B. Hydrolysis with Whole Snake Venom (Phosphodiesterase and 5′-Nucleotidase).—The reaction mixture containing 30 mg. of substrate, 200 mmoles of glycine buffer (pH 8.6), 50 mmoles of magnesium chloride, and 1 ml. of enzyme solution (total volume 2 ml.) was incubated at 37° for 4 hr. Examination of reaction mixture by paper chromatography (solvent B) together with authentic adenosine (R_f 0.19) and α-adenosine (R_f 0.12) showed no differentiation (R_f 0.15) and no spot corresponding to AMP was detected.

Treatment of N-Benzoyl-9-(5′-diphenylphosphoryl)-D-ribofuranosyladenine 2′,3′ Cyclic Carbonate with Sodium Methoxide.—The fully protected nucleotide III (90 mg.) was dissolved in 10 ml. of anhydrous methanol, followed by the addition of 1.5 ml. of 1 *N* sodium methoxide. After refluxing the reaction mixture for 70 min., the pH was adjusted to 4.0–4.5 with IRC-50 (H^+) resin. The resin was removed by filtration and the solvent was evaporated *in vacuo*. The traces of solvent were removed by codistillation with two 20-ml. portions of ethanol and three 30-ml. portions of acetone. A brownish solid material was obtained: 48 mg.; $\lambda_{max}^{H_2O}$ 260 mμ; paper chromatography, R_f 0.48 (solvent A, IO_4^- consuming); paper electrophoresis, R_{AMP} 0.68. This material was slightly contaminated with adenine (R_f 0.32, solvent A).

Enzymatic Hydrolysis of 9-(5′-O-Monophenylphosphoryl)-D-ribofuranosyladenine.—The brownish solid obtained above (30 mg.) was dissolved in a solution consisting of 200 mmoles of glycine–NaCl–NaOH buffer (pH 8.5), 60 mmoles of magnesium chloride, and 0.5 mg. of enzyme.[16] The total volume of the reaction mixture was 2.5 ml. This was incubated at 37° for 30 min. and the reaction was quenched by heating at 100° for 3 min. The whole solution was diluted with 20 ml. of water and one-third of the solution was applied to an ion-exchange column (Dowex IX8, chloride form, 0.8 × 10 cm). Washing with water gave nucleoside (adenosine), $TOD_{260\ m\mu}$ 22.9 (11.9%); elution with 0.006 *N* HCl and 0.2 *M* LiCl gave AMP, $TOD_{260\ m\mu}$ 5.9 (3.1%), and α-AMP, $TOD_{260\ m\mu}$ 54.3 (28.2%) (see Figure II). α-AMP fractions were collected and neutralized with 1 *N* LiOH solution and evaporated *in vacuo* to a small bulk below 25°. Further elution of the column with 0.06 *N* HCl and 0.2 *M* LiCl gave monophenyl-AMP, $TOD_{260\ m\mu}$ 54.3 (28.2%). To the sirupy solution of α-AMP was added 2 vol. of methanol and 30 vol. of acetone. The white precipitate, which appeared after storing the solution in a refrigerator at 0–5° for 3 hr., was collected by centrifugation and washed with ethanol and ether. The dried (3 mm. over P_2O_5 for 5 hr.) material weighed 5.8 mg. The purity estimated photometrically on the weight basis (calculated as having $\epsilon_{260\ m\mu}$ 14,500) was 72.8%. The main contaminant was water of crystallization and lithium chloride: paper chromatography, R_f 0.22 (solvent A), R_{AMP} 0.92; paper electrophoresis, $R_{adenosine}$ 1.5, R_{AMP} 1.03.

Anal. Calcd. for $C_{10}H_{12}LiN_5O_7P \cdot 6H_2O$: P, 6.63. Found: P, 6.91.

(16) Purified as in ref. 13 and tested for its activity to hydrolyze AMP totally to adenosine and inorganic phosphate.

Acknowledgment.—The authors wish to thank Mrs T. Tohma and Miss A. Maeda for elemental analyses

Synthesis and Characterization of 3,6-Diamino-3,6-dideoxy-D-idose[1]

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The stereoselective synthesis of the title compound utilizing the azide group as a potential amine function is described. Versatile intermediates such as methyl 2,3-anhydro-6-azido-6-deoxy-α-D-talopyranoside and 3,6-diazido-3,6-dideoxy-D-idose were synthesized. It is shown that the 3,6-diaminohexose gives a positive reaction in the Elson–Morgan color test and produces 10% the color of D-glucosamine. A study of the opening of epoxides with azide ion in model compounds in the D-talose series is included.

The discovery of several antibiotic and other biological substances possessing substantial therapeutic value has led to the unveiling of some unique types of amino sugars as their components.[2] With the advent of modern techniques, the constitution and gross structural elucidation of many of these new substances has become greatly facilitated. The proof of structure, stereochemistry, and mode of linkage of novel types of amino sugars found in such substances represents a challenge to the chemical investigator.

