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THE NITROUS ACID DEAMINATION OF GLYCOSIDES AND ACETATES OF 2-AMINO-2-DEOXY-D-GLUCOSE*

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ABSTRACT

A procedure is described for the nitrous acid deamination of 2-amino-2-deoxy-D-glucose hydrochloride (**1**), and reduction of the product with buffered borohydride, to afford crystalline 2,5-anhydro-D-mannitol (**3**) in 71% yield. Similar treatment of the methyl α-pyranoside (**4**) of **1** gives 59% of crystalline **3**, and the same product is obtained in 44% yield from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α(or β)-D-glucopyranose hydrochloride (**5** or **6**) by the deamination–reduction sequence with subsequent deacetylation. These results provide a model for a nonhydrolytic, depolymerization technique for structural characterization of glycosaminoglycans.

INTRODUCTION

Nitrous acid deamination of amino sugar-containing polysaccharides is, in principle, an attractive tool for structure determination, as it offers a route for nonhydrolytic depolymerization of these macromolecules[1–3]. It has long been known[4] that 2-amino-2-deoxy-D-glucopyranose (**1**) undergoes ring contraction on treatment with nitrous acid to give 2,5-anhydro-D-mannose (**2**, "chitose")[5,6], and this syrupy, difficultly characterizable, anhydro sugar is also produced from chitosan by the action of nitrous acid[7]. Foster, Martlew, and Stacey showed[8] in 1953 that nitrous acid causes glycosidic scission of methyl 2-amino-2-deoxy-α(and β)-D-glucopyranoside to give mainly chitose, and the reaction has been utilized in structural work with glycosaminoglycans to give fragmentation products having 2,5-anhydro-D-mannose end-groups[2,3]. This reaction with nitrous acid is the basis of the Dische test[9] for 2-amino-2-deoxy sugar residues. The syrupy nature of chitose, and difficulties in preparing crystalline derivatives of it, have hampered both its chemical characterization and the use of nitrous acid in definitive, structural work based on crystalline derivatives. However, in 1956, Bera, Foster, and Stacey[10] were able to reduce chitose with hydrogen and Raney nickel to give, in good yield, crystalline 2,5-

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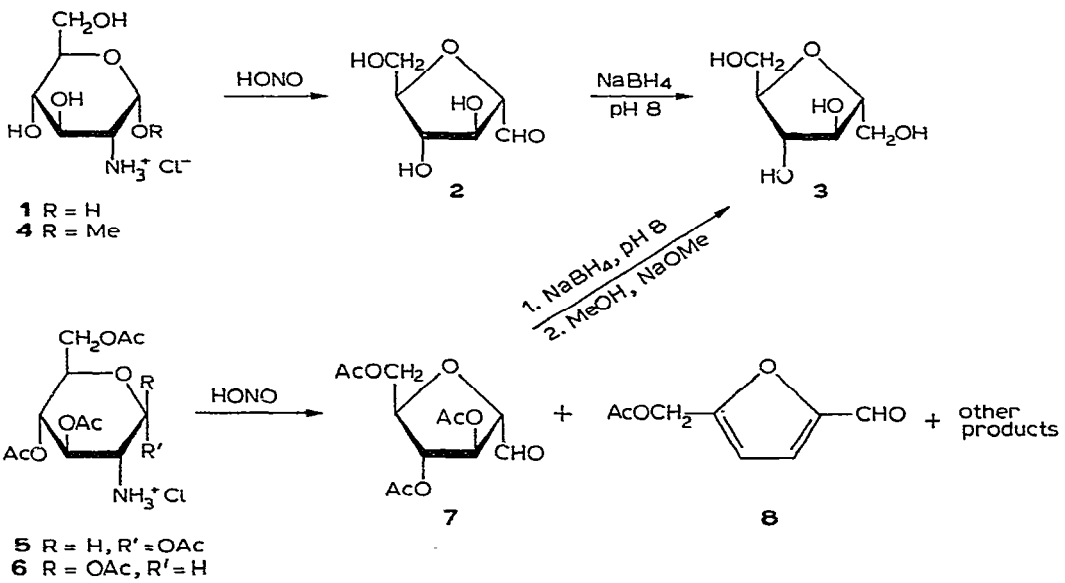
anhydro-D-mannitol (3), whose structure was firmly established by degradative methods.

