by eluting the resin with 1 N HCl, proved to be a mixture of methyl glycosides of a monoaminomonodeoxyhexopyranoside in 75–85% yield. The anomers were separated on Dowex 1 × 2 (OH^-) resin and a comparison of the α anomer with methyl 4-amino-4-deoxy-α-D-glucopyranoside [prepared from methyl-α-D-galactopyranoside by the method of Reist et al.[7] (Figure 2)] confirmed the presence of this sugar in apramycin. Its presence in apramycin accounts for the consumption of the second mole of periodate by penta-*N*-acetylapramycin.

Fraction II was eluted from the resin with 2.4 N HCl. It was predominantly 2-deoxystreptamine in a yield of 8–15 mol %. We could neither purify nor identify the trace amounts of other materials present in this fraction.

Fraction III was obtained by eluting the resin with 4 N HCl. It contained three components, each of which was shown to contain a deoxystreptamine moiety. The major component of this fraction (which was also obtained from *aqueous* acid degradation of apramycin) was a reducing substance which

Scheme II



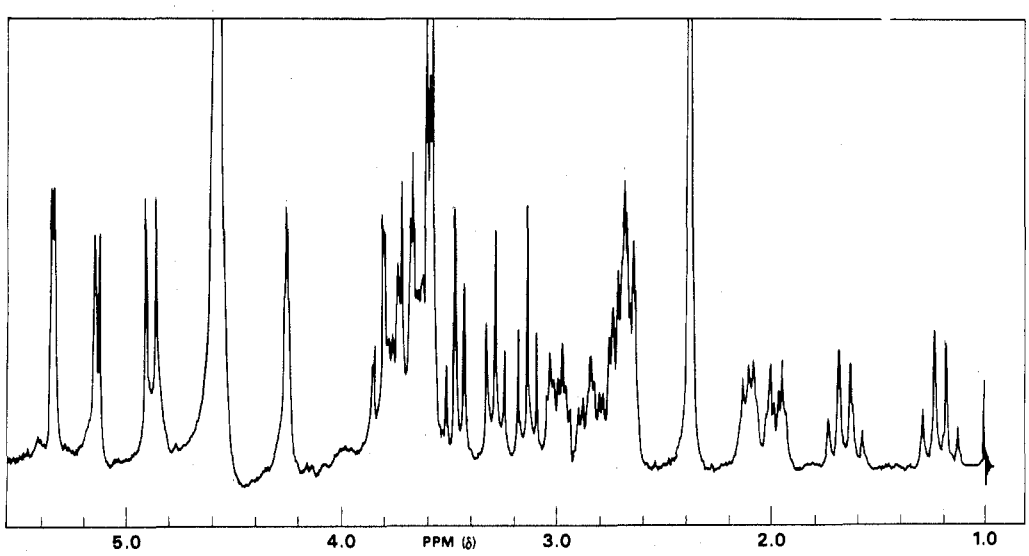


Figure 1. ^1H NMR spectrum (220 MHz) of apramycin in D_2O containing Dowex 1 × 2 (OH^-).

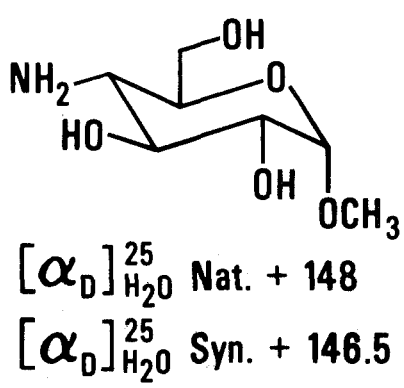


Figure 2. Methyl 4-amino-4-deoxy-α-D-glucopyranoside.

as its crystalline tetrahydrochloride, analyzed for $C_{15}H_{30}N_4O_7 \cdot 4HCl$, suggesting that it was apramycin minus the 4-aminoglucose moiety. We have given this compound the trivial name aprosamine. The ^1H NMR spectrum of aprosamine tetrahydrochloride in D_2O (Figure 3) reveals the presence of two methylene groups (1.8–2.6 ppm), an *N*-methyl group (2.8 ppm), and, downfield from the HOD peak, three anomeric doublets (J = 3.0, 3.5, and 8.4 Hz, respectively). The anomeric resonances integrate for two protons. The two higher field doublets integrate for a single proton and indicate that the anomers are in the ratio 3:1. Reduction of aprosamine with $NaBH_4$ yielded a single amorphous species, found to be $C_{15}H_{32}N_4O_7$ by high-resolution mass spectrometry, whose ^1H NMR spectrum contains only one anomeric resonance (J = 3.0 Hz). A comparison of the mass spectrum of the $NaBH_4$ reduction product with that obtained from reduction with $NaBD_4$ confirmed that the starting material contained only one reducible group in that only one nonexchangeable deuterium was incorporated. The mass spectrum of dihydroaprosamine contained a strong peak at *m/e* 74.06139 (75.06883 in the case of the $NaBD_4$ product). This suggested the presence of the group $HOCH_2CHNHCH_3$ ($HOCHDCHNHCH_3$) and served to locate the *N*-methyl group relative to the group undergoing reduction. Scheme II gives the relevant *m/e* values and summarizes the data on fraction III.