The present synthesis of 3,6-diamino-3,6-dideoxy-D-idose was undertaken for two purposes: firstly, to initiate a synthetic program involving the synthesis of several members of this novel class of diaminohexoses in anticipation of their eventual discovery in biological substances, and, secondly, to study the chemical properties of this representative member of 3,6-diamino-3,6-dideoxyhexoses. The synthesis of a 3,6-diamino-3,6-dideoxyhexose derivative has been recently described.[3] It is noteworthy to mention that the antibiotic kanamycin contains 3-amino-3-deoxy-D-glucose as well as 6-amino-6-deoxy-D-glucose among its components[4] and that the combination of amine functions in one molecule to produce a 3,6-diamino-3,6-dideoxyhexose is biogenetically possible. Furthermore, 3-amino-3-deoxyhexose derivatives are common constitutents of several antibiotics.[2]

The only diaminohexoses of biological origin are 2,6-diamino-2,6-dideoxy-D-glucose (neosamine C),[5–8] 2,6-diamino-2,6-dideoxy-L-idose (neosamine B, paromose),[9–11] and a 2,4-diamino-2,4,6-trideoxyhexose.[12] Owing to the unavailability of 2,6-diamino-2,6-dideoxyhexoses other than the D-gluco analog,[5] considerable time

(1) Preliminary communication, Abstracts of Papers, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, p. 2D.

(2) For a recent comprehensive review on this subject, see J. D. Dutcher, "Advances in Carbohydrate Chemistry," Vol. 18, Academic Press, Inc., New York, N. Y., 1963, p. 259.

(3) M. L. Wolfrom, D. Horton, and Y.-L. Hung, ref. 1, p. 3D. The D-*altro* stereochemistry has been assigned to this diamino sugar derivative by degradative studies (personal communication by Dr. D. Horton).

(4) (a) M. J. Cron, O. B. Fardig, D. L. Johnson, H. Schmitz, D. F. Whitehead, I. R. Hooper, and R. U. Lemieux, *J. Am. Chem. Soc.*, **80**, 2342 (1958); (b) M. J. Cron, O. B. Fardig, D. L. Johnson, D. F. Whitehead, I. R. Hooper, and R. U. Lemieux, *ibid.*, **80**, 4115 (1958); (c) K. Maeda, M. Murase, H. Mawatari, and H. Umezawa, *J. Antibiotics* (Tokyo), **11A**, 73 (1958); (d) H. Ogawa, T. Ito, S. Inoue, and S. Kondo, *ibid.*, **11A**, 166 (1958).

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(6) K. L. Rinehart, Jr., M. Hichens, K. Stiegler, K. P. Rover, T. P. Culbertson, S. Tatsuoka, S. Horii, T. Yamaguchi, H. Hitomi, and A. Miyake, *J. Am. Chem. Soc.*, **83**, 2964 (1961).

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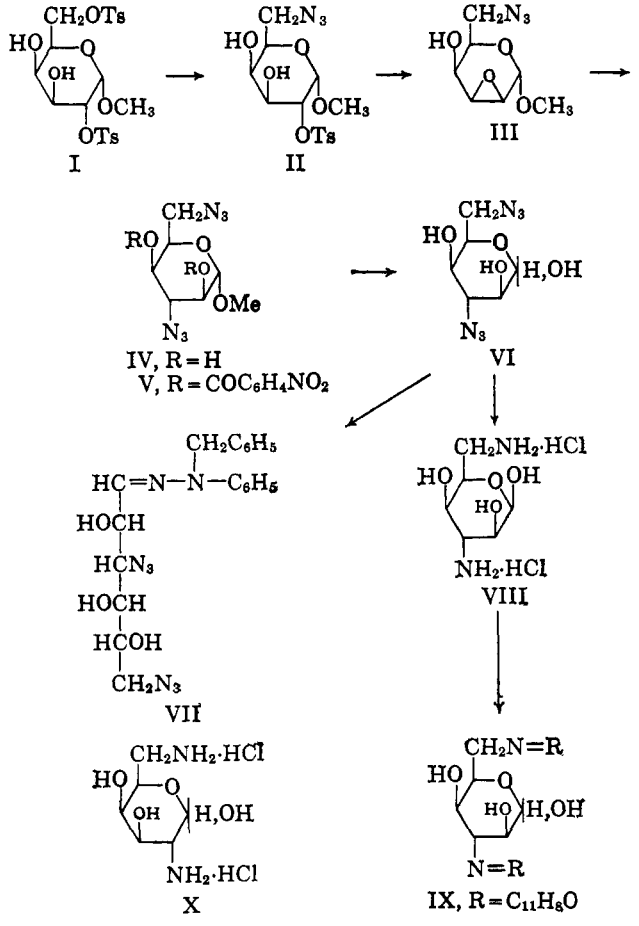
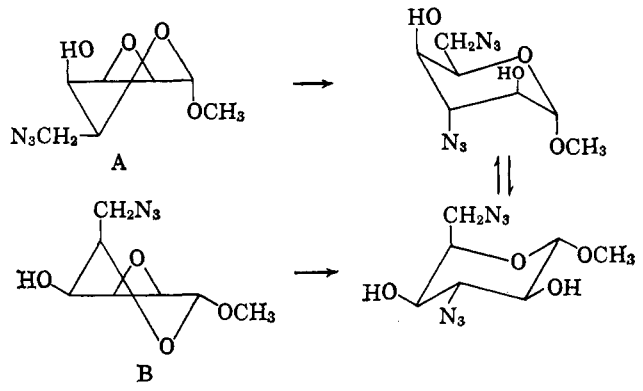
SCHEME I



SCHEME II



elapsed until suitable degradative methods were devised[9,11,13] to eludicate their total stereochemistry. The need for model compounds of this and related classes of unusual amino sugars is thus appreciated.

