[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS]

Mutarotation, Hydrolysis, and Rearrangement Reactions of Glycosylamines[1]

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The mutarotation, hydrolysis, and rearrangement reactions of D-glucosylamine, D-mannosylamine, and D-xylosylamine have been studied, and rationalized on the basis of the formation of an intermediate, acyclic imonium ion. Rate constants are given for the hydrolysis reaction under various conditions. The formation of diglycosylamines is explained as a type of transamination in which a second molecule of the glycosylamine reacts with the first (in the imonium ion form) with the subsequent elimination of ammonia. Unsubstituted D-glucosylamine has been found to undergo an Amadori rearrangement in glacial acetic acid or in some of the methylenic compounds found by Hodge and Rist to be effective in the rearrangement of *N*-substituted glycosylamines. Intramolecular mechanisms are suggested to account for the effectiveness of carboxylic acids and methylenic compounds in promoting the Amadori rearrangement. By these mechanisms an enolic structure can be transferred from a catalyst to the amine.

The following new compounds are reported: β-D-mannopyranosylamine monohydrate; *N*-acetyl-tetra-*O*-acetyl-β-D-mannopyranosylamine; *N*-acetyl-β-D-mannopyranosylamine monohydrate; octa-*O*-acetyl-di-D-mannosylamine; *N*-acetyl-tri-*O*-acetyl-β-D-xylopyranosylamine; *N*-acetyl-β-D-xylopyranosylamine; di-D-xylosylamine; hexa-*O*-acetyl-di-D-xylosylamine; di-L-arabinosylamine. On the basis of periodate oxidation data and comparisons of optical rotation, ring structures and anomeric configurations have been assigned to the above glycosylamines and their acetates (except the diglycosylamines) and also to β-D-glucopyranosylamine, β-D-xylopyranosylamine, and their acetates.

In connection with the development of methods for the synthesis of C^{14}-labeled carbohydrates,[2] a study of some reactions of the glycosylamines was undertaken because these materials can be used as intermediates for the production of labeled amino sugars. Glycosylamines, or *N*-glycosides, formed by the condensation of reducing sugars with ammonia or amino compounds, are of major biological importance.[3] They include nucleic acids, certain coenzymes, some vitamins and other natural products. The reactions of the glycosylamines are also of interest, in relation to the formation and properties of osazones,[4] mucoproteins,[5] riboflavin,[6,7] and other substances.

The importance of the glycosylamine structure lies not only in its widespread occurrence in natural products, but also in its ability to rearrange and to participate in a variety of reactions.[8,9] The versatility of the structure arises from its capacity to supply or remove electrons, or to aid in moving them from one part of the molecule to another. This concept, suggested in 1943,[10] was subsequently applied to the interpretation of the hydrolysis and mutarotation of the aldosylamines.[11–13] It was shown that, in suitable solvents, the glycosylamines, like the sugars, establish equilibrium states involving cyclic and acyclic forms, and it was postulated that the conversion of one modification to another takes place through an imonium ion [R—C(H)=$\overset{+}{\text{N}}$RR′] formed by acid catalysis. C-1 of the ion tends to acquire electrons in various ways. In the mutarotation reaction, the incipient electron deficiency at C-1 is satisfied by a pair of electrons drawn from a hydroxyl group within the molecule, thus generating a cyclic modification of the glycosylamine. The reaction is reversible and ultimately leads to an equilibrium state which includes all modifications of the glycosylamine. In other reactions, the deficiency is satisfied by addition, to the imonium ion, of a nucleophilic group from the environment. The addition is usually followed by elimination of the amine (or ammonia) as, for example, in hydrolysis and transglycosylation. Furthermore, the imonium ion can acquire electrons from the adjacent carbon atom by enolization. The resulting enolic amine is in some respects analogous to the enediols of the sugars, and undergoes a variety of rearrangement, cleavage, and condensation reactions.

In this paper, it is pointed out that the mutarotation, hydrolysis, and rearrangement reactions of

(1) Part of a project on the development of methods for the synthesis of radioactive carbohydrates sponsored by the Division of Research, Atomic Energy Commission.

(2) H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel, and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952); H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **50**, 133 (1953); R. Schaffer and H. S. Isbell, *J. Research Natl. Bur. Standards*, **56**, 191 (1956); H. S. Isbell and R. Schaffer, *J. Am. Chem. Soc.*, **78**, 1887 (1956).

(3) R. Kuhn, *Angew. Chem.*, **69**, 23 (1957).

(4) F. Weygand, *Ber.*, **73**, 1284 (1940).

(5) A. Gottschalk, *Nature*, **167**, 845 (1951); *Biochem. J.*, **52**, 455 (1952).

(6) F. Weygand, *Ber.*, **73**, 1259 (1940).

(7) F. Weygand, *Ber.*, **73**, 1278 (1940); German Patent **727,402** (Oct. 1, 1942); U. S. Patent **2,354,846** (Aug. 1, 1944).

(8) G. P. Ellis and J. Honeyman, *Advances in Carbohydrate Chem.*, **10**, 95 (1955).

(9) J. E. Hodge, *Advances in Carbohydrate Chem.*, **10**, 169 (1955).

(10) H. S. Isbell, *Ann. Rev. Biochem.*, **12**, 205 (1943).

(11) H. S. Isbell and H. L. Frush, *J. Am. Chem. Soc.*, **72**, 1043 (1950).

(12) H. S. Isbell and H. L. Frush, *J. Research Natl. Bur. Standards*, **46**, 132 (1951).

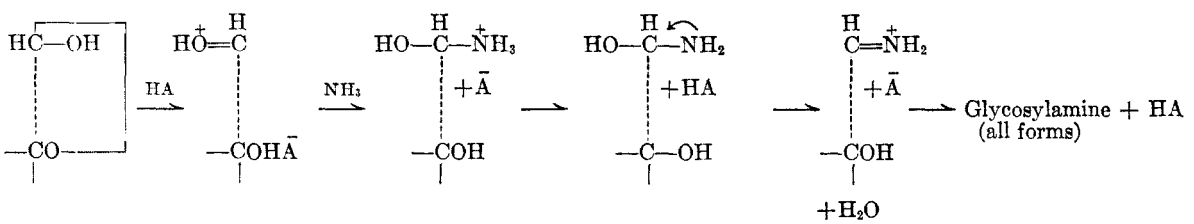
(13) H. L. Frush and H. S. Isbell, *J. Research Natl. Bur. Standards*, **47**, 239 (1951).

the glycosylamines take place concurrently and that the formation of products can be rationalized on the basis of the intermediate imonium ion. It is shown that the mutarotation and hydrolysis of D-glucosylamine, D-mannosylamine and D-xylosylamine, like those of D-galactosylamine and L-arabinosylamine, studied previously, are extremely sensitive to changes in acidity; rate constants are given for the hydrolysis reactions of several glycosylamines under various conditions. An intramolecular mechanism is suggested to account for the effectiveness of certain methylenic compounds and of carboxylic acids in promoting the Amadori rearrangement; evidence is presented for the effectiveness of these reagents in promoting the Amadori rearrangement of unsubstituted D-glucosylamine. Directions are given for the preparation, from D-glucose, D-mannose, and D-xylose, of the free glycosylamines, fully acetylated glycosylamines, *N*-acetylglycosylamines, and diglycosylamines. Some of these are new crystalline compounds. Structures are assigned to the products on the basis of periodate oxidation studies and optical rotation measurements.

