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1943

Reaction of glucose with some amines

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NOTE TO USERS

14

REACTION OF GLUCOSE WITH SOME AMINES

by

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Plant Chemistry

Approved:

In Charge of Major Work

Head of Major Department

Dean of Graduate College

Iowa State College
1943

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INTRODUCTION

Although a considerable amount of work has been done on the nitrogen derivatives of sugars, the literature regarding a great many of these derivatives is very confused. The reason for this confusion is quite evident when it is realized that each of these derivatives may exist, theoretically at least, in a number of isomeric structures.

The following diagram shows the possible structures for the compounds formed by the reaction of an amine, RNH_2, with glucose:

(a) Addition

```
        H                    H
   R    |               R    |
    \    |              |    /
     N-C-OH           HOC-N-H
    /    |              |
   H    |               |
        HCOH            HCOH
        |               |
        HOCH            HOCH
        |               |
        HCOH            HCOH
        |               |
        HCOH            HCOH
        |               |
        HCOH            HCOH
        |               |
        H               H
```
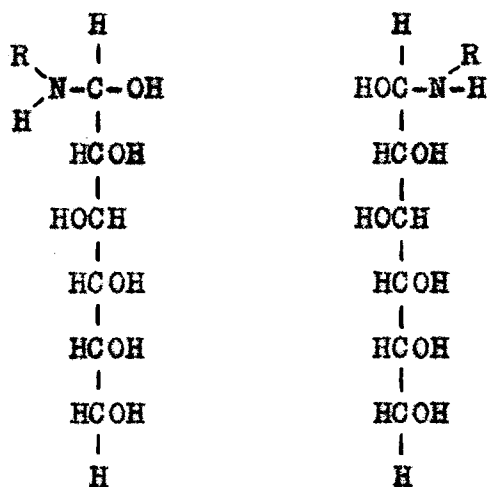
α - and β-Addition Compounds of

Aldehydo-Structure

(b) Water Elimination

```
    H
    |
    C=NR        RN=C-H
    |              |
  HCOH           HCOH
    |              |
  HOCH           HOCH
    |              |
  HCOH           HCOH
    |              |
  HCOH           HCOH
    |              |
  HCOH           HCOH
    |              |
    H              H
```
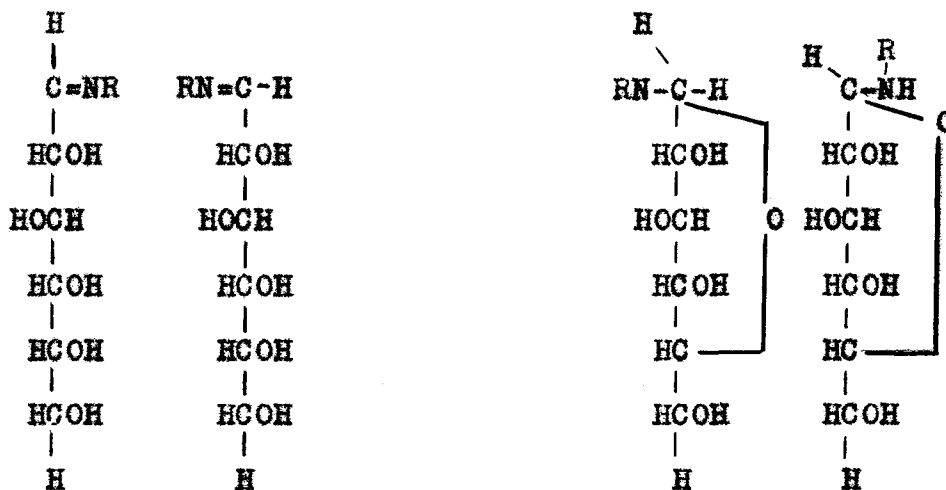
Syn and Anti Imino

Structure

```
    H               H    R
     \               \   |
   RN-C-H             C-NH
      |                |    O
    HCOH             HCOH
      |                |
    HOCH    O        HOCH
      |                |
    HCOH             HCOH
      |                |
    HC                HC
      |                |
    HCOH             HCOH
      |                |
     H                 H
```

α - and β-Pyranoside

Structure

```
      R                    R
   H  |                    |
    \ N    H           H-C-N-H
      C                   |
      |                 HCOH
    HCOH                  |
      |                 HOCH
    HOCH   O              |
      |                 HC
    HC                    |
      |                 HCOH
    HCOH                  |
      |                 HCOH
    HCOH                  |
      |                  H
     H
```
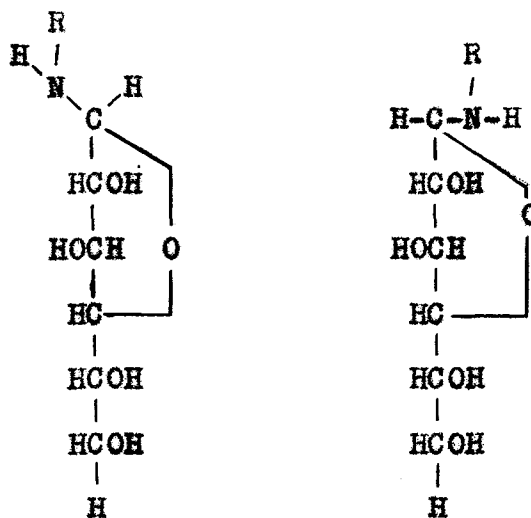
α - and β-Furanoside

Structure

(c) Rearrangement

H
|
N-R
/
HCH
|
C=O
|
HOCH
|
HCOH
|
HCOH
|
HCOH
|
H

H
|
N-R
/
HCH
|
HO-C
|
HOCH   O
|
HCOH
|
HC
|
HCOH
|
H

HH   OH
\   /
RNC-C
|
H
|
HOCH   O
|
HCOH
|
HC
|
HCOH
|
H
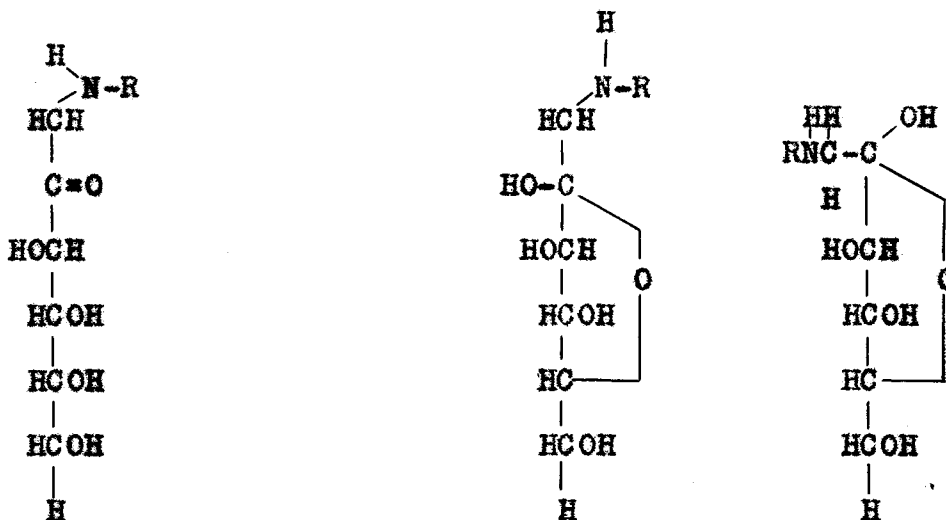
Keto Structure α- and β-2,5-Furanoside Structure
(These may also exist in the
pyranoside structure.)

The keto isomer and the 2,5-furanoside compounds that exist
in equilibrium with it were obtained when the N-glucoside
compound of certain aromatic amines was heated in the presence
of a trace of acid or ammonium salt. The postulated mechanism
of this rearrangement, named after its discoverer, Amadori,
has been advanced by Kuhn and Weygand (77) in the following
diagram:

H H
CH₃-C₆H₄-N-C
HCOH
HOCH
HCOH
HC
CH₂OH

CH₃-C₆H₄-N=CH
HCOH
HOCH
HCOH
HCOH
HCOH
H
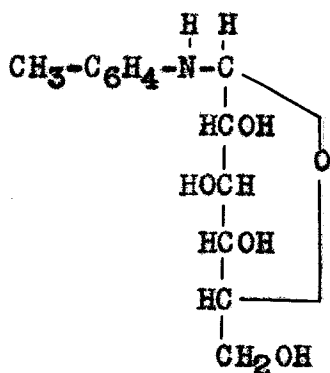
I

p-Toluidine-d-Glucoside
m.p. 115° C.

II

*Schiff's Base Structure

H
CH₃-C₆H₄-N-CH
COH
HOCH
HCOH
HCOH
HCOH
H

H
CH₃C₆H₄NCH₂
C=O
HOCH
HCOH
HCOH
HCOH
H
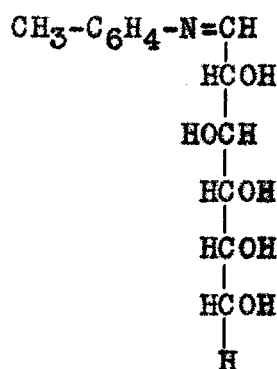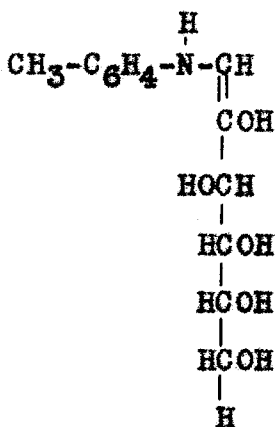
III

*Enol Structure

IV

*Keto Structure

H
CH₃C₆H₄N-CH₂-C-OH
HOCH
HCOH
HC
HCOH
H

H
HO CH₂NC₆H₄CH₃
C
HOCH
HCOH
HC
HCOH
H
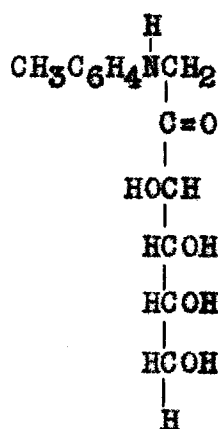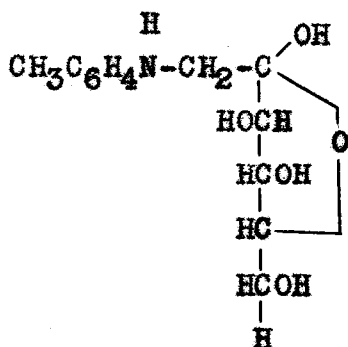
V

*α-2,5-Furanoside Structure
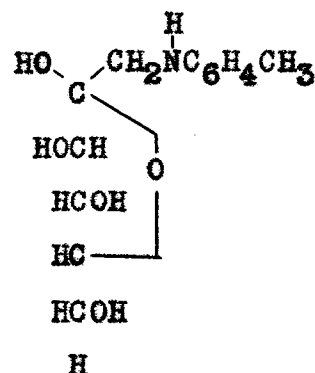
VI

β-2,5-Furanoside Structure
m.p. 152° C.

---

*Postulated intermediate compounds not isolated.

No generalization has been made in the literature, but the data obtained from the different glucosyl amines indicated that the ease of effecting the rearrangement depended upon the relative negativity of the amine and the stability of the glucoside to hydrolysis.

To complicate the study further, the addition compounds and the N-glucosides not only were converted from one structure to another in aqueous solution, but also were dissociated into the free amine and glucose until an equilibrium between the glucosyl amine, glucose and the amine was reached.

Though compounds that have been classified as RNH_2 differed widely in chemical properties, the compounds formed by the reaction of these amines with glucose may be considered as derivatives of glucose ammonia. The differences in the glucosyl amine obtained depended upon the R group substituted on the ammonia. A great deal of work has been done on the glucosyl amines, but the investigations have been isolated studies of a particular amine or group of amines rather than a comprehensive study of the reaction of glucose with amines. A tremendous amount of investigation has been done on the reaction of glucose with phenylhydrazine, yet the literature is very confusing concerning these derivatives. The oximes of sugars have been known for years, yet their relationship to 1-aminoglucose was often not recognized. Recently the synthesis of vitamin B_2 instigated a thorough study of the glucosyl aromatic amines which resulted in elucidation of

the Amadori rearrangement.

The literature concerning the ammonia, phenylhydrazine, hydroxylamine, aromatic and aliphatic amine derivatives of glucose has been reviewed in order to establish a basis of comparison for the reaction of the different amines with glucose.

REVIEW OF LITERATURE

Since the reactions of ammonia and the substituted amines with glucose have been investigated as isolated studies rather than as glucosyl amines differing only in the substitution of the nitrogen, it was necessary to make the review of literature on each type of glucosyl amine. An effort will be made later to correlate these compounds as derivatives of 1-aminoglucose and to show that the differences in the properties and reactions may be explained by the variation of the substituted group.

Ammonia Derivatives of Glucose

1-Aminoglucose

Glucose ammonia was first investigated by Lobry de Bruyn and Franchimont (84), who observed that glucose exhibited an unusual degree of solubility in anhydrous ethyl or methyl alcohol containing ammonia gas. The rotation of the solution of glucose in the ammoniacal methyl alcohol decreased as the glucose reacted. After several days small aggregates of crystals precipitated out of the viscous solution. Upon analysis this compound was identified as an isomer of chitose

amine or 2-aminoglucose reported by Ledderhose (78) and of
the isoglucosamine made by Emil Fischer (29). The properties
of this new isomer differed greatly from the known amino
glucoses in that it was very labile to acids, although the
free amine was quite stable even in aqueous solution. This
stability in water was demonstrated by the fact that the
specific rotation of an aqueous solution changed only from
+19.65° to +13.6° in ten months. The investigators were
unable to prepare the salts of this base. This fact was par-
ticularly noteworthy since the hydrochloride of 2-aminoglucose
was quite stable. The 1-aminoglucose was completely decom-
posed into the ammonium salt and glucose by boiling with an
excess of 1/10 N sulfuric acid. By following the change in
specific rotation, it was found that partially neutralized
aminoglucose was slowly hydrolyzed, the rate being a function
of the concentration of the solution and the amount of acid
added. The recrystallized product melted at 127°-128° and
had the formula $C_6H_{13}NO_5$. Lobry de Bruyn expressed the
reaction as

$$C_6H_{12}O_6 + NH_3 \longrightarrow C_6H_{13}NO_5 + H_2O$$
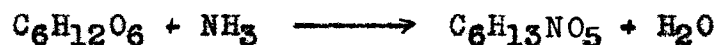
Stone (14), in the same year, studied the reaction of
dextrose with ammonia. The product reported by this investi-
gator was an additive compound in which no molecule of water
had been eliminated. He obtained a lower percentage of
nitrogen and a melting point of 121-122° C. Lobry de Bruyn
(86) discounted these results on the basis that in the

elementary analysis all of the ammonia was not eliminated by distillation of the "glucose ammonia" with potassium hydroxide.

Sjollema (103) refluxed the 1-aminoglucose in absolute methanol for several hours and with ether precipitated the compound $C_{12}H_{22}O_{10}N.2H_2O$. This compound, di-(1-glucosyl)-amine, was easily decomposed by dilute acid to glucose and ammonia, and even the free amine decomposed slowly in aqueous solution.

Lobry de Bruyn (86) termed these as osamine compounds and assigned the structure $CH_2OH-(CHOH)_3-CH-CHOH$ to them.
$$NH$$
Wohl (129) subsequently modified the formula and postulated the gamma-imino ring: $CH_2OH-CHOH-CH-CHOH-CHOH-CHOH$.
$$NH$$

Fischer's (32,33) formula for arabinamine led Irvine, Thompson and Garrett (58) to postulate this structure for 1-aminoglucose:

$$CH_2OH-CHOH-CH-CHOH-CHOH-CHNH_2$$
$$O$$
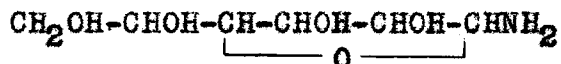
The other formula given was $CH_2OH-CHOH-(CHOH)_3-CH=NH$. The two formulae with the NH ring were rejected on the basis that $\measuredangle$-methyl glucoside will not react with ammonia. Nitrogen was evolved when nitrous acid was added, indicating the presence of an NH_2 group. An attempt by Irvine to obtain glucosamine from $\measuredangle$- and β-pentaacetyl glucose gave the imino-bioses of Sjollema, and this further substantiated the glucosidic linkage of the NH_2 group. Irvine compared the stability of

the 1-aminoglucose and ethylaminoglucose to compounds of the type of 2-aminoglucose, isoglucosamine and fructosazine. The compounds in which the amino group was located on the carbon atom adjacent to the reducing group were remarkably stable to acids; however, substitution on the reducing group or on any other carbon atom not adjacent to the reducing group produced a compound very labile to dilute acids. On the other hand, the 3-aminoglucose (40) and the 6-aminoglucose of Fischer (37) are more stable than the compounds with the amino group on the reducing group of the sugar. These two compounds will be discussed later.

Levene (78) attempted to supply further proof for the glucosidic linkage of the 1-aminoglucose. The two bits of evidence that had to be obtained to prove the structure conclusively were a proof of the oxide ring structure and the presence of an unsubstituted NH_2 group. The presence of the group was tested by a comparison of Kjeldahl and Van Slyke nitrogen determinations. In four minutes all of the N_2 of the NH_2 group was evolved. This could not have been due to dissociated ammonia, since the reaction to evolve N_2 from ammonia was much slower.

Schmuck (99) tested for the presence of an NH ring. If an NH ring were present, water should easily be removed to give a pyrrole ring. Glycerol and $ZnCl_2$ did not remove water and produce substituted pyrrole until the mixture was heated to 200° C. A compound thought to be the heptabenzoate of

aminoglucose produced a nitrosamine on treatment with nitrous acid, showing the presence of a secondary amine and thus confirming the glucosidic linkage postulated by Irvine. This heptabenzoate was later proved to be a pentabenzoate. Marked degradation was produced by heating glucose and ammonia alone or with zinc hydroxide and ammonium hydroxide (128,111). Pyridine and substituted pyrazines were among the recovered products of decomposition.

Ling and Nanji (81,82) made a study of the mechanism of caramelization of sugar by the ammonia process. They reported that the action of an excess of ammonia on dextrose formed an additive compound, glucose ammonia, which gave a negative test for amino compounds. They did not state which test for amino compounds was used. Their experiments showed that no iso-dynamic change occurred and that the reaction was reversible. By removal of the ammonia, they obtained a sugar syrup with a higher reducing value than ordinary glucose, and as a result they postulated the formation of gamma-glucose. Since a red color with Seliwanoff solution was obtained, they attempted to determine the amount of ketose in the presence of aldose. The pH of the solution conditioned this equilibrium. The estimation gave the aldose in the presence of ketose (13,60, 127).

To prove the presence of aldehyde ammonia, Ling and Nanji (82) reduced the compound to the glucamine by catalytic hydrogenation, electrolytic reduction, and reduction with aluminum

amalgam. The glucamine produced was isolated as the oxalate. No yields and only a few experimental details were given. They also condensed glucose ammonia and formaldehyde sodium bisulfite with the production of the omega-sodium sulfonate,

$$CH_2OH.(CHOH)_4.CHOH.NH.CH_2SO_3Na.$$
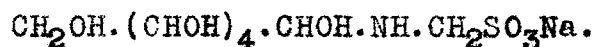
However, there is no reason why the 1-aminoglucose could not also undergo these reactions.

Brigl and Keppler (19) made the pentaacetate of 1-aminoglucose and the α-octaacetate of the diglycosylamine prepared by Sjollema. The pentaacetate, upon saponification with methyl-alcoholic ammonia, formed a monoacetate:

$$CH_2OAc.CH(CHOAc)_3CHNHAc \longrightarrow CH_2OH.CH(CHOH)_3CHNHAc$$

They claimed that the heptabenzoate of Schmuck was an impure mixture and not a heptabenzoate at all.

In an investigation of the action of liquid ammonia on carbohydrates, Muskat (88) reported that the polysaccharides as well as the sugars, their acetone, methylated and acetylated derivatives were quite soluble in liquid ammonia. The ammonia was without effect upon all the carbohydrates except the simple sugars. Glucose was dissolved in liquid ammonia and the residue, upon evaporation of the ammonia, was treated with alcohol. The yield of 1-aminoglucose was practically quantitative. The analysis and the physical constants of the compound were given. He stated that evidence

for the formation of the glucose-ammonia addition compound at first would be given in a later paper, but that paper has not yet appeared.

Wayne and Adkins (122) prepared d-glucamine from d-glucose by hydrogenation of glucose and dry ammonia in anhydrous methanol. The yield of glucamine based upon glucose was 12 per cent. If 1-aminoglucose prepared by the method of Lobry de Bruyn were reduced under the same conditions, a 26 per cent yield was obtained. The glucamine produced was isolated as benzalglucamine. From the neutral equivalent of the crude product, eighty per cent of the glucose had been converted to basic compounds, but the products obtained were mixtures.

Flint and Salzberg (38) took out a patent covering the hydrogenation with nickel of monosaccharides in the presence of ammonia.

2-Aminoglucose

2-Aminoglucose, or chitosamine (77), has long been found in nature in the form of its polymer, chitin. Hydrochloric acid reacted with this amino sugar to form a remarkably stable salt; in fact, chitosamine has generally been prepared by long refluxing of naturally occurring chitin with concentrated hydrochloric acid (50). No attempt has been made to review the literature of this most common amino sugar except for a comparison of the stability of the amine as compared to the other glucosamines. The only other amino sugar that

approached this compound in stability of the salt formed was the 6-aminoglucose. The free amine (18), on the other hand, was quite unstable. Kept in a vacuum desiccator over sulfuric acid for a month, it underwent no decomposition, while in air or in aqueous solution, it decomposed rapidly.

3-Aminoglucose

3-Aminoglucose was prepared (40) from diacetone-3-p-toluenesulfonylglucose on treatment with alcoholic ammonia in a sealed glass tube at 170° C. Hydrolysis by dilute acids gave the free glucosyl-3-amine. A very small yield of the diacetone-3-aminoglucose was obtained when diacetone-3-p-toluenesulfonylglucose was heated with ammonia. The 3-amino sugar was a syrup that forms a phenylosazone. The amino group was stable to two hours' heating at 70° C. in 2 per cent hydrochloric acid. Peat and Wiggins (94) prepared methyl tetraacetyl-3-amino-α-glucoside by the action of ammonia on methyl triacetyl-3-p-toluenesulfonyl-α-glucoside and subsequent acetylation. When methyl 4,6-benzylidene-2,3-anhydro-α-alloside was heated with methyl alcoholic ammonia under pressure and acetylated, two amino sugars were formed, the methyl 4,6-benzylidene-2-amino-α-altroside diacetate and a small amount of methyl 4,6-benzylidene-3-amino-α-glucoside diacetate. On removal of the benzylidene group and acetylation, the methyl tetraacetyl-3-amino-α-glucoside was obtained. The hydrochloride of methyl 3-amino-α-glucoside was made by

treatment in the cold for one hour with 5 per cent solution of hydrochloric acid in methyl alcohol, followed by neutralization with lead carbonate. The amino sugar from the tetra-acetate of methyl 3-amino-α-glucoside was stable to heating on a water bath for thirty-four hours in 6 per cent hydrochloric acid.

6-Aminoglucose

The 6-aminoglucose was prepared by Fischer and Zach (37). Methyl 6-amino-glucoside hydrobromide was prepared by the action of anhydrous liquid ammonia on methyl triacetyl-α-glucoside bromohydrin at 15° C. The methyl 6-amino-α-glucoside was prepared from the hydrobromide by reaction with silver oxide. It was stable to heating for two hours with 2 N hydrochloric acid, but became brown with alkali treatment and liberated ammonia.

Later Ohle and von Vargha (90) prepared the 6-amino-d-glucose from 6-p-toluenesulfonyl-1,2-monoacetone-d-glucose by reaction with methyl alcoholic ammonia at room temperature. The product obtained was the p-toluenesulfonyl salt of the amine compound. This salt was changed into the 6-amino-d-glucose by treatment with normal sulfuric acid.

Phenylhydrazine Derivatives of Glucose

In 1884 Fischer (28) reported that glucose reacted with phenylhydrazine hydrochloride in the presence of sodium acetate to form fine yellow needles that were insoluble in water. Upon investigation he found that a reaction occurred in which an oxidation of glucose as well as a splitting out of water was evident. The resulting compound, the glucose phenylosazone, had the formula $C_6H_{10}O_4 \cdot N_4C_{12}H_{12}$ with the $-NNHC_6H_5$ radicals on the reducing group of the sugar and on the carbon adjacent to it. Upon reduction (29) with zinc and acetic acid, ammonia and aniline were formed along with an isoglucosamine, $C_6H_{13}NO_5$, that was isomeric with the glucosamine of Ledderhose (77).

Later Fischer (30) reported the formation of a "dextrose phenylhydrazine," a product that upon treatment with aqueous acetic acid reacted further to give the phenylosazone (see p.30 for the reaction). When two parts of glucose, two parts of phenylhydrazine, and one part of water were reacted, a product formed that melted at 144-145° C. These colorless needles were purified by dissolving them in warm alcohol and precipitating with ether. These needles, the first prepared phenylhydrazine derivative of glucose, were very soluble in water and hot alcohol.

Since the nomenclature of the phenylhydrazine derivatives

of glucose is so indefinite, especially in the early litera-
ture, and the actual structure of most of these compounds is
still uncertain, table 1 has been formulated from the data on
the compounds reported from the reaction of phenylhydrazine
with glucose.

Table 1

Compounds of Glucose and Phenylhydrazine

Name	Structure Postulated by Behrend	Melting Point (^{o}C.)	Calc. % nitro-gen	Reported by
1. α-Hydrazide	N-Glucoside	159-160	10.37	Behrend, Stempel
2. β-Hydrazone	True Hydrazone	140-141	10.37	Behrend
3. α-Hydrazide	Same as (1)	144-146	10.37	Fischer
4. β-Hydrazone	Same as (2)	115-116	10.37	Skraup, Butler and Cretcher, Jacobi
5. 2:1 Compound	2 mol. hydrazide to 1 mol. phenyl-hydrazine	106-107	12.97	Behrend
6. 1:1 Compound	1 mol. hydrazide to 1 mol. phenyl-hydrazine	86-87	14.81	Behrend

To avoid confusion in the review of literature, the compounds
will be designated as the phenylhydrazine derivatives of
glucose, or as the hydrazone or hydrazide, with the name of
the person who first reported the compound.

Skraup (103) attempted to prepare the phenylhydrazine
derivative reported by Fischer, but in a series of experiments,

amorphous material, rather than the desired crystals, was usually obtained. Only by chance did he obtain a small yield of the needles reported by Fischer. However, out of very dilute solutions a compound crystallized that melted at 115-116° C., which he designated as the β-hydrazone. Further purification raised the melting point to 117-118°. Jacobi (58) prepared the Skraup hydrazone using a bit more water. The specific rotation of this compound ten minutes after solution in water was $[\alpha]_D^{20}$ = -15.3°, which changed to a value of -46.8°. This change occurred in less than twenty-four hours when a 10 per cent solution was used. On evaporation of a solution that had reached equilibrium, followed by purification with alcohol and ether, a compound melting at 114° C. was obtained. From this data he concluded that he did not have a mixture of the Skraup derivative and the Fischer derivative. On a study of the rate of formation of the derivative in a water-acid mixture, the equilibrium point was reached more slowly when a sugar solution that had mutarotated was used than when solid glucose was used.

Simon and Benard (101) obtained Fischer's phenylhydrazine compound only occasionally and then only as a very impure product with a melting point at 125° C. and $[\alpha]_D$ = -66.57°. The β-hydrazone of Skraup, $[\alpha]_D$ = -6.84°, was easily obtained by these workers.

Behrend, on the other hand (14), only occasionally obtained the high-and the low-melting derivatives previously

reported, and in every case these compounds were very impure. In an attempt to prepare the derivative in alcoholic solution using a large excess of phenylhydrazine, snow-white needles with a melting point of 106-107° C. were obtained. On further investigation, Behrend (15) found that the 106-107° C. melting compound that he had identified as the Skraup β-hydrazone was not a pure hydrazone, but a compound of two molecules of the phenylhydrazine derivative with one molecule of phenylhydrazine. This compound decomposed in cold alcohol and liberated phenylhydrazine. The pure β-phenylhydrazone he reported as colorless needles melting at 140-141° C., $[\alpha]_D$ -5° → -54°.

The α-hydrazide of Behrend, that is, the one that melted at the higher temperature and had the highest negative rotation, was prepared by reacting an equal number of moles of the phenylhydrazine and glucose in absolute alcohol containing acetic acid. After washing with alcohol and ether, the pale yellow platelets melted at 159-160° C. The compounds melting at 106-107° C. and at 159-160° C. were also obtained by using glucose that had been dissolved in a small amount of boiling water. These two forms, the α-hydrazide and the β-hydrazone of Behrend, changed in rotation in aqueous solution to approximately the same value, and the two forms were interchanged in alcoholic solution in the presence of acetic acid. The temperature at which the equilibrium and subsequent crystallization occurred seemed to govern the products obtained from the reaction. The α-form was generally crystallized from solution

at room temperature, while lower temperatures were more
expedient for crystallization of the pure β-form.

