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THE SYNTHESIS OF D-GLUCOSE-1-C^{14}

by

Henry Ralph Mahler

Berkeley, California

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The Synthesis of D-Glucose-C^{14} *

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by

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8 September 1948

ABSTRACT

An investigation of the cyanhydrin synthesis as applied to 1,2-acetone-D-xylotrihydroxyglutaric dialdehyde was carried out. It proved to be unsuccessful, probably because of the presence of a mixture of the two diastereomers and of the desacetonated reaction products. Several attempts to deaminate 1,2-acetone-6-desoxy-6-amino-D-glucose and its desacetonated product were made in order to devise a method for obtaining D-glucose through a condensation of the aldehyde named above with nitromethane. These investigations had the aim of arriving at a useful synthesis of D-glucose-6-C^{14}.

In the application of the Sowden-Fischer synthesis of D-glucose, a critical sutdy of the condensation of arobinose with nitromethane was undertaken and the influence of relative reactant and catalyst concentrations was investigated. The influence of several cation exchange resins on the conversion of sodium salts of sugar nitroalcohols to the free nitrosugars was studied.

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* This report is partially based on work performed under Contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California. It is a complete reproduction of the dissertation submitted by H. R. Mahler in partial satisfaction of the requirements for the degree of Doctor of Philosophy in chemistry in the graduate division of the University of California.

Several methods of synthesizing nitromehtane were evaluated in order to arrive at a method suitable for obtaining nitromethane-C^{14}. Brief experimental studies of the thermal isomerization of methyl nitrite and the reaction between alkali nitrites and dimethyl sulfate were undertaken. The Victor Meyer reaction was subjected to critical analysis, the influence of a series of reaction variables was studied, and a practicable synthesis of nitromethane-C^{14} was evolved.

Another reaction studied was the Nef reaction between the sodium salts of aliphatic nitro-compounds and mineral acids. Nitropropane was chosen as the model compound, and an analytical procedure for the assay of propionaldehyde in the reaction mixtures worked out. It was found that temperature had a marked effect on the course of the reaction, with low temperatures leading to the highest yields of desired product. The influence of other variables, such as acid concentration, manner of addition, etc. was less pronounced but should also be carefully controlled. A working hypothesis explaining these various effects was derived and conclusions based on it applied to the synthesis of aldose sugars from sugar nitroalcohols.

Using carbon-14, A modification of the synthesis of methyl iodide-C^{14} had to be found which did not employ phosphorus. This was achieved by the treatment of aqueous methanol with hydrogen iodide of specific gravity 1.96. Nitromethane-C^{14} was synthesized, condensed with arobinose, taken through the steps of the Sowden-Fischer synthesis, and D-glucose-C^{14} and D-mannose-C^{14} isolated, although not in crystalline form so far. It has been possible, however, to prepare these two sugars crystalline, with low specific activities, by the device of co-crystallization with inactive material as carrier.

(no page 5 in report) (error in numbering)

The Synthesis of D-Glucose-1-C^{14}

by

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A. GENERAL

I. Introduction:- Glucose, labelled unambiguously in one of the positions of its carbon skeleton, would be a tool of inestimable value in the investigation of chemical, biological and biochemical processes. Recently it has been possible to synthesize glucose containing radioactive carbon by biological methods (1). Mention need only by made of the photosynthetic experiments of Calvin and co-workers (1-a) and the biological synthesis starting with lactic acid, as carried out by Hastings and his group (1-b). The carbohydrate molecule thus obtained, however, has always been labelled in more than one position and the assignment of definite fragments containing radio carbon has been difficult if not impossible. To achieve the aims ennumerated above, synthetic chemical methods must therefore be employed. The purpose of this research has been to investigate the synthetic tools available; to evaluate them according to ease of adaptation for work with radioactive isotopes, to find conditions for maximum yields, and to make a critical evaluation and choice where two or more methods appeared available.

Positions 1 and 6 in the glucose molecule would seem to be the most desirable for labelling, from the point of view of ease of availability of the five-carbon precursor, the ease of introduction of the additional carbon, and the usefulness of the product thus obtained.

To mention but a few applications: It is the terminal carbons that can be most easily located and their products isolated in the cycle of anerobic

fermentation. The aerobic metabolism also could probably be approached with greatest ease from the carbohydrate side if glucose containing C^{14} in one of the terminal positions were available. The study of the chemical reactions of this most important of carbohydrates as well as rate and mechanism studies in numerous fields of carbohydrate chemistry could be instigated if glucose prepared in this manner were available in quantity.

The succeeding section will be divided into two parts: The first will provide a scheme by which 6-desoxy-6-amino-6-C^{14} glucose can be prepared and will discuss the attempted deamination of this sugar to glucose itself which has so far been unsuccessful. A synthesis of this amino sugar almost identical with the one described below has been carried out by Professor H. O. L. Fischer (2). The second part will describe a scheme and mention experiments leading to a successful synthesis of D-glucose-1-C^{14} and D-mannose-1-C^{14}.

II. Attempted Synthesis of D-Glucose-6-C^{14}

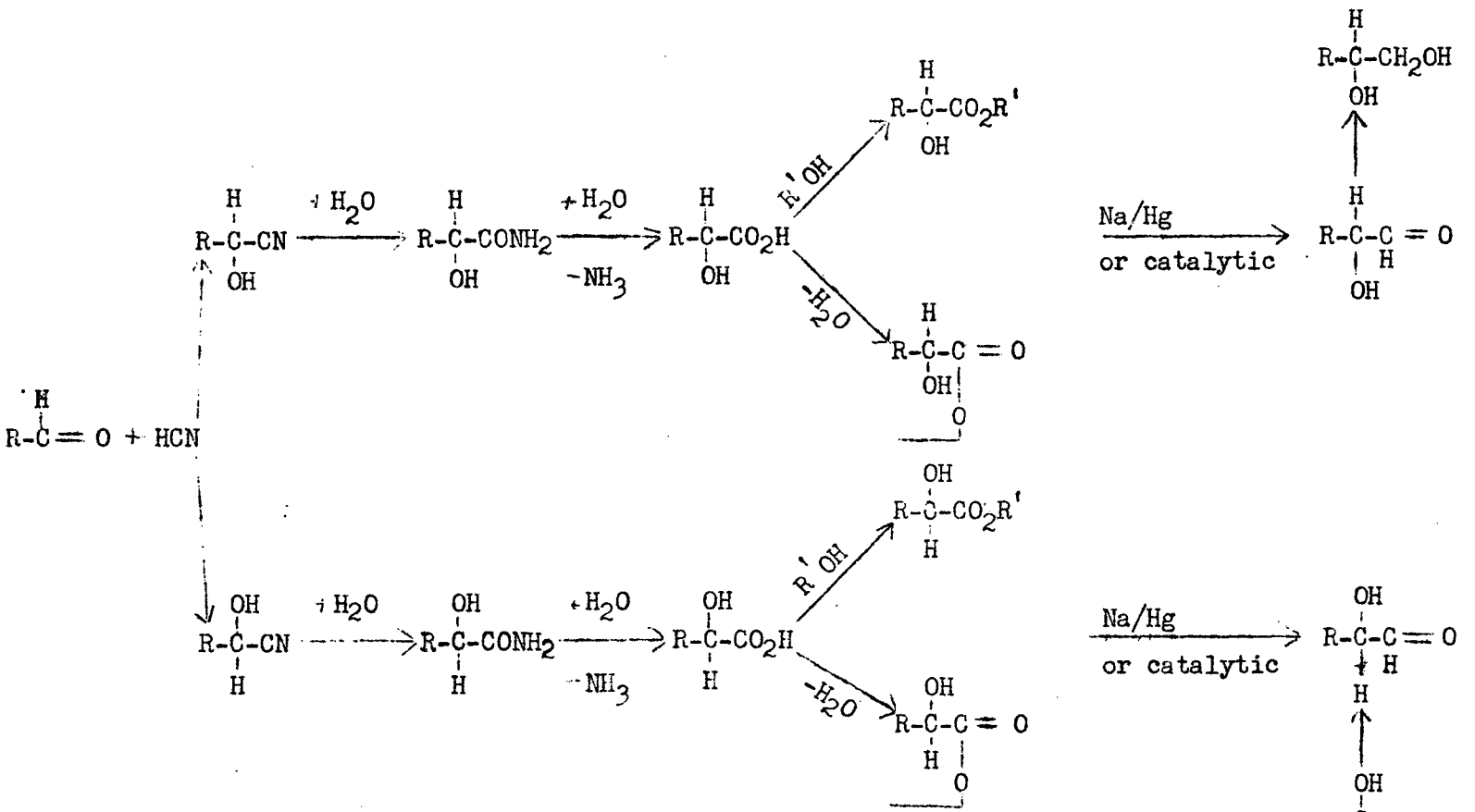
1. Attempts employing the Cyanhydrin Synthesis:- The standard method for the introduction of one carbon atom into a terminal position of a monosaccharide is that using HCN or an alkali cyanide. This method was first discovered by Kiliani (3) and elaborated by E. Fischer (4) during his classical researches into carbohydrate chemistry. Numerous modifications of the original methods are available today, of which only that of Hudson need be mentioned here (5).

All these methods have in common the starting point: An aldose one lower than the desired sugar, an intermediary sugar acid or lactone, and a reduction to the desired aldose or sugar alcohol. This synthesis can be represented schematically as follows in Table I.

It is adaptable to the use of C^{14} in the form of an alkali cyanide, and the last step has been recently investigated critically at least in the case of the sodium amalgam reduction to the aldehyde sugar (6).

The Cyanhydrin (Kiliani) Synthesis

Table I

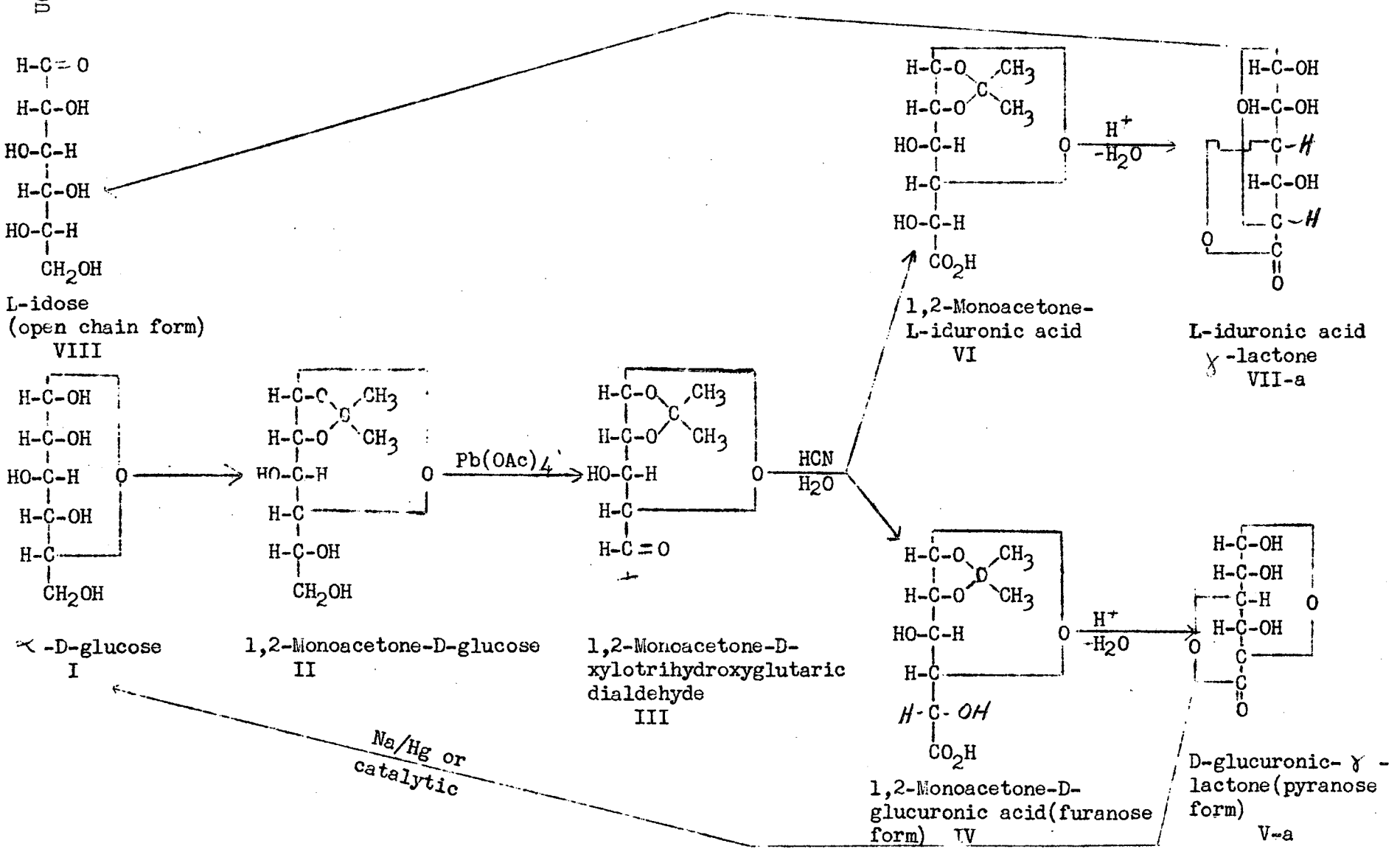


Several disadvantages are inherent in the method. It is of course an a-symmetrical synthesis, and the separation of the two isomers anywhere along the line is usually difficult and attended by considerable losses in yield. (6-b, c). The condensation itself is not one designed to give high yields - thirty percent or so being the optimum total yield so far obtained in previous applications of the synthesis. Nevertheless, it was decided to employ this method in the first attempt to obtain D-glucose-6-C^{14}.

As a starting point in this synthesis we have to look for an aldose with one aldehyde group in the terminal position, such that it will become carbon number five in the glucose molecule, and with another aldehyde group (which must be protected by a glycosidic or lactol linkage so as not to participate in the condensation) in position number one. Such a sugar is available in the 1,2-acetone-D-xylotrihydroxyglutaric dialdehyde, first prepared by Koichi Iwadare (7) and recently re-investigated by Fischer (2). This dialdehyde, in turn, is obtained by the lead tetra-acetate oxidation of monoacetone glucose. Starting with this aldehyde, it was hoped that D-glucose and L-idose could be obtained by the reaction sequence summarized in Table II.

As can be seen from this scheme, the first intermediates that were expected to be isolated would be the monoacetone derivatives of D-glucuronic (IV) and L-iduronic acid (VI), after separation of diastereoisomers. These could then be transformed into the desacetonated lactones (V) and (VII). The lactones in turn should be susceptible to reduction to the corresponding hydroxyl compounds. The classical method to effect this reduction is that employing sodium amalgam, (8). Levene has employed catalytic methods of hydrogenation (9), and the recently discovered reagent lithium-aluminum hydride

Table II


L-idose
(open chain form)
VIII
α-D-glucose
I
1,2-Monoacetone-D-glucose
II
Pb(OAc)4
1,2-Monoacetone-D-xylotrihydroxyglutaric dialdehyde
III
HCN
H2O
1,2-Monoacetone-L-iduronic acid
VI
H+
-H2O
L-iduronic acid γ-lactone
VII-a
1,2-Monoacetone-D-glucuronic acid(furanose form) IV
H+
-H2O
D-glucuronic-γ-lactone(pyranose form)
V-a
Na/Hg or catalytic

might also be used to good advantage (10).

When the Kiliani synthesis was carried out, however, repeated attempts to separate the reaction product proved completely unsuccessful. On consideration of the various compounds possibly present in this mixture, this would not at all appear to be surprising. For not only might we expect to find the D-glucuronic acid (IV), its diastereomer (VI) and the corresponding lactones (VIII) and (X), but also the various desacetonated compounds (V,IX and VII, XI) as shown in Table III.

Although it may be possible to improve the method so as to end up with the lactones exclusively, the possibility of the various equilibria being set up and acetone being removed reversibly is always present whenever we work in aqueous solution, and especially in the presence of mineral acids, the very conditions of the Kiliani synthesis. Consequently, it was decided to abandon this effort, and to look for other means leading to the desired end.

2. The Nitromethane Condensation: - The condensation of carbonyl compounds with nitro paraffins was first discovered by Henry (11). Like all other reactions of nitro paraffins, it has received considerable attention in recent years (12), and was introduced as a synthetic method into carbohydrate chemistry by H. O. L. Fischer and his collaborators (13). Starting with the 1,2-acetone-D-xylo-trihydroxyglutaric dialdehyde, it provides us with an alternative method of going up the series. Table IV shows how this condensation reaction can be used as the initial step towards a synthesis of glucose labelled in the 6 position with C^{14}.

