sulfate. After filtration and evaporation of solution, a crystalline product obtained from a benzene–petroleum ether mixture was identical with 5,6-anhydro-1,2-*O*-isopropylidene-β-L-idofuranose,[9] since the melting point of 74° remained undepressed when admixed with an authentic sample.

Reduction of Compound III.—After 2 g. of III was dissolved in 25 ml. of anhydrous tetrahydrofuran and cooled to 0°, 1 g. of lithium aluminum hydride was added. Within 0.5 hr. the mixture was warmed to 25°, then stirred for 2 hr., and refluxed an additional 48 hr. Excess hydride was destroyed carefully by a slow addition of water. After 100 ml. of a saturated aqueous sodium sulfate solution was added, the mixture was poured into a separatory funnel and mixed with a dilute chilled hydrochloric acid solution until the solids were dissolved. The tetrahydrofuran layer was drawn off, and the remaining aqueous phase was extracted with four 20-ml. portions of chloroform. The chloroform extracts were combined with the tetrahydrofuran layer, washed three times with water, and dried over anhydrous magnesium sulfate. A sirupy product obtained after filtration and evaporation of solvent was dissolved in 25 ml. of absolute ethanol and hydrogenated at 50 p.s.i. for 10 hr. at 25° with palladium on carbon. A portion of the sirupy product obtained after hydrogenation crystallized from a benzene–petroleum ether mxiture as long needles; the yield was 150 mg., m.p. 95°, $[\alpha]^{25}D$ −9.0 (*c* 0.50, chloroform).

A mixture melting point of this product and an authentic sample of 5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose remained undepressed.

Anal. Calcd. for $C_9H_{16}O_5$ (204.22): C, 52.94; H, 7.89. Found: C, 53.14; H, 7.79.

6-*O*-Benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuran-5-enose (IV).—Compound III (2 g.) was dissolved in 15 ml. of alcohol-free chloroform. The mixture was cooled to −5° and 8 ml. of methanol containing 12.5% sodium methylate was added. The solution was stirred for 1 hr. at 0° and then at 25° for an additional 16 hr. A saturated solution of potassium bicarbonate was added, and the mixture was evaporated to remove methanol. The residue was extracted four times with 25-ml. portions of chloroform and dried over anhydrous magnesium sulfate. The solution was filtered and evaporated under reduced pressure to a sirup which crystallized from a mixture of benzene–petroleum ether in long fine needles to yield 1.25 g. (95%) of product with m.p. 113°, $[\alpha]^{25}D$ −47.3° (*c* 1.15, chloroform). Paper chromatograms of IV in irrigants A and B when sprayed with indicator C revealed a single component. Thin layer chromatograms in irrigants E and F indicated that IV was not contaminated with any foreign organic material. It absorbed bromine from a bromine–carbon tetrachloride solution and instantaneously decolorized a potassium permanganate solution indicating unsaturation; ν_{max}^{KBr} 1310 (m) and 975 (s), *trans* double bond; and 810 and 948 cm.$^{-1}$, vinyl ether.

Anal. Calcd. for $C_{16}H_{20}O_5$ (292.33): C, 65.74; H, 6.89. Found: C, 65.92; H, 6.82.

Reductive Ozonolysis of IV.—A 20-ml. portion of purified ethyl acetate was cooled to −70° in a Dry Ice–acetone bath and ozone was bubbled through the solution until a permanent blue color was maintained. A solution containing 30 mg. of IV, dissolved in 1 ml. of cold ethyl acetate, was added to the ozone-saturated ethyl acetate solution. Excess ozone was immediately evaporated and the remaining ethyl acetate solution containing the respective ozonide was concentrated nearly to dryness. After a 10-ml. portion of methylene chloride and 50 mg. of Lindlar catalyst[18] were added to the ozonide, the mixture was agitated for 0.5 hr. in a hydrogen atmosphere. Filtration and evaporation of the hydrogenated solution gave a sirupy product which, on paper chromotography in irrigants A and B, developed with indicator D, migrated identically with 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose[13] (V). The latter was converted to 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose phenylhydrazone[13,14] (VI), m.p. 140°, $[\alpha]^{25}D$ −42.0° (*c* 2.0, chloroform).