A compound that analyzed to correspond to one molecule of
phenylhydrazine combined with one molecule of the phenylhydra-
zine derivative was obtained by treatment of the 106-107° com-
pound with phenylhydrazine. It melted at 85-87° C. The com-
pound melting at 106-107° also formed an addition compound
with pyridine that melted at 100-101°. The pure β-hydrazone
was obtained most easily by dissolving this pyridine compound
in six to seven times its weight of absolute alcohol and
allowing the β-hydrazone to crystallize from the solution.

On further study concerning the change of the α- into the
β- compound and the reverse reaction, Behrend verified that
the α-hydrazide could be obtained from the β- by treatment with
an alcoholic solution acidified with acetic acid at room tem-
perature. In crystallization at lower temperatures, a com-
pound melting at 106-107°, but not the addition compound, was
obtained. The α- was changed into the β-form by refluxing a
dilute alcoholic solution of the α-hydrazide without addition
of acetic acid, and subsequent crystallization of the β-
hydrazone from the solution at low temperatures. On acidifica-
tion, the change of rotation of the β-hydrazone passed through
a minimum, $[\alpha]_D$ = -4.35 ⟶ -48.39 ⟶ -43.45°. In spite of
many attempts, Behrend was unable to isolate a third form of
the hydrazone. He suggested that the two forms were:

```
CH_2OH
|
CHOH
|
CHOH                           CH_2OH
|                              |
CHOH                           CH──O──┐
|                              |      │
CHOH                          (CHOH)_3 │
|                              |      │
CH = NNHC_6H_5                 CH-NHNHC_6H_5
```
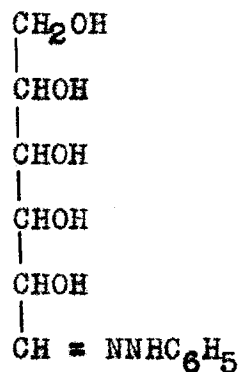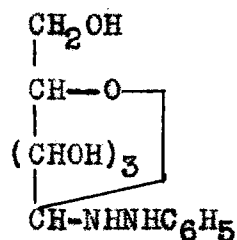
I. True hydrazone ($\underline{\beta}$)   II. Hydrazide ($\underline{\alpha}$)

From the optical rotation of the two forms, it was evident that the isomers are not the $\underline{\alpha}$- and $\underline{\beta}$-forms of the hydrazide structure. The following calculations were given by Behrend[*] to prove this point:

$\underline{\alpha}$-hydrazone $[\alpha]_D$ =	-87°
$\underline{\alpha}$-glucose	+110°
Difference	-197°
$\underline{\beta}$-glucose	+ 19.3°
Difference	+216.3°

α-hydrazone $[\alpha]_D$ =	-87°
β-glucose	+19.3°
Difference	-106.3°
$\underline{\alpha}$-glucose	+110
Difference	+216.3°

$\underline{\beta}$-hydrazone $[\alpha]_D$ =	-5°
$\underline{\beta}$-glucose	+19.3°
	-24.3°
$\underline{\alpha}$-glucose	+110°
Difference	+134.3°

β-hydrazone $[\alpha]_D$ =	-5°
$\underline{\alpha}$-glucose	+110°
Difference	-115°
β-glucose	+19.3°
Difference	+134.3°

Thus Behrend concluded that one has the structure I, the other II.

---

[*]Used rotations given by Armstrong rather than older values given by Behrend.

In cold water and alcohol, the β-hydrazone was less soluble than the α-hydrazide. A trace of pyridine lengthened the time required to obtain an equilibrium mixture from the β-hydrazone, while a trace of acetic acid greatly accelerated the speed of reaction. When accelerated with a trace of acetic acid, the specific rotation passed through a maximum in approximately two hours, $[\alpha]_D^{20} = -4.52 \rightarrow -52.92 \rightarrow -37.44°$.

In an attempt to obtain a dextrorotatory phenylhydrazine derivative from the levorotatory compounds, since both compounds showed initial levorotation in pyridine, the pyridine solutions were heated at different temperatures for varying lengths of time. An increase in the rotation to the right could be observed, but the third form was not isolated nor was its presence proved.

Hofmann (49), reasoning from the fact that these two perplexing isomers of glucose phenylhydrazone existed, decided that a study of the derivatives of phenylhydrazine of a number of other mono- and disaccharides might clarify the confusion. A change in rotation was noted when these derived hydrazine derivatives were dissolved; yet in every case, only one of the isomers could be obtained.

Hofmann acetylated the α-hydrazide of Behrend in pyridine solution at 0° C. and obtained a crystalline acetate with a melting point of 152-153° C., $[\alpha]_D = -12°$ in pyridine. The β-glucose phenylhydrazone of Behrend gave, under the same treatment, an amorphous product that melted from 50° to 70° C.,

$[\alpha]_D = -101°$. The acetates no longer changed from one form to another. This was given as further proof that one form was the hydrazide, the other the hydrazone.

The analysis of the crystalline acetate prepared from the $\underline{\alpha}$-hydrazone corresponded more nearly to the calculated value for the pentaacetate than to the value for the hexaacetate. The amorphous material obtained from the Behrend $\underline{\beta}$-hydrazone in the cold, or from the equilibrium mixture from boiling pyridine, melted from 50-70° C. and when this was analyzed it was found to be the pentaacetate. Hofmann also made the acetates of several substituted phenylhydrazones, both from the solid phenylhydrazone and the equilibrium product, in pyridine. Behrend and Reinsberg (16) investigated the acetates of the phenylhydrazine derivatives still further. Since Behrend had determined that both his $\underline{\alpha}$-hydrazide and $\underline{\beta}$-hydrazone showed an initial rotation to the left when they were dissolved in pyridine, then changed to a dextrorotation, a third form was yet to be isolated. The acetates showed no birotation nor were they interchangeable; thus it appeared unlikely that they were syn and anti forms. Most syn and anti forms of compounds were known to be easily changed from one form to another.

Hofmann had established that the acetates of both of the glucose phenylhydrazide derivatives had the same molecular weight. It had been shown that the nitrogen atom on hydrazones was hard to acetylate, while the nitrogen on a

hydrazide was comparatively easy to acetylate in the cold.

It was proved that a nitrogen had been acetylated in the crystalline phenylhydrazide acetate, since the investigators obtained the acetylphenylhydrazone of benzaldehyde by warming the crystalline acetate with a water-alcoholic solution of benzaldehyde. Likewise, α-acetylphenylhydrazine was recovered by hydrolyzing the crystalline acetate with five per cent hydrochloric acid.

When benzaldehyde was reacted with the amorphous phenylhydrazone, only the phenylhydrazone of benzaldehyde was obtained. An attempt to hydrolyze the acetate of the Behrend β-hydrazone with two per cent hydrochloric acid resulted in the formation of a resinous material. The structure for these two acetates based on the above evidence was

$$
\begin{array}{ll}
\text{CH}_2\text{OCOCH}_3 & \text{CH}_2\text{OCOCH}_3 \\
| & | \\
\text{CH} & \text{CHOCOCH}_3 \\
| & | \\
\text{CHOCOCH}_3 \quad \text{O} & \text{CHOCOCH}_3 \\
| & | \\
\text{CHOCOCH}_3 & \text{CHOCOCH}_3 \\
| & | \\
\text{CHOCOCH}_3 & \text{CHOCOCH}_3 \\
| & | \\
\text{CHNHN-C}_6\text{H}_5 & \text{CH=NNHC}_6\text{H}_5 \\
\quad \text{COCH}_3 &
\end{array}
$$
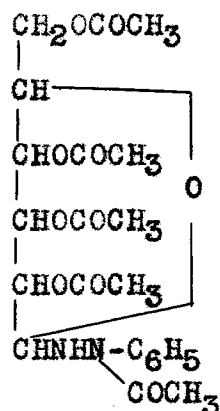
m.p. 152-153°   amorphous
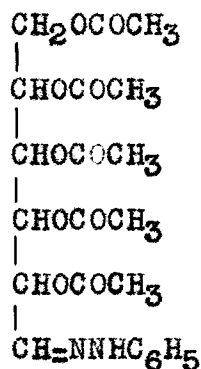
α-pentaacetate β-pentaacetate

Further proof of structure of these compounds was obtained by the preparation of α-acetylphenylhydrazone of glucose. If the compound formed were a condensation product with a double

bonded nitrogen, a hexaacetate should have been formed on acetylation. Since one of the compounds formed on acetylation was identical with the 152° α-pentaacetate, the evidence offered for the glucoside structure of the crystalline acetate was even more conclusive.

The melting point of the Behrend α-hydrazide acetate exhibited an interesting phenomenon. Upon heating, a melting of the compound was evident at about 130°, but upon further heating the compound solidified and melted again at 151°. On cooling this product and heating again, a melting point was no longer observed at 130°, but the compound melted at 151°. If ether were saturated at its boiling point with the crystalline acetate, the lower-melting compound gradually separated upon refluxing gently. It was evident that there was an equilibrium of the two compounds in ether solution, and that as the ether slowly evaporated, the less soluble compound, the lower-melting one, separated. Thus the higher-melting form could be changed completely to the lower-melting form by a sufficiently slow evaporation of the solvent. As both forms were present in solution, the change of melting point could not be explained as polymorphism. In pyridine, the rotation of the two forms was identical, $[\alpha]_D = 17.5°$, and upon analysis no change in molecular weight could be detected. In benzene, both compounds exhibited a change in rotation and approached a limit that, within the limits of experimental error, was identical.

The molecular weight of the pentaacetate was 449 determined in boiling alcohol. This value was rather low as compared to the calculated molecular weight of 480 for the pentaacetate. This discrepancy was attributed to the hygroscopic nature of the acetate.

For condensation of glucose with $\underline{\alpha}$-acetylphenylhydrazine in alcoholic solution, acetic acid was essential. Besides the crystalline pentaacetate, another compound with a rotation of $[\alpha]_D = -143.1°$ was obtained on acetylation of the glucose derivative of $\underline{\alpha}$-acetylphenylhydrazine. The molecular weight of this compound was 489 compared to the calculated value of 522 for the hexaacetate and 480 for the pentaacetate. Nonetheless, hydrolysis of the acetate groups showed the compound to be a hexaacetate. On splitting the compound with five per cent hydrochloric acid in the cold, $\underline{\alpha}$-acetylphenylhydrazine was identified; therefore the hexaacetate compound was a hydrazone.

Hydrolysis of all of the acetates formed was carried out by N/10 and N/5 potassium hydroxide as well as N/1 and N/2 sulfuric acid. The acetyl groups were readily removed from all but the nitrogen atom. It had previously been ascertained that N/1 acid did not hydrolyze the acetyl group from $\underline{\alpha}$-acetylphenylhydrazine in the cold. This group, however, was removed by refluxing the compound with N/1 acid.

As acid did not hydrolyze the amorphous compound prepared from the Behrend $\underline{\beta}$-phenylhydrazone, N/20 and N/4 alkali were

used. The N/4 alkali removed all of the acetate groups. When the hexaacetate was saponified with N/4 alkali, the percentage of acetate groups determined corresponded to the removal of six acetate groups, while the more dilute alkali removed only five acetate groups. The reactions attempting to form the phenylhydrazine acetates from β-acetochloroglucose and phenyl-hydrazine (12) were not fruitful.

Frerejacques (39) hydrolyzed the α- and β-d-glucose phenylhydrazine derivatives reported by Behrend and Lohr (15) and concluded that α-d-glucose was obtained as a product of the hydrolysis. His conclusion was based upon the fact that upon the completion of the hydrolysis, the rotatory power of the hydrolyzed product decreased. He explained this decrease as a decrease caused by the mutarotation of α-d-glucose formed. He catalyzed the hydrolysis with oxalic and picric acid, whose phenylhydrazine salts were nearly insoluble, resulting in near completion of the reaction.

Stempel (109) hydrolyzed α-glucose phenylhydrazide in hope that some quantitative data might be obtained to prove the presence of a certain form of glucose upon hydrolysis. His data showed that the hydrolysis with oxalic acid followed the unimolecular law quite closely, and mutarotation had little if any effect on the velocity constant. The decrease in the rotatory power of the hydrolyzed product that had led Frerejacques to believe that α-d-glucose was formed, Stempel attributed to the removal of glucose by adsorption in the

precipitated phenylhydrazine salt. This view was substantiated by the fact that approximately 80 per cent of the glucose theoretically possible remained in the solution. Before precipitation occurred over 90 per cent of the sugar could be found in the solution. From the rates of reaction of phenylhydrazine with α- and β-d-glucose in water-alcohol solutions, he concluded that it was impossible to detect a difference between the rates of reaction of the two isomers since the mutarotation of glucose was too slow to allow such a comparison to be made. He attributed the differences in the rates of reactions that occurred to the presence of acetic acid in the α-d-glucose. It was noteworthy that the phenylhydrazine derivative alone was formed when hydrochloric acid was used, but that both the hydrazide and osazone were formed when acetic acid was used. In a polarimetric study a few years later, Stempel and Orning (110) verified this lack of correlation between the structure of the sugars and the phenylhydrazine derivatives. They investigated the mutarotation and hydrolysis of the α-d-glucose phenylhydrazine derivative and developed mathematical formulae to express the rotation at time t. The rate of reaction, k^o, depended only upon the phenylhydrazonium ion, $C_6H_5NH-NH_3^+$, but the equilibrium constant, K, was independent of the ionized base. The rate of production of the phenylhydrazine derivative was proportional to the ionized base at low concentrations but reached a maximum at high concentrations. The investigators also found that

no osazone was formed if all the oxidizing agents, including
the adsorbed oxygen, were removed.

Ardagh and Rutherford (8) investigated the effect of pH
on the rates of formation of the phenylhydrazine derivatives.
They found that phosphate buffers were approximately ten times
more effective in catalyzing the formation of the compounds of
phenylhydrazine than the acetate buffers. The value $[\alpha]_D^{22.5} =$
-52.55° was reported for the equilibrium value for phenyl-
hydrazine derivatives of glucose in aqueous solution 0.200
molar.

Butler and Cretcher (22) attempted to study the hydrazine
derivatives of some uronic acids condensed with sugars. As a
preliminary study they investigated the reaction of phenyl-
hydrazine with some simple sugars. The investigators had
considerable difficulty in obtaining the pure isomers by any
of the published methods; so they made a study of the forma-
tion of glucosazone from glucose and fructose and from the
phenylosazones under conditions not ordinarily favorable to
such reactions. A 63 per cent yield of glucosazone was ob-
tained from an attempt to obtain the $\underline{\alpha}$-phenylhydrazide
described by Behrend. They obtained a yield of 60 per cent of
the phenylhydrazine derivative described by Fischer as melting
140-150° C. By precipitation of the mother liquor with ether,
Skraup's phenylhydrazone, melting at 111-112° C., was obtained.
A modification of the procedure was used but only the Skraup
phenylhydrazone was obtained. No mention was made of the fact

that Behrend identified this compound with the 106-107° melting compound that contained 2 molecules of glucose phenylhydrazine derivative with one molecule of phenylhydrazine. The formation of glucosazone from the phenylhydrazone was expressed by the equation

$$3 \begin{array}{l} CH=NNHC_6H_5 \\ | \\ (CHOH)_4 \\ | \\ CH_2OH \end{array} + H_2O \longrightarrow \begin{array}{l} CH=NNHC_6H_5 \\ | \\ C=NNHC_6H_5 \\ | \\ (CHOH)_3 \\ | \\ CH_2OH \end{array} + 2\ C_6H_{12}O_6 + C_6H_5NH_2 + NH_3$$
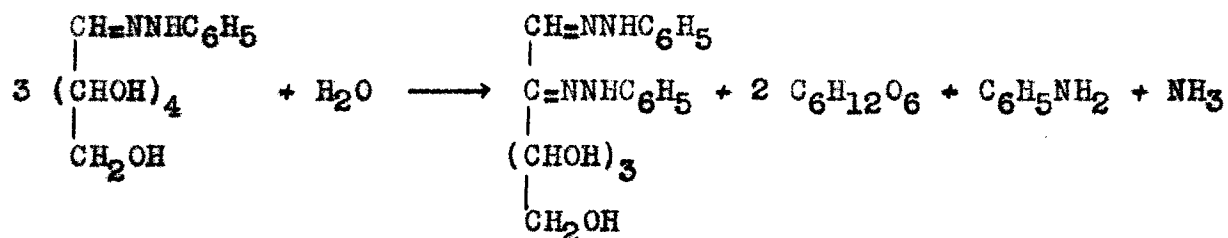
Acetic acid was necessary in order for this reaction to occur.

Stempel (109) gave a method for preparing pure α-d-glucosyl phenylhydrazide of Behrend by slightly decreasing the amount of phenylhydrazine used and stirring vigorously. This material was purified either by repeated washing with acetone or by recrystallization from hot absolute alcohol three times. Cooling below 20° resulted in the precipitation of the Skraup hydrazone.

Knecht and Thompson (69) made a quantitative study of the interaction of glucose and phenylhydrazine. Fischer had obtained a 45 per cent yield of osazone, and Ost (91) had obtained a yield of 50 per cent from hydrolyzed cellulose. Using the amounts represented in the equation

$$C_6H_{12}O_6 + 3\ NH_2NHC_6H_5 \longrightarrow C_6H_{10}O_4(NNHC_6H_5)_2 + C_6H_5NH_2 + NH_3 + 2H_2O$$
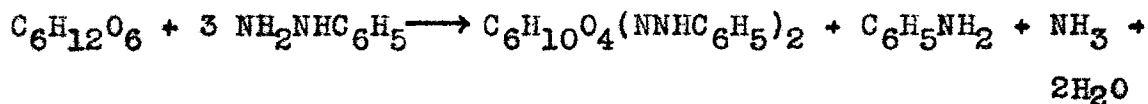
Knecht and Thompson obtained only a 50 to 65 per cent yield.

From the analytical data obtained on the phenylosazones, it
appeared that the equation generally used was justified and
that the sugar that was not converted into the osazone was
present mostly as the phenylhydrazine derivative.

Weygand (126) prepared glucosazone from the aromatic
amine glucosides that had undergone the Amadori rearrangement
and obtained yields up to 95 per cent of the theoretical value.
On the basis of the Amadori rearrangement he suggested a new
mechanism for the osazone reaction. Since phenylhydrazine
itself was known to be a strong reducing agent, it was
thought strange that the CHOH group adjacent to the aldehyde
group in the monosaccharide could reduce phenylhydrazine to
ammonia and aniline, when a strong reducing agent like
titanium trichloride had no effect upon the phenylhydrazine.
p-Tolyl-d-isoglucosamine reacted with phenylhydrazone to give
the following reaction, although the intermediate products
were not isolated:

$$
\begin{array}{ccc}
\overset{\text{H}}{CH_3C_6H_4-N-CH_2} & & CH_3C_6H_4-NH-CH_2 \\
\quad HOC & & \quad C=NNHC_6H_5 \\
\quad HOCH & & \quad HOCH \\
\quad HCOH \quad O \;+\; NH_2NHC_6H_5 \longrightarrow & & \quad HCOH \\
\quad CH & & \quad HCOH \\
\quad CH_2OH & & \quad CH_2OH \\
\;\;\;\; I & & \;\;\;\; II^{*}
\end{array}
$$

---
*Compounds not isolated.

$CH_3C_6H_4-NH-CH$
‖
$C-NHNHC_6H_5$
|
$HOCH$
|
$HCOH$
|
$HCOH$
|
CH_2OH

III*

$\longrightarrow$

$CH_3C_6H_4-N=CH$
|
$C=NH$
|
$HOCH$ + $C_6H_5NH_2$
|
$HCOH$
|
$HCOH$
|
CH_2OH

IV*

IV + 2 $C_6H_5NHNH_2$ $\longrightarrow$

$CH=NNHC_6H_5$
|
$CH=NNHC_6H_5$ + $CH_3C_6H_4NH_2$ + NH_3
|
$HOCH$
|
$HCOH$
|
$HCOH$
|
CH_2OH

V
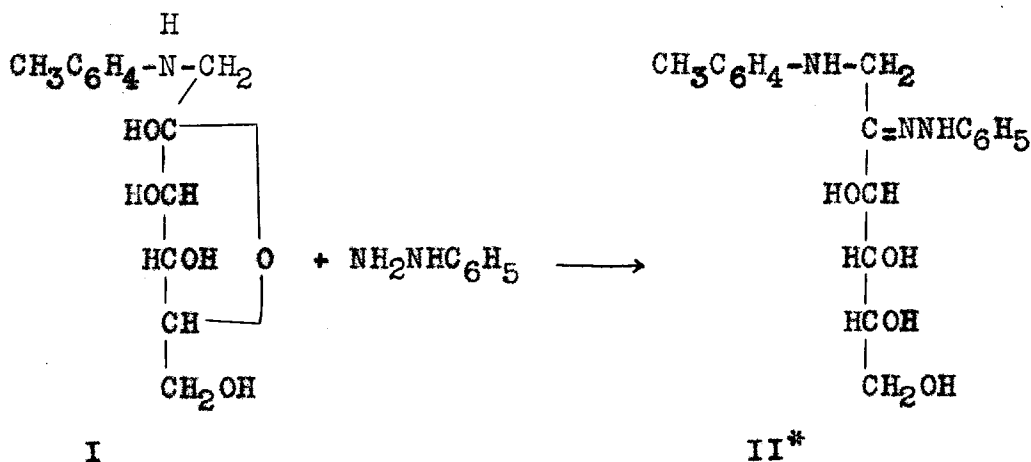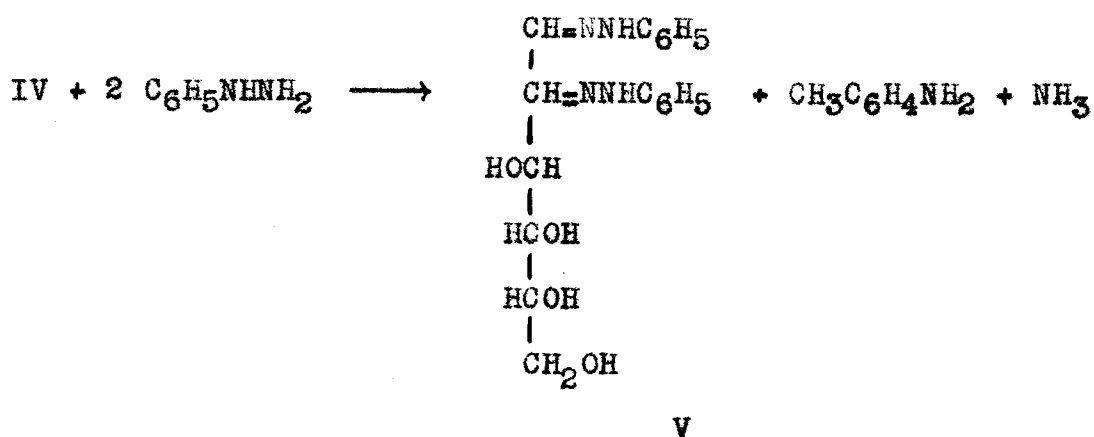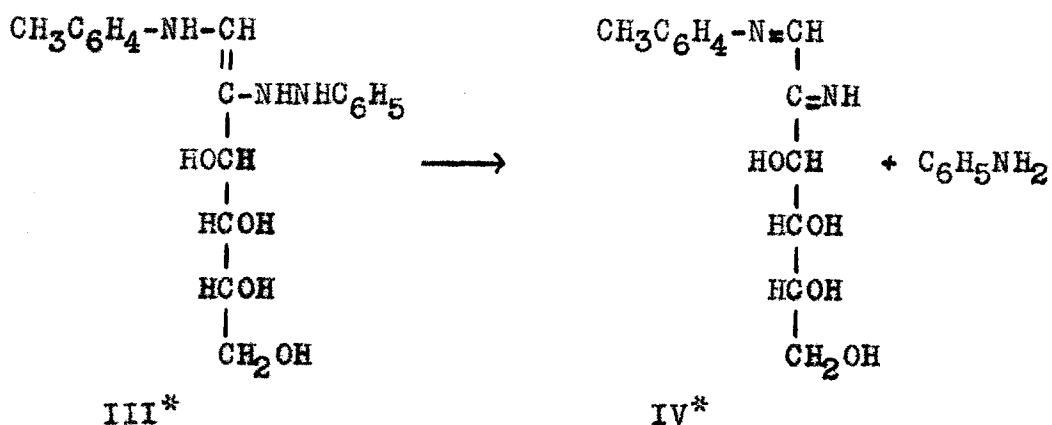
The reaction occurred only in weakly acid solution. This condition did not appear unusual since phenylhydrazine mixed with phenylhydrazine hydrochloride decomposed into aniline, ammonia, benzene, and nitrogen at 165° C., but the free base was stable to 300° C. Likewise o-amino-β-benzylphenylhydrazine hydrochloride was very unstable. It was postulated that the intermediate compound containing ethylene linkage in the above diagram should lose two hydrogen atoms readily and simultaneously the phenylhydrazine would be reduced to aniline and ammonia. Two diagrams were given for the explanation of

*Compounds not isolated.

the mechanism of osazone formation; in one, an intermediate imino-aldehyde was formed; in the other, an Amadori shift was postulated for the hydrazide followed by a reaction of the ketone group with phenylhydrazine, and the subsequent splitting out of aniline to form an imino group. This group reacted with another molecule of phenylhydrazine to form the osazone. The second series of reactions was favored since it appeared that the Amadori rearrangement was the limiting factor in the yield of the osazone. Another point in favor of the latter mechanism was the failure of mannose to form the osazone. It was very difficult to make the mannose derivatives undergo the Amadori rearrangement. The existence of more than one isomer of phenylhydrazone of glucose also supported this mechanism, although none of the existing isomers have been identified as the rearranged glucosidic form.

Hydroxylamine Derivatives of Glucose

Only one isomer of glucose oxime, the glucosidic structure, has been reported in the literature. Evidence for the establishment of the ring structure has been reviewed by Wolfrom and Thompson (132). They have also prepared the pentaacetate and the hexaacetate of the aldehydo-structure of the oxime from pentaacetyl-aldehydo-glucose and hydroxylamine. The pentaacetyl compound on further acetylation gave the hexaacetyl compound that had different physical constants from

the hexaacetate that Wohl had obtained from the mild acetyla-
tion of glucose oxime. Wohl considered the compound he pre-
pared the anti form of glucose oxime, but on further study it
was proved to be the β-glucose oxime hexaacetate.

Glucose oxime on vigorous acetylation also yielded some
of the straight chain hexaacetate.

Further proof of the acyclic and cyclic structures of
the oxime acetates was offered by estimation of the O-acetyl
and N-acetyl groups of the oxime hexaacetates (131).

Aliphatic Amine Derivatives of Glucose

In order to determine the correct structure for 1-amino-
glucose, Irvine, Thomson and Garrett (51) studied the reaction
of some alkyl amines with glucose.

The four structures that were possible for 1-aminoglucose
from data available at that time were the following:

$$CH_2OH.CHOH.CHOH.CHOH.CH-CHOH \qquad I$$
$$NH$$

$$CH_2OH.CHOH.CH.CHOH.CHOH.CHOH \qquad II$$
$$NH$$

$$CH_2OH.CHOH.CH.CHOH.CHOH.CHNH_2 \qquad III$$
$$O$$

$$CH_2OH.CHOH.CHOH.CHOH.CHOH.CH=NH \qquad IV$$
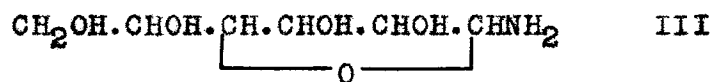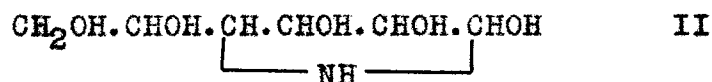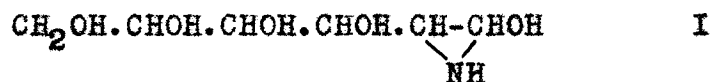
It was evident that the only one of these formulae that
could account for the formation of compound from glucose and

a secondary aliphatic amine was structure III. Glucose
reacted with ethylamine, diethylamine, and dimethylamine with
difficulty, and only in the first case were the authors able
to obtain a crystalline product. Glucose and ethylamine were
shaken together in an ethanol solution and the reaction was
allowed to continue at room temperature for a period of three
months. After removal of the solvent, studies of the rotation
and solubility showed that about thirty per cent of the glucose
was unaltered. A crystalline product was obtained, however,
by mixing the glucose with the amine in a 1:3 ratio and seed-
ing the mixture with a bit of the glucosylethylamine already
obtained. No physical constants were obtained for the com-
pound from the reaction of dimethylamine and glucose, although
evidence for the occurrence of the reaction was unquestionable.
On analysis it was found that the sirupy mass had the correct
nitrogen content. With dimethylamine the same difficulty was
encountered although a few crystals were deposited in the
sirupy residue. With triethylamine there was no evidence of
compound formation, and no change in rotatory power was ob-
served after a considerable lapse of time. Negative results
were likewise obtained with diphenylamine, methylaniline, and
acetanilide.