Although the dialdehyde desired as a starting material had been prepared by Iwadare, no yields were given by him, and it seemed generally desirable

Possible Reversible Interconnections

Table III

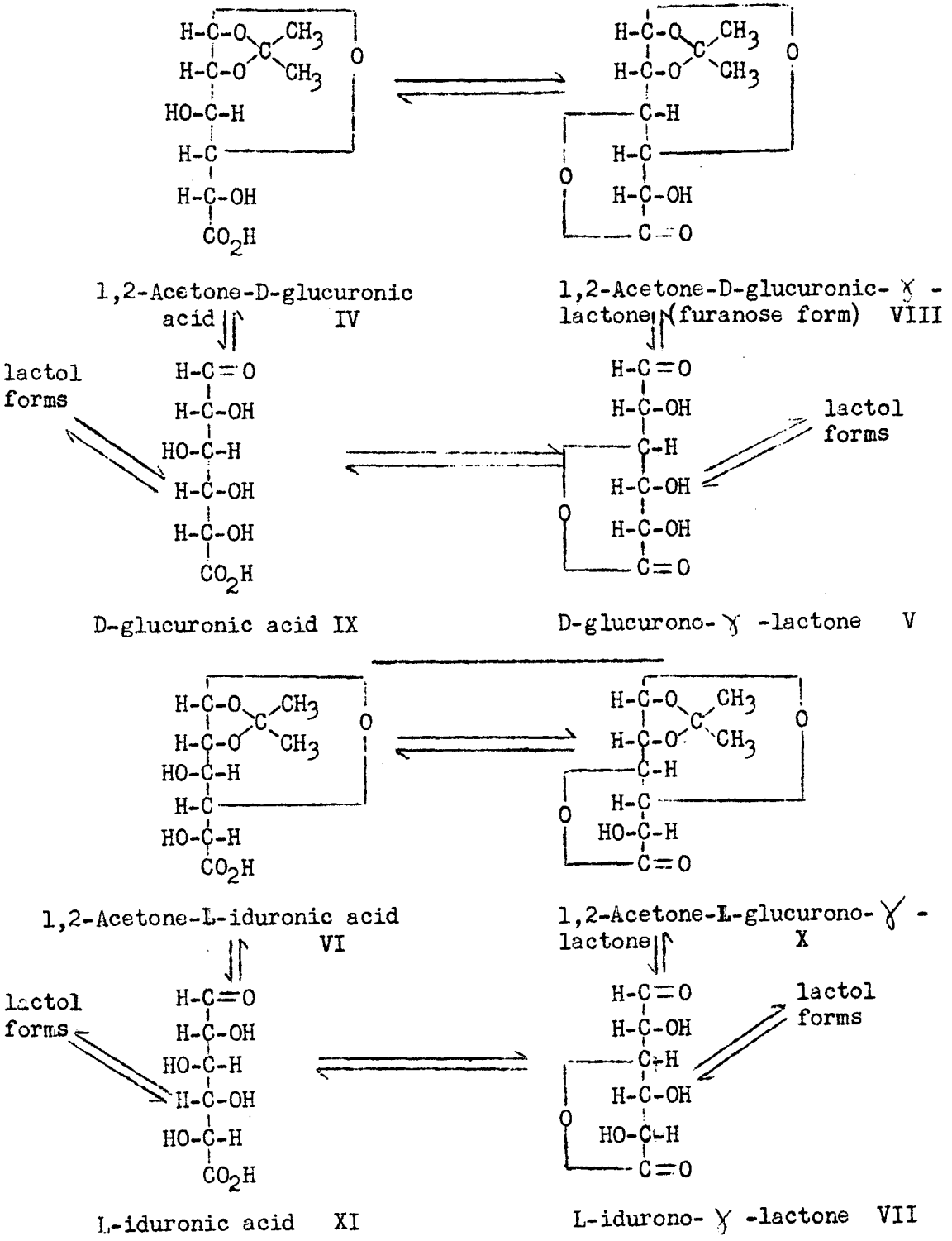
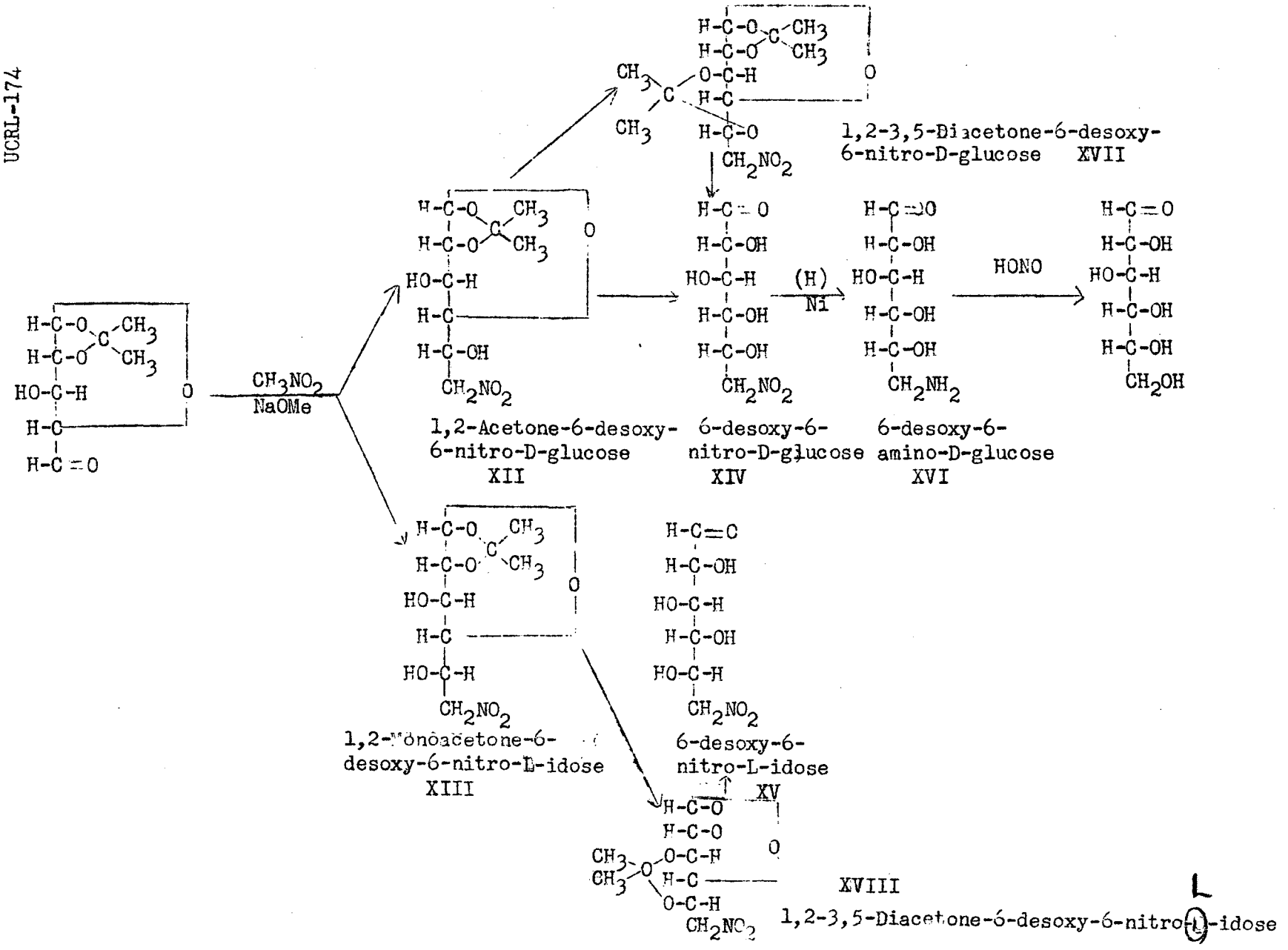


Table IV


CH3NO2
NaOMe
1,2-Acetone-6-desoxy-6-nitro-D-glucose
XII
1,2-3,5-Diacetone-6-desoxy-6-nitro-D-glucose
XVII
6-desoxy-6-nitro-D-glucose
XIV
(H)
Ni
6-desoxy-6-amino-D-glucose
XVI
HONO
1,2-Monoacetone-6-desoxy-6-nitro-L-idose
XIII
6-desoxy-6-nitro-L-idose
XV
XVIII
1,2-3,5-Diacetone-6-desoxy-6-nitro-L-idose

to investigate the variables involved in this oxydation by lead tetraacetate. Directions which lead to yields between 85 and 90% in this oxidation will be found in the Experimental part. Benzene was used as a solvent and the reaction carried out just below its boiling point. Under these conditions the oxidation is practically instantaneous and quantitative. The use of periodic acid (14) was also considered, but since an inert medium such as benzene seemed desirable because of possible reversible desacetonations, this possibility was not investigated further.

Several papers of H. O. L. Fischer (15) discuss the use of nitromethane in the sugar series, and no difficulty was experienced in applying this condensation to our particular case. The reaction was carried out in methanol using sodium methoxide as a catalyst.

This condensation, after working up of the reaction mixture, yields the two diastereomeric compounds, XII and XIII, in a yield of about 45%. Attempts to induce separation by fractional crystallization were unsuccessful. H. O. L. Fischer, however, in his Harvey lecture (16) mentions a process of fractional acetonation as having been used successfully by Grossheintz to effect separation in this particular case without giving any experimental details. It was therefore decided to investigate this process which appears reasonable since the idose configuration of the one should make reaction with acetone to yield the diacetone derivative more feasible than the glucose configuration of the other compound. Such was found to be the case. Acetonation of the mixture yielded almost complete acetonation of XIII and only partial acetonation of XII. The reaction mixture could then be separated into the monoacetone derivative XII and the diacetone derivative XIII. Professor Fischer who kindly con-

sented to apprise us of the work carried out with Dr. Grossheintz on the same compounds prior to the publication of this work (2), and whose remarks in his Harvey lecture first prompted this line of research, came to almost identical conclusions.

On desacetonation of the 1,2-acetone-6-desoxy-6-nitro glucose, the glucose derivative with isopropylidene group removed was obtained as a white crystalline powder. This compound has an initial rotation of $(\alpha)_D^{20} = 45$, in close agreement with the value reported by Fischer. The muta-rotation accelerated by acid was also observed by us.

This compound was quantitatively reduced to the 6-amino derivative using Raney nickel and atmospheric pressure. The sugar then was isolated as the p-toluene sulfonate. It was found to be identical with the same compound prepared previously by Ohle and von Vargha (17).

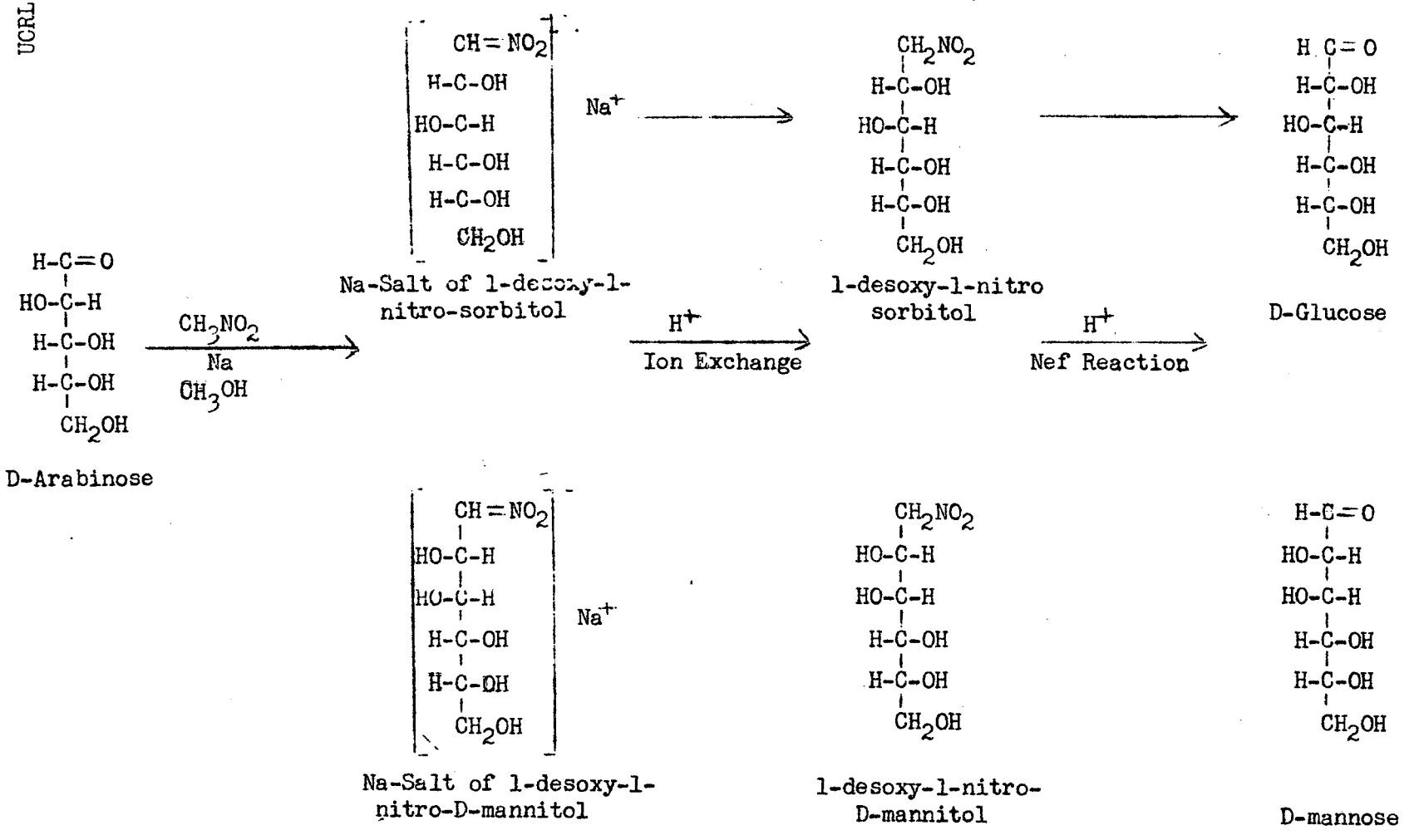
All attempts at desamination of this compound to yield free glucose or any of its derivatives have been unsuccessful so far. It has been found previously that de-aminations in the sugar series yield anomalous results (18) whenever a possibility exists that some of the sugar hydroxyls can enter into side reactions. Opportunity for such reactions certainly exists in the present case. Maybe the use of a partially or completely acetonated derivative can be made to lead to the desired results.

III. Synthesis of D-glucose-1-C^{14} and D-Mannose-1-C^{14}

1. General:- In 1947 Sowden and Fischer published a method for the synthesis of D-glucose and D-mannose or their enantiomorphs starting from D- or L-araginose respectively (15) and employing nitromethane as the means of lengthening the carbon chain by one member. Their experiment may be summarized as follows in

TABLE V

The Nitromethane Synthesis of D-Glucose and D-Mannose



It was decided to investigate this method for its possible applicability to the present problem, to find conditions and experimental methods adaptable to the utilization of carbon-14, and, if possible, to effect the synthesis of the two labelled hexoses.

As can be seen from the table, the problem can be divided into the following four parts: The synthesis of nitromethane-C^{14}, the condensation of nitromethane-C^{14} with arabinose, the conversion of the sodium salts of the nitro-alcohols to the nitro-alcohols proper by means of ion exchange, and the separation of 1-desoxy-1-nitromannitol from 1-desoxy-1-nitrosorbitol, and finally the hydrolysis of the sugar nitro-alcohols to the aldo-hexoses proper. The nature and cost of radioactive carbon makes it necessary to study all reactions involved in a particular sequence so as to insure maximum yield and minimum loss of activity due to mechanical difficulties. To achieve this end the reactions ennumerated above were analyzed from the point of view of all the variables, control of which might further the ends mentioned. The following sections will give a description of these studies.

2. The Synthesis of Nitromethane-C^{14}:- A search of the literature indicates five possible routes to the synthesis of nitromethane:

1) CH_4 or higher paroffins $+ HNO_3 \xrightarrow{\triangle} CH_3NO_2$

2) $CH_2XCO_2H \longrightarrow CH_2NO_2CO_2H \xrightarrow{\triangle} CH_3NO_2 + CO_2$

3) $CH_3ONO \xrightarrow{\triangle} CH_3NO_2$

4) $KNO_2 + (CH_3)_2SO_4 = CH_3NO_2 + KOSO_2OCH_3$

5) $AgNO_2 + CH_3I = AgI + CH_3NO_2 + CH_3ONO$

The five methods can be described as

1) The vapor-phase nitration of paraffins.

2) The Kolbe reaction.

3) The thermal isomerization of methyl nitrite.

4) The reaction between an alkali nitrite and dimethyl sulfate.

5) The Victor Meyer reaction..

Of these five methods number one can be discarded immediately for our purposes. Although it is the most recent (19) and the one employed industrially in the synthesis of nitromethane, the difficulties of obtaining methane-C^{14} and designing a vapor-phase nitrator small enough to accomodate the quantities of reactants used in syntheses employing isotopic carbon preclude the use of this method.

The other four methods bear closer investigation. The Kolbe synthesis is the common laboratory method used today for the synthesis of ordinary nitromethane (20). It is also the method given in Organic Syntheses. The yields have never been reported to exceed 60%. Acetic acid-1-C^{14} has been prepared in this laboratory, as have its halogen derivatives (20a). The sequence from barium carbonate-C^{14} would be

6) $$Ba C^{14}O_3 \xrightarrow[100\%]{H_2SO_4} C^{14}O_2 \xrightarrow[100\%]{\substack{90\% \\ \text{cat or} \\ LiAlH_4}} C^{14}H_3OH \xrightarrow[85\%]{\substack{95\% \\ HI \text{ or} \\ P_4 + I_2}}$$

$$C^{14}H_3I \xrightarrow[100\%]{} C^{14}H_3MgI \xrightarrow[98\%]{CO_2} C^{14}H_3CO_2H \xrightarrow[75\%]{Br_2, PBr_3}$$

$$C^{14}H_2BrCO_2H \xrightarrow[55\%]{(1)\ NaNO_2,\ (2)\ \Delta} C^{14}H_3NO_2 + CO_2$$

The yields for each step are indicated beneath the arrows. These are maximum yields achieved after careful investigation of the reactions and are not likely to be improved. As can be seen, this sequence then would correspond to an overall yield of only 30% from barium carbonate. A yield of starting material this low would not make for efficient utilization of C^{14}.