Reaction of methyl 2,6-di-*O*-*p*-tolylsulfonyl-α-D-galactoside[14] (I) with sodium azide in dimethyl sulfoxide or dimethylformamide overnight at 100° effected a selective displacement of the terminal tosyloxy function to give crystalline methyl 6-azido-6-deoxy-2-*O*-*p*-tolylsulfonyl-α-D-galactoside (II) in good yield. Treatment of the latter in refluxing ethanol with aqueous sodium hydroxide essentially according to a procedure employed by Charalambous and Percival[15] gave crystalline methyl 2,3-anhydro-6-azido-6-deoxy-α-D-talopyranoside (III). This substance was somewhat volatile as observed in samples kept in closed vials. Although its infrared and n.m.r. spectra were compatible with the expected structure and the substance was homogeneous chromatographically, a satisfactory analysis could not be obtained. Variations in the solvent of crystallization did not improve the analytical data but rather produced more ambiguous values. The crystalline product, however, was quite suitable for further use.

The opening of epoxides by azide ion was first described by VanderWerf and co-workers[16] who utilized aqueous dioxane as solvent. While our work was in progress, Guthrie and Murphy[17] reported on the first application of the opening of sugar epoxides with azide ion in Methyl Cellosolve containing ammonium chloride. In the present investigation, Methyl Cellosolve was found to be the most suitable solvent among several that were tried, including aqueous dioxane, with regards to product yield. The reaction of the epoxide III with sodium azide was done essentially according to the above workers.[17] Optimum conversion was attained after 3.5–4 hr. at reflux temperature whereby two components could be detected on thin layer chromatograms. Separation by silicic acid column chromatography afforded methyl 3,6-diazido-3,6-dideoxy-α-D-idopyranoside (IV) (fast component) in approximately 90% yield and presumably methyl 2,6-diazido-2,6-dideoxy-α-D-galactoside (slow component) in less than 5% yield which was not further investigated. Compound IV was obtained in the form of a colorless homogeneous sirup that gave a sirupy diacetate but a crystalline 2,4-di-*p*-nitrobenzoate derivative V (Scheme I). The specific optical rotation of V, $[\alpha]_D$ +70° ($CHCl_3$), offered the first evidence that the fast component was the idose rather than the galactose derivative and was compatible with values reported for methyl 3-amino-D- or -L-idoside derivatives.[18] The corresponding methyl 2-amino-2-deoxy-α-D-galactoside derivatives have considerably higher specific optical rotations. The n.m.r. spectrum of the diester V at 60 Mc. integrated for the correct number of hydrogens, the anomeric proton being a singlet at τ 5.05 ($J_{1,2} < 2$ c.p.s.) which indicates a possible *C1* conformation or a half-chair–boat form.[19] A theoretical consideration of the reaction path (Scheme II) indicates that the product of diaxial opening from the relatively less stable epoxide conformation A (2 axial substituents) is the experimentally obtained ido isomer. The alternative epoxide conformation B (2 equatorial substituents) would give the ido isomer only by a diequatorial opening which is a hindered process. A *1C* conformation has been suggested for α-L-idose pentaacetate[20] on the basis of n.m.r. and optical rotatory dispersion studies. A detailed study on the conformation of methyl idopyrano-

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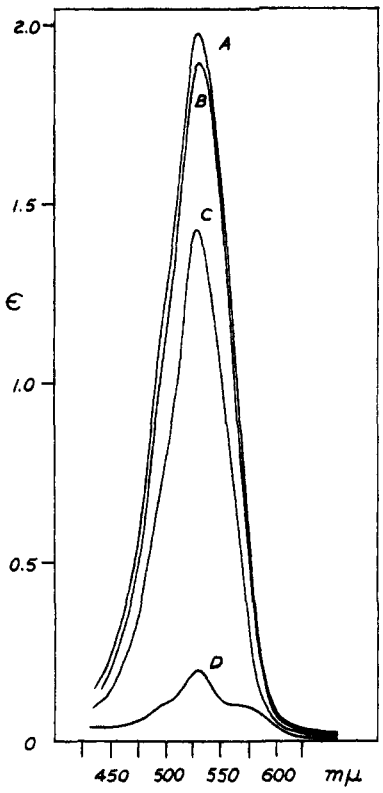

Figure 1.—Elson–Morgan color reaction (Rondle and Morgan modification[37]) of VIII (D), X (C), D-galactosamine hydrochloride (B), and D-glucosamine hydrochloride (A).

side derivatives is in progress in our laboratories. This involves the investigation of acetate esters of such derivatives by n.m.r. to assign axial and equatorial dispositions of the acetoxy groups[21-24] thereby defining the particular conformation as *1C* or *C1*.