3-*O*-Acetyl-6-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuran-5-enose.—A 300-mg. portion of IV was dissolved in 3 ml. of pyridine, cooled to 0°, and 1 ml. of acetic anhydride was added. After 10 hr. at 0°, the reaction mixture was warmed to 25° for an additional 9 hr., then poured into ice–water, and stirred until the gummy acetate settled. A chloroform solution containing the acetyl derivative was washed with water and dried over anhydrous sodium sulfate. After filtration and evaporation of solution, the acetyl derivative, obtained as a semisolid, crystallized from benzene–petroleum ether to give a product with m.p. 78°, $[\alpha]^{25}D$ −34.5° (*c* 1.05, chloroform).

Anal. Calcd. for $C_{18}H_{22}O_6$ (334.36): C, 64.66; H, 6.63; CH_3CO, 12.87. Found: C, 64.83; H, 6.72; CH_3CO, 12.63.

5-Deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose.—Compound IV (200 mg.) was dissolved in 20 ml. of absolute ethanol containing 1 g. of palladium on carbon. The mixture was subjected to 50 p.s.i. of hydrogen pressure in a Parr hydrogenation apparatus and shaken at 25° for 8 hr. Filtration of the reaction mixture and evaporation of solvent gave a sirup, which spontaneously crystallized from a mixture of benzene–petroleum ether to yield 130 mg. (90%) of product with m.p. 95°, $[\alpha]^{25}D$ −10.0° (*c* 0.71, in chloroform). An X-ray powder diffraction pattern of the crystalline product was identical with an authentic sample of 5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose.

Anal. Calcd. for $C_9H_{16}O_5$ (204.22): C, 52.94; H, 7.89. Found: C, 53.05; H, 7.98.

Acknowledgment.—This work was supported in part by the Department of Health, Education, and Welfare.

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6-Deoxy-6-hydrazinoamylitol and 6-Deoxy-6-hydrazinocellulose[1]

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Direct tosylation of amylitol and cellulose to (degree of substitution) 0.65 and 0.64, respectively, and reaction with hydrazine leads to 6-deoxy-6-hydrazinoamylitol of D.S. 0.40 and 6-deoxy-6-hydrazinocellulose of D.S. 0.50. Different oxidants remove the hydrazino groups with formation of 6-deoxyglycans which on hydrolysis produce D-glucose and D-quinovose. Reaction of the 6-deoxy-6-hydrazinoglycans with D-glucose or D-glucono-1,4-lactone produces the appropriate derivatives in quantitative yields.

Introduction of hydrazino groups into the linear polysaccharides amylose and cellulose is undertaken to make available reactive groups that may be used as attachment points for sugars or sugar derivatives. In this way it is possible to construct molecules with sugar size chains of controlled lengths. By insertion of the hydrazino groups, used for coupling, at the C-6 position, side chains are uniformly located. Such definite compounds are of value in establishing the relationship between molecular architecture and physical properties of high polymeric hydrophilic molecules. Previously, this laboratory[2] made 6-amino-6-deoxy-

(1) Journal Paper No. 2222 of the Purdue Agricultural Experiment Station, Lafayette, Ind.