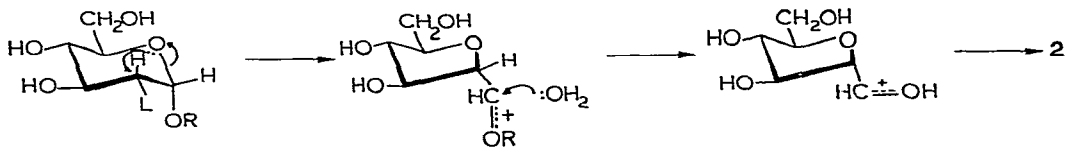
This report describes investigations to (*a*) establish optimal and convenient preparative conditions for crystalline 2,5-anhydro-D-mannitol (3) from 2-amino-2-deoxy-D-glucose hydrochloride (1), (*b*) determine the yield of crystalline 3 obtainable from the methyl α-pyranoside (4) of 1 as a model for the nitrous acid depolymerization of *N*-desulfated heparin, and (*c*) determine whether or not *O*-acetylation alters the course of nitrous acid deamination of 1.

DISCUSSION

To optimize the yield of 2,5-anhydro-D-mannitol (3) from 2-amino-2-deoxy-D-glucose hydrochloride, conditions were sought for minimizing degradative loss of the 2,5-anhydro-D-mannose (2) intermediate, and to circumvent the high-pressure hydrogenation step used by Bera, Foster, and Stacey[10] for preparatively converting 2 into 3. The amino sugar 1, at mutarotational equilibrium, was treated with a 4-molar excess of aqueous nitrous acid at 0° until the starting material had disappeared, whereupon nitrous acid and salts were removed without heating the solution above room temperature. The anhydro sugar 2 was reduced at 0° with sodium borohydride, and side reactions[10] caused by the alkalinity of this reagent were avoided by keeping the solution at pH ~8 through use of carbon dioxide. The nonreducing product was decationized, and freed from boric acid as the volatile methyl borate. Crystallization of the product gave the anhydroalditol 3 in 71% yield from 1. Nucleation was necessary in order to obtain crystalline 3 in the first instance but not thereafter, and the crystalline product was stable on storage without special precautions. The earlier preparation[10] was hygroscopic and liquefied on storage.



By the same procedure as that used for converting **1** into **3**, methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride[11] (**4**) gave 2,5-anhydro-D-mannitol (**3**) in crystalline form in 59% yield. As it had already been shown[12] that the β-D anomer[13] of **4** also gives **3** by the deamination–reduction sequence, it may be concluded on the basis of crystalline products that substitution of **1** at the anomeric center does not alter the course of the principal reaction[6,14]. This process[8] can be visualized as rearside attack by O-5 on C-2 of a diazotized intermediate (diazonium ion or diazohydroxide[15]), with cleavage of the C-1–O-5 bond to generate (from the aldose) the protonated 2,5-anhydroaldose, or (from the glycoside) an oxocarbonium ion that, by



1 or 4
L = $-N_2^+$ or $-N{=}NOH$

solvent capture and loss of ROH, gives the same product. The ring contraction arises as a result of the very low activation-energy for the reaction of the amino group with nitrous acid[15], so that the incipient, "hot" carbonium ion suffers attack by the sterically best-situated nucleophilic center, in this instance O-5. The stereochemistry and type of substitution at C-1 does not appear to affect the reaction course, although the rate may be influenced; it has been observed[8] that the α-glycoside **4** reacts more slowly than the β-D anomer with nitrous acid.

As *O*-acetylated amino sugars having the amino group in a salt form are readily prepared by way of derivatives having a removable, *N*-protecting group, and as such procedures can be applied to oligo-[16] and polymeric, amino sugar-containing carbohydrates, it was considered of value to examine the course of reaction of the anomeric, *O*-acetylated derivatives of 2-amino-2-deoxy-D-glucopyranose with nitrous acid. Both 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride[17] (**5**) and the β-D anomer[18] (**6**) underwent conversion by aqueous nitrous acid at 0° into a water-insoluble gum; the β-D anomer reacted the more rapidly. In each example, t.l.c. on silica gel indicated a major product (R_F 0.65 with 3:1 chloroform–ether) together with five other components, and there was very little detectable difference, by t.l.c. or by preparative product-isolation, between the product from **5** and that from **6**.

