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EXPERIMENTS RELATING TO THE SYNTHESIS
OF
2-AMINO-2-DEOXY-D-IDOSE

A THESIS

Presented to
the Faculty of the Graduate Division
by
William Beveridge Spencer

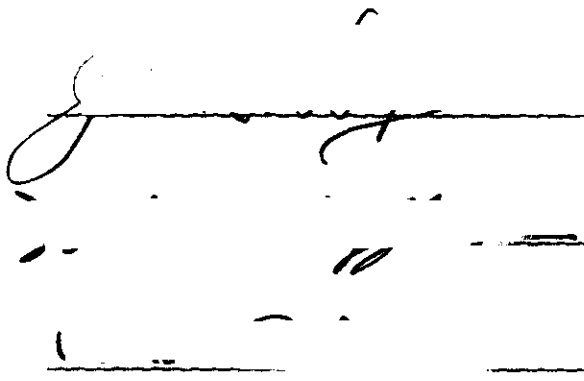
In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Chemistry

Georgia Institute of Technology
June, 1958

EXPERIMENTS RELATING TO THE SYNTHESIS
OF
2-AMINO-2-DEOXY-D-IDOSE

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APPROVED:

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Date Approved by Chairman: May 28, 1958

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ABSTRACT

EXPERIMENTS RELATING TO THE SYNTHESIS

OF

2-AMINO-2-DEOXY-D-IDOSE

(38 pages)

WILLIAM BEVERIDGE SPENCER

Thesis Advisor: John R. Dyer

A synthetic route to 2-amino-2-deoxy-D-idose, one of the few remaining unknown hexosamines, was sought. The route chosen is based on the reaction of D-xylose with an amine, yielding a D-xylosylamine, which yields, by condensation with hydrogen cyanide, an epimeric mixture of nitriles, an idosaminic acid nitrile and a gulosaminic acid nitrile. Reactions of a similar nature recorded in the literature indicate that catalytic hydrogenation of the nitriles would yield the respective 2-amino-2-deoxysugar.

Attempts to synthesis D-idosamine and D-idosaminic acid by a reaction between liquid ammonia and D-xylose, followed by treatment with liquid hydrogen cyanide were unsuccessful.

N-benzyl-D-xylosylamine, a new compound, was prepared by the reaction of benzylamine with D-xylose. Upon reaction of hydrogen cyanide with N-benzyl-D-xylosylamine, no crystalline product could be isolated.

N-phenyl-D-xylosylamine was prepared by the reaction of D-xylose with aniline. The N-phenyl-D-xylosylamine was condensed with gaseous hydrogen cyanide, giving N-phenyl-D-idosaminic acid nitrile and a small amount of a compound $C_{12}H_{14}O_3N_2$, the structure of which has been postulated to be 3,6-anhydro-N-phenyl-D-idosaminic- γ -iminolactone.

Experiments relating to the semi-reduction of N-phenyl-D-idosaminic acid nitrile by catalytic hydrogenation were studied in an attempt to obtain crystalline 2-amino-2-deoxy-D-idose.

Hydrolytic conditions for the conversion of N-phenyl-D-idosaminic acid nitrile to N-phenyl-D-idosaminic acid have been determined but a crystalline product has not been isolated.

A literature search has been made in order to examine a possible correlation of molecular rotation values between x-amino-x-deoxy-sugar derivatives and the corresponding sugar derivatives. It was concluded that a definite correlation of absolute configuration is possible for 2-amino-2-deoxy compounds and x-amino-x-deoxy-1,6-anhydro compounds.

CHAPTER I

INTRODUCTION

A new aminosugar, 2-amino-2-deoxy-D-gulose, has been obtained as a hydrolysis product of the antibiotics streptothricin and streptolin B (1). The chemical evidence presented as a proof of structure for the natural product was sufficient to limit structural possibilities to 2-amino-2-deoxy-D-gulose and 2-amino-2-deoxy-D-idose, neither of which were known compounds. A decision between the two possibilities was reached by comparison of molecular rotation values of the β -1,6-anhydride of the natural product, also present in acid hydrolysis of the antibiotics, with the corresponding non-nitrogenous analogues.

The original purpose of the current work was the synthesis of 2-amino-2-deoxy-D-gulose and the proof of absolute stereochemistry of the synthetic material by conventional degradation reactions. During the past year, two syntheses of 2-amino-2-deoxy-D-gulose have appeared (2, 3), one of which defined the absolute stereochemistry through unambiguous stereospecific reactions (2). The synthetic product was shown to be identical in all respects with that obtained by acid hydrolysis of the antibiotics streptothricin and streptolin B. Accordingly, a synthetic route to 2-amino-2-deoxy-D-idose, one of the few remaining hexosamines which have not been synthesized,

was sought.

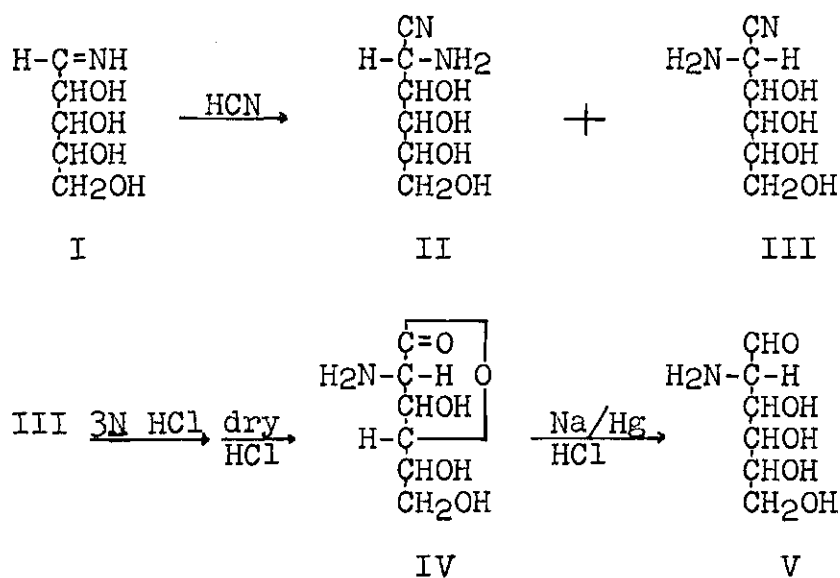
A literature search has been made to obtain data in order to examine a possible correlation of molecular rotation values between x-amino-x-deoxysugar derivatives and the corresponding sugar derivatives.

CHAPTER II

SURVEY OF RELATED WORK

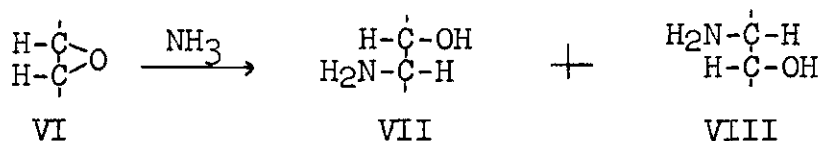
A. Methods of synthesis of aminosugars.--The first aminosugar to be isolated in pure form was obtained by Ledderhose (4) in 1878 by mineral acid hydrolysis of the chitin found in lobster shells and was named "glycosamin". A close structural relationship to D-glucose was inferred from the reducing capacity of the aminosugar and by its conversion to D-glucosazone on treatment with phenylhydrazine. When a second aminosugar, chondrosamine, was isolated (5), a need for a synthesis of these hexosamines became apparent.

