PREFACE

In this thesis a brief account is given of the methods available for the synthesis of nitro sugars, and their reactions known so far are reviewed. Studies undertaken with the intent to contribute to the chemistry of nitrogeneous sugars are then described and their results are discussed.

The thesis is divided into two parts. Part I deals with the dehydroacetylation of nitro sugar acetates. Nucleophilic additions to nitro sugar olefins are discussed in Part II. In Part II.1, the addition of isopropyl lactates is described which lead to the synthesis of positional isomers of muramic acid. In Part II.2, the synthesis of 2,3-diamino-2,3-dideoxy-D-glucose and of its D-*manno* isomer by addition of aminobenzoic acids is discussed.

The results of Part I, and Part II.1, have been published.[a]

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a. H. H. Baer and F. Kienzle, J. Org. Chem., 33, 1823 (1968); H. H. Baer and F. Kienzle, ibid., 32, 3169 (1967).

ACKNOWLEDGMENTS

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ABSTRACT

Part I.

The dehydroacetylation of methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-glucopyranoside (I) and of its D-manno (II) and D-galacto (III) isomers resulted in the loss of two molecules of acetic acid and gave, as a reaction intermediate, a derivative (VI) of 2H-pyran which dimerized spontaneously in a Diels - Alder reaction to an optically inactive, tricyclic product, 3,4,4a,8a-tetrahydro-3,5-dimethoxy-8a,9-dinitro-1,4-ethenopyrano [4,3-c]-pyran-1,7(5H)-dimethanol diacetate (VIII). At lower temperatures an optically active diastereoisomer (XXX) of VIII was isolated in addition. The stereochemistry of the products is discussed, and their structure follows from spectroscopic data and from a facile, thermal conversion into an isochromene, 1-hydroxy-7-nitro-1H-2-benzopyran-3,5-dimethanol 3,5-diacetate (X). Acid-catalyzed acetalization of X furnished the corresponding 1-alkoxy derivatives, XIII, XIV, and XV, whereas phenylhydrazine gave a monophenylhydrazone (XVI) of the ring-open tautomer. Mild oxidation of X led to the corresponding isocoumarin (XVII) and more drastic oxidation, to nitrobenzene-3,4,5-tricarboxylic acid (XVIII) which was characterized also as trimethyl ester (XIX). Sodium borohydride reduction of X furnished a tetraol (XX) that was degraded by periodate to

3,4-dihydro-3-hydroxy-7-nitro-1H-2-benzopyran-5-methanol (XXI). With semicarbazide, XXI yielded 2,6-bis-hydroxymethyl-4-nitrophenylacetaldehyde semicarbazone (XXII), and with sodium borohydride it was reduced to 4-(2-hydroxyethyl)-3,5-bis-hydroxymethyl-nitrobenzene (XXIII).

Reaction of the triacetate I with liquid ammonia resulted in the formation of a methyl 2,3,4-trideoxy-2,4-diacetamido-3-nitro-hexopyranoside (XXXIII) which was hydrogenated and acetylated to give a methyl 2,3,4-triacetamido-2,3,4-trideoxy-hexopyranoside (XXXIV).

Part II.1.

Nucleophilic addition to methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-erythro-hex-2-enopyranoside (I) of isopropyl L-lactate and isopropyl D-lactate, respectively, gave methyl 4,6-O-benzylidene-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-ß-D-glucopyranoside (II) and its D-1-(isopropoxycarbonyl)ethyl epimer (III) in yields of over 80%. The use of isopropyl DL-lactate afforded II and III in a ratio 10:1. By standard methods of hydrolysis, catalytic hydrogenation, and acetylation, II and III were converted into 3-amino-2-O-(L-1-carboxyethyl)-3-deoxy-D-glucose (XII) and its D-1-carboxyethyl epimer (XIX) and various derivatives thereof, including the lactams VIII and XVII.

A second synthesis started from methyl 3-acet-

amido-3-deoxy-ß-D-glucopyranoside (XX) whose 4,6-O-benzylidene derivative (XXI) was condensed with DL-2-chloropropionic acid and debenzylidenated to give methyl 3-acetamido-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside (XXII).

Part II.2.

Nucleophilic addition to the nitro olefin I of o-, m-, and p-aminobenzoic acids, respectively, resulted in the formation of methyl 4,6-O-benzylidene-2-(2-carboxyphenyl)amino-2,3-dideoxy-3-nitro-ß-D-glucopyranoside (VI), its 2-(3-carboxyphenyl)amino (XXII), and its 2-(4-carboxyphenyl)amino isomer (XXIII). Variation of the reaction conditions gave in the case of o-aminobenzoic acid a yellow-colored isomer of VI for which the manno configuration (VIII) was established. Compounds VI and VIII were further converted into the methyl esters VII and IX, respectively. VII was directly obtained by addition of methyl o-aminobenzoate to olefin I. Hydrolysis of VI, XXII, and XXIII gave the benzylidene-free compounds X, XXIV, and XXV, respectively, all of which furnished upon hydrogenation in the presence of palladium oxyhydrate - barium sulfate catalyst, crystalline methyl 2,3-diamino-2,3-dideoxy-ß-D-glucopyranoside dihydrochloride (XII). Known methyl 2,3-diacetamido-2,3-dideoxy-ß-D-glucopyranoside was obtained from XII by standard procedures. Yellow VIII, which

formed a colorless dihydrate (XV), yielded upon hydrolysis and catalytic hydrogenation, methyl 2,3-diamino-2,3-dideoxy-ß-D-mannopyranoside dihydrochloride (XVII). N-Acetylation of XVII gave the diacetamido compound XIX which was hydrolyzed to the free sugar, 2,3-diamino-2,3-dideoxy-D-mannose, isolated as dihydrochloride (XX). The same diacetamido compound XIX was obtained from methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-mannopyranoside (XXI), a by-product in the addition of ammonia to olefin I, by hydrolysis, hydrogenation, and acetylation.

Nucleophilic addition of o-aminobenzoic acid to the threo isomer of the nitro olefin I furnished an addition product (XXVII) whose configuration has not been established.

INTRODUCTION

Numerous nitrogen-containing derivatives of carbohydrates have been isolated from natural sources. They are either glycosylamine-type compounds or amino-deoxy sugars (1), but nitro sugars have not been encountered in nature thus far. Nitro sugar derivatives can, however, be synthesized and are particularly valuable as intermediates for the preparation of rare amino sugars. They also show interesting chemical properties not found in carbohydrates generally. For these reasons they are attractive as subjects of further study.

A. Methods for the Synthesis of Nitro Sugars

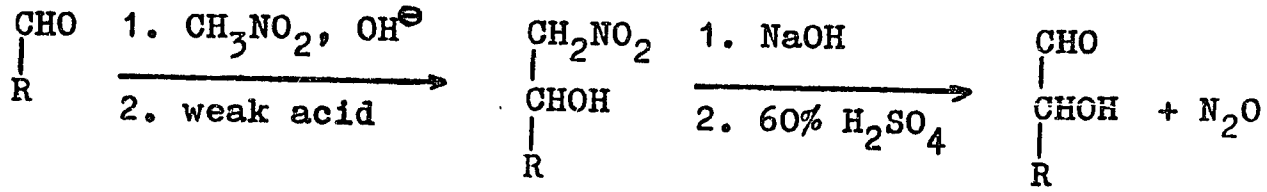
1. Synthesis of 6-deoxy-6-nitro sugars

a. Nitromethane condensation

In 1895, L.Henry (2) discovered that aldehydes undergo a base-catalysed condensation with nitromethane, a reaction analogous to the aldol condensation. In subsequent years this reaction has found widespread applications in aliphatic and aromatic chemistry and has been reviewed in detail (3).

The reaction was introduced into carbohydrate chemistry by Sowden and Fischer (4) in 1944. These authors prepared deoxynitroalditols from aldoses

nitromethane; and they showed that the nitro compounds obtained may be subjected to Nef reaction, i.e., acidic elimination of the nitro group.



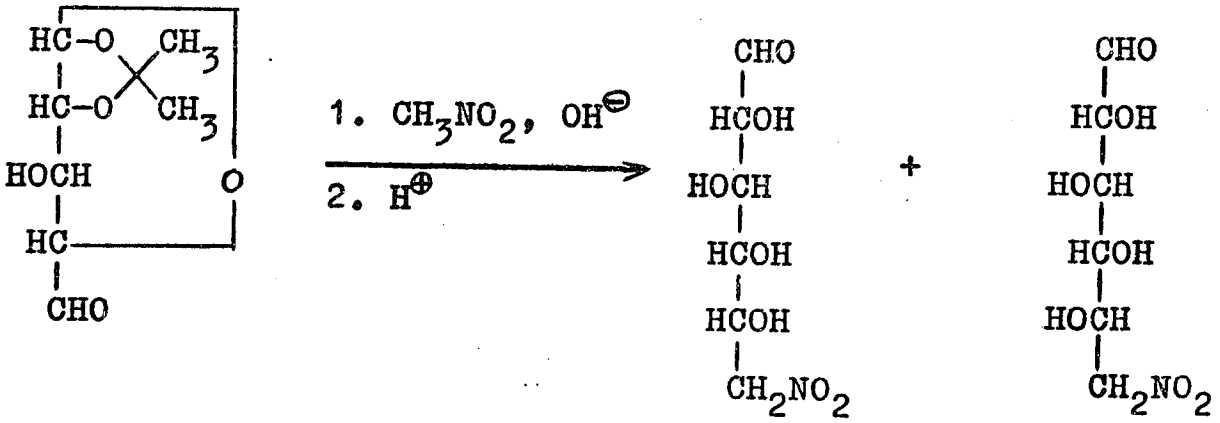
aldopentose

1-deoxy-1-nitro-alditol (two epimers)

aldohexose

The Sowden-Fischer procedure has thence become one widely applied method of lengthening the carbon chain in carbohydrates (5).

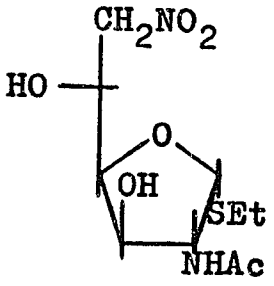
The first reducing 6-deoxy-6-nitro sugars were synthesized in a similar way by Grosheintz and Fischer (6). They condensed nitromethane with 1,2-O-isopropylidene-D-xylodialdose and obtained, upon removal of the isopropylidene blocking group, 6-deoxy-6-nitro-D-glucose and -L-idose.



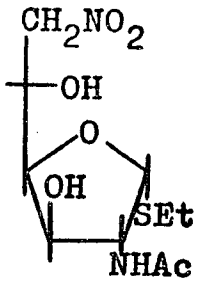
6-deoxy-6-nitro-D-glucose

6-deoxy-6-nitro-L-idose

Using this principle, Wolfrom and coworkers (7) synthesized ethylthio 2-acetamido-2,6-dideoxy-6-nitro-α-D-gluco- (I) and L-idofuranoside (II) as intermediates in the first synthesis of the antibiotics component, streptamine.



I



II

b. Nucleophilic substitution

Sugihara and coworkers (8) were the first who tried to react suitable 6-substituted sugars with sodium nitrite in order to synthesize 6-deoxy-6-nitro sugars. They were able to obtain from 2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside and from 2,3,4,5-di-O-benzylidene-1,6-diiodo-mannitol the 6-nitro and the 1,6-dinitro derivatives, respectively.

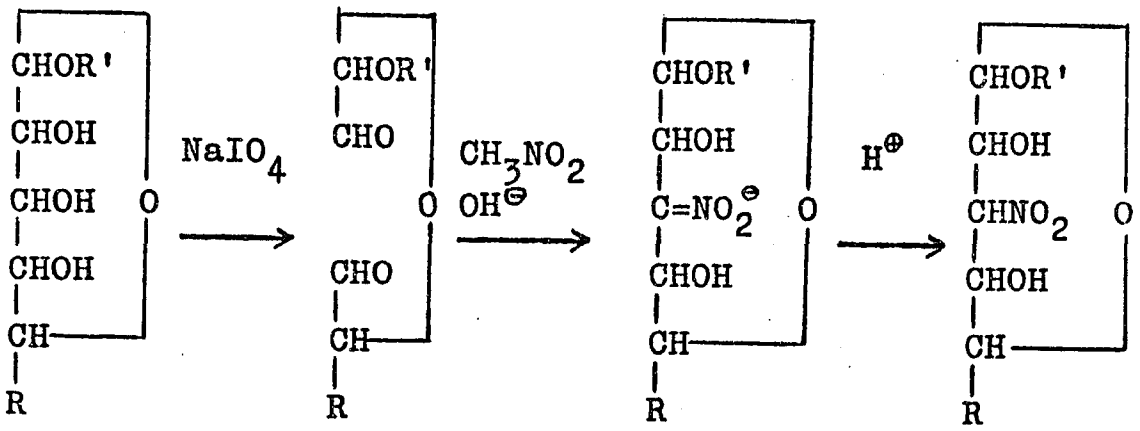
Lindberg and Svensson (9) synthesized in a similar way methyl 6-deoxy-6-nitro-ß-D-glucopyranoside starting from either acetylated 6-iodo or 6-tosyl glucoside. As expected, the yield from the tosylate as compared to that from the iodide was less satisfactory, the tosyl group being a poorer leaving group. These authors could also obtain crystalline, deacetylated methyl 6-deoxy-

6-nitro-ß-D-glucopyranoside, but they were unable to crystallize the corresponding α-isomer. This has since been achieved in our laboratory (10).

Similar reactions in the galactose series have failed so far. The low reactivity of primary tosylates and iodides in sugars with galactose configuration might be caused by steric hindrance by the axial substituent at carbon 4 (8).

2. Synthesis of 3-deoxy-3-nitro sugars

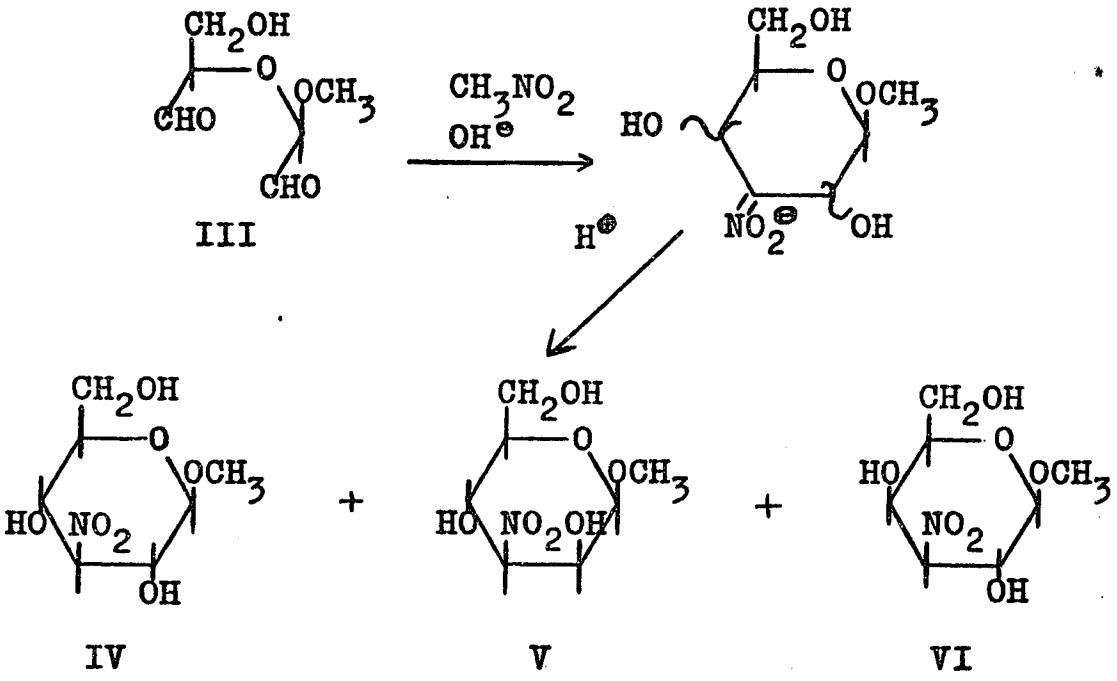
In 1958, Baer and Fischer (11) introduced a novel method of synthesizing nitrogeneous sugar derivatives consisting of the cyclization of sugar dialdehydes with nitromethane in the presence of a basic catalyst. A 'sugar dialdehyde' in this context refers to the product of periodate or lead tetraacetate oxidation of a glycoside. These oxidations have been studied thoroughly and have been reviewed in detail (12). The cyclization reaction with nitromethane has also been the suject of several publications (13). The reaction can be represented by the following general scheme:



R=H, CH_2OH, CH_3

R'=CH_3

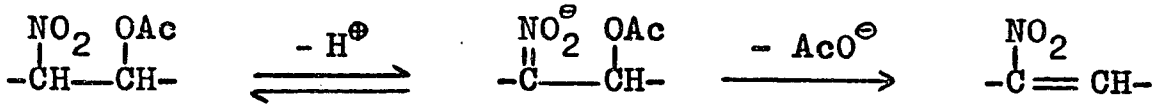
The first step in the cyclization of the dialdehyde is the formation of a nitronate. Two asymmetric centres are thereby generated; subsequent acidification forms a third asymmetric carbon. Hence one might expect the formation of four stereoisomeric nitronates and of eight nitro glycosides. However, a marked stereoselectivity which is due to conformational preferences in the arising products is always observed. For instance, in the nitromethane condensation of L'-methoxy-D-hydroxymethyldiglycolic aldehyde (III) only three isomers were isolated (14). The main product was the gluco isomer (IV) which was obtained in over 40% yield, while the manno (V) and the galacto (VI) isomers were formed in smaller amounts.



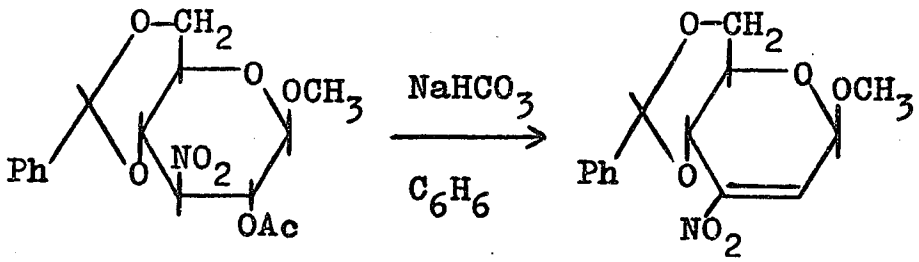
This result agrees with that predicted by Cram's rule (15). Nitropentosides, hexosides, 6-deoxyhexosides, 1,6-anhydrohexoses, and 2,7-anhydroheptuloses have been synthesized by the use of this cyclization reaction. Branched-chain nitro sugars can be obtained when nitroethane is substituted for nitromethane (16).

3. Synthesis of nitro sugar olefins

It is known that ß-acetoxynitroalkanes easily undergo base-catalyzed elimination of acetic acid (Schmidt-Rutz reaction) (17).

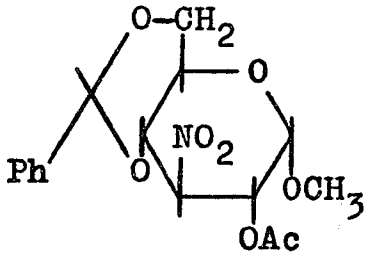


Applying this reaction to 3-nitro sugars, Baer and Neilson (18) synthesized in 1964 the first sugar derivative possessing a nitro olefin grouping as part of the glycoside ring. By refluxing methyl 2-O-acetyl-4,6-O-benzylidene-3-deoxy-3-nitro-ß-D-glucopyranoside (VII) together with solid sodium bicarbonate in benzene they obtained in high yield crystalline methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-erythro-hex-2-enopyranoside (VIII).



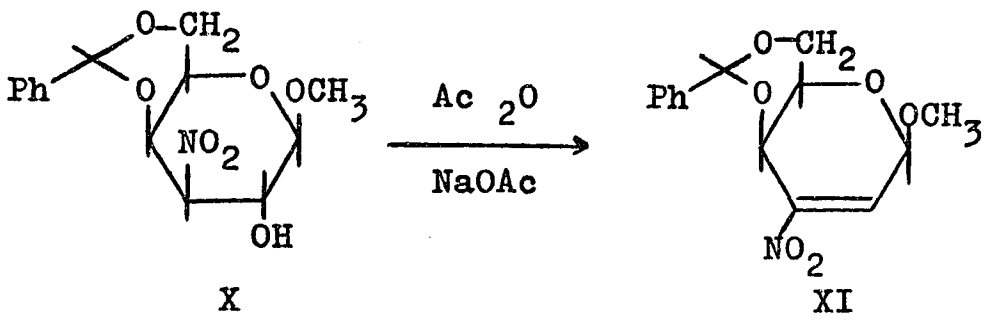
VII VIII

The corresponding nitro olefin in the α-series was synthesized in the same way (19). To prepare the ß-anomer, one to two days of refluxing is sufficient whereas it may take up to nine days to complete the reaction with the α-compound. It appears plausible that the axial methoxy group in the acetate IX may interfere in the approach of the bicarbonate crystal surface to the lower side where the hydrogen at carbon 3 must be abstracted.



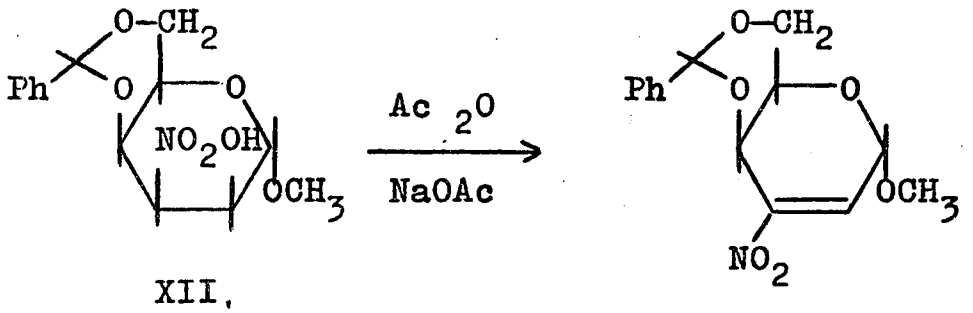
IX

Analogous experiments in the galacto series revealed an interesting configurational effect. In the first place, it was difficult to obtain the 2-O-acetate that was thought to be required as a precursor for the 2,3-unsaturated glycoside. However, when methyl 4,6-O-benzylidene-3-deoxy-3-nitro-ß-D-galactopyranoside (X) was heated for five minutes in acetic anhydride in the presence of sodium acetate, the nitro olefin XI was produced directly and could be isolated in high yield (20).



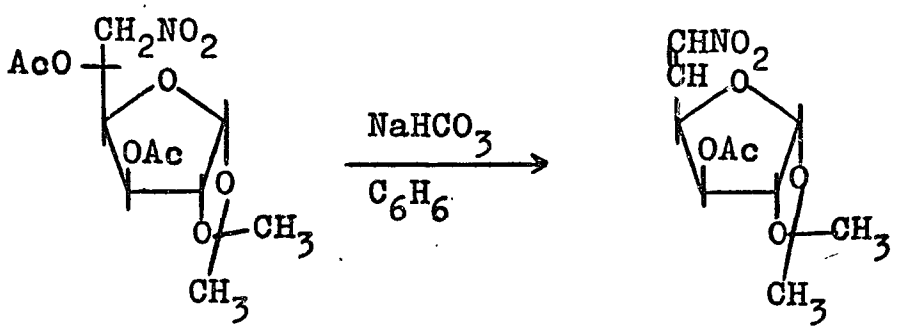
X XI

Methyl 4,6-O-benzylidene-3-deoxy-3-nitro-α-D-talopyranoside (XII) behaved similarly (19).



XII.