Table I. Properties of Apramycin Monohydrate

Melting point, °C	245–247 (DTA)
Space group	$P2_1$
Density	1.414
Mol wt (x-ray)	560 ± 10
pK_a (H_2O)	8.5, 7.8, 7.2, 6.2, 5.4
Mol wt (titration)	552
$[\alpha]^{25}$ (H_2O)	+162.5
Solubility	v sol H_2O, sl sol lower alcohols
Karl Fischer titration	4.1%

By mass spectrometry and ^1H NMR the two minor components of fraction III are anomeric methyl glycosides of aprosamine (termed methyl aprosaminides) and under appropriate conditions all three compounds are interconvertible. This interconvertibility suggested to us that the resin used in

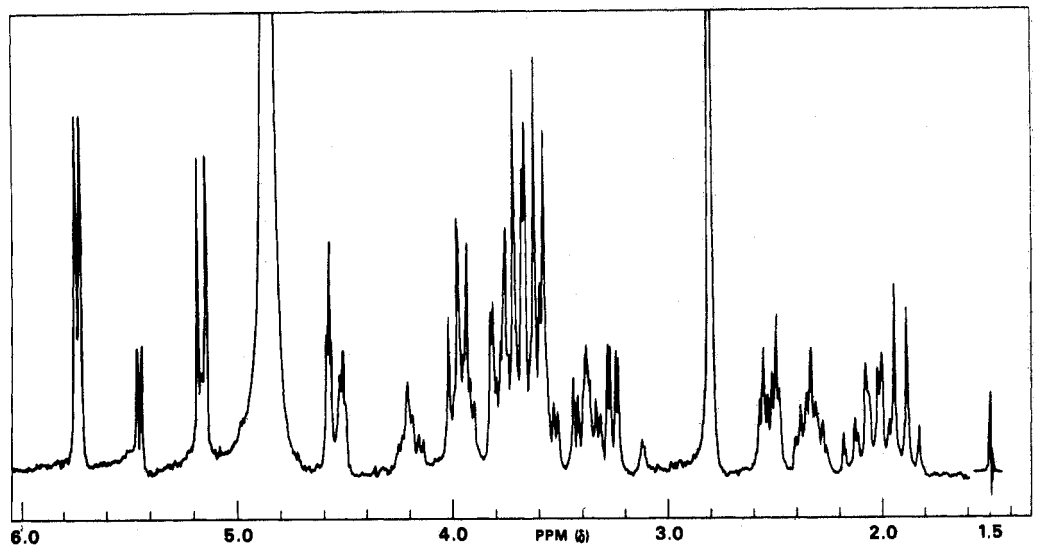


Figure 3. ^{1}H NMR spectrum (220 MHz) of aprosamine tetrahydrochloride in D_2O.