Glucosamine was the first amino-deoxysugar to be obtained synthetically (6). The major part of this early work was performed by P. A. Levene and co-workers. For example, a pentose was reacted with ammonia, yielding the corresponding pentosimine (I), which on condensation with hydrogen cyanide yielded an epimeric mixture of 2-amino-2-deoxyhexonic acid nitriles (II and III). The nitriles were hydrolyzed to the corresponding acids, which were separated by fractional crystallization. The pure 2-amino-2-deoxyhexonic acid was then converted by dry hydrogen chloride gas in alcohol into a γ -lactone (IV), sodium amalgam reduction of which yielded a 2-amino-2-deoxysugar (V). In this way, several aminosugars were prepared in pure form, and derivatives were obtained of others.



The chief disadvantage to this general method of synthesis is that the final step also yields large amounts of sodium chloride, from which the desired, water soluble, 2-amino-2-deoxysugar must be separated. Further, the method is applicable only to the preparation of 2-amino-2-deoxysugars.

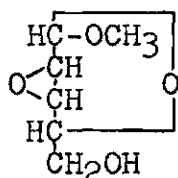
The second general method of synthesis for deoxyaminosugars consists in the ammonolysis of a compound containing an epoxide (VI), yielding a diastereoisomeric (trans-aminoalcohol) mixture of products (VII and VIII). The starting material need not contain the epoxide; a material such as



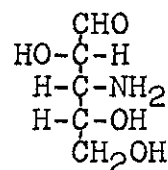
a trans-2-halo-alcohol or trans-2-sulfonyl alcohol may be used. In these cases, reaction proceeds with the intermediate form-

ation of the epoxide.

Anderson and Percival reacted methyl 2,3-anhydro-D-lyxofuranoside (IX) with methyl alcohol and ammonia for forty-eight hours at 120° C. (7). The syrup obtained was acetylated, and this product was hydrolyzed by 3 N hydrochloric acid at 100° for one hour. 3-Amino-3-deoxy-D-arabinose hydrochloride (X) was the product isolated.

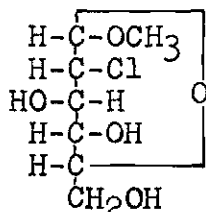


IX

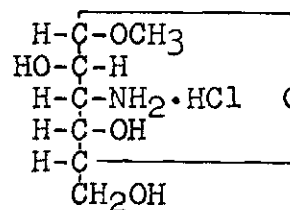


X

Fischer, Bergmann and Schotte (8) found that when methyl 2-chloro-2-deoxy-D-glucopyranoside (XI) was heated at 100° under pressure with 25 per cent aqueous ammonia for twelve to fifteen hours "methyl epiglucoamine hydrochloride" (actually methyl 3-amino-3-deoxy-D-altropyranoside hydrochloride (XII)) was the product obtained.



XI



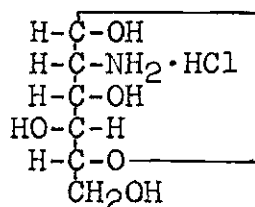
XII

This general method has been used many times for the synthesis of aminosugar derivatives. The products obtained by ammonolysis of the epoxide ring are not formed in equal

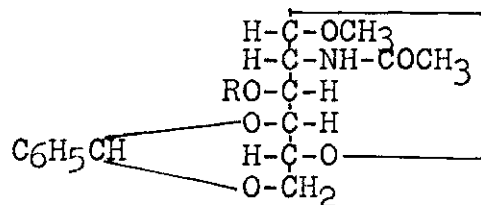
amounts. Usually, one product predominates, frequently practically to the exclusion of the minor product. Although this fact leads to easier isolation and purification of one compound, the other compound, which might be desired, cannot be obtained. The common aminosugars, glucosamine and galactosamine cannot be obtained in practical yields utilizing this type of synthesis.

A method somewhat similar to the above starts with an aminosugar in which the stereochemistry of the amino group is known at the outset and is not changed during the synthesis, but an inversion of stereochemistry of a particular hydroxyl group is caused. An example of this method is the synthesis of 2-amino-2-deoxy-D-gulose hydrochloride recently reported by Tarasiejsha and Jeanloz (2). This article described the synthesis of 2-amino-2-deoxy-D-gulose-hydrochloride (XIII) from methyl-2-acetamido-2-deoxy-4,6-O-benzylidene- α -D-galactopyranoside (XIV). The first step was to transform XIV into the 3-O-methylsulfonyl derivative XV, then to eliminate the benzylidene ring by hydrolysis of XV, giving crystalline methyl-2-acetamido-2-deoxy-3-O-methylsulfonyl α -D-galactopyranoside XVI, after which it was possible to obtain the gulosamine derivative XVII by heating with sodium acetate in methyl cellosolve. Acetylation, followed by alkaline hydrolysis gave crystalline methyl 2-acetamido-2-deoxy- α -D-gulopyranoside (XVII). Acid hydrolysis of this compound gave the desired compound, XIII; the synthetic compound had a decomposition point of 150-

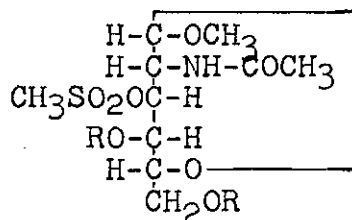
170° and rotation of $[\alpha]_D^{22} +6^\circ$ (10 min.) \longrightarrow -18° (36 hours) in water. This is in excellent agreement with the work of van Tamelen, et al. with the naturally occurring compound.