The thermal isomerization of methyl nitrite has only been reported once in the literature (21). At first glance the method, if it could be duplicated, should give an efficient and elegant way to the desired goal. The path from barium carbonate-C^{14} would then be

$$7)\quad BaC^{14}O_3 \longrightarrow C^{14}O_2 \longrightarrow C^{14}H_3OH \xrightarrow[H^+]{NaNO} C^{14}H_3ONO \xrightleftharpoons{\triangle} C^{14}H_3NO_2$$

The conversion of barium carbonate to methanol is almost quantitative, as is the formation of methyl nitrite. Everything therefore hinges on the last step.

In their original paper, Neogi and Chowdhuri report that they succeeded in isomerizing methyl nitrite to nitromethane, the thermodynamically more stable isomer, by passing the former through a hot tube at 110-130° C. No yields are given, and it is also stated that no reaction occurs below 110° C, and that extensive decomposition of methyl nitrite occurred with formation of acetaldehyde and acetic acid above 150° C. It appeared desirable to repeat this experiment. Table VI summarizes our results.

As can be seen from the data given in Table VI, we have been completely unable to repeat the original experiments. Not only were we not able to isomerize methyl nitrite to nitromethane using the surface and temperature conditions mentioned by them, but even the substitution of more active surfaces and more vigorous temperatures was of absolutely no effect. Decomposition did not set in below 200° C. Thus the conversion of methyl nitrite does not appear to

Table VI

The Thermal Isomerization of Methyl Nitrite

Run	Tube filling	Temp. C	$\%CH_3NO_2$ formed	Remarks
0	asbestos	110-130	some	original experiment
1	asbestos	110-130	none	to duplicate original experiment
1a	glass wool	150	none	no decomposition
1b	glass wool	200	none	slight decomposition
1c	glass wool	220	none	decomposition
1d	glass wool	105	none	
1e	glass wool	125	none	
2	glass beads	125	none	
3a	silica gel	125	none	
3b	silica gel	150	none	
3c	silica gel	175	none	
3d	silica gel	200	none	slight decomposition
4	alumina	125	none	
5	$AlCl_3$	125	none	

hold much promise in the synthesis of nitromethane-C^{14}.

The Reaction between Methylsulfate and Potassium Nitrite

This reaction, summarized by equation 4), was discovered by Kaufler and Pomeranz in 1901 (22a) when a yield of 25% was reported in the literature. It was also used ty Walden in 1907 (22b) who succeeded in synthesizing nitromethane in a yield of 57% based on the methyl sulfate used. The method, if successful conversions could be effected, might be adaptable to the use of C^{14} and was therefore investigated.

In the rather cursory survey of experimental conditions undertaken, the following variables were considered: The temperature, the relative proportions of the two reactants, the concentration of potassium nitrite, and the presence of a solid phase. Our results are summarized in Table VII.

As can be seen from a comparison of runs 1,2 and 4, the effect of temperature on yields is not very pronounced. As a matter of fact it might be said that the lower yield in run 1 was entirely due to the presence of an excess of potassium nitrite (v.i.). Lowering the temperature does, as expected, prolong the time necessary for completion of the reaction.

The concentration of potassium nitrite does seem to have a notable effect on the amount of nitromethane formed. Similar observations have been made by McCombie and Saunders in their investigation of the corresponding synthesis of nitro-ethane from sodium nitrite and ethyl sulfate (23). As an explanation of this phenomenon, let us consider the two simultaneous reactions

8) $KNO_2 + (CH_3)_2SO_4 = CH_3NO_2 + KOSO_2OCH_3$

9) a $(CH_3)_2SO_4 + H_2O = HOSO_2OCH_3 + CH_3OH$

Table VII

Preparation of Nitromethane by Use of Me_2SO_4 and KNO_2

Run	Temperature	Time to complete reaction (approx)[a]	$(CH_3)_2SO_4$ (moles)	KNO_2 (moles)	CCH_2O	CH_3NO_2 mole % (based on Me_2SO_4)	Purity of product[b]
1	c	12 hrs	0.5	1.0	30	66.7	poor
2	25	24 hrs	0.5	0.5	30	70.4	good
3	25	24 hrs	0.5	1.0	30	65	poor
4	0	48 hrs	0.5	0.5	30	69.8	good
5	25	36 hrs	0.5	0.5	150	50.6	good

a as determined by the disappearance of the $(CH_3)_2SO_4$ layer

b as determined by index of refraction and odor (see experimental part)

c no temperature control during addition, warmed on steam bath during addition of excess KNO_2 to complete reaction

9) b $HOSO_2OCH_3 + H_2O \rightarrow H_2SO_4 + CH_3OH$

c $NO_2^- + H^+ \rightarrow HNO_2 \longrightarrow$ further reaction

Reaction 8) is that yielding nitromethane and potassium methyl sulfate. The latter is perfectly stable towards excess potassium nitrite, at all temperatures employed in the reaction, although Neogi has claimed that nitromethane is formed when the two slats are heated above 180° C in the absence of solvent (24). Reaction 9), on the other hand, shows the hydrolysis of methyl sulfate, a slow reaction in the absence of base and under the doncitions of the experiment. It is followed by the attack of the sulfuric acid formed on the potassium nitrite, yielding nitrous acid, which may then undergo decomposition and dismutation reactions, yielding oxides of nitrogen. These oxides of nitrogen are indeed formed in the reaction and are detectable by their odor and color (in certain instances). But it is difficult to decide whether they were formed by the reaction just discussed, or else by the formation and subsequent decomposition of methyl nitrite according to

10) $KNO_2 + (CH_3)_2SO_4 \rightarrow CH_3ONO\uparrow + KOSO_2OCH_3$ or

11) $KNO_2 + H_2SO_4 + CH_3OH \rightarrow CH_3ONO\uparrow + KHSO_4 + H_2O$

Reaction 10) would correspond to the simultaneous formation of methyl nitrite from the original reactants in analogy to the observations reported for the Victor Meyer reaction (q.v.), while reaction 11) shows the formation of methyl nitrite from the methyl alcohol and sulfuric acid all formed in reaction 9).

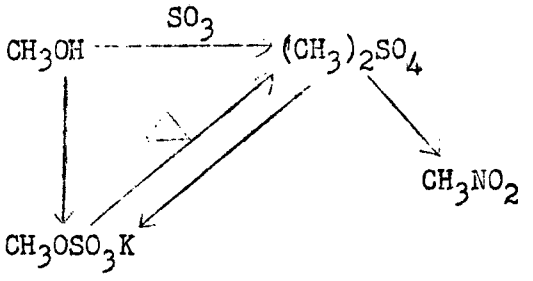
In either case, however, a decrease in the concentration of potassium nitrite (the concnetration of the other reactant, viz. methyl sulfate, is deter-

mined by its solubility in the aqueous phase) would decrease the forward rate of 8) and thus provide time necessary for the side reactions in 9) to become more important.

The addition of a molar excess of potassium nitrite to the reaction mixture after the reaction has somewhat subsided, in accordance with Walden's directions, gives rise to a solid phase of potassium nitrite in addition to the two liquid phases, methyl sulfate and water containing potassium nitrite and methyl sulfate. The overall rate of the reaction is indeed increased, but the amount of nitromethane formed is lower, and the product is considerably less pure and contains noticeable (by odor) concentrations of oxides of nitrogen.

The yield figures, as given in the table, are deceptive, as far as use of the method with isotopic carbon is concerned. For, as equation 8) shows, if we start with a certain activity a contained in the methyl sulfate, the maximum amount of activity found in the desired product can only be $a/2$. Therefore, as far as total activities are concerned, all yields in Table VII must be divided by two.

For this reason it was decided to abandon the method in favor of the Victor Meyer reaction. Nevertheless, the synthesis just discussed does provide a useful path for the synthesis of nitromethane-C^{14}, especially when the reactions leading to almost quantitative conversions of methanol-C^{14} to potassium methyl sulfate-C^{14} and dimethyl sulfate-C^{14} have been studied more fully. For then the activity contained in the latter compound (which is always recoverable) could be immediately utilized in the formation of additional dimethyl sulfate which then in turn could be used in the synthesis, e.g.



The Victor Meyer Reaction

The reaction bearing his name was discovered and vigorously investigated by Victor Meyer and his students towards the close of the last century (25). Since then it has been extended to numerous other aliphatic compounds and to compounds having halogens in aliphatic side chains or aromatic nuclei (26). Very little work concerning the basic nature of the reaction, however, has been undertaken. Reynolds and Adkins have subjected the reaction to a comprehensive treatment (27) but they do not concern themselves with nitromethane, nor with the influence of solvents upon the reaction or several other variables such as temperature. No kinetic investigations have been made at all.

Nevertheless, from their work and that of their predecessors, the following facts applicable to our particular problem emerge:

1) The reaction always yields nitroparaffin and isomeric nitrite, although in varying proportions. These proportions depend on

a) The nature of the halogen atom displaced

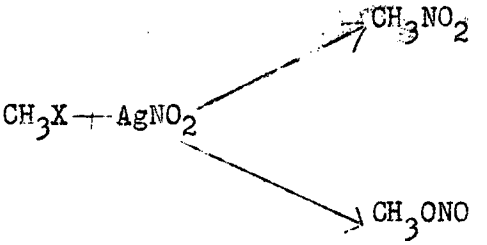
b) The nature of the metal atom

c) The nature of the alkyl radical

2) The total yield of nitrocompound and nitrite varies approximately inversely with the "ordinary" order of reactivity of halides, viz. n-hexyl > ethyl > t-butyl > allyl > 1-propyl > sec-butyl. The so-called ordinary order of reactivity is allyl > 3° > 2° > 1°.

3) Only heavy metal nitrites, expecially those of silver and mercury, can be used in the reaction.

In the case of nitromethane, the primary object of interest of this study, we need not be concerned with rules 1) b), c) and 2), since we are limited to the reaction



We limited ourselves to the use of methyl iodide since this compound is easily available and its manipulation presents no insurmountable difficulties. Although Reynolds and Adkins state that bromides in general yield higher proportions of nitrocompound over nitrite, this effect becomes noticeable only for compounds having more than three carbon atoms in the chain.

Our first concern was the confirmation of rule 3). We found that neither sodium nor potassium nitrite with or without the addition of catalytic amounts of silver nitrite was of any usefulness in the reaction. Mercury nitrite could be used but gave slower reactions and lower yields than silver nitrite, in agreement with previous results. No further studies employing this compound were therefore undertaken and all the experiments discussed below were carried out on methyl iodide with silver nitrite.

In order to achieve maximum yields of nitromethane, two conditions must prevail: The total yield must reach a maximum, and the proportion of nitroparaffin to nitrite also must be made as large as possible. Preliminary experiments showed that in the presence of an excess of ether as a solvent the theoretical amount of silver nitrite added was transformed to the iodide. The study of

reaction conditions was therefore primarily concerned with the sedond of the above conditions. In order to achieve the desired goal the following parameters were varied: Temperature, amount and nature of solvent, mode of addition, and nature of the condensing system. Our results are summarized in Table VIII.

The first variable investigated, which had a profound influence on the course of the reaction, was temperature. Reduction of the reaction temperature from 40°C to 0°C changed the yield of nitromethane formed from 36% to 64%. The reaction also proceeded at a much slower rate, as might be expected from reaction rate theory. This effect of temperature might be due to either or both of the following reasons:

A. consider the formation of the two isomers

12a) $AgNO_2 + CH_3I \underset{K_2}{\overset{K_1}{\rightleftharpoons}} AgI + CH_3NO_2$

12b) $AgNO_2 + CH_3I \underset{K_4}{\overset{K_3}{\rightleftharpoons}} AgI + CH_3ONO$

Assuming that both K_1 and K_3 are considerably larger than K_2 and K_4, the lowering of temperature might then slow down reaction 12a less than reaction 12b. In more technical terms, the temperature coefficient of 12b in the forward direction may be greater than that for 12a. Or, if we consider the Arrhenius equation,

13) $K = PZe^{-\frac{\triangle A}{RT}}$

the activation energy $\triangle Aa$ for 12a may be less than $\triangle Ab$, the corresponding energy for reaction 12b, also in the forward direction.

B. Or the effect of temperature lowering may be due to the experimental circumstances in the first four runs, where no provision was made to keep all the methyl nitrite within the reaction system, other than its solubility in

Table VIII

Preparation of Nitromethane by the Victor Meyer Reaction

Run	Temperature	% CH_3I Reacted	Yield (% CH_3NO_2 formed)	% CH_3NO_2 in mixture[2]	% CH_3ONO in mixture[k]	Time of completion	Solvent	Remarks
1	40 (bath)	100	36	36	64	150 min.	ether	a,b
2	25	100	61	61	39	180 min.	ether	a,b
3	15	90	54	62	38	180 min.	ether	a,b
4	0	12.5	8.0	64	36	90 min.	ether	a,b
5	0	100	64	64	36	72 hrs.	ether	a,b,c
6	-15	10	6.8	68	32	180 min.	ether	a
7	0	100	74	74	26	360 min.	ether	a,d
8	0	100	72	72	28	360 min.	ether	e
9	0	100	60	60	40	90 min.	none	f
10	0	100	73	73	27	360 min.	methanol	g
11	0	100	72	72	28	5 hrs.	methanol	h
12	0	80	69	69	31	5 hrs.	acetone	i
13	0	incompl.	~20	—	—	72 hrs.	methanol	l

For all experimental details see experimental part.

For remarks turn to next page.

Remarks to Table VIII

a Three parts (by volume) ether; one part methyl iodide; $AgNO_2$ added.

b Reflux condenser.

c Addition of $AgNO_2$ complete after six hours, flask at 0° stoppered for remainder.

d Closed system with gas condenser; $AgNO_2$ added.

e Same as d, CH_3I added.

f $AgNO_2$ added.

g Three parts (by volume) methanol; one part methyl iodide; $AgNO_2$ added - average of five hours.

h Ten parts (by volume) methanol; one part methyl iodide; $AgNO_2$ added.

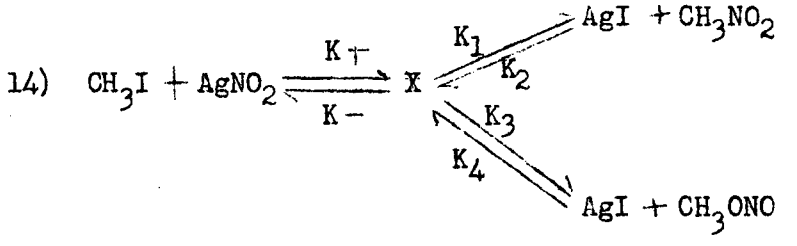
i Three parts (by volume) acetone; one part methyl iodide; $AgNO_2$ added.

k By difference.

l 5% water added.

ether at the temperatures of the various experiments. But if we assume that both reactions mentioned above are reversible, then the presence of methyl nitrite will increase the speed of the reverse reaction by the law of mass action and thus an opportunity for additional formation of nitromethane presents itself. In other words, in all previous investigations of this reaction, including the experiments marked as runs 1, 2, 3 and 4 in our table, we have been operating at conditions removed more or less from equilibrium, due to the exceedingly low boiling point of methyl nitrite (16°C) which thus had continuously been removed from solution. But the equilibrium concentration might be much more favorable to nitromethane.

Incidentally, these two possible explanations would still govern the reaction if we wished to assume a common intermediate on the path to nitrite and nitro compound. For the effect of this assumption is simply the injection of another step which does not change the overall picture in the least, viz.



The results of our experiments would indicate that although both explanations may be necessary, the second effect must be the more important one. For without changing the reaction temperature, the substitution of an experimental setup which insures the presence of the methyl nitrite formed until the end of the reaction, gives rise to greatly improved proportions of nitromethane in the final mixture.

Consequently, all further investigations were carried out utilizing the reaction conditions thus derived. As can be seen, a decrease in the methyl iodide concentration brought about by the addition of more solvent slows down the reaction as expected, but is without effect on yield or proportions. Similarly, the substitution of methanol for ether is without any effect on the overall course of the reaction. This is an extremely fortunate result, for it permitted us to carry out the reaction in a solvent which did not have to be separated from the nitromethane formed, but could be used immediately in the subsequent step of the reaction sequence (methanol is the solvent used in the nitromethane condensation of arabinose.)

The substitution of acetone for methanol or ether has a marked effect on the reaction. There is no ready explanation for this phenomenon, but it is conceivable that it may be due to the presence of minute quantities of water in the solvent. As can be seen from the results of run 13, water in small quantities has exceedingly deleterious effects on the reaction and great care must be taken to insure that all reagents used are completely anhydrous.