(2) R. L. Whistler and D. G. Medcalf, presented in part before the Carbohydrate Division at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1962; *Arch. Biochem. Biophys.*, **104**, 150 (1964).

amylose by nucleophilic displacement of *p*-tolylsulfonyloxy (tosyloxy) groups specifically located at carbons C-6 in 2,3-di-*O*-phenylcarbamoylamylose with azide ions, and then subsequent reduction. For degrees of substitution (D.S.) less than 1.0, initial blocking of secondary hydroxyl groups is unnecessary and direct tosylation of the polysaccharide may be used. As shown in previous work,[3] direct tosylation up to a D.S. of about 0.7 introduces tosyl groups almost entirely on primary positions with attachment of very few at secondary positions. Therefore, in this work tosyl groups were introduced to a D.S. of 0.65 and 0.64 in amylitol and cellulose, respectively. Secondary substitution did not exceed a D.S. of 0.05. While reaction of 6-*O*-tosylcellulose with hydrazine at 100° for 30 min. suffices to remove all tosyl groups, reaction of tosylamylitol in pyridine with hydrazine at 100° for 1 hr. does not completely remove the tosyl groups. However, a second treatment with hydrazine alone, at 17° for 34 hr., suffices. Direct treatment of 6-*O*-tosylamylitol with hydrazine at 17° for 48 hr. removes all tosyl groups, but gives a product with only a low hydrazino content.

Hydrazinolysis of polysaccharides occurs when they are heated with hydrazine,[4] particularly for extended times. Since amylose is especially susceptible to alkaline degradation, it was hydrogenated to amylitol before treatment. Hydrogenated chondroitin sulfate has been found[5] to be more resistant to hydrazinolysis than the reducing polysaccharide. Viscosity measurements indicated that, under the conditions used here, hydrazinolysis is not extensive. The results appear to confirm previous[4] observations.

Reaction with hydrazine does not lead to complete replacement of tosyloxy groups by hydrazino groups. Even those tosyloxy groups on primary positions are not fully replaced, being 83% replaced in the cellulose derivative and 67% replaced in the amylitol derivative. Since the hydrazinoglycans are free of tosyl groups, some tosyl groups must be removed by hydrolysis, a conclusion substantiated by the isolation of hydrazinium tosylate in expected yield from the reaction mixture. The hydrochloric acid salts of 6-deoxy-6-hydrazinocellulose and 6-deoxy-6-hydrazinoamylitol are stable and water soluble, producing clear, nonretrograding solutions. Addition of acetic acid to pH 5 causes a solution of 6-deoxy-6-hydrazinoamylitol to form a stiff gel. The free 6-deoxy-6-hydrazinoglycans are fairly stable under nitrogen at low temperatures. However, at 25° in the presence of air, the white solids change to a light yellow color and the nitrogen content decreases progressively. Hydrolysis of this product yields D-glucose and 6-deoxy-D-glucose, D-quinovose. Various oxidants attack and remove the hydrazine groups from the C-6 position leaving 6-deoxy units. Oxidants which attack the free base in this way are oxygen, hydrogen peroxide, nitrous acid, iodine, and potassium iodate. Iodate ion reacts rapidly and quantitatively with hydrazinoamylitol with completion of the reaction in 15 min.

The 6-deoxy-6-hydrazinoglycans react readily with a concentrated solution of D-glucose to attach one D-glucose unit to each hydrazino group, probably as the hydrazone. These derivatives do not hydrolyze in dilute aqueous solution. Likewise the 6-deoxy-6-hydrazinoglycans react with D-glucono-1,4-lactone to form D-gluconohydrazines in quantitative yields. These hydrazides are stable in neutral aqueous solution but hydrolyze readily on addition of dilute base.

(3) R. L. Whistler and S. Hirase, *J. Org. Chem.*, **26**, 4600 (1961).
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Experimental

Chromatography.—Chromatographic separations were on Whatman No. 1 paper at 20° with the following irrigants: A, ethyl acetate–acetic acid–formic acid–water (18:3:1:4 v./v.); B, ethyl acetate–pyridine–water (10:4:3 v./v.); C, 1-butanol–ethanol–water (40:11:19 v./v.). For location of components, papers were sprayed with silver nitrate-sodium hydroxide solution.[6]

Amylitol was prepared from corn amylose isolated as the 1-butanol complex.[7] After three recrystallizations, the 1-butanol–amylose complex from 120 g. of starch was dissolved in 1 l. of previously boiled 0.2 *N* sodium hydroxide solution. Ten grams of sodium borohydride[8] was added, and the mixture was allowed to stand at 17° for 24 hr. The solution was acidified with acetic acid, and the amylitol precipitated by addition of 1.5 l. of methanol. The precipitate was suspended in 1 l. of water, precipitated with 1.5 l. of methanol, and dried by washing with three successively fresh portions of absolute ethanol in a Waring Blendor followed with dry ether. The remaining ether was removed under reduced pressure over calcium chloride.