Discussion of the reaction mechanisms. Formation of the glycosylamine from the sugar plus ammonia, represented by Equation 1, involves the initial addition of ammonia to one of the acyclic modifications formed from the sugar by the mutarotation reaction.[12] The addition compound decomposes, with elimination of a hydroxyl ion, to form the imonium ion of the glycosylamine. Condensation of the sugar with ammonia can presumably take place either through the neutral, carbonyl modification of the sugar or through the acyclic cation $[R—\overset{H}{C}=OH]^+$ formed by acid catalysis, but the acyclic cation is far more reactive. This hypothesis accounts for the effectiveness of general acid catalysts in promoting formation of glycosylamines. Condensation of the acyclic cation with ammonia does not take place in strongly acidic solution, because, under these conditions, the nucleophilic character of the ammonia is satisfied by combination with hydrogen ion (ammonium salt formation).

basicity of the amino component of the glycosylamine. At a low *p*H substantially all of the glycosylamine exists in the form of ammonium salts II and IV; this accounts for the stability of the glycosylamine in strongly acidic solution.[11–13] When the amino component is strongly basic, the glycosylamine readily forms the ammonium salt II; on the other hand, when the amino component is weakly basic, the glycosylamine yields more of the intermediate III. In the formation of the imonium ion V from III, electrons must move from the nitrogen atom to the glycosidic carbon atom. Strongly basic amino components supply electrons more readily than do weakly basic. Thus, strong basicity of the amino component is favorable for the last step in the production of the imonium ion but unfavorable for the existence of the glycosylamine I under acid conditions. For this reason, the concentration of the imonium ion V is dependent on both the acidity of the environment and the basicity of the amino component of the glycosylamine. The equilibria given in Equation 2 may be present.

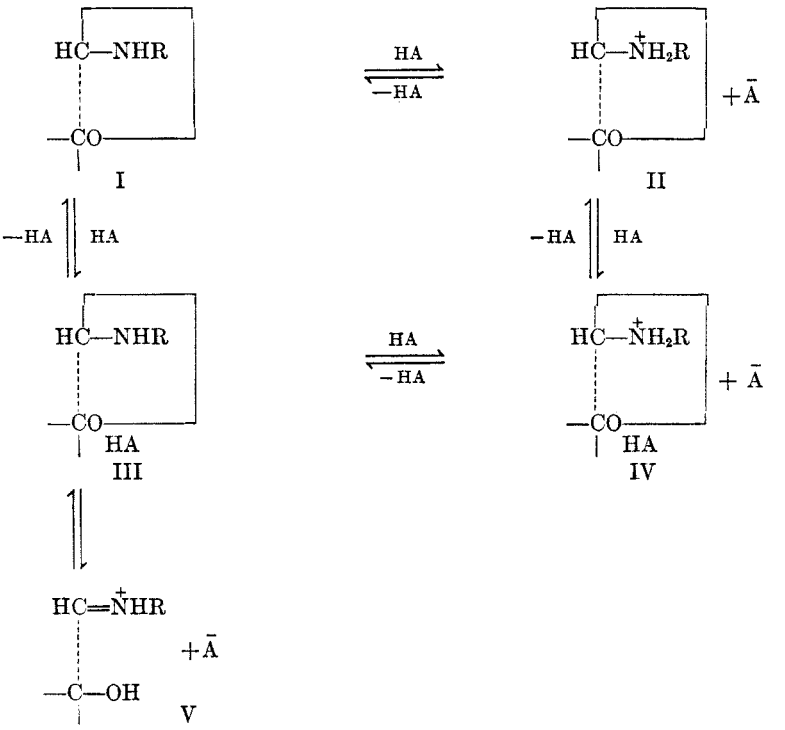
According to the hypothesis previously presented,[12] the mutarotation reaction of a glycosylamine (Equation 3) involves addition of an acid catalyst to the ring oxygen atom, accompanied by a shift of electrons to give the open-chain imonium ion; the anomeric furanoses and pyranoses are then derived by intramolecular condensation with a hydroxyl group of C-4 or C-5, respectively. In this process, the electrophilic property of the carbon atom attached to nitrogen is satisfied by electrons from the oxygen atom of the hydroxyl group. In the present study, it was found that the mutarotation reactions of D-glucosylamine, D-mannosylamine, and D-xylosylamine, like those of D-galactosylamine and L-arabinosylamine, are extremely rapid in neutral and acid solutions but slow in alkaline solutions. The extent of the mutarotation reaction can be estimated from the difference in the optical rotations of freshly prepared solutions of the glycosylamine in aqueous ammonia (in which mutarotation and hydrolysis are slow) and in aqueous acid (in which mutarotation is nearly instantaneous



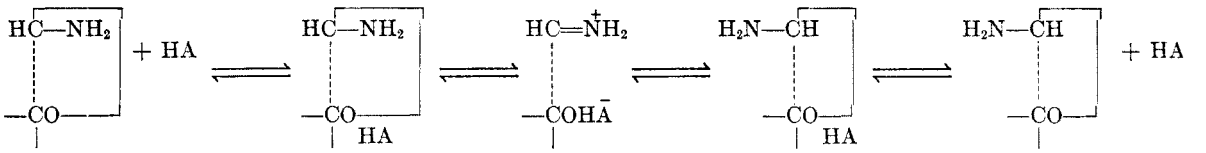
Equation 1. Formation of glycosylamine

Formation of the imonium ion from the glycosylamine I, shown in Equation 2, depends on the presence of an acid catalyst, but the situation is complicated by a side reaction yielding the unreactive ammonium salt II. The extent of the side reaction depends on the *p*H of the solution and on the

and hydrolysis is slow). The changes in specific rotation for β-D-glucopyranosylamine (+20.9° to +20.6°) and for β-D-xylopyranosylamine (−19.5° to −17.6°) are small. This is evidence that D-glucosylamine and D-xylosylamine establish equilibrium mixtures consisting almost entirely of the



Equation 2. Formation of imonium ion



Equation 3. Mutarotation of glycosylamine

beta pyranose modification. The stability of the *beta* pyranose forms of D-glucosylamine and D-xylosylamine presumably arises from their assuming the normal chair conformation, C'1, in which all of the groups attached to the ring, except the hydrogen atoms, lie in equatorial positions.[14–16] The large change in rotation for β-D-mannosylamine (−11.7° to −2.9° for the monohydrate) is evidence that the *beta* form is less stable than β-D-glucosylamine, and that the equilibrium solution contains other, presumably *alpha*, modifications. The difference in behavior of the two glycosylamines may be attributed to the presence of an additional instability factor in the C'1 conformation of the mannose derivative, namely, the axial hydroxyl group at C-2. Since one of the boat forms (B1)[14] for α-D-mannopyranosylamine has an exceptionally stable arrangement (eeeee), it may possibly exist in equilibrium with the β-C'1 form. This would account for the greater mutarotation of β-D-mannopyranosylamine, as well as for the anomalous rotational differences in the mannose series which were previously ascribed to differences in ring conformation.[17]

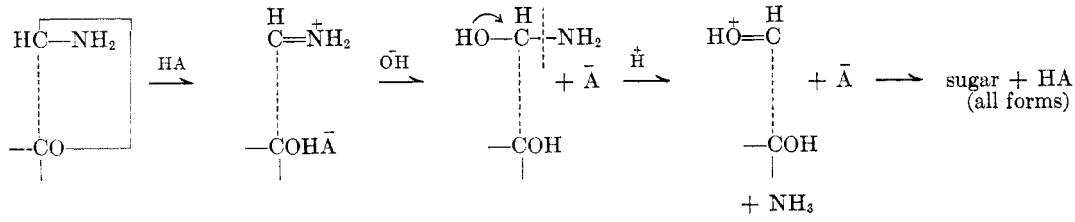
The rate of hydrolysis of glycosylamines was previously found to have a striking and unique dependence on the *p*H of the solution; it attains a maximum in *weakly* acid solution. This marked dependence on *p*H was ascribed to the fact that the reaction requires both an acid catalyst and a hydroxyl ion (Equation 4). As in the other reactions considered here, the hydrolysis mechanism we propose includes the imonium ion intermediate. Addition of a hydroxyl ion forms an aldehyde-ammonia which decomposes to yield ammonia plus the acyclic cation of the sugar. Removal of the ammonia or amine by volatilization or by combination with an acid drives the reaction to completion. As shown in Table II, the rates of hydrolysis for the glycosylamines of D-glucose, D-mannose, and D-xylose reach a maximum in the region of *p*H 5; in strongly acidic or in basic solution the rates are very low. The maximum rate found for D-glucosylamine (0.0081) was only one seventh that for D-mannosylamine (0.060) and one fifth that previously found

(14) R. E. Reeves, *Advances in Carbohydrate Chem.*, 6, 108 (1951).

(15) H. S. Isbell, *J. Research Natl. Bur. Standards*, 57, 171 (1956).