In 1934 Votoček and Valentin (118,119) began an investi-
gation using some aliphatic amines and the sugars. These
workers were interested in the reaction of rhamnose with
ammonia and acetoacetic ester to form "rhamnodiazine" with the

concurrent elimination of three molecules of water. They
attempted to carry out the reaction using a sugar with an
alkylamine and a β-diketone in order to obtain a compound
analogous to the "rhamnodiazine." The compound obtained had
properties that were quite different from those of the com-
pounds expected and upon further experimentation the same com-
pound was obtained when the reaction was run with the β-dike-
tone omitted. The compound was a condensation of the alkyl-
amine and the sugar. This reaction was studied by reacting
a series of the alkylamines from methylamine through heptyl-
amine with some monosaccharides. The compounds obtained
were thought to be N-glucosides similar to the compounds re-
sulting from the condensation of aromatic amines and sugars.
However, in all cases it was not necessary that a molecule of
water was removed, as in the glucosylarylamines, and often
the compounds appeared with water of crystallization. This
water of crystallization could not be removed by heating since
the compounds decomposed at low temperatures but the anhydrous
compound sometimes could be made by preparation in a different
solvent. In one case when the condensation was carried out
in methyl alcohol, a molecule of methyl alcohol of crystalli-
zation was attached to the compound.

It was observed that the rotation of the compounds
changed with temperature, and that in aqueous solution the
rotation slowly changed and finally reached a constant value.
This change was attributed to mutarotation since the value

did not reach the value of sugar and since the investigators believed that the hydrolysis of these compounds was slower than that of the ammonia derivatives prepared by Lobry de Bruyn. The amines were easily displaced by phenylhydrazine and its derivatives. Whether this was the result of difference in solubility of the compounds formed from the sugar, one was unable to deduce since the solvent in which the reaction occurred was not given.

As the compounds were readily hydrolyzed by acid and even in water solution, they gave the usual reactions of the free sugars. It was suggested that these compounds could be used for the separation and identification of the sugars, especially for sugars containing impurities that were so often found in plant chemistry. The authors predicted that the compounds would add hydrogen cyanide which upon hydrolysis should form N-substituted α-aminohydroxy acids. They suggested that substituted glucamines could be prepared from the glucosylalkylamines by reduction.

The adding of hydrogen cyanide to the alkylamine glucosides was accomplished by Votoček and Lukeš (116,117). Miller and Plöchl (86) had reacted hydrocyanic acid with the oximes, hydrazones, and anilides of the sugar. The present investigators prepared the nitriles of the simple glucosylamines and obtained the α-amino substituted glucoheptanoic acids. Later an attempt was made to prepare the aryl substituted acids (121). The nitriles were prepared, but upon hydrolysis

the arylamine was removed and an odor of phenylisocyanide was observed. The nitriles of the arylamines were simply prepared by adding hydrogen cyanide to the condensation product without further purification.

These nitriles as well as the nitrile made from the condensation product with piperidine were readily methylated, acetylated and benzoylated. These compounds with all of the active hydrogen atoms removed were very soluble in ether and reacted with the Grignard reagent, thus opening an entirely new field of organic synthesis.

Karrer and his co-workers (65,66,67,68) had developed a method for preparation of glucamines in the synthesis of vitamin B_2. This is discussed in the review of literature concerning the glucosylarylamines. They applied this method to methyl- and ethylamine (61,65). With ethylamine, they reacted glucose in a methyl alcoholic solution of the amine, and, after refluxing, removed the solvent with suction, washed the residue with ether, and dissolved the product in methanol. On hydrogenation under 20-25 atmospheres of pressure at 100° C. with nickel or palladium as a catalyst, N-ethyl-d-glucamine was obtained and was isolated as the hydrochloride. They prepared N-methyl-d-mannamine and di-l-arabitylamine.

Amide Derivatives of Glucose

No direct condensations have been reported from the amides and glucose except the condensation of urea with glucose (100,44). Fischer (34) prepared silver succinimide and reacted acetobromoglucose with it. Silver bromide was split out, and the resulting tetraacetate of the glucoside of succinimide was hydrolyzed with ammonia in methyl alcohol to prepare the glucoside of succinamide. A similar method was used to prepare the glucoside of saccharine and of benzene sulfamic acid (59). The condensations of acetobromoglucose with the silver salt of some alkaloids (30,44) has also been reported. With urea a condensation occurred by leaving six grams of glucose in six milliliters of warm water with four grams urea and four drops of concentrated hydrochloric acid. The molecular compound $\underline{d}$-glucose urea.$CO(NH_2)_2$ which separated at first was converted into $\underline{d}$-glucosyl urea by treatment with 75 per cent alcohol.

Aromatic Amine Derivatives of Glucose

Schiff (95,96) first prepared an anilide of glucose during his investigation on the derivatives of salicin. No crystalline product was obtained, and when the solution was heated, a dark residue was formed that produced aniline and water upon distillation of the mixture. From analysis it was

determined that the glucosanilide was composed of one molecule of glucose and one molecule of aniline with a molecule of water removed; so a Schiff's base type of compound was proposed.

Sorokin (106,107) prepared the anilide and o- and p-toluidide of glucose by heating glucose with an excess of the base in alcoholic solution until the glucose completely dissolved. On evaporation of the solvent the glucosylamine precipitated. The precipitate was recrystallized from alcohol and an 80 per cent yield was obtained. The anilide decomposed easily by heating above its melting point of 140° C. On heating of the anilide with concentrated hydrochloric acid, a salt of levulinic acid was recovered, but with dilute acid the anilide was split into aniline and glucose.

Strauss (114) supported the double-bonded or Schiff's base type of condensation, since hydrogen cyanide added to the anilide, but Sorokin upheld the ring or glucosidic structure of the compound. Irvine and Gilmour (52) studied the constitution of the anilide, oxime, and phenylhydrazone of glucose. The study was made by preparation of the tetramethylglucose derivatives of aniline, hydroxylamine, and phenylhydrazine.

The anilide of tetramethylglucose was a nicely crystalline solid that precipitated from alcoholic solution and showed no mutarotation in acetone. On further methylation with silver oxide and methyl iodide the methoxy content could

not be increased; so it was concluded that the anilide could not possess a straight chain aldehyde form, since the compound possessing that structure contained a free hydroxyl group that should be easily methylated. The tetramethyl derivative of phenylhydrazine could not be crystallized, and no significant results were obtained on further methylation. From evidence of a study of the condensation in the cold in an ethanol solution, Irvine and Gilmour reported that two stereoisomers of the anilide existed. The oxide constitution of the glucosanilide was further verified by methylation of glucosanilide. The compound obtained possessed the same constants as the compound prepared from condensation of tetramethylglucose and aniline. The dextrorotatory compound that was initially formed was the less soluble compound and changed from the dextro into the levo form. Hydrochloric acid greatly accelerated the mutarotation. Aqueous solutions of glucosanilide underwent mutarotation, but no end value was recorded because hydrolysis occurred.

The p-toluidide, p-phenetide, β-naphthylamide, and o-carboxyanilide of glucose were prepared (53). Three crystalline forms of the toluidide were obtained, the form depending upon the amount of water of crystallization present. The anhydrous form was prepared by Sorokin's method, and subsequent crystallization was carried out by boiling with ethyl acetate. The alcoholic solution was strangely dextrorotatory, but the mutarotation was very rapid, especially when the

reaction was catalyzed with a trace of acid. There was likewise evidence that the β-naphthylamine and o-carboxyaniline condensation products existed in both the α- and β-forms, though only one form was isolated in each instance.

The mutarotation of the compounds of aniline and o-carboxyaniline (55,56) with the different sugars and their tetramethyl derivatives was studied in pyridine, ethanol, methanol, and 90 per cent alcohol. Results much the same as those reported for glucosanilide were obtained. The mutarotation of the oxime and ammonia derivatives of glucose was followed and shown to be slight (54).

Cameron (23,26) reported the action of aniline and acetic acid on glucose. The reaction between the amine and the glucose was catalyzed by the acid. In alcoholic solution a brown coloration occurred, but in aqueous solution the colored material precipitated. Glucosanilide likewise gave a brown coloration in aqueous or alcoholic solution if acid were present. The failure to reduce Fehling's solution after long standing was evidence of the absence of glucose and glucosanilide. The effect of acid in speeding up the reaction was observed; in fact, the maximum value of rotation in the acid solution was reached in four hours, while in aniline-glucose alone, the maximum was not observed for 22 days.

In a later communication (24) the action of o- and p-toluidide on glucose in the presence of acetic acid and in the presence of potassium dihydrogen phosphate was reported.

The solutions, on becoming dark, were more reactive; they were more easily oxidized and gave a positive test with Seliwanoff's reagent. The author attempted to prove that the aldehyde structure was formed from the more stable ring structure of Irvine and Hynd. In order to do this he tested a solution of methylaniline, glacial acetic acid and glucose, and observed that the rates of coloring were considerably slower than in a solution of aniline, glacial acetic acid, and glucose. No conclusions could be drawn, since the reaction between glucose and methyl aniline itself was very slow at room temperature. Benzylamine also gave colored products with glucose and acetic acid. The authors followed the change in rotation of glucose and aniline in aqueous solution in the presence of acetic acid and potassium dihydrogen phosphate, and in both cases the rotation of the solution became negative. The value became more negative when the reaction was effected in the presence of the potassium dihydrogen phosphate than in the presence of acetic acid.

It was found (25) that glucose and benzylamine reacted in water solution to form glucosylbenzylamine. When a molecular proportion of acetic acid was present, no condensation occurred. This was expected since 0.5 gram of glucose benzylamide hydrolyzed almost immediately when a monomolecular proportion of acetic acid was added. If only a small amount of acid were added, a coloration was developed slowly. In methyl alcoholic solution in the presence of acetic acid a

color developed and a quinone-like odor was noticed. Methyl glyoxal was recovered from the solution in small yield and characterized as the osazone. An explanation for the enhanced reactivity of the solution was postulated. This activity was attributed to the formation of the aldehyde isomer that enolized or decomposed to form methylglyoxal. In case of the enol structure, the formation of certain ring nitrogen compounds such as the pyrazines was possible by the condensation of two molecules of the enol and the elimination of two molecules of water. It was known that 2,5-ditetroxybutylpyrazine (112) was formed from fructose and ammonia in alcoholic solution.

Baker (11) studied the mechanism of mutarotation in relation to ring-chain tautomerism. The anilide, p-toluidide, p-anisidide, p-bromoanilide, p-chloroanilide, and the N-methylanilide of tetraacetylglucose were prepared by dissolving the tetraacetylglucose in the amine or in a concentrated ether solution of amine and leaving the solution at room temperature for several hours. On dilution with ether the hydrobromide of the base separated. On concentration of the solution, the residue crystallized, and the products were recrystallized from suitable solvents. The N-methylanilide was the only compound that did not show mutarotation in ethyl acetate with an acid catalyst. Even on fusion no mutarotation occurred. This was given as evidence that the attack of the acid was not merely a coordination of a proton to give an

ammonium complex that then caused mutarotation, since the secondary amine should act fundamentally in the same way.

As it was not possible to study the effect of alkaline catalysts because of the ease of hydrolysis of the acetyl groups from the anilide of tetraacetylglucose, an investigation (10) was carried out on the nitrogen derivatives of tetramethylglucose. The methoxy group was much more resistant to the action of acid and alkali catalysts, so that the effect of both kinds of catalyst on the relative velocity of mutarotation was studied in a series of p-substituted anilides of tetramethylglucose. The anilides of the sugars were prepared by refluxing for several hours a mixture of tetramethylglucose with an excess of the base in alcoholic solution.

In order to test the possibility that various secondary amines that were stronger bases than methylaniline might undergo mutarotation due to an ammonium coordination complex, tetraacetylglucosyl derivatives of piperidine, dimethylamine, diethylamine, and the p-substituted benzylmethylamines were prepared (12). These compounds were isolated as the hydrochlorides. On studying these tetraacetylglucosyldialkylamines polarimetrically, a change in rotation was observed, but this change was attributed to a slow fission of the hydrochlorides into tetraacetylglucose and the hydrochloride of the base rather than to mutarotation. When the free N-glucosides were dissolved in anhydrous ethyl acetate in the presence of a little hydrochloric acid as a catalyst, the slow

crystallization of the amine hydrochloride occurred in the polarimeter tube.

In order to prepare these compounds the reaction was carried out in the cold; the amine hydrobromide crystallized from the solution, and the tetraacetylglucosyldialkylamine was crystallized from the concentrated ethereal extract. The dimethyl and diethylamine tetraacetylglucosides were isolated only in form of their hydrochlorides and these in very small yields. The piperidine glucoside was prepared in two modifications, one of which is quite insoluble in the ether solution. Conversion of one form to the other was not accomplished. The value of k was given but no data concerning the specific rotation of the compounds were recorded.

Perhaps the most valuable of all the research carried on about this time was the isolation of two compounds from the condensation of glucose and p-phenetidine (3). If the condensation were carried out in 83 per cent ethanol, the compound that melted at 118° was obtained. If the reactants were heated together without a solvent and the alcohol added after the solution became homogeneous, another compound was precipitated. This compound, whose melting point was 155° C., had been reported earlier (45). There was a decided difference in the stability of the two isomers (5). In a study of the constitution of these two isomers, since neither compound contained an NH_2 group, the structure of three pairs of isomers was postulated; the N-glucosidic structure, the

double-bonded straight-chain aldehyde condensation type of compound, and the N-ring type of compound. For the isomer made in ethanol solution, it was observed that dilute acid caused the decomposition of the compound into its component parts. The other isomer was stable in acid solution, but decomposed into glucose and p-phenetidine in alkaline solution. The compound that decomposed with acids was l-rotatory and exhibited mutarotation, a fact that supported the structure of the glucosidic linkage. However, the acid stable compound was also l-rotatory and showed mutarotation, but as it was alkali labile, it was believed at that time that this form was the Schiff's base type of compound or an imino-ring structure, both of which exhibit syn-anti isomerism. Two isomers (4) were isolated from the p-anisidine and glucose condensation. They showed the same relative stability to acids and bases as the p-phenetidine compounds demonstrated. The same type of reaction was demonstrated with p-toluidine (6) and later with o-phenetidine, o-anisidine, and o-toluidine (7).

From an entirely different field of investigation came further experimentation which helped elucidate the molecular rearrangement of the N-glucosides. In the synthesis of vitamin B_2, lactoflavin, 1,2-dimethyl-4-amino-5-(d-1'-ribityl-amino)-benzene was condensed with alloxan. Ströble (73) heated 1,2-dimethyl-4-nitro-5-amino-benzene with d-ribose in an alcoholic solution and hydrogenated the condensation product. This whole procedure occurred in only two steps.

On separation of the products by chromatographic adsorption,
he found that the condensation of the substituted o-nitro-
aniline with the pentose was an equilibrium reaction, and by
the recondensation of the reactants that had not undergone
condensation, a 75 per cent yield was obtained. This began a
new series of investigations on the N-glucosides of the sugars
and the so-called "Amadori Rearrangement." The stable isomer
of p-phenitidine that was not hydrolyzed by acid was consid-
ered a Schiff's base compound by Amadori. Kuhn and Dansi
(72) pointed out that the question as to the formation of a
double-bonded linkage of this sort between aromatic amines
and sugars had not been settled, and no proof for the exist-
ence of such a substance had been brought forth. The authors
gave evidence that the acid-stable "Schiff's base" compound
was not a Schiff's base at all but a product produced from
molecular rearrangement of the sugar chain. That the acid-
labile compound was in reality a N-glucoside as Amadori had
suggested was verified. The acid-labile compound of
p-toluidine and d-glucose was acetylated with pyridine and
acetic anhydride, and the compound that resulted was identical
with the compound formed from acetobromoglucose and p-tolui-
dine (54). The constitution of this glucoside was also sub-
stantiated by similar studies with the tetramethylglucose and
the methylated N-glucoside. The acid-stable compound was not
methylated, since the compound was alkali labile, but a tetra-
benzoyl derivative was produced which at once eliminated a

Schiff's base structure. The stable isomer upon hydrogenation gave N-p-tolylglucamine.

The products obtained on oxidation of the two forms with chromic acid were noteworthy. It was at first reported that twice as much acetic acid was obtained on oxidation of the rearranged product than by oxidation of the known N-glucoside. The isomers yielded two different saccharin acids, and it seemed possible that the same sort of rearrangement had occurred as is found in the saccharin acids.

Kuhn and Weygand (76) investigated the Amadori rearrangement still further and found that the stable compound gave also 0.6 mols of acetic acid; so there was no relationship between this rearrangement and the saccharin acid rearrangement. The rearranged p-toluidine-d-glucoside on reduction gave N-p-tolyl-d-mannamine. This compound was also obtained by reducing the normal N-glycoside prepared by the condensation of d-mannose and p-toluidine in alcoholic solution in the presence of ammonium chloride. The normal N-toluidine-d-glucoside upon reduction gave N-p-tolyl-d-glucamine. The rearranged product was a very good reducing agent and was comparable to the action of ascorbic acid. It reduced o-dinitrobenzene in dilute alcohol to o-nitrophenylhydroxylamine.

The rearranged or "stable" p-toluidine-d-glucoside reacted with hydroxylamine to form an oxime. This showed that there must have been a rearrangement from the glucosidic

form to an isoglucosamine form. The mechanism suggested was
given as a diagram on page 4. Compound I from the diagram
(p. 4) upon reduction gave

$$
\begin{array}{c}
\text{H H} \\
| \ | \\
CH_3C_6H_4N-CH \\
| \\
HC-OH \\
| \\
HOCH \\
| \\
HCOH \\
| \\
HCOH \\
| \\
HCOH \\
| \\
H
\end{array}
$$
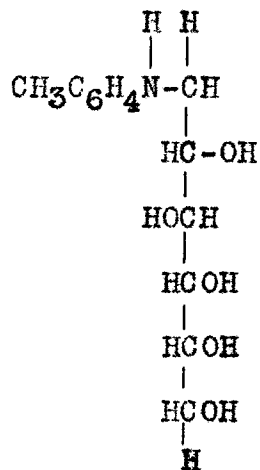
N-p-tolyl-d-glucamine

but reduction of compound VI gave

$$
\begin{array}{c}
\text{H H} \\
| \ | \\
CH_3C_6H_4N-CH \\
| \\
HOCH \\
| \\
HOCH \\
| \\
HCOH \\
| \\
HCOH \\
| \\
CH_2OH
\end{array}
$$
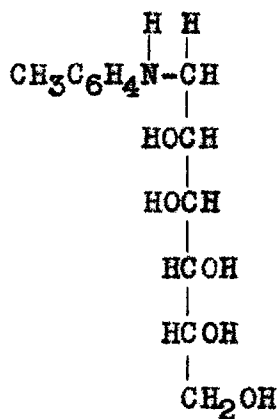
N-p-tolyl-d-mannamine

The rearranged glucoside showed a rapid mutarotation in
pyridine solution. Karrer (64,68) reduced a product that he
designated 6,7-dimethyl-9-d-araboflavin, prepared by the

condensation of the amine and arabinose. The product was not isolated, and upon reduction it was found that rearrangement had occurred and the d-ribityl compound rather than the d-arabityl compound was obtained. 2-Nitro-4,5-dimethylaniline was easily condensed with l-arabinose and d-ribose in alcoholic solution. Of the six possible structures for the simple condensation product (the α- and β-pyrano- or furano-glucosides or the Schiff's base syn and anti isomers), the Schiff's base was eliminated because a triacetyl compound rather than a tetraacetyl compound was obtained on acetylation. That the pentosides possessed a furanoid structure was shown by the formation of a trityl compound on carbon atom five.

o-Nitroaniline (74) was found to be much harder to condense than the p-substituted aniline. A condensation was finally effected by the use of a very large excess of the amine. It was found that the condensation occurred easily in the presence of some ammonium acetate, and that the amount of water present in the alcohol influenced the condensation. Both the α- and β-glucoside of 2-nitro-4,5-dimethyl-aniline-d-glucose were obtained and the acetates were made from these by direct acetylation.

It has long been known that alkalies react to produce a decomposition of sugars. In fact, traces of alkali cause caramelization. When an organic base (115) acted on a sugar, an enol form was produced. This compound was a much more active form of the sugar and acted much the same as ascorbic

acid. The method used for testing the presence of the enol
group was reduction of methylene blue or dichlorophenol-
indophenol, a reagent used in the quantitative determination
of vitamin C.

The tetraacetate of piperidine glucoside was very un-
stable in the presence of acids or bases, but did not affect
the oxidizing agents such as methylene blue and 2,6-dichloro-
phenol-indophenol. An excess of piperidine, when heated with
glucose, gave a solution that reduced methylene blue and
dichlorophenol-indophenol. The reducing property of this
solution was due to some rearrangement of the glucose. Since
repeated precipitations could not free the material from
piperidine, the compound formation as the cause of the reduc-
ing property seemed more plausible. Vogel postulated an enol
formation to explain this ease of oxidation:
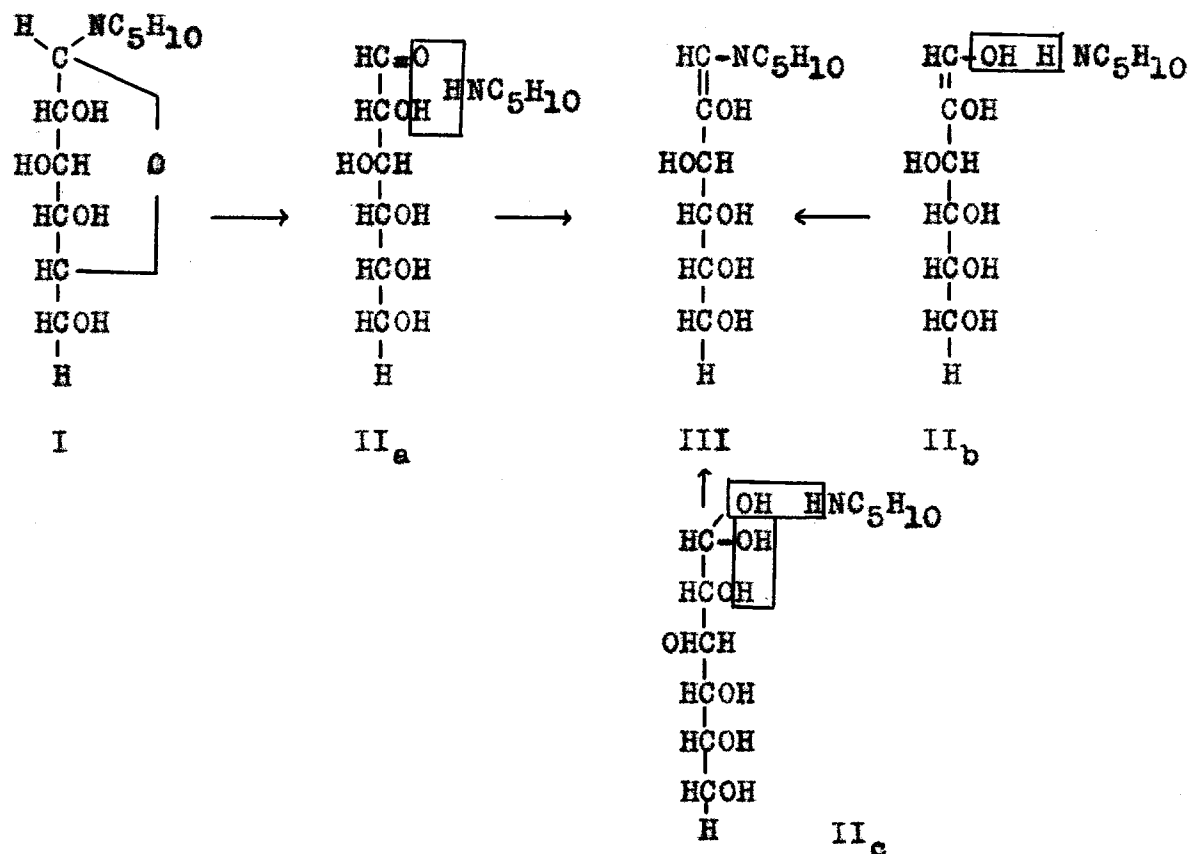
Many other sugars such as mannose, fructose, galactose, and maltose showed this reaction with piperidine. Heating of the water-free components formed a compound that was not cleavable unless it was changed into the active form. In an aqueous solution the dienol was formed; then the 1,2-dienol (enediol) was split into piperidine and 1,2-dienol-glucose-1,5-anhydride.

If piperidine were heated with 1/10 its amount of glucose and then precipitated at once by ether-petroleum ether, a colorless syrup was obtained. This syrup in aqueous solution decolorized dichlorophenol-indophenol rapidly. If acid or alkali were added, the color was no longer destroyed, and even acidification before addition of the oxidizing agent gave no decolorization.

If instead of precipitation the solutions were acidified, they possessed strong reduction property. If cooled first, diluted, then acidified, the reducing value obtained was four times as large as it was in the concentrated solution.

Vogel reported that the acetyl groups from carbon 1 and 2 were removed upon treatment of tetraacetylglucose with piperidine and that the corresponding dienol was formed. The pentaacetylglucose did not form a dienol and it appeared from this that a 1,5- and 2,5-ring structure were concerned with the dienol formation.

Kuhn and Birkofer (71) investigated the action of piperidine on glucose in order to correlate the phenomenon

of mutarotation with the ease of reduction of the glucosides of the secondary amines. Mutarotation, according to the theory of ring-double-bond desmotropy, was caused by a change of the glucosidic form into the double-bonded compound. The mutarotation of the glucosides of the secondary amines offered an anomaly to which it was difficult to give credence. Karrer (62) had discussed the mechanism of reduction of the N-glucosides to N-aryl-$\underline{d}$-glucamines, and since he was unable to reduce the N-methylanilide of tetraacetylglucose, he pointed out that this substantiated the theory that a Schiff's base type of compound was a necessary intermediate for reduction. Kuhn and Birkofer disproved this hypothesis as such by reducing the glucoside of piperidine and benzylamine. These secondary amine glucosides mutarotated slowly in pyridine, but the rate of mutarotation was greatly accelerated by the addition of a small amount of water or hydrochloric acid. The conclusion was drawn that the rearrangement was really concerned with cation formation. The scheme for hydrogenation was represented as follows:

R
R—N   H
 \C/
  |
  R₁   O
  |
 HC——
  |
  R₂

I

R
R—N
  \CH₂
   |
   R₁
   |
  HCOH
   |
   R₂

IIIₐ

R
H   N—R
 \C/
  |
  R₁   O
  |
 HC——
  |
  R₂

V

$-H_2O \uparrow\downarrow +H_2O$ Ni $\uparrow$ $-H_2O$ $-H_2O \uparrow\downarrow +H_2O$
                                      H₂

R H OH⁻
 \ | /
R—N⁺
   \C/
    |
    R₁   O
    |
   HC——
    |
    R₂

II

$\longleftrightarrow$

R   OH⁻
 \   +/
R—N
    \\CH
     |
     R₁
     |
    HCOH
     |
     R₂

III

$\longleftrightarrow$

OH⁻   H
       | R
    H +N—R
     \ /
      C
      |
      R₁   O
      |
     HC——
      |
      R₂

IV
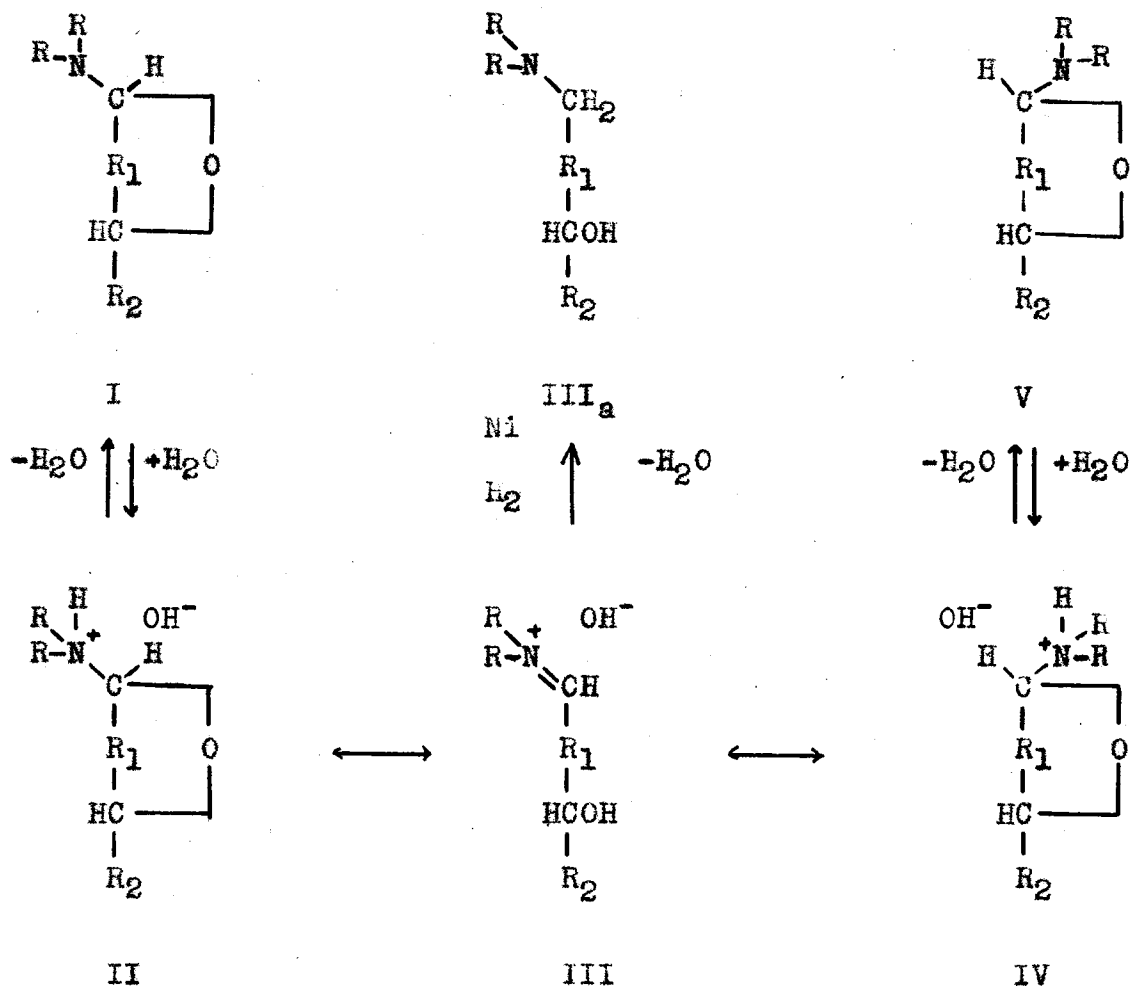
Mutarotation and Reduction

Kuhn and Birkofer (70) condensed the aromatic amines
with a number of pentoses and hexoses and listed the optimum
conditions for the different condensations. Some of the con-
densations proceeded easily in alcoholic solution while
others required a trace of hydrochloric acid or ammonium
chloride to catalyze the reaction. The investigators were
unable to isolate a Schiff's base type of compound or to
offer any proof of its presence. The rearranged compounds

reduced o-dinitrobenzene, 2,6-dichlorophenol-indophenol and methylene blue, but not quantitatively. Weygand (123) found that the N-glucosides formed in concentrated aqueous solution, and that the N-glucosides that were formed were stable in the presence of a bit of alkali but decomposed and browned rapidly in the presence of acid.