6-*O*-Tosylamylitol.—Ten grams of this fluffy powder was stirred in 100 ml. of dry pyridine for 10 min. at 100°. The thick suspension was cooled to 5° and 17.54 g. of tosyl chloride was added with vigorous stirring. After 1 hr. at 5°, the pink mixture was heated to 30° for 1 hr. It then was poured slowly, with stirring, into 2 l. of 80% methanol. On decantation of the methanol and washing the precipitate with water in a Waring Blendor, a powder resulted which was washed free of chloride ion with water and was dried in a vacuum desiccator containing calcium chloride. Sulfur analysis indicated a D.S. of 0.65. Primary tosyl groups were determined[9] by displacement of the primary groups with iodine in 2,5-hexanedione at 120° for 2 hr. Organic iodine was converted[10] to iodine ion by displacement with hydroxyl using potassium hydroxide in ethanol to indicate a D.S. of 0.60, tosyl groups in the amylitol. Tosyl groups located at secondary positions were estimated from sulfur analysis to correspond to a D.S. of 0.05.

6-Deoxy-6-hydrazinoamylitol.—To establish improved conditions for tosyl displacement, 1-g. portions of the derivative were dissolved in 20-ml. portions of dry pyridine and treated at 100° with 0.2, 0.5, 1.0, 2.0, 4.0, and 8.0 ml. of anhydrous hydrazine. Products were precipitated by addition of 150 ml. of methanol, centrifuged, washed through several portions of fresh methanol in a Waring Blendor, washed with diethyl ether in a beaker, filtered, and dried over calcium chloride in a vacuum desiccator. Bound hydrazine estimated by nitrogen analysis[11] indicated D.S. of 0.14, 0.20, 0.25, 0.31, 0.38, and 0.38. Sulfur determinations indicated tosyl contents corresponding to D.S. of 0.36, 0.28, 0.20, 0.14, 0.10, and 0.09.

Since complete elimination of tosyl groups was not obtained, the above products were reacted with anhydrous hydrazine a second time. This effected complete removal of tosyl groups. For example, 0.5 g. of the product having a D.S. of 0.38 in hydrazino groups and 0.10 in tosyl groups when treated with 5 ml. of hydrazine at 17° in oxygen-free nitrogen[12] for 34 hr. was devoid of sulfur and possessed a hydrazino content equivalent to a D.S. of 0.40.

When 5 g. of 6-*O*-tosylamylitol of D.S. 0.65 was treated with 50 ml. of hydrazine at 17° for 48 hr. in an oxygen-free atmos-

(6) W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, **166**, 444 (1950).
(7) T. J. Schoch, *J. Am. Chem Soc.*, **64**, 2957 (1942).
(8) M. Abdel-Akher, J. K. Hamilton, and F. Smith, *ibid.*, **73**, 4691 (1951).
(9) F. B. Cramer and C. B. Purves, *ibid.*, **61**, 3458 (1939); T. S. Gardner and C. B. Purves, *ibid.*, **64**, 1539 (1942); C. J. Malm, L. J. Tanghe, and B. C. Laird, *ibid.*, **70**, 2740 (1948).
(10) J. F. Mahoney and C. B. Purves, *ibid.*, **64**, 9 (1942).
(11) S. J. Clark, "Quantitative Methods of Organic Microanalysis," Butterworth and Co., London, 1956, p. 91.
(12) L. F. Fieser, *J. Am. Chem. Soc.*, **46**, 2639 (1924).

phere, the product was free of sulfur and had a hydrazine content equivalent to a D.S. of 0.15.