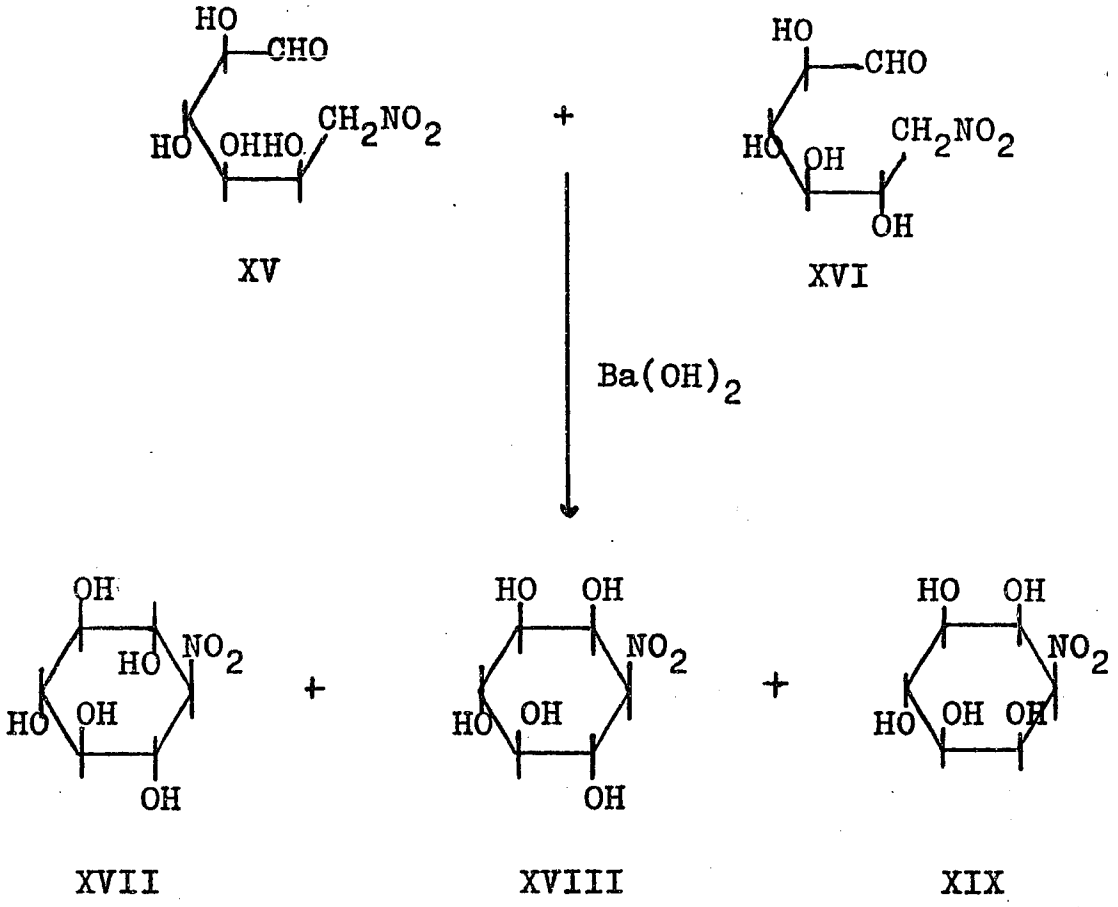
The Schmidt-Rutz reaction was also successfully applied to 6-nitro sugars. Baer and Rank (21) isolated the expected nitro olefin XIV upon refluxing of 3,5-di-O-acetyl-6-deoxy-1,2-O-isopropylidene-6-nitro-α-D-xylo-hexofuranose (XIII) with sodium bicarbonate in benzene. The analogous 1,2-cyclohexylidene derivative has been obtained in amorphous condition only (22).



XIII XIV

B. Reactions of 6-Deoxy-6-nitro Sugars

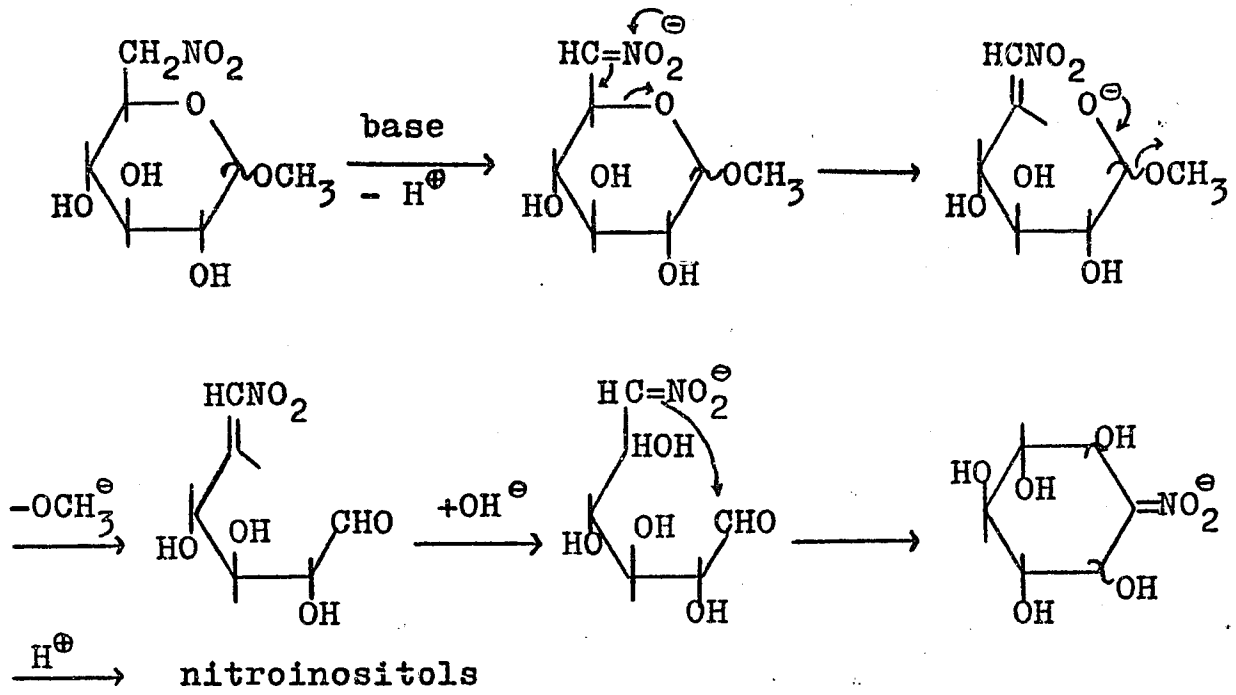
6-Deoxy-6-nitro-D-glucose (XV) and -L-idose (XVI) possess a free aldehyde and a nitro group in the same molecule and are therefore ideally suited to undergo an internal Henry addition. Grosheintz and Fischer (23) demonstrated that these sugars yield, on treatment with barium hydroxide, three stereoisomeric deoxynitroinositols which were later shown (24) to have the scyllo (XVII), D,L-myo-1 (XVIII), and muco-3 (XIX) configurations. Lichtenthaler's (25) detailed studies established that the muco isomer arises by kinetic control; when allowed to remain in an alkaline medium it epimerizes to a thermodynamically more stable equilibrium mixture of the scyllo and myo compounds.



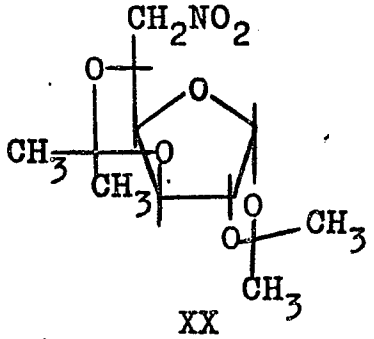
XV XVI

XVII XVIII XIX

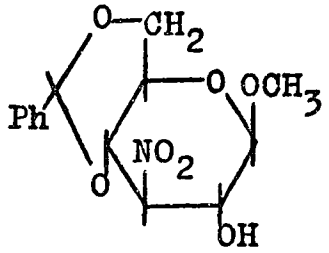
Recently, Baer and Rank (21) observed that the methyl 6-deoxy-6-nitro-D-gluco- and -L-idopyranosides, too, yield the same deoxynitroinositols on treatment with base. The mechanism they propose involves, first, fission of the pyranoside ring, followed by loss of the aglycon and cyclization of the resulting nitro sugars.



The instability of these glycosides towards alkali is remarkable, but a similar lability has been observed on several occasions in acetals or ketals that bear a primary or secondary nitro group in the ß-position. Thus Fischer and Baer (26) observed a facile alkaline removal of the 3,5-isopropylidene group in 6-deoxy-1,2;3,5-di-O-isopropylidene-6-nitro-α-D-glucofuranose (XX). Similarly, methyl 4,6-O-benzylidene-3-deoxy-3-nitro-ß-D-glucopyranoside (XXI) readily loses benzaldehyde on treatment with alkali (27).



XX



XXI

C. Reactions of 3-Deoxy-3-nitro Sugars

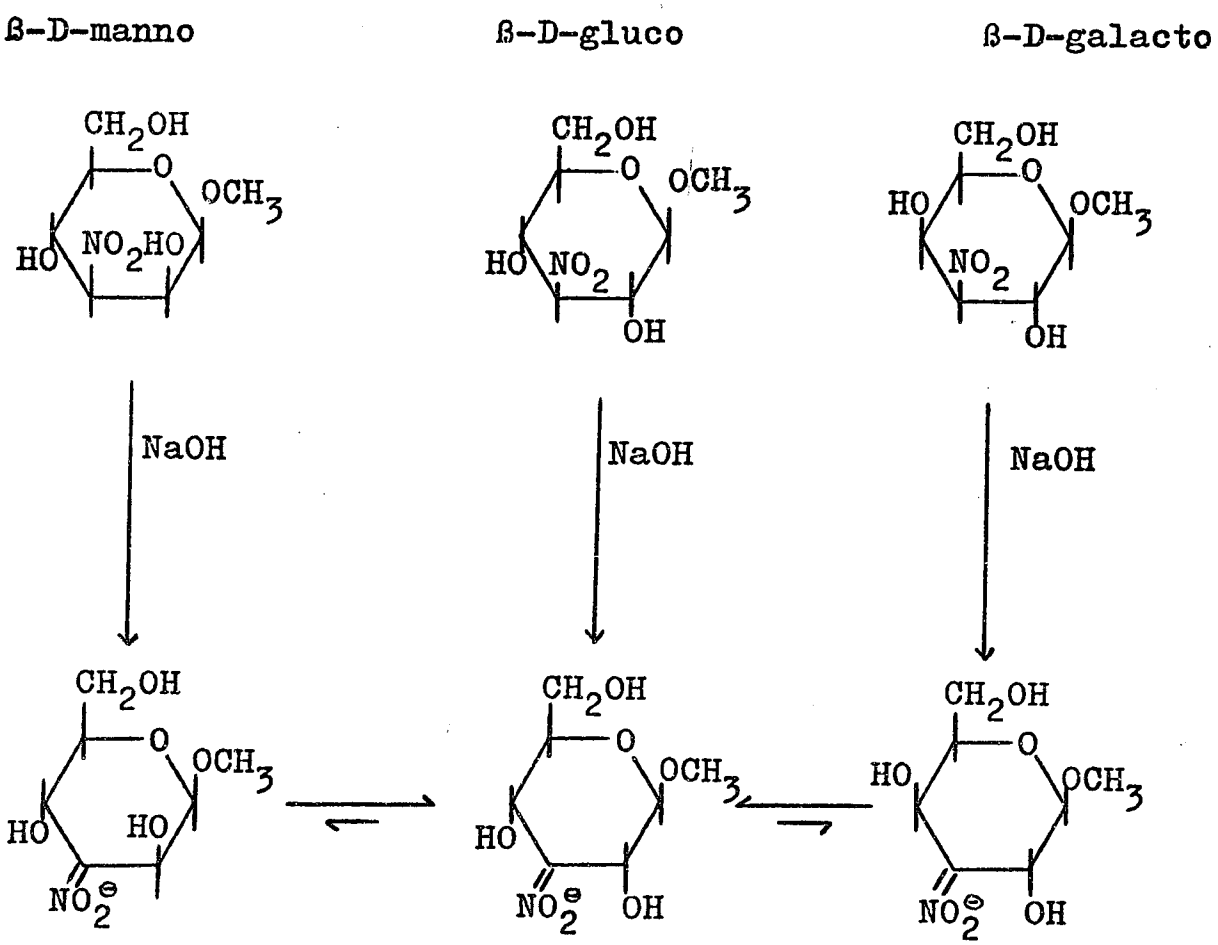
1. Reactions in alkaline medium

One characteristic property of 3-deoxy-3-nitro glycosides is their sensitivity towards alkali which is, however, of a type different from that mentioned of the glycosides and acetals in the preceding paragraph. In the compounds to be discussed now, the activating nitro substituent is not located in ß-position of an acetal oxygen (more specifically, the ring oxygen), and consequently the glycoside ring is not opened by alkali. However, in basic solution these glycosides exhibit spontaneous mutarotations which are due to epimerizations occuring at carbon atoms adjacent to the aci-nitro grouping.

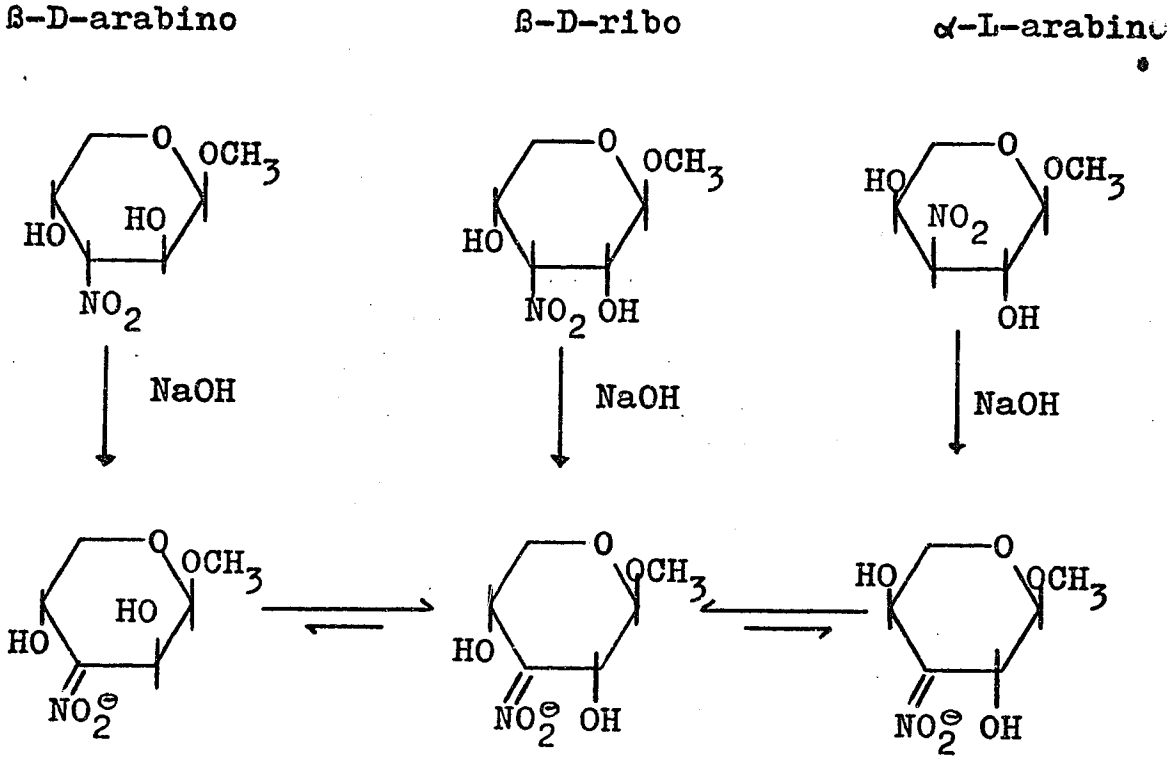
Two sets of crystalline nitroglycosides, namely ß-D-hexosides and pentosides, have been studied in more detail. It was found that in both sets a common value of specific rotation is attained in basic solution within a few hours. Deionization at this point and

chromatographic inspection of the resulting solution, directly as well as following hydrogenation to the amine stage, indicated that an equilibration had taken place and caused identical chromatographic patterns regardless of the configuration of the starting compound. This was also confirmed by isolation in crystalline form of the nitro compounds derived from equilibrated nitronates.

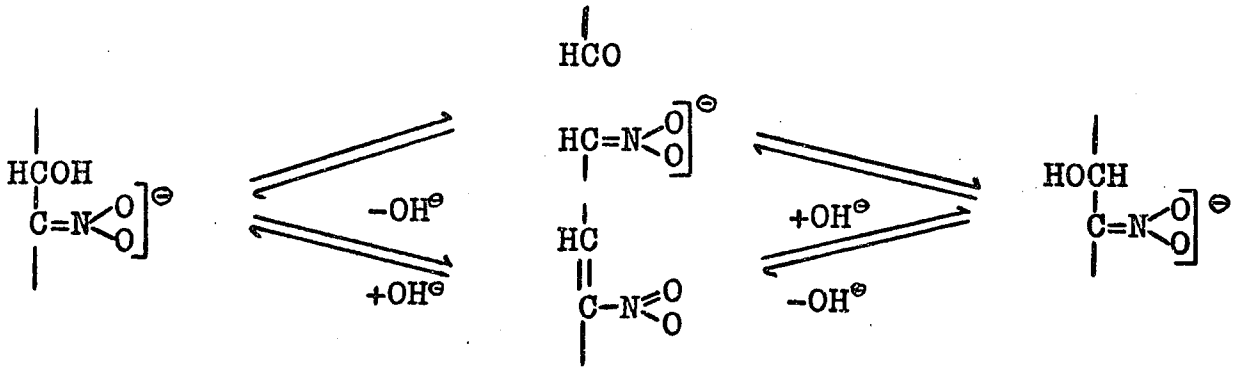
In the ß-D-hexoside series the equilibrium can be represented by the following scheme (27).



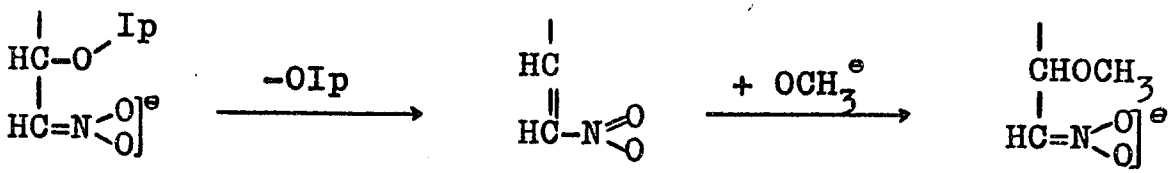
In the pentoside series the epimerization was found to proceed thus (28):



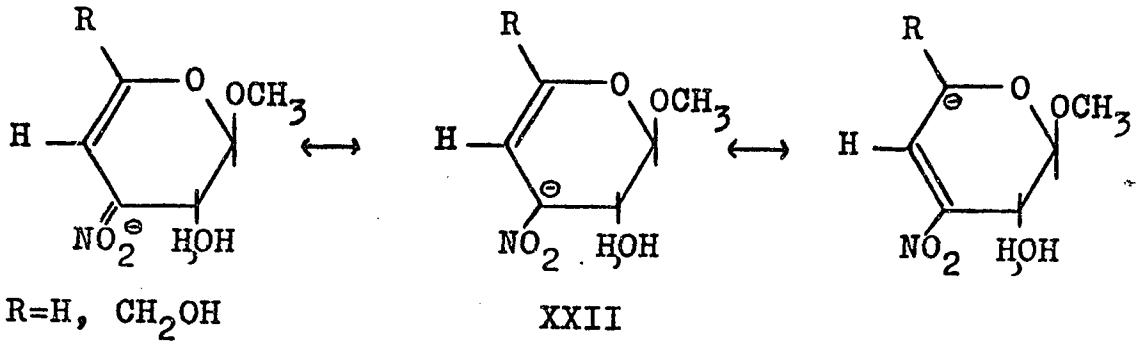
Two possible pathways may be discussed for the epimerization reaction (13). The first one would be a reversal of the Henry condensation; the second mechanism calls for the intermediate formation of a nitro olefin.



At present there is no direct proof available for either mechanism, but the second possibility has an analogy in the related case of 1,2;3,5-di-O-isopropylidene-6-deoxy-6-nitro-α-D-glucofuranose (XX) which easily eliminates in methanolic alkali the C-5 substituent and adds methoxide ion to form two C-5 epimeric methyl ethers (26).

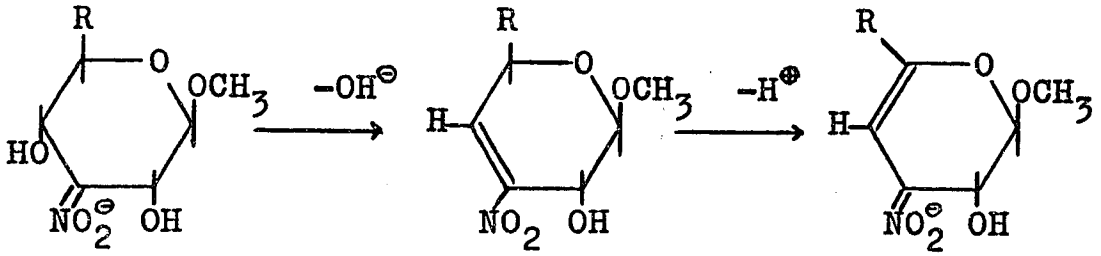


An intermediate nitro olefin would also help to explain a further reaction which the pentoside and hexoside nitronates undergo. When these compounds, which show the characteristic nitronate absorption (29) in the ultraviolet at 248-250 mμ, are allowed to stand in aqueous solution for several days at room temperature (or at 98° for 15 min.), a secondary reaction takes place which is characterized by the disappearance of the nitronate peak with concurrent development of an absorption peak of similar strength at 296mμ. Work-up on completion of the spectral transformation gave in over 70% yield a solid sodium nitronate for which the mesomeric structure XXII was established by Baer and Kienzle (27).



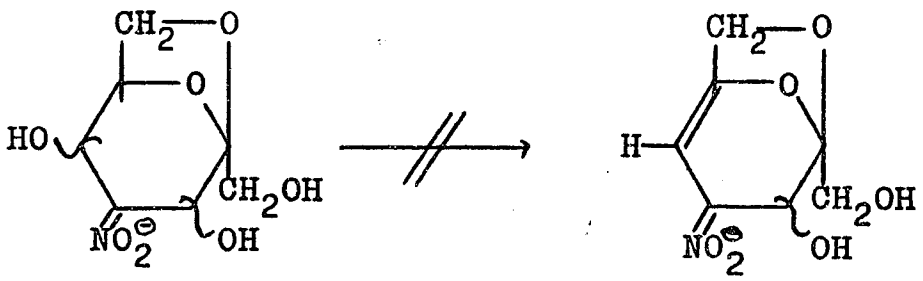
R=H, CH_2OH XXII

The formation of such an endocyclic allylic system could easily be explained by assuming an intermediate nitro olefin (13).



R=H, CH_2OH

Solutions of the sodium salts of 2,7-anhydro-4-deoxy-4-nitro-ß-D-heptulopyranoses (XXIII) do not show any absorption in the ultraviolet at longer wavelengths, even on prolonged heating (27).

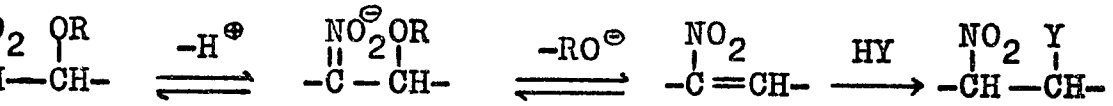


XXIII

The non-occurrence of the reaction was expected in this case since an allylic system would necessitate the formation of a double bond at a bridgehead carbon.

2. Nucleophilic additions and elimination-additions

As is known from aliphatic nitro compounds, the nitro group possesses an activating effect that permits the introduction or exchange of substituents in the ß-position. The key role in these reactions is played by an α-nitro olefin which may either be employed as such or generated in situ from a suitable precursor.

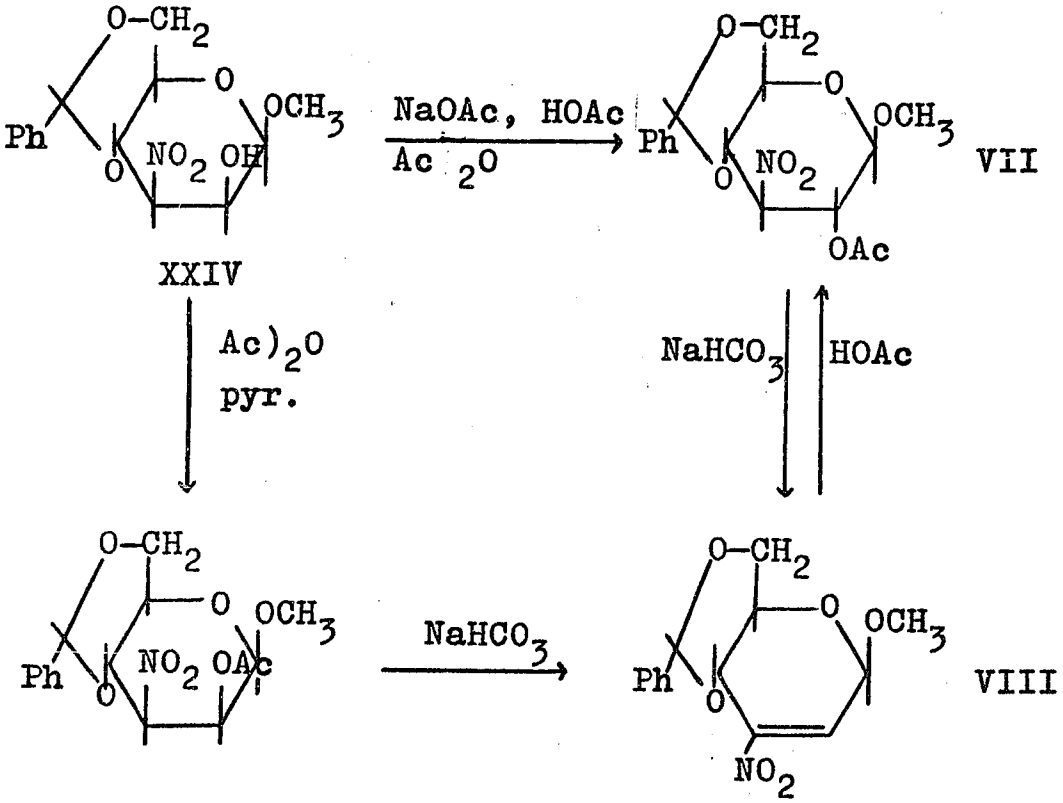


R=$COCH_3$, H; Y=nucleophile

There are many examples of these types of reactions in aliphatic chemistry (30), but so far they have found only little application in carbohydrate chemistry.

Paulsen (22) successfully added ammonia to 3-O-acetyl-1,2-O-cyclohexylidene-5,6-dideoxy-6-nitro-D-xylo-5-hexeno-furanose and obtained a mixture of crystalline 5-amino-5,6-dideoxy-6-nitro-D-gluco- and -L-ido derivatives which he was able to separate.