The diazido glycoside IV was hydrolyzed to 3,6-diazido-3,6-dideoxy-D-idose (VI) using Amberlite IR-120 (H^+). Optimum conditions as indicated by thin layer chromatography experiments were found to be 2 hr. at 98°. Mineral acid hydrolysis caused some discoloration with the appearance of slower spots due to side products. The diazido hexose VI was obtained as a stable colorless sirup. It gave a crystalline benzylphenylhydrazone VII. A point of theoretical significance arose when the optical rotation of VII was measured. The so-called hydrazone rule as discussed by Hudson[25] and Votocek[26] assigns the configuration of C-2 of an aldose hydrazone depending on the sign of rotation. This rule agrees well with all published rotational data on sugar hydrazones and has been successfully used as a tool for the assignment of configuration at C-2 in several instances.[27] The hydrazone VII should have a positive rotation; the observed rotation, however, was $[\alpha]_D$ −35° in methanol. The same discrepancy has been reported in an enantiomorphic idose hydrazone, namely, 3-*O*-benzyl-6-deoxy-L-idose benzylphenylhydrazone by Wolfrom and Hanessian[28] which had a rotation of $[\alpha]_D$ +44° in ethanol. The opposite sign but comparable magnitude in the rotation of these hydrazones corroborates the validity of the argument. It appears therefore that idose hydrazones having certain substituents at C-3 do not conform with the hydrazone rule.[25,26] That the hydrazone VII was in an acyclic form and not in a cyclic modification was ascertained from a study of its n.m.r. spectra. The C-1 hydrogen appeared as a doublet centered at τ 3.30 ($J_{1,2}$ = 3 c.p.s.) in agreement with the results of Wolfrom and coworkers[29] who recently studied the n.m.r. spectra of several hexose *p*-nitrophenylhydrazones. It is noteworthy that galactose methylphenylhydrazone tetraacetate and glucose benzylphenylhydrazone used as model compounds in our work, both with a D-*glycero* configuration at C-2, showed the C-1 hydrogen at τ 3.50 and 3.08, respectively, but with identical J values of 4.5 c.p.s. In addition to the conclusive evidence concerning the acyclic nature of the hydrazone VII, the present n.m.r. study afforded further evidence that in this hydrazone the C-1 hydrogen is in a different environment from those in the model hydrazones.

The diazido hexose VI was hydrogenated in aqueous methanol containing hydrochloric acid and 20% Pd–C. The resulting 3,6-diamino-3,6-dideoxy-D-idose dihydrochloride (VIII) was obtained as a colorless crystalline solid. It was stable in the dry state, but acquired a yellow color when exposed to air, and eventually decomposed. Its homogeneity was confirmed by thin layer chromatography. A stable crystalline 3,6-di(2-hydroxynaphthylidenamino) derivative IX was obtained in the usual way. The optical rotational properties, melting point, and other properties discussed below showed VIII to be different from 2,6-diamino-2,6-dideoxy-D-galactose[30] (X), although both compounds gave similar mobilities in at least one solvent system.

When subjected to the ninhydrin degradation,[31] VIII remained mostly unchanged while X was degraded to several components as evidenced by thin layer chromatography.

Although the Elson–Morgan color reaction[32] has been widely used in the characterization of 2-amino sugars,[33] its application to 3-amino sugars has been limited. In fact, 3-amino sugars were considered to be unreactive in the test since 3-amino-3-deoxy-D-altrose gave no color.[34] Ogawa and co-workers[35] claimed that 3-amino-3-deoxy-D-glucose isolated from kanamycin gave as much color as glucosamine when measured at 530 mμ but that the color faded on standing overnight. Very recently Baer[36] discussed a brief study of the behavior of 3-amino sugars in the Elson–Morgan reaction. Only 3-amino-3-deoxy-D-talose was

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(26) E. Votocek, *Collection Czech. Chem. Commun.*, **3**, 250 (1931).
(27) See, for example, E. Walton, J. O. Rodin, C. H. Stammer, F. W. Holly, and K. Folkers, *J. Am. Chem. Soc.*, **80**, 5168 (1958).
(28) M. L. Wolfrom and S. Hanessian, *J. Org. Chem.*, **27**, 1800 (1962).
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(31) P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem. Biophys.*, **52**, 373 (1954).
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(33) A. B. Foster and D. Horton, "Advances in Carbohydrate Chemistry," Vol. 14, Academic Press, Inc., New York, N. Y., 1959, p. 264.
(34) M. W. Whitehouse, P. W. Kent, and C. A. Pasternak, *J. Chem. Soc.*, 2315 (1954).
(35) H. Ogawa, T. Ito, S. Kondo, and S. Inoue, *Bull. Agr. Chem. Soc. Japan*, **23**, 289 (1959).
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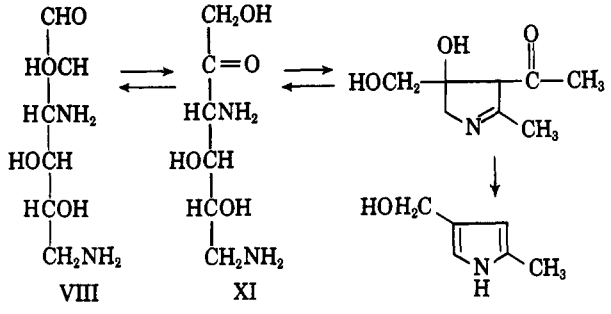
found[36] to give the same color production as glucosamine while 3-amino-3-deoxy-D-mannose, -D-galactose, and -D-glucose analogs gave only 10% color. The variation in results in the Elson–Morgan color reaction with different modifications is discussed in the literature.[33]

It was of interest to compare the behavior of VIII and X under the conditions of the Elson–Morgan reaction (Rondle and Morgan modification[37]). As depicted in Figure 1, VIII showed a maximum at 530 mμ in addition to two humps at 493 and 580 mμ and produced only about 10% the color of glucosamine hydrochloride. A normal curve with a diminished absorption (73% color production) was given by X.