After removal of the polysaccharide derivative, the reaction mixture from the detosylation and the methanol washings, were concentrated to dryness under reduced pressure and the residue was dissolved in ethanol. After filtration, the solution was concentrated to about 10 ml., and 3 ml. of dry ether was added. On standing at 25° for 1 hr., white crystals appeared which were filtered and washed with dry ether. Further concentration of the mother liquor to about 2 ml., addition of ether to slight turbidity, and cooling in a refrigerator gave a second crop of crystals. The total yield was 2.2 g., with m.p. 122°, undepressed on mixture with hydrazinium tosylate.

Anal. Calcd. for $C_7H_8N_2H_4SO_3$: C, 41.13; H, 5.87; N, 13.75. Found: C, 40.99; H, 5.45; N, 13.62.

Authentic hydrazinium tosylate was prepared from 200 mg. of toluenesulfonic acid by reaction with 2.0 ml. of hydrazine hydrate. After heating for a few minutes on the steam bath, the mixture was concentrated to dryness. Upon standing at 25° for 1 hr., a white crystalline material appeared which was filtered and washed with dry ether and had a melting point of 122°.

Oxidation with Hydrogen Peroxide.—To 0.5 g. of 6-deoxy-6-hydrazinoamylitol of D.S. 0.40, 15 ml. of 10% hydrogen peroxide solution was added. The temperature increased from 25 to 35°, and some foaming occurred. The mixture was cooled to 25° and a solution of 1 *N* sodium hydroxide was added to adjust the pH to 11. After 15 min. the solution was poured into 50 ml. of methanol, filtered, washed with methanol, and then washed with dry diethyl ether. The product was soluble in cold water, gave a blue color with iodine, and reduced Fehling's solution which indicated that some depolymerization had occurred also.

Oxidation with Potassium Iodate.—6-Deoxy-6-hydrazinoamylitol (6 g.) of D.S. 0.4 was dissolved in 100 ml. of 2% acetic acid solution by stirring and heating at 100° for several minutes. The solution was cooled to 25° and 5 ml. of 98% formic acid solution was added with 20 ml. of a water solution containing 1.1 g. of potassium iodate. An immediate reduction of the iodate to iodine was indicated by the development of a dark blue color. The temperature increased to 35° and some foam appeared. After 15 min., 10 g. of potassium iodide was added, followed by enough sodium thiosulfate to remove the color of the iodine complex. After 2 days of dialysis against distilled water, the solution was concentrated to 50 ml., and the polysaccharide was precipitated and washed as before.

The yellowish nitrogen-free product was soluble in water, gave a blue color with iodine, a negative test with Fehling's solution, and did not gelatinize with acetic acid.

Determination of the Hydrazino Groups by Oxidation with Potassium Iodate.—6-Deoxy-6-hydrazinoamylitol (26.6 mg.) of D.S. 0.40 was dissolved in 10 ml. of 1% acetic acid solution, and 10.0 ml. of a solution of 0.01 *M* potassium iodate was added with 1 ml. of concentrated formic acid. After 15 min., 200 mg. of potassium iodide was added and the excess of iodine was titrated with 0.05 *N* sodium thiosulfate solution. Under these conditions 1 mole unit of hydrazinoamylitol was oxidized by 0.35 mole of potassium iodate.

Oxidation with Iodine.—6-Deoxy-6-hydrazinoamylitol (241 mg.) of D.S. 0.40 was dissolved in 50 ml. of 1% acetic acid solution and titrated with 0.12 *M* iodine solution. The pH was brought to 7.4 and kept constant throughout the titration by addition of sodium bicarbonate solution. A total of 14.7 ml. of iodine solution was added. One mole of 6-deoxy-6-hydrazinoamylitol was oxidized by each 1.34 moles of iodine. The oxidized solution was dialyzed, concentrated, and precipitated as before. The product was soluble in water, reduced Fehling's solution slightly, and did not gelatinize with acetic acid.