The mode of addition is without any significant influence on the reaction. Equally good results could be obtained by the addition of the silver salt to the methyl iodide solution, the procedure used in the majority of experiments, or by droping the solution slowly onto the silver nitrite as in run 9. This completely confirms the results of Reynolds and Adkins, obtained with higher homologues of methyl iodide. Probably better control can be achieved if the solid is added slowly to the liquid when small quantities are used. In large batches either method is equally suitable. Care must always be taken, however, to add one of the reagents as slowly as possible, in order to avoid local overheating and decomposition of the silver nitrite according to the equation:

15) $2\ AgNC_2 = AgNO_3 + Ag + NO \uparrow$

Sufficient agitation is maintained by swirling, when the quantities used do not exceed 50 millimoles. For larger runs stirring is indicated.

Mention must be made here of the action of methyl iodide prepared in the manner usual for the synthesis of methyl-iodide-C^{14} (28). This reagent has the most pronounced influence on the Victor Meyer reaction. Upon addition of the halide to the silver nitrite there immediately commenced deposition of large amounts of colloidal silver and violent decomposition of the silver salt could occasionally be noted even in the absence of light. Even the addition of a large excess (5 moles/1 mole methyl iodide) of silver nitrite did not lead to yields as high as t ose usual in the reaction of methyl iodide prepared by other methods. We believe this to be due to small traces of phosphine or other reducing phosphorous compound present in the methyl iodide, even after the most careful purification of the latter. The phosphine then must act in a catalytic manner either in the photodecomposition of silver nitrite, or in some other dismutation reaction of this unstable salt.

It is always dangerous to hypothesize about possible mechanisms for a reaction in the absence of reliable kinetic data. This becomes especially true if we are dealing with a heterogenous reaction as in the present case. Nevertheless, it might be interesting to sepculate a little about the path that would give a consistent explanation for the experimental facts discovered in this and previous investigations.

The electronic theory of the chemical bond, together with resonance theory, does at least suggest certain avenues of approach:

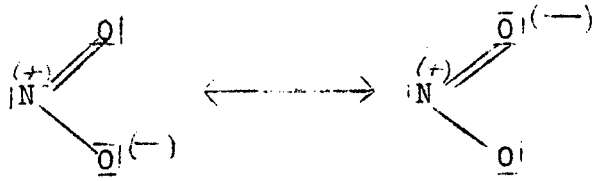
(a) Only silver and mercury nitrite undergo the Victor Meyer reaction.

But it is fairly certain that silver and mercury nitrite differ fundmentally from the alkali nitrites in other ways as well. They are for instance a great deal more unstable towards the influence of heat and light. It seems safe to assume that the heavy-metal nitrites are covalent in their crystal lattice, while the corresponding alkali compounds are ionic.

(b) The alkyl halides react with silver nitrite following an order of reactivity inverse from that commonly associated with a carbonium ion or equivalent mechanism (i.e. one involving previous or instantaneous ionization).

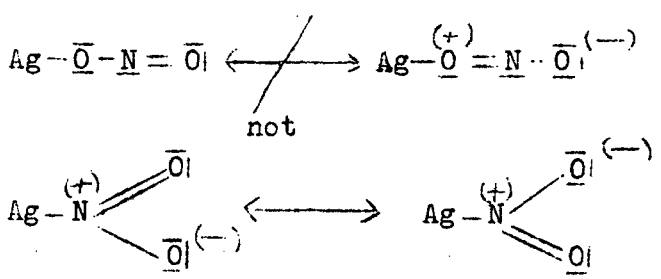
(c) The above two facts seem to exclude all mechanisms involving prior ionization of either of the two reactants.

(d) The nitrite ion is a resonance hybrid with the following two structures contributing to the largest extent:

16) 

No true double bond, open to attach by electrophilic reagents therefore exists in the nitrite ion and its inactivity toward alkyl halides, and its relative stability becomes explicable in view of the resonance stabilization involved. The exact structure of silver nitrite has never been determined adequately. Considerable controversy exists as to the nature and the location of the bonds in this compound (29). Until X-ray or diffraction data become available we are limited to conjecture.

(e) The most likely structure for silver nitrite is

17) 

the one with a covalent link between silver and oxygen, not that with this link between silver and nitrogen. For the latter would not impair the corresponding resonance of the nitrite group, and the sharp discontinuity between heavy metal and alkali nitrites can best be explained assuming the absence of this nitrite resonance in the former case. (a similar situation exists in the case of the azides, with the alkali azides being ionic and relatively stable, while the heavy metal azides are covalent and unstable.) Thus silver nitrite should have a lowered resonance energy and higher reactivity.

(f) This structure (described in (e)) has a true double bond as a path of attack and unshared electron pairs at both nitrogen and terminal oxygen. This should make the compound open to attack by electrophilic reagents at either position, due to relatively small difference in electronegativity between oxygen and nitrogen, thus giving rise to the two isomers. Alkyl halides are electrophilic reagents: although their reactions are usually regarded as nucleophilic displacements on carbon, they can of course be equally appropriately described as electrophilic displacements on the central atom of the base involved.

(g) The role of solvents in increasing the proportion of nitro compound over nitrite is obscure. It may be that certain solvents coordinate primarily around the exposed oxygen. This heavily solvated oxygen then would be in a more hindered position towards incoming reagents than the relatively unhindered nitrogen.

To sum up the results of this investigation, the following may be stated to be the conditions designed to insure maximum conversion of methyl iodide to nitromethane. These are consequently the conditions utilized in the successful

preparation of nitromethane in consistent yields of 70% based on the methyl iodide used.

The methyl iodide is dissolved in three times its own volume of solvent (either ether or methyl alcohol - completely anhydrous) and the finely divided silver nitrite (freshly prepared and vacuum dried) is added slowly, so as to control the reaction carefully. Care is taken not to permit the temperature of the reacting system to rise above 0°C and to insure that all the methyl nitrite produced is contained within the system by the use of an efficient condensing system.

3. The Condensation of Arabinose with Nitromethane. - Before the reaction between nitromethane and arabinose, first used by Sowden and Fisher, could be employed with nitromethane-C^{14}, the usual investigation of the effect of several controlling variables upon the course of the reaction had to be undertaken.

Two parameters would appear to have the greatest influence in this ragard: the relative proportion of the two reactants proper, and the amount of sodium added to effect the condensation.

Table IX shows the effect of varying the nitromethane arabinose ratio, holding the amount of sodium (1.20 mole per mole) and solvent constant.

Run 1 was undertaken under conditions similar to those reported by the original workers. As can be seen, the yield based on arabinose is very good, precisely the situation aimed for in the original paper. This, however, is completely unsuitable in terms of use of isotopic carbon, for only 8.6% of the total activity originally in the nitromethane would be found in the condensation product (the specific activity, of course, would not under go a corresponding change.)

Table IX

Run	Nitromethane / Arabinose	Yield of Na-Cpd based on Nitromethane (% of theoretical moles)	Yield based on Arabinose	Remarks
1	10:1	8.6	86	
2	4:1	12.5	50	
3	2:1	17.5	35	
4	1.2:1	25.8	31	
5a,b,c, R-2	1:1	30	30	a
6a,b,c,d R-3	1:1.2	34	28.3	b
7	1:1.5	36	24	c
8	1:2	39	26	c
9	1:4	44	11	c
10	1:8	46	5.8	

a Average of four determinations 5% (including one with G-14

b Average of five determinations 3% (ditto)

c Considerable uncertainty in yield data due to the presence of variable amounts of unreacted arabinose in products; yields back-calculated from the amount of crude nitroalcohols formed from the Na-cpd.

The subsequent runs, therefore, were designed to determine that area of relative concentrations which would provide for the most efficient utilization of C^{14}. Evidently, we have to use an excess of arabinose. We must be concerned with that part of the yield curve (based on nitromethane) which shows the steepest slope. In that region, small changes in relative concentration will have the largest effect on yield of product. As can be seen, a ratio of arabinose to nitromethane of 1:1.2 gives an average yield of 34%. An increase of the excess of arabinose to 1:1.5 only increases the yield by 2%, and using an eightfold excess of arabinose, the yield based on the potentially active compound is only boosted by an additional 10%. In all the runs employing large excesses of arabinose, great difficulties were experienced in purifying the nitroalcohols and in certain cases, mixtures were obtained that defied separation of the epimeric pairs. Consequently, in all the runs utilizing active nitromethane, twenty mole percent was the maximum excess of arabinose employed.

As to the reasons for the observed results which show an increase of conversion with mounting excess of nitromethane and a less pronounced increase with rising excess of arabinose, the following facts must be borne in mind:

1) $CH_3NO_2 + R\text{-}CHO \underset{NaOMe}{\rightleftharpoons} RCHOHCH_2NO_2 \underset{NaOMe}{\rightleftharpoons} [RCHOHCHNO_2]^{(-)}\,Na^{(+)}$

2) $CH_3NO_2 + nCH_3NO_2 \underset{NaOMe}{\rightleftharpoons}$ Condensation products

3) Arabinose $\rightleftharpoons$ decomposition products

Reaction 1) is the desired reaction, reaction 2) appears reasonable in light of the known chemical facts about nitromethane, such as its possession of an acidic hydrogen and, as a matter of fact, its ability to condense with aldehydes

such as arabinose. In view of these and other known enolic properties it is quite likely that it may undergo auto-condensation reactions analogous to the aldol condensation. In one of the runs using nitromethane C^{14} this hypothesis was tested. About one third of the total activity was found in the sodium salts of 1-desoxy-1-nitromannitol-1-C^{14} and 1-desoxy-1-nitrosorbitol-1-C^{14} as expected, while two thirds of the activity remianed in the methanolic solution. This activity could not be removed by the addition of more arabinose, and permitting the reaction to proceed for an additional 72 hours, indicating that it was not due to free, unreacted nitromethane. Almost all the activity could be extracted with ether, and upon removal of the solvent in vacuo, the reddish solution turned into a dark brown, glassy mass. This mass defied all attempts at crystallization, was insoluble in water but completely soluble in 6 N sodium hydroxide, and gave a positive ferric chloride test. A test for nitrogen was, as expected, positive.

Reaction 3) summarizes the well known reactions of sugars in a strongly basic medium.

In the presence of an excess of nitromethane, such as in run 1, reaction 3) is held to a minimum. Now if we assume that the speed for the cross-condensation between a nitro compound and a reactive carbonyl compound, such as arabinose, is somewhat faster then the auto-condensation reactions designated as reaction 2), the approach to theoretical yields becomes understandable.

On the other hand, in the presence of an excess of arabinose, reaction 3) becomes of increasingly greater importance and the yield curve would be expected to level off, as it does, and with even further increase in the excess of arabinose over nitromethane it might even eventually assume a negative slope.

All the experiments discussed above have been carried out using an excess of twenty mole percent sodium over the number of moles of the critical

reagent. This quantity of metal should be sufficient to transform all of the nitro-alcohol formed into the corresponding sodio derivative, and also provide the necessary quantity of catalyst for the condensation. Variation of this catalyst ratio and its effect on the reaction is described in Table X.

As the table shows, an increase in the amount of sodium beyond the 20 mole per cent excess necessary to effect conversion and condensation leads to progressively more apparent losses in yield regardless of whether the experiment is carried out in the presence or absence of an excess of nitromethane. This must mean that the decomposition of arabinose, that summarized as reaction 3) in our scheme, must be the most important side reaction in these strongly basic solutions. In all these cases the solution turned from a light pink to dark brown, depending on the amount of sodium used.

On the other hand, a decrease of the amount of sodium towards the minimal quantity necessary to effect transformation of nitro alcohol to sodium salts only, is evidently also instrumental in decreasing the yield. This must be due to an inability of this small quantity of base to catalyze the reaction effectively.

4. The Conversion of the Sodium Salts to 1-Desoxy-1-Nitro-D-Glucose and 1-Desoxy-1-Nitro-D-Mannose. - The first stage of this conversion is the transformation of the sodium salts of the nitro-alcohols to the corresponding free sugar nitro-alcohols. This transformation has been stated to occur in the presence of weak acids, such as acetic or carbonic (29), but in the present case it was effected by the use to cation exchange resins. Since three different resins were available, it was decided to compare their efficiency in carrying out the reaction under discussion. The cation exchangers investigated

Table X

Effect of Sodium Concentration on Condensation

Run	Nitromethane / Arabinose	Sodium / Arabinose	Yield based on Arabinose
A	10:1	1.2:1	86
B	10:1	1.1:1	75
C	10:1	2:1	79
D	10:1	3:1	53
E	10:1	4:1	36
F	1:1	1.1:1	28
G	1:1	1.5:1	25
H	1:1	2:1	19

are known as Dowex 50, Amberlite IR 100-H and Duolite C-5. Table XI shows their respective action.

As can be seen, there is no noticeable difference in the action of the first two resins tested. The third one, however, gave consistently lower yields in this particular conversion. All three compounds are sulfonated high polymers and their action can invariably be expressed by the relation

$$1)\quad RH + MA = HA + RM$$

where RH is the acid form of the resin, MA is any salt, HA is the corresponding acid, and RM the salt form of the resin. The explanation therefore is not due to any fundamental difference in resin properites but is to be sought for in the action of comparatively strong acids on nitro compounds. As will be shown in a subsequent section (5, the Nef Reaction), nitro compounds can undergo a variety of reactions in acid solution. The Duolite resin probably favors one or several of these side reactions, either because of the higher pH at which its interchange occurs, or because of other more obscure factors. That the first of these effects is an ever present one can be seen from the results summarized in the table. If the sodium compound is allowed to remain on the ion exchange column for a significant period of time, the amount of nitro alcohol drops off with length of time exposed. In experiment 5, it was even possible to isolate a detectable amount of hexose (in the form of glucosazone) from the syrup obtained after crystallization of the two diastereomeric nitro-sugars.

The separation of the two isomers from the eluate after the column operation is brought about by fractional crystallization from methanol-ethanol mixtures and absolute ethanol. For details reference is made to the experimental part. It must be mentioned here, however, that this separation becomes increas-

Table XI

Experiment	Type of Resin	Time before elution was started	Conversion (both isomers)
1	Dowex	none	96% a
2	Amberlite	none	95% a
3	Duolite	none	74% b
4	Dowex	1 hour	88%
5	Dowex	3 hours	75%
6	Duolite	1 hour	70%

a Average of five experiments.

b Average of three experiments.

ingly difficult and frequently impossible if great care is not taken to eliminate all other organic material prior to the attempted crystallization. Unreacted arabinose, although bothersome, is removed fairly easily, but methanol-insoluble products due to side reactions in the nitromethane condensation which have been carried through the column are severe handicaps in this step. This case occurs especially if traces of phosphine or its precufsors are present in the original methyl iodide-C^{14} preparation. These phosphorus compounds make the course of the condensation reaction almost completely unpredictable due to catalytic effects on certain side reactions not elucidated so far. The products of these reactions then interfere in all subsequent operations.

5. The Nef Reaction.-- In 1894 Nef discovered that the treatment of salts of nitroparaffins with solutions of mineral acids did not lead back to the original nitro-compounds, as might be expected, but gave rise to carbonyl compounds instead. (30). This hydrolytic reaction, which leads to the formation of aldehydes in the case of primary nitro-compounds and to that of ketones in the case of secondary ones, and which bears the name of its discoverer, has remained rather little used and investigated until recently, probably because of the relative unavailability of nitro-compounds. It has been studied only once since its discovery (31) and since the results of this investigation are not readily available, a fairly comprehensive study seemed necessary in order to discover the feasability of using the reaction as the last step in our proposed synthesis as first described by Fischer.

In his original paper, the acid used was approximately 15 N and the addition of the sugar nitroalcohol was effected either in the form of a solution of the sodium salt, or else, if prior separation of diastereomers was desirable,

in the form of an alkaline solution of the sugar nitroalcohol proper. The experimental conditions which appeared to me most likely in affecting the course of the reaction are: Temperature, the concentration of acid used, the nature of the acid employed, the mode of agitation and the order of addition. These, therefore, were the variables studied. Table XII summarizes our results as obtained with nitropropane as model compound. Since temperature was shown by far to be the most important factor, two of the sets of experimental conditions were repeated in the case of 1-desoxy-1-nitro-mannitol. The observations on this compound as well are included in the table.

It is evident from Table XII, that temperature exerts a very marked effect upon the course of the reaction. Lowering of the temperature from 25°C to -15°C helps to more than double the yield. It is also apparent that this effect appears to be general, since it is evident in the case of two such widely different nitro-compounds, as the simple nitroparaffins, nitro-propane, and the sugar nitroalcohol 1-desoxy-1-nitro-mannitol.

The effect of acid concentration is less pronounced. Within a rather wide range, i.e. between acid concentrations of about 8 N and 25 N, there is no significant change in yield. Outside of this area, however, the yields decrease markedly, and this effect is observed, both in acids more concentrated and those more dilute than the ones falling within the range described.

There appears to be no effect due to individual acids. Acids of the same strength can be used interchangeably, provided they fall within the optimum concentration range.

A decrease in the speed of agitation, or an increase in the rate of addition have the same effect, viz. to decrease the yield of desired product.