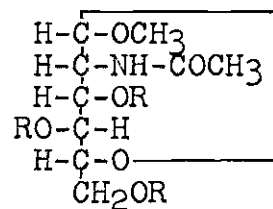
XIII



XIV, R=H

XV, R=CH₃SO₂

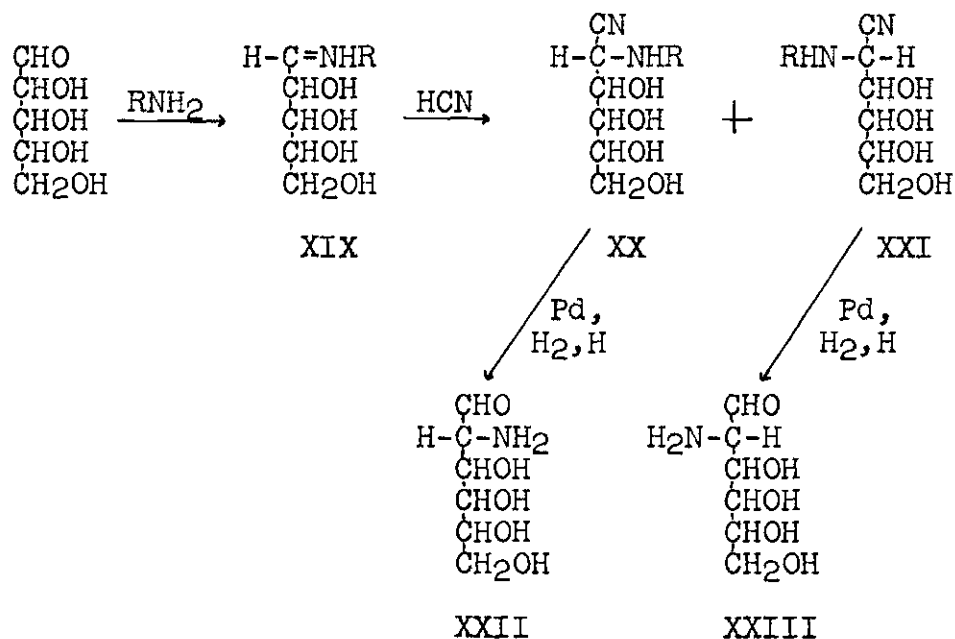
XVI



XVII

The most recently reported method for synthesis of 2-amino-2-deoxysugars has been described in some detail in a series of articles by Kuhn and co-workers (3,9,10,11). For example, a pentose is reacted with an amine (XVIII) (ammonia, aniline, or benzylamine were commonly used), yielding a pentosimine (XIX). This compound was then reacted with hydrogen cyanide, giving a mixture of epimeric nitriles. The nitriles (XX) (XXI) were purified by fractional crystallization; they were then catalytically reduced, using hydrogen and palladium. The 2-aminosugars (XXII) (XIII) were isolated in good yields from the reaction mixture. The amount of hydrogen required for the reduction step varied with the original amine used.

If ammonia was used, one mole of hydrogen was required; if aniline was used, three moles of hydrogen were required; if benzylamine was used, two moles of hydrogen were required.



In this way, L-arabinose was converted into L-glucosamine hydrochloride, D-Lyxose was converted to D-galactosamine hydrochloride, and D-xylose to D-gulosamine hydrochloride.

The disadvantages of the method are that the procedure is applicable only for the synthesis of 2-aminosugars and that epimeric mixtures of nitriles are formed which may be difficult to separate. In addition, ammonium chloride is a product of the hydrogenation step and must be separated from the 2-aminosugar.

B. Comparison of molecular rotations of aminosugars and sugars.

--Correlation of the absolute stereochemical configuration of

one compound with another by means of numerical relationships has frequently been attempted (12). So that the values used may be independent of molecular size, the molecular rotation, and not the simple specific rotation, is used. The method requires a suitable number of compounds of known absolute stereochemistry. If, then, a simple type of substitution is performed, the absolute stereochemistry of the product may be determined, assuming that a correlation of molecular rotations exists.

A preliminary survey of the literature has revealed that a correlation of absolute configuration of aminosugars, with sugars of known absolute configuration serving as reference compounds, may be possible (1). A more complete examination of the literature has been made in an effort to test the applicability and limitations of this particular example of correlation. In the following table, pairs of compounds are listed, an x-amino-x-deoxysugar of known absolute configuration and the parent sugar, also of known absolute configuration. The molecular rotations of the compounds have been calculated from the specific rotations given in the literature.

TABLE I

Pair No.	Name of Compound	$[M]_D$	Ref. No.
1.	β -D-Allose	0 \rightarrow +27	13
	2-Amino-2-deoxy-D-allose hydrochloride	+62	14

Pair No.	Name of Compound	$[M]_D$	Ref. No.
2.	α -D-Galactose	+271 \rightarrow +144	15
	2-Amino-2-deoxy-D-galactose hydrochloride	+261 \rightarrow +172	16
3.	β -D-Galactose	+95 \rightarrow +144	15
	2-Amino-2-deoxy- β -D-galactose hydrochloride	+96 \rightarrow +172	16
4.	α -D-Glucose	+202 \rightarrow +95	17
	2-Amino-2-deoxy- α -D-glucose	+179 \rightarrow +85	18
5.	α -D-Glucose	+202 \rightarrow +95	17
	2-Amino-2-deoxy- α -D-glucose hydrochloride	+215 \rightarrow +156	19, 20
6.	β -D-Glucose	+34 \rightarrow +95	17
	2-Amino-2-deoxy- β -D-glucose hydrochloride	+45 \rightarrow +130	21
7.	β -D-Mannose	-31 \rightarrow +25	17
	2-Amino-2-deoxy-D-mannose hydrochloride	-10	22
8.	α -D-Xylose	+140 \rightarrow +28	17
	2-Amino-2-deoxy-D-xylose	+140	23
9.	α -D-Xylose	+140 \rightarrow +28	17
	2-Amino-2-deoxy-D-xylose hydrochloride	+147 \rightarrow +74	23
10.	1,6-Anhydro- β -D-altropyranose	-345	1
	3-Amino-3-deoxy-1,6-anhydro- β -D-altro- pyranose hydrochloride	-340	24
11.	1,6-Anhydro- β -D-galactopyranose	-35	25
	2-Amino-2-deoxy-1,6-anhydro- β -D-galacto- pyranose	-44	20
12.	1,6-Anhydro- β -D-galactopyranose	-35	25
	2-Amino-2-deoxy-1,6-anhydro- β -D-galacto- pyranose hydrochloride	-31	20
13.	1,6-Anhydro- β -D-gulopyranose	+82	1
	2-amino-2-deoxy-1,6-anhydro- β -D-gulo- pyranose hydrochloride	+88	1

Pair No.	Name of Compound	$[M]_D$	Ref. No.
14.	1,6-Anhydro- β -D-mannopyranose	-206	25
	4-Amino-4-deoxy-1,6-anhydro- β -D-manno- pyranose hydrochloride	-198	20
15.	1,6-Anhydro- β -D-galactopyranose	-35	25
	2-Acetamido-2-deoxy-1,6-anhydro- β -D- galactopyranose	-10	26
16.	α -D-Allose	+27	13
	2-Acetamido-2-deoxy- α -D-allose	-126 \rightarrow -106	14
17.	α -D-Galactose	+271 \rightarrow +144	15
	2-Acetamido-2-deoxy- α -D- galactose	+254 \rightarrow +177	25
18.	α -D-Glucose	+202 \rightarrow +95	17
	2-Acetamido-2-deoxy- α -D- glucose	+142 \rightarrow +90	18
19.	α -D-Xylose	+140 \rightarrow +28	17
	2-Acetamido-2-deoxy- α -D- xylose	+107 \rightarrow +17	23
20.	Methyl β -D-altropyranoside	-101	27
	Methyl 3-amino-3-deoxy- β -D-altro- pyranoside hydrochloride	-342	28
21.	Methyl α -D-glucopyranoside	+308	29
	Methyl 3-amino-3-deoxy- α -D- glucopyranoside	+279	30
22.	Methyl β -D-glucopyranoside	-67	29
	Methyl 3-amino-3-deoxy- β -D- glucopyranoside	-91	30
23.	Methyl β -D-glucopyranoside	-67	29
	Methyl 3-amino-3-deoxy- β -D-gluco- pyranoside hydrochloride	-80	30
24.	Methyl α -D-altropyranoside	+244	27
	Methyl 2-amino-deoxy- α -D- altropyranoside	+208	28
25.	Methyl α -D-altropyranoside	+244	27
	Methyl 2-amino-2-deoxy- α -D-altro- pyranoside hydrochloride	+91	31
26.	β -D-Altrose	-124 \rightarrow +60	32
	3-Amino-3-deoxy-D-altrose	-232	33