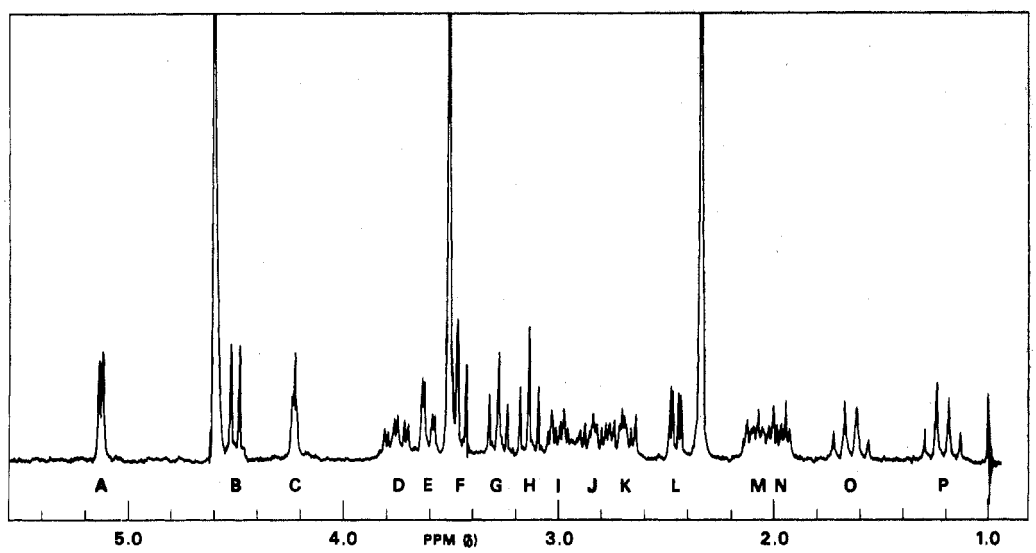


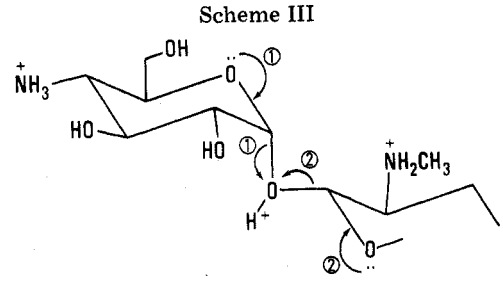
Figure 4. ^{1}H NMR spectrum (220 MHz) of methyl β-aprosaminide in D_2O. Resonances of individual protons are labeled alphabetically.

the acid degradation was not water free. No change in the ratio of aprosamine to the combined methyl aprosaminides (~8:1) occurred on more rigorous drying of the resin and it was at this point that we realized that a 1–1 link had to be present in apramycin since only this feature accounts for the fact that methanolysis of a *single* glycosidic bond results in methyl glycosides (methyl 4-amino-4-deoxyglucosides) from one moiety and a reducing sugar (aprosamine) from the other. Our suggested mechanism for the methoanolysis is outlined in Scheme III. Path 1 is favored owing to the difficulty inherent in generating an oxonium ion so close to a protonated nitrogen. The small amount of methyl aprosaminides obtained in the reaction may be due either to some reaction via path 2 or to glycosidation of aprosamine on the acid resin. This latter reaction does, in fact, occur but at a very low rate, owing, presumably, to the protonated nitrogen on the adjacent carbon.

Separation of the methyl aprosaminides is extremely tedious. We shall be concerned here with only the β anomer (Scheme II). This compound has the same anomeric stereochemistry at the relevant centers as has apramycin (see below), and is given the trivial name methyl β-aprosaminide.

By decoupling (at 100 MHz) it was found that H_A, Figure 4, is coupled to H_I (J = 3.6 Hz) and H_B to H_L (J = 8.6 Hz). A

Scheme III



comparison with the spectrum of 2-deoxystreptamine[6] allows the assignment of protons F (partially obscured triplet), G, and H (on oxygen-bearing carbons), J, and K (on nitrogen-bearing carbons) and the methylene protons N and P to this moiety although the assignment of the latter protons is not critical to the argument. Figure 5 is a spectrum from which, for reasons of simplicity, the 2-deoxystreptamine resonances have been *manually* removed.

The remaining protons were subjected to first-order analysis. The proposed structure (Figure 5) satisfies the following requirements.

(a) The low-field position of H-6′ is explained. It is an equatorial proton on an oxygen-bearing carbon.