The Elson-Morgan reaction requires the presence of an α-aminoaldehyde or carbonyl function in the sugar portion, since the initial step is the condensation with 2,4-pentanedione. In the case of VIII (and other 3-amino sugars) where the requisite structural requirements are absent a preliminary partial transformation to a 3,6-diamino-3,6-dideoxyketose (XI) under the influence of the strong alkaline conditions must take place (Scheme III). The ketose derivative would

SCHEME III

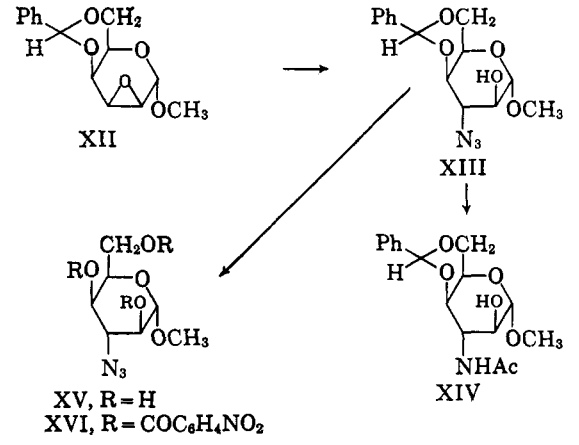


then be capable of condensing with the reagent in the usual manner to give the possible intermediates shown in Scheme III which are presumably responsible for the color produced.

In view of the apparent stereoselective ring opening of the 2,3-anhydro taloside derivative III with sodium azide, it was of interest to investigate the same reaction in a conformationally more rigid system such as methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-talopyranoside[38] (XII). Reaction of XII with sodium azide in 95% Methyl Cellosolve afforded a crystalline azido sugar (XIII) in 92% yield. Reduction of the azide function followed by N-acetylation gave the known methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-idopyranoside (XIV)[18a] (Scheme IV). The predominant attack at C-3 and the formation of the 3-azido derivative by diaxial opening of the epoxide ring is not surprising since such fused-ring systems do not have the freedom of conformational changes as in the unsubstituted analogs. The initially formed product must assume a *C1* or a half-chair conformation. An examination of the n.m.r. spectrum of XIII showed the anomeric hydrogen at τ 5.13 ($J_{1,2} < 2$ c.p.s.) indicating a dihedral angle in the vicinity of 90° such as would be the situation in a diequatorial C-1–C-2 arrangement of hydrogens (*C1* conformation) or in a quasi boat (half-chair) conformation with a staggered arrangement of hydrogens at C-1 and C-2.

(37) C. J. M. Rondle and W. T. J. Morgan, *Biochem. J.*, **61**, 586 (1954).
(38) M. Gyr and T. Reichstein, *Helv. Chim. Acta*, **28**, 226 (1945).

SCHEME IV



Selective acid hydrolysis of the benzylidene group in XIII afforded sirupy methyl 3-azido-3-deoxy-α-D-idopyranoside (XV) which was converted to the crystalline tri-*p*-nitrobenzoate derivative (XVI). The n.m.r. spectrum of XVI also showed a very small coupling constant $J_{1,2} < 2$ c.p.s. for the anomeric hydrogen at τ 4.80 indicating a *C1*, half-chair, or a boat conformation that would satisfy both steric and geometrical requirements. These tentative arguments concerning the conformation of the various methyl 3- and 3,6-substituted idopyranosides must await further confirmation through the study of a larger number of derivatives by more detailed n.m.r. investigations and other physical methods.

Experimental

Melting points are uncorrected. The ion-exchange resins used were products of Rohm and Haas Co., Philadelphia, Pa. Silica gel for thin layer chromatography was type G obtained from Brinkman Instruments, New York, N. Y. MN-Cellulose 300G powder was used for thin layer chromatography, a product of Machery, Nagel and Co., Dürem, Germany. Unless otherwise stated, components were detected with a spray containing 5% each of ammonium molybdate, sulfuric acid, and phosphoric acid after heating the plate for 5–10 min. at 110° or 1% potassium permanganate in 0.1 *N* sulfuric acid. N.m.r. spectra were obtained from deuteriochloroform solutions except for glucose benzylphenylhydrazone where the solvent was d_6-DMSO–$CDCl_3$ (2:3).

Methyl 6-Azido-6-deoxy-2-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (II).—A solution containing 25 g. (0.05 mole) of methyl 2,6-di-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside[14] and 21.4 g. (0.33 mole) of sodium azide in 288 ml. of dry dimethylformamide was heated at 100° overnight. The cooled solution was diluted with a mixture of acetone and ether (1:1), the salts were filtered, and the filtrate was evaporated to a mobile yellow liquid. This was dissolved in 100 ml. of ethanol and added dropwise with rapid stirring to 1500 ml. of ice–water when the product precipitated as a colorless crystalline solid. The product was washed with cold water and dried to give 13 g. (70%) of colorless crystals, m.p. 120–122°. Recrystallization from a mixture of acetone, ether, and petroleum ether (b.p. 30–60°) afforded pure product: m.p. 124–125°; $[\alpha]^{23}D$ 90° (*c* 1, chloroform); infrared absorption spectral data, λ_{max}^{KBr} 2110 (azide), 1180, 1190 cm.$^{-1}$ (sulfonate).