The product mixture was not stable, and the content of the least polar component (R_F 0.95) increased on storage, at the expense of the principal component (R_F 0.65). Column-chromatographic resolution of the mixture gave the pure, R_F 0.95 component, identified as 5-(acetoxymethyl)-2-furaldehyde (**8**) by comparison with an authentic sample[19]. The yield of **8** was 12% from the original mixture, and greater if the mixture was kept for several days at ~25° before chromatography. The major

product (R_F 0.65) was evidently 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose (**7**) or its hydrate; it was not characterized as such, but was immediately reduced with buffered borohydride, and the product was deacetylated with methanolic sodium methoxide to afford 2,5-anhydro-D-mannitol (**3**), obtained crystalline in 44% yield.

A very minor product (R_F 0.82) from the column was not characterized, but it was probably formed from **7** by loss of one molecule of acetic acid per molecule, as samples of **7** stored at ~25° became progressively contaminated with the component having R_F 0.82, as well as with that (**8**) having R_F 0.95.

The products of mobility lower than that of **7** were deacetylated, and paper chromatography in several solvent-systems showed components indistinguishable from D-glucose, D-mannose, and 2-acetamido-2-deoxy-D-glucose. Together, they amounted to <20% of the product mixture. By visual estimation, the ratio of D-glucose to D-mannose was approximately unity for the product from the β-D anomer (**6**), but the product from the α-D anomer (**5**) contained rather more D-mannose than D-glucose.

These results for the acetates **5** and **6** indicate that formation of the acetylated 2,5-anhydro-D-mannose (**7**) is the major reaction-pathway, and that this product eliminates acetic acid very readily to give the furan derivative **8**, possibly, in part, during the actual deamination reaction, and certainly during isolation procedures involving heating or storage. The small proportion of 2-acetamido-2-deoxy-D-glucose formed (after *O*-deacetylation) can be ascribed to O→N acetyl migration[17] in the deamination mixture. Formation, from **5** and **6**, of acetylated derivatives of D-glucose and D-mannose as minor products can be attributed to competitive attack, on an intermediate, diazotized species, by the solvent (water) or by an adjacent acetoxyl group, to give an acyloxonium-ion intermediate[16] that then reacts with water.

The course of the deamination reaction for 2-amino-2-deoxyaldohexopyranose derivatives by cold, aqueous nitrous acid is evidently dependent on the ground-state, conformational orientation of the amino group and the group antiparallel to it. When the bond to the 2-amino group is equatorial in the favored conformation, the ring-contracted product resulting from attack by O-5 can be expected as the preponderant, if not exclusive, product. If the bond to the amino group is axial in the favored conformation, direct attack by water to give the 2-hydroxy derivative with inversion can be expected, as shown[20] in the conversion of 2-amino-2-deoxy-D-mannose into D-glucose. If the bond to an adjacent hydroxyl group is also axial, an epoxide product or intermediate can be expected[21].

Nitrous acid deamination is an unusual reaction, in that its energy of activation is low compared with the energy required for major, conformational change (such as ring inversion) in the substrate. The products formed reflect, therefore, the ground-state conformation of the substrate, and the reaction may be regarded as a useful probe for studying conformations. For most other reactions of sugars, the activation energy for attainment of the transition state for reaction considerably surpasses that needed to populate even rather unfavored conformational states, so that the ground-state conformation may have no direct bearing on the product distribution.

As a model for a depolymerization procedure for structural elucidation of glycosaminoglycans[22], the foregoing experiments establish, on the basis of characterized products, the following.

(*a*) Glycosidically linked 2-amino-2-deoxy-D-glucose residues (having the 2-amino group equatorial in the favored conformation) are converted by cold, aqueous nitrous acid into 2,5-anhydro-D-mannose residues in high yield, with concomitant detachment of the glycosidic link.

(*b*) The base-labile 2,5-anhydro-D-mannose (**2**) can be reduced by borohydride in buffered solution to give the stable 2,5-anhydro-D-mannitol (**3**), isolable crystalline in high yield.