Pair No.	Name of Compound	$[M]_D$	Ref. No.
27.	α -D-Arabinose	-286 \rightarrow -157	17
	3-amino-3-deoxy-D-arabinose hydrochloride	-201	34
28.	D-Ribose	-35 \rightarrow -36	35
	3-amino-3-deoxy-D-ribose hydrochloride	-46	36
29.	β -D-Glucose	+34 \rightarrow +95	17
	3-amino-3-deoxy- β -D-glucose	+33	37,30
30.	Methyl β -D-altropyranoside	-101	27
	Methyl 3-acetamido-3-deoxy- β -D-altropyranoside	-289	38
31.	Methyl β -L-xylopyranoside	+107	39,40
	Methyl 3-acetamido-3-deoxy- β -L-xylopyranoside	+132	41,36
32.	Methyl α -D-arabinofuranoside	+202	42
	Methyl 3-acetamido-3-deoxy- α -D-arabinofuranoside	+209	34
33.	L-Xylose	-138 \rightarrow -29	43
	3-Acetamido-3-deoxy-L-xylose	0	36
34.	Methyl β -L-xylopyranoside	+107	39,40
	Methyl 3-acetamido-3-deoxy- β -L-xylopyranoside	+132	36
35.	Methyl β -L-xylopyranoside	+107	39,40
	Methyl 3-amino-3-deoxy- β -L-xylopyranoside hydrochloride	+115	36

The compounds in the tables have been assigned to groups according to their particular type of derivative: Group I, 1 to 9, are the 2-amino-2-deoxy-compounds; Group II, 10 to 15, the 1,6-anhydro-compounds; Group III, 16 to 19, the 2-acetamido-2-deoxy-compounds; Group IV, 20 to 25, the methyl pyranosides of 2 and 3-amino-derivatives; Group V, the remaining compounds examined.

If no mutarotation values were found in the literature, it was assumed that the value given was for the equilibrium (α/β) mixture.

For comparison purposes, a scale has been arbitrarily set up to determine the degree of correlation. If the amino compound and its parent sugar have a difference of ten or less units in molecular rotation, the agreement is said to be "very good", 11-25 "good", 26-40 "satisfactory", 41-60 "poor", and if greater than 60, no correlation is considered possible.

In Group I, 2-amino-2-deoxysugars, all but one initial value is either "good" or "very good", however the final values vary over the entire range. Therefore, it appears that the correlation holds for initial rotations of 2-amino-2-deoxysugars but does not hold after mutarotation starts.

The aminosugars that give only satisfactory values, allosamine and mannosamine, are the only two in the group for which mutarotation values have not been recorded. This is undoubtedly a contributing factor to the lack of correlation. Also, allose and mannose are the only compounds in the groups that change sign on mutarotation.

Group II, the 1,6-anhydro compounds give excellent agreement without exception. None of them mutarotates, so comparison is easy and undoubtedly more accurate than for the other compounds.

Not much can be said for Group III, the 2-acetamido-2-deoxy compounds, except that it appears that the final mutarotation values are in better agreement than the initial values.

Group IV, the 3-amino derivatives, gave "satisfactory" agreement with two exceptions.

The remaining compounds, Group V cannot be placed into any common groups because they are either isolated compounds or else the variation within the derivative group is too great. Many more compounds will have to be isolated and synthesised before a complete correlation can be made.

It does appear, however, that within certain groups, namely, the 2-amino-2-deoxy compounds and the 1,6-anhydro-x-amino-x-deoxy compounds, where a satisfactory number of comparison compounds is available, a definite correlation of absolute configuration is possible.

CHAPTER III

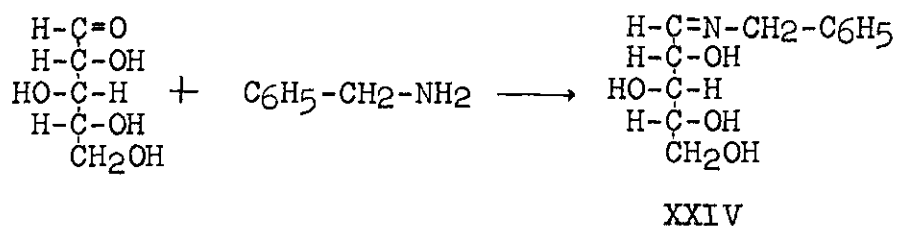
DISCUSSION OF RESULTS

A. D-xylosylamine.--The reaction between ammonia and D-xylose was attempted originally because it was believed that a more favorable proportion of isomers might result due to steric factors.

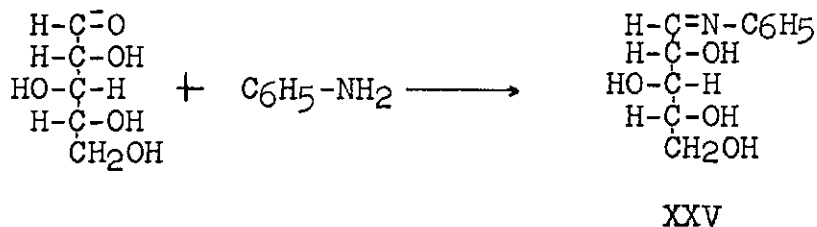
The product from the initial reaction of ammonia and D-xylose was never isolated. This may have been a source of trouble in the isolation of the hydrogen cyanide reaction product. If the D-xylosylamine was contaminated with D-xylose, a hydrogen cyanide reaction product would also be formed from this unreacted sugar. All of these products, except D-xylose could then be hydrogenated, giving an epimeric mixture of each reactant, further complicating the results. When this impure syrup was acetylated, purified by chromatography, and then subjected to acid hydrolysis, paper chromatographic analysis indicated a very complex mixture of products.

B. N-Benzyl-D-xylosylamine (XXIV).--This product (XXIV), not previously reported in the literature, was obtained in crystalline form by the reaction of D-xylose and benzylamine in ethanol solution. The epimeric mixture that resulted from the reaction of N-benzyl-D-xylosylamine with liquid hydrogen cyanide gave a syrup that could not be separated into its

components.