Baer and coworkers (20) observed in 1965 that the nitro olefin VIII easily added acetic acid when refluxed for a few minutes in acetic anhydride containing acetic acid, to yield exclusively the 2-O-acetate with gluco configuration. They also found that acetylation of methyl 4,6-O-benzylidene-3-deoxy-3-nitro-ß-D-manno-pyranoside (XXIV) in refluxing acetic anhydride in the presence of sodium acetate yielded the same gluco 2-O-acetate, a result that can only be explained by assuming an intermediate olefin which instantaneously adds acetic acid to form the observed product.



It is interesting that under the same conditions the olefin with an axial substituent at carbon 4 did not add acetic acid but was recovered unchanged.

The reactivity of the benzylidene nitro olefin (VIII) is further exemplified by the ease of addition of ethanol (20), methanol and benzylalcohol (31); 2-O-alkyl or aryl ethers are thereby obtained.

It is not always necessary to start with the olefin in such syntheses. It was found (31) that the 2-O-acetyl glucoside (VII), when heated for one hour with sodium acetate in alcohol, formed smoothly in over 90% yield the same 2-O-alkyl ethers. Refluxing of the non-acetylated precursor of VII in toluene in the presence of basic aluminum oxide and a nucleophile will also give rise to a 2-substituted product. By use of the latter technique 2-deoxy-2-benzylthio-, 2-deoxy-2-diethylamino-, 2-deoxy-2-piperidino-, and 2-deoxy-2-carbethoxymethylamino derivatives were prepared (31).

An interesting application of these reactions is the recent synthesis of 2,3-diamino-2,3-dideoxy-D-glucose (32) which was achieved by adding ammonia to the benzylidene nitro olefin VIII, to give the amino-nitro and,upon hydrogenation and hydrolysis, the diamino sugar.

The steric course of these reactions nearly

always leads to compounds with the all-equatorial (i.e. gluco) configuration. Only in the case of the ammonia addition a small quantity of a second isomer has been found, but its configuration has remained unelucidated. Such a steric preference is to be expected since axial substitution would give rise to strong interactions which are greatly diminished in the case of an equatorial arrangement of the substituents.

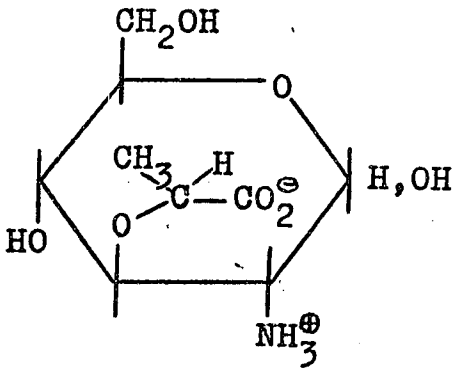
3. Nitro sugars as intermediates for amino sugars

Since reduction of a nitro group to an amino group can be easily accomplished, most of the nitro derivatives of carbohydrates have been synthesized with the intention of preparing suitable intermediates for amino sugars that are not readily accessible otherwise. In many cases the isolation of the nitro sugar is not necessary or not feasible. Nevertheless, the reaction mixtures obtained by nitromethane cyclization can be hydrogenated in the presence of platinum catalyst to yield a mixture of stereoisomeric amino sugars which may then be separated at that stage. A great variety of rare amino sugars have been synthesized using this method, for instance: D and L 3-amino-3-deoxy-ribose (33), D and L 3-amino-3-deoxy-arabinose (28), 3-amino-3-deoxy-D-glucose, -mannose, -talose,

and-galactose (34,35,36), 3-amino-2,3-dideoxy-D-arabino-hexose (18), 3-amino-2,3-dideoxy-D-lyxo-hexose (37), 3-amino-1,6-anhydro-3-deoxy-D-gulose, -altrose, and -idose (38), 3-amino-3,6-dideoxy-L-glucose and -talose (39), and 2,7-anhydro-4-amino-4-deoxy-ß-D-gulo-, -altro-, and -allo-heptulopyranose (40).

D. Muramic Acid

In 1954, muramic acid, a derivative of 2-amino-2-deoxy-D-glucose was detected in the hydrolysate of spore peptides produced by Bacillus megaterium (41). The same compound has since been found in cell walls of numerous Gram-positive and Gram-negative bacteria (42) and in an alga (43). Muramic acid was first isolated by Strange and Dark (44) in 1956 and subsequently shown by degradation (44) and synthesis (45) to be 2-amino-3-O-(D-1-carboxyethyl)-2-deoxy-D-glucose (XXV).

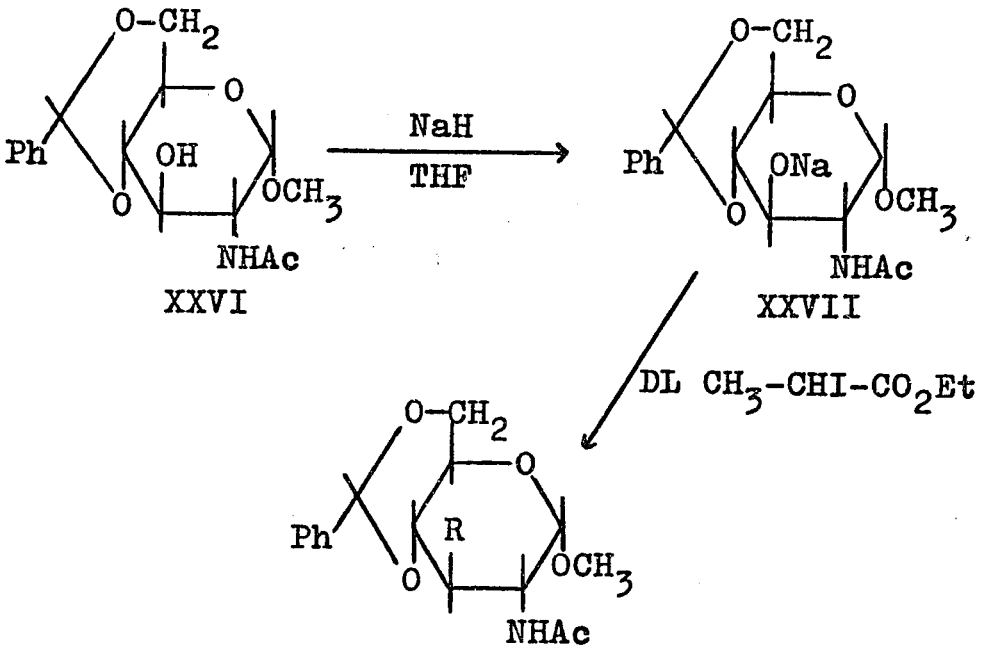


XXV

The structural assignment was made on the basis of molecular weight determination, periodate oxidation and acetylation studies, and the fact that boiling with hydroiodic acid liberated propionic acid. A purple color reaction with p-dimethylaminobenzaldehyde (Morgan-Elson reaction) was taken as evidence that the substituent was at carbon 3 rather than at carbon4, since it is known (46) that 4-O-substituted derivatives of N-acetylhexosamines do not respond to this color test. The D-configuration for the lactic acid moiety was assumed on biological grounds. The final synthesis confirmed the correctness of the proposed structure.

The first synthesis (45) as well as later improvements (47,48,49) were based on condensations between derivatives of D-glucosamine and an α-halo-propionic acid. Strange and Kent (45) started from methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (XXVI) which was condensed as a sodio derivative (XXVII) with α-DL-iodopropionic acid ethylester. This resulted in a mixture of D and L ethers which, after hydrolysis, had to be separated by column chromatography. The more strongly dextro-rotatory isomer was found to be identical with the naturally occuring muramic acid. The higher rotation supported the assignment of D configuration to the lactic acid moiety since, as infrared spectroscopy

had shown, muramic acid exists as a 'zwitterion', i.e. an internal salt, and since it is also known that the salts of D(-)lactic acid are dextrorotatory.



XXVI XXVII

R = D or L lactic acid residue, $\begin{matrix} CH_3 \\ | \\ CHO- \\ | \\ COOH \end{matrix}$

The above condensation takes place with inversion of configuration (S_N2 reaction); therefore a final proof of the D configuration of the side chain was given by the use of pure L-α-chloropropionic acid which upon condensation yielded the natural product (48).

E. Specification of the Goals of the Present Thesis

As shown in the preceding paragraphs, the presence of a nitro group in a sugar molecule induces properties which open new ways for the synthesis of carbohydrate derivatives hitherto inaccessible. The scope of our knowledge of nitro sugars is narrow at present, and it therefore appeared to be of interest to broaden it by investigating the following problems:

1. The synthesis of nitro sugar olefins is achieved by dehydroacetylation of ß-acetoxy nitro compounds. It seemed to be of interest to study that process, known as the Schmidt-Rutz reaction, on sugars that have not one but two neighboring acetyl groups, to see which of these would be more favored in the elimination.

2. In nucleophilic additions to 3-nitro olefins there have been used, thus far, amines, alcohols, and thioalcohols. It was desirable to study whether other nucleophiles would add with the same ease, and also, whether such addition reactions might result in new ways for the synthesis of naturally occuring products or analogs thereof.

PART I

The Dehydroacetylation of Methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-glycosides: A Novel Synthesis of the Isochromene System via a Diels-Alder Reaction

DISCUSSION

Methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-gluco- (I)[a], manno- (II), and galactopyranoside (III) contain a nitro group flanked on both sides by acetoxy functions. The base-catalyzed dehydroacetylation (Schmidt-Rutz reaction) of these sugar acetates may, therefore, be expected to lead to the formation of either the 2,3- or the 3,4-unsaturated derivatives or to a mixture of both. In addition, the elimination of two molecules of acetic acid, resulting in the formation of dienes, had to be considered as a possible way of reaction, since it has been reported (50) that 3-nitro-1,3-pentadiene arises upon heating of 2,4-diacetoxy-3-nitropentane with sodium acetate[b].

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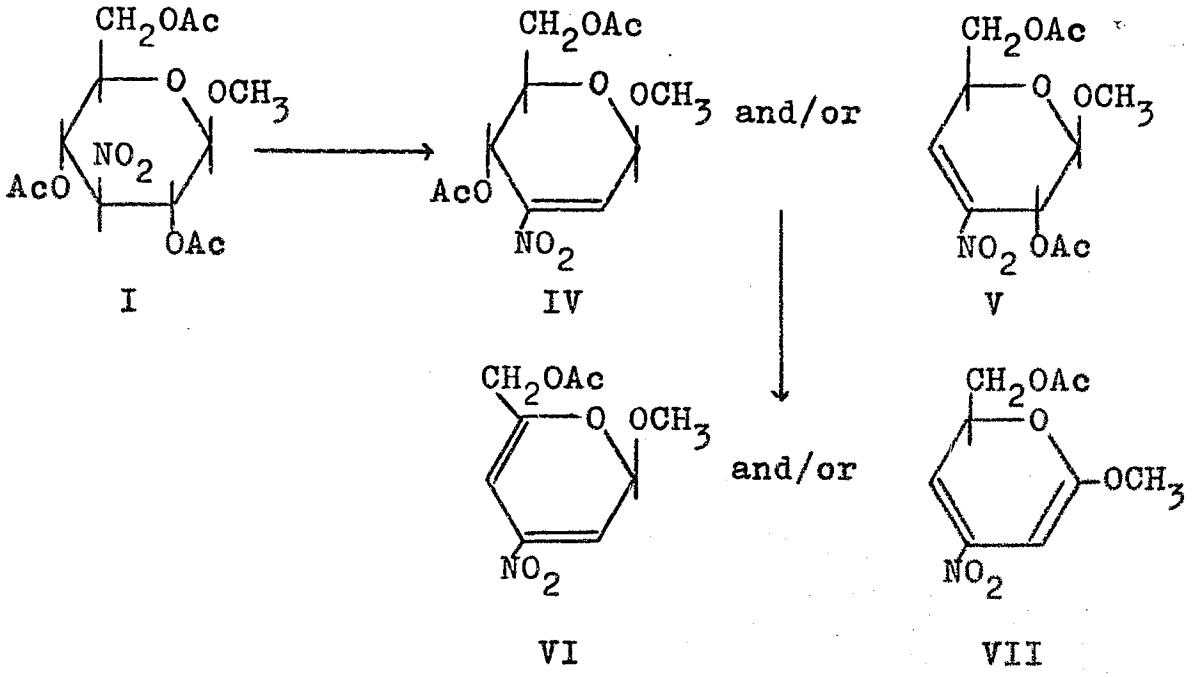
a. For convenience, compounds in this chapter are numbered using a new set of Roman numerals.

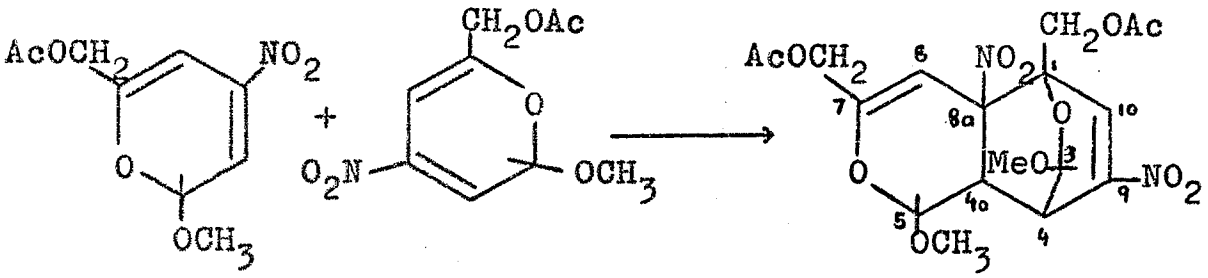
b. The possibility that a diene such as VI might be engendered did not come to mind immediately, however. The formation of 3-nitropentadiene cited (50) appears to be an isolated instance in the literature and does not represent a widely known reaction.

The following equation exemplifies for the gluco isomer these expectations:



When methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-glucopyranoside (I) was heated under reflux in dry benzene in the presence of sodium hydrogen carbonate, a colorless crystalline product of mp 131° was isolated in 43% yield. Elemental analysis and molecular weight determination established the formula $C_{18}H_{22}N_2O_{12}$ which does not correspond to any of the structures in the above formula scheme. A dimer of the diene VI (or of VII, or a combination of both) possesses, however, such a composition, and it was therefore considered that the dehydroacetylation of I had in fact led to VI (and/or VII) which then immediatly underwent an intermolecular Diels-

Alder addition. If one would assume that all the double bonds in the hypothetical intermediates VI and VII had dienophilic character, one would arrive at sixteen structural possibilities for such a dimer. In the following equation, which exemplifies the formation of one of these, one molecule of VI serves as the diene, and the 2,3-double bond of a second molecule of VI serves as the dienophile.



VI VI VIII

The reaction product of mp 131° was shown to possess indeed the structure VIII. It is to be designated as 3,4,4a,8a-tetrahydro-3,5-dimethoxy-8a,9-dinitro-1,4-ethenopyrano [4,3-c] pyran-1,7(5H)-dimethanol diacetate.

Deduction of the Structure of Compound VIII by Spectroscopy

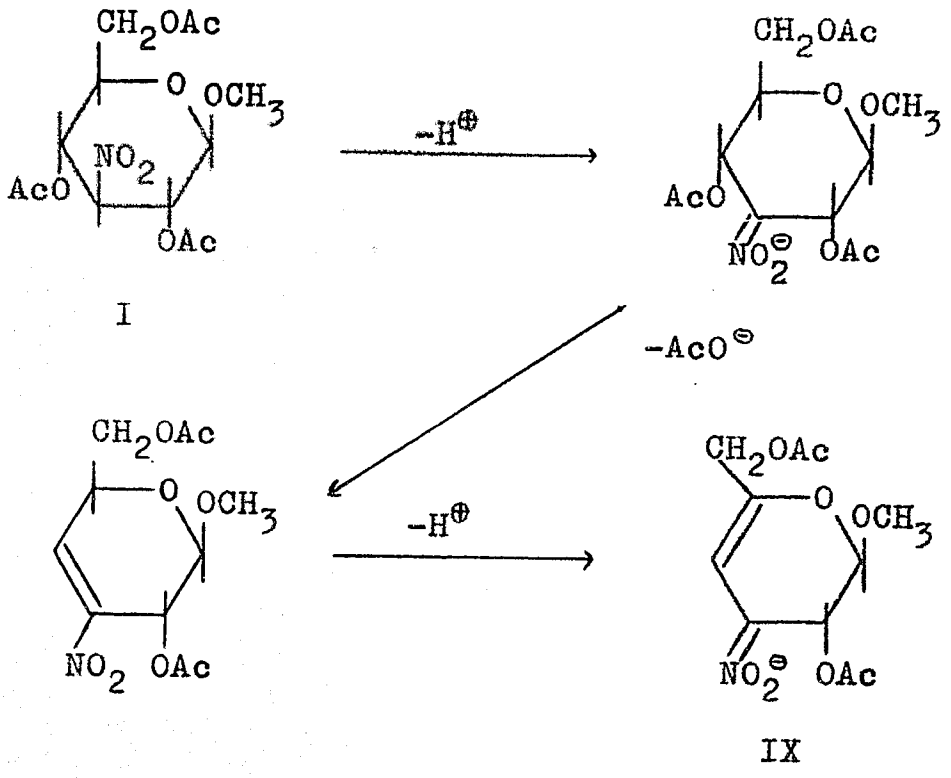
The infrared spectrum (Figure 1 and Table 1) of compound VIII exhibited olefinic C-H and C=C stretching vibrations as well as strong bands for ester carbonyl and for an unconjugated and a conjugated nitro group. There was no hydroxyl absorption.

The ultraviolet spectrum of an ethanolic solution of VIII revealed no high-intensity, long wave-length absorption. Addition of alkali produced, however, a nitronate chromophore absorbing strongly at λ_{max} 255mμ. This behaviour conforms with the presence of an α-nitroalkene structure that can readily add base to form a ß-substituted alkanenitronate. A secondary nitroalkane grouping in VIII would likewise explain such a behaviour. However, the triacetate I under the same conditions gave rise to a strong absorption of λ_{max} 302mμ (ϵ, 19000) ascribed to an unsaturated nitronate (IX)(51), and the failure of VIII to produce that particular absorption can be taken as evidence for the absence of the moieties

$$-O-CH(CH_2OAc)-CH(OAc)-CH(NO_2)-$$

and

$$-O-CH(CH_2OAc)-CH=C(NO_2)-$$



I

IX

The nmr spectrum of VIII is shown in Figure 2. Sharp singlets, each of three proton intensity, are seen for two acetoxy groups (τ 7.93, τ7.87) and for two methoxy groups (τ 6.70, τ 6.67). The signal centered at τ 5.59, with an intensity of four protons, is ascribed to the methylene parts of the two acetoxymethyl groups. The signal has the appearance of a triplet but, as will be seen below, it really consists of two virtually coinciding AB quartets with geminal coupling of 13 cps, a feature that rules out the presence of vicinal hydrogen.

The remaining signals in the spectrum, all of one proton intensity and well separated, are a narrow multiplet at τ6.40 (H-4a), a quartet at τ5.97 (H-4), a doublet with a splitting of 0.7 cps at τ4.92 (H-5), a doublet with a splitting of 2.5 cps at τ4.80 (H-3), a singlet at τ4.18 (H-8), and a doublet with a splitting of 2.6 cps at τ2.81 (H-10). The assignments given in parentheses could be made, in a tentative way, after chemical degradation had pointed to formula VIII as the most probable structure. One discrepancy, however, seemed to exist in the doublet character of the signal at lowest field. This signal, because of its chemical shift (τ 2.81), doubtless was due to a nitroolefinic proton (H-10), but formula VIII contains no proton vicinal to H-10, and for allylic coupling with H-4 the splitting of 2.6 cps appeared to be rather too large considering the possible geometry of the molecule. The H-4 bond must lie nearly in the plane of the nitroolefinic double bond as can be seen with the help of a molecular model. For such a case, allylic coupling would be expected to be at a minimum. No alternative formula could be written that would have satisfied the apparent structural requirement $\rangle$CH-CH=C(NO_2)- without at the same time causing other serious inconsistencies, both spectroscopic and chemical. The problem was resolved by spin decoupling data obtained from a 100 Mc spectrum (52), which verified the assignments

made above and, in particular, proved that allylic coupling actually existed between H-10 and H-4.

Irradiation of H-4 caused the H-10 doublet to collapse to a singlet; at the same time, the H-3 doublet became a singlet also, and the signal for H-4a narrowed to a doublet with a splitting of 0.7 cps. Irradiation of H-4a removed the splitting (0.7 cps) of H-5 and changed the H-4 quartet into a triplet. The H-4 quartet was likewise converted into a triplet by irradiation of either H-3 or H-10. In the 100 Mc spectrum, the coinciding signals for the two acetoxymethylene groups (τ 5.59) appeared as an AB quartet with clearly separated center lines. Spin decoupling showed that there was a weak allylic coupling between the protons of the methylene group at C-7 and the olefinic proton H-8 whose signal, described above as a singlet, did have fine structure.

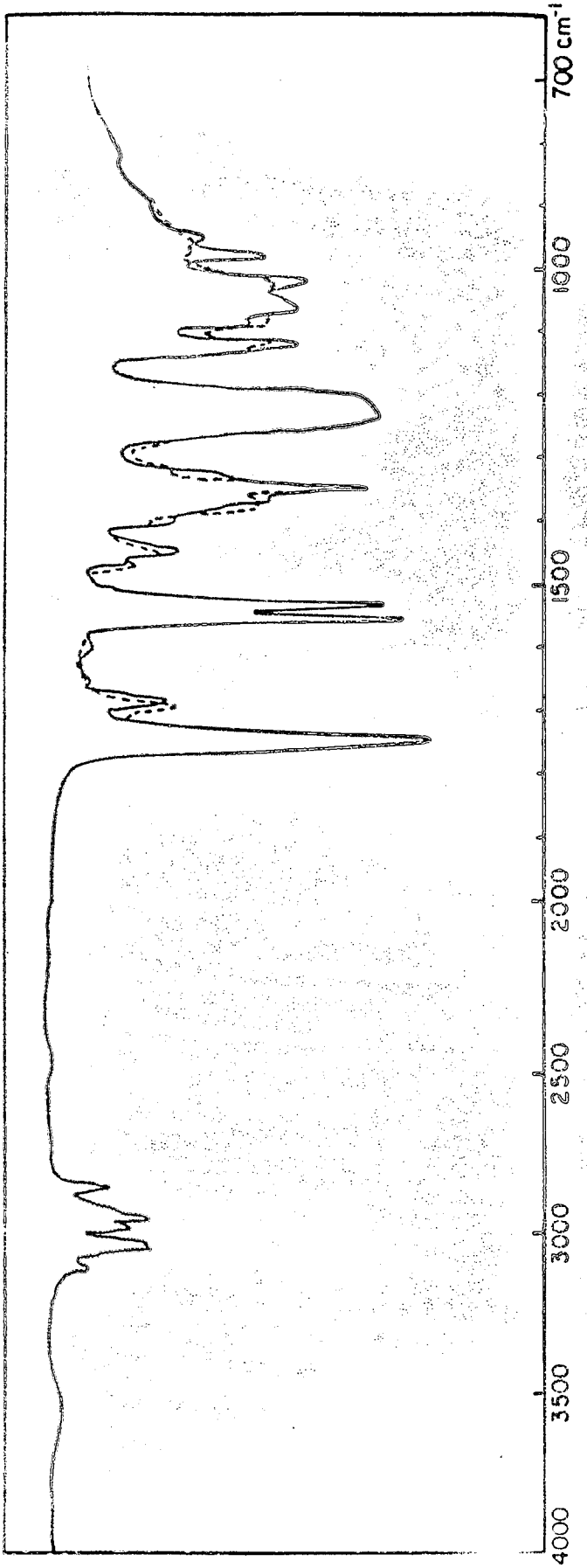


Figure 1. - Infrared spectra of compounds VIII (solid line) and XXX (broken line where it deviates from VIII), in chloroform.