Reaction with Nitrous Acid.—6-Deoxy-6-hydrazinoamylitol (1 g.) of D.S. 0.4 was dissolved in 60 ml. of 2% aqueous acetic acid, and 2 g. of sodium nitrite was added at 30°. After 5 min., during which time gas evolved, the solution was neutralized with 1 *N* sodium hydroxide and the polysaccharide was precipitated with methanol. After centrifugation, the yellowish precipitate was washed with methanol followed by dry ether. The product was soluble in water, reduced Fehling's solution slightly, and did not gelatinize with acetic acid.

Isolation and Identification of Quinovose.—Acid hydrolysates from the oxidized products, after neutralization with barium carbonate and filtration, were concentrated to dryness. Each was examined as follows. Absolute methanol (10 ml.) was added and any colored precipitate was discarded. The clear methanolic solution was concentrated under reduced pressure and chromatographed on Whatman No. 3 MM paper, using either irrigant A or B. The zone corresponding to D-quinovose was eluted with water, and the extract was concentrated and heated with 200 mg. of *p*-nitrophenylhydrazine in 20 ml. of 1 *N* hydrochloric acid. The red precipitate was recrystallized from 50% ethanol and the melting point and mixture melting point with authentic *p*-nitrophenyl-D-quinovosazone[13] was 222°. The number of D-quinovose units produced by oxidation of the hydrazoamylitol was estimated by acid hydrolysis, oxidation of the product with periodate, and quantitative determination of the acetaldehyde produced.[14] For the iodate oxidized hydrazinoamylitol of D.S. 0.40, the resulting 6-deoxy content was 0.16.

Retrogradation.—A 250-mg. portion of 6-deoxy-6-hydrazinoamylitol of D.S. 0.4 was dissolved in 50 ml. of 1% hydrochloric acid solution, the pH was adjusted to 5, and the mixture was held at 4° for 8 days. Likewise, a solution of 250 mg. of amylitol in 25 ml. of 1 *M* potassium hydroxide solution was neutralized with an equal volume of 1 *M* hydrochloric acid solution, the pH was adjusted to 5, and the solution was held at 4° for 8 days. The solution of 6-deoxy-6-hydrazinoamylitol remained clear, but the amylitol solution quickly clouded and developed a precipitate.

Hydrazinocellulose.—Cut cotton linters (230 g.) were stirred with 4 l. of acetic acid in a Waring Blendor. Then 1.5 ml. of concentrated sulfuric acid was added and the reaction mixture was kept at 25° for 2 hr. Acetic anhydride (1 l.) was added and the mixture was stirred frequently for 5 hr. The thick solution was poured in a small stream into about 20 volumes of water. After standing at 25° for 2 hr. with frequent change of water, the filamentous precipitate was stirred in a Waring Blendor and washed with an excess of water. The acetyl content was 44.5%.