The rearrangement (125) to the isoglucosamine structure was greatly catalyzed by the presence of a small amount of hydrochloric acid. If too large an amount of acid were added, the N-glucoside was cleaved, although a very small amount of acid greatly accelerated the velocity of formation of the compound.

When the rearranged compounds were reduced catalytically, various products were obtained. When one mol of the compound in alcoholic solution was reduced in the presence of two mols of acid, the aromatic kernel was reduced but the ketone group remained intact. In neutral solution no prediction could be made concerning the products of reduction. Sometimes the benzene ring was reduced, sometimes the ketone group, and sometimes there was complete reduction. In the presence of 2.8 mols of alkali, only the keto group was reduced.

In view of these experiments and because of the different isomers obtained on reduction, it was concluded that the rearrangement and subsequent reduction proceeded only in the presence of an acid or salt. In view of this concept, the mechanism of the rearrangement was modified in the following

manner:

CH$_3$	CH$_3$	CH$_3$	CH$_3$
C$_6$H$_4$	C$_6$H$_4$	C$_6$H$_4$	C$_6$H$_4$
N$^+$H$_2$ X$^-$	NH$^+$ X$^-$	N$^+$H$_2$ X$^-$	NH$_2^+$ X$^-$
HCOH	CH	CH	CH$_2$
HCOH	HCOH	COH	C=O
HOCH	HOCH	HOCH	HOCH
HCOH	HCOH	HCOH	HCOH
HCOH	HCOH	HCOH	HCOH
HCOH	HCOH	HCOH	HCOH
H	H	H	H
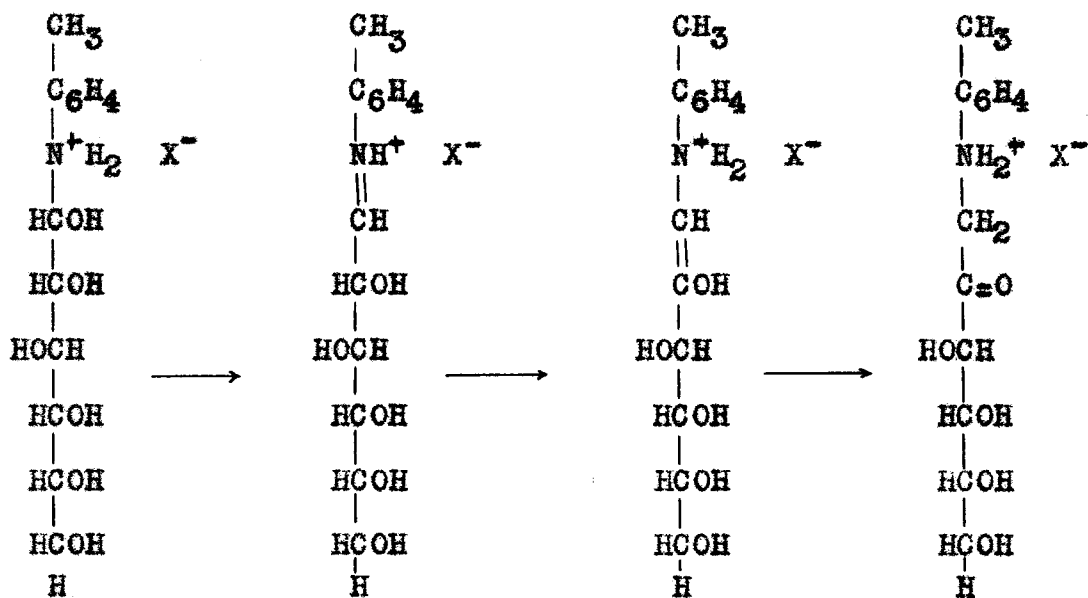
It was not determined whether it is possible to reverse the
equilibrium. Weygand (124) further studied the steric
course in the hydrogenation of the isoglucosamines.

Karrer and his co-workers (66,67) had already studied
the reduction of the aromatic amine condensation products
with pentoses. In a synthesis of flavine, N-monoacetyl-o-
phenylenediamine was reacted with sugar and reduced catalyti-
cally in the presence of nickel. In this method the inter-
mediate condensation product was not isolated. They also
condensed d-2-ribodesose (63) with some aromatic amines and
upon reduction produced the type of compound found in thymus
nucleic acid.

A specific type of aromatic amine condensations was
evidenced in the aromatic diamines. Griess and Harrow (41,
42) reacted some sugars with the aromatic diamines and

obtained the diamine condensation products. However, if the condensation of glucose with phenylenediamine were carried out in acid solution, two different types of compounds were obtained. One of these was a quinoxaline type of compound which reduced Fehling's solution and ammoniacal silver nitrate. The other was a benzimidazole compound that showed entirely negative reactions to those reagents. Ohle (89) attempted to clarify the confusion resulting from this paper and showed that with arabinose and o-phenylenediamine, a benzimidazole instead of a quinoxaline structure was obtained, and also that there were four hydroxyl groups that could be acetylated, thus disproving the keto structure offered by Griess and Harrow.

Adler (1) also made a dicondensation product of the sugars but did not study the compounds formed in acid solution.

Weygand (126) explained the formation of the (d-arabo)-tetraoxybutylquinoxaline in view of the Amadori rearrangement. First the glucoside was formed, and upon the action of acid, the Amadori rearrangement occurred. The compound then underwent a further condensation between the keto group and the o-amino group, whereupon a dihydroquinoxaline compound was formed. Two hydrogen atoms were then obtained to form the quinoxaline compound. It is possible that the sugar was reduced to an alcohol and furnished the two necessary hydrogen atoms as was the case when isobutylaldehyde was condensed

with o-phenylenediamine and isobutanol isolated as a product.

Amine Derivatives of 2-Methylglucose

Hickinbottom (46) prepared 2-methylglucose from methyl 3,4,6-triacetyl-α-glucoside by methylation of the compound by silver oxide and methyl iodide. He then removed the acetyl groups and hydrolyzed the glucosidic linkage. 2-Methylglucose was characterized by the phenylhydrazone that melted at 175-176° C. Brigl and Schinle (20) prepared 2-methylglucose and obtained the phenylhydrazone that contained one methoxyl. By heating 2-methylglucose for one and one-half hours with a solution of phenylhydrazine and acetic acid, glucose phenyl-osazone was obtained. This compound gave no methoxyl group by a micro-Zeisel analysis. For further proof the phenyl-hydrazone containing one methoxyl group was treated with the phenylhydrazine and acetic acid mixture. The glucosazone that resulted gave no value for the methoxy determination. The methoxyl group in the 2- position had evidently been removed during the reaction.

A few years earlier Pacsu (92) had reported the prepara-tion of a monomethylglucose as 4-methylglucose. Levene (79) attempted to prepare the 4-methylglucose and observed that the physical constants for the compound obtained corresponded very well to those that had recently been reported by Brigl and Schinle. Levene prepared the phenylhydrazone that melted

at 176° C. and verified the formation of glucose phenylosa-
zone that gave an entirely negative micromethoxy determina-
tion. The yield he obtained was very small but Brigl and
Schinle (21) obtained 0.08 gram of the phenylosazone from
0.10 gram of the pure hydrazone; so the yield of osazone was
not from glucose in an impure product.

Pacsu (93) published a paper in answer to this correc-
tion. At the time of his original work all the monomethyl-
glucose compounds except the ones in position four and five
on the carbon atom had been established. The compound formed
a phenylosazone; therefore, the substitution of a methyl on
position four was arbitrarily assigned as the structure of
the compound. The analysis corresponded quite closely to the
calculated value for a methylhexosazone, but no methoxy analy-
sis was made. The phenylosazone for the compound that was
believed to be 4,5,6-trimethylglucose on analysis was found
to possess only one methoxyl group. Pacsu believed that a
new investigation of the whole problem should be undertaken.

Schinle (97,98) prepared the Pacsu monomethylglucose by
Pacsu's method and reported that a mixed melting point with
the 2-methylglucose prepared earlier by Brigl and Schinle gave
no depression of the melting point. The phenylhydrazone was
also prepared from both samples and they were proved identical.
The glucose phenylosazone was prepared and the test for the
methoxyl group was negative.

Thus it was well established that in the formation of

the phenylosazone from 2-methylglucose, a methyl group was
removed. If it were necessary that the compound underwent an
Amadori rearrangement before osazone formation as Weygand
suggested, then the methyl group was necessarily removed in
this rearrangement or in some intermediate form.

EXPERIMENTAL

Phenylhydrazine Derivatives of Glucose

Bergmann and Machemer (17) prepared phenylhydrazine derivatives of acetylated degradation products of cellulose by heating the dextrins in liquid phenylhydrazine at 130° C. for one hour. Staud and Gray (108) had reacted cellulose and its degradation products with phenylhydrazine in 10% acetic acid. They concluded from the results of the nitrogen analysis that this method was more reliable than the copper number for the determination of the molecular size of the dextrin. Bergmann and Machemer stated that the phenylhydrazine was chemically bound to the carbohydrate.

This method was applied to the corn syrup dextrins in this laboratory in an attempt to find another measure for molecular size of the dextrins (80). It was hoped that the phenylhydrazine derivatives might also be useful in a simple fractionation of the dextrins; that is, in the removal of the molecules with reducing groups from the material that does not contain reducing groups.

The phenylhydrazine derivatives of Bergmann and Machemer were made by dissolving the cellulose dextrin in phenylhydrazine and heating the solution in an oil bath. The compound formed was isolated by pouring the material into anhydrous

ether. The precipitate was purified by solution in 50% acetic acid and reprecipitated with anhydrous methanol. For the corn syrup dextrins a modification of this method was used. One gram of the dextrin was heated in 5 ml. of redistilled phenylhydrazine under reflux at 130° C. for two hours. After cooling the brown-red reaction mixture was poured into 50 ml. of anhydrous benzene. The precipitate, formed on vigorous stirring, was recovered, dried, and extracted in a Soxhlet extractor with ether for twenty-four hours. At the end of this time all the free phenylhydrazine had apparently been removed.

The dried derivatives were amorphous powders with a yellow color. When they were dissolved in water an odor of phenylhydrazine was apparent at once. Ether extraction of the water solution removed as much as 85% of the nitrogen. However, there was fairly close agreement between the molecular size as determined by the nitrogen analysis of these derivatives, by the iodine titration or copper reducing value of the original dextrin, and by the potassium analysis of the dextrinic acid salt.

The phenylhydrazine derivatives of glucose and maltose were studied to obtain evidence regarding structure of the dextrin compounds. Preliminary work showed that the reaction of glucose with phenylhydrazine under the conditions that were used to prepare the dextrin derivatives produced a non-homogeneous product melting at 111-112° C. whose nitrogen

analysis was approximately that of the pure hydrazide. Maltose gave a compound with phenylhydrazine that melted over a wide range and possessed an even greater degree of instability in water than the glucose compound. The nitrogen analysis agreed quite closely with that calculated for maltose phenylhydrazide. The literature regarding the reaction of phenylhydrazine with glucose is very confused. The reported derivatives as named in the literature, the structure postulated by Behrend, the melting point, and nitrogen analyses are arranged in tabular form in table 1, page 17.

The reactions were much more complicated than was first supposed in that several isomers of the compound formed by the reaction of phenylhydrazine with glucose were possible. From theoretical considerations the derivatives may be expected to have any of the following structures: The α- and β-glucopyranyl phenylhydrazide, the α- and β-glucofuranyl phenylhydrazide, the true hydrazones which could be either in syn or anti forms, and possibly the enol or keto form of the compound that has undergone a complete or partial Amadori rearrangement.

Preparation of the α-hydrazide reported by Behrend

Twenty grams (0.11 mole) of glucose was dissolved in 15 g. (0.40 mole) of glacial acetic acid mixed with 5 g. (0.28 mole) of water (15). This mixture was heated on the water bath until the glucose went into solution, cooled, then poured

with vigorous stirring into 160 ml. of absolute alcohol containing 15 g. (0.139 mole) of redistilled phenylhydrazine. This faintly yellow solution was allowed to stand at room temperature and after two days a fine golden yellow precipitate covered the bottom of the flask. After two more days the phenylhydrazide was filtered by suction and the precipitate was washed with a mixture of 25 ml. of anhydrous ether and 25 ml. of absolute alcohol. After drying in a vacuum desiccator, 9 g. of material with a melting point of 150-155° C. was obtained.

Another crop of crystals was obtained from the mother liquor after two days. After filtering and washing with alcohol and ether mixture, the yellow crystals were dried. On investigation it appeared that this 5 g. of material was the osazone, melting point 197-205° C. Recrystallization from pyridine gave a product melting from 200-205° C. A mixed melting point with a prepared sample of glucose phenylosazone gave no depression.

The yield of the impure hydrazide was 30.3%. On repeating the preparation and seeding the reaction mixture, a light yellow crystalline precipitate was obtained that melted from 135-137° C. In an effort to purify the compound, 1 g. of the hydrazide was dissolved in 3 g. of pyridine. To this a bit of alcohol was added and the compound precipitated with ether. The color was considerably lighter but the melting point was about 125° C. All efforts to obtain a colorless product that

melted at 159-160° C. were without success. Rotation in
aqueous solution was obtained, but in several of the products
a faint yellow flocculent precipitate appeared when the
material was dissolved in water. Specific rotation in pyri-
dine after eight minutes gave a value $[\alpha]_D^{25} = -80.8°$ (0.3378 g.
dissolved in 10 ml. anhydrous pyridine; 1 dm. tube; $\alpha = -2.73$
after eight minutes). The specific rotation of a 2.5% aqueous
solution was $[\alpha]_D^{25} = -74°$ after eight minutes, which changed
to a value of -40.4° in thirty-three hours.

A modification of this method was used by Stempel (109)
since Butler and Cretcher (22) had reported that they obtained
no α-hydrazide at all by using the above method. An attempt
to obtain the pure α-hydrazide was made by the use of their
modification.

Twenty grams (0.11 mole) of glucose was added to 15 g.
(0.4 mole of acetic acid in 5 g. (0.28 mole) of water. The
glucose-acid-water mixture was heated until the glucose dis-
solved, and this solution was added to 14 g. (0.131 mole) of
phenylhydrazine diluted with 160 ml. of absolute alcohol. The
only modification was in the amount of phenylhydrazine added.
The solution was stirred vigorously for three hours, then left
in a stoppered flask at room temperature for twelve hours. On
filtering by suction 12 g. of precipitate was obtained. After
washing six times in acetone, the precipitate was nearly white.
It possessed a low melting point, 134-135° C. A second reac-
tion was run identical with the first except that the mixture

was seeded. A large part of the precipitation occurred within three hours from the time of mixing the solutions. At the end of this time the light yellow precipitate was removed by filtering with suction and 14.5 g. of a nearly white product was obtained after repeated acetone washing and subsequent drying. (On standing for two days, 2 g. of glucose phenyl-osazone precipitated from the mother liquor.) The hydrazide, though it looked quite pure, melted at 128-130° instead of 159-160°, as reported. A 1% solution of this product was so murky that the mutarotation could not be followed. The material prepared in the first reaction gave a rotation of 67° after eight minutes in a 1.5% aqueous solution. However, after 240 minutes the change in rotation did not stop at $[\alpha]_D^{20}$ = -52.5°, as Stempel indicated. Even after twenty-four hours the rotation of the solution still slowly changed and became less negative. It may be that in more dilute solution hydrolysis as well as mutarotation occurred. After further washing with acetone, the initial rotation after ten minutes was 79° (c., 4% in water). Two grams of the product obtained by the method of Behrend, m.p. 145-149° C., was repeatedly washed with acetone and the specific rotation determined. After five minutes $[\alpha]_D^{25}$ = -75.5° (c., 4% in water) and this value changed to -35.2 in sixteen hours. Ardgath (8) found that in 5.4% solution the equilibrium value was $[\alpha]_D^{25}$ = -52.44° at 22.5°, and Behrend and Lohr obtained approximately the same value by using 3-4% solutions, but the rotation became

less negative after the solutions were left for a few days.
It is evident from the difficulty of duplicating the preparation of this form, known as the α-hydrazide, that no satisfactory method for the preparation has been found. The fact that the compound slowly decomposed upon standing, even in a vacuum desiccator, complicated the study.

Although no satisfactory constants were obtained from the Behrend α-hydrazide preparation, nitrogen analysis by the micro Dumas method showed that the compound was homogeneous as far as the nitrogen content was concerned. The difficulty in obtaining the pure compound seemed to be the result of the ease of transformation of one isomer to the other.

%N   Calc. for $C_{12}H_{18}O_5N_2$, 10.37%   Found, 10.27%
                                                                    10.25%

Preparation of the β-hydrazone reported by Behrend

Compound of two molecules of the phenylhydrazide with one molecule of phenylhydrazine. Fifteen grams (0.083 mole) of glucose were dissolved in 7.4 g. (4.1 moles) of boiling water. This viscous syrup after cooling was poured into a solution of 30 g. (0.027 mole) of phenylhydrazine in 100 ml. of absolute alcohol. This mixture was left at room temperature for twenty-four hours; then the flask was scratched to facilitate crystallization and placed in the refrigerator. In twelve hours a light yellow crystalline product was obtained. The mixture was filtered by suction and washed with an alcohol-

ether mixture. The white mat obtained was left to dry in a vacuum desiccator and 8.6 g. of the needles melting at 106-108° C. was obtained. This compound corresponded in properties to the compound designated by Behrend as two molecules of the hydrazide with one molecule of phenylhydrazine. Another preparation yielded 15.4 g. of the compound that melted at 107-108° C. This represented a 57% yield. This compound turned pink in a few days in the vacuum desiccator, and red in just a few hours if it were left in the air. The smell of phenylhydrazine was very apparent even after repeated extraction with ether.

Phenylhydrazide-pyridine complex. This compound was prepared by dissolving 6.4 g. (0.010 mole) of the compound obtained above in 12.8 g. (0.16 mole) of hot pyridine. The compound crystallized on cooling but for complete deposition of the crystals the compound was left over night. The crystals were filtered from the mother liquor, washed with a very little pyridine, with alcohol and finally with anhydrous ether. Four grams of the pyridine compound was obtained with a melting point of 101-102° C. This compound was quite stable even in air. The yield was about 57%.

β-Hydrazone. Two grams of the compound that melted at 101-102° C. was dissolved in 12 g. of absolute ethanol. After standing for twenty-four hours, beautiful white platelets were obtained. These platelets melted at 134-136° C., while the needles reported by Behrend melted at 141-142° C. Several small amounts of this compound were prepared; the yields

usually were from 40-50 % of the theoretical. It was some-
times quite difficult to obtain the crystals from the gelatin-
ous mass first obtained. The compounds had different melting
points according to their purity, for example, 134-136°, 132-
135°, 140-141° C. This compound was remarkably stable and
after nine months the color was only slightly darker and the
melting points were approximately the same. The only reports
concerning the preparation of this compound was given by
Behrend (15). A mixed melting point with the α-hydrazide com-
pound gave 121-124°.

A microrotation was determined on the platelets, m.p. 134-
136° C., about eight months after their preparation. After
twenty-five minutes $[\alpha]_D^{25} = -14.7°$ in water. This value
changed to $-47.3°$ after twelve hours (0.0986 g. in 1.694 ml.;
1 dm. tube). A rotation at the time of preparation in a 2.5%
aqueous solution gave $[\alpha]_D^{25} = -3.9°$, changing to $-40.4°$ in
thirty-three hours. Nitrogen analysis of platelets by the
micro Dumas method gave the following results:

%N Calc. for $C_{12}H_{18}O_5N_2$, 10.37% Found, 10.45%
10.46%

Some of the platelets were washed with a little acetone,
since acetone was used as the wash solution in purification of
the α-hydrazide. The crystal form was at once changed to
needle form. These beautiful white needles were washed with
acetone and dried in the Abderhalden drying pistol with
acetone as the heating liquid. The melting point was 105-106°C.

This compound has a lower melting point than the one described by Skraup. It is possible that another form has been made since its melting point after washing and drying in the Abderhalden tube over phosphorus pentoxide is entirely different from that of the platelets.

The rotation of a 4% aqueous solution was determined on a sample prepared from a less pure β-hydrazone sample in a 1 dm. tube, and $[\alpha]_D^{25} = -34^\circ$ after five minutes, changing to -42° in three hours and one half. The rotation began to descend toward the positive values and had reached a value of -34.5° in twenty-four hours.

Preparation of phenylhydrazine derivatives of glucose corresponding to derivatives of corn syrup dextrins

In the preliminary experiment the compound obtained by heating commercial glucose in phenylhydrazine melted at 111-120° C. and the specific rotation, although it could not be determined accurately because of the dark color, was approximately -40°. The nitrogen analysis gave 10.2% as compared to 10.37% for the calculated value. In a duplicate preparation, the compound obtained had a lower melting point, 105-107°. In an effort to obtain isomers of the phenylhydrazine derivative, α-d-glucose and β-d-glucose were reacted with phenylhydrazine. The procedure used in the preliminary experiments was repeated. The β-d-glucose was made by the method of Hudson and Dale (51), $[\alpha]_D^{11} = 22^\circ$ after 1-1/2 minutes (0.8509 g.

dissolved in 25 ml. water. Rotation, α = 1.50 in 2 dm. tube).

The $\underline{\alpha}$-$\underline{d}$-glucose used was anhydrous $\underline{\alpha}$-$\underline{d}$-glucose prepared from purified cerelose and recrystallized from strong acetic acid, washed with ethyl alcohol and dried in the vacuum oven at 70° C. $[\alpha]_D^{15}$ = 106° after three minutes (0.5505 g. dissolved in 25 ml. of water, reading taken in 2 dm. tube, α = 4.66).

One gram of $\underline{\beta}$-$\underline{d}$-glucose was added to 5 ml. of redistilled phenylhydrazine and refluxed in an oil bath at 125° C. for two hours. The solution was cooled and poured into 75 ml. of benzene. The precipitate was recovered and washed with anhydrous ether. After drying, the compound was extracted with 35 ml. anhydrous ether in a Soxhlet extractor for twelve hours. The residue was left in a vacuum desiccator over night.

The method was exactly duplicated for the preparation of the derivative of the $\underline{\alpha}$-$\underline{d}$-glucose. It was interesting to note that in every case the derivative formed from the $\underline{\alpha}$-$\underline{d}$-glucose was darker than that from the $\underline{\beta}$-$\underline{d}$-glucose.

The melting point of the $\underline{\beta}$-$\underline{d}$-glucose compound was 105-108° C.; for the $\underline{\alpha}$-$\underline{d}$-glucose compound it was 105-110° C. No value for the optical rotation could be recorded since the compounds were too dark in aqueous or alcoholic solution to obtain a reading. A mixed melting point of the two showed no depression.

It was found that if 1 g. of $\underline{\beta}$-$\underline{d}$-glucose was added to 5 g. of water-clear phenylhydrazine and left at room

temperature, a gel formed within an hour. When this material was poured into 75 ml. of benzene, a precipitate formed. This solid material was washed well with anhydrous ether and dried in the vacuum oven at 50° C. The dried residue was extracted in a Soxhlet extractor with anhydrous ether for twelve hours and then dried in a vacuum desiccator over night. The α-d-glucose was treated in exactly the same manner. The reaction with β-d-glucose set to a gel more easily than the reaction with α-d-glucose, and the excess phenylhydrazine was more readily extracted from the β-d-glucose derivative.

Both preparations were nice crystalline needles. While in the phenylhydrazine, transparent platelets were formed that appeared very birefringent when viewed under the polarizing microscope. On addition of alcohol, these platelets dissolved and a mass of white needle-like crystals appeared. When these needles were isolated, washed, and kept in a vacuum desiccator in a nitrogen atmosphere, they remained white for several days. If, on the other hand, the compounds were left in the air, they were colored red-brown in a very few hours. No differences of the α- and β- derivatives could be detected under the polarizing microscope.

Both preparations appeared to soften about 60-70° and gave off phenylhydrazine, but they did not melt until the temperature reached 105-110° C. A series of nitrogen analyses and vigorous drying showed progressive removal of the phenylhydrazine (table 2). The data certainly indicate that the

hydrazine derivatives of glucose formed at ordinary temperatures exist in many forms in which there is phenylhydrazine of solvation. The phenylhydrazine comes off rather easily although it cannot be removed by extraction with ether alone. Apparently the melting point of these addition products is indefinite and approaches 105-110° as phenylhydrazine is volatilized.

Table 2

Change in Nitrogen Content Produced by

Various Drying Procedures

Drying Treatment	Per cent Nitrogen of Phenyl-hydrazine Derivative Prepared at Room Temperature from:	
	β-d-Glucose	α-d-Glucose
Product after ether extraction and drying in the vacuum desiccator	16.56% 15.53%	16.13% 15.53%
Product dried 12 hours at 50° in vacuum oven	13.87% 13.53%	14.74% 14.89% 13.18%
Product dried 12 hours more at 70° in vacuum oven	11.98% 11.73%	11.75% 11.66%
Product made by refluxing components	10.56%	10.70%
Calculated for the phenylhydrazide	10.37%	10.37%

The compound formed by refluxing the components appeared to be similar to the compound reported by Skraup, although it was so dark in color that a value for the rotation could not

be obtained. The melting point, nevertheless, was rather sharp, 105-107° C.; although it might be expected from the melting point that this compound was the phenylhydrazine solvated compound or the compound reported by Skraup, and the nitrogen analysis indicated that it probably was the latter.

The rotation of α- and β-glucose and a mixture of α- and β-glucoses was followed in pure phenylhydrazine. Since the solutions had to be warmed to effect solution of the α- and the mixture of glucoses, a rather empirical group of readings was obtained. The conditions were kept as constant as possible, and the results of two runs were plotted in fig. 1. The data are given in table 3.

One-half gram of α-, β-, and a mixture of α- and β-glucose whose initial rotation was the equilibrium value of $[\alpha]_D$ = -52.5°, were dissolved by heating the mixture to 80° in 10 ml. of redistilled phenylhydrazine. The observed readings were plotted against time since the other values were constant. The rotations were observed by placing the solution in a 1 dm. tube and using a mercury lamp with filters to give approximately the D-line of sodium (correction 1/1.04).