(16) H. S. Isbell, F. A. Smith, E. C. Creitz, H. L. Frush, J. D. Moyer, and J. E. Stewart, *J. Research Natl. Bur. Standards*, 59, 41 (1957).

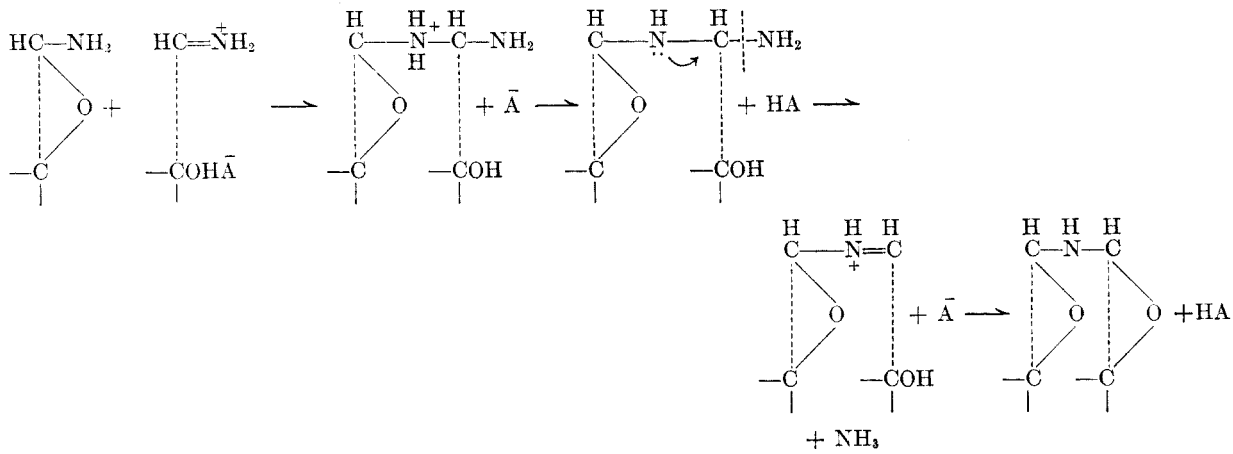
(17) H. S. Isbell, *J. Research Natl. Bur. Standards*, 18, 505 (1937); 24, 125 (1940).



Equation 4. Hydrolysis of glycosylamine

for D-galactosylamine (0.043). The maximum rate for D-xylosylamine (0.112) does not differ widely from that previously found for L-arabinosylamine (0.108).

Transglycosylation reactions[18-20] and the formation of diglycosylamines may also be interpreted in terms of the imonium ion. In transglycosylation reactions, a second amino compound adds to the imonium ion of the first (Equation 5). The resulting diamino compound decomposes with formation of a new glycosylamine and liberation of the amino group originally present (as the free amine or ammonia). When an unsubstituted glycosylamine is heated in a suitable solvent, two molecules condense to form one of a diglycosylamine. This is a type of transglycosylation in which one molecule of the glycosylamine adds to the imonium ion of another molecule. The addition product, like the aldehyde-ammonia formed in the hydrolysis reaction, is unstable and decomposes with the elimination of ammonia. In the absence of an acid catalyst, the reaction is slow for lack of the imonium ion, and in acid solution it is slow for lack of the free amine. Each of the glycosyl groups of the diglycosylamine might have a pyranose or a furanose structure and an *alpha* or a *beta* configuration for the glycosidic carbon atom. Various combinations of these structural features make a number of isomers possible for each glycosylamine, and, in fact, two modifications of di-D-glucosylamine have been isolated.[21] In the present study, it was found that the reaction in methanol or in methyl Cellosolve (ethylene glycol monomethyl ether) is accelerated by the addition of phenol. This catalyst provides some imonium ion without converting the major part of the glycosylamine to the ammonium salt. The conditions do not differ widely from those used for the Amadori rearrangement, except that volatilization of ammonia tends to drive the transglycosylation to completion.



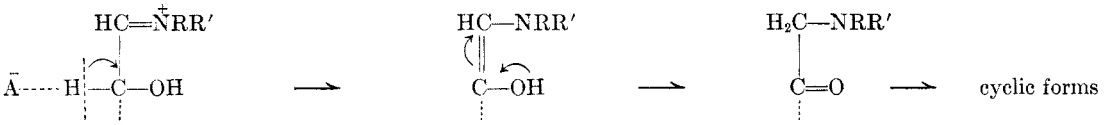
Equation 5. Formation of diglycosylamine

The character of the Amadori rearrangement,[22] the conversion of a *N*-substituted aldosylamine to a *N*-substituted 1-amino-1-deoxyketose, was first recognized by Kuhn and Weygand[23] who clarified the mechanism[6,23] and showed the general application of the reaction. Until fairly recently, the Amadori rearrangement was believed to take place only with *N*-arylglycosylamines. However, Hodge and Rist[24] greatly extended the scope of the reaction by the discovery that members of all classes of *N*-substituted glycosylamines can be rearranged by treatment with a compound having an activated methylene group, in the presence of a

(18) R. Kuhn and A. Dansi, *Ber.*, **69**, 1745 (1936).
(19) Y. Inoue and K. Onodera, *J. Agr. Chem. Soc. Japan*, **22**, 119 (1948).
(20) R. Bognar and P. Nanasi, *J. Chem. Soc.*, 189, 193 (1955).
(21) P. Brigl and H. Keppler, *Z. physiol. Chem.*, **180**, 38 (1929).
(22) M. Amadori, *Atti. reale accad. nazl. Lincei*, [6] **2**, 337 (1925); [6] **9**, 68, 226 (1929); [6] **13**, 72 (1931).
(23) R. Kuhn and F. Weygand, *Ber.*, **70**, 769 (1937).
(24) (a) J. E. Hodge and C. E. Rist, *J. Am. Chem. Soc.*, **74**, 1494 (1952); (b) J. E. Hodge and C. E. Rist, *J. Am. Chem. Soc.*, **75**, 316 (1953).

catalytic quantity of a secondary amine. More recently, it has been found that *N*-alkyl-4,6-*O*-benzylidene-D-glucosylamines can be readily rearranged,[25] presumably because substitution on carbon 6 stabilizes the reactive carbonyl form of the ketose. We have found that rearrangement of unsubstituted glycosylamines can be effected by some of the methylenic compounds of Hodge and Rist, as well as by glacial acetic acid.[26]

Weygand's interpretation of the mechanism of the Amadori rearrangement[6] involves the addition of a proton from an acid catalyst to the nitrogen atom of the glycosylamine. The resulting ammonium salt II is converted to the open-chain, imonium ion V. Subsequent release of a hydrogen atom from the adjacent carbon atom to a base catalyst yields a 1,2-enolic amine. This, on deenolization, presumably gives the two epimeric aldosylamines and the corresponding 1-amino-1-deoxyketose. We consider that the rearrangement of the glycosylamine involves addition of the acid catalyst to the ring oxygen atom rather than to the nitrogen atom[12,13] (see Equation 2) and thus avoids the stable ammonium salt II as a reaction intermediate.[27] As shown in Equation 6, enolization of



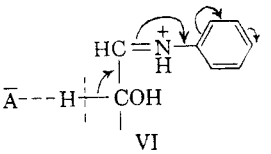
Equation 6. Amadori rearrangement

the resulting imonium ion then takes place by removal of the hydrogen atom of C-2. This hydrogen atom is susceptible to elimination, because a flow of electrons toward the positive nitrogen atom leaves C-1 transiently positive, and a secondary flow of electrons from C-2 to C-1 weakens the C—H bond of C-2. Elimination of the hydrogen atom, with a shift of electrons, yields the enolic amine of Kuhn and Weygand. By tautomeric shift, the enolic glycosylamine is then converted to the 1-amino-1-deoxyketose and presumably to the corresponding epimeric aldosylamines.

Removal of the hydrogen atom of C-2 requires a proton acceptor (base catalyst). Heretofore, this has been considered to derive from the environment. However, the exceptionally strong catalytic effect of methylenic compounds[24] and of the carboxylic acids[28] suggests that more than simple acid-base catalysis is involved, in some cases. Intramolecular mechanisms seem possible for these reactions. Rearrangements effected by methylenic compounds may take place through the addition of the enolic form of the methylenic compound to the imonium ion (Equation 7). The transitory intermediate would then decompose intramolecularly, with regeneration of the methylenic compound in the keto form and formation of the enolic form of the glycosylamine. The process is a type of transenolization, whereby an enolic structure is transferred from one substance to another. It may be considered a model for certain biological enolizations.