Anal. Calcd. for $C_{14}H_{19}N_3O_7S$: C, 45.03; H, 5.12; N, 11.25; S, 8.58. Found: C, 44.87; H, 5.29; N, 11.61; S, 8.93.

Methyl 2,3-Anhydro-6-azido-6-deoxy-α-D-talopyranoside (III).—To a refluxing solution of II (4.5 g.) in 20 ml. of ethanol containing 1 drop of phenolphthalein was added 2 *N* NaOH dropwise until a permanent pink color was observed. The solution was refluxed a few more minutes after which time it was evaporated to dryness. The residue was extracted with ether, the salts were

removed, and the solution was evaporated to a sirup which crystallized. The crystals were triturated with a mixture of ether and pentane, filtered, and washed with the same mixture to give 1.2 g. of product, m.p. 61–63°; a further 265 mg. was obtained from the mother liquors. A sample was recrystallized from ether and pentane by allowing the solution to evaporate slowly to a small volume to give sticky crystals: m.p. 65–67°; infrared absorption spectra data, λ_{max}^{KBr} 2100 cm.$^{-1}$ (azide), no sulfonate absorption. A sample was homogeneous on silica gel plates in benzene–ethanol (10:2) and showed a single spot of medium velocity. The n.m.r. spectrum of III (dried *in vacuo* at room temperature) in $CDCl_3$ integrated for the correct number of hydrogens and agreed with the structure. The product was somewhat volatile as observed in stoppered glass vials.

Methyl 3,6-Diazido-3,6-dideoxy-α-D-idopyranoside (IV).—A solution containing 450 mg. (2.25 mmoles) of III, 240 mg. (2.5 mmoles) of ammonium chloride, and 500 mg. (8 mmoles) of sodium azide in 10 ml. of Methyl Cellosolve containing 10% water was refluxed for 4 hr. The solution was cooled and diluted with 150 ml. of acetone, the salts were filtered, and the filtrate was evaporated to a pale yellow sirup (0.5 g.). This product showed essentially two spots of medium mobility on silica gel plates in benzene–ethanol (10:3). The sirup was fractionated on a silicic acid[39] column (2.5 × 30 cm.) using the same solvent to give the fast moving major component IV as a homogeneous sirup (477 mg.). A slower minor component was also obtained as a sirup (10 mg.) and was not investigated further.

Methyl 3,6-Diazido-3,6-dideoxy-2,4-di-*O*-*p*-nitrobenzoyl-α-D-idopyranoside (V).—A solution of IV (75 mg.) in 2 ml. of pyridine was treated at 0° with180 mg. of *p*-nitrobenzoyl chloride in 1 ml. of pyridine, and the resulting suspension was stirred overnight at room temperature. The mixture was poured into 70 ml. of ice–water, and the solid was washed with cold water and petroleum ether and dried to give 135 mg. of product. Recrystallization from a mixture of acetone, ether, and pentane gave pure material: m.p. 156–157°; $[\alpha]^{24}D$ 70° (*c* 0.94, chloroform); infrared absorption spectra data, λ_{max}^{KBr} 2200 (azide), 1740 (ester), 1530, 1350 cm.$^{-1}$ (nitro).

Anal. Calcd. for $C_{21}H_{18}N_8O_{10}$: C, 46.50; H, 3.34; N, 20.69. Found: C, 46.81; H, 3.48; N, 20.36.

3,6-Diazido-3,6-dideoxy-D-idose (VI).—A solution of V (265 mg.) in 10 ml. of water containing 5 ml. of Amberlite IR-120 (H^+) was refluxed with stirring for 2 hr. The filtered solution was evaporated to a pale yellow sirup which was contaminated with a trace of starting material as shown by thin layer chromatography (silica gel, benzene–methanol, 10:1.5). Purification by silicic acid column chromatography gave VI as a homogeneous, colorless, ether-soluble sirup (208 mg.). This material which failed to crystallize was stored at 5°.

3,6-Diazido-3,6-dideoxy-D-idose Benzylphenylhydrazone (VII).—A solution of sirupy VI (46 mg.) in 6 ml. of ethanol containing 100 mg. of sodium acetate, 50 mg. of benzylphenylhydrazine hydrochloride, and enough water to give a homogeneous solution, was refluxed gently for 2 hr. The cooled solution was evaporated to dryness, the residue was extracted with chloroform, and the extracts were washed with water and dried. Evaporation of the extracts gave a pale yellow sirup which crystallized from a mixture of ether and pentane at 5° to give 44 mg. of product. Recrystallization was effected from the same mixture to give pure product: m.p. 96–97°, $[\alpha]^{24}D$ −35° (*c* 0.43, methanol).

Anal. Calcd. for $C_{19}H_{22}N_8O_3$: C, 55.60; H, 5.40; N, 27.30. Found: C, 56.05; H, 5.25; N, 27.44.