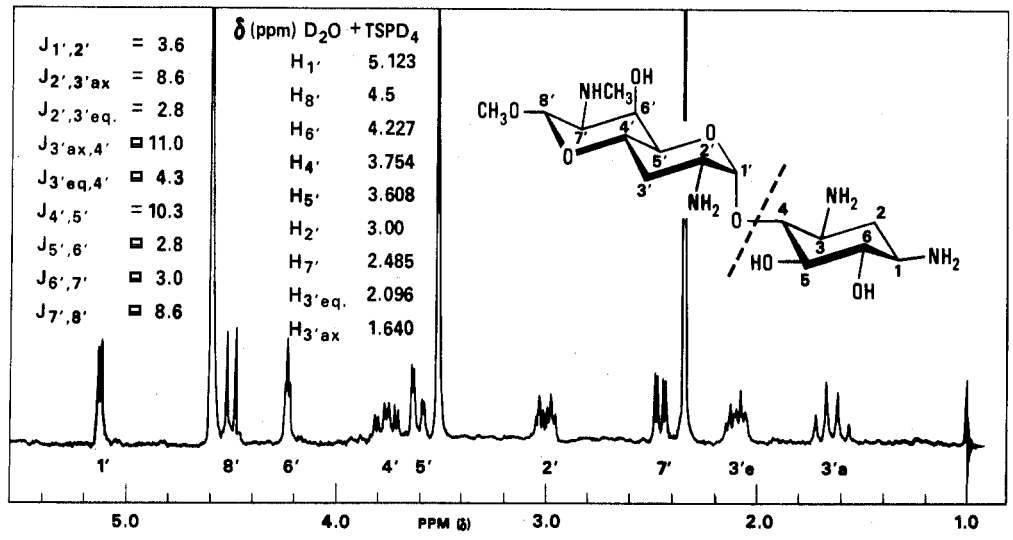


Figure 5. ^{1}H NMR spectrum of methyl β-aprosaminide (cf. Figure 4) from which the 2-deoxystreptamine resonances have been removed.

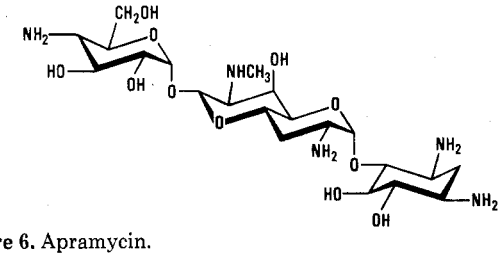


Figure 6. Apramycin.

Table II. Crystal Data for Apramycin Hydriodide, $C_{21}H_{41}N_5O_{11}\cdot HI\cdot H_2O\cdot C_2H_6O$

Mol wt	731.6
a	9.367 (2) Å
b	14.785 (4) Å
c	23.449 (5) Å
Space group	$P2_12_12_1$
Molecules/cell	4
Obsd density	1.52 g cm^{-3}
Calcd density	1.50 g cm^{-3}

(b) A 1,3-diaxial interaction with the axial hydroxyl group on C-6′ accounts for the rather low-field position of the H-4′ resonance.

(c) The asymmetry of the H-5′ signal requires that it be coupled to at least one lower field proton. In fact, it is coupled to both H-4′ and H-6′.

(d) The *N*-methyl group is α to the anomeric carbon. The mass spectrometric data on dihydro- and monodeuteriodihydroaprosamine supports this.

(e) There is only one hydroxyl group in the bicyclic moiety as required by the mass spectrum of *N,O*-permethylapramycin penta-*N*-acetate which indicated a total of six hydroxyl groups, three in 4-aminoglucose, two in deoxystreptamine, and one in the octadiose.

(f) Protons 4′ and 5′ cannot be on carbons bearing hydroxyl groups and must bond to carbons bearing ether oxygens. This, in turn, requires the presence of a bicyclic moiety. Such a system satisfies the requirement that apramycin contain four rings.

The intact antibiotic was assigned the structure shown in Figure 6. The stereochemistry at the anomeric centers follows from the previous decoupling study on apramycin. The anomeric doublet at highest field, J = 8.5 Hz, is coupled to a proton, δ 2.67 ppm, on a nitrogen-bearing carbon. This doublet cannot, therefore, be assigned to H_1 in the 4-aminoglucose ring. Since this signal is not present in the ^{1}H NMR spectrum of dihydroaprosamine, it is not involved in bonding to 2-deoxystreptamine. It follows that in the 1–1 link the oxygen linking the 4-aminoglucose to the bicyclic sugar is equatorial to the octadiose since only this arrangement results in an anomeric coupling of 8.5 Hz. In view of the small coupling constants found for the remaining anomeric protons, the anomeric oxygens must be axial in both these cases.

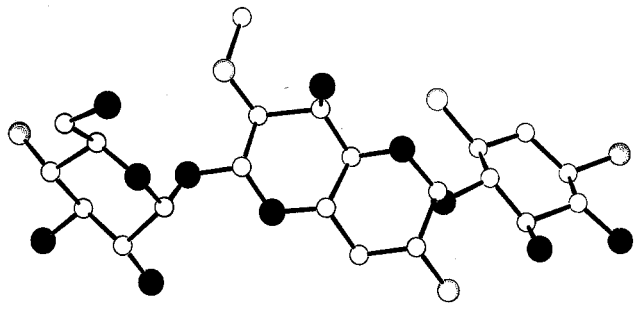


Figure 7. The conformation of apramycin in the crystalline state. Open, stipled, and solid spheres represent carbon, nitrogen, and oxygen atoms, respectively.