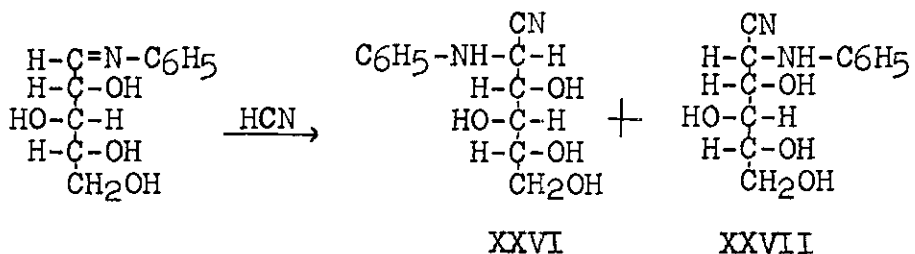


C. N-Phenyl-D-xylosylamine (XXV).--The reaction between D-xylose and aniline in ethanol solution, gives almost a quantitative yield of N-phenyl-D-xylosylamine (XXV).



This reaction will proceed even in the presence of slight amounts of water. The product can be recrystallized from warm alcohol. The physical constants obtained agree with the reported literature values.

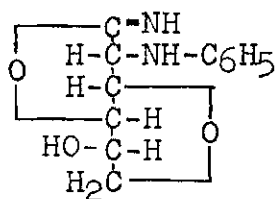
When this product is reacted with gaseous hydrogen cyanide, an epimeric mixture of N-phenyl-D-idosaminic acid nitrile (XXVI) and N-phenyl-D-gulosaminic acid nitrile (XXVII) should be formed.



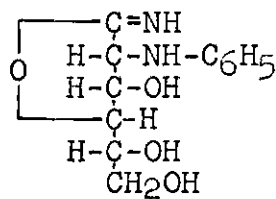
To insure total reaction, the method of adding the hydrogen cyanide is important. The addition of a gas dispersion tube and a trap to solidify the hydrogen cyanide permits a slow addition of the gas by allowing it to liquify and vaporize before bubbling through the alcoholic solution of N-phenyl-D-xylosylamine. This addition method would probably be advantageous in the preparation of the hydrogen cyanide products of D-xylosylamine and N-benzyl-D-xylosylamine.

The total yield in this step is very good; however, in isolating the isomers by fractional crystallization, some product is sacrificed. Both of these materials give the same R_f values on papergrams and give melting points of 118-118.5 and 109-110° for the idose and gulose derivatives, respectively.

In addition to these compounds, a higher melting material was also isolated. The compound had an empirical formula of $C_{12}H_{14}O_3N_2$. A possible structure consistent with the analytical and infrared data is (XXVIII), 3,6-anhydro-N-phenyl-D-idosaminic- γ -iminolactone.



XXVIII



XXIX

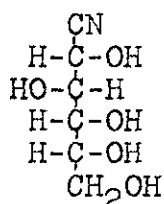
The hydroxyl band in the infrared spectrum of this material was very weak, the nitrile band was missing and a band at

6.05 μ indicated the presence of a C=N grouping. The analytical data are not consistent with a structure such as XXIX (C₁₂H₁₆O₄N₂), which might be expected to be present in small amounts.

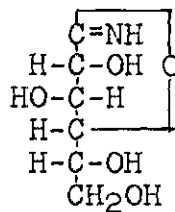
The compound XXVIII is slightly soluble in water and could be recrystallized from hot water as fine long needles. However, these crystals did not have the same properties as the material originally isolated, but did resemble the N-phenyl-D-idosaminic acid nitrile in melting point and R_f value on papergrams. The infrared spectra, however, were distinctly different.

An attempt was made to convert a small portion of the N-phenyl-D-idosaminic acid nitrile to the higher melting compound, but only a syrup was obtained which could not be crystallized.

It has been reported by Papadakis and Cohen (45) (44) that glucono-nitrile (mp 143°) when recrystallized from glacial acetic acid gave a product with a melting point of 145°. If the higher melting product was recrystallized from absolute ethanol, a product with a melting point of 120.5° resulted. These two products could be interchanged easily by recrystallization from the appropriate solvent. The products also had different rotations. On spectral evidence, the product of m.p. 120.5° was assigned a nitrile structure (XXX), and the product of m.p. 145° was assigned an iminolactone structure (XXXI).



XXX



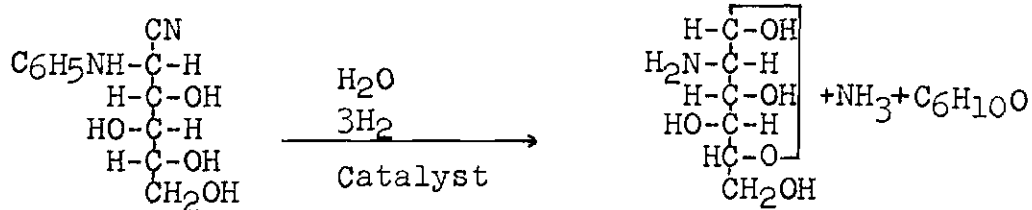
XXXI

The products formed by hydrogenation of N-phenyl-D-idosaminic acid nitrile apparently depend on the catalyst used. Numerous hydrogenation experiments were performed; they all can be grouped into four categories: palladium with acetic acid, platinum with acetic acid, platinum with hydrochloric acid and palladium with hydrochloric acid. A typical result with each type is shown in Table II.

TABLE II

<u>Trial</u>	<u>Mmoles. of sample</u>	<u>Catalyst and acid used</u>	<u>Mmoles of Hydrogen uptake per mmole of sample</u>
1	1.31	Pd/C acetic acid	1.57
2	1.19	PtO ₂ acetic acid	5.47
3	2.10	Pt/C hydrochloric acid	4.69
4	2.01	Pd/C hydrochloric acid	3.25

It is evident from these data that platinum is a much stronger catalyst for reduction. The desired reaction would be the uptake of three moles of hydrogen, forming 2-amino-2-deoxy-D-idose, XXXII, ammonia and cyclohexanone.



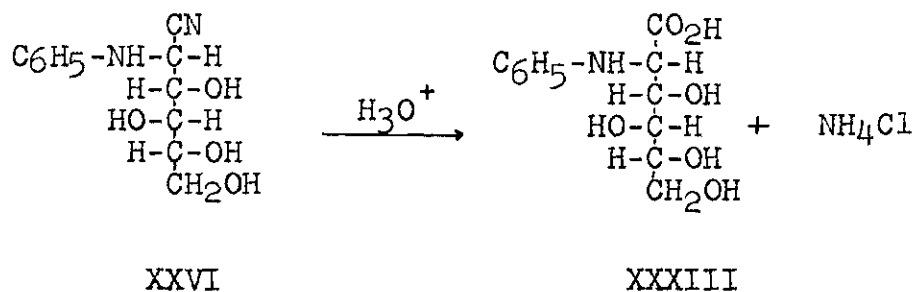
XXXII

This appears to be the reaction that occurs with palladium and dilute hydrochloric acid. Palladium with acetic acid probably is not as vigorous a reducing medium and does not remove the N-phenyl group by breaking the carbon nitrogen bond, therefore utilizing only two moles of hydrogen. The explanation for the reduction using the platinum catalyst might be that the nitrile group reacts with one mole of hydrogen followed by acid hydrolysis to give the aldehyde and ammonia. At the same time the phenyl group could be saturated, consuming three moles, followed by a hydrogenolysis of the nitrogen carbon bond.