In rearrangements effected by carboxylic acids there may be condensation of the imonium ion with the carboxylate ion, followed by intramolecular decomposition of the intermediate and liberation of the carboxylic acid.[29] The process is illustrated in Equation 8.

The facility with which *N*-substituted glycosylamines undergo the Amadori rearrangement is strikingly dependent on the character of the amino component. *N*-Arylglycosylamines, in particular, show a wide variation in reactivity. As shown in Equation 9, both imonium ion formation and subsequent enolization are affected by the nature of the *N*-substituent. It was pointed out in a previous paragraph that strongly basic amino compounds (although tending in acid solution to enter into a side reaction, ammonium ion formation) supply electrons for the formation of the imonium ion more readily than do weakly basic amino components. In this step, the electron shift is away from the aromatic nucleus. However, in the next step (enolization) the electron shift is toward the aromatic nucleus as shown in VI. The electron shift is facilitated by the tendency of the ring (particularly



VI

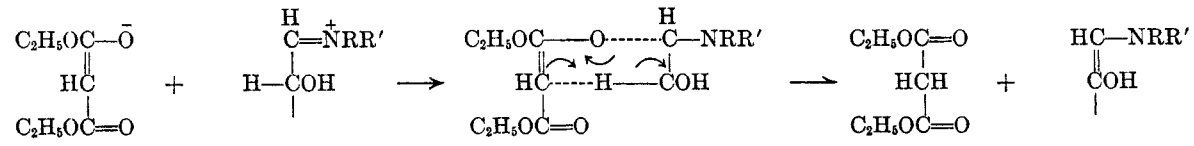
(25) F. Micheel and A. Frowein, *Chem. Ber.*, **90**, 1599 (1957).

(26) See W. Pigman, E. A. Cleveland, D. H. Couch, and J. H. Cleveland, *J. Am. Chem. Soc.*, **73**, 1976 (1951). These workers found that *N*-substituted D-glucosylamines, when dissolved in glacial acetic acid, develop a brown color, and that D-glucosylamine and *N*-butyl-D-glucosylamine show changes in optical rotation from positive to negative values. They suggested that an Amadori-type rearrangement is involved.

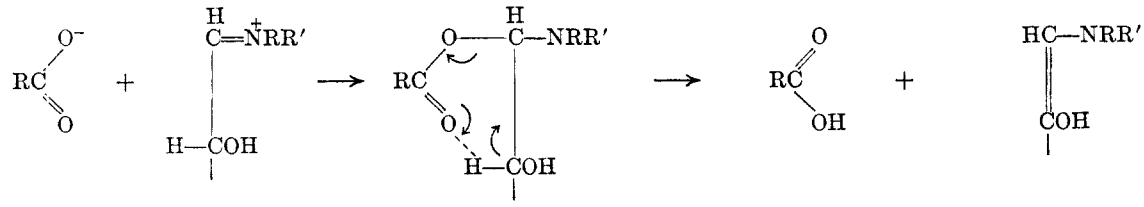
(27) The stability of the salt is evidenced by the inertness of the glycosylamine in strongly acidic solution. The ammonium salt, in the equilibrium, is therefore considered to be only a collateral product.

(28) L. Rosen, J. W. Woods, and W. Pigman, *Chem. Ber.*, **90**, 1038 (1957).

(29) Presumably the transitory intermediate can also be formed by the addition of the carboxylic acid, followed by elimination of a proton. This accounts for the effectiveness of glacial acetic acid in the rearrangement.



Equation 7. Suggested mechanism for effect of methylenic compounds in promoting Amadori rearrangement

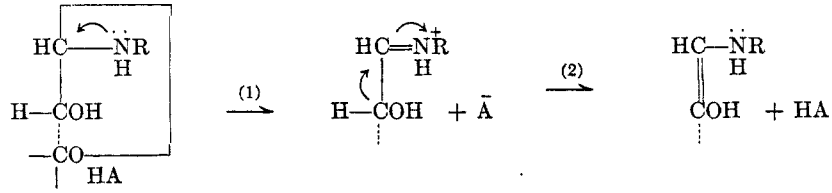


Equation 8. Suggested mechanism for effect of carboxylic acids in promoting Amadori rearrangement

in weakly basic arylamino components) to take up electrons, thus enhancing the electron deficiency at carbon 1. The opposite requirements involved in these two steps are not mutually exclusive, but they do limit the conditions under which the Amadori rearrangement can most favorably occur. Thus, when the attraction of R in Equation 9 for the electrons of the nitrogen atom is too great (as in *N*-acetylglucosylamine[30] and *N*-glycosides of *p*-nitroaniline[28,31]) step 1, namely imonium ion formation, is inhibited; when the attraction of R for these electrons is too small (as in the more strongly basic alkylamines), there is little or no tendency for the enolization, step 2, to occur by the mechanism given. However, the intramolecular mechanisms suggested in Equations 7 and 8 depend upon decomposition of an intermediate formed from the imonium ion, and not upon an electron shift toward R, as given in step 2 of Equation 9. It seems significant that the methylenic compounds of Hodge and Rist are effective in promoting rearrangement of strongly basic alkylamines, compounds for which the substituent R does not promote step 2.

Amadori rearrangement proceeds more or less completely from the *N*-substituted glycosylamine to the corresponding substituted 1-amino-1-deoxyketose. The process is complicated by side reactions arising from the tendency of the intermediate enolic amine to decompose in a variety of ways as summarized by Hodge.[9] Some of these may involve migration of electrons from points of higher electron-density to points of lower electron-density, together with the addition and elimination of ions by steps analogous to those described for the formation of 2-furaldehyde and reductic acid from pentoses.[33]

It was mentioned above that the original concept of the Amadori rearrangement has been broadened to include rearrangement of both *N*-aryl- and *N*-alkylaldosylamines to the corresponding substituted 1-amino-1-deoxyketoses. It is suggested here that the name should also be applied to the so-called "reverse Amadori rearrangement" of ketosylamines to the corresponding 2-amino-2-deoxyaldoses, a type of rearrangement that has received relatively little attention. Such use of the term, Amadori rearrangement, would recognize the similarity of the intermediates and of the reaction mechanisms. The similarity is shown by the conversion of D-fructosylamine to 2-amino-2-deoxy-D-glucose (D-glucosamine) in liquid ammonia containing ammonium hydroxide.[34] Here, the ammonium



Equation 9. Effect of basicity of amino component on imonium ion formation and subsequent enolization

The labile, enolic amine obtained by these mechanisms should yield an equilibrium mixture of the two epimeric aldosylamines plus the corresponding 1-amino-1-deoxyketose, but this equilibrium condition has not been realized experimentally.[32] The

(30) E. Mitts and R. M. Hixon, *J. Am. Chem. Soc.*, 66, 483 (1944).

(31) F. Weygand, W. Perkow, and P. Kuhner, *Chem. Ber.*, 84, 594 (1951).

(32) See K. Heyns, H. Breuer, and H. Paulsen, *Chem. Ber.*, 90, 1374 (1957). These authors recently reported that, in an Amadori-type rearrangement of D-fructosyl compounds of certain amino acids, the 2-amino acid derivatives of both D-glucose and D-mannose were obtained.

(33) H. S. Isbell, *J. Research Natl. Bur. Standards*, 32, 45 (1944).