Table XII

Effect of Experimental Conditions of the Nef Reaction

Run	Cpd hydrolyzed	Temp. C	Acid used	Yield of Aldehyde	Remarks
1	Nitropropane	25	17N H_2SO_4	35%	a
2	Nitropropane	5	17N H_2SO_4	41%	a
3	Nitropropane	-15	17N H_2SO_4	75%	a
4	Nitropropane	-15	17N H_2SO_4	65%	a,b
5	Nitropropane	-15	HCl (conc.)	74%	a
6	Nitropropane	-15	36N H_2SO_4	63%	a
7	Nitropropane	-15	28N H_2SO_4	70%	a
8	Nitropropane	-10	8N H_2SO_4	72%	a,c
9	Nitropropane	- 5	4N H_2SO_4	58%	a,c
10	Nitropropane	-15	17N H_2SO_4	52%	a,d
11	Nitropropane	- 5	17N H_2SO_4	48%	a,e
12	f	-15	17N H_2SO_4	62%	g
13	f	25	17N H_2SO_4	36%	g

Turn to next page for remarks.

Remarks to Table XII

a Yield determined by hydroxylamine titration.

b All the nitropropane solution was added rapidly within two minutes instead of drop-wise as in the other runs.

c Temperature had te be raised to avoid appearance of solid phase.

d No stirring.

e Order of addition reversed, i.e. acid added drop-wise to alkaline solution of nitroparaffin.

f 1-desoxy-1-nitro-mannitol.

g Yield determined by amount of phenylhydrazone formed.

For all additional details see experimental part.

The same effect is also observed if the order of addition is changed, i.e. if the acid is dropped into the alkaline solution of the nitroparaffin instead of the ordinary mode of addition which is the reverse one.

To explain this varied set of observations, we must consider the several reactions which salts of nitro-compounds can undergo in acid solution. They are outlined in Table XIII and may be described as

(1) gives the three most important mesomeric structures of the resonating anion of the alkali salts of nitrocompounds.

(2) shows that on the addition of a proton this anion can be transformed into (a) the aci-form of the nitro-compound or (b) the nitro-compound itself.

(3) indicates the tautomeric equilibrium existing between these two forms.

Equations (1), (2), and (3) are entirely analogous to the corresponding relations in keto-enol systems. The reality of the aci-form of nitroparaffins, which of course corresponds to an enolic structure, has been conclusively demonstrated by its isolation and by physical-chemical measurements (32). Similar measurements have also been instrumental in determining the equilibrium constant for reaction (3) in several cases (33).

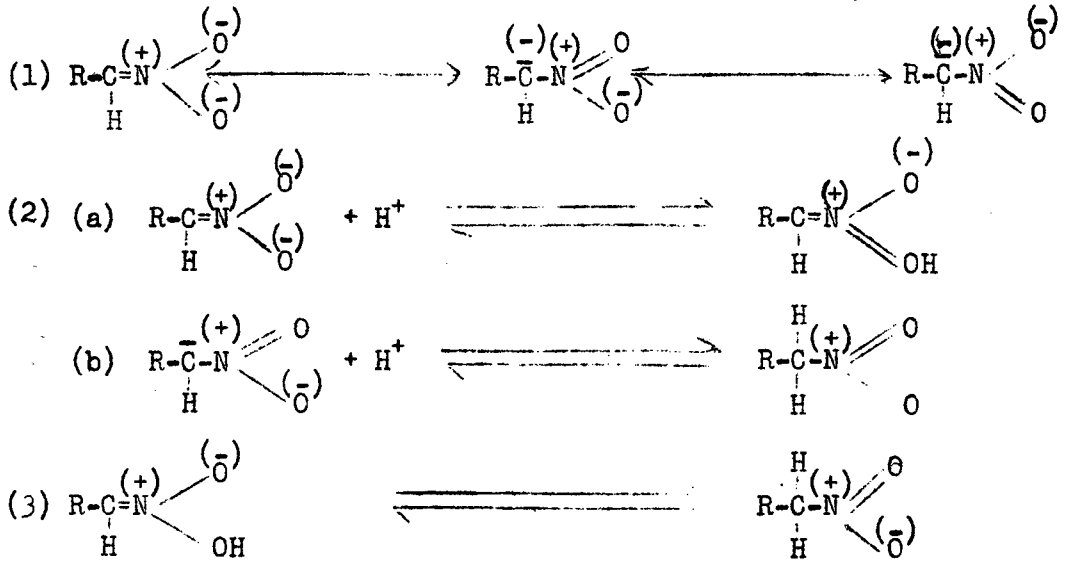
Reactions (4) and (5) are peculiar hydrolytic reactions only found in aliphatic nitro-compounds. Of these, reaction (4) is the Nef reaction, while reaction (5) leads to carboxylic acids and salts of hydroxyl amine, with hydroxamic acids as isolable intermediates. It proceeds through the following steps, according to a mechanism proposed recently (34):

a) Sulfuric acid is added across the nitrogen-carbon double bond of the aci-nitrocompound, with nitrogen, as the more electronegative element, accepting the proton.

b) A nitroso derivative is formed with loss of water. This is entirely

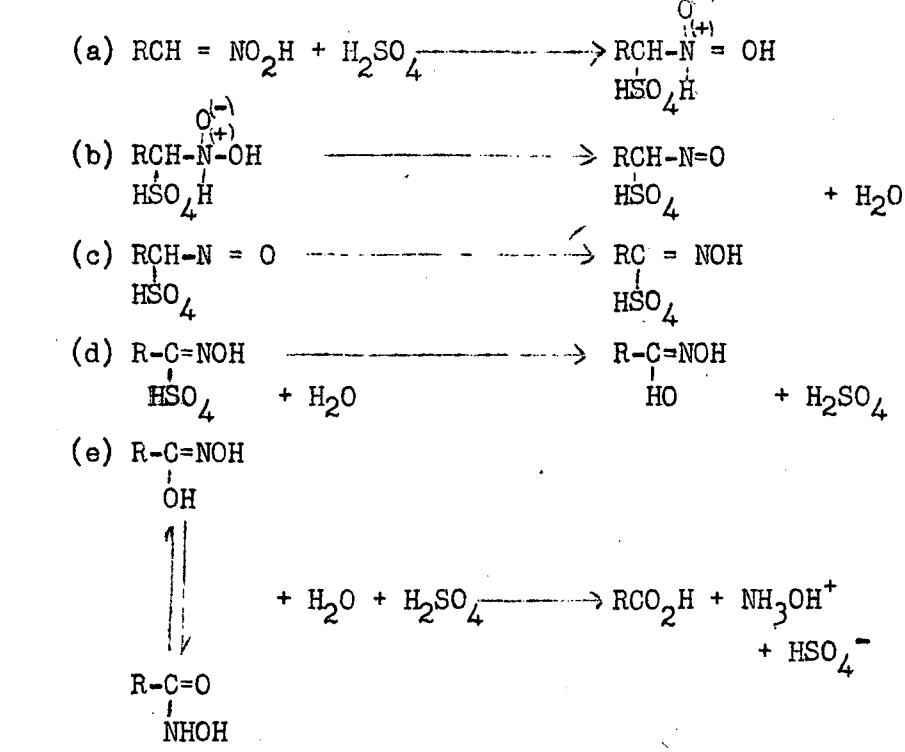
Table XIII

Reactions of Sodio-Nitrocompounds in Acid Solution



(4) $2\ [RCHNO_2]^-Na^+ + 2H^+ \longrightarrow 2\ RCHO + N_2O + 2\ Na^+ + H_2O$

(5) $RCH = NO_2H + H_2SO_4 + H_2O \longrightarrow RCO_2H + [NH_3OH]^+ [HSO_4]^-$



reasonable reaction.

c) The nitroso derivative undergoes a prototropic rearrangement ot yield the mixed anhydride of sulfuric acid and of a hydroxamic acid. This is another example of a prototropic triad.

d) The mixed anhydride is hydrolyzed into its two component acids.

e) Hydroxamic acids, under the influence of mineral acids, yield carboxylic acids and hydroxylamine salts.

Assuming that the hydrolytic sequence in the Nef reaction is similar to that proposed in the preceding paragraph, at least as far as the initial stages are concerned, the effect of temperature becomes understandable. For the first step in any proposed mechanism must be the conversion of the sodium salt to the aci-nitrocompound, which can then undergo further changes. The nitro-compound itself is incapable of undergoing similar transformations. But the tautomeric change between aci- and true nitro form is temperature dependent, in analogy to similar prototropic changes between keto and enol, with low temepratures foavoring the enol and aci-forms. A raise in temperature, therefore, makes unavailable a larger proprotion of compound. Similar effects can be observed if local overheating is not prevented and thus the influence of stirring rate as well as rate of addition find an easy explanation.

The concentration effects can be explained by means of reactions (2) and (5). The conversion of nitrocompounds to acids has been shown to be most efficient in 80% sulfuric acid. (34). Therefore a rise in acid concentrations to high values (the effect only becomes noticeable in rather more concentrated solutions because of the presence of the nitro-paraffin as the sodio derivative in alkaline solution) will decrease the yield of aldehyde by increasing the amount lost due to side reactions alogn path (5). Very low acid contrations, on the

other hand, are not sufficient for the steps of reaction (4) and therefore only serve to transform the sodium salt back to the original nitro-compound.

The effect of inverting the order of addition can probably be explained in a similar manner. The acid added initially would be too dilute to effect the desired hydrolytic reaction and would only serve to form the nitro-compound. As more acid is added, the acid concentration eventually rises to the level necessary for the Nef reaction, and so the formation of an aldehyde becomes noticeable, although in reduced yield.

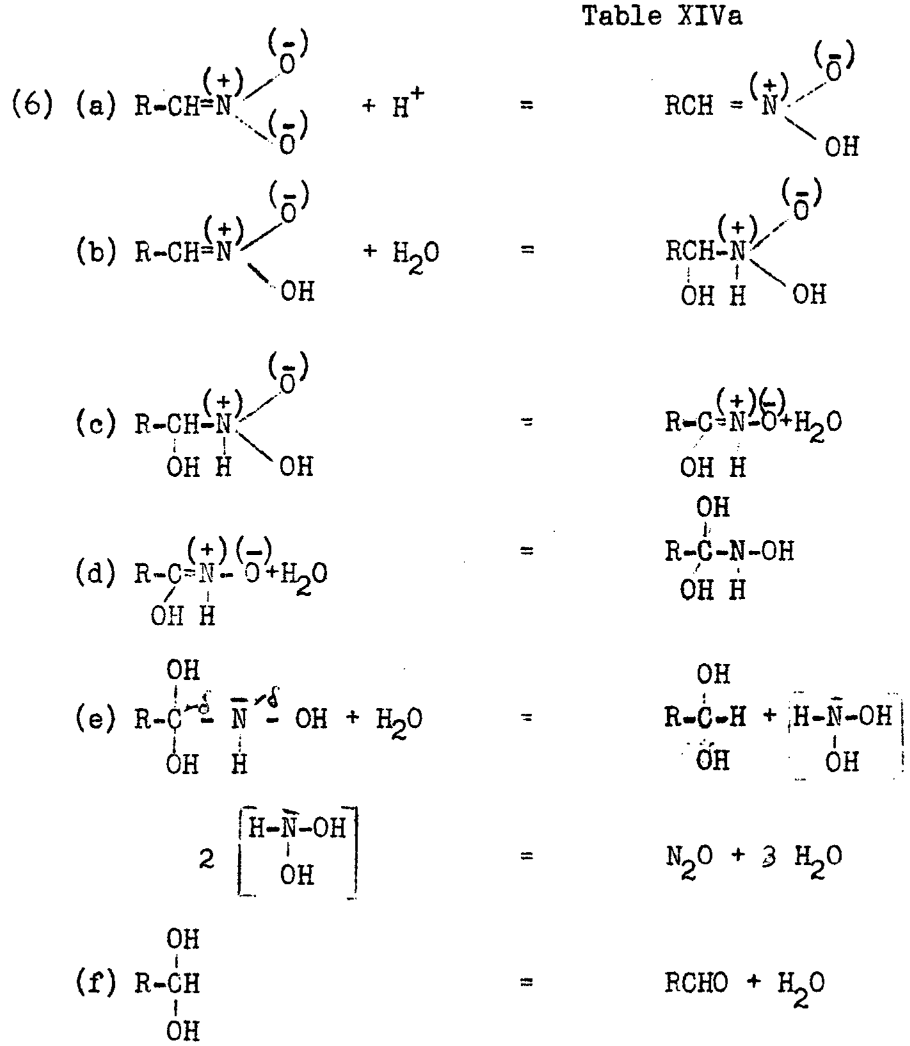
Finally, a highly tentative mechanism for the reaction may be proposed as outlined in Table XIVa.

Steps (a) and (b) are the formation of the aci-nitrocompound and the addition of water across its carbon-nitrogen double bond respectively. This reaction corresponds to the initial stage of reaction (5).

Step (c) indicates a loss of a molecule of water giving again rise to a carbon-nitrogen double bond but now with the carbon atom holding the hydroxyl group. The close proximity of carbon and nitrogen in the scale of electronegativities makes this step not appear too unlikely.

Steps (d) and (e) represent the addition of another molecule of water across the triad oxygen-nitrogen-carbon, with oxygen as the most electronegative accepting the proton. This is followed by scission of the carbon-nitrogen bond concurrent with acceptance of a proton by the negative carbon-fragment and of hydroxyl by depositive nitrogen, followed by combination of two now neutral nitrogen containing fragments. The product of this reaction sequence is nitrous oxide, as observed experimentally. Step (f) is self-explanatory as the loss of water of the aldehyde-hydrate to yield the aldehyde proper.

Table XIVa



overall reaction

(7) $$2\left[R\text{-}CH{=}NO_2\right]^{(-)} + 2\,H^+ = 2\,RCHO + N_2O + H_2O$$

identical with

$$2\left[R\text{-}CH{=}NO_2\right]^{(+)}\,Na^{(-)} + H_2SO_4 = 2\,RCHO + N_2O + H_2O + Na_2SO_4$$

To summarize: In order to achieve maximum yields in the Nef reaction, the alkaline solution of the nitrocompound must be added slowly under constant stirring to an aqueous solution of a mineral acid approximately 10 to 25 N in concentration. The acid should be cooled to the minimum temperature possible without creating a solid phase.

B. EXPERIMENTAL

I. SYNTHESES NOT INVOLVING ISOTOPIC CARBON

1. Preparation of 1,2-Acetone-D-Xylo-Trihydroxyglutaric-Dialdehyde

A. Preparation of Lead Tetraacetate

Fieser's directions have been found to be very satisfactory for the preparation of this reagent with the exception of the temperature conditions suggested by him. It has been observed by us that at temperatures not exceeding 55° C less decomposition of the acetic anhydride occurs.

A three-necked flask was fitted with mercury-seal stirrer and thermometer. In the container were placed 150 ml. of acetic anhydride and 200 ml. of glacial acetic acid. Efficient stirring was started and maintained throughout the course of the reaction. The reaction mixture was heated to 45° C where 200 grams of red lead were added in ten to fifteen gram portions, care being taken not to commence the addition of a new portion until the color due to the preceding one had faded. The flask was cooled when necessary to keep the temperature below 55° C especially towards the end of the reaction. The dark colored, thick liquid on cooling separates the lead tetraacetate in fine needles. The precipitate is immediately redissolved in a large excess of warm acetic acid (care must be taken here to avoid elevated temperatures since these give rise to very great losses in

yield). Some activated charcoal is added and the solution filtered rapidly. It is then placed in the refrigerator overnight. The white needles of lead tetra-acetate are filtered out the next day and dried in a vacuum dessiccator over anhydrous potassium hydroxide. In the absense of air the reagent has been found to be stable over a period of months. Yield 120 grams, i.e. 68% based on the Pb_3O_4 used.

It has been found that the best results in the subsequent oxydation could be achieved by first suspending the compound to be oxydized (monoacetone glucose) and then adding the theoretical amount of lead tetra-acetate in small portions. If this procedure is reversed significant losses in yield may occur. If the amount of monoacetone glucose to be oxydized is small. the oxydation can be allowed to take place either at elevated temperatures, in which case it is instantaneous, or at room temperature. In the case of large batches our observation has been that the test results are achieved when the reaction is allowed to proceed slowly over a period of hours at room temperature.

B. Large Scale Oxydation

100.0 grams of monoacetone glucose supplied to us by Dr. Cantor of Corn Products Refining Corporation and recrystallized to constant rotation were very finely ground and suspended in two liters of benzene. Rapid stirring was started. 200.0 grams of lead tetra-acetate were then added in small portions over a period of one hour. After the addition of the oxydizing agent has been completed the mixture was allowed to stand for an additional three hours. At the end of this period tests showed the lead tetra-acetate to have been completely used up. Inorganic lead salts were eliminated by suction filtration and washed with 1000 ml. of dry acetone. The filtrate and washings were combined and the organic solvents removed under reduced pressure. To the syrupy residue 1000 ml.

of absolute ethanol were added. On shaking, a fine, white precipitate separated out which was removed by filtration. The solution was again taken to a syrup under the pump, and the addition of alcohol repeated. No precipitate separated out this time, The solution was then quickly saturated with gaseous hydrogen sulfide, the tracers of black lead sulfide removed by filtration, and the solvents again distilled off in vacuo. To the thick syrup thus formed 500 ml. of absolute ether were added and the dialdehyde dissolved. The ether was then removed in vacuo and after complete removal of all solvents the product remained behind as a very thick, pale amber syrup which could be transformed into a glass by further treatment in high vacuum. The yield was 87 grams corresponding to 89% based on the monoacetone glucose.

The aldehyde could be distilled at pressures of approximately 10^{-3} mm. mercury. No change in rotation, however, attended this step and therefore this purification is not considered necessary.