Table 3

Rotation of α-, β-, and $\alpha+\beta$-Glucose in Phenylhydrazine

Time (min.)	α-d-Glucose 0.5000 g.	β-d-Glucose 0.5000 g.	Mixture of glucose (α- + β-) to read 52.5° 0.5000 g.
41	-0.50	-1.37	-0.75
60	-1.15	-1.97	-1.20
120	-2.52	-2.18	-2.05
470	-2.90	-2.65	-2.83
1310	-3.03	-2.77	-3.05
1610	-3.09	-2.85	-3.10
	0.2500 g.	0.2500 g.	0.2500 g.
24	-0.05	-0.55	-0.10
30	-0.10	-0.65	-0.30
60	-0.45	-0.80	-0.45
120	-0.90	-1.02	-0.90
390	-1.40	-1.37	-1.35
1180	-1.47	-1.52	-1.47
1360	-1.52	-1.50	-1.50

The only difference that could be observed was the higher negative value of the β-d-glucose in the first few hours. This may be due to the original difference in rotation of the α- and β-d-glucose; in that case the mixture should be intermediate between the two forms instead of nearly duplicating the α- form. Whether the original sugar mutarotated or the compound formed mutarotated, or the difference in readings at the first was due to the rate of formation of the hydrazide, it appeared that after six hours the three sugars reached an equal rotatory power.
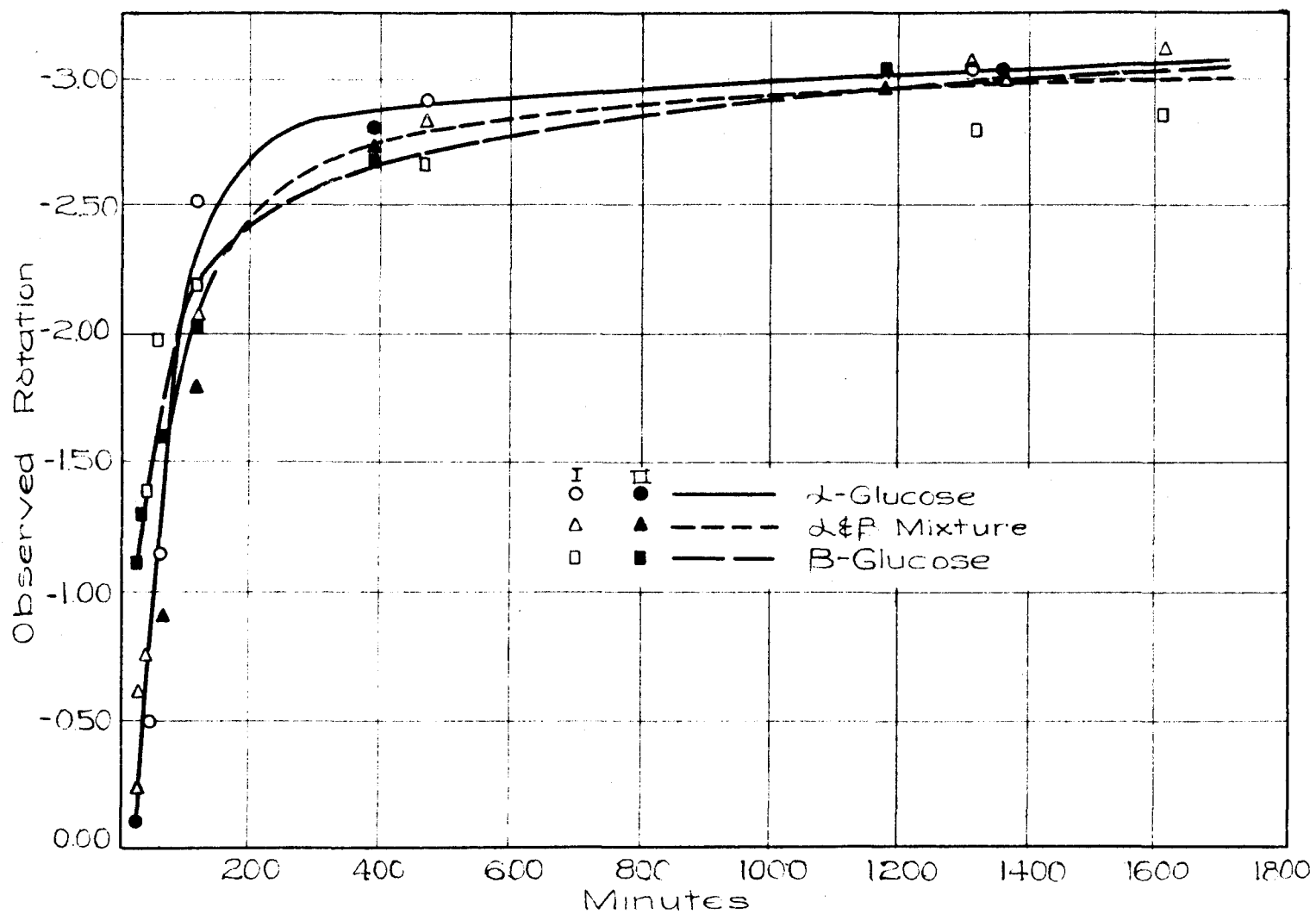
Fig.1 Change in rotation with Time of ᐊ,-β-, and ᐊ,β-glucose in phenylhdrazine

Hydrolysis of phenylhydrazine derivatives of glucose and maltose

From the literature it appeared that the hydrolysis of glucose-phenylhydrazine compounds in water solution was negligible. The change in rotation and final equilibrium value was attributed to the equilibrium between the different forms. There was some evidence that a slow hydrolysis did occur, since the readings for the α-hydrazone rapidly decreased to about $[\alpha]_D = -50°$, then slowly decreased to a value about $[\alpha]_D = -40°$ or less. The Behrend β-hydrazone increased to about $[\alpha]_D = -50°$ in approximately four hours and then decreased to a smaller negative value. One gram of the α-hydrazide prepared by the method of Stempel, dissolved in 25 ml. of water, was extracted with 75 ml. of ether after forty minutes, and 0.01 g. of phenylhydrazine was obtained. This represented about 2% hydrolysis.

The hydrolysis of the maltose derivative was more rapid than the hydrolysis of the glucose derivative. In fact, the maltose compound was very difficult to prepare because of its hygroscopic nature. A crystalline compound, melting point 95-110° C., was obtained. When the hydrazide was dissolved in water, phenylhydrazine could be extracted with ether. The phenylhydrazine was removed by repeated extraction with with ether, but upon standing the equilibrium between the derivative and its components was again reached and more phenylhydrazine could be extracted, showing that the hydrolysis was fairly slow.

The phenylhydrazides of the dextrins were even more un-
stable than the maltose derivatives. It appeared that the
larger the molecule the more unstable the phenylhydrazide and
the greater the ease of hydrolysis. Levine, Foster and Hixon
(80) found that as much as 85% of the nitrogen could be removed
from the dextrin derivatives by ether extraction. The ease of
hydrolysis of these compounds indicated that they were primar-
ily in the hydrazide form.

Aliphatic Amine Derivatives of Glucose

Preparation of glucosylalkylamines

Glucosyl-n-butylamine. To 3 g. (0.0167 mole) of glucose
was added 3.7 g. (0.037 mole) of n-butylamine. The n-butyl-
amine used was an Eastman Kodak Company product that had been
dried over potassium hydroxide for twenty-four hours. It dis-
tilled at 77-78° C. The mixture of the glucose and amine was
heated on a water bath until all the glucose dissolved and the
solution was left at room temperature over night. By morning
there appeared white needle-like crystals, which were filtered
by suction and washed with anhydrous ether. After the product
was dried in a vacuum desiccator, the melting point was 83-84°
C. In an effort to obtain two forms of this compound, 2 g.
(0.011 mole) of β-glucose was added to 2.5 g. (0.034 mole) of
n-butylamine. The solution was warmed until the glucose dis-
solved. The needles that were formed after a few hours were

filtered, washed in anhydrous ether and dried. The melting

point of these needles was 83-84° C. α-d-Glucose was treated

in a similar manner and the product obtained had a melting

point of 81-82° C. To determine whether the two isomeric

glucosides of the sugar might be expected by this method, the

optical rotation of α-, β-, and an equilibrium mixture of α-

and β-d-glucose in n-butylamine was determined. The glucose

dissolved after fifteen minutes and the readings of 1.000 g.

samples of the sugar in 10 ml. of n-butylamine were identical

within experimental error. Thus it appeared that there was

practically instantaneous mutarotation of the sugars to one

value. There was a slow decrease in rotation during twenty-

four hours or more which probably indicated that compound

formation was not completed very rapidly. The values for ni-

trogen analysis by the Kjeldahl method were low. The compound

could be recrystallized from water, ethanol-water, or dioxane,

but was quite soluble in anhydrous methanol. The recrystal-

lized product had a slightly higher percentage of nitrogen

than the crude product but the value still was considerably

lower than the calculated value.

After trying several modifications, a more satisfactory

method for the preparation of the compound was obtained. To

a flask containing 10 g. (0.056 mole) of glucose and 2 ml. of

N/2 hydrochloric acid, 9 g. (0.12 mole) of n-butylamine was

added, and the solution, with occasional shaking, was refluxed

on a water bath at 70° C. for ten minutes. At this time the

solution was diluted with 20 ml. of ethanol, cooled and pre-
cipitated with 100 ml. of ether. If the ether were added
slowly and the solution seeded, needles usually crystallized
out. Sometimes, however, a gelatinous substance was formed
to which a little more alcohol had to be added and the gel
broken up mechanically by shaking for a couple of hours.

The needles were filtered by suction and repeatedly
washed with ether. The yield was usually around 11 g. or 79%
of the theoretical amount. Analysis of the dried material by
the Kjeldahl method was reported below:

%N   Calc. for $C_{10}H_{23}O_6N$, 5.53%   Found, 5.39%
      for $C_{10}H_{21}O_5N$, 5.96%   5.47%

Another portion of glucosyl-n-butylamine was washed again
with ether and dried under reduced pressure over phosphorus
pentoxide with acetone as the heating liquid. After five
hours the compound appeared slightly darker than before drying.
The melting point was 86-87° C. Kjeldahl analysis gave 5.48%,
5.48%, 5.49%, and 5.46% for nitrogen content. When the com-
pound was repeatedly crystallized from water and dried in the
drying pistol, the melting point was raised to 96-97° C., with
softening earlier. The optical rotation in ethanol was ob-
served using a 2% solution and a 2 dm. tube. After five min-
utes $[\alpha]_D^{25} = -22°$ and the specific rotation reached a constant
value $[\alpha]_D^{25} = -7.8°$ after thirty-seven minutes.

Glucosyl-n-amylamine. The same procedure as the above was

used for the preparation of the glucosyl-n-amylamine. The n-amylamine was an Eastman Kodak Company product which, after drying over potassium hydroxide, distilled at 103° C. Eleven grams of the pure white crystals was obtained, representing a 74% yield. The melting point of the compound after thorough drying was 96-97° C. The same conditions were used for determination of the specific rotation as were used with the glucosyl-n-butylamine. After 12 minutes, $[\alpha]_D^{25} = -22^\circ$, which changed to a constant value of -8.0° in one hour. The compound was analyzed by the titration method. Twenty-five milliliters of 0.1884 N sulfuric acid was added to 0.5371 and 0.5344 g. of glucosyl-n-amylamine. The solutions were boiled gently for 20 minutes. The first weight required 28.10 ml. and the second 28.20 ml. of 0.0955 N sodium hydroxide for back titration to a methyl red end point.

%N   Calc. for $C_{11}H_{25}O_6N$, 5.25%   Found, 5.28%
        for $C_{11}H_{23}O_5N$, 5.62%   5.28%

Glucosyl-n-heptylamine. The heptylamine was an Eastman Kodak Company product which, after drying over potassium hydroxide, had a boiling point of 154-155° C. The same procedure was used for the preparation of the n-heptylamine compound as for the n-butyl and n-amylamine condensation products. Thirteen grams of white needles, melting point 97-98° C., was obtained. This represented a 79% yield of the theoretical value. The product was easily recrystallized from water or

methanol-water mixture. A 2% solution of the compound in ethanol gave a rotation of $[\alpha]_D^{25} = -13^\circ$ after 12 minutes which rapidly mutarotated to a value of -7.0°. After drying in the Abderhalden pistol over phosphorus pentoxide, the Kjeldahl analysis was as follows:

%N   Calc. for $C_{13}H_{29}O_6N$, 4.77%   Found, 4.70%
                                                  4.63%

<u>Glycosyldicyclohexylamine</u>. The cyclohexylamine, an Eastman Kodak Company product, was dried over potassium hydroxide and distilled at 131-132° C. To 9 g. (0.09 mole) of cyclohexylamine was added 10 g. (0.056 mole) of glucose and 2 ml. of N/2 hydrochloric acid. The mixture was heated on a water bath at 75° C. until the solution became homogeneous and then for ten minutes thereafter. After cooling, 20 ml. of ethanol was added, then 150 ml. of ether. The slightly yellow solution was left in the refrigerator to crystallize. Large needle-like crystals began to precipitate from the solution in twenty-four hours. After thirty-six hours, 6 g. of crystals was recrystallized from a very small amount of water. This preparation was peculiar in that the crystals dissolved upon long standing and could not be recovered. If more ether were added to precipitate the crystals, a syrupy layer separated from which no crystals could be obtained. The crystals darkened much more rapidly than the crystals of the other glucosylalkylamines. The specific rotation in a concentration of 2% in ethanol was $[\alpha]_D^{25} = -23.5^\circ$, which changed to a constant

value of -11.6° in less than six hours. On analysis it was found that two molecules of cyclohexylamine had condensed with the monosaccharide rather than one molecule, as in other aliphatic amine condensations.

%N   Calc. for $C_{18}H_{36}O_5N_2$, 7.77%   Found,

	Kjeldahl	Dumas
	7.38%	7.60%
	7.45%	7.75%

The compound after recrystallization from water and drying melted at 97-98° C., but softened earlier. An attempt was made to prepare the 1:1 condensation product by reacting 5 g. (0.05 mole) of the amine with 7 g. (0.04 mole) of glucose. This mixture was heated gently and left over night to crystallize. The product obtained was the same as the one above as shown by nitrogen analysis. Using molecular proportions of the reactants, no crystals of any sort were obtained, even when the solution was seeded. The yield of this product was much lower than the yield from the condensation of other alkylamines with glucose, often only about 30% of the theoretical value. This may be the result of the difficulty in crystallizing the compound.

Since it was thought that the extra molecule of cyclohexylamine might be removed by slight warming under reduced pressure, if phosphorus pentoxide were present to remove the amine vapors as they formed, a weighed amount of dried cyclohexylamine condensation product was heated three hours under reduced pressure at the boiling point of acetone. Only 2% of the total weight was lost. The heating liquid was changed to

alcohol and after four hours the material was dark and gummy, and a loss of 13.89% of the original weight was observed. Heating with water at reflux temperature for fourteen and one-half hours resulted in the loss of 20.44% of the total weight. However, instead of a decrease in nitrogen content, an increase was noted, N = 8.26%. Water was evidently removed from the sugar even more easily than the cyclohexyl group.

An attempt to prepare the di-n-butylamine condensation product, in hope of obtaining a compound similar to the cyclohexylamine condensation product, was made. The method used was the general procedure for the preparation of the alkylamines except that 20 ml. of methanol was added to promote solution of the reactants. After a few days glucose began to precipitate from the solution. No crystalline condensation product could be obtained, although the solution of glucose in the methanol solution gave evidence of compound formation.

2-Aminooctane condensation product. The 2-aminooctane was an Eastman Kodak Company product. The method of preparation was the same as that used for the n-heptylamine condensation product, but on addition of ether no precipitate was formed. The solution was rather dark. After standing several days, water was added, and an oil separated, but still no crystals of the condensation product were obtained.

Two grams (0.011 mole) of glucose dissolved in a very little water were added to 2 g. (0.016 mole) of 2-amino-octane. The solution was heated on a water bath until it was

homogeneous and shaken on a shaker for twenty-four hours.
After leaving in the refrigerator two days, 10 ml. of methanol
was added. On refluxing with methanol, no crystals or pre-
cipitate formed.

Glucosylisopropylamine. The isopropylamine was an
Eastman Kodak Company product. Five grams of glucose (0.028
mole) and 5 g. (0.084 mole) of isopropylamine were gently
refluxed until the glucose dissolved. After five minutes,
10 ml. of anhydrous methanol was added and heating continued
for ten minutes longer. The solution was cooled; 125 ml. of
anhydrous ether was added. On standing, the material precipi-
tated in small clumps. The precipitate was filtered, dis-
solved in ethanol and reprecipitated with ether. After stand-
ing over night the solution was filtered and dried in a vacuum
desiccator. The melting point was approximately 46° C., but
softened somewhat earlier indicating that the substance was
not very pure. No crystals were obtained by the procedures
used for the preparation of the other glucosylalkylamines. A
mixture of 3 g. isopropylamine and 1 g. glucose was heated and,
although solution occurred, no crystals were obtained. The
analysis of the product obtained by the first method gave the
following results:

%N   Calc. for $C_9H_{21}O_5N$, 5.85%   Found, 6.70%
      for $C_9H_{19}O_5N$, 6.33%   6.65%

Glucosyl-n-octadecylamine. The octadecylamine, furnished
by Armour and Company, was recrystallized from dioxane,

filtered and washed with ether. After drying over sulfuric acid, the melting point of the compound was 75-77° C.

The condensation product was made by refluxing 15 g. (0.056 mole) of n-octadecylamine with 10 g. (0.056 mole) of glucose in 100 ml. of 98% ethanol. This mixture was heated for an hour on the water bath. After a few minutes, all of the glucose went into solution and the glucosylamine formed precipitated on cooling. The precipitate was filtered and washed with ethanol. The material was recrystallized from dioxane, washed with ether and then ligroin. The material crystallized in clumps. Another 3 g. sample of the glucosyl-amine was dissolved in butanol and after standing for a few days in the refrigerator the precipitate was removed by suction filtration. This material looked quite amorphous under the microscope. The preparation was repeated using 27 g. of the amine dissolved in 50 ml. of absolute ethanol to which was added 18 g. of glucose and an additional 50 ml. of ethanol. After the glucose was all dissolved, the solution was refluxed for fifteen minutes. On cooling, the compound that precipi-tated from the solution was filtered, washed with ether, and dried over phosphorus in a vacuum desiccator. A yield of 37.5 g. or 83.3% of the theoretical value was obtained. The compound was recrystallized from ethanol. The melting point was 104-105° C. with softening earlier. The specific rotation was not determined because of the low solubility of the com-pound in ordinary solvents at room temperature. The nitrogen

content was determined by the Kjeldahl method.

%N   Calc. for $C_{24}H_{51}O_6N$, 3.12%   Found, 3.01%
                                                      3.06%
                                                      3.01%

Glucosyl-n-hexadecylamine. The n-hexadecylamine, fur-
nished by Armour and Company, was dissolved in hot dioxane and
precipitated from this solvent on cooling. The reprecipitated
amine melted at 81-82° C. with a slight softening earlier. The
condensation product of the amine with glucose was prepared by
dissolving 24 g. (0.056 mole) of the amine in 50 ml. of ethanol,
and adding to this solution 18 g. (0.056 mole) of glucose in
an additional 50 ml. of ethanol. All of the glucose went into
solution after a few minutes of refluxing on a boiling water
bath. The solution was refluxed fifteen minutes after it
became homogeneous; the dark red-brown liquid was cooled and
the precipitate that formed was filtered with suction and
washed with alcohol and ether. After drying over sulfuric acid
in a vacuum desiccator, the product weighed 31 g. This repre-
sented a 74% yield. The compound was quite soluble in hot
ethanol, and was recrystallized from this solvent. The powdery
product did not have a sharp melting point, but melted at 106-
107° C. with a noticeable softening at a lower temperature.
The product was insoluble in most solvents, both organic and
inorganic in the cold, but dissolved in the alcohols and
dioxane on heating. No rotation was determined, but nitrogen
analysis by the Kjeldahl method gave the following results:

%N   Calc. for $C_{22}H_{47}O_6N$, 3.31%   Found, 3.28%
                                                               3.26%

<u>Diglucosylethylenediamine</u>. The ethylenediamine was dried over potassium hydroxide and refluxed for two hours over sodium. The amine distilled at 114-115° C. To 3.5 g. (0.06 mole) of ethylenediamine dissolved in 50 ml. of absolute methanol was added 21 g. (0.12 mole) of glucose. The methanol solution was refluxed gently on the water bath until the solution became homogeneous. After a short time, a precipitate appeared on the side of the flask and the amount slowly increased as the solution was refluxed. After two hours the refluxing was stopped, the flask cooled, and placed in the refrigerator over night. After filtration, w shing with cold methanol and drying, 11 g. of a nearly white compound was obtained. This compound melted with decomposition at 152-154° C. A white crystalline product was obtained by careful recrystallization from methanol-water mixture. The specific rotation of a 2% solution in 50% ethanol was $[\alpha]_D^{25} = -17°$, which changed to $[\alpha]_D^{25} = +14.5°$ in thirty-nine hours. Slow hydrolysis probably occurred. Analysis of the compound by the Kjeldahl method gave the following results:

%N Calc. for $C_{14}H_{30}O_{12}N_2$, 6.66% Found, 7.35%

   for $C_{14}H_{26}O_{10}N_2$, 7.29%   7.39%

<u>Diglucosylpropylenediamine</u>. Propylenediamine, Eastman Kodak practical (80-85%), was dried over potassium hydroxide and refluxed for two hours over sodium. It distilled at 119° C.

The method of preparation used in preparing the ethylene-diamine condensation product was not satisfactory since the

propylenediamine compound would not precipitate out of the methanol. On precipitation with ether, a dark, gummy material was obtained. An impure product was obtained by heating for one-half hour 5.2 g. (0.07 mole) of propylenediamine and 25 g. (0.14 mole) of glucose in 40 ml. of ethanol. On leaving over night, a non-crystalline solid appeared. This was filtered, washed with ether, dissolved in methanol and reprecipitated by addition of ethanol. The dried residue melted above 88° C., and Kjeldahl analysis of the product gave the following results:

%N   Calc. for $C_{15}H_{32}O_{12}N_2$, 7.04%   Found, 7.19%
         for $C_{15}H_{28}O_{10}N_2$, 6.45%   7.23%

An effort to obtain a better product was made by the following procedure: to 25 ml. of ethanol containing 2.6 g. (0.035 mole) of propylenediamine, 12.5 g. (0.07 mole) of glucose was added. After heating for about fifteen minutes all the glucose went into solution. The material was refluxed for another half hour to complete the reaction. The solution became a dark brown and, upon cooling, a gummy material was obtained, but no crystalline product could be obtained from it.

Catalytic reduction of glucosylalkylamines.

The catalytic reduction of the glucosylalkylamines was carried out in a high pressure bomb of the Parr type. The catalyst used in every case was Raney nickel activated by the method given in "Organic Syntheses" (87).

<u>N-Butyl-d-glucamine</u>. In the bomb was placed 2 g. of glucosyl-<u>n</u>-butylamine (m.p. 86-87° C.) dissolved in 100 ml. of methanol. To this was added 6.5 g. of wet Raney nickel suspended in 100 ml. of water. The reduction was carried out at 1000-1100 lbs. pressure and the temperature was kept between 60 and 78° C. The solution was removed from the bomb after fourteen hours. An aliquot of the original material gave a ferricyanide reducing value (27) of $R_{cu} = 2029$, and the calculated value was $R_{cu} = 2140$ based on the equivalents of glucose contained in the glucosylamine. (For glucose, $R_{cu} = 2800$.) After reduction, the reducing value was again determined and found to be about $R_{cu} = 891$. It was concluded that some reduction had occurred. The catalyst was filtered off and the solvent removed from the clear solution by reduced pressure at temperatures below 60° C. The residue solidified after the water and methanol were removed. The distilling flask was washed out with 10 ml. of ethanol and the compound crystallized from the ethanol solution on standing overnight. The melting point of the crude product was 123-126° C. To determine the purity of the product, 0.0904 g. of the reduced compound was dissolved in water and 50 ml. of 40% potassium hydroxide added. The solution was distilled for twenty minutes and the vapors passed into 15 ml. of 0.0950 N acid. On analysis it was found that no amine was distilled over. The original glucosylamine was treated in a similar manner and the butylamine distilled over into the standard acid. In twenty

minutes, 98% of the calculated amount of butylamine present had distilled.

Ten grams of glucosyl-n-butylamine was dissolved in 200 ml. of 50% methanol and 9 g. of activated Raney nickel was added. The reduction was continued for ten hours at a pressure of 800 lbs. and a temperature range of 66-82° C. A pure white compound was obtained on filtering off the catalyst and removing the solvent. The melting point of the compound was 127-128° C. The yield amounted to 73% of the theoretical value. The specific rotation of the reduced compound was$[\alpha]_D^{25}$ = -14° (c., 1% in 50% ethanol). This product had the property of lowering the surface tension and causing a remarkable amount of foaming. It was stable to heating with 50% alkali and formed salts with mineral acids. Analysis of the compound by the Kjeldahl method gave the following results:

%N   Calc. for $C_{10}H_{23}O_5N$, 5.91%   Found, 6.02%
                                                     5.87%

N-Amyl-d-glucamine. The same procedure was used to reduce the glucosyl-n-amylamine. In this preparation, however, 50 ml. of ethanol, 50 ml. of methanol and 100 ml. of water were used as the solvent. The reduction was made in the presence of 9 g. of Raney nickel. The pressure was approximately 850 lbs., the temperature was regulated between 60 and 80° C., and the time of reduction was fourteen hours. Eight grams of a white crystalline product that was easily recrystallized from methanol was recovered. This represented an 85% recovery of

the product. The melting point of the compound was 129-130° C.
The specific rotation of the reduced compound was $[\alpha]_D^{25} = -13.8°$
(c., 1% in 50% ethanol). Nitrogen analysis gave the following
values:

		Kjeldahl	Dumas
%N Calc. for $C_{11}H_{25}O_5N$, 5.58%	Found,	5.38%	5.42%
		5.16%	5.52%

N-Heptyl-d-glucamine. The same procedure was used for
the reduction of a 10 g. sample of glucosyl-n-heptylamine,
except that 75 ml. ethanol, 75 ml. methanol and 100 ml. of
water were used as the solvent. The glucosylamine still had a
tendency to solidify but was all in solution at 40° C. Hydro-
gen pressure of 800 lbs. was applied and the temperature
thermostated between 70-83° C. The product crystallized as
soon as the solvent was removed. After drying in the Abder-
halden pistol the platelets melted at 126-127° C. A 96%
recovery (9 g.) of the platelets was obtained.

It was interesting to note that a high reducing value was
obtained even on the reduced compound which had been recrys-
tallized from methanol. A sample (0.0363 g.) of the compound
(m.p. 126-127° C.) was found to have a reducing value of
R_{cu} = 1365, while with the original condensation product the
value was 1700. Evidently the grouping $-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-H$ may be
$\ HO\ NHR$
rather easily oxidized. This accounts for the fact that in
the preliminary experiments reducing values of considerable
magnitude were always obtained after the reduction as well as
before.
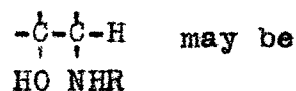
The simplest criterion of reduction was distillation of a known weight of the compound with 40% potassium hydroxide. The condensation product turned yellow at once and the amine distilled over into the standard acid. The reduced compound remained water-clear and was perfectly stable. The reduced material formed a suds that was stable for over twenty-four hours. The specific rotation was determined and $[\alpha]_D^{25} = -14°$ (c., 1% in 50% ethanol). The nitrogen was determined by Kjeldahl analysis:

%N   Calc. for $C_{13}H_{29}O_5N$, 5.02%   Found, 5.08%
                                                     4.99%

N-Cyclohexyl-d-glucamine. To 6.5 g. of glucosyl cyclohexylamine dissolved in 200 ml. of 50% methanol, 6 g. of activated Raney nickel was added. The reduction was carried out at 60-74° C. under 1200 lbs. of hydrogen pressure. The recovery of the compound was carried out as reported above. Only 3 g. of material was recovered, representing a 63% yield based on the weight of glucosylamine. The white needles melted rather sharply at 145-146° C. The specific rotation was $[\alpha]_D^{25} = -11°$ (c., 1% in 50% ethanol).