(34) K. Heyns and K. H. Meinecke, *Chem. Ber.*, 86, 1453 (1953).

ion presumably serves as acid catalyst to form the imonium ion, and either the hydroxyl ion or the ammonia molecule serves as base catalyst. Several *N*-substituted D-fructosylamines prepared from aliphatic amines or amino acids have also been found to undergo rearrangement to the corresponding D-glucosamine derivatives.[32,35–37]

Assignment of Ring Structure. The structures for the *N*-acetylglycosylamines were assigned from the changes in optical rotation found on oxidation of the material with sodium metaperiodate (Table IV). Niemann and Hays[38] had previously reported that one mole of *N*-acetyl-D-glucosylamine reacts with 2 moles of sodium metaperiodate to yield a product characteristic of a pyranose structure. On the basis of the specific rotation (−22°) they assigned the *beta* pyranose structure to *N*-acetyl-D-glucosylamine. The later preparation of an anomeric pair of *N*-acetyl-D-galactopyranosylamines by Frush and Isbell[13] made possible a comparison of the optical rotations of the dialdehydes formed by periodate oxidation, and hence an assignment of ring structure for other *N*-acetylglycosylamines. Thus, the *alpha* and *beta* *N*-acetyl-D-aldohexopyranosylamines, on periodate oxidation, give products having specific optical rotations, respectively, of about +60° and −96°, based on the weight of the glycosylamine; all *N*-acetyl-pentopyranosylamines having the same absolute configuration for C-1 as *N*-acetyl-α-L-arabinopyranosylamine give a product with an optical rotation of about −49°. The ring structures and anomeric configurations of the *N*-acetylglycosylamines listed in Table I were assigned on the basis of these values. Three of these assignments were made from the data of Table IV; the others had been made previously.

The structures for the fully acetylated glycosylamines of Table I are based on the structures of the corresponding *N*-acetyl derivatives, which were obtained by their deacetylation with barium methylate. This mild treatment would presumably not lead to a change in structure or configuration; hence, the fully acetylated glycosylamines have been assigned the same structures as the corresponding *N*-acetyl derivatives.

The structures for the free glycosylamines (Table III) were assigned by application of Hudson's principle of isorotation.[39a,39b] According to this principle, the approximate rotation of the *alpha* pyranose is given by B + A and that of the *beta* pyranose by B − A, where A is the rotational contribution of C-1, and B is that of the rest of the molecule. The molecular rotations of the glycosylamines were calculated on the assumption that the values of B for the glycosylamines and the sugars are alike, and that the value of A, obtained from the rotations of the *alpha* and *beta* D-galactopyranosylamines, is applicable to all of the glycosylamines listed, except D-mannosylamine. Because the values of A in the mannose series are anomalous, the molecular rotation of β-D-mannosylamine was calculated from that of β-D-glucosylamine by application of Hudson's rule for epimeric difference.[40] The agreement of the calculated molecular rotations with the observed values supports the structures listed. The marked similarity in the optical rotations of the sugars and the corresponding glycosylamines is noteworthy.

Several diglycosylamines are also listed in Table I. Although a detailed study of their structures is contemplated, this report is restricted to a description of their properties.

EXPERIMENTAL

β-D-Mannopyranosylamine monohydrate. To 50 ml. of methanol were added 20 g. of D-mannose and 0.5 g. of ammonium chloride, and the mixture was treated with ammonia gas at 0° until the sugar had dissolved. The solution was stored at 0° until satisfactory crystallization had occurred. The first crystallization was slow, and required storage of the solution for about a month. Subsequent crystallizations with the aid of seed crystals were more rapid, but maximum yields required storage for a week or two. The crystals were separated, washed with methanol, and dried over sodium hydroxide in a vacuum desiccator. The crude product, 17 g., was dissolved in 17 ml. of water and the solution was diluted with 170 ml. of methanol and sufficient ethanol to produce turbidity. After 48 hr., the crystalline material was separated, washed with methanol, and stored over sodium hydroxide in an atmosphere of ammonia for 2 days.

Anal. (after removal of excess ammonia by evacuation): Calcd. for $C_6H_{13}NO_5.H_2O$: C, 36.54; H, 7.67; N, 7.10. Found: C, 36.9; H, 7.7; N, 7.1. M.p. 93–94°; $[\alpha]_D^{20}$ −11.6° (H_2O, c = 2).

The new, crystalline D-mannosylamine can be kept for short periods in an atmosphere of ammonia. However, when stored for several weeks in a vacuum desiccator over phosphoric anhydride, it is converted to an amorphous material from which the previously known di-D-mannosylamine[41] can be crystallized.

N-Acetyl-tetra-O-acetyl-β-D-mannopyranosylamine. Finely powdered, crystalline β-D-mannopyranosylamine (10 g.) was added to a previously cooled mixture of 100 ml. of pyridine and 50 ml. of acetic anhydride in a flask that was equipped with a mechanical stirrer and immersed in a mixture of ice and salt. Stirring was continued in the ice-salt bath until the crystals had dissolved. After standing a few hours at room temperature, the solution was poured into a liter of ice and water; the mixture was stirred for 30 min. and then extracted with chloroform. Evaporation of the chloroform gave a crude product, 15.6 g., which was recrystallized twice from chloroform, with the addition of petroleum ether, to give pure *N*-acetyl-tetra-*O*-acetyl-β-D-manno-

(35) J. F. Carson, *J. Am. Chem. Soc.*, **77**, 1881, 5957 (1955); **78**, 3728 (1956).

(36) K. Heyns, R. Eichstedt, and K. H. Meinecke, *Chem. Ber.*, **88**, 1551 (1955).

(37) K. Heyns, H. Paulsen, and H. Breuer, *Angew. Chem.*, **68**, 334 (1956).

(38) C. Niemann and J. T. Hays, *J. Am. Chem. Soc.*, **62**, 2960 (1940).

(39) (a) C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909); (b) F. J. Bates and Associates, *Natl. Bur. Standards Circ.* **C440**, 428 (1942).

(40) C. S. Hudson, *J. Am. Chem. Soc.*, **48**, 1434 (1926).

(41) C. A. Lobry de Bruyn and F. H. Van Leent, *Rec. trav. chim.*, **15**, 81 (1896).

TABLE I

GLYCOSYLAMINES, DIGLYCOSYLAMINES, AND THEIR ACETATES INCLUDED IN THIS STUDY

Compound	Reference to Preparation	Formula	Molecular Weight	$[\alpha]_D^{20}$	Molecular Rotation	Melting[a] Point, °C.
α-L-Arabinopyranosylamine	b,c	$C_5H_{11}NO_4$	149.15	+ 86.3° (H_2O)	+12,870	124–125
β-D-Xylopyranosylamine	b	$C_5H_{11}NO_4$	149.15	− 19.6° (H_2O)	− 2,920	128–129
α-D-Galactopyranosylamine ammonia complex	d,b	$C_6H_{16}N_2O_5$	196.21	+138° (H_2O)	+27,080	107–109
β-D-Galactopyranosylamine	d,b	$C_6H_{13}NO_5$	179.18	+ 62.2° (H_2O)	+11,140	134–136
β-D-Glucopyranosylamine	e,f	$C_6H_{13}NO_5$	179.18	+ 20.8° (H_2O)	+ 3,730	125–127
β-D-Mannopyranosylamine monohydrate	New	$C_6H_{15}NO_6$	197.20	− 11.6° (H_2O)	− 2,290	93–94
N-Acetyl-tri-*O*-acetyl-α-L-arabinopyranosylamine	c	$C_{13}H_{19}NO_8$	317.30	+ 89.6° ($CHCl_3$)	+28,430	177–178
N-Acetyl-tri-*O*-acetyl-β-D-xylopyranosylamine	New	$C_{13}H_{19}NO_8$	317.30	+ 28.5° ($CHCl_3$)	+ 9,040	172–173
N-Acetyl-tetra-*O*-acetyl-α-D-galactopyranosylamine	d	$C_{16}H_{23}NO_{10}$	389.36	+117.4° ($CHCl_3$)	+45,710	172–173
N-Acetyl-tetra-*O*-acetyl-β-D-galactopyranosylamine	d	$C_{16}H_{23}NO_{10}$	389.36	+ 34.7° ($CHCl_3$)	+13,510	173–174
N-Acetyl-tetra-*O*-acetyl-β-D-glucopyranosylamine	f	$C_{16}H_{23}NO_{10}$	389.36	+ 17.4° ($CHCl_3$)	+ 6,770	163–164
N-Acetyl-tetra-*O*-acetyl-β-D-mannopyranosylamine	New	$C_{16}H_{23}NO_{10}$	389.36	− 16.5° ($CHCl_3$)	− 6,420	188–189
N-Acetyl-α-L-arabinopyranosylamine	c	$C_7H_{13}NO_5$	191.18	+ 69.7° (H_2O)	+13,330	222–224
N-Acetyl-β-D-xylopyranosylamine	New	$C_7H_{13}NO_5$	191.18	− 0.7° (H_2O)	− 130	213–214
N-Acetyl-α-D-galactopyranosylamine	d	$C_8H_{15}NO_6$	221.21	+194.9° (H_2O)	+43,110	179–180
N-Acetyl-β-D-galactopyranosylamine	d	$C_8H_{15}NO_6$	221.21	+ 9.8° (H_2O)	+ 2,170	233
N-Acetyl-β-D-glucopyranosylamine	f	$C_8H_{15}NO_6$	221.21	− 22.8° (H_2O)	− 5,040	260[g]
N-Acetyl-β-D-mannopyranosylamine monohydrate	New	$C_8H_{17}NO_7$	239.23	− 47.4° (H_2O)	−11,340	203–204
Di-L-arabinosylamine	New	$C_{10}H_{19}NO_8$	281.27	+ 50.6° (H_2O)	+14,230	145
Di-D-xylosylamine	New	$C_{10}H_{19}NO_8$	281.27	− 44.3° (H_2O)	−12,460	159–161
"β"-Di-D-glucosylamine dihydrate	f	$C_{12}H_{27}NO_{12}$	359.49	− 21.1° (H_2O)	− 7,590	106–109[h]
Di-D-mannosylamine	i	$C_{12}H_{23}NO_{10}$	341.33	− 36.8° (H_2O)	−12,560	157–158
Hexa-*O*-acetyl-di-D-xylosylamine	New	$C_{22}H_{31}NO_{14}$	533.48	+ 16.8° ($CHCl_3$)	+ 8,960	218–219
Octa-*O*-acetyl-di-D-mannosylamine	New	$C_{28}H_{39}NO_{18}$	677.60	− 68.0° ($CHCl_3$)	−46,080	146–147