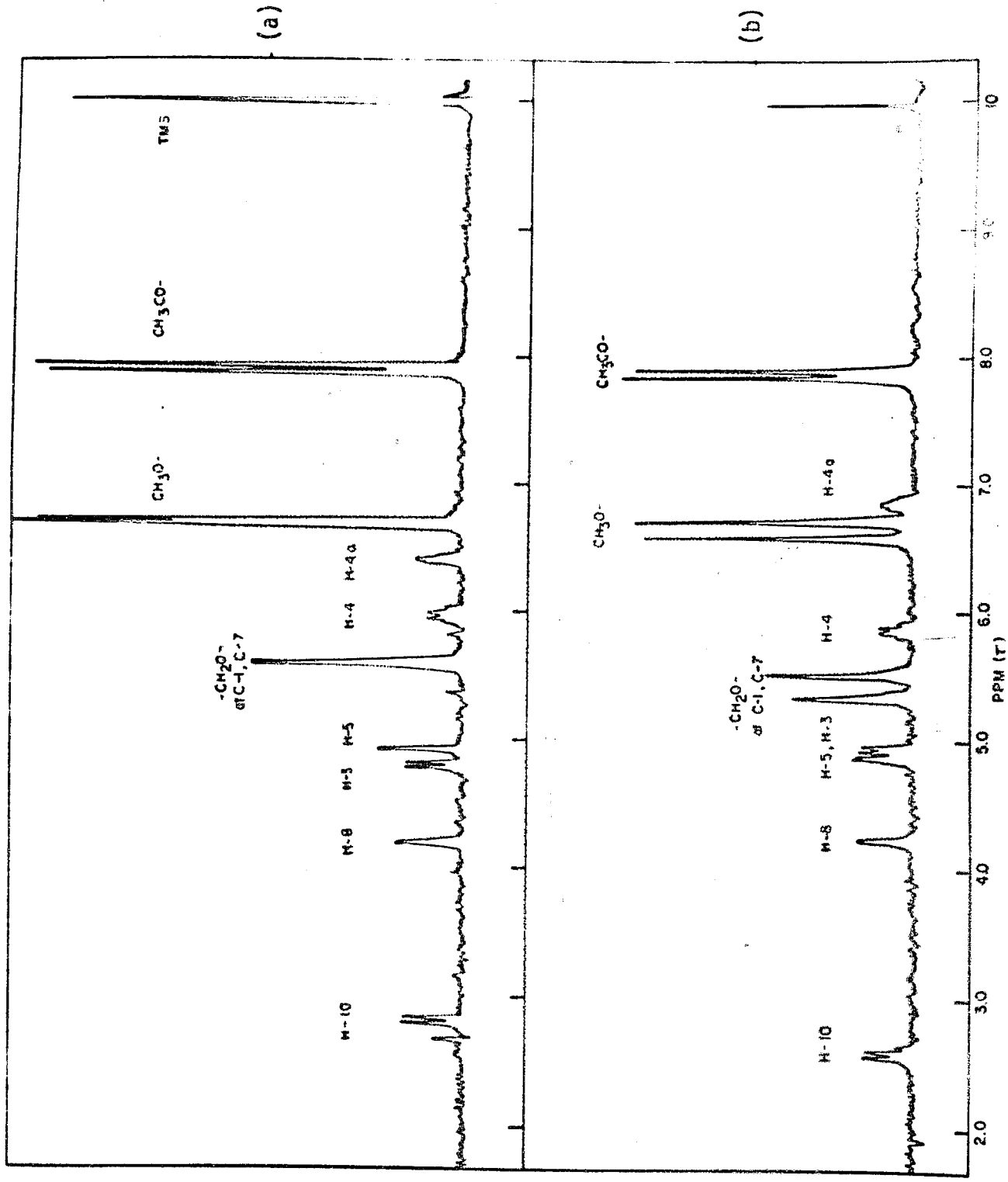
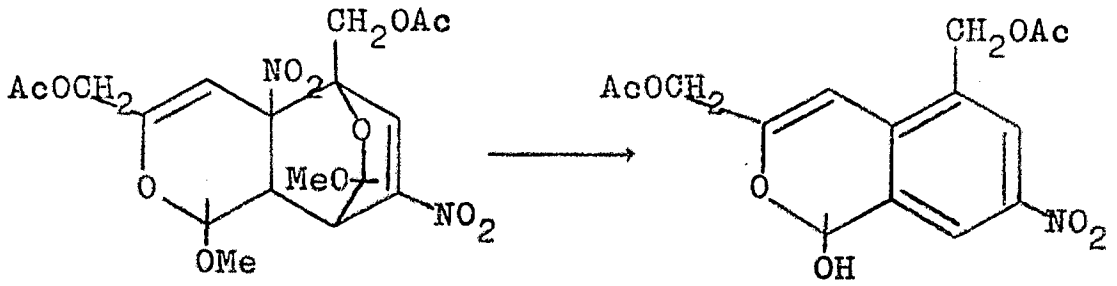


Figure 2. - Nmr spectrum in deuteriochloroform of (a) compound VIII and (b) compound XXX.

Chemical Degradation of Compound VIII

It has been observed earlier (51) that in the preparation of the triacetate I, by acetylating its parent deoxynitro glycoside in the presence of pyridine and recrystallizing the crude acetate from ethanol, small amounts of three crystalline by-products were formed. Two of them were yellow and will be shown to be identical with transformation products of VIII which, in turn, was identical with the third (white) by-product.

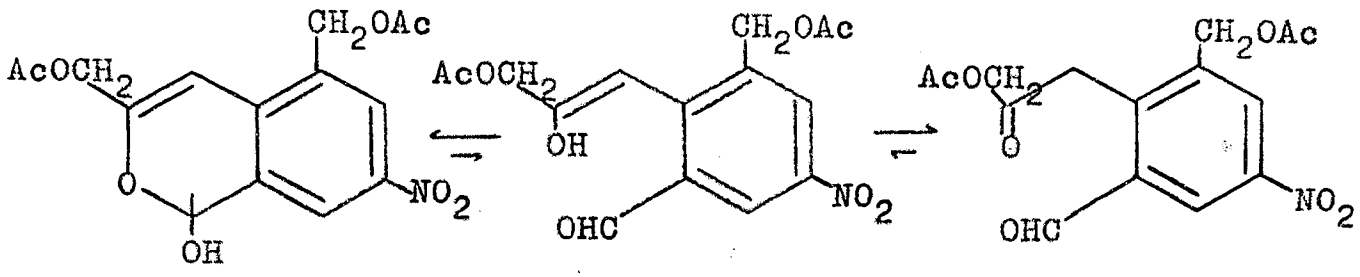
When VIII was heated in the dry state above its melting point, or when it was boiled in benzene or dioxane in the presence of small amounts of pyridine, a yellow coloration developed. Lemon yellow crystals could be isolated in a low yield. The same substance was obtained in a facile manner by fusion of VIII in acetamide for three minutes on a steam bath. Elemental analysis of the optically inactive product, of mp 151-152°, indicated the composition $C_{15}H_{15}NO_8$. The structure was firmly established by spectroscopical and chemical evidence as that of an isochromene derivative, 1-hydroxy-7-nitro-1H-2-benzopyran-3,5-dimethanol 3,5-diacetate (X). This compound, so unexpectedly engendered from a carbohydrate under mild reaction conditions, furnished chemical proof for the structure of its precursor (VIII).



VIII X

It is seen that X can arise from VIII by a fragmentation involving the loss of the -O-CH(OCH_3)- bridge, elimination of nitrous acid, and hydrolysis of the remaining methyl acetal. The latter hydrolysis is presumably linked with the production of nitrous acid for which there was direct evidence in an evolution of oxides of nitrogen during the reaction.

The solid state infrared spectrum of compound X (Table 1) exhibited a broad peak in the 3300 cm^{-1} region indicating hydrogen-bonded hydroxyl. Bands attributable to ester carbonyl and to an aromatic nitro group were at 1735 and 1515 cm^{-1}, respectively, and C=C vibrations occured at 1658 and 1600 cm^{-1}. At 1710 cm^{-1} an additional carbonyl peak appeared, which was presumed to be due to an aldehyde group stemming from a partial tautomerization of X according to the following scheme:

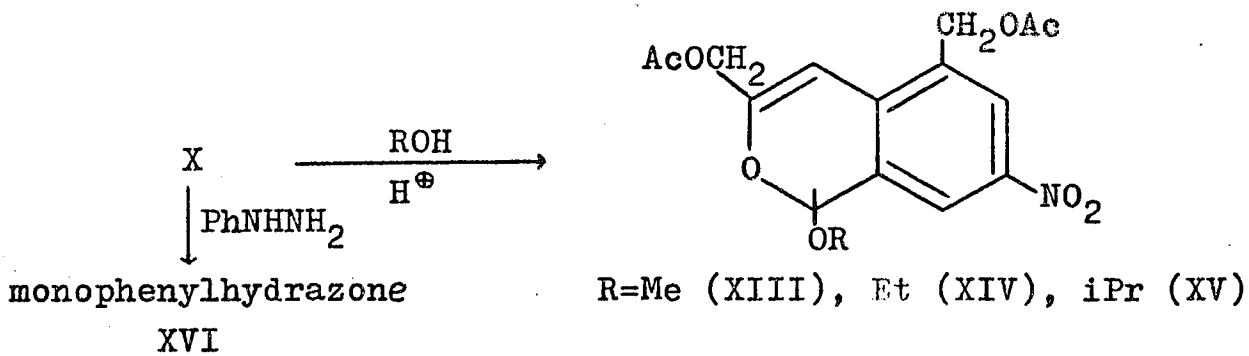


In chloroform solution, the infrared spectrum showed a sharp hydroxyl band at 3590 cm^{-1} and the carbonyl peak at 1710 cm^{-1} was absent, which suggested that in solution the enolic lactol XI is the prevalent tautomeric form. This view was supported by the strong ultraviolet absorption showing a maximum at 346 mμ(ϵ, 11000) and extending into the visible region. Although no spectral data for comparably substituted isochromenes could be found in the literature, this spectrum is believed to be consistent with a substituted p-nitrostyrene system such as X. p-Nitrostyrene itself shows λ_{max} 303 mμ (ϵ, 14400 in methanol) (53). The substituents present in X, especially the enolic ether bridge are thought to cause the observed bathochromic shift. This absorption at a long wave-length would not favour the dicarbonyl tautomer that bears structural resemblance to colorless m-nitrobenzaldehyde (λ_{max} 262 mμ, in methanol) and p-nitrophenylacetone which is described (54)

as forming 'almost colorless crystals'.

The nmr spectrum of X (Figure 3, Table 2) was in full agreement with the isochromene structure.

Both the cyclic hemiacetal and the potential carbonyl character of X were also revealed by chemical reactions. Treatment of X with cold alcohols containing hydrogen chloride resulted in facile acetalization giving crystalline methyl (XIII), ethyl (XIV), and isopropyl (XV) derivatives. Their nmr data (Table 2) could be interpreted readily on the basis of the assigned structures. Phenylhydrazine gave a monophenylhydrazone (XVI) of the dicarbonyl tautomer, the second carbonyl function remaining free despite the use of excess reagent. The location of the phenylhydrazone grouping has not yet been determined with certainty. In the infrared spectrum (Table 1) a carbonyl band at 1727 cm^{-1} would suggest a free keto rather than an aromatic aldehyde group. (m-Nitrobenzaldehyde absorbs at 1707 cm^{-1}). Unfortunately, nmr spectra of XVI were of poor quality owing to insufficient solubility, and they did not yield reliable information to confirm this assignment.



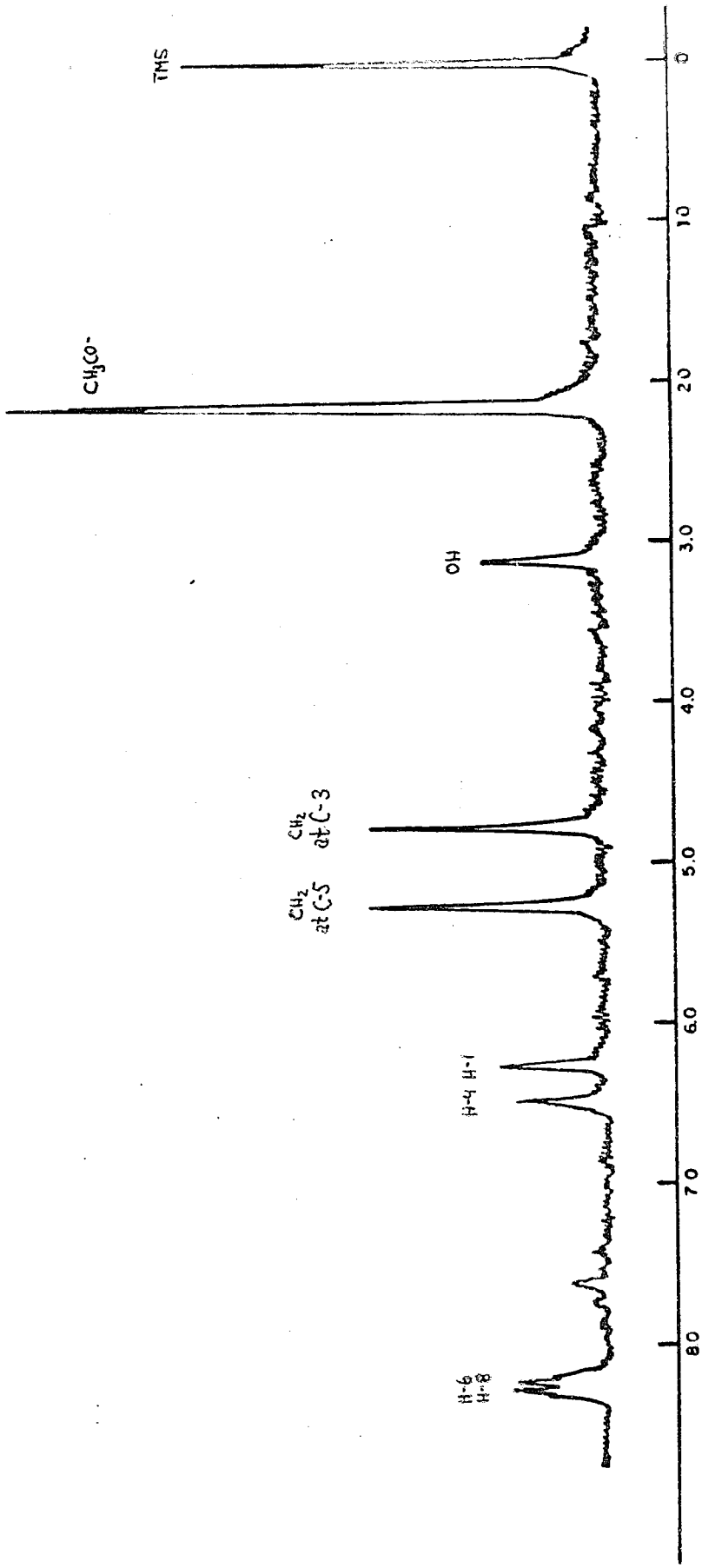
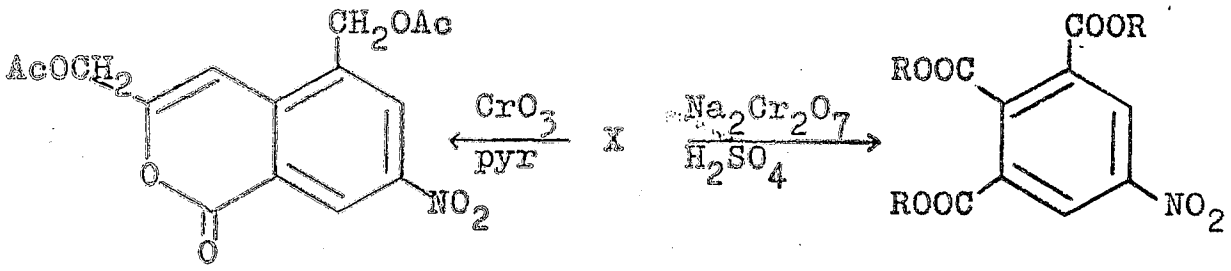


Figure 3. - ¹Hmr spectrum in deuteriochloroform of compound X.

Oxidation with chromium trioxide in pyridine converted the isochromene (X) into the isocoumarin derivative XVII, 7-nitro-1H-2-benzopyran-1-one-3,5-dimethanol diacetate. This product exhibited ultraviolet maxima at 264 mμ (ε, 7400) and 326 mμ (ε, 13500) in methanolic solution. This is in accordance with values reported (55) for various isocoumarines. The band at longest wavelength occurs usually in the region between 318 and 340 mμ. The infrared spectrum of XVII lacked hydroxyl absorption and showed carbonyl bands at 1747 and 1730 cm^{-1}. This is also in agreement with known carbonyl absorptions for isocoumarines which have been found to occur in the range of 1745 to 1725 cm^{-1} (56). All hydrogen atoms of XVII were readily accounted for in the nmr spectrum (Figure 4, Table 2).



XVII

XVIII, R=H

XIX , R=CH_3

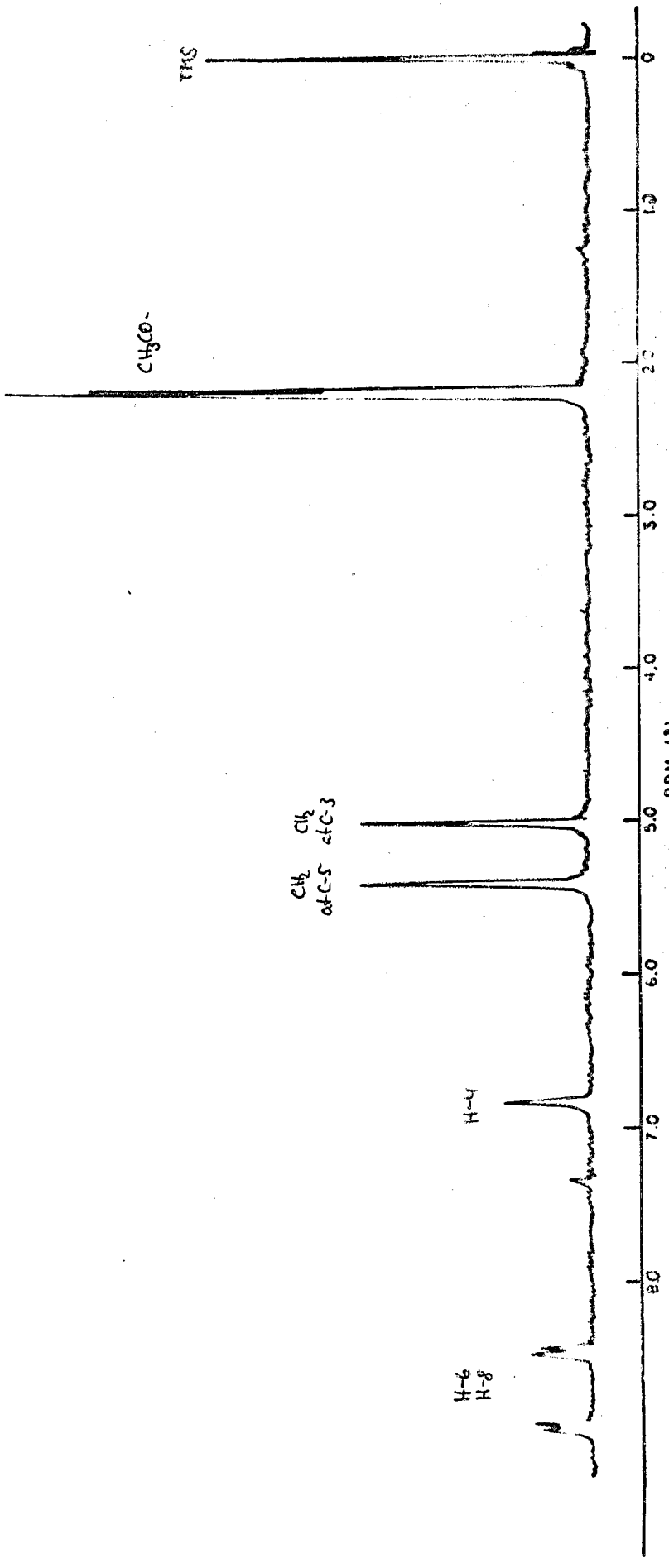


Figure 4. - Nmr spectrum in deuteriochloroform of compound XVII.

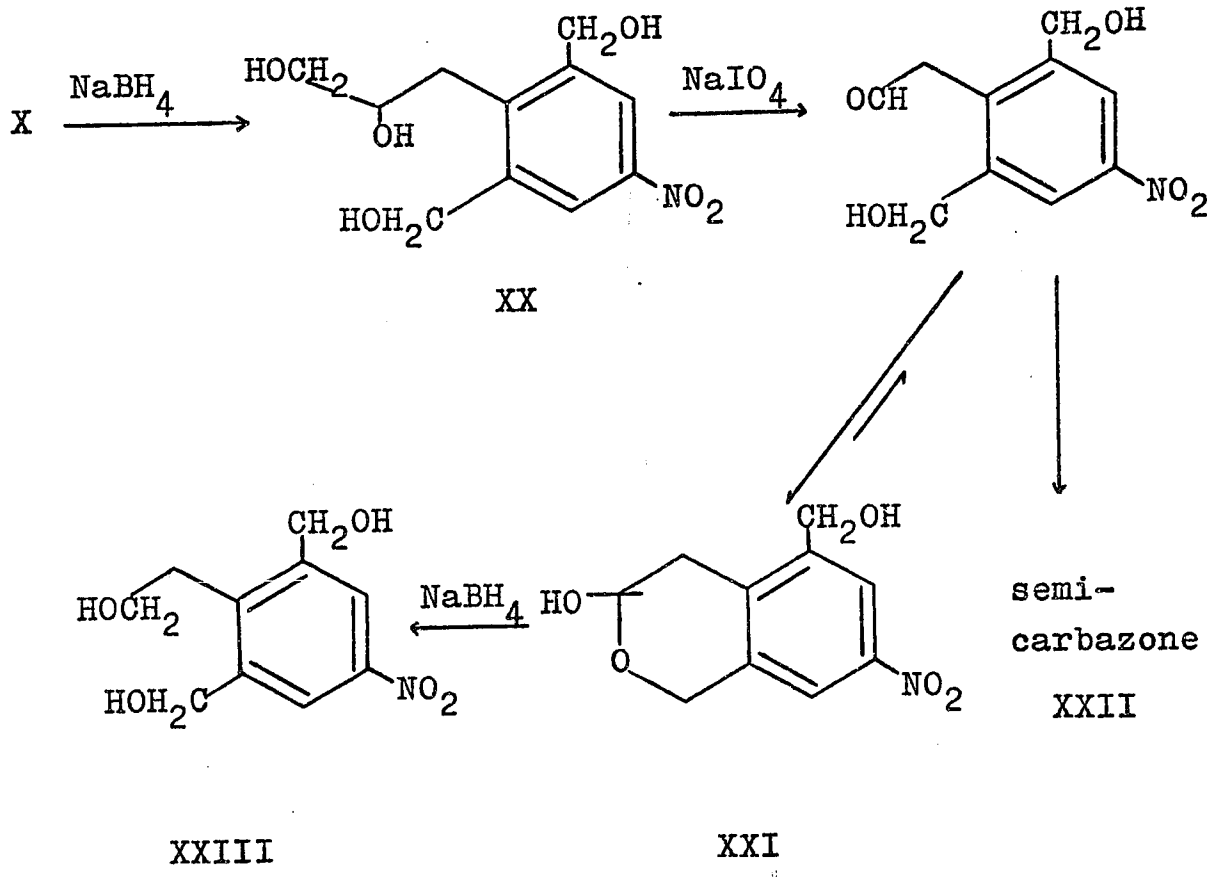
With ethanolic sodium hydroxide, XVII gave an intense, purple coloration (λ_{max} 540 mμ); similarly, addition of sodium hydroxide to X caused a deep, orange red coloration (λ_{max} 490 mμ). According to Rose and co-workers (57), 7-nitro-isocoumarins that are substituted in position 3 with o- or p-hydroxyaryl residues develop a dark violet color with alkali. Compound XVII, even though it lacks a hydroxyaryl substituent, gives a similar color reaction, which is presumably due to a rapid hydrolysis of the lactone ring to form a resonance-stabilized, purple enolate. Analogously, the color produced by X in alkaline medium is believed to originate from the enolate of the open-chain tautomer (XI). In agreement with this assumption, the alkylated derivatives XIII - XV do not give the color reaction.

A more drastic, chromic acid oxidation of X led to nitrobenzene-3,4,5-tricarboxylic acid (XVIII, 5-nitrohemimellitic acid). This acid has been reported to melt 'above 190°' and to yield upon two sublimations an internal anhydride of mp 182-183° (58). No analysis was recorded for the free acid. Its trimethyl ester melts at 144° (58). Our acid XVIII was obtained as a monohydrate of mp 208-210°, it gave an anhydride that melted at 178° after one sublimation, and with diazomethane it formed a trimethyl ester (XIX) of mp 144°. The nmr spectrum of XVIII in deuterium oxide consisted

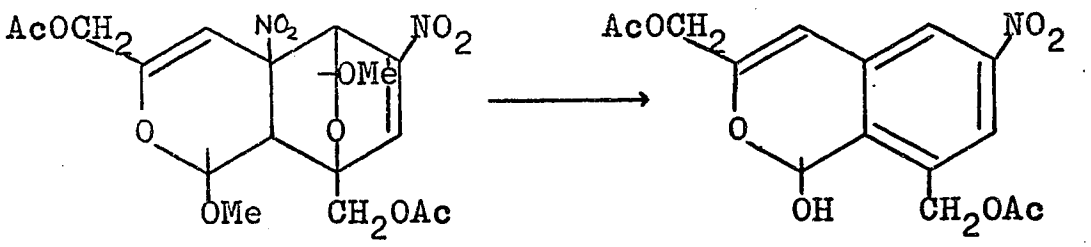
of only one signal, a sharp singlet in the aromatic resonance region, as expected for this symmetrically substituted benzene derivative. Similarly, the ester (XIX) in deuteriochloroform solution produced only one signal, a sharp singlet corresponding in intensity to two protons, in the aromatic resonance region (τ 0.96); the signals for the three methyl ester groups were two singlets at τ5.98 and 6.02 (total intensity, 9H). The only positional isomers of XVIII and XIX, respectively, that would also be compatible with these spectral features are nitrobenzene-2,4,6-tricarboxylic acid (nitrotrimesic acid) and its trimethyl ester. Their melting points (59) are 290° and 107-108°, respectively, and the acid is incapable of internal anhydride formation. Therefore, no doubt exists about the identity of XVIII. The degradation of X to XVIII proved that carbon was attached to the benzene ring of X in the three positions meta and para to the nitro group.