$[\alpha]_D^{25} = 48 \pm 2$ (c = 1.67) from water.

C. Small Scale Oxydation

5 grams of monoacetone glucose were suspended in 200 ml. of benzene and maintained at 60° C; 10 grams of lead tetra-acetate were then added rapidly and the solution was allowed to come to room temperature. The separated lead salts were removed by filtration and a procedure similar to that given under B. was followed for purification of the product.

Yield: 4.95 grams, i.e. 90% based on monoacetone glucose.

D. Preparation of Derivatives

1) Semicarbazone

1.64 grams of aldehyde, 2 grams of semicarbazide hydrochloride and

1.5 grams of sodium acetate were dissolved in 20 ml. of water forming a cloudy solution. The flask was immersed in boiling water for five minutes, whereupon the mixture became homogeneous. On cooling white plates were obtained. They were filtered, dried and weighed. The weight was 1.12 grams, and after two recrystallizations from ethanol, the crystals were found to melt at 207 - 208° C, with decomposition.

Iwadare gives 209 - 209.5° C (decomp.).

2) Phenylhydrazone

1.5 grams of aldehyde were treated with one ml. of phenylhydrazine and 5 ml. of 30% acetic acid. About one gram of sodium acetate was then added and the mixture warmed. On standing in the cold, yellow needles began crystallizing out.

They were collected by filtration, recrystallized twice from ethyl acetate.

Melting point: 140 - 141° C, $[\alpha]_D^{25} = -42° \pm 2$ (c = 3.0),

in chloroform

Literature values:

Fischer: mp 140.5 - 141° C $[\alpha]_D^{25} = -42° \pm 2$ (c = 5.0)

Iwadare: mp 140.5 - 141° C $[\alpha]_D^{10} = -41° \pm 1$

both in chloroform

2. The Kiliani Synthesis.

A modification of Hudson's procedure (5) was used as follows: 100 ml. of an aqueous solution containing 11.3 grams, (0.060 moles), of dialdehyde, 4.0 grams of anhydrous calcium chloride (0.036 moles), and 3.26 grams of sodium cyanide (0.066 moles) was used. The temperature of the solution rose quite rapidly to 35° C. The reaction mixture was allowed to stand for 24 hours at room temperature; in a parallel experiment it could be shown that 95% of the theoretical amount of

ammonia could be isolated within that period by distillation. 8.88 grams (0.060 moles) of calcium hydroxide was then dissolved in the solution and in a short while a white precipitate of the basic calcium salts began separating out. After precipitation was complete, the salts were filtered off and washed with cold lime water until only a faint chloride test could be observed. The theoretical amount of sulfuric acid was then used to decompose the precipitate. Concentration of the acid used was 6 N.

In one experiment the above solution was concentrated to dryness in order to isolate the lactones in the usual manner. On concentrating the solution, however, only a syrup could be obtained which defied crystallization and separation by all methods attempted.

In a second set of experiments isolation and separation was attempted through the barium salts; after complete removal of calcium ions by sulfuric acid, the solution was concentrated to 25 ml. An equal volume of alcohol was added and the residual calcium sulfate removed by centrifugation. The alcohol was removed by fractional distillation, and the alcohol-free solution neutralized to phenolphthalein with warm barium hydroxide. Upon concentration of the solution a white precipitate could be obtained in some experiments which should be shown to contain barium. It did not have a sharp melting point however, and could not be separated into diastereomers by fractional crystallization from any of the solvents tried. In other experiments only syrups were obtained at this stage.

3. Condensation of the Dialdehyde with Nitromethane.

50.0 grams of 1,2-acetone-D-xylo-trihydroxyglutaric dialdehyde was dissolved in 200 ml. of 95% ethanol. To this were added 100 ml. of nitromethane. The solution was made alkaline to litmus by the addition of two normal sodium

methoxide (in ethanol) and then a slight excess of methoxide added. The solution was shaken for 24 hours and then concentrated to about one fourth of its original volume. About 250 ml. of carbon tetrachloride were added and this solution extracted about seven times with 10 ml. portions of water. On concentrating the organic solvents in vacuo a viscous residue remained. By the slow addition of a small portion of absolute ether accompanied by stirring the nitro-compounds could be dissolved. On slow evaporation crystallization could be induced by scratching the walls of the vessel. The crystals were filtered by suction and washed five times with ether. The mother liquor and combined washings could be made to yield additional product on slow evaporation. The mixed 1,2-acetone-6-desoxy-6-nitro-D-glucose and its L-idose isomer were found to melt over a range of 100-120° C. The optical rotation in different preparations varied from -21 to -26°. Recrystallization was found to be possible from ethyl carbitol or chloroform, but separation by fractional crystallization could not be effected. The yields of mixture varied from 40 to 50 % of the theoretical.

4. Separation of 1,2-Acetone-6-Desoxy-6-Nitro-D-Glucose.

10 grams of mixed nitrosugars as prepared in 3. were dissolved in 500 ml. of absolute acetone containing two ml. of concentrated hydrochloric acid. The mixture was placed in the icebox and allowed to stand for one week. The acid was then just neutralized with concentrated ammonia, the ammonium chloride was centrifuged off, and the solution concentrated in vacuo to about 15 ml. The syrupy residue was taken up in 250 ml. of ether and extracted four times with 5 ml. portions of water. The solid residue was filtered and the ether solution concentrated in vacuo at room temperature. The resulting crystalline mass was dissolved in 25 ml. of hot ethanol and the solution slowly diluted with ice-cold

distilled water. The diacetone products largely consisting of 1,2-3,5-diacetone-6-nitro-6-desoxy-L-idose began separating out almost immediately and crystallization was complete after standing in the icebox overnight. The crystals were filtered with suction and washed with a little water. The brownish mother liquor after concentration in vacuo to half its volume was decolorized by brief heating with activated charcoal. On complete removal of the solvent under the vacuum pump the unchanged 1,2-acetone-6-desoxy-6-nitro-D-glucose was obtained in a crystalline form and could be recrystallized from benzene and ethyl carbitol to constant physical properties. Yield about 10% of theoretical. Melting point: 124 - 126° C, $[\alpha]_D^{23} = -18° \pm 2$ (c = 3.5)

Values given by Fischer are mp 126 - 127° C,
$[\alpha]_D^{24} = -16.8°$.

5. 6-Desoxy-6-Nitro-D-Glucose

The 1,2-acetone-6-desoxy-6-nitro-D-glucose obtained in the previous step was dissolved in 100 ml. of 0.1 N sulfuric acid and kept for approximately one hour in a water bath at 80° C. 80 ml. of 0.1 N barium hydroxide were then added and the remainder of the sulfate neutralized with a 0.1 N solution of barium acetate. The barium sulfate was removed by centrifuging. On evaporation of the solution in vacuo the free nitro sugar was obtained as a white powder with a melting point of 153 - 155° C and an optical rotation of $[\alpha]_D^{25} = +43° \pm 2$ (after about five minutes after dissolution in water). The rotation dropped to 37° after six hours.

Equilibrium rotation as given by Fischer = 36.7°.

6. 1,2-Acetone-6-Desoxy-6-Amino-D-Glucose-p-Toluene-Sulfonate.

2.0 grams of 1,2-acetone-6-desoxy-6-nitro-D-glucose was dissolved in

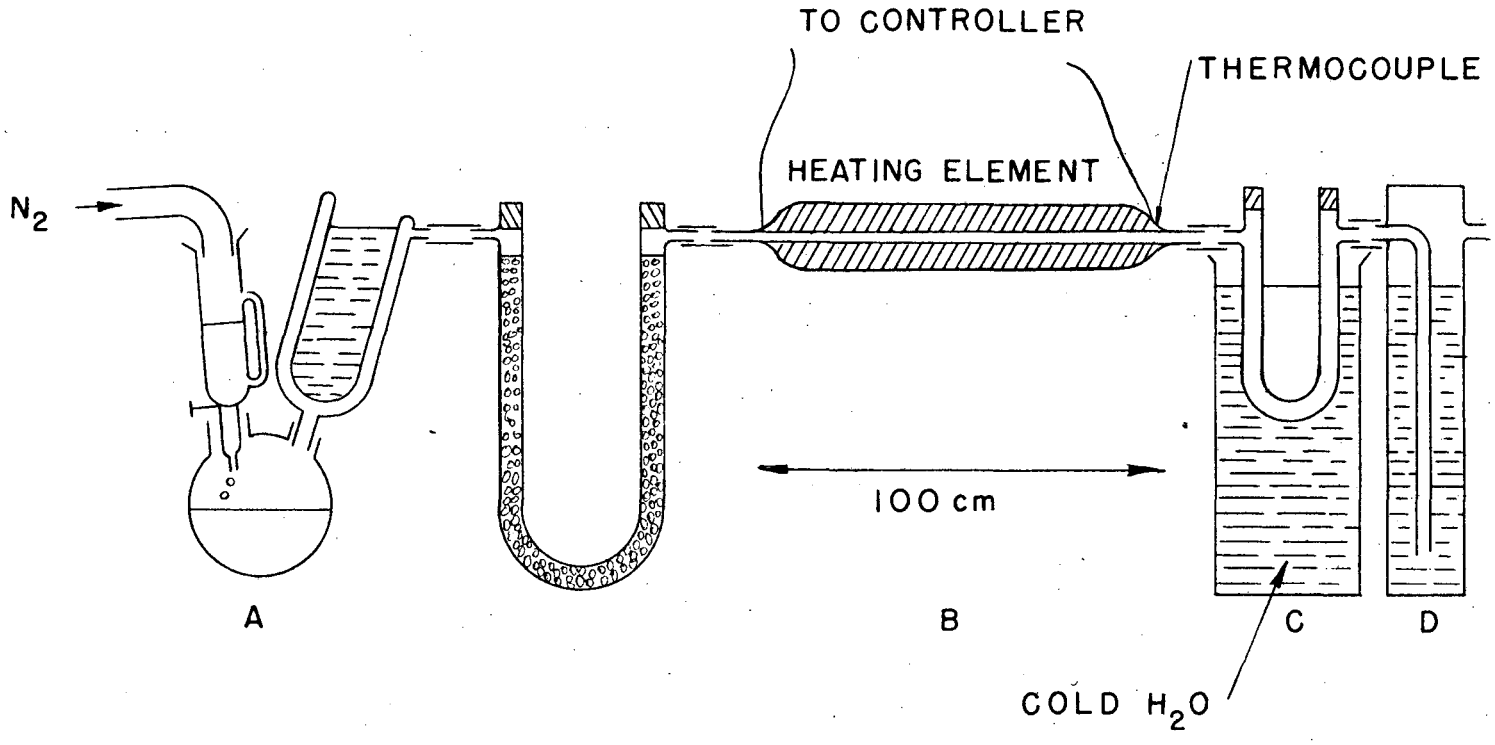

TO CONTROLLER
THERMOCOUPLE
HEATING ELEMENT
N_2
100 cm
A
B
C
D
COLD H_2O

FIG. 1

50 ml. of water and subjected to hydrogenation at ordinary temperature and pressure in the presence of 15 grams of freshly prepared Raney nickel catalyst. Within 60 minutes 600 cc. of hydrogen were absorbed. The theoretical value is 605 cc. at 25° C. and 750 mm. of pressure. The catalyst was then centrifuged off and the solution neutralized with 0.5 N p-toluene-sulfonic acid and the solvent removed in vacuo. The amorphous residue was dissolved in 15 ml. of absolute ethanol. On gradual addition of a large excess of absolute ether 2.0 grams (i.e. 80% of theory) of a crystalline compound was obtained with a melting point of 176 - 177° C and an optical rotation of $[\alpha]_D^{25} = 7^\circ$ (c = 3.5).

These values agree exactly with those reported in the literature.

Then a similar experiment was carried out with the desacetonated 6-desoxy-6-nitro-D-glucose, almost quantitative reduction could be observed in the hydrogenation step. So far it has not been possible, however, to isolate crystalline paratoluenesulfonate.

7. Attempted Deaminations.

0.5 grams of the 1,2-acetone-6-desoxy-6-amino-D-glucose-p-toluene-sulfonate obtained in 6. were treated with 3.5 ml. of water containing 0.7 grams of silver nitrite. The temperature was kept below 0° C by means of an ice salt bath. When the vigorous reaction ensuing appeared to have ceased, the reaction mixture was allowed to come to room temperature and was kept at that temperature overnight. The solution was then made just acid by means of 2 N hydrochloric acid, and the precipitated silver chloride centrifuged off. Immediately afterwards the clear solution was passed first through an anion and then through a cation exchange column. Water was then removed in vacuo, leaving behind a golden yellow syrup. All attempts to induce crystallization or separation by means of various solvents failed.

In a second experiment the deamination was carried out as described above, but immediately after the removal of the silver chloride about 0.5 grams of phenylhydrazine hydrochloride and 0.5 grams of sodium acetate were added. The mixture was then warmed on the steam bath and eventually kept in a beaker of boiling water for one hour. No solid osazone could be isolated from the reaction.

As an alternative method the procedure of Armbrecht (35) was used to effect the deamination. 0.5 grams of the p-toluene-sulfonate was dissolved in 2.5 ml. of water and the solution, while being kept at 30 - 35° C, saturated with vapors of N_2O_3. The latter was generated in a flask connected to the reaction vessel by means of rubber tubing. Nitrogen was used to sweep the gas from the generator to the reactor. Again evidences of reaction could be observed, but deionization by means of ion exchange, followed by removal of solvent in vacuo, again did not lead to a crystalline product. On treatment of the deamination mixture with pehnylhydrazine hydrochloride and sodium acetate at 100°, about 1 mg. of yellow precipate was obtained which melted indistinctly over a wide range between 180 and 200° C. The quantity of osazone thus isolated was not sufficient for isolation or purification experiments.

Similar negative results were obtained in all attempts at deamination starting with the desacetonated product 6-desoxy-6-nitro-D-glucose syrup.

8. Thermal Isomerization of Methyl Nitrite.

In order to duplicate as closely as possible the experimental conditions of the original investigators the following setup was employed:

As can be seen from Figure 1 methyl nitrite is produced in generator A, consisting of a two-necked roundbottom flask equipped with dropping funnel and gas condenser by the addition of dilute sulfuric acid to a solution of sodium nitrite

in aqueous methanol. The gas thus generated is swept through the system by a slow stream of nitrogen or carbon dioxide, dried over calcium chloride and subjected to thermal isomerization in the glass tube surrounded by a heating jacket (B). Temperature is maintained and controlled by a Foxboro automatic controller. Any nitromethane formed would be collected in the U tube (C) cooled to 15° by means of cold water while unreacted methyl nitrite is absorbed in tower D by means of aqueous methanol.

In a typical experiment, 50 ml. of 12 N sulfuric acid were added slowly to 290 grams of 95% sodium nitrite dissolved in 180 ml. of methanol and 170 ml. of water so as to give a slow and continuous evolution of methyl nitrite.

In run 1 the hot tube was filled with asbestos as in the original reference. This tube filling was later replaced by glasswool, glass beads, silica gel and the other sufraces shown in Table VI. This table also shows the various temperatures maintained in the experiments.

9. The Reaction between Methylsulfate and Potassium Nitrite.

In run 1, 45 grams of potassium nitrite (90% pure, 0.5 mole) was placed in a round bottom flask and 30 ml. of water were added to dissolve the solid. The solution was brought to ice-bath temperature and then 63 grams (15 moles) of dimethylsulfate were added slowly from a dropping flask. No reaction appeared to take place. After the addition was complete, the flask was equipped with a reflux condenser and was allowed to come to room temperature under constant and vigorous shaking. Again no reaction was observed. The vessel was then transferred to a steam bath and warmed gently. Almost immediately a vigorous exothermic reaction could be observed and maintained for three hours. Towards the end of that period, the vigorous evolution of gas appeared to cease and the liquid again separated into two layers. The top layer was clear and consisted of dimethylsulfate, while the

sodium nitrite solution in the bottom layer gave a turbid and brownish appearance. An additional 45 grams of potassium nitrite was then added but again agitation alone was not sufficient to restart the reaction. Warming was therefore again instituted and maintained at such a rate as to give a constant evolution of bubbles. This process was continued for two and a half hours at the end of which period the dimethylsulfate layer had almost completely disappeared. Upon standing for an additional six hours at room temperature the reaction mixture appeared completely homogenous.

The flask was then equipped with a Vigreaux column and distillation of the mixture undertaken. The fractions boiling between 80 - 90° and 90 - 100° C were collected, dried over calcium chloride over night, and examined separately. Both fractions appeared to boil between 99 and 101° C. The lower boiling had a refractive index of 1.3780, the higher boiling of 1.3775. Pure nitromethane has a $n_D^{20} = 1.3818$.

The liquid thus formed had a light straw color and a noticeable odor of oxides of nitrogen. Its purity is therefore doubtful. The yield of product (combined fractions) was 20.52 grams, corresponding to 66.7% of theory.

In all subsequent runs the following modifications were employed: A three-necked flask equipped with thermometer, mercury sealed stirrer and condenser was used. The dropping funnel was attached to the top of the condenser by means of a ground glass joint, and the addition of the dimethyl sulfate was made through the condenser.

In run 2, the temperature was kept constant at 25° C, the addition was complete after 10 hours, but stirring was continued for an additional 14 hours. The product was isolated in the manner already described, and after drying was found to weigh 21.40 grams (70.4%). It had a boiling point of 100-101°, was

colorless and odorless, and showed an $n_D^{25} = 1.3810$.