[a] In some cases the specific rotations and melting points given differ somewhat from those reported in the literature cited. [b] Ref. 44. [c] Ref. 12. [d] Ref. 13. [e] Ref. 43. [f] Ref. 21. [g] Gradual decomposition from about 230°. [h] Brigl and Keppler[21] reported 125–126°. [i] Ref. 41.

pyranosylamine. M.p. 188–189°; $[\alpha]_D^{20}$ −16.5° ($CHCl_3$, c = 2).

Anal. Calcd. for $C_{16}H_{23}NO_{10}$: C, 49.35; H, 5.95; N, 3.60. Found: C, 49.3; H, 5.9; N, 3.6.

N-Acetyl-β-D-mannopyranosylamine monohydrate. *N*-Acetyl-tetra-*O*-acetyl-β-D-mannopyranosylamine (10 g.) was dissolved in 100 ml. of anhydrous methanol containing 10 ml. of 0.2*M* barium methylate. After the solution had stood for 1 hr. at room temperature, dilute sulfuric acid, exactly equivalent to the barium methylate, was added. The barium sulfate was removed by filtration and the filtrate was concentrated under reduced pressure to a sirup from which *N*-acetyl-β-D-mannopyranosylamine was separated by crystallization. The crude product weighed 6 g. and had a specific rotation of −44.9° in water. It was recrystallized several times by dissolving in 2 parts of warm water, and adding 12 parts of methanol and 3 parts of ethanol. The properties of the new *N*-acetyl-β-D-mannopyranosylamine were not altered by further recrystallization. M.p. 203–204°; $[\alpha]_D^{20}$ −47.4° (H_2O, c = 2).

Anal. Calcd. for $C_8H_{15}NO_6.H_2O$: C, 40.16; H, 7.16; N, 5.86. Found: C, 40.2; H, 7.2; N, 5.8.

Di-D-mannosylamine and octa-O-acetyl-di-D-mannosylamine. A sample of the new D-mannosylamine hydrate, when recrystallized after long storage over phosphorus pentoxide, was found to have been converted to the previously known di-D-mannosylamine.[41] The latter substance was also prepared by heating crude D-mannosylamine hydrate in methanol. The material was recrystallized several times from 1 part of aqueous ammonia by the addition of 5 parts of methanol. M.p. 157–158°; $[\alpha]_D^{20}$ −36.8° (5 min.) (H_2O, c = 5). This value is somewhat higher than that (−28.3°) reported by Lobry de Bruyn and Van Leent.[41] When di-D-mannosylamine was hydrolyzed in a hydrochloric acid–sodium acetate buffer (*p*H 5) the optical rotation, $[\alpha]_D^{20}$, reached a constant value of +13.8°, based on the weight of the D-mannose formed. This is in approximate agreement with the accepted value for D-mannose (+14.2°).

Di-D-mannosylamine (4.0 g.) was acetylated in a mixture of 60 ml. of pyridine and 30 ml. of acetic anhydride by the

TABLE II

MUTAROTATION AND HYDROLYSIS OF GLYCOSYLAMINES[a]

Composition of Solvent	pH	Optical Rotation: First reading Time (min.)	First reading $[\alpha]_D^{20}$	Last reading Time (min.)	Last reading $[\alpha]_D^{20}$	pH of Mixture	$k_{hydrol.}$[b]
β-D-Glucosylamine							
Concd. NH_4OH + water (1:9)	11.4	4.0	+20.9°	4,320	+23.9°[c]	11.6	0.00001
Water (CO_2 free)	7.0	3.0	+20.8°	5,760	+23.0°[c]	10.0	0.00002
1*M* KH_2PO_4 + 1*N* NaOH (5:1)	5.9	6.4	+23.8°	1,440	+51.1°	6.4	0.0017
1*N* Acetic acid + 0.4*N* NaOH (1:1)	4.3	4.8	+24.7°	300	+52.8°	4.8	0.0081
1*N* Acetic acid + 0.2*N* NaOH (1:1)	4.0	4.4	+23.0°	300	+52.7°	4.4	0.0080
1*N* Acetic acid	2.2	3.7	+22.2°	1,400	+52.8°	3.7	0.0032
2.5*N* HCl	—	—	+20.6°	7,200	+22.7°[c]	—	0.00001
β-D-Mannosylamine							
Concd. NH_4OH + water (1:9)	11.4	2.0	−11.7°	1,110	− 9.7°[c]	11.4	0.00003
Water (CO_2 free)	7.0	4.0	−11.6°	1,140	−10.7°[c]	10.5	0.00002
1*M* KH_2PO_4 + 1*N* NaOH (5:1)	5.9	2.3	− 5.1°	36	+ 9.0°[c]	6.3	0.040
1*N* Acetic acid + 0.4*N* NaOH (1:1)	4.3	2.1	− 0.7°	30	+12.4°	4.9	0.060
1*N* Acetic acid + 0.2*N* NaOH (1:1)	4.0	2.0	+ 0.8°	160	+12.4°	4.6	0.032
1*N* Acetic acid	2.2	2.2	− 2.5°	1,180	+12.5°	4.3	0.025
2.5*N* HCl	—	9.0	− 2.9°	1,180	− 2.4°[c]	—	0.00001
β-D-Xylosylamine							
Concd. NH_4OH + water (1:9)	11.4	3.4	−19.5°	15,840	− 8.7°[c]	11.6	0.00004
Water (CO_2 free)	7.0	1.9	−19.6°	5,760	−10.5°[c]	10.0	0.00003
1*M* KH_2PO_4 + 1*N* NaOH (5:1)	5.9	1.9	− 1.3°	240	+18.0°	6.4	0.020
1*N* Acetic acid + 0.4*N* NaOH (1:1)	4.3	1.6	− 6.0°	40	+19.0°	4.8	0.112
1*N* Acetic acid + 0.2*N* NaOH (1:1)	4.0	1.5	− 7.9°	60	+18.9°	4.5	0.110
1*N* Acetic acid	2.2	1.9	− 9.9°	180	+19.1°	3.7	0.070
2.5*N* HCl	—	4.0	−17.6°	7,200	−11.8°[c]	—	0.00001

[a] The glycosylamine (0.5 g.) was dissolved in a sufficient quantity of the solvent to give a volume of 25 ml. [b] The rate constant was calculated from a series of readings taken between the first and last readings given. The following equation was used:

$$k_{hydrol.} = \frac{1}{t_2 - t_1} \log \frac{r_{t_1} - r_\infty}{r_{t_2} - r_\infty}$$

where t_2 and t_1 are points in time after a steady state has been obtained for the modifications of the amine in solution, r_{t_1} and r_{t_2} are the optical rotations observed at times t_1 and t_2, respectively, and r_∞ is the optical rotation of the solution after hydrolysis was complete. [c] When the hydrolysis reaction was not complete at the last reading, the optical rotation for the completely hydrolyzed material was used in the calculation.