(*c*) *O*-Acetylation of the amino sugar does not alter the principal course of the deamination reaction, but the product very readily eliminates acetic acid, and side-reactions leading to the corresponding aldose and its 2-epimer occur to some extent.

EXPERIMENTAL

General methods. — Solutions were evaporated under diminished pressure at ~25°. Melting points were determined with a Thomas–Hoover "Unimelt" apparatus and are uncorrected. N.m.r. spectra were recorded at 60 MHz with a Varian A-60A spectrometer, with solutions (~10%) in chloroform-*d* at ~30°, and with tetramethylsilane (τ 10.00) as the internal standard. T.l.c. was performed with 0.25-mm layers of Silica Gel G (E. Merck, Darmstadt, Germany) activated at 110° as the adsorbent, and sulfuric acid as the indicator. Paper chromatography was performed on Whatman No. 1 paper by the descending method; the detecting reagents were silver nitrate–sodium hydroxide and (for amino sugars) ninhydrin. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation (camera diameter 114.59 mm). Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The three strongest lines are numbered (1, strongest).

Nitrous acid deamination of 2-amino-2-deoxy-D-glucose hydrochloride (**1**). — A solution of **1** (10.8 g, 50 mmoles) in water (150 ml) was allowed to attain mutarotational equilibrium (~5 h at ~25°) and was then cooled to 0° in an ice–salt bath. Sodium nitrite (13.8 g, 200 mmoles) was added in several portions with mechanical stirring, at such a rate that the temperature of the solution remained at 0°. Acetic acid (8.3 ml, 150 mmoles) was added at such a rate that the temperature did not exceed 2°. The mixture was stirred for 4 h at 0°, at which point, the amino sugar was no longer detectable (ninhydrin). The solution was brought to room temperature, nitrogen was bubbled through it for 30 min to remove the excess of nitrous acid, and it was then lyophilized without delay. The resultant, tacky solid was dispersed in methanol (~150 ml), and the methanol was evaporated off. The addition and subsequent evaporation of methanol was repeated several times, and then a subsequent extract with methanol, containing most of the salts as a granular suspension, was filtered and the salts were washed with methanol. The combined filtrate and washings were evaporated, to afford syrupy 2,5-anhydro-D-mannose (**2**), which was used at once in the following step.

Preparation of 2,5-anhydro-D-mannitol (3). — A solution of the foregoing syrupy **2** in water (200 ml) was cooled to 0°, and sodium borohydride (1.9 g, 50 mmoles, 4 equivs.) was added in small portions. The pH of the solution was kept near 8 by the simultaneous addition of small chips of Dry Ice. After the addition had been completed, the solution was kept for 1 h at 0°, at which point a Fehling test was negative, and was then made neutral by dropwise addition of acetic acid [Amberlite IR-120 (H^+) resin was equally effective]. Methanol was added to, and evaporated from, the solution until a sample of the distillate no longer burned with a green flame (methyl borate absent). The remaining aqueous solution was now evaporated, and the yellow, syrupy residue was dispersed in methanol, and the dispersion treated with charcoal. The insoluble salts and charcoal were removed by filtration through a Celite pad, and the filtrate was passed through a short column of Amberlite MB-3 (H^+, OH^-) resin. The effluent was evaporated to a mobile syrup that crystallized readily after nucleation. The crystals were filtered off and dried: yield of analytically pure, nonhygroscopic, large, clear prisms, 5.8 g (71% from **1**); m.p. 101–101.5°, $[\alpha]_D^{25}$ +57° (*c* 1.2, water) [lit.[10] m.p. 100–101°, $[\alpha]_D^{20}$ +58.2° (*c* 1.37 ,water)]; R_F 0.70 (t.l.c., methanol); X-ray powder diffraction data: 6.46 m, 5.81 m, 5.19 w, 4.73 vs (1), 4.10 vw, 4.01 w, 3.74 s (2), 3.51 s (3), 3.25 vw, 3.04 w, 2.84 s, 2.70 w, 2.60 m, and 2.44 m.