Treatment of compound X with sodium borohydride resulted in reduction with concurrent loss of the acetyl groups and gave a tetraol (XX). Glycol cleavage of the tetraol with periodate afforded an aldehyde that existed in the tautomeric, cyclic hemiacetal form (XXI), both in solution and in the crystalline state. This was evident from the absence , in its infrared spectrum, of a carbonyl peak and the presence of a sharp absorption at 1130 cm^{-1}

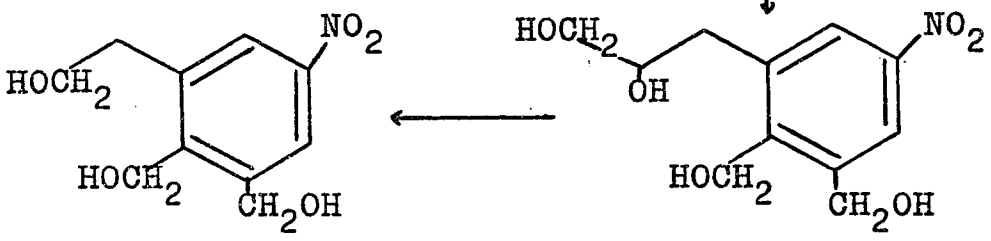
that is probably associated with the cyclic C-O-C bond; furthermore, the nmr spectrum (Table 2) did not show a low-field signal attributable to an aldehydic proton but possessed a one-proton triplet near τ5.0 due to the anomeric H-3. The nature of XXI as a potential aldehyde was ascertained by the preparation of a semicarbazone (XXII). Finally, sodium borohydride reduction of XXI yielded a triol, 4-(2-hydroxyethyl)-3,5-bishydroxymethyl-nitrobenzene (XXIII).



The chemical degradation of X discussed so far constitutes unambiguous proof of the structure depicted if one alternative isomer, namely XXIV which also is compatible with the results, can be ruled out. This may be done on spectroscopic grounds. Let us compare the tetraol XX and the triol XXIII with their positional isomers XXV and XXVI that would arise from hypothetical XXVII.



XXVII XXIV

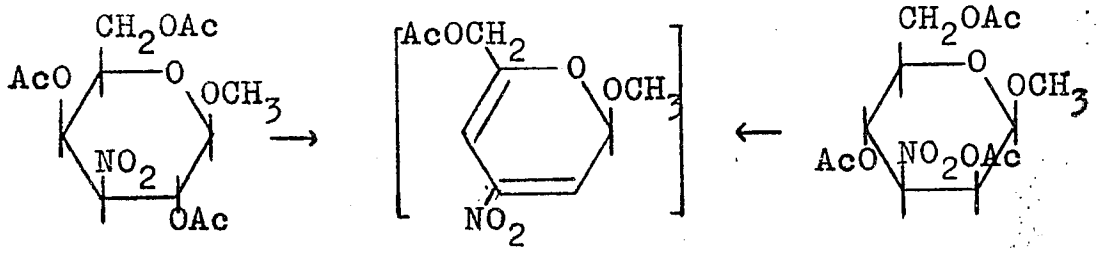


XXVI XXV

The tetraol that was actually obtained showed in its nmr spectrum a single sharp peak (τ 1.8, in D_2O) for its aromatic protons, indicating equality of chemical shifts and therefore suggesting a symmetrical substitution pattern. The same was true for the triol whose signal for its two aromatic protons was a singlet near τ1.9. Formulas XX and XXIII were consequently preferred over the alternative structures XXV and XXVI where the unsymmetrical substitution patterns would be expected to cause chemical shift differences for the aromatic protons, and, therefore, pairs of doublets due to meta coupling. This argument could be disputed if the magnetic differences in the structurally rather similar side chains of XXV and XXVI were insufficient to affect the aromatic protons noticeably. At any rate, all the unsymmetrically substituted nitrobenzenes encountered in this study did show, without exception, the expected multiplicity (see compounds X, XIII, XIV, XV, and XVII in Table 2). A compelling reason, however, for discarding the structure XXIV in favor of X is that XXIV would require its precursor, the Diels-Alder adduct, to be formulated as XXVII, and this would be entirely inconsistent with the nmr spectrum of the adduct (VIII) discussed above.

Stereochemistry

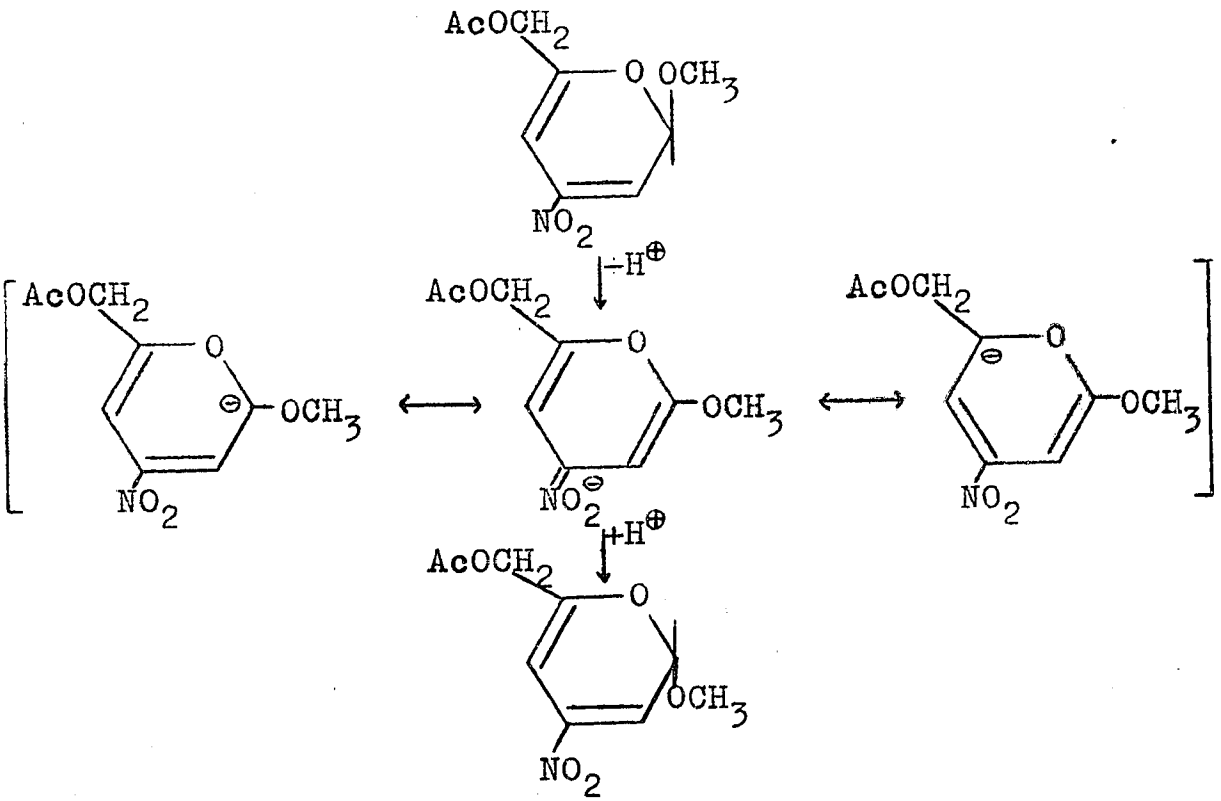
When methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-mannopyranoside (XXVIII) and the corresponding galacto isomer XXIX were dehydroacetylated with sodium hydrogen carbonate in refluxing benzene, the products were identical with compound VIII obtained from I. Formation of the diene VI as a common intermediate in all three cases readily explains this fact.



XXIX VI XXVIII

There was, however, another stereochemical feature requiring special discussion. Compound VIII proved optically inactive although the reaction sequence I → VI → VIII did not affect the anomeric center structurally and was not at first sight expected to involve it mechanistically. The observed optical inactivity imposed the conclusion that racemization at the anomeric carbon had nonetheless occurred at some stage during the reaction, perhaps in the intermediate

VI, which on abstraction of the anomeric proton would give a resonance-stabilized anion. Protonation could then furnish the enantiomer. An attempt to prove this proposition by using sodium deuterium carbonate in a dehydroacetylation of I failed inasmuch as no deuterium was incorporated in VIII. A possible explanation might be dissociation of VI into an ion pair, with the proton having no opportunity of being exchanged.



For this reason it was of interest to examine whether changes in the reaction conditions might have some influence upon the nature of the product.

When, for the dehydroacetylation of I, potassium carbonate was substituted for sodium hydrogen carbonate the reaction went to completion within five hours, even at the lower temperature of 25 - 30°. The isolated crystalline product was very similar to VIII in its infrared spectrum, but it showed a lower, usually unsharp melting point which was difficult to bring to constancy by recrystallization. Nmr spectroscopy revealed that the crude product contained besides VIII approximately 10 - 20% of another, similar substance (XXX) which could be enriched to some extent by repeated recrystallizations. Mixtures of VIII and XXX were readily converted into the isochromene, no product other than X being thereby encountered. A mixture in which XXX predominated over VIII was obtained by dehydroacetylation of I with potassium carbonate in tetrahydrofuran for 53 hr at -70° followed by two days at -5°. Pure XXX (mp 115°) was finally isolated and, in contrast to VIII, XXX was optically active ($[\alpha]_D$ -4.5°). Analytical and spectral data indicated that XXX was a diastereoisomer of VIII. Thus, the infrared spectra of the two pure isomers virtually coincided in some spectral regions and showed but slight divergencies in others (Figure 1 and Table 1). Acetamide fusion of both compounds produced the same ultraviolet band at 346 mμ. The nmr spectrum of XXX exhibited all the general

features of the spectrum of VIII, with slight variations in the values of certain chemical shifts and coupling constants (Figure 2). Although spin decoupling experiments have not yet been done with XXX, the close kinship of the two spectra leaves little doubt in the validity of the proton assignments that correspond to those established for VIII.

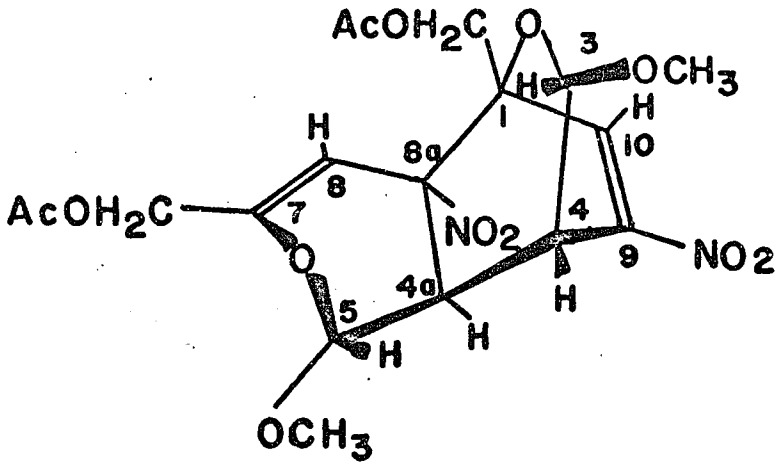
As for a stereochemical representation of VIII, one has to take into account its optical inactivity and must proceed from the assumption that VI, the intermediate diene, is capable of racemization. When a molecule of VI acts as dienophile to combine with a molecule of its enantiomer acting as diene, and if Alder's rule of preferential endo addition is obeyed, the configuration depicted in Figure 5 arises. There are many exceptions known to Alder's rule (60) but it has been stated (61) to be 'quite closely obeyed in the additions of cyclic dienes to cyclic dienophiles'. Also, the additions to cyclopentadiene of nitroethylene, 1-nitropropene, 1-nitro-1-butene, and various 2-aryl-1-nitroethylenes have been shown to follow predominantly the endo path, with endo:exo ratios 9:1 (one case), 6:1 (three cases), 4:1 and 3:1 (one case each) (62).

Such a structure is in accord with the magnitude of the splittings observed in the nmr signals for H-3, H-4, H-4a, and H-5. Inspection of a Dreiding model

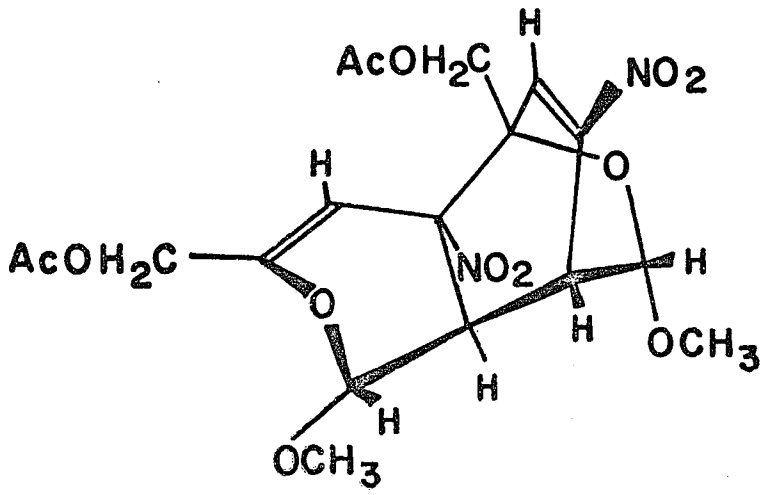
shows that the dihedral angles between H-3 and H-4, and between H-4 and H-4a, in the rigid, bridged system approximate 60°, consistent with the splittings of about 2.5 cps. The ring comprising C-5, C-6, C-7, and C-8 is constrained in a twist boat conformation in which the dihedral angle between H-5 and H-4a is about 75°, causing these protons to be coupled by less than 1 cps. In the model, the twist boat can be flipped so as to make the methoxyl group equatorial and H-5 - H-4a trans diaxial, but such a conformation would cause a large coupling in the latter protons and is therefore ruled out.

While we favor the endo configuration for VIII in view of Alder's rule and in the absence of contradictory evidence, it is recognized that the exo configuration (Figure 5), if more stable, might have arisen by thermodynamic control of the reaction. Since such a structure would also satisfy the nmr data just discussed, a decision cannot be made on this basis.

Regarding the stereochemistry of optically active XXX, one may assume that, at low temperatures, the racemization of intermediate VI is retarded relative to the diene addition, and that consequently an adduct arises in which both anomeric centers (C-3 and C-5) have retained the original absolute configuration. The stereochemical representations for an endo and exo adduct,



ENDO



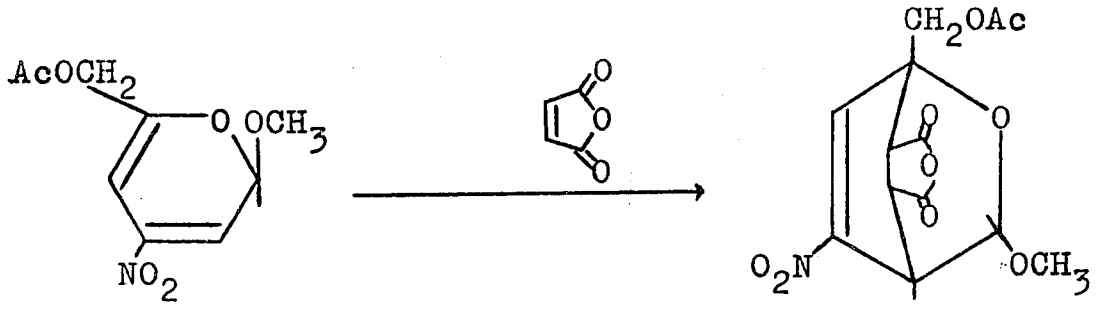
EXO

Figure 5

Possible configurations of compound VIII.

respectively, would then be the same as those of VIII except for inverted C-3 configurations.

In the Diels-Alder reaction leading to VIII two molecules of the intermediate VI are involved, one acting as the diene, the other as the dienophile. It was thought, therefore, that if the reaction were to be carried out in the presence of an excess of another olefin which could replace the dienophile, it should be possible to obtain a 'mixed' adduct. For instance, with maleic anhydride one would expect:



VI

Unfortunately, attempts with maleic anhydride, acrylonitrile, and azobenzene were unsuccessful as no crystalline product could be isolated.

The investigations reported in this section may be summarized as follows. The dehydroacetylation of methyl 3-deoxy-3-nitro-hexopyranose triacetates leads to products that had not really been anticipated, namely to tricyclic Diels-Alder products resulting from self-

addition of an intermediate nitrodiene. The reaction takes place with great ease, and the aromatization of the product into a derivative of isochromene, particularly by a brief treatment with acetamide, has opened a novel approach to that heterocyclic system.

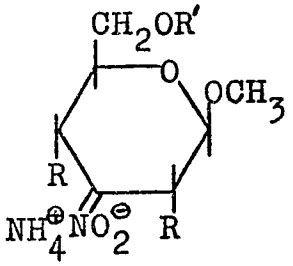
Reaction of the Triacetate I with Ammonia

As was discussed in the introduction (page 18), it is possible to replace under suitable conditions an acetoxy grouping vicinal to a nitro group by a nucleophile. It was therefore interesting to investigate the behaviour of the triacetate I towards ammonia. In the light of our previous findings three products could be expected:

1. Formation of an ammonium nitronate followed by elimination of acetic acid to give a nitroallyl system.
2. Elimination of acetic acid with subsequent addition of ammonia to give aminonitro sugars.
3. Formation of the diene VI which would then undergo the Diels-Alder addition discussed above.

Methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-glucopyranoside (I) was dissolved in liquid ammonia at -70°. Subsequent evaporation of the ammonia in vacuo at this low temperature left a yellow, solid residue which exhibited strong absorptions in the ultraviolet at 251

and 306 mμ. These two bands may be attributed to at least two different species; the lower wavelength absorption should be due to an aci-nitro salt of the type XXXI, and the absorption at the longer wavelength suggests [(51), see also introduction page 16] the presence of an allylic structure such as XXXII.



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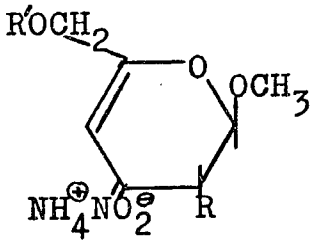


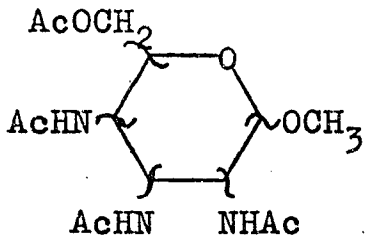
XXXI R = OAc, NH_2
R′ = Ac, H

XXXII R = OAc, NH_2
R′ = Ac, H

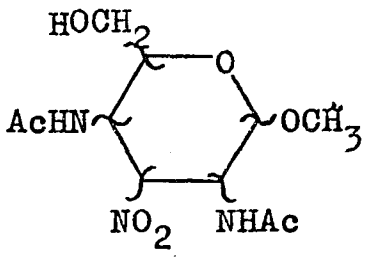
Upon laborious recrystallizations and attempts at purification, whereby decomposition of some part of the mixture seems to have occured as indicated by the evolution of a gas, a white solid could be isolated in a very low yield. In its infrared spectrum the substance showed several peaks of medium intensity at 3360 - 3100 cm^{-1} (NH, OH stretch), strong absorptions at 1662 (amide I), 1568 (amide II), and 1550 cm^{-1} (NO_2). Addition of alkali caused a strong ultraviolet absorption at 255 mμ which changed only slightly upon heating the alkaline solution for ten minutes at 98°. A structure that would be compatible with these spectral data and which is also sup-

ported by elemental analysis is that of a methyl 2,3,4-trideoxy-2,4-diacetamido-3-nitro-hexopyranoside (XXXIII). 3-Nitrohexosides in alkaline solution have been shown (see Introduction page 16) to be converted rapidly into a nitroallyl system (XXXII, R=OH); similarly the triacetate I gives XXXII (R=OAc) instantaneously when dissolved in alkali. The stability of the alkaline solution of XXXIII is not surprising, however, the acetamido group being a poorer leaving group than either hydroxyl or acetoxyl.

Hydrogenation of XXXIII followed by acetylation furnished a methyl 2,3,4-trideoxy-2,3,4-triacetamido-6-O-acetyl-hexopyranoside (XXXIV). This compound gave a correct elemental analysis and showed in its infrared spectrum a medium peak at 3350 cm^{-1} with a shoulder at 3300 cm^{-1} (NH stretch), strong peaks at 1740 cm^{-1}(ester CO), 1650 cm^{-1}(amide I), 1560 and 1535 cm^{-1} (amide II). Addition of alkali did not produce any ultraviolet absorption, which indicated the absence of a nitro group. The stereochemistry of compounds XXXIII and XXXIV has not been determined due to lack of material. The possibility exists that the configurations of all the asymmetric carbons have been subject to change since the olefins IV and V, as well as the dienes VI and VII (Formulae page 28) must all be considered as potential intermediates in the formation of XXXIII.



XXXIV



XXXIII

Compound XXXIV represents the first methyl glycoside containing three amino nitrogen functions. In view of the interest that is being focused on diamino sugars in the realm of the chemistry of microorganisms, it appears worthwhile to continue studies on triamino analogs. In concurrent work in this laboratory, 1,2,3-triamino cyclohexane and cyclitol derivatives have been synthesized by use of the same method (63), and in similar studies Lichtenthaler (64) has very recently obtained a triamino 1,6-anhydrohexose.

Table 1

Infrared frequencies (cm^{-1}) in Nujol[a]

Compd.	OH	-C=CH	C=O	C=C	NO_2	other prominent peaks
VIII		3120w	1735s	1690m	1550s	1400w, 1368ms, 1350ms,
		3100m			1530s	1330w, 1250s, 1210w,
						1123m, 1090w, 1070m,
						1025ms, 980-700w[b]
XXX		3120w	1740s	1680m	1550s	1405w, 1368ms, 1350ms,
					1525s	1320w, 1270m, 1240s,
						(sh 1220), 1125m, 1093m,
						1030m, 980-700w[b]
X	3300s,bd	3100w	1735s	1658ms	1515s	1410w, 1365m, 1340s,
			1710s	1600ms		1300ms, 1260s, 1240s,
						1210m, 1100w, 1080m,
						1055ms, 1035ms, 990m,
						918m, 870-700w[b]
XIII		3100w	1740s	1658m	1520s	1405w, 1360ms, 1340s,
				1600m		1315w, 1230s,bd,
						1100-900ms
XIV		3100w	1738s	1650m	1525s	1408w, 1340s, 1330sh,
				1600m		1315sh, 1275m, 1250m,
						1225s, 1080ms, 1025ms,
						1000-700w,m[b]

Table 1 (continued)

Compd.	OH	-C=CH	C=O	C=C	NO_2	other prominent peaks
XV		3100w	1735s	1650m	1525s	1405w, 1338s, 1325sh,
				1595m		1280m, 1250m, 1225s,
						1075ms, 1030-700w, m[b]
XVI			1760s	1600m	1518s	3320w, 1535w, 1498w,
			1740s	1550ms		1415w, 1350s, 1310w,
			1727s			1290w, 1260w, 1225s,
						1160-670w, m[b]
XVII		3115w	1750s	1665m	1530s	1438ms, 1370ms, 1350s,
			1730s	1603m		1330ms, 1245s, 1225s,
						1100m, 1050-700w, m[b]
XVIII	3300s, bd		1715s	1605m	1530ms	1420w, 1363ms, 1300m,
	2700-2500w[b]					1245m, 1215m, 1170m,
						1133w, 1080w, 820-650w[b]
XIX		3125w	1735s	1610m	1540ms	142ow, 1365m, 1310m,
						1275s, 1240s, 1115w,
						1070w, 1000m, 950-700w[b]
XX	3250s, bd			1610w	1510s	1345s, 1250w, 1090 ms,
				1595w		1020s
XXI	3350m	3100w		1610w	1533s	1415w, 1350s, 1320w,
				1595w	1520sh	1260w, 1130s, 1085s,
						1025ms, 1000-670w[b]

Table 1 (continued)

Compd.	OH	-C=CH	C=O	C=C	NO_2	other prominent peaks
XXII	3600-3200s[b,c]	3100w	1665s	1590ms[d]	1515ms	1350s, 1263w, 1175w, 1145w, 1075s, 1000-700w[b]
XXIII	3300s,bd	3100w		1600w	1515s	1340s, 1290-1170w[b], 1080s, 1025s, 900-700w[b]

[a]Designations are s (strong), ms (medium strong), m (medium), w (weak), bd (broad), sh (shoulder). [b]Several bands. [c]OH and NH stretching vibrations. [d]C=C stretching and NH bending vibrations.