%N   Calc. for $C_{12}H_{25}O_5N$, 5.37%   Found, 5.18%

N,N'-Ethylenediglucamine. The reduction of 10 g. of di-glucosylethylenediamine was carried out using 50% methanol as solvent and 6 g. of activated Raney nickel as catalyst. The hydrogen pressure was approximately 1300 lbs. and the

temperature remained between 69 and 74° C. Six grams of the
reduced compound with a melting point of 136-137° C. was
recovered on evaporation of the solvent. The compound was
insoluble in ethanol but very soluble in water. The specific
rotation was determined $[\alpha]_D^{25}$ = -15.5° (c., 1% in 50% ethanol.
The compound was analyzed for nitrogen content:

%N   Calc. for $C_{14}H_{30}O_{10}N_2$, 7.22%   Found, 6.74%
                                                         6.92%

N-Hexadecyl-d-glucamine. A 10 g. sample of glucosyl-n-
hexadecylamine was reduced using 200 ml. of 95% ethanol and
9 g. of Raney nickel as catalyst. The hydrogen pressure was
approximately 1300 lbs. and the temperature was kept below 98°
C. for ten hours. The solvent was evaporated from the reduced
compound and a jelly-like product was obtained. From this
3.2 g. of the reduced compound was recovered. The yield was
probably much higher but the recovery was poor. The compound
was pure white rather than the light tan color of the original
condensate. The melting point was 123-124° C. with softening
earlier. In the preparation of this compound it would be
advantageous to be certain that no n-hexadecylamine is present
in the condensation product since the solubilities of the
amine and the glucamine are so similar that it is almost im-
possible to separate the two by extraction. Specific rotation
of the compound was not determined because it was only
slightly soluble in the common solvents at room temperature.
Nitrogen analysis corresponded fairly closely to the theoreti-
cal value:

%N   Calc. for $C_{22}H_{47}O_5N$, 3.45%   Found, 3.42%
                                                    3.25%

N-Octadecyl-d-glucamine. The reduction of 10 g. of the
octadecylamine condensation product was carried out in a solu-
tion of 160 ml. ethanol, 10 ml. water and 25 ml. butanol. The
reduction proceeded under 1200 lbs. of hydrogen pressure with
a temperature from 70-82° C. Only 1.5 g. was recovered,
although the reduction seemed complete. The compound was
quite insoluble in water and other solvents at room temperature.
The difficulty in the preparation lay in obtaining the solid
material from the gelatinous precipitate after the solvent
had been removed. The melting point was 118-119° C. with
softening about 115° C. No reduction of Fehling's solution
was observed, even on heating. Rotations were not taken
because of the difficulty in dissolving the compound. Nitro-
gen analysis of the white product gave the following results:

%N   Calc. for $C_{24}H_{51}O_5N$, 3.23%   Found, 3.19%
                                                    3.14%

N-Isopropyl-d-glucamine. To 9 g. of isopropylamine, 10 g.
of glucose and 2 ml. N/2 hydrochloric acid were added. This
solution was refluxed on the water bath until the glucose went
into solution. Seventy-five milliliters of methanol was added
and the solution refluxed one-half hour. This solution was
cooled, 100 ml. of 50% methanol and 3 g. of Raney nickel added
and the mixture reduced under 1100 lbs. pressure at 70-82° C.
for eight hours. On evaporation of the solvent, 7.5 g. of a

white product, which softened a bit about 120° and melted at
126-127° C., was obtained in 61% yield. On analysis by titra-
tion it was found that only 73% of this white solid was the
N-alkylglucamine. The titration curve was very similar to
that obtained from the N-heptyl-d-glucamine. The impurity in
the N-alkylglucamine was assumed to be sorbitol. In an at-
tempt to recrystallize the product from ethanol a gel was
formed, and no crystals were obtained. However, on complete
evaporation of a methanol solution, needle-like crystals that
melted at 125-127° C. were deposited on the sides of the flask.
The material was completely reduced since a negative test to
Fehling's solution was obtained even when heated. The conden-
sation product as well as glucose reduced Fehling's solution
immediately on heating. The rotation of the N-isopropyl-d-
glucamine was determined as $[\alpha]_D^{25}$ = -13.0° (c., 1% in 50%
ethanol).

Reduction of the diglucosylpropylenediamine. A mixture
of 12 g. of glucose, 2.5 g. of propylenediamine and 60 ml. of
methanol were refluxed for an hour. To this 150 ml. of 50%
methanol and 3 g. of Raney nickel were added and the mixture
reduced under 1100 lbs. pressure for seven hours at 62-90° C.
On removal of the solvent, a viscous liquid remained. It was
precipitated by methanol but remained gummy. No analyses were
made on the product, but reduction had occurred, as was shown
by the failure of the syrup to reduce Fehling's solution on
heating.

Potentiometric titration of the N-alkyl-d-glucamines

The N-alkylglucamines are all strong amines and may be titrated potentiometrically. A Coleman pH meter made by the Webster Electric Company at Racine, Wisconsin, was used for these titrations. The titration curves for N-heptyl-d-glucamine (0.2720 g. in 200 ml. of water) and N-butyl-d-glucamine (0.2447 g. in 200 ml. of water) are shown in fig. 2, p. 99. The percentage of nitrogen calculated from the end-point determined on the curve was 5.87% for the butylamine compound, as compared to the theoretical value of 5.92%; for the heptylamine compound the percentage of nitrogen calculated from the curve was 5.04%, while calculated from the formula it was 5.02%. The pOH as given for the two compounds from these curves is 4.8 for the N-heptyl-d-glucamine and 4.6 for the butyl. Thus K_b for N-heptyl-d-glucamine was determined as 1.6×10^{-5} and for N-butyl-d-glucamine as 2.5×10^{-5}. From the curves in fig. 2 it appeared that Congo red or brom-cresol green might serve best as indicators and even Alizarin red S might be used if the titration were stopped on the red side of the indicator change. Since the drop was rather gradual, the most satisfactory method of determination was by potentiometric titration.

Hydrolysis of glucosylalkylamines

Several of the compounds formed by the condensation of the alkylamines and glucose were recrystallized from a small
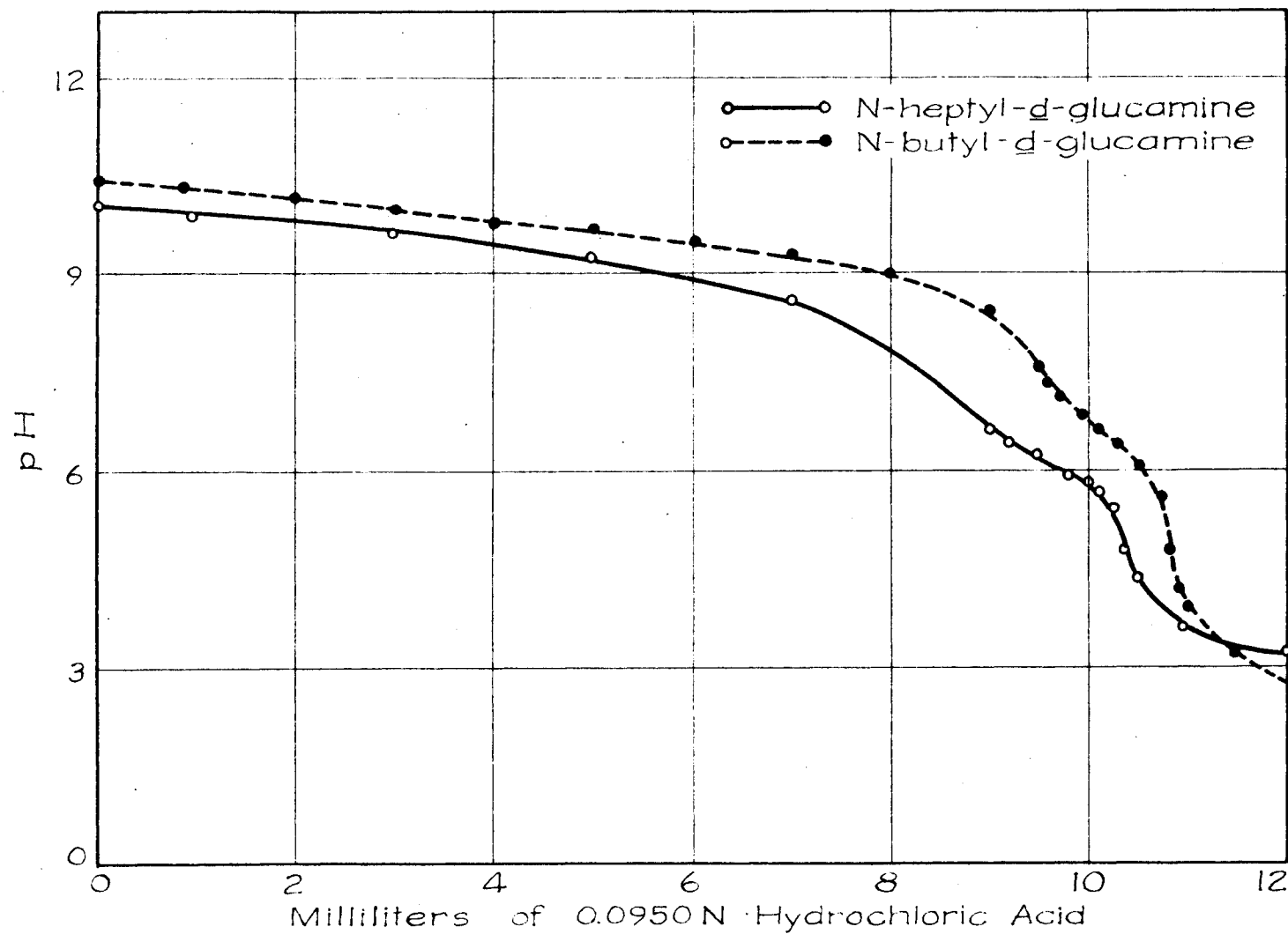
Fig. 2   Potentiometric titration of N-alkyl-d-glucamines.

amount of water. These compounds, however, when dissolved in water, dissociated and after a few hours reached an equilibrium between the amine, glucose and some form of the glucosylalkylamine. This equilibrium was followed by the change in optical rotation and by potentiometric titration and checked by extraction of the free amine at equilibrium.

Glucosyl-n-butylamine was selected as a representative compound of this series, and the equilibrium of 0.5000 g. of the compound in 25 ml. of water was followed. From fig. 3, it is evident that equilibrium in aqueous solution was reached in 22 hours. The values for the optical rotation were obtained by passing the light from a sodium lamp through the solution in a 2 dm. tube. The values for methanol and 50% methanol solutions were obtained under identical conditions.

By assuming that only one form of the glucosylamine was present, and that it dissociated into glucose and the amine, the amount of the compound that hydrolyzed was calculated. The specific rotation for the glucosyl-n-butylamine in water calculated from zero time in fig. 3 is $[\alpha]_D^{20}$ = -21.5°.

$$\alpha_e = \alpha_{glucose} + \alpha_{glucosyl-butylamine} \qquad (I)$$

At equilibrium, α_e = 0.48, and substituting known values into (I), the second equation is obtained:

$$0.48 = \frac{52.5° \times 2 \times (180/253)\ X}{25} + \frac{-21.50° \times 2 \times (0.5 - X)}{25} \qquad (II)$$

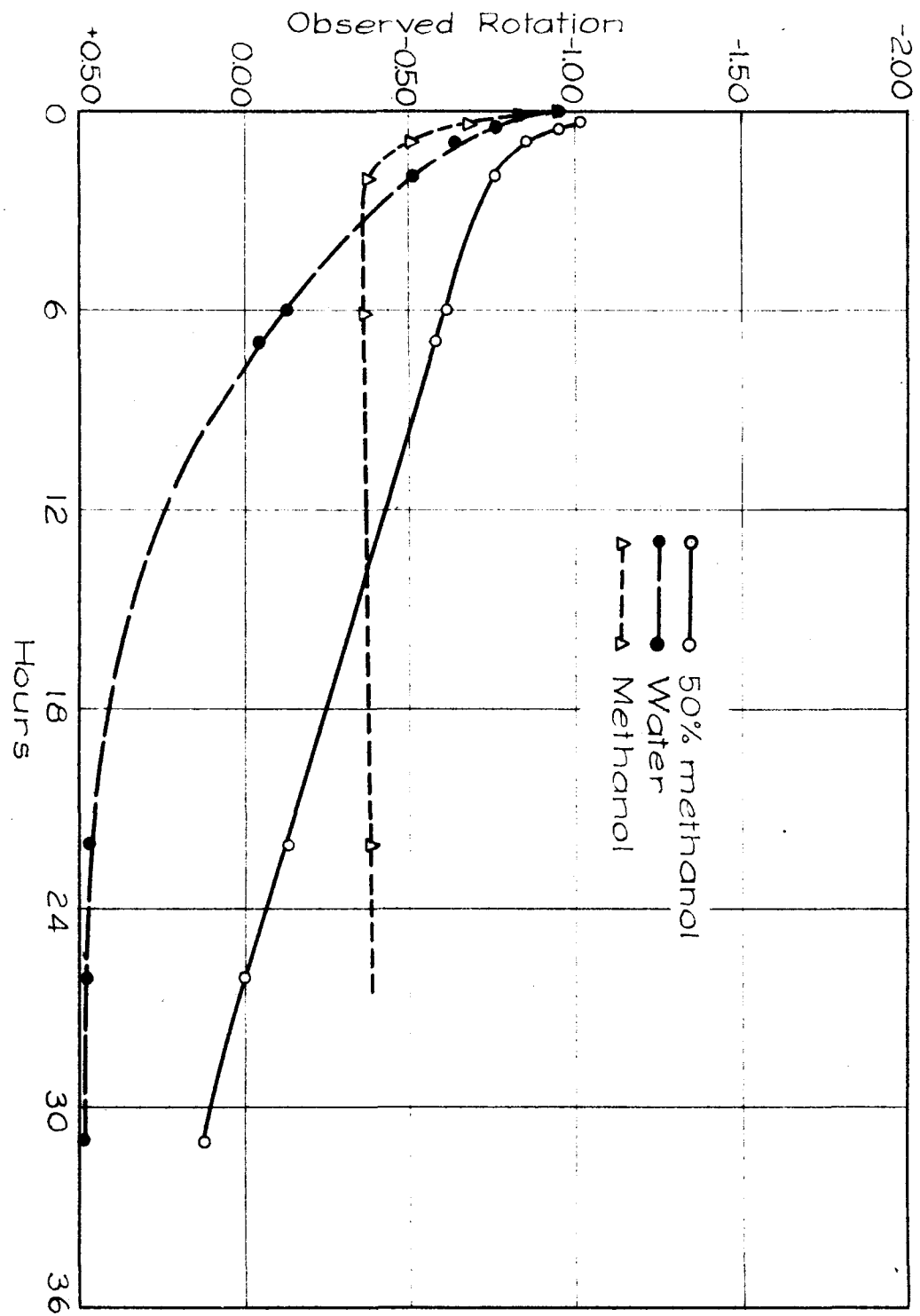Fig. 3 Change in rotation with time of glucosyl-n-butylamine
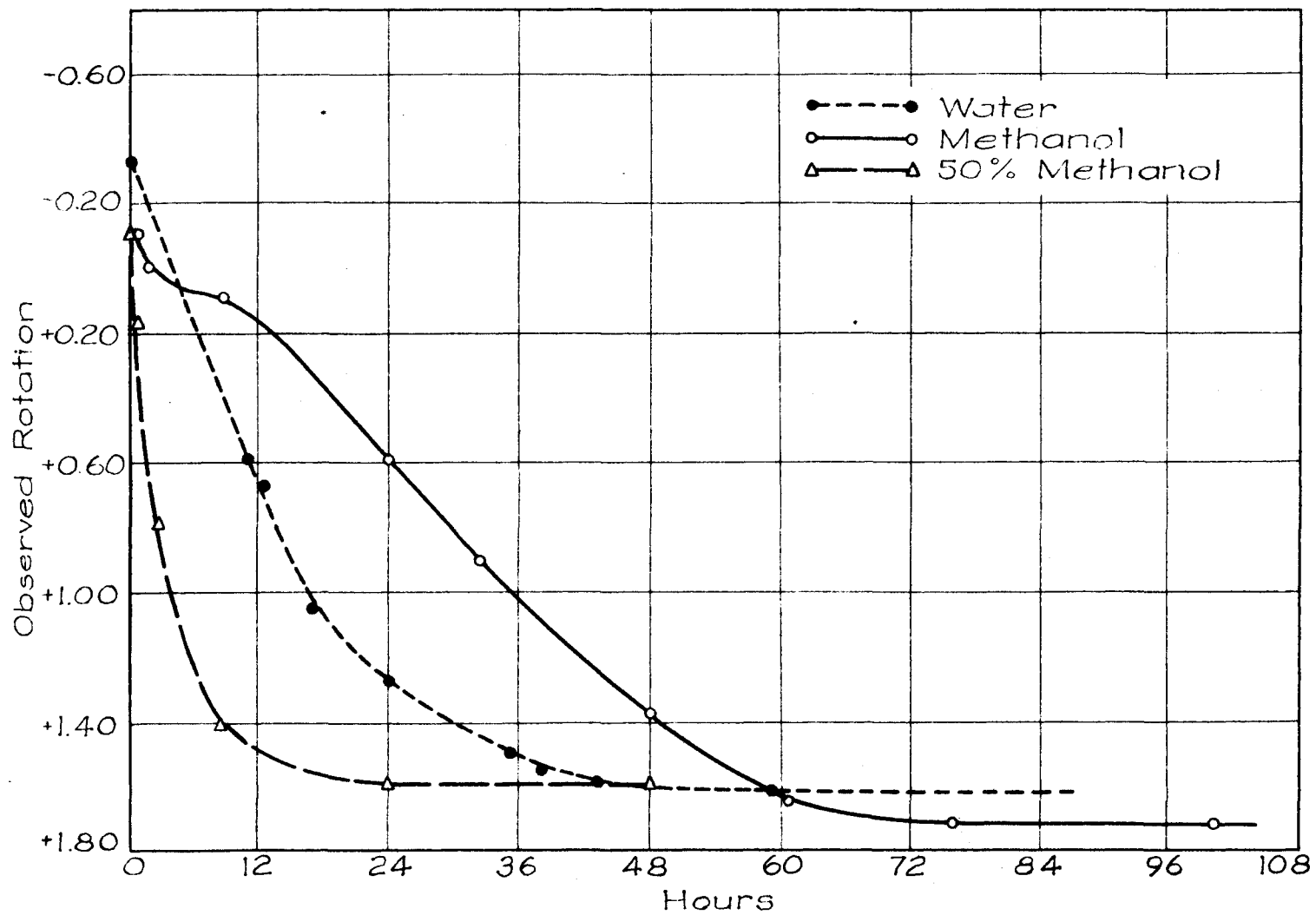
Fig. 4   Change of rotation with time of glucosyl-n-butylamine
acidified with HCl.

Fig. 5   Potentiometric titration of glucosyl-n-butylamine
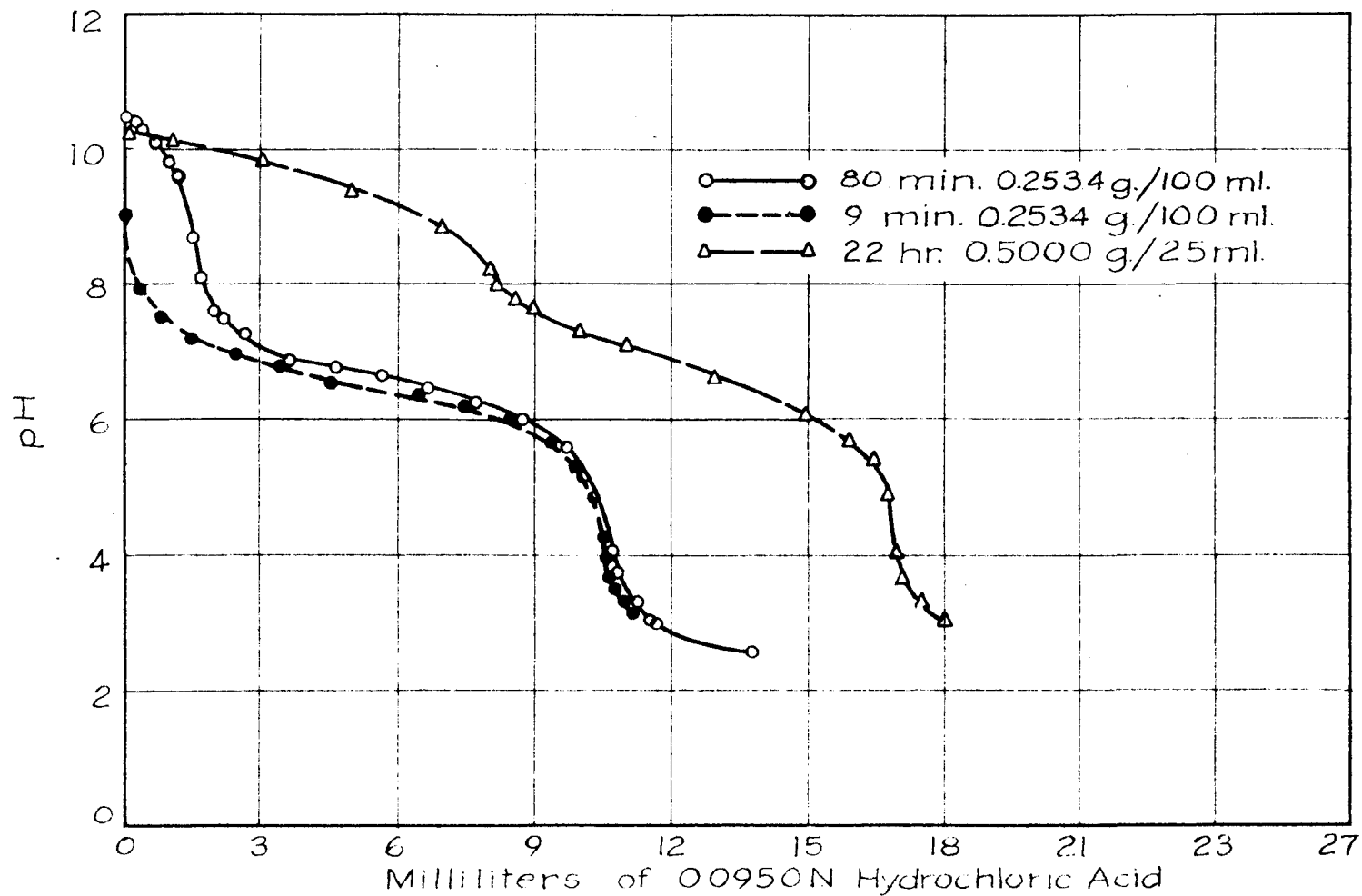
The legend within the figure reads:

○———○ 80 min. 0.2534 g./100 ml.
●----● 9 min. 0.2534 g./100 ml.
△----△ 22 hr. 0.5000 g/25 ml.

Axis labels: pH (vertical), Milliliters of 0.0950 N Hydrochloric Acid (horizontal)

Calculation of the amount of hydrolysis of the glucosyl-n-butylamine from equation II gave 56.9%. The value was considerably higher than 39.5%, as determined by potentiometric titration, fig. 5, of an equal amount of glucosyl-n-butylamine in 25 ml. of water at equilibrium after 22 hours. By extraction of the dissociated amine at equilibrium (0.5 g.) approximately 44% (8.90 ml. of 0.0950 acid used to neutralize extracted amine) of hydrolysis was calculated. The amount of butylamine in glucosyl-n-butylamine used in each experiment was equal and was determined by boiling a 0.5000 g. sample with 25 ml. of 0.1884 N sulfuric acid for one-half hour, then back titration of the excess acid with 29.00 ml. of 0.0955 N base to a methyl red end-point. The nitrogen content calculated from this experiment was 5.46%, which checked rather closely with the theoretical value of 5.53%.

It was evident from the different values obtained for the amount of hydrolysis by rotation and titration that negative mutarotation of the glucosyl-n-butylamine must have taken place as well as hydrolysis. Using the values for hydrolysis obtained by titration, calculations gave $[\alpha]_D^{20}$ = -4.4° for the mutarotated glucosyl-n-butylamine.

From the values for the rotation of the compound in methanol (fig. 3), it was observed that the rotation became constant in less than two hours. As there was no odor of butylamine and since the specific rotation at equilibrium possessed such a large negative value, it was concluded that

little, if any, hydrolysis occurred in absolute methanol.
From the curve for the change in optical rotation in 50% methanol, it appeared that rapid mutarotation occurred at first, followed by much slower hydrolysis.

The compounds evidently form salts, although hydrolysis in the presence of mineral acids is so rapid that no salts have been isolated. In fig. 4 the change in rotation of 0.5 g. glucosyl-n-butylamine, with one equivalent of hydrochloric acid added, was followed in methanol, 50% methanol, and water. It was observed that even in acid solution, the hydrolysis was not instantaneous; in fact, in aqueous solution, the hydrolysis required more than two days in order to be completed. On completion the rotation was approximately that calculated for the glucose liberated from 0.5 g. of the glucosyl-n-butylamine ($[\alpha]_D$ = +50.3° calculated on the glucose liberated). It was calculated that 20.84 ml. of 0.0950 N acid would be required to neutralize the liberated amine. On neutralization of the solution and extraction with ether, the extracted amine required 19.25 ml. of 0.0950 N acid for neutralization. This represented about 92% of the theoretical value.

The other glucosylalkylamines behaved in much the same manner as the n-butylamine compound and the values for rotation and potentiometric titration are given in fig. 9 and fig. 10. No data were obtained for the higher amine glucosides because of the low solubility in ordinary solvents at room temperature.

Attempted Amadori rearrangement of the glucosylalkylamines

The general method for the condensation of the glucosyl-
alkylamines given previously is conducive to the formation of
the rearranged compound for certain glucosyl aromatic amines.
Nevertheless, in no case was a compound formed from the alkyl-
amines and glucose that reduced methylene blue in dilute
alkaline solution.

In a further attempt to cause the rearrangement or par-
tial rearrangement of the glucosylalkylamines, 1 g. of glucose
was refluxed for three hours in 10 g. of butylamine. When one
ml. of the solution was diluted, a cloudiness appeared which
was removed on acidification. This solution decolorized methy-
lene blue in dilute alkali but not in proportion to the amount
of glucose present. Seven milliliters of the solution was
rapidly evaporated with suction on a water bath to remove the
excess n-butylamine and the dark residue was dissolved in
alcohol and diluted with ether. A gelatinous precipitate was
formed on standing from which on drying a small amount of
brownish needle-like crystals melting at 70-75° C. were ob-
tained. Although these crystals had the property of reducing
methylene blue not possessed by the pure glucoside, the
appearance and melting point as well as stability in aqueous
solution indicated that the crystals were for the most part
the glucoside.

The solution of 2-aminooctane condensation product that
had been refluxed and left for several days became quite dark

and showed a remarkable ability for reduction of methylene
blue, as did the glucose cyclohexylamine crystals that had
darkened on long standing. This property of reduction may be
from the action of the alkali on glucose itself rather than
as a result of the formation of a small amount of rearranged
glucoside.

Ammonia Derivatives of Glucose

Preparation of 1-aminoglucose

1-Aminoglucose, or glucose ammonia, was prepared by the
method of Ling and Nanji (82). To 40 ml. of 95% methanol
saturated with ammonia, 50 g. of commercial glucose was added.
The glucose dissolved in a few hours and at the end of two
weeks a few rosettes of crystals began to appear in the viscous
solution. Fifteen grams of white crystals was removed on
filtration of the liquid. This material was washed with meth-
anol and dried; the melting point was 120-121° C. Nitrogen
analysis by the Kjeldahl method on the crude product gave the
following results:

%N   Calc. for $C_6H_{15}O_6N$, 7.10%   Found, 7.12%
         for $C_6H_{13}O_5N$, 7.87%   7.32%

Fourteen grams of 1-aminoglucose was recovered from the mother
liquor and the rest of the solution discarded.

On recrystallization of the compound from 90-95% methanol,
crystals were obtained that had a melting point of 120-121° C.

Values ranging from $120°$ to $131°$ C. have been reported for the melting point of this compound. The crystals were usually recovered easily, but occasionally the material would not recrystallize, even when the solvent was partially removed by vacuum and the remaining solution was seeded. The same difficulty was encountered in the recrystallization of glucose oxime and the cyclohexylamine condensation product. The recrystallized material was analyzed in the following manner. To 0.3816 g. of 1-aminoglucose, 25 ml. of 0.1884 N acid was added, the solution heated for 30 minutes and cooled. For back-titration to a methyl red end-point, 29.40 ml. of 0.0955 N base was required. The per cent nitrogen calculated from these data was 7.01%. Using a 1 dm. tube (0.1001 g. in 1.694 ml. of water), the microrotation was determined, $[\alpha]_D^{25} =$ $-19.1°$. Values ranging from $-19.5°$ to $-22.6°$ have been reported for the rotation of 1-aminoglucose and glucose ammonia.

Hydrolysis of 1-aminoglucose

The stability of 1-aminoglucose in aqueous solution was demonstrated by the data shown in fig. 6. That ammonium hydroxide may be determined in the presence of 1-aminoglucose was shown by the titration curves of 5 ml. of ammonium hydroxide alone and 5 ml. of ammonium hydroxide added to 0.001 mole of 1-aminoglucose made up to 100 ml. of solution. The titration curve for 0.1791 g. (0.001 mole) of the compound in 100 ml of water showed that the glucose amine did not

dissociate appreciably into ammonia and glucose, even after several hours. This stability over a period of time was demonstrated by the titration curves of 0.001 mole of 1-aminoglucose that had been left for different lengths of time in aqueous solution. This observation was verified by the study of the change in rotation of 1-aminoglucose in fig. 10. When 0.1434 g. of 1-aminoglucose dissolved in 100 ml. of water there was no measurable change in rotation over a period of forty-eight hours. The titration curve for 60 ml. of this solution has been plotted in fig. 9. This curve also shows that no appreciable dissociation occurred. The data for fig. 10 have been placed in table 4.