Run 3 was identical with run 2 in all respects, except that an additional 0.5 mole of potassium nitrite was added after the completion of the dimethylsulfate addition. The yield was 19.85 grams (65%) of a yellow liquid of intense odor with an $n_D^{25} = 1.3794$.

Runs 4 and 5 respectively differed only by the temperature of the reaction and the concentration of the potassium nitrite, as summarized in Table VII. The two refractive indeces were 1.3814 for run 4 and 1.3808 for run 5. All other experimental details are given in Table VII and the reaction discussed in the general part.

10. The Victor Meyer Reaction.

In the preliminary experiment, run 1, 7.10 grams (0.05 moles) of methyl iodide and 10 ml. of ether were placed in a round bottom flask equipped with a small retort for adding solids and an efficient refulx condenser. The system was protected from light as much as possible and the reaction carried out in the absence of direct illumination to minimize photodecomposition of either the silver nitrite or the silver iodide formed. 11.52 grams (0.075 moles) were added in small portions to the ethereal solution which was kept at a constant bath temperature of 40° C. A vigorous reaction started immediately and appeared to be complete after 30 minutes. But to insure completion of the reaction, the same temperature was maintained for an additional 120 minutes. At the end of this period the yellow silver iodide was removed by filtration, washed quickly with several portions of anhydrous ether and was dried in a vacuum oven at 60°. It was found to weigh 16.50 grams. The ethereal filtrate plus washings was placed in a round bottom flask equipped with a Vigreaux column and fractionated. The fraction boiling from

100-101° was found to weigh 1.10 grams and to have a refractive index of n_D^{25} = 1.3814. This corresponds to a yield of 36% of theory of pure nitromethane.

Since the wieght of the silver residue is knwon, the above data are sufficient to show that all the methyl iodide used must have been converted to either nitromethane or its isomer. For they must satisfy the equation a - 153.89x + 234.80x = b, where a = the experimentally determined number of grams of silver nitrite used, b = the total amount of silver residue (silver nitrite plus silver iodide), and x = the amount (in moles) of silver nitrite reacted. In subsequent runs the completion of the reaction was also tested by treating a small portion of the solution both with silver nitrate and with trimethylamine in methanol. The latter, of course, reacts with any methyl iodide present to form a light precipitate of tetramethyl ammonium iodide.

Run 2 was carried out in a manner similar to that just described except for the introduction of a thermometer and careful control of the temperature to 25°. Under these conditions the silver residue was found to weigh 16.88 grams, and the nitromethane obtained by distillation and of a similar purity as that just described weighed 1.87 grams. This corresponds to a yield of 61.3% of theory. Under these conditions the reaction appears to be complete in one hour but an additional two hours were again allowed to insure 100% reaction. Except for the variations shown in Table VIII, no changes were made in runs 3 and 4.

In run 5, where the temperature was maintained at 0° C, the addition of silver nitrite was extended over six hours. After that period the reaction flask was stoppered and placed in the icebox at 0° C in the freezing compartment for an additional 66 hours. Under these conditions reaction goes to completion.

From reaction 7 on the following experimental setup was used: A three-necked conical flask was equipped with an induction stirrer, a gas condenser cooled

with isopropyl alcohol - dry ice mixture and either an addition retort or a dropping funnel as the case demanded. The reaction flask was immersed in an ice-salt bath and the temperature inside the flask was kept below 0° C. The addition of silver nitrite (or of methyl iodide in run 8) was carried out slowly so as to maintain a constant slow evolution of gas inside the reaction vessel. When ether was used as a solvent the separation procedure was similar to that already indicated and the pure nitromethane was identified by its boiling point and its refractive index.

In the case of run 9 where no solvent was employed the reaction flask after the time indicated was transferred to the vacuum line and the nitromethane distilled off directly. It was again identified by its boiling point at atmospheric pressure and its refractive index.

In runs 10 and 11, where methanol was used as a solvent, separation could not be effected by distillation since nitromethane and methanol form a minimum boiling azeotrope. Another method had to be devised therefore in order to estimate quantitatively nitromethane in the presence of methanol. It was found that the qualitative test described by Manzoff (36) could be adapted to give quantitative results within 1%. Known solutions of nitromethane varying by 1% were made up in the concentration ranges from one to twenty volume percent of nitromethane and methanol. To ten ml. of each of the above solutions were added two mg. of vanillin and two drops of concentrated ammonia. Under these conditions a red color is obtained which varies with concentration and does not fade over a period of months. Within a fairly wide range neither shade no intensity is dependent on vanillin or ammonia concentration. Tests against known concentrations of nitromethane in methanol showed the method to be accurate within 1% by simple matching.

Consequently in run 10 one ml. of the solution was withdrawn, made up to five ml. by means of methanol and the concentration then determined. In run 11 the nitromethane concentration was such that 10 ml. of the solution could be withdrawn directly, the vanillin plus ammonia added, and the concentration determined against a known standard.

11. The Condensation of Arabinose with Nitromethane.

Three procedures will be given, one with a very large excess of nitromethane, the second with nitromethane and arabinose in approximately equal proportions, and the third with the arabinose in excess. All the other experimental conditions summarized in Table IX are variations of these three basic procedures.

A. With nitromethane in excess.

23.0 grams of L arabinose (0.151 moles) were suspended in 48 ml. of absolute methanol and 82.5 ml. of nitromethane (75 grams, 1.19 moles). To this was added a solution of 4.85 grams of sodium (0.21 gram atoms) in 161 cc. of methanol. The reaction mixture was placed on the shaking machine and shaken for 24 hours. The white amorphous precipitate which had separated out by the end of that period was filtered by suction and washed with absolute methanol, ether and benzene. It was then dried in a high vacuum over phosphorus pentoxide and was found to weigh 40.0 grams. On the basis of 95% conversion on the column (see below) this corresponds to an 86% yield of theory.

B. Approximately equimolar quantities.

0.98 grams (16 millimoles) of nitromethane were dissolved in 12 ml. of methanol. To this solution was then added 3.00 grams (20 millimoles) of D-arabinose. This arabinose had been obtained from the Thomas chemical company and it showed a rotation of $[\alpha]_D^{20} = 105^\circ$. 0.50 grams of sodium (22 millimoles)

were then dissolved in an additional 12 ml. of methanol and the two solutions combined. Shaking was then started and continued for 20 hours. At the end of that period the reddish mother liquor was separated from the yellowish residue by suction filtration. The amorphous solid was then washed three times with 25 ml. portions of methanol and three times with 10 ml. portions of anhydrous ether. It was placed in a vacuum dessiccator and dried over phosphorus pentoxide. After processing through the column and concentration of the eluate as described below, about one gram of arabinose separated out on the addition of methanol. Taking into consideration this amount of unreacted arabinose and calculating on the basis of 95% conversion in the Dowex-5 column, the yield of pure nitrosugar (sodium salts) corresponds to 36% of theory. The addition of more arabinose to the mother liquor obtained after filtration of the sodium salts and shaking for an additional 48 hours is without effect on the yield.

C. With arabinose in excess.

0.85 ml. of nitromethane (15 millimoles) is dissolved in 25 ml. of methanol. To this solution is added 9.00 grams of arabinose (60 millimoles) and 0.50 grams of sodium dissolved in an additional 25 ml. of methanol. Condensation was allowed to proceed for 36 hours. The residue isolated after the usual washing procedure was pink in color while the mother liquor remaining behind was red. After the usual sequence of reactions the supposed sodium salts can be shown to contain over 50% of unreacted arabinose. This arabinose must be removed before the nitrosugars can be separated.

12. Conversion of Sodium Salts to Sugar Nitroalcohols.

The experiments summarized in Table XI were carried out with samples of sodium salts obtained under the conditions discussed in 12a). Under those

conditions the sodium derivatives are quite pure and no interference by unreacted arabinose has to be dealt with. In a typical experiment 2.00 grams of sodium salts was dissolved in 15 ml. of water and passed through a column containing 100 grams of moist Amberlite-IR100-H. The column was eluted with 200 ml. of water. The pH at the end of the elution period was about 3.5. The eluate was concentrated under reduced pressure to a syrupy consistency. Absolute ethanol was then added and the solvent removed in vacuo. On repetition of this procedure, crystallization could be induced in the residue which after vacuum drying over concentrated sulfuric acid was found to weigh 1.75 grams. This corresponds to 95% of theory. Almost identical results were achieved by using Dowex-50 cation resin. In the case of Duolite cation exchanger only 74% of mixed nitrosugars could be recovered (run 3). In order to investigate whether this was due to slower action of the last named resin run 6 was undertaken. Here the solution of sodium salts was put on the resin and maintained there for one hour before elution was started. Under those conditions the yield drops to 70%. Quite plainly, a side reaction must occur under the pH maintained by the resin and in order to test this hypothesis runs 4 and 5 were made using Dowex resin which had heretofore given satisfactory results. In run 4 an hour was allowed to elapse before elution was begun, while in run 5 this period was extended to three hours and in an additional run to 24 hours. 88% conversion was the total amount of crystalline nitrosugars isolated in the former case, and around 75% in either of the two latter cases. The most likely side-reaction under the conditions of the experiment would be the hydrolytic aciton of an acid on a nitro-compound yielding a carbonyl derivative (see also the discussions of the Nef reaction.) If that is indeed the course of reaction that we have to contend with then it should be possible to isolate at least a derivative of the corresponding carbonyl compound, in this

case of either D-glucose or D-mannose. Consequently the syrup remaining behind after the separation of the mixed nitro-derivatives was dissolved in aqueous ethanol, 1.0 grams of phenyl hydrazine hydrochloride and 0.5 grams of sodium acetate added and the solution placed in a testtube in a steam bath. After about one hour the testtube was examined and indeed on cooling a yellow precipitate was found. It was filtered out and washed and found to weigh about 50 mg. It was identified as glucosazone by its melting point of 205° (with decomposition). It could have arisen from either D-glucose or D-mannose since the latter, although at first yielding mannose phenylhydrazone, would also give the ozazone under the vigorous conditions of the experiment.

13. Separation of Diastereoisomers.

Usually the procedure about to be described was followed: Between one and five grams of sodium salts, synthesized as described above, were converted to the free nitroform by passage through the column and fairly rapid elution. The water was removed under reduced pressure, between 15 and 50 ml. of absolute methanol added and the solvent removed in vacuo. If a small amount of unreacted arabinose was mixed up with the sodium salts it usually separates out at this point and can be removed by flitration. On the addition of a second portion of methanol and evaporation a crystalline residue is usually obtained although in some cases only syrups have been observed. If it is over a wide range between 95 and 130°, larger amounts of arabinose are pr sent (the smaller the range and the closer to 158°, the more arabinose). The removal of the arabinose is achieved by placing the mixed solids into the thimble of a Soxhlet apparatus and extracting continuously with acetone for periods ranging up to 48 hours. The mixed nitrosugars become dissolved in the acetone and separate slowly on cooling while the

arabinose remains behind. After the 1-desoxy-nitro-sorbitol and the 1-desoxy-nitromannitol have been obtained free from arabinose, they are separated by fractional crystallization from ethanol. The best results have been obtained by dissolving the nitrosugars in the minimum amount of hot absolute ethanol and allowing slow evaporation to take place in the desiccator over concentrated sulfuric acid or phosphorous pentoxide. Under these conditions the 1-desoxy-1-nitro-D-mannitol separates out first and can be obtained pure after but one additional recrystallization.

It melts at 134° C and shows a rotation of $[\alpha]_D^{25} = -7.0 \pm 2$ (c=5.3). From the mother liquor and washings on continued slow evaporation the 1-desoxy-1-nitrosorbitol with a melting point of 106° and a rotation of $[\alpha]_D^{25} = -10.0$ can be obtained. These physical constants are in perfect agreement with those given by H. O. L. Fischer.

The yields in representative runs are: 92% of theory to the mixed nitrosugars from the sodium salts, and 40% to the pure 1-desoxy-1-nitromannitol, with the 1-desoxy-1-nitrosorbitol obtainable in a yield of 36% of theoretical. This means that the pure nitrosugar alcohols can be synthesized in an overall yield of 70% from the sodium salts. The minimum scale on which these separations have been carried out was 25 millimoles.

14. The Nef Reaction.

Since very little work had ever been carried out concerning this reaction, it was decided to study the conditions controlling it in a rather more thorough fashion (see page 45.)

a. Analytical

1) Nitropropane

Since Fischer's studies had shown the sugar nitroalcohols to behave simi-

larly to ordinary nitroparaffins it seemed reasonable to investigate the Nef reaction by means of a simple nitroparaffin as a model compound. Nitropropane was chosen because of its ready availability, ease of purification, and the higher boiling point of propionaldehyde (the reaction product), as compared to formaldehyde or acetaldehyde. The commercial brand of C.P. 1-Nitropropane (supplied by Commercial Solvent Corp.) was fractionated at atmospheric pressure, the fractions boiling below 130° C discarded, and only the fraction boiling at 131° C and having a refractive index of 1.4005 collected.

2) Propionaldehyde

The success of the method hinged, of course, on the possibility of measuring the propionaldehyde produced accurately and efficiently. The vapor pressures of this aldehyde are such that with care and experience in manipulation special precautions do not have to be taken in titration. Several volumetric methods are available, of which those using hydroxyl amine as the specific reagent appeared to hold the greatest promise. Kolthoff and Stenger describe several procedures (37), of which the method described below can be regarded as a simiplified modification.

The changes introduced are: Aqueous (or aqueous-alcohol) reagents are used throughout. Also, since it has been found that the condensation between aldehydes and the so-called carbonyl reagents (such as bisulfite, hydroxyl amine, phenylhydrazine, etc.) used in quantitative determinations is most rapid in the buffer regions determined by these nitrogenous bases, previous methods have introduced a base (such as pyridine) which together with the salt of the reagent permitted one to operate within a buffer region. In our method no extraneous base is necessary, since the solution is buffered by the use of the hydroxylamine hydrochloride-hydroxylamine system itself. This result is achieved by adding one half the number of equivalents of a strong base to a given number of equivalents of

hydroxylamine hydrochloride. Finally, bromophenol blue (0.1% in alcohol) is used as the indicator.

To test the method against known concentrations of aldehyde the following procedure was used:

First, 0.5 N solutions of hydrochloric acid, sodium hydroxide and hydroxylamine hydrochloride are prepared. The first two named are standardized and their exact concentration determined, while the last need only be approximate.

Then hydroxylamine hydrochloride-hydroxylamine base solutions were prepared in two series: In series A, 40 ml. of the hydroxylamine salt solution were treated with 20 ml. of the known base, while in series B, 80 ml. of salt solution and 40 ml. of base were used. Thus the two series only differed in the amount of hydroxylamine freed, but not in their pH. To the stoppered flasks containing these solutions was then added 1.00 ml. of propionaldehyde (0.807 grams, 13.92 millimoles) by careful pipetting. The flask - five in series A, four in series B - were quickly stoppered, shaken occasionally, and permitted to stand for the lengths of time indicated in Table XIV. As has already been mentioned, the two series differed by the amount of free hydroxylamine base available for condensation with the aldehyde immediately upon addition of the latter. In series A, the amount of aldehyde corresponds to an excess of aldehyde over free hydroxylamine, while in series B the situation was reversed. This investigation was undertaken in order to insure, or avoid, the presence of excess hydroxyl amine base. Our results are summarized in Table XIV. Backtitrations were with the known base in series A, and with the known acid in series B, since excess free acid is produced in the former and excess free base in the latter.

Table XIV

Aldehyde Titrations

Time	Series A	% Deviation	Series B	% Deviation
2 min	25.68	-8.01		
30 min	26.70	-4.08	26.81	-3.56
40 min			27.40	-1.22
60 min	27.76	0.10	27.48	-1.15
120 min	27.97	0.52	26.49	-4.32
16 min	26.87	-3.48		

$$NH_2OH \cdot HCl + NaOH \rightleftharpoons NH_2OH + NaCl + H_2O$$

$$NH_2OH \cdot HCl \rightleftharpoons NH_3OH^+ + Cl^-$$

$$RCHO + NH_2OH \rightleftharpoons RCH = NOH + H_2O$$

$$RCHO + NH_2OH \cdot HCl \rightleftharpoons RCH = NOH + H_2O + HCl$$

The numbers given in Table XIV refer to ml. of 0.500 N hydroxylamine used up in condensation with the aldehyde. They are derived from the titrimetric results as described above, with acid or base, after subtracting an indicator correction determined by running an indicator blank. As can be calculated, 27.84 ml. of the reagent would be needed to react with the amount of aldehyde used. The percentage deviations are calculated from the experimental and this theoretical value. As the table shows, either procedure can be used to give results accurate within about one percent, although procedure A. appears to yield slightly more accurate results. The optimum times are after the reaction has proceeded between 16 and 120 minutes. In reaction times shorter than these, the condensation reaction apparently has not yet reached equilibrium, while in the long reaction time, side-reactions, probably of an oxydative nature, combine to make the results inaccurate.

After it has thus been determined that propionaldehyde could be analyzed quantitatively by hydroxylamine titration, the method was adapted for analysis of product in the Nef reaction. Several portions of propionaldehyde were run through the procedure described below, neutralized at 0° C with sodium hydroxide to a bromphenol blue endpoint and then subjected to hydrox lamine titration as described with a 60 minute reaction time. All these blanks fell within one percent of the theoretical values.