Tobramycin[4] contains a 2-amino-3-deoxyhexose moiety linked to O^4 of deoxystreptamine. The occurrence of a similar substitution pattern in the bicyclic sugar of apramycin prompted us to suggest that this position is the point of attachment of deoxystreptamine in apramycin also.[8]

In order to confirm the proposed structure and to determine the absolute configuration of the molecule, an x-ray diffraction study of apramycin was undertaken. Apramycin hydriodide crystallized from ethanol–water as colorless plates containing one molecule each of water and ethanol per molecule of antibiotic. The crystal parameters are given in Table II. The intensities of 2540 unique reflections were measured with copper radiation on a four-circle diffractometer. Four crystals were used since they deteriorated rapidly when exposed to x rays. The data were scaled together and an approximate absorption correction was made. The iodide ion was readily located from a sharpened Patterson map. The structure was solved by the heavy atom method and was partially refined by least squares.

The results of the x-ray study fully confirm the previously proposed structure, shown in Figure 7. The absolute configuration of the entire molecule may be deduced by correlating the x-ray structure with the known absolute configuration of the 4-aminoglucose moiety. All four six-membered rings in the molecule are in the chair conformation with all substitu-

ents equatorial, with the exception of the axial hydroxyl at C6′ and the glycosidic linkages at C1′ and C1″. The molecule is fully extended in the solid state and there appears to be a hydrogen bond joining the amino group at C2′ and the hydroxyl function at C5. Complete details of the crystal structure will be published elsewhere.

Experimental Section[9]

Apramycin Monohydrate. Apramycin was produced and isolated by modifications of previously described methods.[5] Final purification was by chromatography on Bio-Rex 70 (NH_4^+) resin with 0.1 N NH_4OH. The product crystallized from EtOH/H_2O (7:1) as the monohydrate, $[\alpha]^{25}D$ −164° (*c* 1, water). Differential thermal analysis indicated a fairly sharp melting point, 245–247 °C, not preceded by loss of water. Karl Fischer titration gave a value of 4.1%. Prior to microanalysis the monohydrate was dried to constant weight under vacuum at 110 °C.

Anal. Calcd for $C_{21}H_{41}N_5O_{11}$: C, 46.75; H, 7.66; N, 12.98; O, 32.62. Calcd for $C_{21}H_{43}N_5O_{11}$: C, 46.56; H, 8.00; N, 12.93; O, 32.50. Found: C, 46.47; H, 7.99; N, 13.05; O, 32.29.

Apramycin was recovered unchanged after refluxing with sodium borohydride and after attempted hydrogenation with either Pt or Pd. The ir spectrum showed no evidence of multiple bonds.

Penta-*N*-acetylapramycin. Apramycin monohydrate (10 g) was stirred overnight with methanol (1 l.) and acetic anhydride (100 ml). After removal of the volatiles penta-*N*-acetylapramycin was crystallized from acetonitrile–water–methanol (100:10:1) as an acetonitrile solvate which rapidly lost solvent of crystallization on air drying, $[\alpha]^{25}D$ +131.6° (*c* 1.255, water).

Anal. Calcd for $C_{31}H_{51}N_5O_{16}$: C, 49.66; H, 6.86; N, 9.34; O, 34.14. Found: C, 49.49; H, 7.07; N, 9.18; O, 34.30.

***N,O*-Permethylpenta-*N*-acetylapramycin.** Vacuum dried (24 mmHg, 100 °C) penta-*N*-acetylapramycin (7.5 g) was dissolved in dry (Linde 4A) DMF (500 ml) and treated at 5 °C (ice bath) with a 10% excess, 9.6 g, of a 50% suspension of NaH in mineral oil. After addition was complete, the reaction vessel was fitted with a gas drying tube and stirred magnetically for 60 min. Methyl iodide (28 g) was added dropwise (90 min) so that the internal temperature did not exceed 10 °C and the reaction mixture was then stored overnight at 5 °C. The reaction mixture was taken to dryness and the residue in water, 500 ml, was extracted with ether.

Lyophilization of the aqueous layer, after deionization with Amberlite MB3 resin, yielded *N,O*-permethylpenta-*N*-acetylapramycin (8.1 g) as an amorphous powder. ^1H NMR (Me_2SO-d_6) of the product indicated no *C*-methyl resonances.

Anal. Calcd for $C_{41}H_{71}N_5O_{16}$: C, 55.53; H, 8.04; N, 7.87; O, 28.76. Found: C, 55.31; H, 7.94; N, 7.78; O, 29.02.

High-resolution mass spectrum molecular ion *m/e* 889.4897 (calcd for $C_{41}H_{71}N_5O_{16}$, *m/e* 889.48955).