3,6-Diamino-3,6-dideoxy-β-D-idose Dihydrochloride (VIII).—To a solution containing 151 mg. of VI and 50 mg. of 20% palladium on carbon in a mixture of methanol and water (30 ml., 6:1) was added 3 ml. of methanol containing 50 mg. of hydrogen chloride, and the whole was hydrogenated through a medium-pored sparger for 1 hr. The filtered colorless solution was evaporated to dryness, the residue was dissolved in a small volume of water, and excess alcohol was added. A small amount of oily residue was separated, and the turbid supernatant was evaporated to a small volume whereby the product crystallized. Attempted filtration afforded a gummy residue. The cooled solution containing the crystalline product was transferred to a centrifuge tube, and the crystals were washed with ethanol by decantation and finally dried in a stream of nitrogen; yield 20 mg. Addition of ether to the alcoholic supernatants gave a further 55 mg. of crystalline product: total yield 75 mg. (45%); m.p. 140° dec.; $[\alpha]^{24}D$ 12.85° → 16.5° (30 min.) → 18.4° (1.5 hr.) → 16.5 ° (5 hr.) → 7.35° (16 hr.); X-ray powder diffraction pattern data,[40] 2.988 w, 2.655 m, 2.318 s, 2.19 w, 2.094 w, 2.071 w, 2.029 w, 1.946 w, 1.887 w, 1.855 vw, 1.738 w br, 1.69 w br. The product was homogeneous on cellulose thin layer plates (*t*-butyl alcohol–acetic acid–water, 2:1:1) and showed a single spot of slow mobility. It could be detected with the ninhydrin, silver nitrate, and aniline hydrogen phthalate reagents. The crystals were hygroscopic, acquired a yellow color when exposed to air, and eventually decomposed.

Solutions of VIII (1 mg.), X (1 mg.), D-galactosamine hydrochloride (1 mg.), and D-glucosamine hydrochloride (1 mg.) in a mixture of 0.96 ml. of water and 0.04 ml. of pyridine containing 2% of ninhydrin were applied to cellulose thin layer plates. The plates were heated at 100° for 80 min.[41] The plates were then allowed to cool to room temperature and placed in the *t*-butyl alcohol–acetic acid–water (2:2:1) solvent system. Only VIII remained mostly unchanged, whereas the other compounds were degraded.

Solutions of VIII (1.5 mg.), X (1.5 mg.), D-glucosamine hydrochloride (0.5 mg.), and D-galactosamine hydrochloride (0.5 mg.) were used in the Rondle and Morgan modification[37] of the Elson–Morgan[32] reaction (See Figure 1).

3,6-Di(2-hydroxynaphthylideneamino)-3,6-dideoxy-D-idose (IX).—To a solution of VIII (20 mg.) in a mixture of 0.5 ml. of water and 5 ml. of methanol was added 25 mg. of sodium acetate and 76 mg. of 2-hydroxynaphthaldehyde. After standing in the dark for 3.5 hr., the yellow solution was diluted with cold water, and the yellow solid was filtered and washed with cold water and then pentane, to give 40 mg. of crude product. The latter was purified by preparative thin layer chromatography (silica gel, benzene–methanol, 10:2) and a faster moving product was thus separated from a slower moving dark yellow-brown impurity. The product zone was eluted with methanol and the solution was evaporated to give the yellow crystals. Recrystallization from a mixture of methanol, ether, and pentane at 5° gave 8 mg. of pure product, m.p. 175–177° dec.

Anal. Calcd. for $C_{28}H_{26}N_2O_6$: N, 5.76. Found: N, 5.54.

Methyl 3-Azido-4,6-*O*-benzylidene-3-deoxy-α-D-idopyranoside (XIII).—A solution containing 530 mg. of XII, 107 mg. of ammonium chloride, and 1 g. of sodium azide in 50 ml. of Methyl Cellosolve was refluxed 4 hr. The cooled solution was diluted with excess acetone, the salts were filtered, and the filtrate was evaporated to a crystalline residue. Recrystallization of the product from a mixture of acetone, ether, and pentane gave the pure product in two crops: 615 mg. (90%), m.p. 153–154°, $[\alpha]^{27}D$ 105° (*c* 0.94, chloroform).

Anal. Calcd. for $C_{14}H_{17}N_3O_5$: C, 54.72; H, 5.58; N, 13.68. Found: C, 54.75; H, 5.44; N, 13.57.

Methyl 3-Acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-idopyranoside (XIV).—To a refluxing ethereal suspension of lithium aluminum hydride (0.2 g.) was added XIII (190 mg.) in 30 ml. of ether dropwise, and refluxing was continued for 2 hr. Excess reagent was decomposed with the addition of 2 ml. of ethanol, and the suspension was evaporated to dryness. The residue was extracted with methanol several times (50 ml.), and the extracts were treated with 3 ml. of 2 *N* acetic acid. The filtered clear solution was treated with 3 ml. of acetic anhydride, kept at room temperature overnight, and diluted with 5 ml. of water; the solution was evaporated to a crystalline mass. Trituration with cold water gave the crude product which was recrystallized from methanol to give 166 mg. of pure product: m.p. 224–225°, $[\alpha]^{24}D$ 50° (*c* 1, chloroform); lit.[18a] m.p. 230–231°, $[\alpha]^{24}D$ 51° (*c* 0.5, chloroform).