TABLE 2

60 Mc Nmr Data[a]

Compd.	Arom. Protons	H-4[b]	H-1[b]	$-CH_2-O$	CH_3-CO	Others
X[r]	q 1.74(2)[c]	s 3.50(1)	s 3.72(1)	s 4.72(2), s 5.21(2)	s 7.85(3), s 7.87(3)	s 6.88(1)[d]
XIII[r]	q 1.78(2)[c]	s 3.74(1)	s 3.96(1)	s 4.80(2), s 5.25(2)	s 7.87(3), s 7.89(3)	s 6.43(3)[e]
XIV[r]	q 1.80(2)[f]	s 3.68(1)	s 3.81(1)	s 4.75(2), s 5.21(2)	s 7.85(6)	q 6.10(2)[g], t 8.73(3)[h]
XV[r]	q 1.80(2)[f]	s 3.72(2)[i]		s 4.75(2), s 5.23(2)	s 7.86(6)	m 5.70(1)[j], m 8.73(6)[k]
XVII[r]	q 1.20(2)[c]	s 3.15(1)		s 4.60(2), s 4.99(2)	s 7.80(3), s 7.85(3)	
XVIII[s]	s 1.5 (2)					s 1.2 (3)[l]
XIX[r]	s 0.96(2)					s 5.98, s 6.02 (9)[m]
XX[t]	s 1.8 (2)			5.3 - 6.5[o]		m 7.0 (2)[p]
XXI[s]	q 2.2 (2)			s 5.35(2), s 5.65(2)		m 7.4 (2)[p], s 4.9 (1)[v], s 4.7 (1)[d], s 3.4 (1)[d]
XXIII[u]	s 1.9 (2)			s 5.45(4), m 6.6 (2)		m 7.25(2)[p]

TABLE 2 (continued)

[a]Designations are s for singlet, t for triplet, q for quartet, and m for multiplet. Figures in parentheses are proton intensities obtained by integration. Chemical shifts are given as τvalues, relative to TMS = 10 for compounds measured in $CDCl_3$ solution and relative to DOH = 5.25 for XX. Chemical shifts for XVIII, XXI and XXIII are without standardization and therefore approximate only. [b]In compounds X-XVII. [c]J_{meta} ca. 2 cps. [d]Hydroxyl signal, disappearing on deuterium exchange. [e]Methoxyl. [f]J_{meta} 2.3 -2.5 cps. [g]$-CH_2-$ in ethoxyl. [h]CH_3- in ethoxyl. [i]Signals for H-4 and H-1 coincide. [j]-CH- in isopropoxyl. [k]CH_3 in isopropoxyl. [l]Broad peak due to carboxyl H. [m]Estimated ratio of signals, 1:2. [o]Poorly resolved signals, partially obscured by DOH peak. [p]Unsymmetrical multiplet presumably due to benzylic CH_2. [r]Solvent $CDCl_3$. [s]Solvent DMSO-6d. [t]Solvent D_2O. [u]Solvent DMSO-6d/D_2O. [v]Anomeric proton C-3.

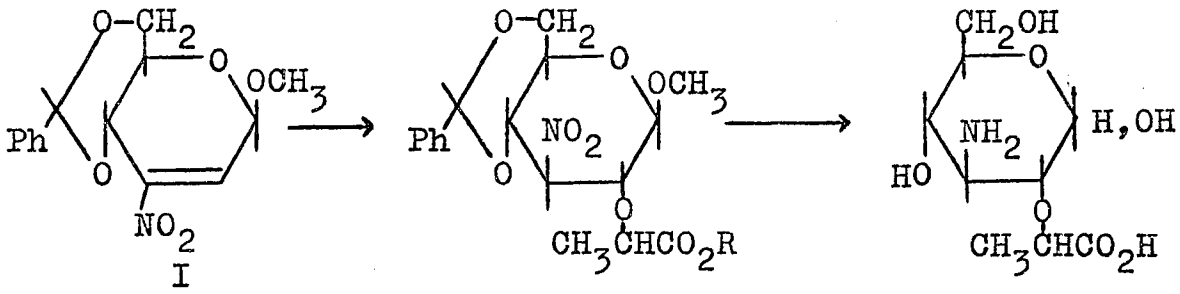
PART II

Nucleophilic Additions to Methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-erythro-hex-2-enopyranoside

DISCUSSION

1. Addition of Isopropyl Lactate: The Synthesis of Positional Isomers of Muramic Acid.

Owing to its capability of undergoing nucleophilic additions, the nitro sugar olefin I[a] suggested itself as a suitable starting material for the synthesis of positional isomers of muramic acid, whose availability might be desirable for future structural and biochemical studies on microorganisms. In an abbreviated form, the project may be outlined as follows:



Thus, 3-amino-3-deoxy-2-O-carboxyethyl hexoses would be obtained, i.e. sugars that differ structurally from muramic acid by transposition of the substituents at C-2 and C-3.

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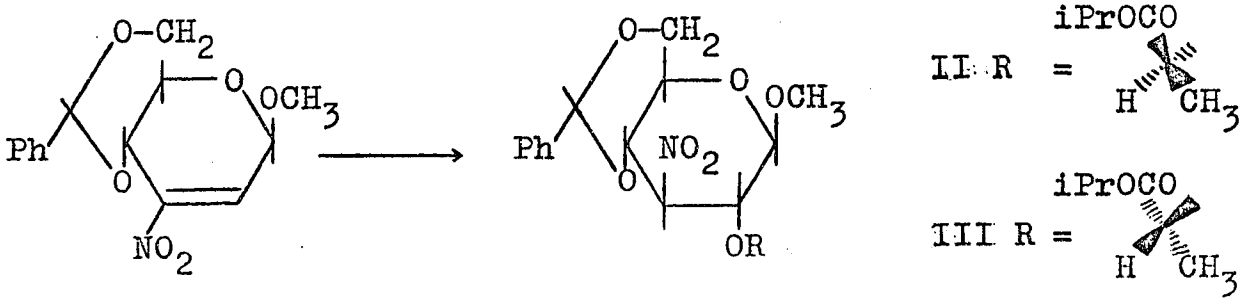
a. For convenience, compounds in this chapter are numbered using a new set of Roman numerals.

When methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-erythro-hex-2-enopyranoside (I) was heated with isopropyl DL-lactate in benzene in the presence of a trace of potassium hydroxide, a mixture of epimeric addition products was formed from which pure methyl 4,6-O-benzylidene-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-ß-D-glucopyranoside (II) and its D-1-(isopropoxycarbonyl)ethyl epimer (III) were obtained by fractional crystallization in yields of 56 and 5.5%, respectively. Separate reactions were performed under identical conditions with the optically active isopropyl L- and D-lactates in place of the racemate. They furnished the L and D adducts, respectively, in practically identical yields of about 83%. The 10:1 ratio of II and III isolated in the addition of the racemate could in part be due to steric factors favoring a faster reaction of the L ester. However, the difficulty of separating the more soluble III in pure condition from the epimeric mixture certainly contributed to the observed ratio.



I

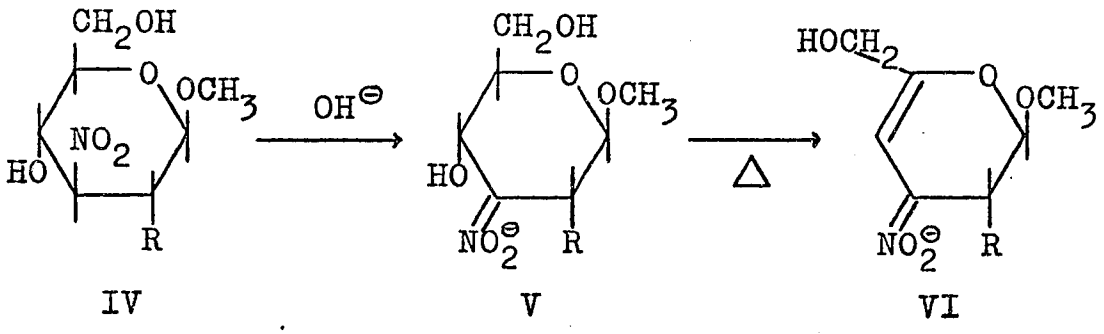
Since the reactions leading to II and III did not involve any cleavage of bonds at the asymmetric carbon atoms in the isopropyl lactates, the side-chain configurations in the adducts were unequivocally defined. The gluco configuration of the hexose moiety could a priori be assumed, as similar nucleophilic additions to I had preferentially or exclusively (see Introduction page 18) led to trans diequatorial disposition of the substituents at C-2 and C-3. Definite proof was nonetheless deemed necessary and was obtained as shown in a subsequent paragraph.

The L-1-Carboxyethyl Series

The L adduct II was prepared, from readily available racemic isopropyl lactate, rather more conveniently than the D adduct III. It was, therefore, expedient to use the former for a more detailed pursuit of the planned path of synthesis.

Debenzylidenation of II furnished methyl 3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl] -3-nitro-ß-D-gluco-pyranoside (IV). This nitro glycoside, like others with related structures, exhibited the characteristic behaviour in alkaline medium that has been discussed (page 16). In 0.01 N sodium hydroxide solution it showed an ultraviolet absorption peak at 255 mμ(ε, 11000) which is due

to the nitronate ion (V). The peak disappeared on heating and, after 15 minutes at 98°, was replaced by a new peak at 298 mμ (ε, 20000). This change is attributed to the loss of a molecule of water resulting in the formation of a double bond between C-4 and C-5, in conjugation with the aci-nitro group (VI).



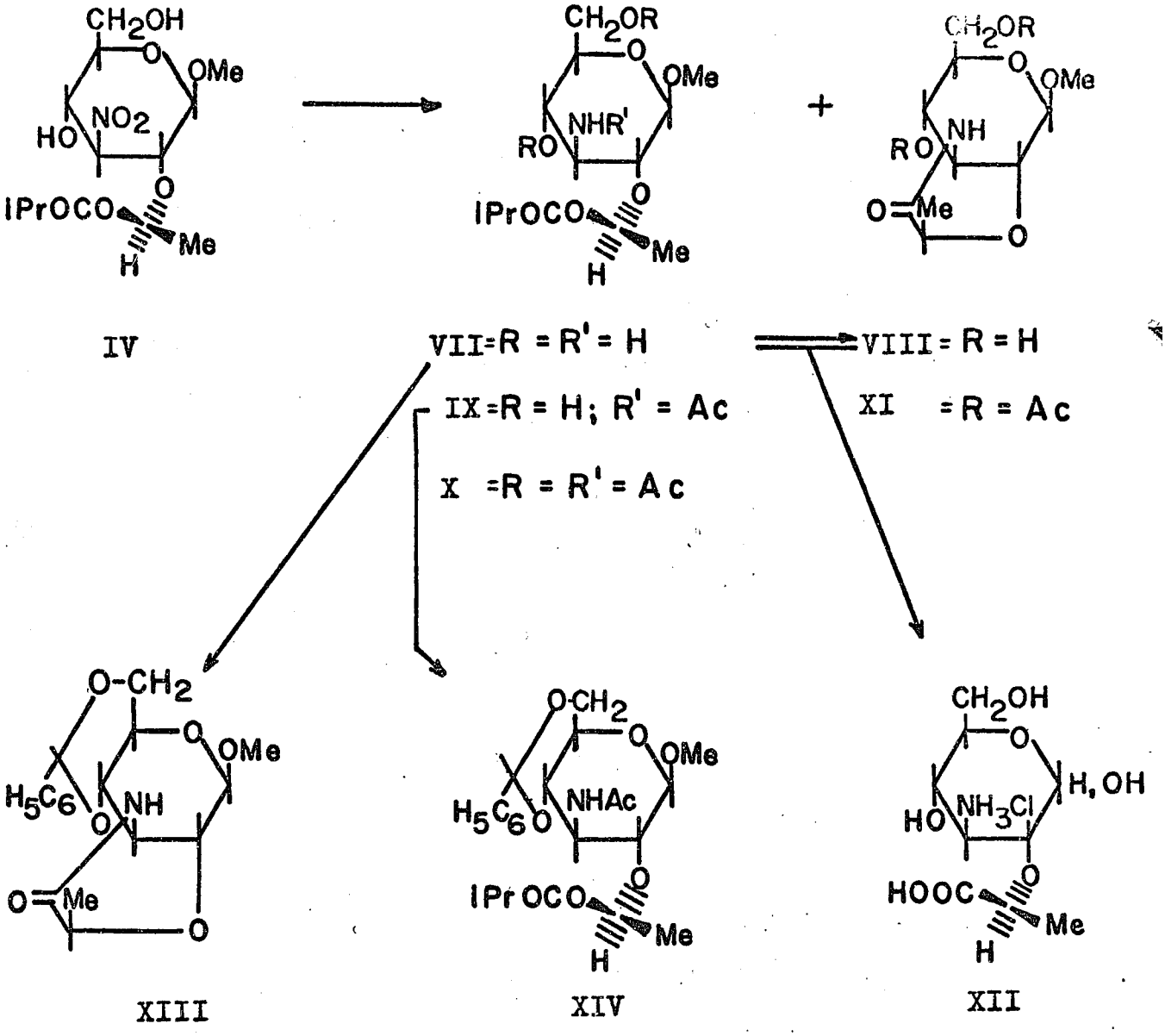
IV V VI

R = isopropyl 2-L-lactyl

The platinum-catalyzed hydrogenation of IV to the corresponding amine proved to be not quite straightforward, the product composition varying to some extent with the reaction conditions. Pressure (70 psi) and a reaction time of 3-4 days were required, and even then a considerable quantity of unchanged starting material usually remained. This contrasts with the facile catalytic reductions that can normally be performed with nitro sugars. Hydrogenation in 2-propanol produced the expected amine, methyl 3-amino-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (VII) in a

29% yield, and in addition it gave about 10% of a crystalline by-product which has not yet been identified. When the hydrogenation was carried out in a 2-propanol-water mixture (1:1), only about 15% of the amine VII and 9% of the by-product were isolated, but a third product was obtained in a 23% yield. The latter compound proved to be the lactam (VIII) of methyl 3-amino-3-deoxy-2-O-(L-1-carboxyethyl)-ß-D-glucopyranoside; it obviously had arisen in a secondary reaction from the amino ester VII. When an aqueous solution of crystalline VII was heated on a steam bath for 3 hr, a nearly complete conversion into VIII occurred. The isolation of VIII in the hydrogenation of IV also offered a clue to the nature of the by-product mentioned above. Analytical data of the latter lay between those of VIII and of the corresponding free amino acid, and also the infrared spectrum, melting point, and behaviour in an acetylation (see Experimental) suggested that the by-product was a mixture of the two. The material gave a negative ninhydrin test, but that does not necessarily rule out the presence of the amino acid which, under the test conditions, might well be converted rapidly into the lactam.

The amino ester glycoside VII was further characterized by preparing its N-acetyl derivative IX and its N-acetyl-4,6-di-O-acetyl derivative X. The lactam VIII gave a 4,6-di-O-acetyl derivative (XI). Spectral data


CH2OH
OMe
HO
NO2
IPrOCO
Me
H
IV
CH2OR
NHR'
RO
VII=R = R' = H
IX=R = H; R' = Ac
X =R = R' = Ac
+
NH
Me
VIII = R = H
XI = R = Ac
O-CH2
H5C6
XIII
NHAc
XIV
NH3Cl
H, OH
HOOC
XII

of these compounds were in accord with the structures depicted. Thus, the infrared spectra of the isopropyl esters VII and IX exhibited sharp carbonyl stretching bands at 1745 cm^{-1}. In the spectra of the acetates X and XI these peaks occurred at 1745 and 1735 cm^{-1}, respectively, and they were broader because of the multiple ester groupings. No ester carbonyl absorption was present in VIII. The acetamido compounds IX and X had amide-I bands at 1660 and amide-II bands at 1570-1560 cm^{-1}. The lactam diacetate XI showed a sharp amide carbonyl stretching band at 1670 cm^{-1} which in the parent lactam VIII was shifted to 1640 cm^{-1} and broadened owing to hydrogen bonding. Neither lactam gave an amide-II band. The nmr spectrum of the triacetyl compound X showed two sharp singlets corresponding to six and to three protons at τ7.95 and 8.05, respectively, which could be assigned to the two equatorial acetoxy groups and an equatorial acetamido group (40,65-68). The methoxyl resonance was a singlet at τ6.57, and the C-methyl group of the lactic acid moiety gave a doublet centered at τ8.63 and split by 7 cps. In addition, there was a series of signals in the 8-9τ region which in part overlapped with the methyl signals of the acetyl and lactic acid moieties. Integration showed that they corresponded to six protons; they must be due to the methyl protons of the isopropyl ester group. Evidently,

the methyl parts of that group were magnetically non-equivalent in their particular structural environment, for otherwise one would have expected them to give a doublet rather than the observed multiplet. In the lactam diacetate XI the acetoxy resonances appeared as separate singlets at τ7.87 and 7.93; the methoxy signal was at τ6.46, and the C-methyl group gave a doublet at τ8.53 with a splitting of 7 cps.

Acid hydrolysis of the amino ester glycoside VII produced 3-amino-2-O-(L-1-carboxyethyl)-3-deoxy-D-glucose which crystallized as the hydrochloride XII. The same reducing amino sugar was shown by paper chromatography to arise from the lactam VIII by acid hydrolysis.

Treatment of the amino ester glycoside VII with benzaldehyde and anhydrous zinc chloride led to simultaneous O-benzylidenation and lactam formation, yielding the lactam (XIII) of methyl 3-amino-4,6-O-benzylidene-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside. Similar benzylidenation of the N-acetyl derivative IX proceeded without lactamization and afforded methyl 3-acetamido-4,6-O-benzylidene-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (XIV). The preparation of the latter compound was undertaken because it provided a link with a second synthesis of muramic acid isomers (p.75) and hence would be a key to the proof of the gluco configuration of its precursors.

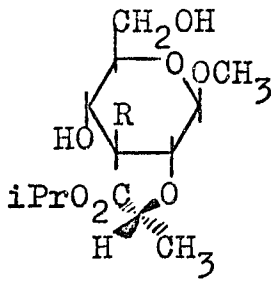
The D-1-Carboxyethyl Series

Debenzylidenation of the D-lactate adduct III gave methyl 3-deoxy-2-O-[D-1-(isopropoxycarbonyl)ethyl]-3-nitro-ß-D-glucopyranoside (XV). The catalytic hydrogenation of XV in 2-propanol was difficult like that of its L epimer IV, and no crystalline product could be isolated in this case. However, the syrup obtained gave only one ninhydrin-positive (R_{GN}[a] value 2.0) spot on paper chromatograms, and, since its R_{GN} value was close to that of the amine VII (R_{GN} 1.9), it was concluded that the D epimer XVI was present. Hydrogenation of XV in 2-propanol - water (1:1) afforded in a 45% yield the crystalline lactam XVII of methyl 3-amino-2-O-(D-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside, which was characterized further by its 4,6-di-O-acetyl derivative. XVIII. The infrared spectrum of XVII showed a strong carbonyl stretching band at 1645 cm^{-1} as well as a series of sharp bands (OH and NH) in the 3600 - 3200 cm^{-1} region. There was no ester carbonyl, nitro, or amide-II absorption. The diacetate XVIII lacked hydroxyl absorptions and gave a weak band at 3230 cm^{-1} (NH) and strong peaks at 1740 (ester carbonyl) and 1665 cm^{-1} (amide carbonyl). In the region from 1400 to 700 cm^{-1} both compounds exhibited the same general patterns as their corresponding epimers VIII and XI, though with distinct differences in band positions

a. R_{GN}= speed relative to glucosamine hydrochloride

and intensities. In the nmr spectrum of XVIII, the methoxyl and the two O-acetyl signals appeared at τ 6.44, 7.89, and 7.94, respectively, and the C-methyl group gave a doublet centered at τ 8.55 with a splitting of 7 cps.

Both the crystalline lactam XVII and the syrup containing the amino ester glycoside XVI were hydrolyzed by hydrochloric acid. Although the expected reducing sugar, 3-amino-2-O-(D-1-carboxyethyl)-3-deoxy-D-glucose (XIX), was not isolated from the hydrolysis solution, its presence was indicated by paper chromatography. The hydrolysates presumed to contain XIX gave two ninhydrin-positive spots (R_{GN} 0.8 and 1.3), the faster spot being much stronger than the slower one. In the case of the L epimer XII (R_{GN} 0.8) the presence of a trace of a faster moving material (R_{GN} 1.25) was detected, too, which became more intense when a sample of XII was heated in vacuo for 8 hr at 80° prior to chromatography. One possible explanation is that these faster moving components were lactones of XII and XIX, respectively.



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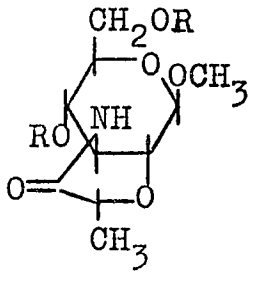

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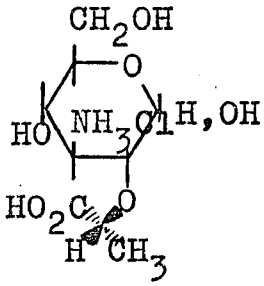


XV = R = NO_2
XVI= R = NH_2

XVII = R = H
XVIII= R = Ac

XIX

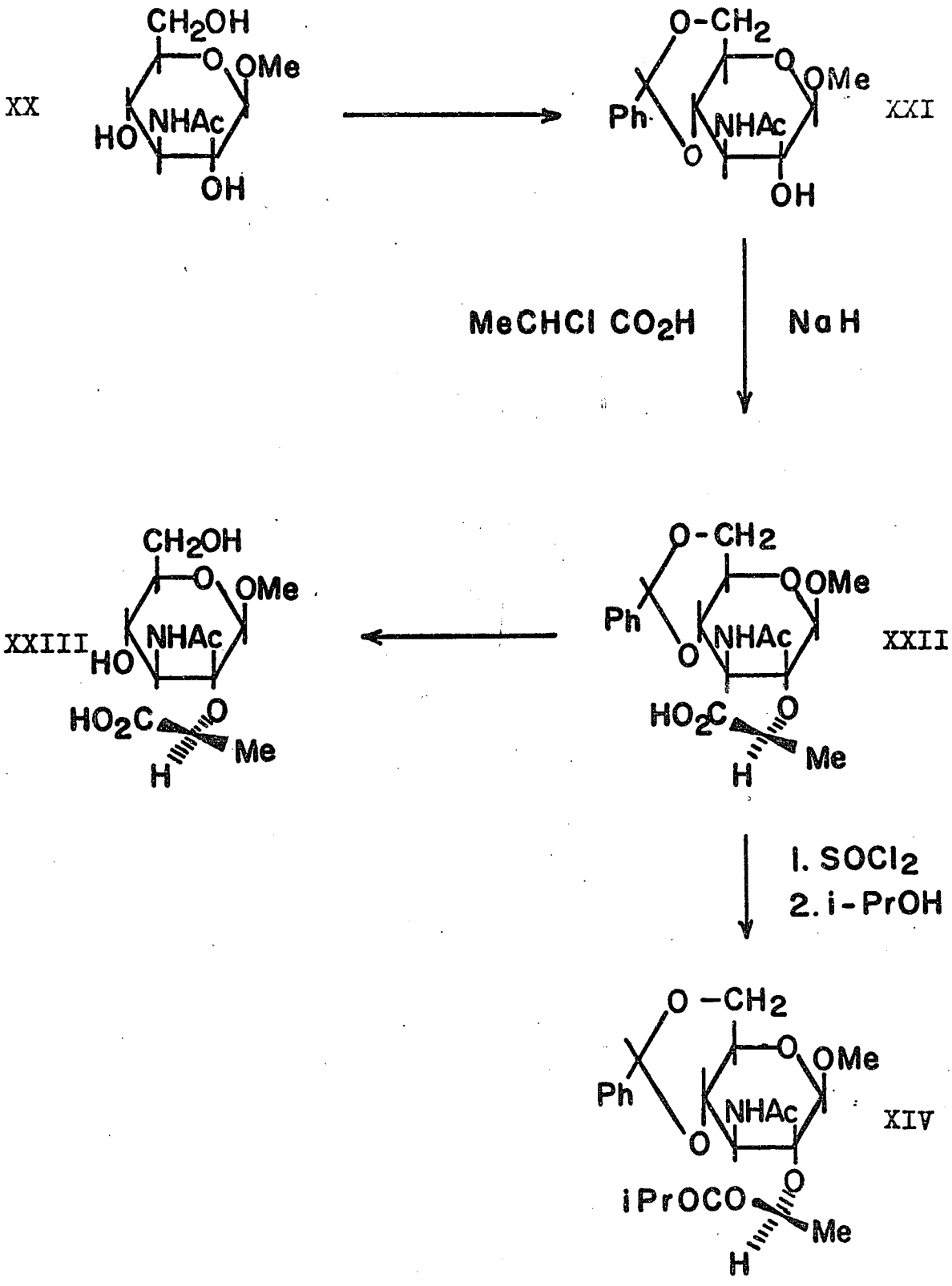
Synthesis of Positional Isomers of Muramic Acid by an Independent Route

As has been pointed out (p.67), it was reasonable to assume on the basis of experiences with similar nucleophilic additions to I (p.18) that the products described in the preceding sections would possess the gluco configuration. Supporting evidence had arisen, at least for an equatorial disposition of the acetamido group in X, from the chemical shift of that group (p.71). Rigorous proof for the gluco configuration of XIV was adduced by an independent synthesis that started from a glucose derivative and did not involve any chemical changes at the asymmetric centers of the sugar chain.

This second entry into the series of 2-O-carboxyethyl derivatives of 3-amino-3-deoxy-D-glucose was patterned after the syntheses (45,47-49) of muramic acid. The starting point was known methyl 3-acetamido-3-deoxy-ß-D-glucopyranoside (XX), which was blocked in position 4 and 6 by benzylidenation. The benzylidene derivative XXI was to be condensed with isopropyl DL-2-chloropropionate in the hope that the 2-O-[L-1-(isopropoxycarbonyl)ethyl] derivative (XIV) or its D epimer (or both) would be obtained. This seemed to

be the most convenient way of ascertaining by direct comparison the gluco configuration of the products from the first synthesis. Unfortunately, no condensation took place under the conditions specified.

However, when DL-2-chloropropionic acid and XXI were refluxed in dioxane in the presence of excess sodium hydride, methyl 3-acetamido-4,6-O-benzylidene-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside (XXII) was obtained in a yield of 30%. The corresponding D-1-carboxyethyl epimer may have been formed also in this reaction but it could not be isolated. The acid XXII was then converted into its isopropyl ester by treatment with thionyl chloride followed by 2-propanol, and the ester so prepared proved to be identical with XIV. Finally debenzylidenation of XXII, which was effected by heating in water, led to methyl 3-acetamido-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside (XXIII).