This compound was, at first, not obtained crystalline, despite repeated efforts. The original sample[10] had liquefied and was not available for nucleation. A nucleus obtained from Dr. J. W. LeMaistre of Atlas Chemical Industries, Wilmington, Delaware, caused rapid crystallization of the syrup and of all other preparations of **3** obtained in this laboratory. In subsequent preparations, nucleation of the syrup was not found necessary.

Nitrous acid deamination of methyl 2-amino-2-deoxy-α-D-glucopyranoside[11] (**4**). — Following the procedure used for the deamination of **1**, a solution of **4** (2.9 g, 20 mmoles) was treated with sodium nitrite (5.5 g, 80 mmoles) and acetic acid (4.6 ml, 80 mmoles). After 2 h at 0°, no trace of amino sugar remained (papergram, ninhydrin). After an additional 2 h at 0°, the solution was processed as described for **1**, to give **2** as a pale-yellow, viscous syrup. Reduction as before with sodium borohydride (0.76 g, 20 mmoles) gave crystalline **3**; yield 1.9 g (59% from **4**).

Before reduction, paper-chromatographic examination of the deamination product **2**, from **1** or from **4**, showed no trace of components migrating with the characteristics of reference samples of D-glucose or D-mannose.

Nitrous acid deamination of 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride[17] (**5**) *and its β anomer*[18] (**6**). — A solution of **5** or **6** (3.8 g, 10 mmoles) in water (100 ml) was kept at 0° by means of an ice–salt bath, and sodium nitrite (2.8 g, 40 mmoles) was added. The solution was stirred magnetically and kept at 0° while acetic acid (1.7 ml, 30 mmoles) was added dropwise. A yellow, water-insoluble gum appeared immediately in the reaction from the β anomer **6**, and a similar gum appeared several minutes later when the α anomer **5** was used. After 2 h at 0°, the mixture was allowed to warm to ~25°, and a stream of nitrogen was passed

through it for 30 min to remove the excess of nitrous acid. The mixture was extracted with three 10-ml portions of dichloromethane, and the combined extracts were washed with saturated, aqueous sodium hydrogen carbonate (10 ml) dried (magnesium sulfate), and evaporated to give a pale-yellow syrup. The syrup darkened if kept for several days at ~20°, but it could be kept essentially unchanged for several weeks at −20°. T.l.c. (3:1 chloroform–ether) indicated that the syrup contained at least 6 components (R_F 0.95, 0.82, 0.65, 0.15, 0.10, and 0.05), with no detectable difference between the product from **5** or from **6**. Column chromatography on silica gel (No. 7734, Merck) with the t.l.c. solvent yielded 0.2 g (1.19 mmoles, 12%) of the component having R_F 0.95, identified as 5-(acetoxymethyl)-2-furaldehyde (**8**) by comparison with an authentic sample prepared by acetylating 5-(hydroxymethyl)-2-furaldehyde with pyridine–acetic anhydride; n.m.r. data (chloroform-*d*): τ 7.90 (s, 3 H, OAc), 4.88 (s, CH_2), 3.43 (d, $J_{3,4}$ 4.0 Hz, H-4), 2.79 (d, H-3), and 0.39 (s, CHO) in agreement with literature[19] values.

The major product (R_F 0.65) was separated from the mixture, and was presumed to be 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose (**7**); yield 1.45 g (51%). It was not characterized as such because of its instability, and it was immediately reduced by treatment with sodium borohydride (0.2 g, 5 mmoles) in water (50 ml) at 0°, maintained at pH ~8 by the simultaneous addition of Dry Ice. After 1 h, the solution was made neutral by the dropwise addition of acetic acid, and borate salts were removed by repeated addition of methanol and evaporation from the solution. The final solution was evaporated to a thin syrup that was treated with mM methanolic sodium methoxide (20 ml). After 2 h at ~25°, this solution was neutralized with Amberlite IR-120 (H^+) resin, and evaporated to a thin syrup that crystallized spontaneously to give 2,5-anhydro-D-mannitol (**3**); yield 44%, m.p. and mixed m.p. 101–101.5°.

The component having R_F 0.82 was present only in trace amount; it was not investigated further.