Methyl 3-Azido-3-deoxy-2,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-idopyranoside (XVI).—A solution of XIII (150 mg.) in a mixture of 20 ml. of acetone and 5 ml. of water containing 10 ml. of Amberlite IR-120 (H^+) was refluxed for 1.5 hr. The turbid solution was filtered; the filtrate was evaporated to a semicrystalline residue which was filtered from water to give 12 mg. of starting material. The residual solution which contained a slow moving impurity in addition to the product moving as a medium spot

(39) Silicic acid, Grade 12, a product of Davison Chemical Co., Baltimore 3, Md.

(40) Interplanar spacing, Cu Kα radiation. Relative intensity estimated visually: s, strong; m, medium; w, weak; v, very; br, broad.

(41) "Methods in Carbohydrate Chemistry," Academic Press, Inc., New York, N.Y., Vol. 2, 1963, p. 218.

was evaporated to a colorless sirup (125 mg.). The latter was fractionated on a silicic acid column (2 × 25 cm.) using benzene–methanol (10:3) to give 120 mg. of a product (XV) as a homogeneous sirup, which was dissolved in 3.5 ml. of pyridine, cooled to 5°, and treated with 350 mg. of *p*-nitrobenzoyl chloride. The suspension was kept at room temperature overnight and poured into ice–water, and the solid was washed with water, aqueous bicarbonate, and finally with water to give 150 mg. of crude product. Recrystallization from a mixture of acetone, ether, and pentane gave pure material: m.p. 149–150°, $[\alpha]^{24}D$ 15° (*c* 2, chloroform).

Anal. Calcd. for $C_{28}H_{22}N_6O_{14}$: C, 50.50; H, 3.34; N, 12.65. Found: C, 50.79; H, 3.52; N, 12.01.

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Nuclear Magnetic Resonance Spectroscopy of Acetylated Methyl Glycopyranosides of Aminohexoses. Characterization of an Aminohexose from Septacidin

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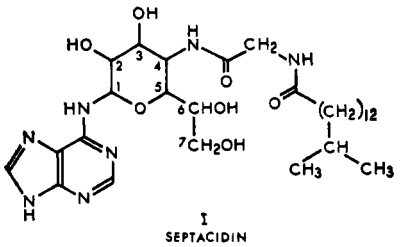
Acid hydrolysis of septacidin (1) yielded a monoaminoheptose which was subsequently converted to the monoaminohexose. Chemical proof demonstrated the aminohexose was a 4-amino-4-deoxy-L-glucose. N.m.r. study of the β anomer of the methyl glycoside tetraacetate derivative (2) of this aminohexose, utilizing proton–proton spin decoupling at 60 Mc. and solvent effect studies at 100 Mc., established the position of the acetamido group and the configuration of the ring protons. The 100-Mc. spectra of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-β-L-glucopyranoside (2) in deuteriochloroform and acetonitrile exhibited selective solvent–solute interactions.

The cytotoxic and antifungal agent, septacidin (1),[2] contains a monoaminoheptose moiety, $C_7H_{15}NO_6$.[3] Periodate oxidation of a suitable derivative of this



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SEPTACIDIN

aminoheptose cleaved off a terminal hydroxymethyl group, and subsequent reduction with sodium borohydride followed by acidic hydrolysis yielded a monoaminohexose, $C_6H_{13}NO_5$. The crystalline β anomer of the methyl glycoside tetraacetate derivative of this aminohexose was subjected to n.m.r. spin-decoupling studies.

Prior to this work, chemical studies and n.m.r. spectra of derivatives of the aminohexose indicated that this sugar had an unbranched aldose structure with the amino group at position 3 or 4 and suggested that it had a *gluco* or *ido* configuration. Thus, these proton decoupling studies sought (1) to establish the position of the amino group in the aminohexose and (2) to determine the configuration of this sugar.

Simultaneously with this examination by n.m.r., a chemical proof of the structure and configuration of this aminohexose was obtained by a comparison of the physical properties of the α anomer of the methyl glycoside tetraacetate derivative with those of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-D-glucopyranoside.[4a] These two compounds were identical (melting point, $[\alpha]D$, infrared and n.m.r. spectra, and X-ray diffraction pattern) except for the sign of rotation.[4b] Thus, the aminohexose was shown to be 4-amino-4-deoxy-L-glucose. This conclusion agrees with that arrived at by the n.m.r. studies described below.

Since previous n.m.r. studies[5-16] have demonstrated the utility of this spectroscopic technique in carbohydrate chemistry, the configuration of the ring protons and the positions of the various substituents of the methyl glycoside tetraacetate derivative 2 of the aminohexose were established by these methods, *i.e.*, proton–proton spin decoupling and the use of different solvents at 60 Mc. and 100 Mc. The magnitude of the coupling constant[5,6,17] was the criterion for determining whether adjacent methine protons on carbons 1 through 5 are coupled axial–axial or axial–equatorial. Although the possibility of the methyl glycoside tetraacetate derivative 2 being a furanose structure does exist, the proton n.m.r. spectrum indicates the pyranose

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(4) (a) This sample was kindly provided by Dr. E. J. Reist, Stanford Research Institute, Menlo Park, Calif. The unequivocal synthesis of this compound has been reported: E. J. Reist, R. R. Spencer, B. R. Baker, and L. Goodman, *Chem. Ind.* (London), 1794 (1962). (b) M. H. von Saltza, J. Reid, and J. D. Dutcher, in press.

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