The cellulose acetate was deacetylated by placing it in an 8-l. solution of 0.5 *N* sodium hydroxide in ethanol at 25° for 2 days. The product was filtered through cheese cloth, washed with water until neutral, and air-dried for 4 days. Drying this product to constant weight under reduced pressure at 60° gave a yield of 225 g. Ten grams of the regenerated cellulose was activated[15] in a mixture of 50 ml. of water and 150 ml. of pyridine. It was then washed three times with anhydrous pyridine, filtered, and 23.5 g. of tosyl chloride (2 moles of tosyl chloride per mole of D-glucose unit) was added in 200 ml. of anhydrous pyridine. After shaking the mixture at 25° for 24 hr., the mixture was poured into a 2 l. of methanol, filtered, and stirred with methanol in a Waring Blendor. After filtration, the tosyl derivative was washed with methanol until the filtrate gave a negative test for chlorine. The product was light brown and the yield was 15.5 g. of D.S. 0.64 (of which 0.60 was primary). Under the same conditions used for the preparation of 6-deoxy-6-hydrazinoamylitol, cellulose gave a white fluffy compound which was insoluble in water or dilute acid solutions. A soluble 6-deoxy-6-hydrazinocellulose was obtained by a slightly different procedure. For this 1 g. of 6-*O*-tosylcellulose (D.S. 0.64, 40 mesh) was heated and stirred at 100° with 10 ml. of anhydrous hydrazine in the absence of oxygen for 25 min. It then was poured into 200 ml. of methanol. The 6-deoxy-6-hydrazinocellulose precipitated as a white fibrous mass which was broken up by stirring for 1 min. in a Waring Blendor. It was filtered and washed again in a Waring Blendor for 1 min. with 80% aqueous methanol. The product was filtered then and washed with dry methanol. All excess hydrazine must be washed out as soon as possible. The 0.5 g. of final product was soluble in dilute acids as long as it was not subjected to prior drying. The D.S. was 0.50 and sulfur was absent.

Reaction with D-Glucose.—Both 6-deoxy-6-hydrazinoamylitol and 6-deoxy-6-hydrazinocellulose reacted similarly when subjected to reactions involving hydrazino groups. 6-Deoxy-6-hydrazinocellulose (D.S. 0.50), freshly prepared from 1.0 g. of 6-*O*-tosylcellulose while still wet with methanol, was transferred to a 2.3 × 20 cm. test tube which contained 4 g. of D-glucose dissolved in 15 ml. of water. Glacial acetic acid (1 ml.) was added, and the mixture was shaken thoroughly and heated at 45° for 30 min. No change in color was observed but higher temperatures for longer times produced a brown color. After addition of 20 ml. of 1 *N* sodium hydroxide solution, the mixture was poured into 200 ml. of methanol stirred in a Waring Blendor. A fine white

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(14) B. H. Nicolet and L. A. Shinn, *J. Am. Chem. Soc.*, **63**, 1456 (1941).
(15) C. Malm, L. Tanghe, and B. Laird, *ibid.*, **70**, 2747 (1948).

precipitate developed. The mixture was heated to boiling for 10 min. and centrifuged. The precipitate was suspended twice in 100-ml. portions of hot 80% aqueous methanol and centrifuged to remove traces of D-glucose. The polysaccharide was washed with methanol and dried over calcium chloride to yield 0.7 g.

The amount of D-glucose combined with the polymer was determined by periodate oxidation. An 18.3-mg. portion was mixed with 80 ml. of 5% potassium chloride solution and 20 ml. of 0.3 *M* sodium metaperiodate. The mixture was shaken in the dark at 18°. Aliquots were taken every 12 hr., and the formic acid present was titrated by 0.01 *N* sodium hydroxide solution using methyl red–methylene blue as indicator. 6-Deoxy-6-hydrazinocellulose was used in a blank determination. The oxidation was complete in about 2 days. The amount of formic acid obtained indicated that the number of D-glucose units which had become attached were equivalent to the number of hydrazino groups present.

The product formed by treating D-glucose with the 6-deoxy-6-hydrazinocellulose of D.S. 0.5, when freshly prepared, was readily soluble in water. When 100 mg. of the polymer was shaken in 10 ml. of water for 48 hr., no free D-glucose was evident on paper chromatography of solution aliquots.