Kuhn (9) (11) reports that if the nitrile is present in similar compounds the aminosugar will be formed. However, if the iminolactone isomer was used, the product of reduction was the amino acid.

It is also interesting to note that when a hydrogenation experiment was performed on glucosamine hydrochloride with platinum oxide and fifty per cent acetic acid, only 30 per cent reduction was noted in fifty hours. This was a linear uptake, so the possibility of reducing the idosamine compound would not proceed to any significant extent.

A variety of conditions were attempted in the hydrolysis of N-phenyl-D-idosaminic acid nitrile to yield N-phenyl-D-idosaminic acid (XXXIII). However, no crystalline product other than ammonium chloride was isolated.



Numerous time studies were performed at room temperature and at 100° in attempts to hydrolyze this compound. Paper chromatography has shown that the nitrile reacts immediately on contact with dilute hydrochloric acid at room temperature; more than one product is always formed, as indicated by the development of three spots. Upon addition of the nitrile to the acid, a yellow solution forms immediately. After about one-half hour at room temperature, a product starts to precipitate and upon standing, the color of the solution darkens. Attempted isolation by evaporation in vacuo or by freeze drying techniques, followed by attempts to crystallize a product from solvent, have all failed.

CHAPTER IV

EXPERIMENTAL PROCEDURE

A. Apparatus and Techniques

Silicic acid adsorption (46) chromatographic columns were used often as a method of separation and purification of products. The usual way of preparing a column is to slurry acetone-dried silicic acid in chloroform until a homogeneous mixture is obtained. The slurry is then poured with stirring into a chromatographic column and bubbles of air are removed by stirring. The silicic acid is allowed to settle by gravity until a firm surface is obtained. A translucent column of silicic acid is obtained.

The sample that is to be chromatographed is dissolved in a minimum amount of chloroform and added dropwise to the surface of the column, followed by chloroform in small portions to wash the material onto the column. The material adsorbed on the column is eluted with chloroform containing increasing amounts of alcohol; usually ten per cent alcohol is sufficient to elute all materials adsorbed on the column.

Paper chromatographs (47) have been very useful in the present work as a means of characterizing various products obtained. Several solvent mixtures were used to irrigate the paper chromatograms. The following mixtures (parts by volume)

(and their abbreviations) are used throughout the text: t-butyl alcohol: acetic acid: water (2:1:1), BAW; n-propyl alcohol: acetic acid: water (10:1:9), PAW; ethyl acetate: ethyl alcohol: water (6:3:1), EEW-1; ethyl acetate: ethyl alcohol: water (15:3:2), EEW-2; acetone: ethyl alcohol: water (8:1:1), AEW; and ethyl alcohol: cyclohexane: water (585:400:15), ECW.

Three spray reagents were used to develop the papergrams. Ninhydrin (47), a 0.2 per cent solution of ninhydrin (triketohydrindene hydrate) in fifty per cent pyridine, which gave reddish or purple spots on a colorless background upon warming. Tollen's reagent (47), consisting of equal volumes of 0.1 N silver nitrate and 5.0 N ammonium hydroxide mixed immediately before using, gave black spots on a whitish background unless heated. A periodate (48) spray reagent is made up in two parts. First, the papergram was sprayed with a saturated aqueous solution of potassium metaperiodate and allowed to stand at room temperature for five to ten minutes. It was then developed, giving colorless spots on a blue background, by a benzidine reagent, made by mixing ten volumes of 0.1 M benzidine in fifty per cent aqueous ethanol with one volume of 0.2 N hydrochloric acid and two volumes of acetone.

The atmospheric pressure hydrogenation (49) apparatus used had a capacity of five hundred and fifty milliliters of hydrogen. It contained a mercury manometer, an oil burette for storage, and had outlets to nitrogen and hydrogen tanks. The entire system could be evacuated by a water aspirator,

and the reaction could be continually stirred by a magnetic stirrer. Platinum and palladium catalysts were used in either dilute hydrochloric acid or acetic acid solutions.

The ninhydrin reagent was used as a qualitative test for compounds containing an amino group. A drop of the test solution was mixed with about 0.5 ml. of the ninhydrin reagent and the solution was heated at 100° for three minutes. The development of a purple color constituted a positive test. Nessler's reagent was used to detect the presence of ammonia or any compound which would yield ammonia under the conditions of the test. The reagent (50) was prepared by dissolving 100 g. of mercuric iodide, 70 g. of potassium iodide, and 100 g. of sodium hydroxide in water to give one liter of solution. A drop of the test solution was mixed with several drops of the reagent. The immediate appearance of an intense orange color or an orange-brown precipitate constituted a positive test.

Infrared spectra were obtained using a Perkin Elmer Infracord Model 137. All measurements of optical rotation were obtained by using a Bellingham and Stanley polarimeter. Melting points were taken on a Kofler microscope hot stage and are uncorrected.

Because of the extreme thermal instability of the compounds described, evaporation and drying techniques were suitably altered. All evaporations were performed in vacuo, the temperature not exceeding 45°. A conventional freeze-drying

apparatus was used to evaporate to dryness at 0°.

B. Studies with D-xylosylamine

Ammonia gas was passed through a stirred mixture of 10.0 g. of D-xylose and 750.0 ml. of absolute alcohol. A homogeneous solution was not obtained, and after one and one-half hours, approximately 50 ml. of liquid ammonia were added. After one and one-half hours, the mixture was filtered; 1.5 g. of unreacted D-xylose (mp. 143-4; R_f 0.77 (PAW) with ninhydrin and 0.75 (PAW) with periodate spray) was obtained.

To the chilled filtrate was added 4.0 ml. of liquid hydrogen cyanide, prepared in the conventional way (51). The solution was placed in an ice bath overnight; an amorphous gum (A) separated. The supernatant liquid was decanted and the residual gum was washed with three 10 ml. portions of absolute ethanol. The combined supernatant liquid and washes were evaporated to dryness in vacuo, yielding a second amorphous gum (B).

Both gums were acetylated by adding to the solid material 80 ml of dry pyridine, with cooling to 0°. At this temperature, 80 ml. of acetic anhydride were added in portions during ten minutes. The mixture was allowed to stand at 0° for three hours with occasional vigorous shaking and was then allowed to warm to room temperature. The solid material dissolved completely only after standing several days at room temperature. The solution was neutralized with sodium bicarbonate solution and extracted with chloroform. The

chloroform solution was extracted with hydrochloric acid, water, and then dried with magnesium sulfate and evaporated to dryness. Gum A gave 16.2 g. of amorphous acetylated product while gum B gave 4.0 g. of amorphous acetylated product.