XX
CH2OH
O
OMe
HO
NHAc
OH
O-CH2
Ph
XXI
MeCHCl CO2H
NaH
XXIII
HO2C
Me
H
XXII
1. SOCl2
2. i-PrOH
XIV
iPrOCO

2. Addition of Aminobenzoic Acids: The Synthesis of 2,3-Diamino-2,3-dideoxy-D-mannose

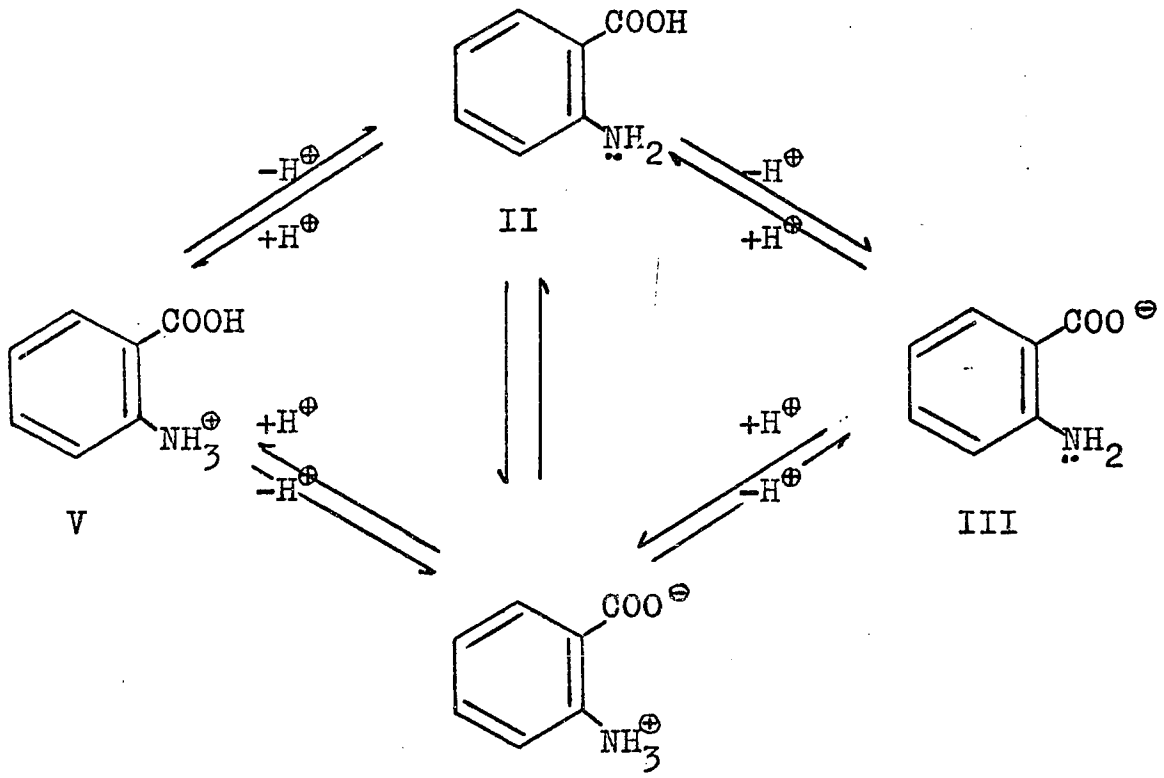
Carbohydrate derivatives of o-aminobenzoic acid have recently been found to occur in nature. Thus, cultures of Aerobacter aerogenes (69) and Escherichia coli (70) synthesize 1-(o-carboxyphenylamino)-1-deoxy-D-ribulose; in cultures of Salmonella typhimurium (71) and Neurospora crassa (72), 1-(o-carboxyphenyl)-D-fructosamine has been detected. These compounds, which are supposed to be intermediates in the biosynthesis of tryptophane (70,72), have also been obtained synthetically (73).

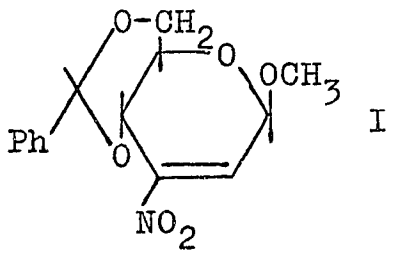
So far no sugars have been found in nature that contain an aminobenzoic acid substituent at a carbon atom other than C-1, but this might well become the case in the future. Synthetic access to such carbohydrate derivatives would then be desirable, and we therefore undertook to study the feasibility of the nucleophilic addition of aminobenzoic acids to the olefin I[a].

---

a. Compounds in this chapter are numbered using a new set of Roman numerals.

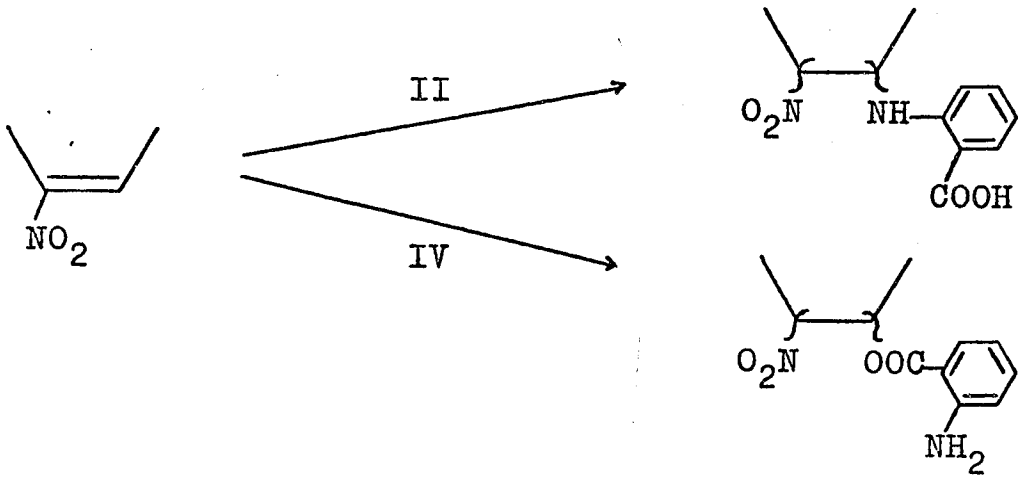
In nucleophilic additions to olefin I of aminobenzoic acids one has to take into account the possibility that either the amino or the carboxyl group could act as the nucleophile, since it is known (74) that these compounds may exist in four forms in an acid-base equilibrium. In the case of anthranilic acid the following equilibrium would be present:





I

Under our reaction conditions the equilibrium II ⇌ IV would be the most important since the reactions are carried out in a non-aqueous medium in the absence of proton sources other than the aminobenzoic acid itself. If II should be the reacting species the resulting compound would be an acid; if IV acted as the nucleophile an ester should be obtained.



As will be shown below, only acids were isolated, i.e. only II reacted as the nucleophile.

When the olefin I was refluxed for 5 hr in dry benzene together with a 2-3 molar excess of o-aminobenzoic acid in the presence of catalytic amounts of solid potassium hydroxide, a colorless crystalline acid (VI) was isolated in 83% yield. This acid could be converted into its methyl ester (VII) by reaction with diazomethane. The same methyl ester was obtained by refluxing the olefin I in benzene in the presence of methyl anthranilate

(Formulas see p. 87).

However, when the olefin was refluxed in benzene with an excess of o-aminobenzoic acid but without the potassium hydroxide catalyst, only 41% of the acid VI could be isolated. In addition a lemon yellow, crystalline compound (VIII) was found in 22% yield. This compound, also an acid, was shown to be an isomer of VI by elemental analysis and molecular weight determination (mass spectrometry). The use of an equivalent amount, rather than an excess, of anthranilic acid increased the yield of the yellow isomer VIII to 56% and no VI was isolated in this case. However, it should be noted that the separation of small amounts of VI and VIII can be difficult due to their tendency to form gels. Reaction of VIII with diazomethane afforded smoothly a methyl ester (IX) which also formed yellow crystals.

A comparison of the physical data (Table III) of the two isomeric acids shows great similarities in the melting points and ultraviolet absorptions but large differences in the optical rotations. The esters differ strongly in their melting points as well as rotations. It may be noted that neither the yellow acid nor its ester exhibit any discernible absorption in the visible spectral region. Even fairly concentrated solutions appear colorless, so that the yellow color seems to be associated only with the crystalline state. This phenomenon will be discussed further on page 101.

TABLE III

Physical Data of VI and VIII and Their Methyl Esters

Compound	mp	$[\alpha]_D^{23}$ in acetone	UV in methanol
Colorless VI	220-221°	-25.5°	λ_{max} 255 mμ (ε, 10100), λ_{max} 340 mμ (ε, 5000)
Yellow VIII	222°	-173.0°	λ_{max} 254 mμ (ε, 11100), λ_{max} 343 mμ (ε, 5300)
Methyl ester of VI	225°	-22.0°	
Methyl ester of VIII	167-168°	-188.0°	

The infrared spectra of the two isomeric acids (Figures 6 and 7) also exhibit considerable differences. The spectrum of the acid VI shows a sharp peak at 3320 cm^{-1} (NH) which goes over into a broad absorption reaching from 3300 - 2500 cm^{-1}. The latter is the usual, characteristic bonded O-H stretching absorption which is diagnostic of the carboxylic acid functional group. Other absorptions in the spectrum of VI which can be assigned to specific functional groups are strong peaks at 1675 (CO), 1555 (NO_2), and 1095 cm^{-1} (C-O-C). Bands of medium intensities at 1590, 1525, 750, and 700 cm^{-1} are indicative of aromatic structure.

The yellow acid VIII, in contrast, does not exhibit the broad peak which would be expected for a carboxylic acid OH. It shows instead a sharp peak at 3470 cm^{-1} and a broader absorption consisting of several bands between 3320 and 3000 cm^{-1}. Interestingly, the carbonyl absorption consists of two bands at 1685 and 1700 cm^{-1}, respectively. In a solution spectrum (THF) only a single carbonyl peak at 1685 cm^{-1} is found. The sharp absorption at 3470 cm^{-1} is also absent in the solution spectrum. Other predominant features in the infrared spectrum of VIII are a strong peak at 1560 cm^{-1} (NO_2), medium absorptions at 1590, 1525, 750, and 700 cm^{-1} (aromatic structure). At 1200 cm^{-1} the acid VIII shows a strong band which is not present in the spectrum of VI.

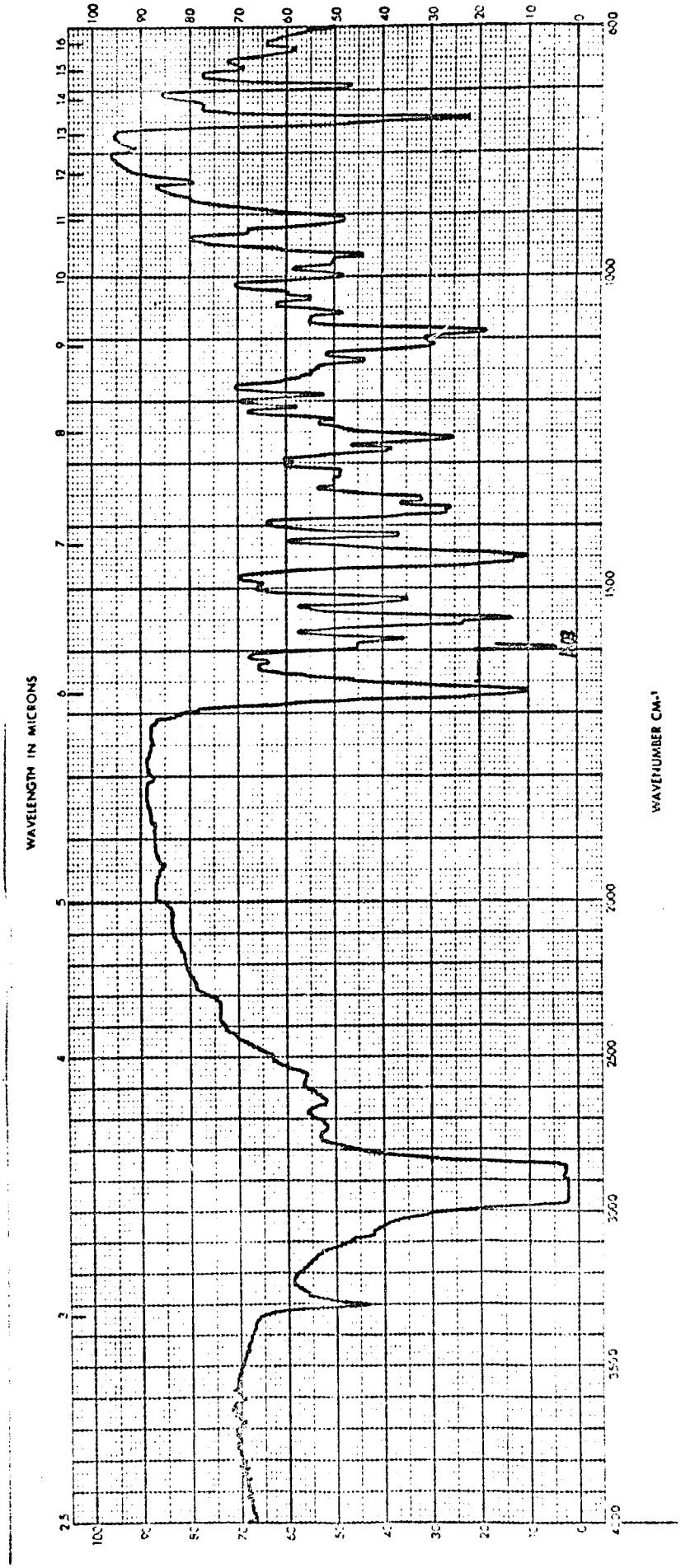


Figure 6. - Infrared spectrum of compound VI (Nujol mull).

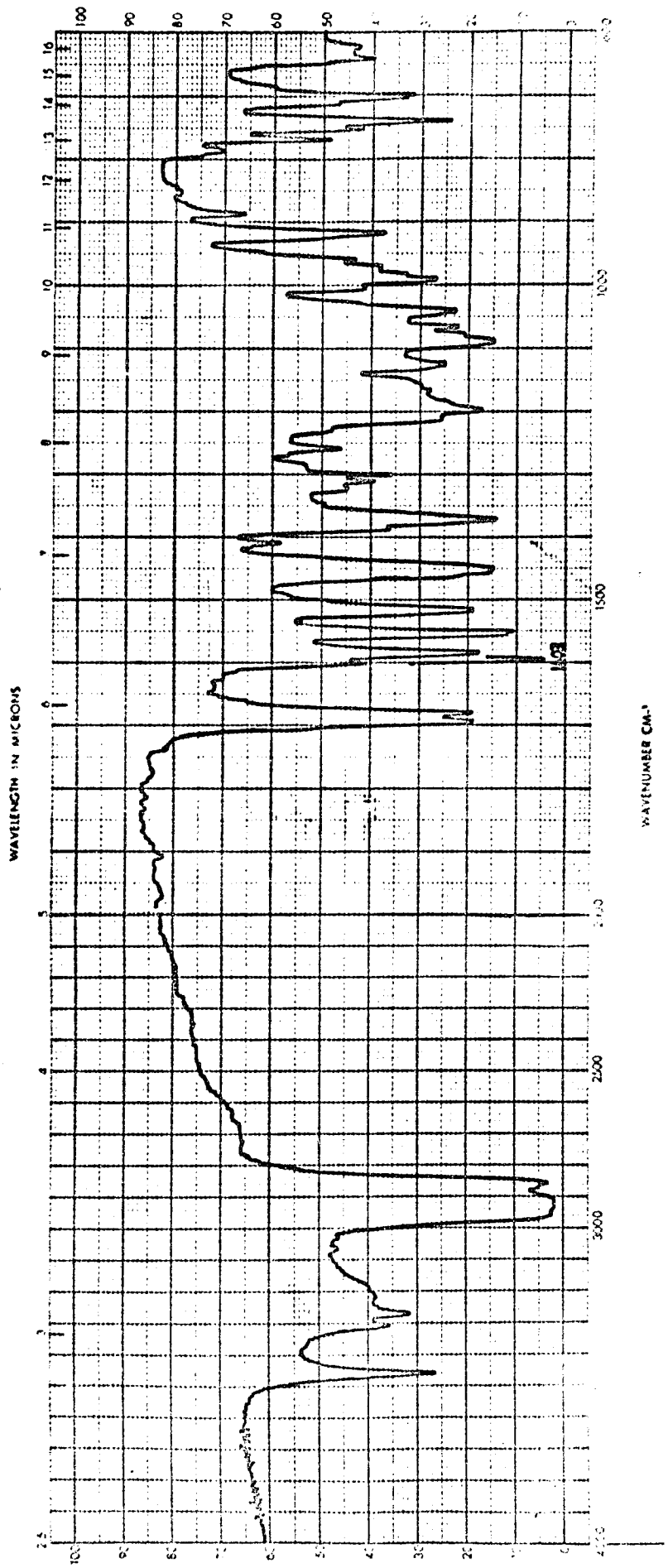


Figure 7. - Infrared spectrum of compound VIII (Nujol mull).

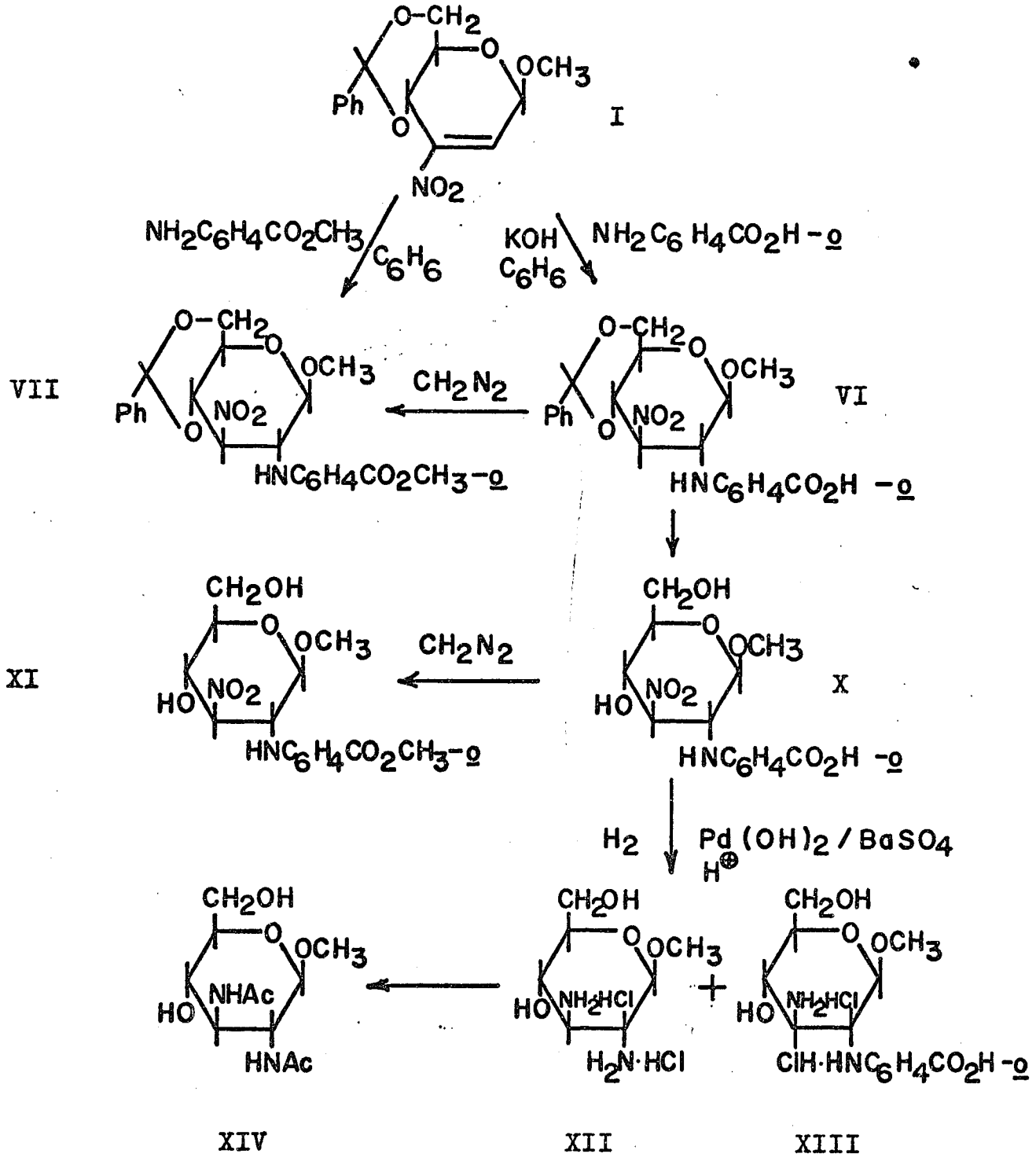
Addition of a nucleophile to the olefinic double bond in I will generate two new asymmetric centres at C-2 and C-3 of the sugar, respectively. It was therefore necessary to provide a proof of configuration for VI and VIII.

The Structure of the Colorless Acid VI

Subsequently, the colorless isomer will be shown to be methyl 4,6-O-benzylidene-2,3-dideoxy-2-(2-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (VI). This was established as follows:

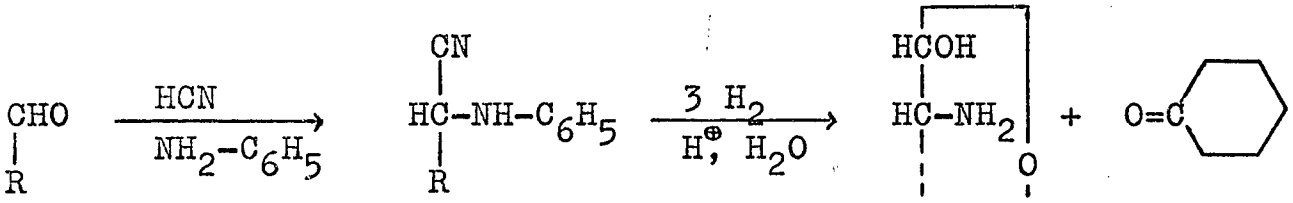
Debenzylidenation of VI in 70% acetic acid furnished methyl 2,3-dideoxy-2-(2-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (X) which was further characterized by conversion into its methyl ester (XI). Hydrogenation of X in dilute hydrochloric acid in the presence of palladium oxyhydrate-barium sulfate catalyst (75) gave two compounds, methyl 2,3-diamino-2,3-dideoxy-ß-D-glucopyranoside (XII) and methyl 3-amino-2-(2-carboxyphenyl) amino— 2,3-dideoxy-ß-D-glucopyranoside (XIII) in yields of 62 and 6%, respectively. Both compounds were obtained in the form of crystalline dihydrochlorides.

N-Acetylation of the diamino sugar XII yielded a diacetamido derivative which was found to be identical


O-CH2
O
OCH3
Ph
O
I
NO2
NH2C6H4CO2CH3
C6H6
KOH
C6H6
NH2C6H4CO2H-o
VII
CH2N2
VI
HNC6H4CO2CH3-o
HNC6H4CO2H-o
CH2OH
XI
X
HO
NO2
H2
Pd(OH)2 / BaSO4
H⊕
NHAc
NH2HCl
H2N·HCl
ClH·HNC6H4CO2H-o
HNAc
XIV
XII
XIII

with known (32) methyl 2,3-diacetamido-2,3-dideoxy-ß-D-glucopyranoside (XIV) with respect to its infrared spectrum, melting point, and optical rotation. This reaction sequence established the gluco configuration of compounds VI, VII, X, XI,XII, and XIII.

The reductive cleavage of N-aryl compounds under normal pressures and temperatures in the presence of $Pd(OH)_2/BaSO_4$ catalyst was discovered accidentally in 1956 by Kuhn and Kirschenlohr (76) who on hydrogenation of N-arylamino nitriles (obtained from aldoses, hydrogen cyanide and an arylamine) isolated amino sugars that had lost the aryl substituent.