Isolation of 2-Deoxystreptamine from Apramycin. Apramycin monohydrate (5.6 g, 10 mmol) was refluxed for 16 h in 48% hydrobromic acid. The black residue, after removal of the volatiles, was shaken with 50 ml of 3 N HCl and filtered. The filtrate, after stirring for 30 min with 5 g of carbon, was again filtered and the filtrate taken to dryness. Lyophilization of an aqueous solution of the residue yielded a yellow powder which was chromatographed on a column of Bio-Rad AG 1 × 2 (OH^-) resin with water as eluent. The ninhydrin positive fractions were pooled and lyophilized yielding 2-deoxystreptamine, 1.45 g (90%), characterized as its crystalline 1,3-di-*N*-acetyl derivative, mp 290 °C dec (lit.[10] mp 292–293 °C).

Aqueous Acid Hydrolysis of Apramycin. Apramycin monohydrate (5.6 g, 10 mmol) was heated at 95 °C for 6 h in 4 N hydrochloric acid. Decolorizing carbon (10 g) was added and the heating continued for 30 min. The mixture was filtered and the filtrate reduced to a gum in vacuo (bath temperature <30 °C). The gum was dissolved in hot (70 °C) 6 N hydrochloric acid and hot 2-propanol added to slight turbidity. After standing at room temperature the solution deposited crystals, 2.5 g, of aprosamine tetrahydrochloride, $[\alpha]^{25}D$ +21.6° (*c* 1.27, water).

Anal. Calcd for $C_{15}H_{30}N_4O_7 \cdot 4HCl$: C, 34.36; H, 6.54; N, 10.69; Cl, 27.05. Found: C, 34.21; H, 6.68; N, 10.71; Cl, 27.32.

Sodium Borohydride Reduction of Aprosamine. A solution of aprosamine tetrahydrochloride (4.0 g) in water (50 ml) was adjusted to pH 9.0 (1 N NaOH) and to the cooled (5 °C) solution was added sodium borohydride (500 mg). After stirring for 4 h the mixture was adjusted to pH 1 (1 N HCl) and taken to dryness. Passage of the residue in water (10 ml) through a column of Bio-Rad 1 × 2 (OH^-) resin and elution of the column with water yielded, on lyophilization of the ninhydrin positive fraction, 2.4 g of dihydroaprosamine as a colorless powder. The mass spectrum showed a molecular ion at *m/e* 380. The material failed to crystallize.

Anal. Calcd for $C_{15}H_{32}N_4O_7$: C, 47.36; H, 8.48; N, 14.73; O, 29.44. Found: C, 47.59; H, 8.21; N, 14.52; O, 29.71.

Methanolysis of Apramycin on Dowex-50 (H^+). Dowex 50 × 4 (H^+) resin (100–200 mesh) was washed with 1 N hydrochloric acid and then with water. The washed resin was stirred for 1 h with three volumes of methanol followed by settling and decantation. This process was repeated three times and the resin finally washed with absolute (Linde 3A) methanol, filtered, and stored under absolute methanol. Apramycin monohydrate (2.7 g) was suspended in 500 ml of absolute methanol and 100 ml (bed volume) of the resin added to the suspension (equivalent to five sulfonic acid residues per amino group based on a stated capacity of 1.2 mequiv/ml). The supernatant was free of apramycin (ninhydrin) after stirring for 15 min. After packing into a glass Soxhlet thimble, the resin was extracted with refluxing absolute methanol (1 l.) for 14 days. (The reaction may be conveniently monitored by removing samples of resin at intervals and adding them to concentrated aqueous ammonia. TLC analysis of the supernatant reveals the extent of reaction.) The reaction was terminated and the methanol evaporated in vacuo yielding a trace (~15 mg) of polystyrene. Isolation of the compounds resulting from cleavage of apramycin on the resin is detailed below.

Methyl 4-Amino-4-deoxy-α-D-glucopyranoside (Fraction I). The resin from the Soxhlet thimble, after slurrying in water, was packed in a column (diameter 2.5 cm) containing 25 ml of acid-washed Dowex 50W × 4 resin (100–200 mesh). After washing with water (500 ml) the column was eluted with 1 N hydrochloric acid (1 l.). Removal of the volatiles from the pooled eluate yielded crude methyl 4-amino-4-deoxy-α-D-glucopyranoside hydrochlorides. This material was chromatographed on Dowex 1 × 2 (OH^-) resin. Elution with water yielded two well-separated fractions. The first fraction yielded the α anomer, mp 159–160 °C from ethanol–acetonitrile (lit.[7] mp 158–159 °C), $[\alpha]^{25}D$ +148° (*c* 1.1 water), *m/e* 193.