Both acetylated products were chromatographed on silicic acid columns. A number of fractions were taken which yielded only syrups which could not be crystallized. Several of these fractions were hydrolyzed at 100° for one hour with 2 N hydrochloric acid. Ion exchange resin IR-45 (OH⁻) was added to raise the pH of the hydrolysis mixture to 5 at the end of the reaction. The ion exchange resin absorbed all the color produced during hydrolysis. After removing the ion exchange resin by filtration, the filtrates were evaporated to dryness in vacuo; paper chromatograms run on these various fractions all indicated a very complex mixture of products present. As many as four spots could be located on the papergram.

C. Studies of N-Benzyl-D-xylosylamine

A mixture of 5.0 g. of D-xylose, 15 ml. of absolute ethanol and 4 ml. of redistilled benzylamine was boiled under reflux for twenty minutes. The xylose dissolved in about ten minutes and as the reaction proceeded, the color of the solution became deep maroon. The solution was allowed to cool to room temperature and was then placed in an ice bath for twelve hours. On cooling to 0° the solution formed a thick jelly, which liquified upon warming to room temperature. Twenty

milliliters of redistilled absolute ethanol were added to the solution and five volumes of ether were added. A large quantity of amorphous product precipitated, which was collected by filtration and washed with small portions of chloroform. The white product was recrystallized from five milliliters of redistilled absolute ethanol and fifty milliliters of cyclohexane. Fine needles were formed, mp. 85-88, $[\alpha]_D -44.3$ (3 min.) $\longrightarrow -23.8$ (48 hrs.), (c , 2.3, methanol). The material showed R_f 0.73 in PAW with a periodate spray and 0.91 (PAW) with ninhydrin.

A solution of 0.8 g. of N-benzyl-D-xylosylamine, 10.0 ml. of absolute ethanol and 0.5 ml. of liquid hydrogen cyanide was allowed to stand at room temperature for twelve hours. The solution was then cooled in an ice bath for several days. No crystalline material could be obtained; the solution was concentrated by distillation in vacuo and the resulting syrup acetylated in the usual manner, yielding 2.5 g. of product. The product was chromatographed on a silicic acid column made from 38.0 g. of silicic acid slurried with chloroform. Crystalline material could not be obtained from any of the fractions taken.

D. Studies with N-Phenyl-D-xylosylamine

1. N-Phenyl-D-xylosylamine

A mixture of 50 g. of D-xylose, 35 ml. of redistilled aniline, and 750 ml. of absolute ethanol was boiled under reflux for three hours. Complete solution was observed at the

end of the first hour of refluxing. The reaction solution was allowed to cool to room temperature and allowed to stand for twelve hours. It was then filtered and placed in an ice bath until crystallization appeared to be complete. The first crop weighed 29 g.. The filtrate was evaporated to one-half volume two times at 50° in vacuo, and two more crops were isolated, 17.0 g. and 13.0 g. respectively. Each of the three crops was washed two times with absolute ethanol. The total yield of crystalline material was 59 g. (73.0%) mp., 140-1; R_f 0.75 (PAW) with ninhydrin and 0.74 (PAW) with periodate, $[\alpha]_D$ -70 (3 min.) \rightarrow -20 (36 hrs.),* (c , 0.856, methanol).

2. Addition of Hydrogen Cyanide to N-phenyl-D-xylosylamine

Fifty-nine grams of N-phenyl-D-xylosylamine was dissolved in 1.2 l. of redistilled absolute ethanol at 50° C. Hydrogen cyanide was prepared as usual, however it was solidified in a trap that was cooled to dry ice-acetone temperature. The solid hydrogen cyanide was liquified in a water bath and then aerated into the reaction flask with a slight amount of heat. A gas dispersion tube was used for better absorption. A minimum of 73 ml. of liquid hydrogen cyanide was added and the solution was allowed to stand at room temperature for twenty-four hours. The solution was then placed in an ice bath for thirty-six hours. The first crop was collected by filtration at 0° C. and washed with cold absolute ethanol,

*G. P. Ellis and J. Honeyman, J. Chem. Soc. 1490, (1952), report m.p. 143-144, $[\alpha]_D$ -34.1 \rightarrow -21.9, (c , 1.0, methanol).

wt., 33 g., m.p. 90°-112°, R_f 0.92 (PAW), using the periodate spray, $[\alpha]_D$ -127 (c , 1.27 methanol). A second crop was collected by filtration at 0° after the mother liquor was concentrated in vacuo to 300-400 ml. and washed with cold alcohol, wt., 11 g., m. p. 90-101°, R_f 0.89 (PAW), using the periodate spray.

Both of the above crops were recrystallized from warm ethyl acetate, yielding 28 g. (A), m. p. 115-120°; R_f 0.89 (PAW), using the periodate spray, $[\alpha]_D$ -113 (c , 1.01 ethanol) and 9.1 g. (B), m. p. 100-110° R_f 0.89 (PAW), using the periodate spray, $[\alpha]_D$ -132, (c , 0.97, methanol).

Product A was dissolved in 240 ml. of absolute ethanol at 60°. The solution was allowed to cool to room temperature. The product, N-phenyl-D-idosaminic acid nitrile was collected by filtration and washed with alcohol, yielding 7.5 g., m.p., 118-118.5°, R_f 0.87 and 0.92 (PAW), using the periodate spray, $[\alpha]_D$ -40 (c , 0.86, methanol).

This material had initially been recrystallized from ethyl acetate, but was now only slightly soluble.

The filtrate from the collection of the N-phenyl-D-idosaminic acid nitrile was cooled to 0°; the crystalline material which separated was collected and washed with cold alcohol, yielding 11.5 g. (C), m. p. 90-110°. The filtrate from the collection of product C was evaporated in vacuo to one-half volume and 1.5 g. of crystalline material, m. p., 106-116°, was obtained, R_f 0.93 (PAW) with ninhydrin and 0.94

(PAW) with periodate spray. Product C was mixed with 95 ml. absolute ethanol at 70° and an insoluble material, 1.6 g. (D) was collected by filtration; it showed m.p. 139-146°, R_f 0.93 (PAW), using the periodate spray. On cooling the filtrate to room temperature, a crystalline material separated which was collected by filtration and washed with alcohol, wt., 3.3 g. (E) m.p. 75-95°, R_f 0.95 (PAW), using the periodate spray, $[\alpha]_D$ -78, (c , 1.2 methanol). The filtrate from the collection of product E was evaporated to one-half volume in vacuo, and the crystalline material collected by filtration and washed with alcohol. The yield was 0.85 g., (F), m.p., 109-110°, R_f 0.93 (PAW), using the periodate spray, $[\alpha]_D$ -120.7 (c , 1.21, methanol).

Product E was washed three times with acetone, giving 1.5 g. of material, m.p. 139-148°. Product D and E were combined and mixed with 300 ml. of water at 80°: an insoluble (0.71 g.) was removed by filtration; the filtrate was cooled slowly to room temperature. Product G crystallized as fine long needles and was collected, washed with water, and dried, yielding 0.41 g. This material was crystallized from hot water, giving 0.15 g., m.p. 120-121° R_f 0.94 (PAW) with periodate spray.