TABLE III

CALCULATED AND OBSERVED OPTICAL ROTATIONS OF THE GLYCOSYLAMINES

Structure	Glycosylamine: Observed values $[\alpha]_D^{20}$	Glycosylamine: Observed values [M]	Glycosylamine: Calculated value[a] [M]	Sugar: Observed values[b] $[\alpha]_D^{20}$	Sugar: Observed values[b] [M]	Sugar: 2B
α-L-Arabinopyranose	+ 86.3°	+12,870	+12,200	+ 77.0°[c]	+11,560	+40,170
β-D-Xylopyranose	− 19.6°	− 2,920	− 2,360	− 20°	− 3,060	+11,050
α-D-Galactopyranose	+151°[d]	+27,080	+26,210	+150.7°	+27,150	+36,660
β-D-Galactopyranose	+ 62.2°	+11,140	+10,450	+ 52.8°	+ 9,510	+36,660
β-D-Glucopyranose	+ 20.8°	+ 3,730	+ 3,910	+ 18.7°	+ 3,370	+23,580
β-D-Mannopyranose	− 12.8°[e]	− 2,290	− 2,860	− 17.0°	− 3,060	—

[a] Molecular rotation M = B ± A, where A (obtained from the anomeric galactosylamines) is +7,880, and B is assumed to be the same as for the corresponding sugar. [b] Values are from Table 55 of Ref. 39b except the indirect value for β-D-xylose; for this, see C. S. Hudson and E. Yanovsky, *J. Am. Chem. Soc.*, **39**, 1013 (1917). [c] α-L-Arabinose crystallizes as a calcium chloride complex $C_5H_{10}O_5.CaCl_2.4H_2O$. The optical rotation is given on the basis of the sugar only. [d] α-D-Galactopyranosylamine crystallizes as a complex with one molecule of ammonia. The optical rotation is given here on the ammonia-free basis. See also Table I. [e] β-D-Mannopyranosylamine crystallizes with one molecule of water. The optical rotation is given here on the water-free basis. See also Table I.

procedure previously described. The crude crystalline product (4.1 g.), on repeated crystallization from hot ethanol, yielded 3.5 g. of a new octa-*O*-acetyl-di-D-mannosylamine. M.p. 146–147°; $[\alpha]_D^{20}$ −68.0° ($CHCl_3$, *c* = 2).

Anal. Calcd. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.8; H, 5.9; N, 2.0.

β-D-Glucopyranosylamine. D-Glucose (40 g.) was converted to D-glucosylamine by the procedure described for the preparation of β-D-mannosylamine. The product was

TABLE IV

PERIODATE OXIDATION OF *N*-ACETYLGLYCOSYLAMINES

Reaction Time (min.)	Optical Rotation[a] $[\alpha]_D^{20}$	Moles Periodate per Mole Amine
N-Acetyl-β-D-glucosylamine		
2.8	−52.2°	—
15.9	−89.3°	—
30.	−94.8°	2.05
60.	−95.6°	2.06
N-Acetyl-β-D-mannosylamine		
2.5	−90.°	—
20.	−101.°	—
60.	−99.9°	2.05
120.	−98.9°	2.06
N-Acetyl-β-D-xylosylamine		
2.5	−16.2°	—
20.6	−43.6°	1.93
60.	−44.4°	2.03
120.	−39.3°	2.05

[a] The *N*-acetylglycosylamine (1 g.) was dissolved in sufficient 0.3*M* sodium metaperiodate to give a volume of 50 ml.; the solution was read in a 2-dm. tube, and $[\alpha]_D^{20}$ was based on the weight of the original *N*-acetylglycosylamine.

recrystallized from an equal weight of a 1:10 mixture of concentrated ammonium hydroxide and water, by the successive addition of 2 volumes of methanol and 2 volumes of ethanol. The yield was 25 g. The properties of the compound, given in Table I, are in substantial agreement with those previously described.[42]

N-Acetyl-tetra-O-acetyl-β-D-glucopyranosylamine. β-D-Glucopyranosylamine was acetylated by the method described above. The crude product was recrystallized from ethanol, and then from chloroform with the addition of heptane. M.p. 163–164°; $[\alpha]_D^{20}$ +17.4° ($CHCl_3$, c = 2) in substantial agreement with the values of Niemann and Hays.[38]

N-Acetyl-β-D-glucopyranosylamine. *N*-Acetyl-tetra-*O*-acetyl-β-D-glucopyranosylamine was *O*-deacetylated with barium methylate, and the crude crystalline product was recrystallized several times from aqueous acetone. Brigl and Keppler[21] reported a mutarotation from −22° to −23° in the course of 5 hr. Our product failed to show this mutarotation, but had a constant specific rotation (−22.8°) in substantial agreement with the final value previously reported.

β-Di-D-glucosylamine. This compound was prepared by the method of Sjollema[43] as described fully by Brigl and Keppler.[21] After recrystallization of the material from a concentrated water solution, by the addition of methanol, $[\alpha]_D^{20}$ was −21.1° (H_2O, c = 2). This value is in approximate agreement with that previously reported, but the melting point (106–109°) is considerably lower than that of Brigl and Keppler (125–126°). The compound will be investigated further.

β-D-Xylopyranosylamine and N-acetyltri-O-acetyl-β-D-xylopyranosylamine. D-Xylosylamine, previously reported by Lobry de Bruyn and Van Leent,[44] was prepared from D-xylose, and purified by the procedure described for D-mannosylamine. The specific rotation, $[\alpha]_D^{20}$, of the pure β-D-xylosylamine (−19.6°, 2 min., H_2O, c = 2) is somewhat higher than that previously reported (−18.1°). Acetylation of 10 g. of this material by the procedure previously described yielded 1.4 g. of silky needles, $[\alpha]_D^{20}$ +13.7°, which proved to be the new hexa-*O*-acetyl-di-D-xylosylamine described in a later paragraph. From the mother liquors, 12 g. of prismatic crystals were obtained, which, after several recrystallizations from chloroform with the addition of petroleum ether, gave a pure new *N*-acetyl-tri-*O*-acetyl-β-D-xylopyranosylamine. M.p. 172–173°; $[\alpha]_D^{20}$ +28.5° ($CHCl_3$, c = 2).

Anal. Calcd. for $C_{13}H_{19}NO_8$: C, 49.21; H, 6.04; N, 4.41. Found: C, 48.9; H, 6.0; N, 4.3.

N-Acetyl-β-D-xylopyranosylamine. *N*-Acetyl-tri-*O*-acetyl-β-D-xylopyranosylamine (10 g.), when *O*-deacetylated by barium methylate, yielded a crude, crystalline product that weighed 5.8 g. and had a specific rotation of −0.6° in water. After several recrystallizations from water, with the addition of methanol, a pure new, *N*-acetyl-β-D-xylopyranosylamine was obtained. M.p. 213–214°; $[\alpha]_D^{20}$ −0.7° (H_2O, c = 2).

Anal. Calcd. for $C_7H_{13}NO_5$: C, 43.97; H, 6.85; N, 7.33. Found: C, 44.1; H, 6.9; N, 7.4.

Di-D-xylosylamine and hexa-O-acetyl-di-D-xylosylamine. D-Xylosylamine (10 g.) was refluxed in 20 ml. of methyl cellosolve until evolution of ammonia had ceased (about 1 hr.). After the straw-colored solution had stood for several hours at room temperature, 2.3 g. of crystals were separated and then recrystallized from water with the addition of methanol. In a separate experiment, di-D-xylosylamine was obtained in approximately the same yield by refluxing a solution of β-D-xylosylamine in methanol containing a small amount of phenol, as described later for the preparation of di-L-arabinosylamine. The new di-D-xylosylamine melts at 154–155°; $[\alpha]_D^{20}$ −44.3° (10 min.), −40.2° (20 min.), −38.0° (90 min.) (H_2O, c = 1.4).