Reaction with D-Glucono-1,4-lactone.—6-Deoxy-6-hydrazinocellulose of D. S. 0.50, freshly prepared from 1.0 g. of 6-*O*-tosylcellulose, was placed in a 2.3 × 20 cm. test tube which contained 4 g. of D-glucono-1,4-lactone dissolved in 15 ml. of water. The mixture was shaken thoroughly and heated at 45° for 30 min. After centrifugal removal of a small amount of insoluble material, the centrifugate was mixed with 30 ml. of 1 *N* sodium hydroxide solution. The mixture, when poured into 200 ml. of methanol and stirred in a Waring Blendor, produced a fine white precipitate. The methanol suspension was heated, centrifuged, and the precipitate was washed twice with 100-ml. portions of 60% aqueous methanol. It then was washed with methanol and dried over calcium chloride to yield 0.7 g. The amount of combined D-gluconic acid was estimated by periodate oxidation of the product and measurement of the amount of formaldehyde released. The amount found corresponded to the attachment of one D-gluconic acid unit to each hydrazino group.

The product was alkaline hydrolyzed by suspending 200 mg. in 15 ml. of hot ethanol, adding 5 ml. of 0.1 *N* sodium hydroxide solution, and heating at 100° for 5 min. The mixture was filtered while hot, and the filtrate was neutralized with diluted acid and chromatographed using irrigants A, B, and C. On spraying with silver nitrate solution a single strong spot appeared, corresponding to D-gluconate.

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2,2′:5′,2″-Terpyrrole[1a]

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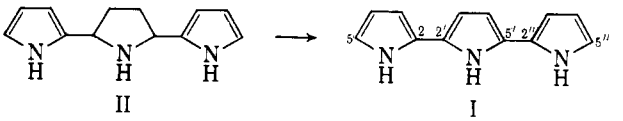
The synthesis of 2,2′:5′,2″-terpyrrole has been accomplished by dehydrogenation of pyrrole trimer and decarboxylation of a terpyrrole dicarboxylic acid. The properties found for this compound differ appreciably from those reported previously for material assigned the terpyrrole structure. A general procedure has been developed for the synthesis of terpyrroles from the condensation of a 2,2′-bipyrrole and a 2-pyrrolidinone to give a pyrrolinylbipyrrole, and dehydrogenation of the latter.

It has been suggested that pyrrole is an intermediate in the formation of naturally occurring melanins and that pyrrole black, generated when pyrrole is oxidized in acetic acid, is structurally similar to melanins.[2] Because of the polymeric character of pyrrole black, Chierici and co-workers attempted to synthesize 2,2′:5′,2″-terpyrrole (I) to determine if this compound is an intermediate in the formation of pyrrole black and of possible significance in relation to melanin.[3] From the reaction of 1,4-di-2′-pyrrylbutane-1,4-dione and ammonia, they reported the isolation of a compound assigned structure I, m.p. 100° dec., too unstable for further characterization. These properties were quite unexpected in view of our experience with 2,2′-bipyrroles.[4] Therefore, we have investigated the synthesis of terpyrroles and describe here several representatives of this system.

Pyrrole, treated with acid, undergoes trimerization[5] to form pyrrole trimer, 2,5-di-2′-pyrrylpyrrolidine (II).[6] When II was subjected to catalytic dehydrogenation, the product obtained had the molecular formula of the desired terpyrrole but was clearly not the same material as previously reported[3b] since it melted at 242°. The following spectral evidence established the structure of this compound as terpyrrole (I). (a) The n.m.r. spectrum showed six β-protons (δ 6.2) and two α-protons (δ 6.7) as complex signals, while integration established the approximate location of the three protons attached to nitrogen (δ ~10). (b) The ultraviolet spectrum retained the same fine structure as found in 2,2′-bipyrrole (III)[4] and exhibited the anticipated bathochromic shift (~45 mμ) relative to bipyrrole. A methanolic solution of terpyrrole exposed



II I

to air and light slowly turns green and, after 24 hr., a black precipitate forms. These changes occur instantaneously when hydrogen peroxide is added to a solution of I in acetic acid. However, crystalline material is stable for long periods when stored in the cold in the absence of air and light. From a comparison of these properties with those reported by Chierici and Serventi,[3b] it is clear that the material they had isolated was not terpyrrole.

A more general synthesis of terpyrroles was effected by condensation of a 2,2′-bipyrrole with a 2-pyrroli-

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