Anal. Calcd for $C_7H_{15}NO_5$: C, 43.52; H, 7.83; N, 7.25; O, 41.41. Found: C, 43.31; H, 7.78; N, 7.23; O, 41.57.

Tetraacetate from chloroform–hexane, mp 139–140 °C (lit.[7] mp 138–138.5 °C), $[\alpha]^{25}D$ +147.2° (*c* 1.0 $CHCl_3$).

Anal. Calcd for $C_{15}H_{23}NO_9$: C, 49.35; H, 5.95; N, 3.60. Found: C, 49.16; H, 6.20; N, 3.71.

The β anomer, although pure by TLC, could not be crystallized and was characterized as the tetraacetate, mp 196–197 °C from tetrahydrofuran, $[\alpha]^{25}D$ +7.9° (*c* 0.95, $CHCl_3$).

Anal. Calcd for $C_{15}H_{23}NO_9$: C, 49.35; H, 5.95; N, 3.60. Found: C, 59.20; H, 6.02; N, 3.76.

The combined yield of methylglycosides (α/β 3/2) was 91%.

2-Deoxystreptamine from Methanolysis of Apramycin (Fraction II). Elution of the methanolysis resin with 2.4 N hydrochloric acid (500 ml) yielded crude 2-deoxystreptamine dihydrochloride characterized as before, by conversion to its di-*N*-acetate, yield 15% based on apramycin. A number of minor components of the pooled elutate could not be characterized.

Methyl α- and β-Aprosaminides (Fraction III). On eluting the methanolysis resin with 4 N hydrochloric acid (1.5 l.), an 8:1 mixture of aprosamine and methyl aprosaminides was obtained. The bulk of the aprosamine was removed by crystallization, from 6 N hydrochloric acid–2-propanol. The mother liquor, containing aprosamine and the methyl aprosaminides, was taken to dryness, dissolved in water (2 ml), and chromatographed on a column of Dowex 1 × 2 (OH^-) resin (200–400 mesh), eluting with water. Aprosamine was bound irreversibly to the resin under these conditions. Methyl α-aprosaminide contaminated with ~10% of the β anomer eluted first followed by a mixture of both anomers. Finally almost pure (93+% by ^1H NMR) methyl β-aprosaminide was obtained. Neither compound could be induced to crystallize. Chromatography on a column of silica gel with methanol–chloroform–ammonium hydroxide mixtures led to essentially the same result as those with Dowex 1 × 2 resin. Rotations were obtained on the best available material and are not corrected for contamination by the other anomer.

Methyl α-aprosaminide, $[\alpha]^{27}D$ −14.5° (*c* 1.0, water).

Anal. Calcd for $C_{16}H_{32}N_4O_7$: C, 48.97; H, 8.22; N, 14.28; O, 28.54. Found: C, 49.10; H, 8.12; N, 14.22; O, 28.50.

High-resolution mass spectrum molecular ion *m/e* 392.22710 (calcd for $C_{16}H_{32}N_4O_7$, *m/e* 392.22707).

Methyl β-aprosaminide, $[\alpha]^{27}D$ +104° (*c* 1.1, water).

Anal. Calcd for $C_{16}H_{32}N_4O_7$: C, 48.97; H, 8.22; N, 14.28; O, 28.54. Found: C, 49.15; H, 3.09; N, 14.11; O, 28.70.

High-resolution mass spectrum molecular ion *m/e* 392.22730 (calcd for $C_{16}H_{32}N_4O_7$, *m/e* 392.22707).

Aprosamine from Methyl Aprosaminides. Aqueous acid hydrolysis of either the α or β anomer under conditions identical with those used for apramycin yielded aprosamine in almost quantitative yield.

Methyl Aprosaminides from Aprosamine. Absorption of aprosamine from a neutralized aqueous solution onto Dowex 50W × 4 (H^+) resin, washing the resin with methanol, and extraction of the resin with refluxing methanol for 72 h led to a 7% yield of methyl aprosaminides.

Apramycin Hydriodide. Apramycin monohydrate (5.6 g) was dissolved in water (100 ml) and titrated to pH 8.3 with aqueous hydriodic acid. The solution was lyophilized and the residue added to refluxing 90% ethanol (250 ml). After 10 min the solution was filtered, the small amount of solids being discarded. On cooling, the filtrate deposited crystals, 3.5 g, of apramycin hydriodide monoethanolate monohydrate.

Anal. Calcd for $C_{23}H_{50}N_5O_{13}I$: C, 37.75; H, 6.88; N, 9.57; O, 29.88; I, 17.35. Found: C, 37.54; H, 7.01; N, 9.48; O, 29.54; I. 17.62.