Anal. Calcd. for $C_{10}H_{19}NO_8$: C, 42.7; H, 6.45; N, 4.98. Found: C, 43.0; H, 6.9; N, 5.0.

When di-D-xylosylamine was hydrolyzed by bubbling carbon dioxide through the solution, the specific rotation, $[\alpha]_D^{20}$, became +18.4°, based on the weight of D-xylose formed. This is in good agreement with the equilibrium value, +18.8°, for pure D-xylose.

The di-D-xylosylamine was acetylated with pyridine and acetic anhydride as previously described. The product crystallized as a mixture of long, hair-like crystals that formed gelatinous clumps, and fine needles that separated in brush-like clusters. The substances were fractionally separated by successive recrystallizations from hot ethanol and then from chloroform with the addition of petroleum ether. The hair-like product is a new hexa-*O*-acetyl-di-D-xylosylamine. M.p. 218–219°; $[\alpha]_D^{20}$ +16.8° ($CHCl_3$, c = 2).

Anal. Calcd. for $C_{22}H_{31}NO_{14}$: C, 49.52; H, 5.86; N, 2.63. Found: C, 49.6; H, 5.8; N, 2.7.

The second product has not yet been identified.

Di-L-arabinosylamine. A mixture consisting of 5 g. of β-L-arabinopyranosylamine,[12,44] 25 ml. of methanol, and 1 ml. of phenol was heated and stirred under a reflux condenser. When the evolution of ammonia had subsided (about 1 hr.), the mixture was cooled to room temperature and set aside for crystallization. Two types of crystals formed, bipyramids and needles. By repeated recrystallization of the crude product from aqueous ammonia, with the addition of methanol, 3.1 g. of the pure bipyramid product, a new di-L-arabinosylamine, was obtained. M.p. 145° (dec.); $[\alpha]_D^{20}$ +50.6° (H_2O, c = 1.6).

Anal. Calcd. for $C_{10}H_{19}NO_8$: C, 42.70; H, 6.45; N, 4.98. Found: C, 42.3; H, 6.9; N, 4.9.

On treatment of di-L-arabinosylamine with 0.1*N* hydrochloric acid, the specific rotation, $[\alpha]_D^{20}$ (c = 2), of the substance reached a maximum of +94.2° in 6 min., decreased to +90.4° in 21 min., and finally increased to +109.3° in 60 hr. It seems probable that the initial rapid change arises from establishment of an equilibrium state for the di-L-arabinopyranosylamine; the decrease in rotation results

(42) C. A. Lobry de Bruyn, *Rec. trav. chim.*, 14, 98 (1895).

(43) B. Sjollema, *Rec. trav. chim.*, 18, 292 (1899).

(44) C. A. Lobry de Bruyn and F. H. Van Leent, *Rec. trav. chim.*, 14, 134 (1895).

from cleavage of the diglycosylamine, thus forming L-arabinosylamine and L-arabinose; and the final slow increase indicates hydrolysis of L-arabinosylamine to L-arabinose.

The substance that separated in needle-like crystals has not yet been isolated in the pure state.

Amadori rearrangement of D-*glucopyranosylamine in acetic acid.* β-D-Glucopyranosylamine (0.5 g.) was dissolved in 10 ml. of glacial acetic acid and the solution was kept at 20°. The optical rotation, $[\alpha]_D^{20}$, changed from a positive value to −18° in 1 hr. and −65° in 18 hr.; the solution had then become amber-colored. The acetic acid was removed by the repeated addition of toluene and evaporation in a rotary vacuum still. The residue, containing a small amount of acetic acid, was dissolved in 10 ml. of water, and the solution, after standing for several hours for hydrolysis to take place, was again concentrated; this treatment caused decomposition of any remaining D-glucosylamine. The residue was dissolved in 10 ml. of water and passed through a column containing 20 ml. of cation exchange resin (Amberlite IR120-H. Rohm & Haas Co., Philadelphia, Pa.); the resin was then washed with water and the wash liquor discarded. The basic materials held on the resin were eluted with 20 ml. of *N* hydrochloric acid. The eluate and wash liquor were combined, concentrated and then adjusted to a volume of 10 ml. The specific rotation, $[\alpha]_D^{20}$, was −58° on the basis of the original D-glucosylamine. Production of the stable basic substance having a levorotation indicates the presence of 1-amino-1-deoxy-D-fructose. The material is being investigated further.

Amadori rearrangement by a modification of the Hodge and Rist method. D-Glucosylamine (0.2 g.) was dissolved in 5 ml. of dimethylsulfoxide[45] and 5 ml. of diethyl malonate was added. The mixture was heated for 90 min. at 80° and kept at room temperature overnight. The brown solution was diluted with water and allowed to stand for several hours to effect hydrolysis of the remaining D-glycosylamines. The solution was then extracted several times with chloroform, and the aqueous portion was filtered through decolorizing carbon, concentrated in a rotary still, and adjusted to a volume of 10 ml. The optical rotation, $[\alpha]_D^{20}$, was −43.5°, based on the weight of the original D-glucosylamine. The levorotatory product, presumably containing 1-amino-1-deoxy-D-fructose, is being studied further.

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(45) Dimethyl sulfoxide has been found to be an exceptionally useful solvent for glycosylamines.

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC ELECTROCHEMISTRY, DEPARTMENT OF CHEMICAL ENGINEERING, TOKYO INSTITUTE OF TECHNOLOGY]

Guanidination of D-Glucosamine*

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Since 1942, quantitative studies on the preparation of several naturally occurring guanidine compounds have been carried out and reported by K. Sugino, K. Odo, and their collaborators.

Both the *S*-methylisothiourea method and improved cyanamide condensation methods were applied for this purpose. The latter involves the following two methods which were newly established by the same authors as the result of researches made on the mechanism of the guanidination of amines with cyanamide. (1) The reaction of amine salt with cyanamide in the fused state.[1,2] (2) The reaction of an amine salt with cyanamide in aqueous solution in the presence of a small amount of free amine.[3]

As of this writing, quantitative studies on the preparation of diguanidines [ethylene-,[2] tetramethylene-(arcain),[2] hexamethylene-,[4] decamethylene-[4]], aminoalkyleneguanidine (agmatine),[5] guanidino amino acid or diguanidino acid [arginine and diguanidino valerianic acid,[6] homoarginine and diguanidino caproic acid[7]), and creatine[8] were completed successfully using one of these methods selectively. The detailed description of each special procedure suitable to each compound has been given in the preceding papers.

In the present work, the synthesis of 2-deoxy-2-guanidino-D-glucose from D-glucosamine has been studied. In regard to this problem, only one paper which turned out to be erroneous has been reported.[9]

Among the three methods of guanidine preparation described above, the cyanamide condensation in the fused state appeared to be out of the question in view of the thermal stability of D-glucosamine. Therefore, the *S*-methylisothiourea method and the cyanamide condensation in aqueous solution were both tried. The condensations of D-glucosamine with S-methylisothiourea were unsuccessful. Instead of a guanidino compound, a resinous product was obtained due to the effect of alkali on D-glucos-

* Cyanamide Derivatives, XLVII.

(1) K. Sugino, *J. Chem. Soc. Japan*, **60**, 421 (1939).

(2) K. Sugino, K. Shirai, and K. Aoyagi, *Bull. Chem. Soc. Japan*, **17**, 126 (1942).

(3) K. Odo, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **71**, 394 (1950).

(4) K. Odo and K. Sugino, *J. Chem. Soc. Japan*, **63**, 336 (1942).

(5) K. Odo, *J. Chem. Soc. Japan*, **67**, 132 (1946).

(6) K. Odo, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **74**, 1, 774 (1953).

(7) K. Odo and E. Ichikawa, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **76**, 228 (1955).

(8) K. Odo and E. Ichikawa, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **77**, 1413 (1956).

(9) J. Miller, *J. Chem. Soc.*, 2722 (1949).