The slower-moving components (R_F 0.15, 0.10, and 0.10) could not be separated by column chromatography. The combined fractions (0.3 g) were dissolved in mM methanolic sodium methoxide (15 ml), and, after 3 h at ~25°, the solution was made neutral with Amberlite IR-120 (H^+) resin. Paper-chromatographic examination of the product in three solvent systems (4:1:1 butyl alcohol–ethanol–water; 3:1:1 ethyl acetate–acetic acid–water; and 5:5:1:3 pyridine–ethyl acetate–acetic acid–water) revealed the presence of three components, respectively indistinguishable chromatographically from standards of D-glucose, D-mannose, and 2-acetamido-2-deoxy-D-glucose.

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REFERENCES

1 A. B. FOSTER AND A. J. HUGGARD, *Advan. Carbohyd. Chem.*, 10 (1955) 335.
2 A. B. FOSTER, R. HARRISON, T. D. INCH, M. STACEY, AND J. M. WEBBER, *J. Chem. Soc.*, (1963) 2279.
3 M. L. WOLFROM, P. Y. WANG, AND S. HONDA, *Carbohyd. Res.*, 11 (1969) 179.
4 G. LEDDERHOSE, *Z. Physiol. Chem.*, 4 (1880) 139; E. FISCHER AND F. TIEMANN, *Ber.*, 27 (1894) 138.
5 F. TIEMANN AND R. HAARMANN, *Ber.*, 19 (1886) 49; E. FISCHER AND E. ANDREAE, *ibid.*, 36 (1903) 2587; P. A. LEVENE AND F. B. LAFORGE, *J. Biol. Chem.*, 20 (1915) 433; 21 (1915) 345; P. A. LEVENE, *ibid.*, 36 (1918) 89; P. A. LEVENE, *Hexosamines and Mucoproteins*, Longmans–Green, London, 1925.
6 D. HORTON, *Monosaccharide Amino Sugars*, in R. W. JEANLOZ (Ed.), *The Amino Sugars*, Vol. IA, Academic Press, New York, 1969.
7 K. H. MEYER AND H. WEHRLI, *Helv. Chim. Acta*, 20 (1937) 361; see also, ref. 96a cited in ref. 1.
8 A. B. FOSTER, E. F. MARTLEW, AND M. STACEY, *Chem. Ind.* (London), (1953) 825.
9 Z. DISCHE AND E. J. BORENFREUND, *J. Biol. Chem.*, 184 (1950) 517.
10 B. C. BERA, A. B. FOSTER, AND M. STACEY, *J. Chem. Soc.*, (1956) 4531.
11 A. NEUBERGER AND R. PITT RIVERS, *J. Chem. Soc.*, (1939) 122; A. B. FOSTER, D. HORTON, AND M. STACEY, *ibid.*, (1957) 81; M. FUJINAGA AND Y. MATSUSHIMA, *Bull. Chem. Soc. Jap.*, 37 (1964) 468.
12 B. C. BERA, Ph. D. Thesis, University of Birmingham, England, 1956.
13 J. C. IRVINE AND J. C. HYND, *J. Chem. Soc.*, 103 (1913) 41.
14 S. PEAT, *Advan. Carbohyd. Chem.*, 2 (1946) 37; F. SHAFIZADEH, *ibid.*, 13 (1958) 9.
15 R. A. MOSS, *Chem. Eng. News*, (1971) Nov. 22, p. 28, and references cited therein.
16 Compare, T. H. HASKELL AND S. HANESSIAN, *J. Org. Chem.*, 28 (1963) 2598.
17 W. E. MAST, D. HORTON, AND K. D. PHILIPS, *J. Org. Chem.*, 32 (1967) 1471.
18 M. BERGMANN AND L. ZERVAS, *Ber.*, 64 (1931) 975.
19 T. FUKUZUMI, S. SAKUMA, H. TAKAHASHI, K. TOMITA, K. FUJIHARA, Y. ISOME, AND T. SHIBAMOTO, *Holzforschung*, 20 (1966) 51.
20 D. HORTON, K. D. PHILIPS, AND J. DEFAYE, *Carbohyd. Res.*, 21 (1972) 417.
21 L. F. WIGGINS, *Nature*, 157 (1946) 300.
22 E. A. BALASZ AND R. W. JEANLOZ (Eds.), *The Amino Sugars*, Vol. IIA, *Distribution and Biological Role*, Academic Press, New York, 1968.