Hydrolysis in the presence of acid was also quite slow. When a solution of 0.1791 g. of 1-aminoglucose in 100 ml. of water was partly neutralized and left over night, the pH of the solution had risen considerably by morning, but the curve, fig. 7, still showed that complete hydrolysis was not approached. With a similar sample the same conditions were used except that 0.535 g. of ammonium chloride was added. The first portion of the titration curve was at a lower pH than the other sample because of the acidity of the salt. Hydrolysis occurred in the presence of the ammonium salts, but the amount was hard to predict because of the buffer action of ammonium chloride.

A derivative of 1-aminoglucose was made and is described under the preparation of acyl derivatives of 1-aminoglucose.
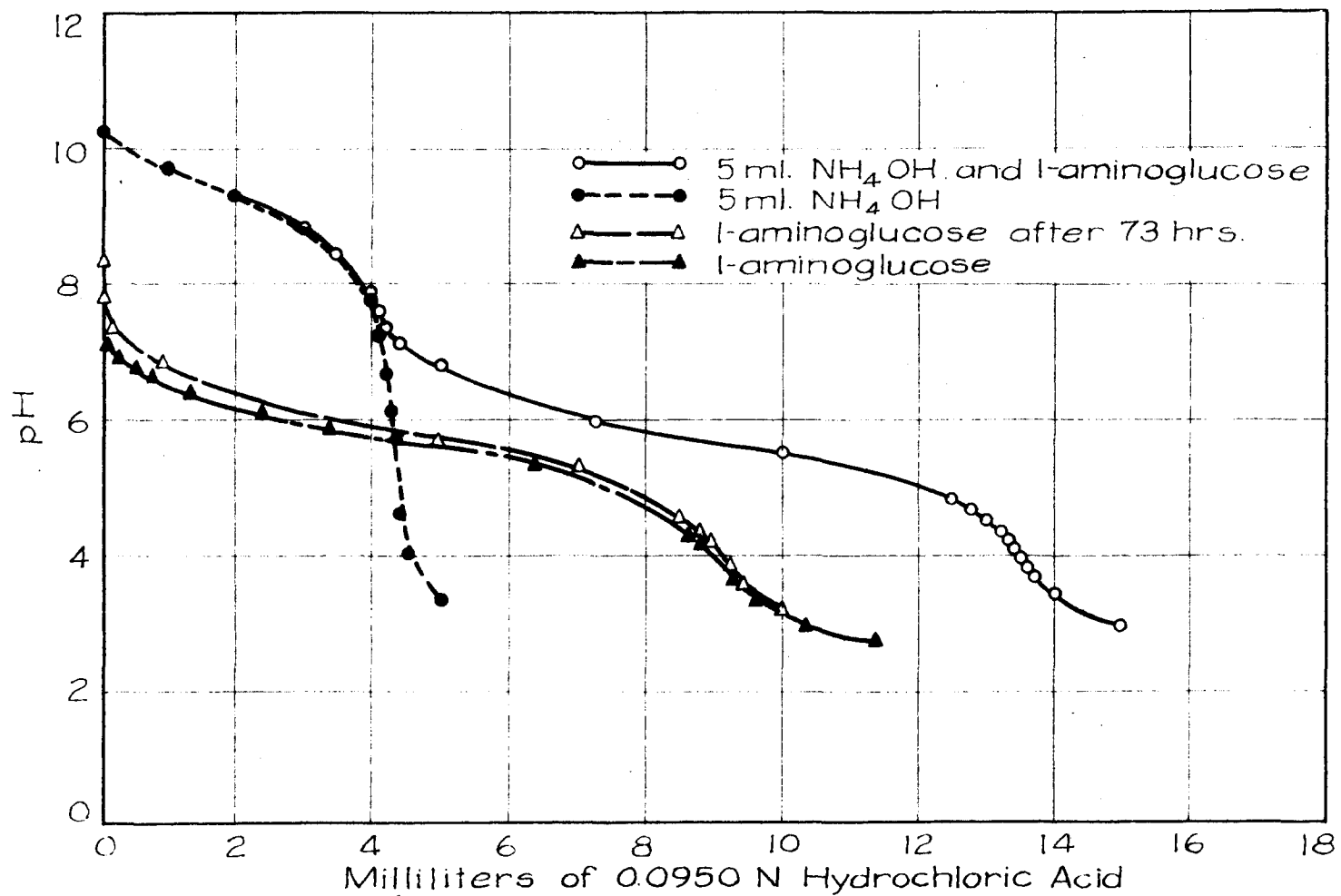
Fig. 6   Potentiometric titration of l-aminoglucose.

Legend:
- ○—○ 5 ml. NH₄OH and l-aminoglucose
- ●---● 5 ml. NH₄OH
- △—△ l-aminoglucose after 73 hrs.
- ▲---▲ l-aminoglucose

X-axis: Milliliters of 0.0950 N Hydrochloric Acid
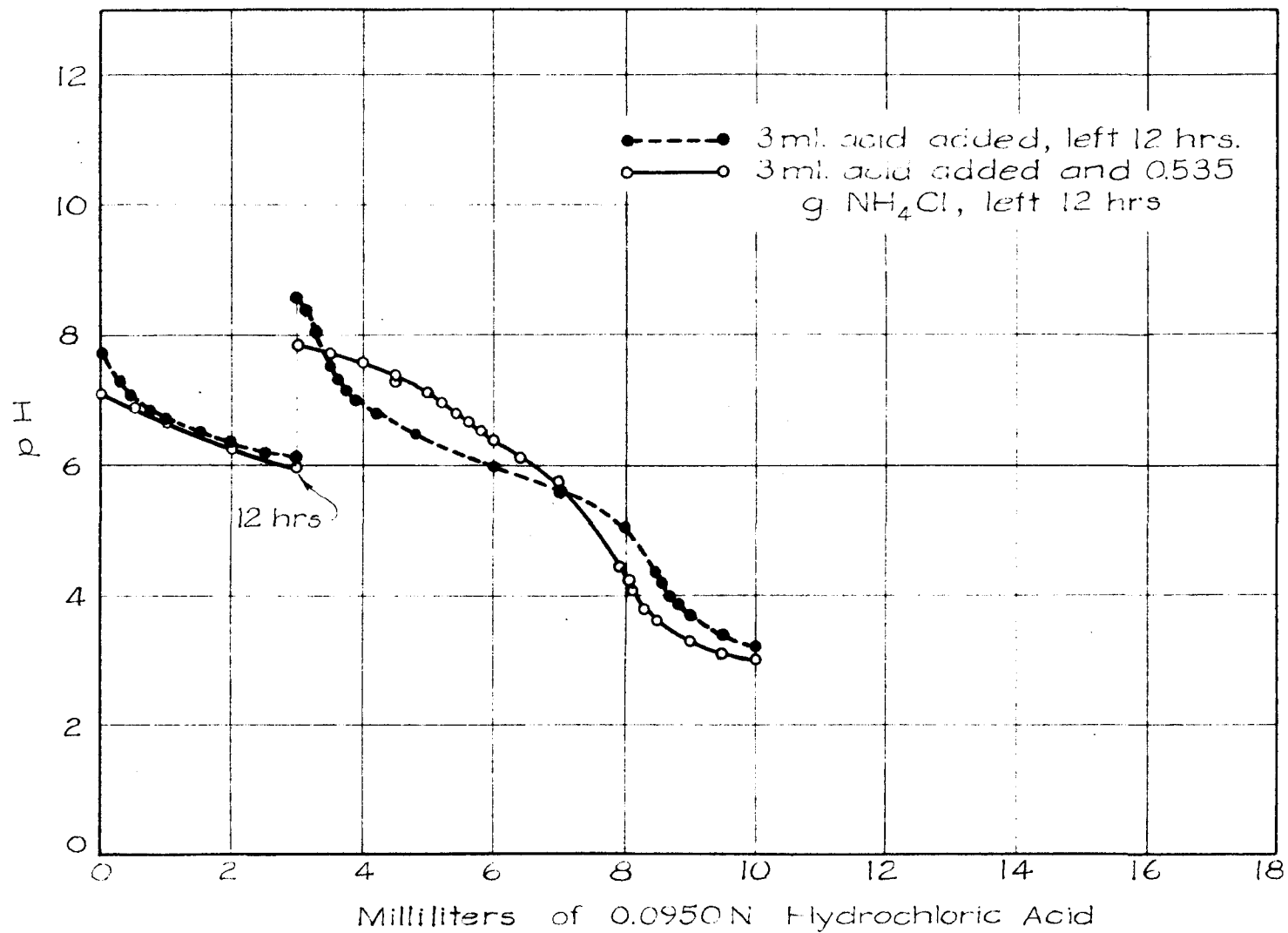Y-axis: pH

Fig. 7 Potentiometric titration of 1-aminoglucose left 12 hours in the presence of 3 ml. of acid.

Preparation of 2-aminoglucose

2-Aminoglucose was prepared from dried lobster shells by
the method of Hudson and Dale (50). The decalcified shells
were dreated by refluxing with concentrated hydrochloric acid
for six to eight hours. Norite was used to decolorize the
solution. After filtration the hydrochloric acid solution was
concentrated to a small volume by distillation under reduced
pressure at 60-70° C. Pure white crystals of 2-aminoglucose
hydrochloride were obtained after filtering the precipitate
and washing with 70% ethanol. The hydrochloride had an
$[\alpha]_D^{21}$ = +72.1° (0.5023 g. in 25 ml. of water, 2 dm. tube,
α = +2.92) as compared to +72.5° reported in the literature.
The titration curve for the neutralization of 0.3259 g. with
0.0882 N sodium hydroxide showed that 17.11 ml. was required.
The calculated amount of sodium hydroxide was 17.14 ml.

The free amine was prepared by the method of Breuer (18)
by shaking 5 g. of the hydrochloride with 2.5 g. of diethyl-
amine in 60 ml. of absolute alcohol. After twenty-four hours
the free amine was removed by filtration, washed with alcohol
and a bit more diethylamine added to an alcoholic solution
and the shaking repeated. This procedure was repeated until
no test for chloride ion was obtained in the alcoholic wash-
ings. The melting point of the dry crystals was 107-110° C.
The hydrochloride was perfectly stable and the dry amine did
not decompose when left in a vacuum desiccator for two months.

Attempted preparation of the p-toluidide and the N-tolyl-d-iso-glucosamine from 2-aminoglucose

An attempt was made to prepare the toluidide from 2-amino-glucose hydrochloride by refluxing 1.5 g. of p-toluidine with 2 g. of the hydrochloride in 25 ml. of methanol. The hydro-chloride was so insoluble in methanol that no reaction occurred, and 1.2 g. of the hydrochloride, $[\alpha]_D^{20} = +72°$, was recovered.

One gram of the free amine was then heated with 0.8 g. of p-toluidine in 25 ml. of methanol. The amino sugar went into solution at once, and an odor of ammonia was evident. After ten minutes the solution was cooled and allowed to stand over night. After several days, 0.3 g. of a yellow, partly crystalline material was obtained that melted with softening around 130° C. It was not basic, showing that the amino group had been removed from the 2-position. No toluidide of glucose was obtained.

One gram of 2-aminoglucose was heated with 0.8 g. of p-toluidine, 25 ml. of water and 0.05 ml. of acetic acid. The solution was homogeneous after twenty minutes, but the solution became very dark. Heating for an hour resulted in a tarry mass from which no N-p-tolyl-d-isoglucosamine could be isolated. This dark solution reduced methylene blue readily, however.

Amide Derivatives of Glucose

Preparation of the N-acyl derivatives of l-aminoglucose

l-Aminoglucose pentaacetate. l-Aminoglucose penta-
acetate was made according to the method of Brigl and Keppler
(19). To 12 g. of l-aminoglucose 125 ml. of pyridine and 45
ml. of acetic anhydride were added. The mixture was kept in
the refrigerator for forty-eight hours, then poured into an
equal volume of chloroform. The solution was extracted sev-
eral times with a sodium carbonate solution to remove the
excess acetic anhydride and dried. The chloroform was re-
moved at 40° C. under reduced pressure and the residue dried
over sulfuric acid in a vacuum desiccator. This residue was
dissolved in 80 ml. of chloroform and filtered. A double
volume of petroleum ether was added and the mixture left at
room temperature for twenty-four hours. The crystals were
filtered off, washed with methanol and dried. The crystals
were recrystallized from 97% methanol and the white rhombic
crystals melted at 162° C. Ten grams of the recrystallized
material was obtained. Upon recrystallization from
chloroform-ether, needle-like crystals were obtained that
melted at 162° C.

N-Acetyl-l-aminoglucose. The monoacetyl compound was
made from the pentaacetate by the method of Brigl and Keppler
(19). Five grams of pentaacetyl-l-aminoglucose was added to

a saturated ammoniacal solution of 60 ml. of methanol. The solution was saturated with ammonia at room temperature, then cooled before the sugar derivative was added. All of the pentaacetyl-1-aminoglucose dissolved after a few minutes and a precipitate formed about ten minutes later. Brigl and Keppler did not obtain a precipitate until the solution had stood three hours and was diluted with ether. After three hours the precipitate was removed and the mother liquor was diluted with 300 ml. of ether. Only a small amount of the feathery needles reported by Brigl and Keppler precipitated after dilution. The crystals that were first precipitated had the same crystalline form as the pentaacetyl-1-aminoglucose, but upon melting, the compound sintered about 237° C. and completely melted by 257° C. The specific rotation of 1.5 g. in 120 ml. of aqueous solution was $[\alpha]_D^{25} = -21.3^\circ$ (2 dm. tube, $\alpha = -0.53$). A mixed melting point of the feathery crystals and the rhombic crystals gave no depression of the melting point. This compound was reported to have a specific rotation of $[\alpha]_D^{25} = -22^\circ$ $\longrightarrow -23^\circ$, and to melt at 257° C. with darkening about 230° C.

Hydrolysis of N-acetyl-1-aminoglucose

This compound was quite stable to hydrolysis as shown in fig. 8, where hydrolysis was followed by change in rotation.

To effect the hydrolysis, 120 ml. of 1 N sulfuric acid was added to 1.500 g. of N-acetyl-1-aminoglucose. No change in rotation was observed until the compound was refluxed with
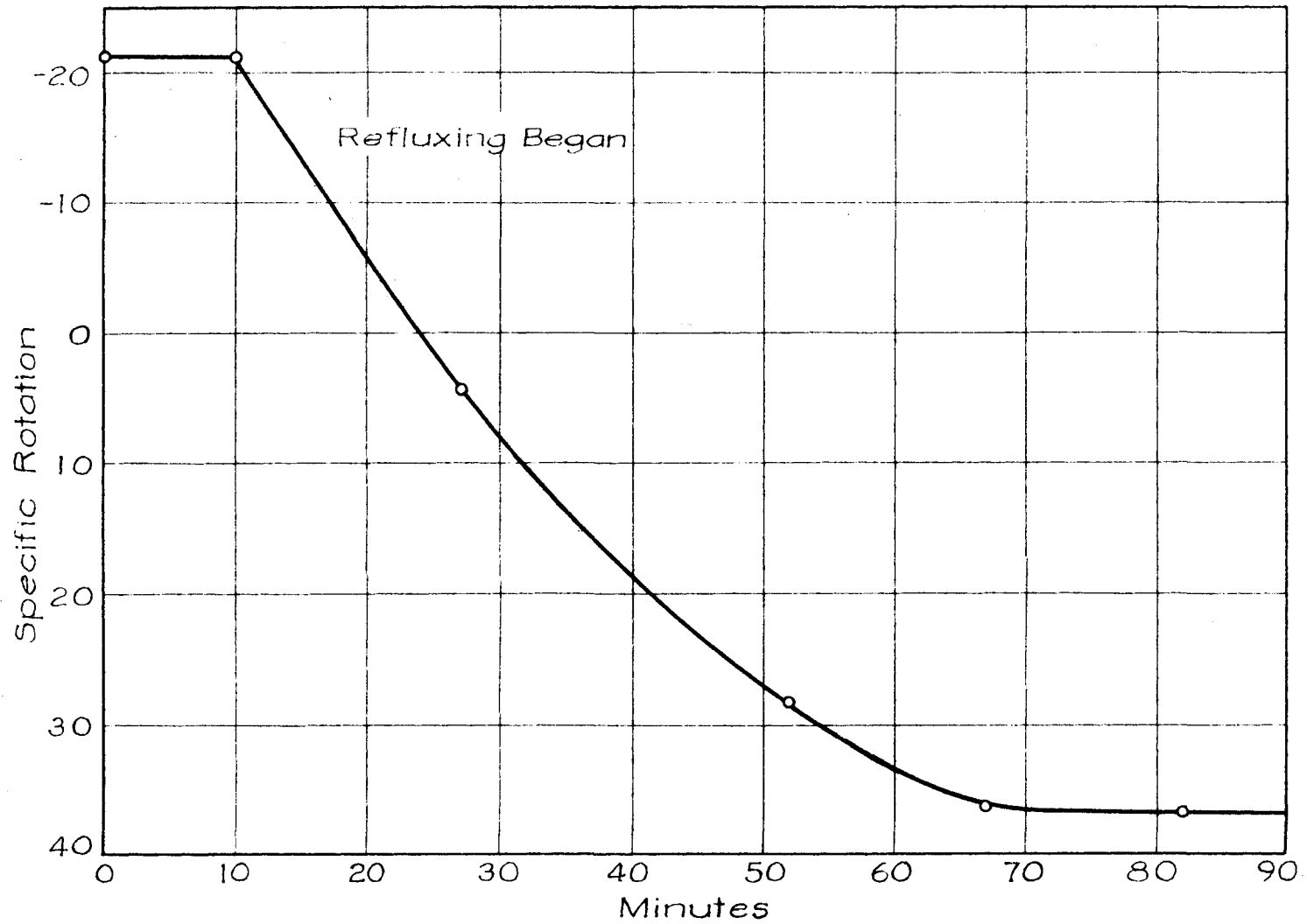
Fig. 8   Change in rotation of N-acetyl-l-aminoglucose with time on refluxing with IN sulfuric acid.

1 N acid. The value for the rotation became constant after fifty-seven minutes of refluxing. The value did not reach the value of the glucose liberated, since the specific rotation calculated from the assumption that glucose was liberated gave a value of +44.7°.

The stability of N-acetyl-1-aminoglucose was further demonstrated when 1 g. was dissolved in 30 ml. of 83% ethanol and 4 ml. of 0.5 N hydrochloric acid was added. The solution was refluxed for two hours and on cooling 0.68 g. or 68% of the starting material was recovered. The solution gave no decolorization of dilute methylene blue, and no evidence of a rearrangement was noted.

Since the compound formed was so stable to hydrolysis, an attempt was made to condense acetamide with glucose.

Attempted condensation of acetamide with glucose and acetobromoglucose

Two grams (0.034 mole) of acetamide was added to 6 g. of glucose (0.033 mole) in 100 ml. of methanol. After a few minutes the glucose went into solution. After refluxing two hours most of the methanol was removed by distillation. The glucose still did not precipitate. In a similar experiment the same amount of material was used except that the amount of methanol was reduced to 25 ml. The solution was still effected in a very short time. This solution after a short refluxing was seeded with the N-acetyl-1-aminoglucose, but no

crystals were obtained. An attempted condensation was made using the acetamide and glucose but no solvent. The solution was heated for one hour and then 25 ml. of methanol was added. Some of the glucose had precipitated by morning, but no other crystals were obtained. The ease of solution could not be accounted for by the polar nature of acetamide alone, since water has a higher dielectric constant and the solubility of glucose in 98% methanol is slight.

A condensation between acetobromoglucose and acetamide was attempted. Ten grams of acetobromoglucose (0.021 mole), made by the method of Fischer (35), was heated under reflux with 3 g. (0.051 mole) of acetamide in 20 ml. of chloroform. After thirty minutes crystals appeared in the solution, which, upon analysis of the unpurified product for bromine, were proved to be $(CH_3CONH_2)_2 \cdot HBr$.

%Br   Calc. for $(CH_3CONH_2)_2HBr$, 40.0%   Found, 41.31%
                                                          41.80%

The melting point was 138-139° C. with sublimation, about 95-100° C. The reported melting point for the hydrobromide of acetamide was 139° C.

After this product was identified, another gram (0.017 mole) of acetamide was added and the solution refluxed for one hour. The crystals were removed by filtration, and the chloroform solution was diluted with an equal volume of petroleum ether. Two layers separated and crystals of the acetamide hydrobromide appeared in the bottom layer after a

few days. The solution was evaporated to a small volume, leaving a syrupy substance mixed with crystals of the acetamide hydrobromide. Three grams of the bromide was recovered. The syrupy layer was diluted with chloroform after separation of the crystals. A very small amount of crystals that had an indefinite melting point about 170-190° C. was obtained, but not enough of the crystals were isolated for analysis. For the most part the syrup could not be crystallized. The residue was stable in air for two months, a fact which showed that no acetobromoglucose was present as such, since it decomposed readily in air. From the results it appeared that the pentaacetyl derivative was not obtained, at least not with the structure of the pentaacetyl-1-aminoglucose described, as it should have crystallized under the above conditions, especially since the syrup was seeded with a crystal of pentaacetyl-1-aminoglucose.

Aromatic Amine Derivatives of Glucose

Preparation of aryl-N-glucosides and N-aryl-d-isoglucosamine

p-Toluidine-d-glucoside. Ten grams of glucose was heated with 8 g. of p-toluidine and 3 ml. of water. The mixture became homogeneous after a few minutes and the solution was heated for five minutes more to insure complete reaction. After the solution was cooled 5 g. of white needles were obtained, melting at 115-116° C., as reported by Weygand (126).

No attempt was made to recover another crop of crystals from the solution.

N-p-tolyl-d-isoglucosamine. The N-p-tolyl-d-isoglucos-amine was made by the method of Weygand (126) by refluxing 10 g. of glucose with 8 g. of p-toluidine, 2.5 ml. of water, and 0.5 ml. of 2 N acetic acid. Twenty milliliters of ethanol was added, and the dark solution was left in the refrigerator over night. Six grams of platelets that melted 152-153° C. was obtained. On further crystallization of the mother liquor, a crop of the low-melting needles was recovered. These showed a slight reduction of methylene blue, but on recrystallization no decolorization of the reagent was produced by the needles. These crystals melted at 115-116° C., and gave no depression of the melting point when mixed with the p-toluidide described above. One drop of the mother liquor, however, caused pro-nounced reduction of methylene blue.

o-Toluidide-d-glucoside. The o-toluidide was also pre-pared by a method of procedure exactly like the p-toluidide except that double the amount was used. The solution did not become homogeneous until it had been refluxed for forty-five minutes, after which the solution was heated fifteen minutes longer to complete the reaction. Fifty milliliters of ethanol and 10 ml. of water were added and the solution was left over night to crystallize. No crystals were obtained after forty-eight hours; so part of the alcohol was removed, and crystals of the toluidide appeared. After the crystals were filtered

and washed, 19 g. was obtained. This represented a 63% yield as compared to the 47% yield reported by Weygand (123).

An attempt was made to obtain the rearranged product of this compound by the method used for the rearrangement of the p-toluidide. The crystals obtained were the N-glucoside, however, and showed no reduction of methylene blue.

Hydrolysis of the N-glucosides

On hydrolysis of the N-glucoside, dilute acid gave the toluidide and glucose. In aqueous solution the change in the specific rotation of 0.002 mole of the N-glucoside and 0.002 mole of N-p-tolyl-d-isoglucosamine, each in 250 ml. of water, was followed. The N-p-tolyl-d-isoglucosamine was less soluble in water, and the rotation changed only slightly. The p-toluidide of glucose showed a change of rotation that approached a constant after about 90 hours, but the change of rotation in the closed polarimeter tube was not equal to that of the solution left in the flask in air. Evidently there was an oxidation or a shift in structure due to the presence of some constituent in the air. This change was also evident in the slight darkening of the solution after a few days. The solution of the N-p-tolyl-d-isoglucosamine was quite dark in color after two days. The equilibrium of the N-glucoside with the amine and glucose could not be determined by potentiometric titration since the pH of the solution was about 6.8 at the initial reading and no sudden drop in the pH could be

observed at the end-point. Hydrolysis of the N-glucoside had occurred to some extent since the odor of p-toluidine was evident after a few days. The equilibrium was determined by extracting the free amine with ether, drying the ether extract with anhydrous sodium sulfate, filtering and evaporating the solvent at room temperature. The dried residue weighed 0.0185 g. This amount was extracted from 0.002 mole in 250 ml. of water and represented about 8% hydrolysis.

The mutarotation and hydrolysis of the glucosylalkylamines, the N-glucoside of p-toluidine, the N-p-tolyl-d-isoglucosamine, and l-aminoglucose in aqueous solutions were followed by change in specific rotation. In each case the amount used is indicated in table 4. Although the dilute solutions have relatively low rotations, a concentration of 0.002 mole in 250 ml. of solution was selected to obtain a basis of comparison. The solubility of the compounds in water prevented the selection of a higher concentration. The change in specific rotation of these compounds was plotted against time in fig. 10.