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Registry No.—Apramycin, 37321-09-8; apramycin hydriodide, 58617-23-5; penta-*N*-acetylapramycin, 58581-37-6; acetic anhydride, 108-24-7; *N,O*-permethylpenta-*N*-acetylapramycin, 58581-38-7; methyl iodide, 74-88-4; 2-deoxystreptamine, 2037-48-1; aprosamine tetrahydrochloride, 58581-39-8; dihydroaprosamine, 58581-40-1; methyl-4-amino-4-deoxy-α-D-glucopyranoside, 4097-95-4; methyl-4-amino-4-deoxy-α-D-glucopyranoside tetraacetate, 2595-35-9; methyl-4-amino-4-deoxy-β-D-glucopyranoside tetraacetate, 21209-55-2; methyl α-aprosaminide, 58617-24-6; methyl β-aprosaminide, 58068-66-9.

References and Notes

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(8) We thank Dr. K. F. Koch of these laboratories for bringing this similarity to our attention.
(9) Melting points are uncorrected. NMR spectra were measured in D_2O with $TSPD_4$ as internal reference on Varian HA-100 and HR-200 spectrometers. In several of the spectra the signal due to the internal reference has been offset so as to appear in the spectrum. TLC was carried out on Brinkmann silica gel G using MeOH, $CHCl_3$, NH_4OH (28%) mixtures. Visualization was by ninhydrin (0.3% in EtOH) for amines and by hypochlorite, ethanol, starch–iodide for both amines and amides. All solvents were reagent grade and were used as received except where noted. Where given oxygen analyses are by direct measurement. Mass spectra were measured on a CEC 21-110, EI, 70 eV. High-resolution spectra were obtained using photoplate recording. Periodate oxidations were monitored by treatment of aliquots of the reaction mixture with 2 N KI solution and titrating the liberated iodine with standardized sodium arsenite solution.
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Nitrones as Intermediates in the Synthesis of *N*-Hydroxyamino Acid Esters

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A new method of synthesis of *N*-hydroxyamino acid esters as substrates for *N*-hydroxy peptides is reported. Esters of amino acid *N*-benzylidene *N*-oxides (nitrones) were obtained by alkylation of *anti*-benzaldoxime with corresponding bromo acid esters or by reaction of triethylammonium salts of nitrone amino acid derivatives with benzyl or *p*-nitrobenzyl bromides. The nitrones were converted into *N*-hydroxyamino acid esters by treatment with hydroxylamine salts (or, in the case of *tert*-butyl esters, with free hydroxylamine), without hydroxylaminolysis of the ester group.

Hydroxyamic acids are known to occur in nature.[1] Some of them are structurally related to peptides. New compounds with *N*-hydroxyamide bonds are still being isolated.[2] They have various physiologic activities, but their biochemical function is not quite clear. They usually occur as metabolites of microorganisms,[1] although the presence of oxidized peptide bonds in cancer protein[3] has also been reported.

Therefore, the synthesis of *N*-hydroxy peptides seems to be of interest. In our program, which aims at elaborating special methods, we are using two routes.

The first consists of an unambiguous synthesis through *O*-benzyl hydroxylamine derivatives.[4] Since the second makes use of standard methods of peptide synthesis for selective N-acylation of *N*-hydroxyamino acid esters, it is, therefore, necessary to obtain these esters.

To date several synthetic methods have been described. Esterification of the *N*-hydroxyamino acid molecule is possible in alcohol in the presence of sulfuric acid,[5] hydrogen chloride[6] or, better, by using diazomethane.[6] All these methods call for substrates that are not readily available, or do not permit the synthesis of the widely employed *tert*-butyl, benzyl, and *p*-nitrobenzyl esters.

Another possibility of obtaining these esters is through formation of the hydroxylamine group in the ester molecule, as, e.g., nitro ester reduction[6] or amino acid ester oxidation.[7]

Of the numerous methods employed in the synthesis of hydroxylamine derivatives, the ones using nitrones seem to be most convenient.[8]

N-Hydroxyglycine was obtained as far back as 1896 from the nitrone prepared from chloroacetic acid and *anti*-benzaldoxime.[9] In 1967, Buehler[10] extended this procedure to other *N*-hydroxyamino acids and found sodium *syn*-benzaldoximate to be O-alkylated by various alkyl halides, whereas *anti*-benzaldoximate was readily N-alkylated.[11] He obtained several nitrones from α-bromocarboxylic acids and hydrolyzed these compounds under drastic conditions with concentrated hydrochloric acid to the corresponding *N*-hydroxyamino