3) D-Mannose

In the applications of the procedure to the reaction producing D-mannose from 1-desoxy-1-nitro-D-mannitol, the former was precipitated as the phenylhydrazone, by reaction with phenylhydrazine hydrochloride and sodium acetate at room temperature. The precipitated phenylhydrazine was washed with cold water, 95% ethanol and cold absolute ethanol, and was then dried in a vacuum oven at 60° C. Since this procedure, when run on known samples of D-mannose, gave uniform yields of 88-90%, the latter value was taken as a constant and the yield back calculated from the weight of dried phenylhydrazone formed.

b. The Reaction Proper

1) With Nitropropane.

The sulfuric acid solution used in experiments 1, 2, 3, 4, 10, 11, 12 and 13 was prepared by diluting 650 ml. of concentrated sulfuric acid with 730 ml. of distilled water. The acids used in the other runs were made up to have the concentrations shown in Table XII.

The sodium hydroxide, used to dissolve the nitroparaffin, was made up of 300 ml. of 6 N sodium hydroxide, 200 ml. of 95% ethanol and 400 ml. of water. All runs were carried out in an identical manner with the only modification as shwon in the table, in order to insure maximum uniformity.

2.00 ml. of 1-nitropropane were dissolved with shaking in 10.00 ml. of the sodium hydroxide solution. (Standardization against known acid showed this solution to be 3.70 N.) This alkaline solution, presumably containing the sodium salts of the nitroparaffin, was placed in a pressure-equalizing dropping funnel, connected by means of standard taper joints to a three-necked flask, also equipped with mercury sealed stirrer and thermometer, containing 20.00 ml. of the sulfuric

Acid solution. The reaction flask was cooled to the temperature indicated by means of suitable cooling baths and then stirring and drop-wise addition of the alkaline solution was started. Great care was taken not to subject the reaction system to local overheating. This was accomplished by careful control of the rate of addition and stirring. When the addition was completed the reaction mixture was allowed to stand for an additional 15 minutes at the temperature indicated, whereupon the dropping funnel was replaced by one containing 6 N sodium hydroxide. A few drops of bromphenol blue indicator solution were then added, and the sulfuric acid neutralized to the bromphenol blue endpoint. Neutralization was allowed to proceed at a rate designed not to raise the temperature above 0° C.

After the neutral point had been reached, 80.00 ml. of the hydroxylamine hydrochloride and 40.00 ml. of the 0.500 N sodium hydroxide solution were then added as previously described. The flask was stoppered tightly and permitted to stand at room temperature for approximately 60 minutes. At the end of this period the solution was back titrated with known hydrochloric acid. The results summarized in Table XII are derived from these titrations by subtracting the appropriate indicator blank corrections.

2) With 1-Desoxy-1-Nitro-D-Mannitol

In the case of runs 12 and 13 the results obtained with nitropropane are applied to 1-desoxy-1-nitro-D-mannitol. The directions for run 12 utilizing conditions giving maximum yield are as follows:

0.1303 grams of nitrosugar (0.610 millimoles) were dissolved in 1.00 ml. of 1.00 N sodium hydroxide and the solution precooled to -25° C. This solution was added slowly with constant stirring to 0.75 ml. of the sulfuric acid solution described above. An ice-acetone bath was used to keep the temperature of the reaction substances below -10° C. After completion of the hydrolysis the excess acid was neutralized with solid sodium carbonate. A slight excess of phenylhydra-

zine hydrochloride (0.620 millimoles) and 0.25 grams of solid sodium acetate were then added. The solution turned cloudy after a few minutes at room temperature and was placed in the icebox overnight. The next day the separated crystals were filtered, washed throughly with water, 95% alcohol and absolute ethanol. They were dried in a vacuum oven at 60° C. Examination showed them to be colorless and to have a melting point of 186-188° C. The weight of mannose phenylhydrazone was 0.0814 grams. This corresponds to a yield of 62% in the Nef reaction.

II. EXPERIMENTS WITH CARBON 14

1. Plating Techniques

In the assay of radioactive samples two techniques only were used in the course of this research. If solutions of the material to be counted could be prepared, aliquots of these solutions were plated directly on glass or aluminum plates and counted, using the Geiger-Mueller counters already deseribed (28, 38). This technique is known as "direct" plating. Where solids, liquids or gases, of which a solution could not be prepared, had to be counted, or where greater accuracy was desired, the assay was carried out in the form of barium carbonate deposited on aluminum plates.

a. Direct Plating

As has already been stated, this method was used whenever a solution of the material to be assayed could be prepared. Successful plates have been made using such varied solvents as absolute ethanol, various ethanol-water mixtures, methanol, ethyl acetate, chloroform, ether and water. In the case of the last named solvent it was found advantageous to add a small quantity of detergent ("Vel") to the plate before deposition of the solution was begun in order to decrease surface tension and to achieve a more even deposit.

The equipment necessary for the successful preparation of direct glass plates and the plates themselves have already been described (38). To summarize, the glass disks used had an overall diameter of 4.5 cm. with the covered area being limited to 11.5 cm^2 by a ground in groove. Sample sizes used were 0.030, 0.10 or 0.20 ml., depending on the activity of the sample and the dilution necessary to effect solution. This volume of solution was spread on the plate in a thin spiral line starting from the center and progressing towards the periphery. It was delivered from micropipet attached to a syringe. The solvent was evaporated by means of a hot air blower. The speed of evaporation was adjusted to give the most uniform layer of solid possible. During sample deposition and evaporation of the solvent the glass plate revolved on a small turntable. At least two plates were prepared for each sample, counted independently, and the average taken as the count reported.

b. Barium Carbonate Plates

Of the various techniques available, the settling technique has been adopted as the most accurate. The organic material was combusted to carbon dioxide, the latter absorbed in alkali, and precipitated as barium carbonate. The diameter of the aluminum plates used is four cm., their thickness 0.006". They were prepared from 2 S, 3/4 hard sheet with one polished face flat strip rolled.

A typical mounting assembly is shown in Fig. 21 of reference 38. The barium carbonate sample was slurried with 95% ethanol by means of a mortar and pestle. It was then allowed to settle briefly. Immediately thereafter the slurry was transferred to the assembly by pouring or pipetting onto the cup formed by a tared disk and the thick concentric sleeve. A tight seal was provided by the pressure ring. The whole setup was heated on a hotplate for a few seconds and then placed under a heat lamp. When the precipitate had been thououghly dried under the lamp, the sample plate was removed from the cup and put on a hotplate at about

140°C for ten or fifteen seconds to remove the last traces of vol tile material. Plate and sample were then weighed and were now ready for counting.

2. Synthesis of D-Glucose-1-C^{14} and D-Mannose-1-C^{14}

a. Nitromethane-C^{14}

1) Methyl Iodide-C^{14}

As has already been stated, the methyl iodide obtained by the method customarily employed in this laboratory (I_2 and P_4 on aqueous methanol) (28) is unsatisfactory for purposes of the Victor Meyer reaction and subsequent transformations becuase of the presence of traces of phosphorus-containing impurities. It therefore became necessary to devise a synthetic method which did not make use of phosphorus. Practice runs both without and with radioactive carbon showed the feasibility of treating the aqueous methanol, obtained by the high pressure, catalytic hydrogenation of CO_2-C^{14}, with a calculated quantity of hydrogen iodide of specific gravity 1.96 such that the concentration of hydrogen iodide at the end of the reaction would not be less than that corresponding to constant boiling HI (specific gravity 1.70). If this scheme is followed quantities of the acid are used which can easily be accomodated in the bomb tube customarily employed here for iodinations.

The experimental procedure consisted of pipetting the calculated amount of HI into the methanol solution cooled to isopropyl alcohol-dry ice temperature, jacketing the bomb tube for refluxing, and refluxing the reaction mixture for approximately one hour on the steam bath. During this period the formation of methyl iodide could be observed as the appearance of a second phase along the walls of the vessel. At the end of the reaction period the mixture is again cooled, the tube transferred to the vacuum line, and the methyl iodide fractionally distilled

off. It is first washed with sodium carbonate, then with water and finally dried over phosphorus pentoxide. All transfer operations are carried out on the vacuum line at pressures of approximately 10^{-3} mm. Hg. In various runs utilizing non-isotopic and isotopic methanol as the starting material, of a scale between 20 and 30 millimoles, the yields were around 90% with a maixmum of 95% based on the methanol used.

2. The Victor Meyer Reaction

1.95 grams (13.75 millimoles) of methyl iodide obtained in the manner just described and containing 1.093 millicuries of activity (this corresponds to $1.093 \times 2.2 \times 10^9/17$, i.e. 142,000,000 counts on our thin mica window GM counters) was transferred on the vacuum line to a three—necked flask containing 4.0 ml. of methanol. The mixture was cooled to isoptopyl alcohol-dry ice temperature and removed from the vacuum line. The flask was equipped with thermometer, an efficient two part condenser, (consisting of a water jacketed condenser connected to a gas condenser with dry ice isopropyl alcohol as a coolant), and an addition retort containing 4.00 grams (26 millimoles) of freshly prepared, vacuum dried silver nitrite. The system was allowed to come to icebath temperature and slow addition of the solid was then commenced adn continued at such a rate as to maintain a steady slow reaction, as evideneed by the appearance of silver iodide. This took approximately two and one half hours. At the end of this period the icebath was removed and replaced by a waterbath kept at a constant temperature of 45° for an additional hour. The system was then permitted to come to room temperature overnight. Agitation was provided by shaking and swirling during the first stages of the reaction. The next morning the flask was cooled to dry ice temperature once more, connected to the vacuum line, and the methanolic solution of nitromethane

transferred to a one-necked, pear-shaped flask. Completeness of removal of methyl iodide was tested for by means of trimethylamine in ethanol.

b. Condensation with Arabinose

To the nitromethane solution were added 1.14 grams (18.70 millimoles) of inactive nitromethane, 3.75 grams (25 millimoles) of arabinose, and a solution of 0.72 grams of sodium in 30 ml. of methanol. The flask was placed on the shaking machine and shaken for 18 hours. At the end of this period the separated sodio-nitro sugars (plus some unreacted arabinose) were removed by filtration washed with ether and petroleum ether, and dried overnight over phosphorus pentoxide. Counts were then taken of the white, amorphous solid and of the yellowish methanolic motherliquors and washings.

The combined sodio-nitro sugars were found to have a total activity of 23,200,000 counts/minute (average of four determinations, direct plates, from water), while the methanolic solution showed an average total activity of 66,700,000. This activity remained constant, even after shaking the solution with an excess of 10 grams of arabinose for an additional 72 hours. These activity data show that of the methyl iodide used approximately 90,000,000 counts, i.e. 65% were converted to nitromethane, while 34% escaped as methyl nitrite, but could be recovered if a higher scale of activities makes this step advisable. The condensation with arabinose, from the same data, appears to have proceeded in a yield of 39%, based on nitromethane. The overall yield of sodium salts from methyl iodide-C^{14} is therefore 25.2%.

c. Separation of Diastereomers

This step was carried out in the manner already described. After passage through the Amberlite column and removal of water, followed by treatment with methanol, the arabinose settled out and could be removed by filtration. On re-evapora-

tion in vacuo with absolute ethanol 0.7258 grams of a solid residue were obtained. It was continuously extracted with acetone for 24 hours. Almost the total solid went into solution, showing complete removal of arabinose. The latter had shown a melting point of 158° C, also indicating absolute purity. The solid residue obtained by cooling the acetone was filtered and redissolved in absolute ethanol. On slow evaporation of the solvent there were obtained 0.3472 grams of 1-desoxy-1-nitro-D-mannitol-1-C^{14}, melting from 128 to 132° C. After two recrystallizations the melting point was found to be 134°C, and the specific activity remained constant at 19,000 cts./min./mg. The total activity of this compound obtained independently was 6,340,000 counts per minute from water. Since the total weight of recrystallized material was 0.3088 grams, this compares with a calculated total activity of 5,900,000 counts per minute, a deviation of about 7%.

Continued slow evaporation yielded 0.2120 grams of 1-desoxy-1-nitro-sorbitol-1-C^{14}, which melted at 92 to 100° C. After two recrystallizations from ethanol this material was found to weigh 0.1740 grams and to have a constant, sharp melting point at 106° C and a constant specific activity of 17,800 counts per minute per mg. Its total activity was found to be 2,997,000 counts per minute as against a calculated total of 3,020,000 counts per minute.

After the removal of the nitrosorbitol there remained behind a syrup with an activity of about 12 million counts, which could not be crystallized. This activity, however is completely recoverable. The yields in this step are as follows: 26% to pure nitromannitol-1-C^{14} and 15% to pure nitrosorbitol-1-C^{14}. Part of the low yield is due to the exceedingly high purity of the products required.

e. The Nef Reaction.

The 0.308 grams of 1-desoxy-1-nitro-D-mannitol-1-C^{14} obtained above were dissolved in 0.82 ml. of 2 N sodium hydroxide and added dropwise from a syringe to

0.94 ml. of 16.76 N sulfuric acid, kept at a temperature of -10° C or below. The reaction mixture was then allowed to come to room temperature and was neutralized with warm barium hydroxide to a pH of 3. The barium sulfate was then centrifuged off, washed with water until no more activity was discernible in the washings, and the combined filtrate and washings were concentrated to about 25 ml. They were then passed through a column of Amberlite IR 100-H and eluted until no additional activity could be found in the eluate. The water was then removed under reduced pressure, absolute ethanol added to the syrupy residue, and again removed in vacuo. The residue was transferred for evaporation to a vacuum desiccator over concentrated sulfuric acid and slow evaporation was undertaken repeatedly. So far all attempts to induce crystallization have been unsuccessful.

Similarly 0.1740 grams of 1-desoxy-1-nitrosorbitol were dissolved in 0.500 ml. of 2 N sodium hydroxide and added to 0.55 ml. of the sulfuric acid solution under similar experimental conditions. Working up of the glucose followed the same scheme as that described above. It is hoped that slow evaporation of the sugar syrups will lead to the desired crystalline sugars containing C^{14} in the 1-position. A similar procedure led to positive results in several inactive practice runs. The yield in the Nef reaction in these runs averaged about 65%, with an overall yield of 7% for the mannose and 5% for the glucose, based on the methyl iodide used.

f. Glucose-1-C^{14} and Mannose-1-C^{14}

In order to have available a certain amount of these sugars for biological experiments, it was decided to co-crystallize aliquot portions of the syrups with excesses of inactive sugars as carriers.

Thus 0.1500 grams of inactive D-glucose and a small portion of D-glucose-

1-C^{14} syrup were dissolved in hot absolute alcohol and crystallized from this medium. After two recrystallizations the resulting sugar was found to have a constant specific activity of 650 counts/minute/mg. and to weigh 0.1270 grams.

Similarly 0.060 grams of mannose were co-crystallized with a small portion of D-mannose-1-C^{14} syrup. The resulting material after recrystallization showed a specific activity of 570 counts/minute/mg.

D. Summary

The original contributions included in this dissertation can be summarized as follows:

An investigation of the cyanhydrin synthesis as applied to 1,2-acetone-D-xylotrihydroxyglutaric dialdehyde was carried out. It proved to be unsuccessful, probably because of the presence of a mixture of the two diastereomers and of the desacetonated reaction products. Several attempts to deaminate 1,2-acetone-6-amino-D-glucose and its desacetonated product were made in order to devise a method for obtaining D-glucose through a condensation of the aldehyde named above with nitromethane. These investigations had the aim of arriving at a useful synthesis of D-glucose-6-C^{14}.

In the application of the Sowden-Fischer synthesis of D-glucsoe, a critical study of the condensation of arabinose with nitromethane was undertaken and the influence of relative reactant and catalyst concentrations was investigated. The influence of several cation exchange resins on the conversion of sodium salts of sugar nitroalcohols to the free nitrosugars was studied.

Several methods of synthesizing nitromethane were evaluated in order to arrive at a method suitable for obtaining nitromethane C^{14}. Brief experimental studies of the thermal isomerization of methyl nitrite and the reaction between alkali nitrites and dimethyl sulfate were undertaken. The Victor Meyer reaction was subjected to critical analysis, the influence of a series of reaction variables was studied, and a practicable synthesis of nitromethane-C^{14} was evolved.

Another reaction studied was the Nef reaction between the sodium salts of aliphatic nitro-compounds and mineral acids. Nitropropane was chosed as the model compound, and an analytical procedure for the assay of propionaldehyde in the reac-

tion mixture worked out. It was found that temperature had a marked effect on the course of the reaction, with low temperatures leading to the highest yields of desired product. The influence of other variables, such as acid concentration, manner of addition, etc. was less pronounced but should also be carefully controlled. A working hypothesis explaining these various effects was derived and conclusions based on it applied to the synthesis of aldose sugars from sugar nitroalcohols.

Using carbon 14, a modification of the synthesis of methyl iodide-C^{14} had to be found which did not employ phosphorus. This was achieved by the treatment of aqueous methanol with hydrogen iodide of specific gravity 1.96. Nitromethane-C^{14} was synthesized, condensed with arabinose, taken through the steps of the Sowden-Fischer synthesis, and D-glucose-C^{14} and D-mannose-C^{14} isolated, although not in crystalline form so far. It has been possible, however, to prepare these two sugars crystalline, with low specific activities, by the device of co-crystallization with inactive material as carrier.

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