Table 4

Change in Rotation with Time of Some Glucosides

Compound	Concentration	Observed rotation	Specific rotation 2 dm. tube	Time (hrs.)
Glucosyl-n-butylamine	0.5036 g. in 250 ml.	-0.06	-13	0.17
		-0.03	- 7	1
		+0.08	+17	7
		+0.09	+20	24
		+0.09	+20	31
Glucosyl-n-amylamine	0.5365 g. in 250 ml.	-0.03	- 7	0.5
		-0.02	- 5	1
		+0.02	+ 5	4
		+0.09	+21	12
		+0.11	+26	24
		+0.11	+26	36
Glucosyl-n-heptylamine*	0.5904 g. in 250 ml.	+0.08	+17	1
		+0.09	+19	1.8
		+0.09	+19	7
		+0.10	+21	24
1-Aminoglucose	0.1434 g. in 100 ml.	+0.07	+24	0.17
		+0.07	+24	0.5
		+0.07	+24	7
		+0.07	+24	24
		+0.07	+24	42
p-Toluidine-d-glucose	0.5312 g. in 250 ml. closed tube	-0.35	-83	0.05
		-0.32	-75	0.63
		-0.30	-71	1
		-0.28	-66	4
		-0.28	-66	24
		-0.27	-64	36
		-0.26	-62	65
		-0.25	-60	87
p-Toluidine-d-glucose	0.5312 g. in 250 ml. open to air	-0.35	-83	0.05
		-0.17	-40	4
		-0.07	-15	40
		-0.03	- 2	48
		+0.02	+ 5	65
		+0.05	+12	87
N-Tolyl-d-iso-glucosamine	0.5328 g. in 250 ml.	-0.26	-61	0.75
		-0.22	-52	1
		-0.21	-50	12
		-0.20	-47	62

*Heated to get into solution.
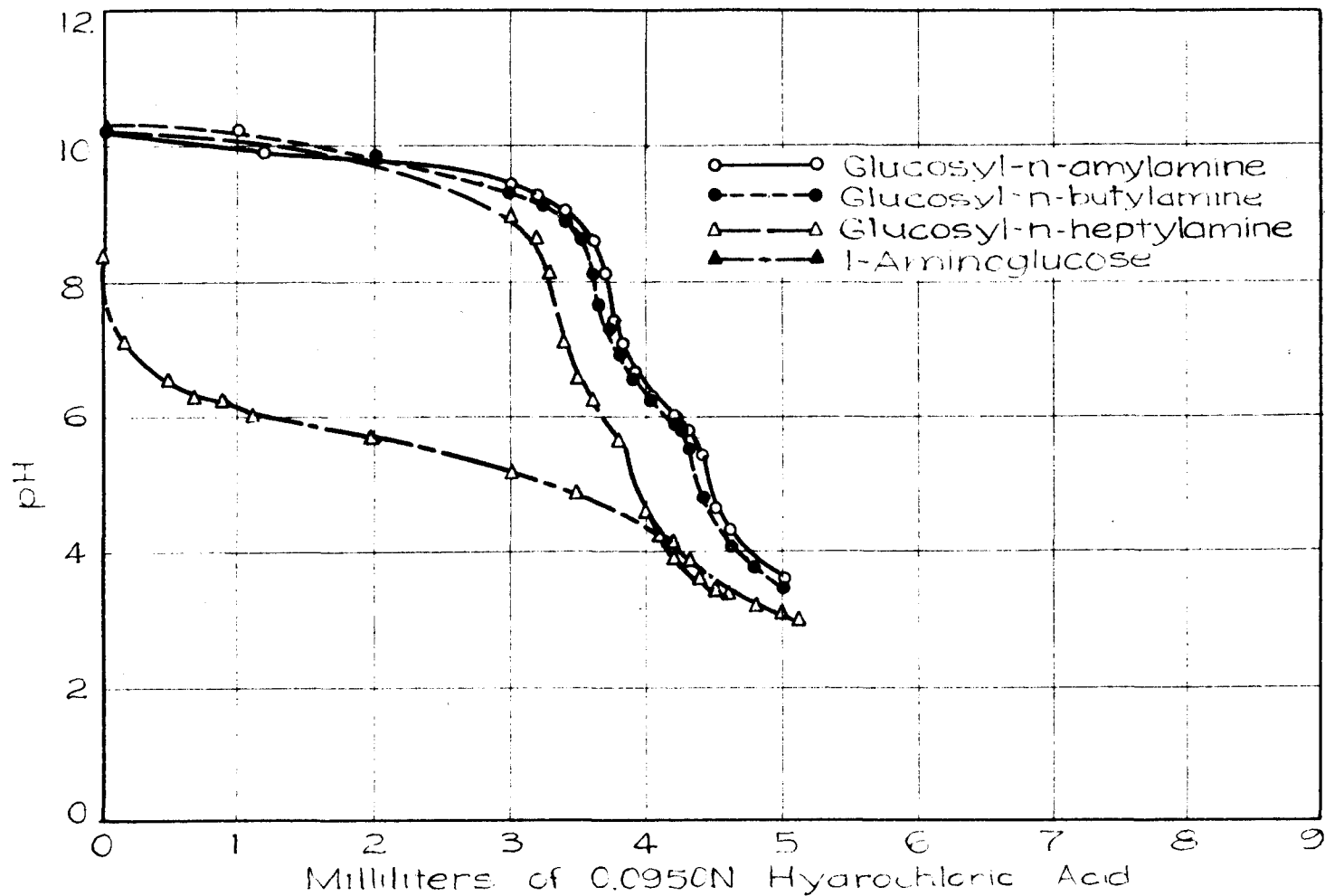
Fig. 9 Potentiometric titration of 0.002 moles of glucoside in 250 ml. H₂O at equilibrium. 60 ml. portions used.
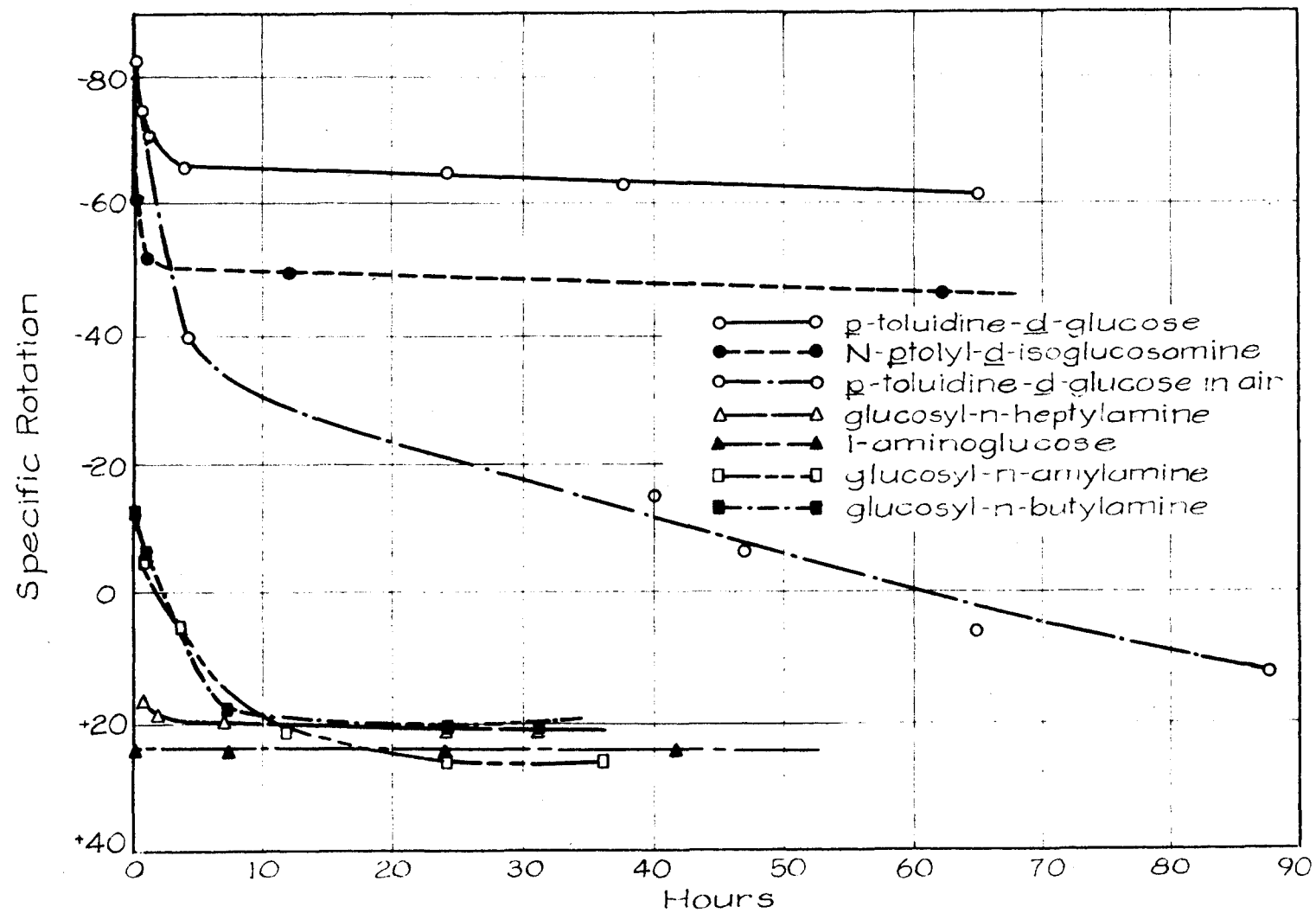
Fig. 10   Change in rotation with time of some N-glucosides.

Legend (right of plot):

○——○ p-toluidine-d-glucose
●----● N-ptolyl-d-isoglucosomine
○-·-○ p-toluidine-d-glucose in air
△——△ glucosyl-n-heptylamine
▲-·-▲ l-aminoglucose
□----□ glucosyl-n-amylamine
■-·-■ glucosyl-n-butylamine

Y-axis: Specific Rotation (-80, -60, -40, -20, 0, +20, +40)
X-axis: Hours (0, 10, 20, 30, 40, 50, 60, 70, 80, 90)

In fig. 9, the equilibrium of the glucosylalkylamines and 1-aminoglucose was also determined by titration. Curves are plotted from pH obtained on titration of 60 ml. of the above solutions with 0.0950 N hydrochloric acid. The glucosyl-alkylamines showed pronounced hydrolysis, about 67% in the amyl- and 69% in the butylamine compound; the heptylamine derivative was limited by difficulty of solubility. The dissociation of ammonia from 1-aminoglucose appeared to be practically negligible.

Amine Derivatives of 2-Methylglucose

Phenylhydrazine derivative

The phenylhydrazine derivative of 2-methylglucose was prepared by dissolving 0.55 g. of the monosaccharide, with a melting point 150-155° C., in 1.65 g. of phenylhydrazine diluted with 1 ml. of water. To this solution 1 drop of glacial acetic acid was added, the mixture shaken until the glucose dissolved, and the mixture allowed to stand over night at room temperature. After sixteen hours, no crystals had appeared in the reddish-brown liquid; so the sides of the flask were scratched to promote crystallization. In a few hours the entire solution had solidified. The small amount of solvent in the crystals was removed by suction, and the nearly white broad needles washed with anhydrous ether and dried. A yield of 90% (0.80 g.) of the phenylhydrazine

derivative, with a melting point of 176-177° C., was obtained.

A methoxyl determination by the micro-Zeisel method gave 11.1% OCH$_3$ as compared to calculated value of 10.93%.

Phenylosazone

Glucose phenylosazone was prepared from 2-methylglucose by refluxing for one and one-half hours 2.55 g. of phenyl-hydrazine and 0.75 g. of 2-methylglucose in 37 ml. of water containing 2.5 ml. of glacial acetic acid. The 2-methylglucose used melted at 152-155° C. and had a methoxyl value of 15.35%. After the solution was heated on the water bath for approximately one-half hour, a flocculent precipitate appeared. The solution was heated one hour longer, cooled and filtered. The dark brown color of the precipitate was removed by washing with a small amount of methanol. After drying 0.275 g. of yellow needles was procured which melted at 208-210° C. A sample of this osazone mixed with a sample of glucosazone gave no depression of the melting point.

The mother liquor, on additional heating for two hours, yielded 0.075 g. of the glucose phenylosazone. The yield of the osazone was about 25.4% of the theoretical value and could not have been produced from glucose in the 2-methylglucose since the methoxyl value of the monosaccharide would have been less than 12% if it had had 0.18 g. of glucose present. (The value 0.18 g. of glucose was found by calculation from the osazone, assuming 100% conversion.)

Analysis of the compound for nitrogen and methoxyl was made.

%N   Calc. for $C_{18}H_{22}O_4N_4$, 15.64%   Found, 15.93%
                                                         (Micro-Dumas)
%OCH$_3$ for $C_{18}H_{22}O_4N_4$, 0.00%   0.40%
                                                      (Micro-Zeisel)

For a further check on the removal of the methoxyl group the phenylosazone was made from the phenylhydrazine derivative of 2-methylglucose. To 0.375 g. of the phenylhydrazine derivative of 2-methylglucose, 0.75 g. of phenylhydrazine dissolved in 12 ml. of water containing 0.85 ml. of glacial acetic acid was added. The crystals dissolved on heating in a water bath and the solution became yellow. A small amount of flocculent yellow precipitate was first observed after forty-five minutes of refluxing. After the solution was refluxed two hours it was cooled and filtered; 0.030 g. of light yellow needles, melting 208-210° C., were recovered. The mother liquor was heated for one and one-half hours longer, and 0.065 g. was obtained. A methoxyl determination was made by the micro-Zeisel method.

% OCH$_3$ Calc. for $C_{18}H_{22}O_4N_4$, 0.0%   Found, 0.40%

p-Toluidide of 2-methylglucose

The p-toluidide of 2-methylglucose (methoxyl content 15.27%) was prepared by heating 1.04 g. of the methyl sugar with 0.72 g. of p-toluidine and 0.30 ml. of water. The mixture was heated on the water bath until the solution became

homogeneous. This required fifteen to twenty minutes, and the solution was heated ten minutes thereafter to insure complete reaction. To the dark brown solution 2 ml. of 95% ethanol was added, and the solution was placed in the refrigerator over night. As no crystals had appeared after forty-eight hours, 4 ml. of ether was added. A precipitate, which weighed 0.15 g. was recovered. The dried material melted at 145-155° C. and gave no depression of melting point when mixed with a sample of 2-methylglucose.

The solution was then evaporated to a volume of approximately 1.5 ml. and left in the refrigerator over night. Needle-like crystals of the toluidide appeared by morning, which on filtering, washing with a small amount of ethanol, then ether, and drying, gave a melting point of 144-145° C. Only 0.08 g. of these needles was obtained from the first crop, but an additional 0.47 g. was recovered from the mother liquor on the second recrystallization. By repeated recovery from the mother liquor, a yield of 0.35 g. was obtained. The recrystallized p-toluidide gave a melting point of 150-151° C.

Analysis for the methoxyl content was made by the micro-Zeisel method.

% OCH_3 Calc. for $C_{14}H_{21}O_5N$, 10.95%   Found, 10.7%.

Attempted rearrangement of the p-toluidide

In an attempt to effect a rearrangement of the p-toluidide of 2-methylglucose and concurrent removal of the

methoxyl group, 1 g. of 2-methylglucose, melting point 150-153° C., was heated on a boiling water bath with 0.72 g. of p-toluidine, 0.25 ml. of water, and 0.05 ml. of 2 N acetic acid. The mixture became homogeneous after a few minutes and soon the glucoside of the p-toluidine crystallized as a solid mass. One milliliter of absolute alcohol was added; the crystals dissolved, and the solution was heated for one and one-half hours. At this time the dark solution was tested for reducing ability with methylene blue. One drop showed a slight reduction for methylene blue. After two days a pre-cipitate was obtained that looked like the p-toluidide of 2-methylglucose; so a few crystals were removed, washed and dried. These crystals melted at 150-151° C. The crystals gave no depression of melting point on mixing with a sample of the previously prepared N-glucoside. The purified crys-tals gave no reduction with alkaline methylene blue.

To the remainder of the crystals 0.05 ml. of 2N hydro-chloric acid and 1 ml. of ethanol were added. This mixture was gently refluxed for 2 hours and after evaporation to half volume, 0.20 g. of a precipitate was obtained that melted at 149-150° C. These crystals gave no depression of the melting point when mixed with a known sample of the p-toluidide. The mother liquor was further concentrated, and 0.10 g. of the toluidide was recovered from this source. Analysis of the crystals melting at 150-151° C. was made.

| % OCH$_3$ | Calc. for C$_{14}$H$_{21}$O$_5$N, 10.95% | Found, 10.70% |
| % N | Calc. for C$_{14}$H$_{21}$O$_5$N, 4.95% | Found, 4.83% |

No crystals of p-tolyl-d-isoglucosamine were obtained,
even when the solution was seeded with a sample of the crystals.
From this it appeared that the Amadori rearrangement had not
occurred and that more than a rearrangement was necessary in
order that the methoxyl group be removed.

DISCUSSION OF RESULTS AND CONCLUSIONS

The purpose of this investigation was to study the reaction of glucose with a number of amines and to correlate the isolated investigations reported in the literature concerning certain amines and glucose. The nitrogen derivatives of the carbohydrates are of particular importance in biological investigations. Coenzyme I or cozymase has been identified as a nicotinamide-ribose-(phosphate)$_2$-ribose-adenine and Coenzyme II as the triphosphopyridine nucleotide (105). Vitamin B_2 or riboflavin is an aromatic amine condensation product of d-ribose that has been reduced and reacted further to form an iso-alloxazine ring. In order to understand the behavior of the more complicated nitrogen derivatives of the carbohydrates such as these oxidation enzymes, vitamins and nucleic acids, it is expedient that derivatives formed from simpler amines with glucose be studied.

The tendency for all of the substituted ammonia derivatives to exist as glucosides is quite pronounced. The only exception to this generalization found in the literature is the β-hydrazone reported by Behrend (15) and the structure of this compound as a true hydrazone has been questioned (130). The nitrogen glucosides vary as to the amount of hydrolysis in water, but all hydrolyze in the presence of dilute acids.

It has been shown that the glucosylarylamines, when heated above their melting points or catalyzed by a trace of acid in alcoholic or concentrated aqueous solution, undergo a rearrangement known as the Amadori rearrangement. It has been proposed that the shift of the two hydrogen atoms from carbon two is accomplished through enolization of the glucosylarylamine salt and subsequent formation of a 2-keto carbon atom. The possibility of effecting this rearrangement in N-substituted aminoglucose other than the aryl type was investigated. Under the conditions that produced the N-aryl isoglucosamines, the alkylamines gave only the N-glucosides. In fact, this procedure was found to be the best method for the preparation of the n-butylamine, n-heptylamine, n-amylamine, and the dicyclohexylamine condensation products. It is interesting to note that the above compounds formed in every case without elimination of a molecule of water, even though the compounds were recrystallized from a mixture of ethanol and Skelly D. The removal of this water of hydration could not be accomplished by heating at 100° C., since the compounds darkened and decomposed at much lower temperatures. No removal of the extra molecule of water was accomplished by heating the crystals in an Abderhalden drying pistol with phosphorus pentoxide for five hours at the boiling point of acetone. Longer heating was impossible, since the compounds began to darken slightly.

A further attempt to effect the rearrangement was made

by heating glucose in an excess of n-butylamine for several hours. After the refluxing, the golden-colored liquid decolorized methylene blue rapidly in dilute alkaline solution, but the only compound obtained from the solution after removal of the excess butylamine was identified as the N-glucoside.

Noticeable reduction of methylene blue (as a test for the keto form) was observed especially with the solution of the 2-aminooctane condensation product. After the solution had been left in the refrigerator for several days and had become quite dark, a small amount of impure precipitate, soluble in water but insoluble in organic solvents, was obtained which reduced methylene blue readily. However, upon washing with alcohol, the compound lost the brown color and the ability to reduce methylene blue rapidly.

It appeared that the cause for the rapid reduction of methylene blue in each of these cases was the brown decomposition product of the glucose. It should also be noted that the solutions of aromatic amines and glucose when catalyzed with acid became quite dark; this may be due to an enolization and decomposition of the glucose rather than to the rearrangement product. If the alkylamine glucosides do form intermediate enolized compounds, it would be predicted that the shift is not to the completely rearranged keto form, since no acid-stable compound was obtained.

It is noteworthy that the isopropylamine and the 2-aminooctane condensations darkened most easily, even at low

temperatures, and that no crystalline N-glucosides were obtained from the solutions. These results indicate that enolization or some other type of isomerization had occurred, thereby preventing the crystallization of the N-glucoside from the solution.

Since the alkylamine compounds did not undergo rearrangement to form easily isolated N-alkyl-d-isoglucosamines, it was decided to see what effect the substitution of an acyl group on the nitrogen atom might have on the tendency for rearrangement. One of the simplest of these derivatives, N-acetyl-1-aminoglucose, was prepared. This compound may be considered a derivative of glucose in which an amide is condensed with glucose. No evidence of rearrangement of this compound was observed after two hours of refluxing of N-acetyl-1-aminoglucose in the presence of acid in seventy-five per cent ethanol. The solution was perfectly colorless and sixty-eight per cent of the starting material was recovered on cooling the solution. From these results it is indicated that the N-acetyl-1-aminoglucose is much too stable to undergo even an enolization under normal conditions.

Since rearrangement of the N-glucosides to the isoglucosamine structure is dependent upon enolization, it is evident that the substituent put on the nitrogen atom governs the ease of rearrangement. The positive alkyl radicals furnish electrons to the nitrogen atom and lessen the possibility of enolization, but the aryl and substituted aryl compounds are

much lower in positivity and subsequently the tendency for enolization is greater.

The dissociation constant for phenylhydrazine is only slightly greater than that for the arylamines; yet no phenylhydrazine derivative has been identified as the isoglucosamine derivative. The ease of hydrolysis in water and instability to acid of the phenylhydrazine derivatives indicate that the N-glucoside structure rather than an isoglucosamine is obtained.

Weygand, however, postulated the formation of the osazone through an Amadori rearrangement, p. 31. Since glucose phenylosazane has been reported from the action of phenylhydrazine on 2-methylglucose in acetic acid solution, the question arose as to the possibility of the removal of the methoxyl group by the Amadori rearrangement. The p-toluidide of 2-methylglucose was prepared, but even after long periods of refluxing in the presence of hydrochloric or acetic acids, the p-toluidide was recovered unchanged. The phenylhydrazide, on the other hand, was prepared and from this derivative the glucose phenylosazone was obtained. The phenylhydrazide had the correct methoxyl value for one methoxyl group, and the osazone contained no significant amount of methoxyl.

2-Aminoglucose was prepared in an attempt to determine the ease of removal of the NH_2 group by this same mechanism. 2-Aminoglucose (2,48), of course, is not comparable to the N-glucosides studied and shows the reverse stability to acids and bases, but the removal of the NH_2 group of the p-toluidide

of 2-aminoglucose from the second carbon atom was expected through enolization. However, the p-toluidide of 2-aminoglucose was not obtained since the free glucosamine liberated ammonia so readily that decomposition of the sugar occurred on slight heating.

A study of the hydrolysis of the N-glucosides in aqueous solution was made. It was found that the glucosylalkylamines were less stable in aqueous solution than glucosylamine itself. The first-reported glucosylalkylamines (118) were thought to be more stable in water than the glucose ammonia compound and the compounds were occasionally crystallized from water solution. Glucosyl-n-butylamine slowly hydrolyzed in a two per cent aqueous solution, the equilibrium being reached in about twenty-two hours at room temperature. This hydrolysis was shown quite plainly by the titration curve of the glucose alkylamines as well as by the change in optical rotation. This hydrolysis was confirmed by extraction of the free amine at equilibrium. The recrystallization of these glucosides from water has been accomplished but the recrystallization had to be carried out from a very small amount of water and by rapid cooling before the slow hydrolysis had occurred to any extent. Methanol and ethanol are to be preferred as solvents for recrystallization but in the case of the larger molecular-weight amine compounds, butanol and petroleum ether mixtures can be used. The rotations of all the glucosylalkylamines were observed in ethanol when solubility would permit. The

rotation of diglucosylethylenediamine was measured in fifty per cent ethanol since the compound was insoluble in absolute alcohols. The value in this case does not represent the mutarotation alone but hydrolysis as well. All the recorded rotations showed a change from a high to a low negative value.

Several of the glucosylamines showed a decided tendency to go into solution and could not be recrystallized. This disturbing property was evident especially with glucose oxime and less often in the diglucosylethylenediamine, glucosyldicyclohexylamine, and 1-aminoglucose. The suggested explanation for this occurrence is the isomerization of the N-glucosides in solution. This isomerization would result in a mixture of isomers from which it would be difficult to obtain a crystalline compound.

The hydrolysis of the different glucosylamines of the alkyl type is comparable as shown in fig. 9 and fig. 10. However, the hydrolysis of 1-aminoglucose in aqueous solution is in sharp contrast to the above compounds as shown by the titration curve and lack of change in rotation of an aqueous solution of the 1-aminoglucose. The change in rotation over a period of months was shown to be very slight by Lobry de Bruyn (84). This may be explained by recognizing 1-aminoglucose as the first member of the series, and as such its abnormal behavior is not unexpected.

The arylamine glucosides showed a decided change of rotation, but extraction after ninety hours showed that less than

ten per cent of free amine was present. An interesting result
was obtained in a study of the hydrolysis of p-toluidide of
glucose. The change in rotation (fig. 10) of the toluidide
in a closed polarimeter tube did not parallel that of the
solution left in a flask in air. The solution left in the
air slowly decreased over a period of days, and a slight dark-
ening of the solution was observed. The only explanation for
this anomaly that could be offered was that the air oxidized
the compound, causing a rearrangement in aqueous solution, or
that the carbon dioxide in the air caused some type of salt
formation which catalyzed rearrangement. It is conceivable
that some intermediate of the Amadori rearrangement was formed
which caused a drift in the rotation. These compounds would
probably be double-bonded, open-chained compounds which are
known to possess slight rotations. A fact which paralleled
this phenomenon is the failure of a phenylhydrazide to form
an osazone even in acetic acid solution if all oxidizing
agents including dissolved oxygen are removed (110).

The N-p-tolyl-d-isoglucosamine shows a mutarotation in
aqueous solution and becomes quite dark after two days but no
further change in rotation was observed. The change in color
of this compound is not surprising when it is remembered that
the compound is easily oxidized and reduces methylene blue
rapidly.

The N-acyl-glucosamine, on the other hand, does not
hydrolyze in water and is quite resistant to hydrolysis by

acids. The N-acetyl group was removed by refluxing with 1 N sulfuric acid as shown in fig. 8. The stability of this compound to hydrolysis suggests that it might be formed by a condensation of an amide and glucose in the same manner as the condensations of the alkyl- and arylglucosylamines. Although repeated efforts were made to condense acetamide and glucose by heating without a solvent, or in methanol, with or without hydrochloric acid as a catalyst, no N-acetyl-1-aminoglucose was obtained. The ready solubility of the reaction products in methanol and the inability to extract with hot chloroform from the gummy residue after solvent removal more than one-third of the acetamide is evidence of reaction. Acetobromoglucose reacted with an excess of acetamide on refluxing the reactants in chloroform solution. The crystals that appeared after several minutes were identified as diacetamide hydrobromide. The other products of the reaction were soluble in chloroform, and as yet no crystalline compound has been obtained.

In preparation of the phenylhydrazine derivatives of glucose it was found that compound formation between glucose and phenylhydrazine occurred at room temperature, but that the compounds obtained, though crystalline, were phenylhydrazine solvated compounds of varying composition. The solvated phenylhydrazine could not be removed by extraction with ether, but on drying at temperatures between 50 and 100° C., the solvation molecules could be removed, the composition

approaching the 1:1 ratio and a melting point of 105-107° C. for the compound.

The preparation of the phenylhydrazine derivatives of glucose was unsatisfactory as evidenced by the difficulty in duplication of preparations reported in the literature, and also by the variation in the product obtained from using any one procedure. An example of this difficulty is observed particularly in the preparation of the α-hydrazide reported by Behrend, in that, although numerous attempts were made to obtain the pure isomer, the physical constants of the products obtained varied greatly, even after repeated attempts at purification. The ease of isomerization of these derivatives is illustrated by the formation of at least three modifications of the compound in recrystallization of the derivative from alcohol. Further illustration of the uncertainty of obtaining the reported compound is the fact that platelets were obtained in the preparation of the β-hydrazone rather than the needles reported by Behrend. The crystalline form and melting point of these platelets may be changed merely by washing with acetone.

This isomerization along with the ease of formation of the solvated phenylhydrazine derivatives indicates that the identity of any of these isomers by chemical means is questionable, but the ease of hydrolysis and isomerization suggests that these compounds are of the N-glucoside type.

It was also shown that the ease of hydrolysis of the

phenylhydrazine derivatives increased with the increase in size of the carbohydrate attached. The possibility of securing a non-hydrated form of a phenylhydrazine derivative of a dextrin that possessed definite constants was slight, though compound formation was probable. Certain of the alkylamines seem to offer a better possibility of forming a nitrogen derivative of the dextrins. The dextrins are soluble in these amines and as long as nonaqueous solvents are used, the derivatives should be stable. The alkylamine derivatives also offer the advantage that only one isomer was obtained, even when different modifications of the sugars were used.

The glucosyl-n-alkylamines were reduced to the N-alkyl-d-glucamines by hydrogenation in a Parr type bomb under high pressure and temperatures below 100° C.

Since alkylglucosylamines would not undergo the Amadori rearrangement, the reduction evidently occurred from the N-glucoside form and only the d-glucamines are obtained. In reduction of the N-glucosides of aromatic amines, both the glucamines and the mannamines were obtained if conditions were such that rearrangement to the isoglucosamine occurred.

The glucamines of the lower molecular weight amines are very soluble in water but insoluble in ethanol, but all the N-alkyl-d-glucamines except the hexadecyl- and octadecyl-compounds are soluble in fifty per cent ethanol; so this solvent was selected for measuring the specific rotation. The salts of these amines are easily formed and, in most cases,

nicely crystalline. The amines are strong enough bases to be titrated potentiometrically, as shown in fig. 2. These compounds also lower the surface tension of water and cause a remarkable amount of foaming. The low solubility of the N-octadecyl- and N-hexadecyl-d-glucamine limited their usefulness in this respect.

The reduction has been effected on the condensation products of isopropylamine and 2-aminooctane without isolation of the glucosylamine. In the case of the isopropyl glucosylamine, about thirty per cent of some reduced product other than the N-alkyl-d-glucamine was obtained. This compound was assumed to be sorbitol, but purification was difficult since a gel formed when an attempt was made to recrystallize the product from ethanol.

The reduction of the glucosylalkylamines was carried out in different solvents; the ones used included methanol, ethanol and aqueous mixtures of each. Results indicated that reduction occurs in most cases before hydrolysis forms any significant amount of glucose and the free amine.

The reduction of 1-aminoglucose is more difficult and only about fifty per cent or less of the glucosylamine is changed to glucamine. The glucamine obtained is hard to isolate in the presence of sorbitol. After reduction the solution smells strongly of ammonia, and since the 1-aminoglucose was stable in aqueous solution at room temperature,

it is suggested that the rise in temperature results in the liberation of the ammonia. It is conceivable that reduction might occur with much better results at room temperature.

SUMMARY

1. The phenylhydrazine derivatives of glucose were investigated. The procedures for the preparation of these derivatives as reported in the literature were found to be unsatisfactory. The phenylhydrazine derivatives of glucose made at room temperature without a solvent were highly solvated with phenylhydrazine. These compounds approached a monophenylhydrazine derivative as phenylhydrazine was removed progressively at higher temperatures. The phenylhydrazine derivatives of the carbohydrates hydrolyzed more rapidly in water solution as the size of the carbohydrate increased.

2. Some glucosylalkylamines were prepared and their physical properties are reported.

3. The reduction of these glucosylalkylamines was carried out in a Parr type reducing bomb using Raney nickel as a catalyst. These compounds were characterized and were found to be good wetting agents.

4. The hydrolysis of the glucosylalkylamines, glucosylamine, glucosylarylamines, and the phenylhydrazine and amide derivatives of glucose in dilute aqueous solution was followed by change in rotation, potentiometric titration, or extraction. The amount of hydrolysis was found to be dependent on the substitution on the nitrogen atom.

5. An attempt was made to effect the Amadori rearrangement of the glucosylalkylamines and the acyl derivative of 1-aminoglucose. No rearranged product was obtained in either case.

6. Evidence for reaction of amides with glucose was obtained by the ease of solubility of glucose and acetamide in methanol and the failure to extract the components from the residue. Acetobromoglucose reacted with acetamide in chloroform solution to give diacetamide and some unidentified product.

7. The p-toluidide and phenylhydrazide derivative of 2-methylglucose were prepared, and, although the methoxyl group on the second carbon prevented the Amadori rearrangement in the case of the toluidide, glucose phenylosazone was prepared from the phenylhydrazine derivative of 2-methylglucose.

ACKNOWLEDGMENTS

The writer wishes to express her sincere
appreciation to Dr. R. M. Hixon for his interest
and guidance in the development of this problem.

Miss B. Brimhall made the original 1-amino-
glucose and the pentaacetylglucosamine.

The 2-methylglucose was prepared and the
methoxyl determinations were made by Doy Howland.

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