Anal: $C_{12}H_{14}O_3N_2$ Calcd.: C, 61.60; H, 5.98; N, 11.97; O, 20.50
(220)

$C_{12}H_{16}O_4N_2$ Calcd.: C, 57.20; H, 6.36; N, 11.10; O, 25.40
(252)

Found: C, 61.45; H, 6.14; N, 11.25.
C, 61.51; H, 5.95; N, 11.49.

3. Catalytic Hydrogenation of N-phenyl-D-idosaminic Acid Nitrile

Trial 1.--A solution of 0.3314 g. (1.31 mmoles.) of N-phenyl-D-idosaminic acid nitrile in 25 ml. of distilled water was added to a suspension of 0.3 g. of ten per cent palladium on carbon in 10 ml. of 0.2 N acetic acid. The mixture was stirred in an atmosphere of hydrogen for fourteen hours; 48.0 ml. (2.05 mmoles, corrected to standard conditions) of hydrogen were consumed.

The catalyst was removed by filtration and the filtrate was evaporated to approximately 2 ml. at 50° in vacuo. Ion exchange resin IR-45 (OH⁻) was added to raise the pH to 6.5. The resin was filtered and the filtrate evaporated as before to dryness. To insure complete dryness, the amorphous material that formed was dissolved in absolute ethanol and distilled to dryness three times. It was then dissolved in 35 ml. of distilled water, filtered and passed over an IR-45 (Cl⁻) column. The eluate was evaporated to dryness in the usual way and dried in high vacuum for fifteen hours.

The weight of amorphous material obtained was 0.07 g. and gave two spots in PAW of R_f 0.93 and 0.99 when developed with ninhydrin. Authentic 2-amino-2-deoxy-D-gulose showed R_f 0.94 when run on the same paper chromatogram. No crystalline material could be isolated.

Trial 2.--A solution of 0.300 g. (1.19 mmoles.) N-phenyl-D-idosaminic acid nitrile in 25 ml. of distilled water was added

to 0.242 grams of platinum oxide suspended in 10 ml. of 0.2 N acetic acid. The suspension was stirred for eighteen hours in an atmosphere of hydrogen and consumed 146 ml. (6.53 mmoles., corrected to standard conditions) of hydrogen. The same method as in Trial One was used to isolate the product; amorphous material that weighed 0.16 g. was obtained. The product showed two spots on papergrams in PAW, R_f 0.93 and 0.98, when developed with ninhydrin. No crystalline material could be isolated.

Trial 3.--A solution of 0.530 g. (2.1 mmoles., corrected to standard conditions) N-phenyl-D-idosaminic acid nitrile in 25 ml. of distilled water was added to a suspension of 1.07 g. of five per cent platinum on carbon in 10 ml. of 0.5 N hydrochloric acid. The mixture was stirred in an atmosphere of hydrogen for two hours; 221 ml. (1.07 mmoles., corrected to standard conditions) of hydrogen were consumed.

The catalyst was removed by filtration and the filtrate was placed on the freeze drying apparatus and evaporated to approximately one milliliter. One milliliter of distilled water and 13 ml. redistilled methanol were added. By addition of acetone, 50 mg. of ammonium chloride was precipitated and removed from solution by centrifugation and decantation. The solution was evaporated to dryness, giving a semi-crystalline product weighing 0.338 g. The papergrams were very complicated.

Trial 4.--A solution of 0.506 g. (2.01 mmoles.) N-phenyl-D-idosaminic acid nitrile in 25 ml. of distilled water was added to 1.015 g. of palladium on carbon (five per cent) suspended in 10 ml. of 0.5 N hydrochloric acid. The suspension was stirred for five hours in an atmosphere of hydrogen and consumed 146 ml. (6.53 mmoles., corrected to standard conditions) of hydrogen. The isolation procedure for this preparation was performed in the same way as in Trial Three and yielded 0.385 grams of a semi-crystalline product. The papergrams run on this material were also very complicated. In PAW, the R_f (ninhydrin) were 0.60, 0.71, 0.80, 0.90 and (periodate) 0.59 and 0.72.

E. Hydrolysis of N-phenyl-D-idosaminic Acid Nitrile

Trial 1.--A solution of 0.2846 g. of N-phenyl-D-idosaminic acid nitrile in 25 ml. of 2 N hydrochloric acid was heated on a steam bath for four hours. Samples (5 ml.) were removed every hour and neutralized with IR-45 (OH^-). After removal of the ion exchange resin by filtration, each filtrate was evaporated to dryness in vacuo. All samples were chromatographed and gave complicated patterns (periodate spray). The one-hour sample showed (BAW) R_f 0.83, and (PAW), R_f 0.76, 0.63 and 0.55; two-hour (BAW), R_f .86 and (PAW), 0.76, 0.63 and 0.55; three-hour (BAW), R_f 0.85 and (PAW), 0.76, 0.63 and 0.55; four-hour (BAW), 0.84 and (PAW), 0.76, 0.63 and 0.55.

Trial 2.--A solution of 0.353 g. of N-phenyl-D-idosaminic acid nitrile in 30 ml. of 2 N HCl was allowed to stand at room temperature. Samples were withdrawn every few hours for fifty-six hours and dried as in Trial One. The R_f of the original nitrile (BAW) with a ninhydrin spray was 0.92. All the hydrolysis fractions including the first (one hour) were complicated, but very similar. There was no spot with an R_f value greater than 0.70; a crystalline product could not be obtained from the fractions.

Trial 3.--A solution of 0.5116 g. of N-phenyl-D-idosaminic acid nitrile in 30 ml. of 2 N hydrochloric acid was allowed to stand for twelve hours at room temperature. It was placed on the freeze dry apparatus and taken to complete dryness. Ten milliliters of absolute ethanol was added to the product. The precipitate of ammonium chloride (50 mg.) was removed by filtration. The filtrate was taken to dryness and 0.3 g. of an amorphous product was obtained that could not be crystallized by conventional solvent extraction methods.

SUMMARY

Attempts to synthesize D-idosamine and D-idosaminic acid by a reaction between liquid ammonia and D-xylose, followed by treatment with liquid hydrogen cyanide were unsuccessful.

N-benzyl-D-xylosylamine, an unreported compound heretofore, was isolated from the reaction of benzylamine with D-xylose. Upon addition of hydrogen cyanide to N-benzyl-D-xylosylamine, no crystalline product could be isolated.

Aniline was reacted with D-xylose in the preparation of N-phenyl-D-xylosylamine. This compound was condensed with gaseous hydrogen cyanide, giving N-phenyl-D-idosaminic acid nitrile and a small amount of a compound $C_{12}H_{14}O_3N_2$, the structure of which has been postulated to be 3,6-anhydro-N-phenyl-D-idosaminic- γ -iminolactone.

Experiments relating to the semi-reduction of N-phenyl-D-idosaminic acid nitrile by catalytic hydrogenation were studied in an attempt to synthesis crystalline 2-amino-2-deoxy-D-idose.

Hydrolytic conditions for the conversion of N-phenyl-D-idosaminic acid nitrile to N-phenyl-D-idosaminic acid have been determined but a crystalline product has not been isolated.

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