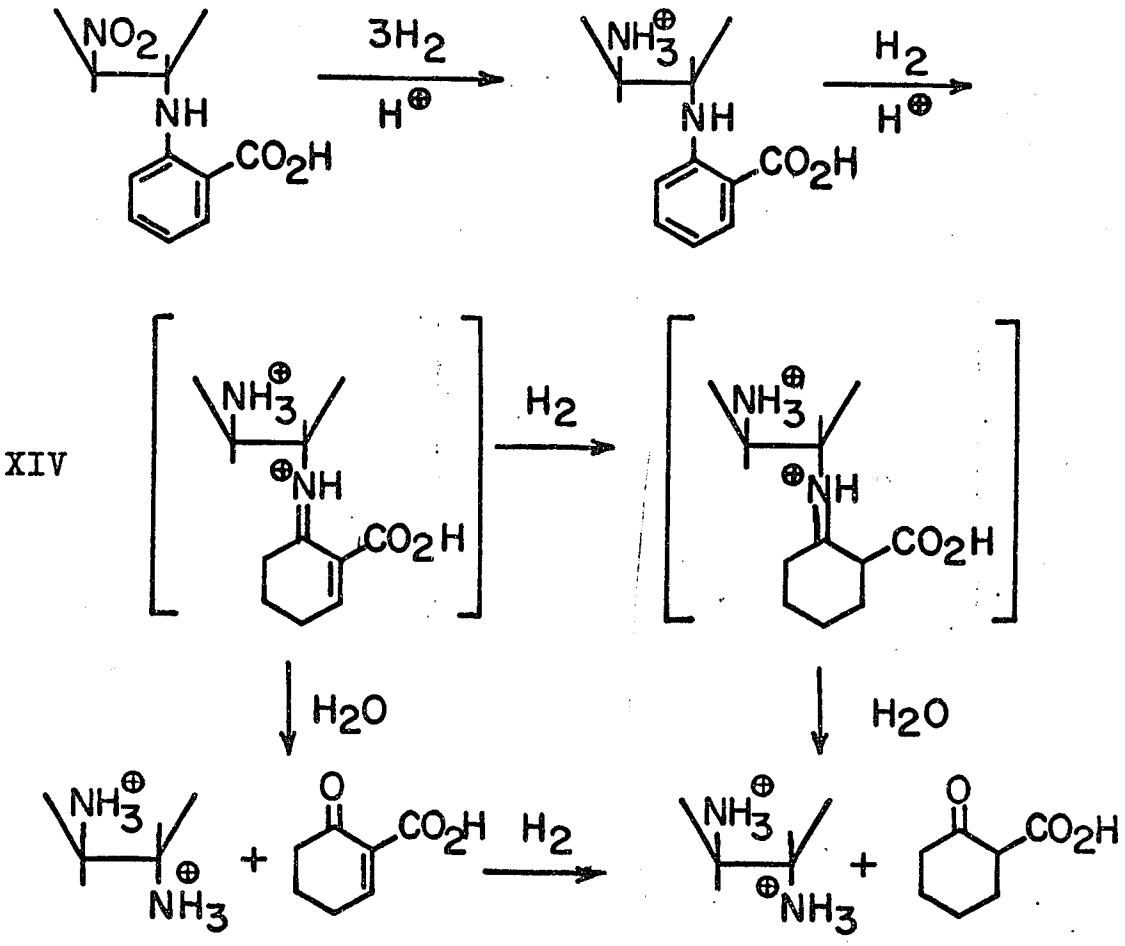


Kuhn and Haas (77) investigated this reaction thoroughly and found that, while the nitrile group was 'hemihydrogenated' to the imine stage to furnish the reducing sugar structure by spontaneous hydrolysis, the aryl moiety was cleaved off after uptake of two moles of hydrogen and was thereby converted into the corresponding hydroaromatic ketone. They proposed for this cleavage a mechanism which involves first the addition of one mole of hydrogen and of one proton to the arylamine group to

give a diene cation, which then can either take up another mole of hydrogen and be cleaved hydrolytically, or decompose immediately to $H_3N^{\oplus}R$ and a cyclohexenone derivative which is then hydrogenated further to give a cyclohexanone.

In the present case the first step of the reaction is the reduction of the nitro group. This is shown by a relatively fast uptake of three moles of hydrogen necessary for the reduction of NO_2 to NH_2, and also by the isolation of XIII which can be thought to be an intermediate in the conversion of X to the diamino compound XII. The next step in the reaction, indicated by a slow uptake of an additional two moles of hydrogen, is then according to Kuhn's mechanism the formation of a diene cation (XIV) which is converted to the endproduct via the two possible routes.

XIV

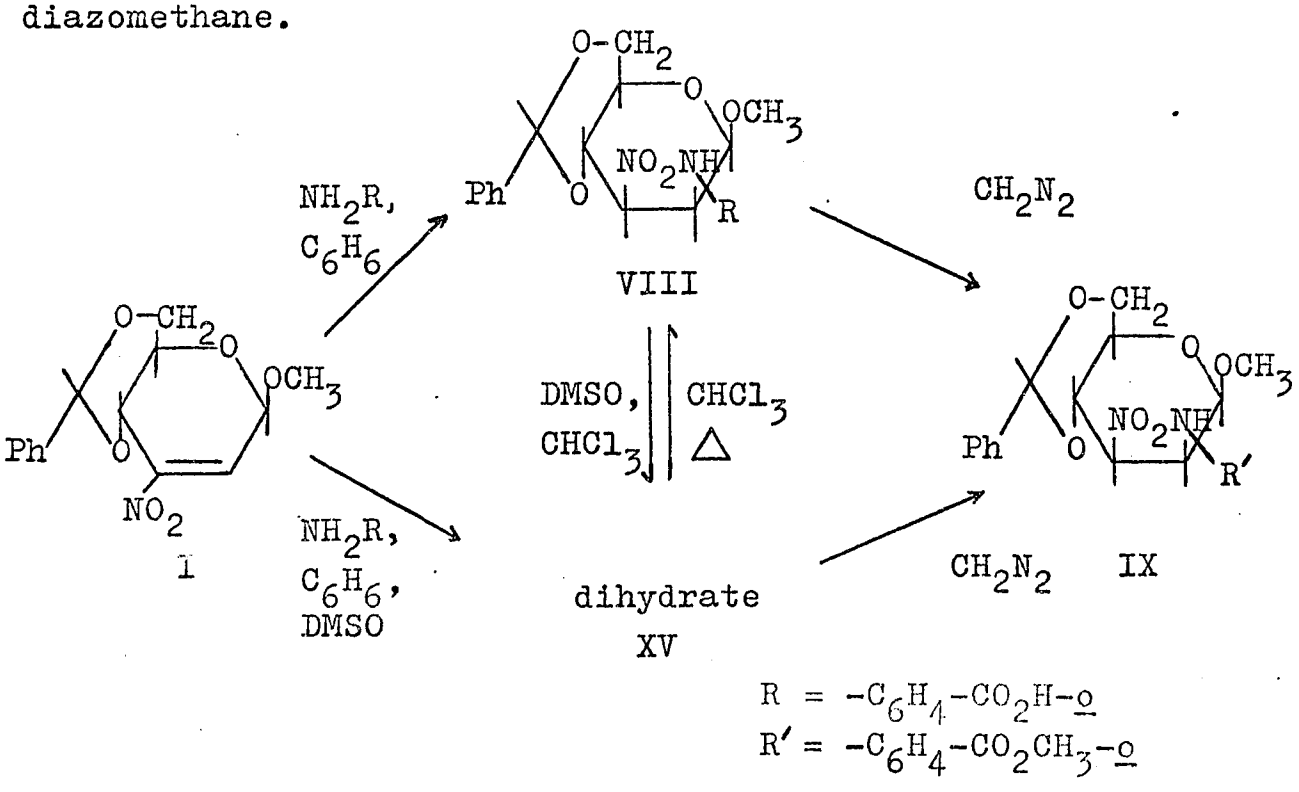


The Structure of the Yellow Acid VIII

The yellow color of compound VIII did not immediately suggest that we were dealing with a simple stereoisomer of VI, i.e., with a compound differing only with respect to the configurations at one or both of the newly generated asymmetric centers. It will be shown, however, that the yellow isomer VIII differs from VI only in the orientation of the anthranilic acid substituent at C-2; the yellow isomer was found to be methyl 4,6-O-benzylidene-2,3-dideoxy-2-(2-carboxyphenyl)amino-3-nitro-ß-D-mannopyranoside (VIII). This finding came as a surprise, there being no readily apparent reasons for the color of VIII and some of its derivatives. A possible explanation will be offered below.

When the yellow acid VIII was dissolved in a mixture of chloroform and dimethyl sulfoxide and petroleum ether was added, a colorless product crystallized. The same product was obtained directly in nucleophilic additions of anthranilic acid to the olefin I in the presence of dimethyl sulfoxide. Elemental analysis suggested this compound to be a dihydrate (XV) of VIII. This view was supported by mass spectrometry where both the yellow acid and its white dihydrate gave rise to the same parent peak (m/e 430) and to a very similar cracking pattern. They also exhibited nearly identical molecular

rotations, M_D 74300 and 73900, respectively (in acetone). Furthermore, the presence of water of crystallization in XV was revealed by an nmr signal at τ7.98 (intensity 4H) in a spectrum taken in deuteriochloroform solution. The signal disappeared upon deuterium exchange. Compound VIII, whose spectrum otherwise was the same, did not give this signal. It appears that the water of crystallization was provided by a small amount of moisture present in the solvents employed. However, the dimethyl sulfoxide seems to play a necessary role, for the dihydrate was readily reconverted into the anhydrous substance by recrystallization from hot chloroform or ethanol even though these solvents were not dried especially. Finally, it was established that VIII and XV give the same (yellow) methyl ester (IX) by reaction with diazomethane.



Debenzylidenation of the methyl ester IX in 70% acetic acid afforded pale yellow methyl 2,3-dideoxy-2-(2-methoxycarbonylphenyl)amino-3-nitro-ß-D-mannopyranoside monohydrate (XVI). The presence of one mole of water of crystallization was established by elemental analysis and by mass spectrometry where at low temperature and low ionization voltage the water peak at m/e 18 was the only major peak in the spectrum.

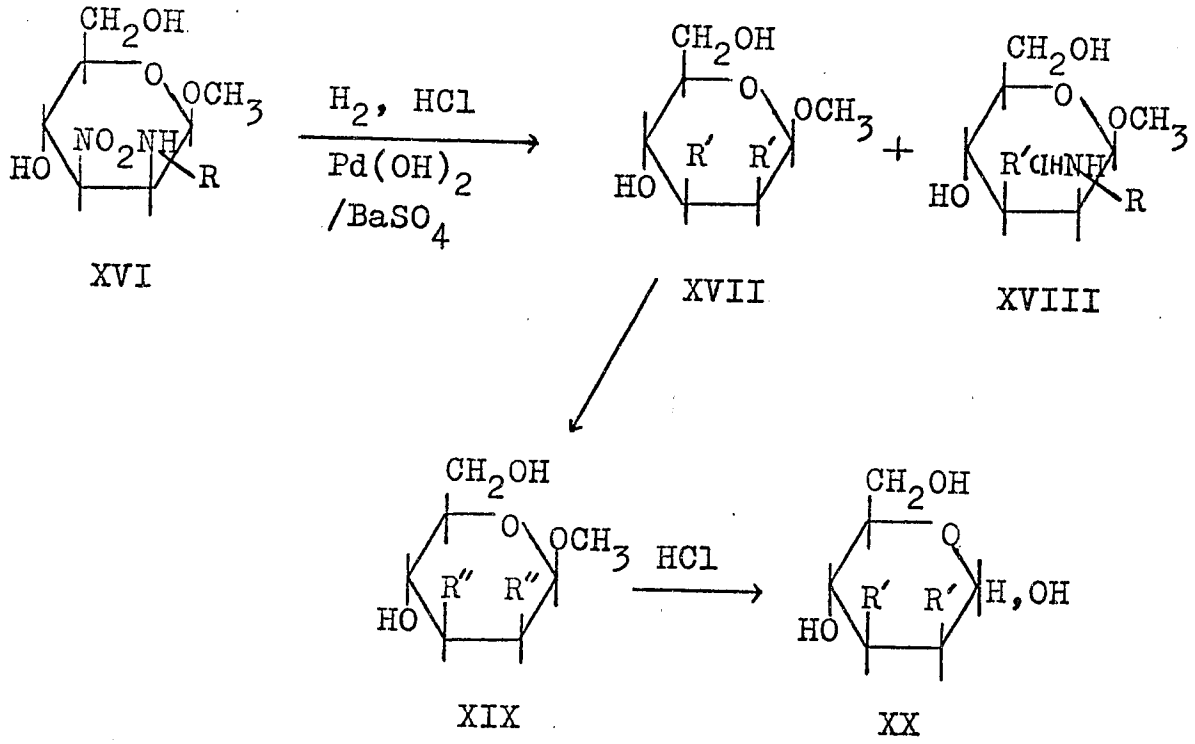
Hydrogenation of XVI in the same manner as described above for X yielded methyl 2,3-diamino-2,3-dideoxy-ß-D-mannopyranoside dihydrochloride (XVII). This diamino sugar was also obtained directly from the acid VIII by debenzylidenation with 70% acetic acid followed by catalytic hydrogenation without isolation of intermediates. The latter method was the preferred one because it was not only shorter but yielded a purer product as well. Methyl 2,3-diamino-2,3-dideoxy-ß-D-mannopyranoside dihydrochloride (XVII) was very similar to its gluco isomer (XII) with respect to optical rotation ($[\alpha]_D^{23}$ -46.5° for the manno, $[\alpha]_D^{23}$ -41° for the gluco isomer) and paper chromatography in which they were practically indistinguishable. They differed, however, in their crystallization behaviour; the gluco compound crystallized readily from absolute ethanol in which the manno isomer is very soluble. Methyl 2,3-diamino-2,3-dideoxy-ß-D-mannopyranoside dihydrochloride (XVII) could only be obtained as a white

powder from 2-propanol or acetone and its elemental analysis suggested that one mole of these solvents was present in the solid. Nmr spectra of XVII in deuterium oxide supported this finding; in one case there was a singlet at approximately τ7.9 (acetone), in another case a doublet centered at τ8.9 (2-propanol).

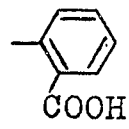
Paper chromatographic investigation of the mother liquor of XVII indicated that besides considerable amounts of XVII another ninhydrin-positive product was present which travelled with about twice the speed of XVII. No attempt was made to isolate this particular compound which was probably methyl 3-amino-2-(2-carboxyphenyl) amino-2,3-dideoxy-ß-D-mannopyranoside (XVIII), an intermediate in the formation of XVII. In the gluco series discussed above, the isomer (XIII) of XVIII had been isolated and shown to behave on a paper chromatogram in a similar way.

N-Acetylation of the diamino mannoside (XVII) furnished methyl 2,3-diacetamido-2,3-dideoxy-ß-D-mannopyranoside (XIX) whose optical rotation was also found to be quite similar to that of its gluco isomer (XIV) ($[\alpha]_D^{23}$ -105° for the gluco, $[\alpha]_D^{23}$ -108° for the manno compound). Its melting point is, however, considerably lower than that of XIV (116° vs. 270°), and its migration speed on a thin layer chromatogram was slower than that of XIV.

Heating of the diacetamido compound XIX in 5 N hydrochloric acid for 2 hr afforded the free sugar, 2,3-diamino-2,3-dideoxy-D-mannose dihydrochloride (XX), as a white solid powder. The free sugar reduced Fehling solution and showed an optical rotation of $[\alpha]_D^{23}$ +1° ⟶ $[\alpha]_D^{23}$ -3° (1 hr).[a]



XVI XVII XVIII XIX XX

R =  , R′ = NH_3Cl, R″ = NHAc

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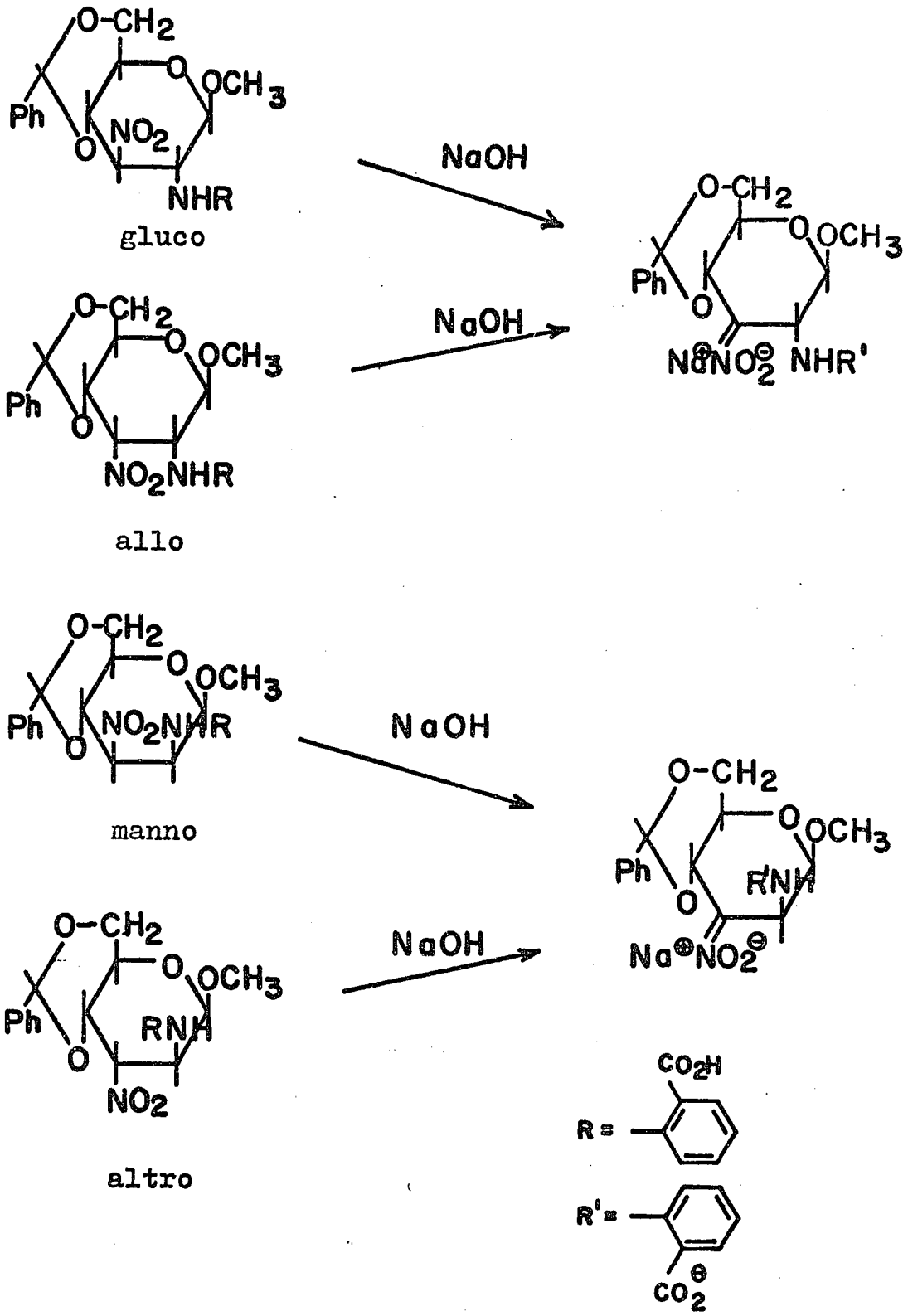
a. The value reported (78) for 2,3-diamino-2,3-dideoxy-D-allose dihydrochloride, the only free 2,3-diamino sugar known besides 2,3-diamino glucose, is $[\alpha]_D^{23}$ +50°.

Since none of the compounds derived from VIII was known, there remained the task of establishing their configuration. This was done on the basis of the following considerations.

In the nucleophilic addition of anthranilic acid to the olefin I, two new asymmetric centres are created, at C-2 and C-3. Consequently, four stereoisomers are possible. In our experiments we isolated two isomers, one of which was shown by chemical means to possess the gluco configuration. This leaves three possibilities, namely the manno, allo, and altro configurations, to be considered for the second isomer.

Inspection of the four diastereoisomeric structures reveals that they can be divided into two pairs, with the members of each pair expected to possess a common aci-nitro anion in alkaline solution. This is indicated by the scheme on the next page.


gluco
allo
manno
altro
NaOH
NaOH
NaOH
NaOH
R =
R' =

Alkaline solutions of the two isomeric acids that were isolated would therefore be expected to exhibit the same value of optical rotation if the second isomer possessed the allo configuration, but different values if it had either the manno or the altro configuration.

As it turned out, solutions of VI and VIII in 0.01 N sodium hydroxide showed a striking difference in their rotational values; $[\alpha]_D^{23}$ -13.4° for compound VI (of proven gluco configuration), $[\alpha]_D^{23}$ -243° for its isomer. By acidification of its alkaline solution, the isomer VIII was recovered unchanged in nearly quantitative yield. It was thereby ascertained that the observed rotation was due to the anion and not to products of some secondary reactions[a].

---

a. Secondary reactions did occur on prolonged standing. The specific rotation changed very slowly at room temperature, from $[\alpha]_D$ -243° to $[\alpha]_D$ -100° in the course of 20 days, and then remained constant. An ultraviolet spectrum at that point showed two strong absorptions, one at λ_{max} 302 mμ, the other at λ_{max} 252 mμ. A strong smell of benzaldehyde was noticed. These observations suggested that probably elimination of benzaldehyde, concomitant with formation of a 4,5-unsaturated nitronate structure, had taken place. A similar process has been reported (27) to occur slowly in alkaline solutions of methyl 4,6-O-benzylidene-3-deoxy-3-nitro-ß-D-glucopyranoside (see also p. 16).

These results eliminated the allo configuration as a possibility for VIII, in accordance with the observation (p.95) that the diamino sugar dihydrochloride XX exhibited a specific rotation quite different from that of the corresponding allo derivative. The compounds of the series were finally recognized to possess the manno configuration by nmr studies of the diacetamido derivative XIX.

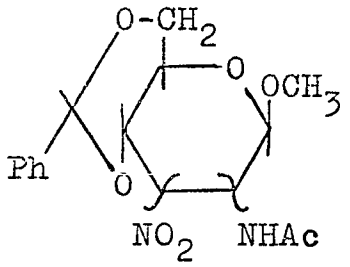
An nmr spectrum of methyl 2,3-diacetamido-2,3-dideoxy-ß-D-glucopyranoside (XIV) in methanolic solution showed two signals at τ8.07 and τ8.09, respectively. These may be attributed to the two equatorial acetamido groupings. The values agree well with those reported (40, 65-68) for equatorial N-acetyl (τ 8.02 - τ 8.11). By contrast, the nmr spectrum of XIX (in methanol) exhibited the signals for the two acetamido groups at τ7.96 and τ8.06, respectively, which is compatible only with the presence of one axial (τ 7.96) and one equatorial (τ 8.06) N-acetyl group in the molecule, values reported (65,66,68) for the former being in the range of τ7.92 - τ 7.96.

This finding ruled out the altro configuration for XIX which would require two axial acetamido signals. Both the manno and the allo configuration possess one axial and one equatorial acetamido group each. Since the allo configuration had already been eliminated by the rotational data discussed above (p.98), compound XIX and

hence its precursor VIII must belong to the manno series,

Baer and Neilson (32), in their work leading to 2,3-diamino-2,3-dideoxy-D-glucose via ammonia addition to the nitro olefin I, have described the isolation of a configurationally undetermined methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-hexopyranoside (XXI) that was formed in small proportion besides the main product, the gluco derivative of that structure.

A supply of XXI was made available to this author, and the material was debenzylidenated, hydrogenated, and N-acetylated. The diacetamido compound thus obtained was identical with XIX as shown by identical rotations, infrared spectra, migration on thin-layer plates, and undepressed mixed melting point. Hence, the manno configuration can be assigned to the by-product of Baer and Neilson.



XXI

1. 70% AcOH
2. H_2/Pt
3. Ac_2O/MeOH

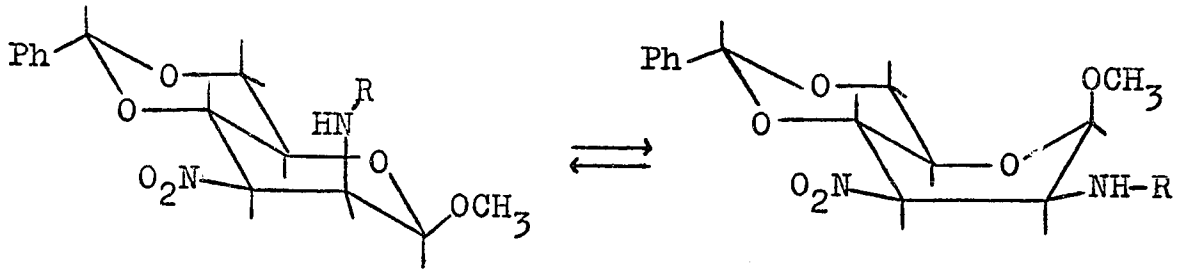
XIX

A point which has not been explained yet is the yellow color of VIII. There is nothing in the manno configuration that would seem to render it essentially different from the gluco configuration with respect to light absorption. The mere fact that the substituent at C-2 is axially oriented could hardly cause any coloration.

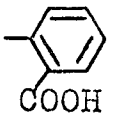
A comparison of the ultraviolet absorption data of VI and VIII (Table III) shows that in solution there exists practically no difference between the two isomers; they both absorb in the near ultraviolet at about the same wavelength with similar intensity, and there is no noticeable absorption in the visible region. This leads to the conclusion that the difference in color must be due only to some differences in the crystalline state, for otherwise one would expect VIII to absorb considerably stronger or at longer wavelength, with at least some discernible trailing into the visible region.

Color originates in unsaturated systems where π-electrons are loosely held and can easily be excited. Therefore we need to postulate some unsaturated structure which could be present in the solid state but would be absent in solution, that is, it should be a 'labile' unsaturation that may be stabilized when the compound is in a fixed conformation.

Comparison of the chair conformation of VIII with its boat conformer reveals that in both cases one can expect steric factors to be important. The bulky axial anthranilic acid substituent would encounter considerable steric interactions if VIII were in the chair form. Conversion into the boat form would remove this particular constraint but would give rise to new interactions since now the methoxy group at C-1, and the hydrogens at C-2 and C-3 would be placed in an axial position.



R =



It can be shown on a molecular model of VIII that the conformation with the least steric interactions is a twisted boat where carbons 1, 2, and 3 of the pyranoside ring lie in the same plane. The oxygen of the ring would be a little above this plane. In such a struc-

ture C-2 and C-3 of the sugar ring, the nitrogen and the oxygen atoms of the nitro group, and the nitrogen and the hydrogen atom of the anthranilic acid NH, are also arranged in a nearly flat six-membered ring so that internal hydrogen bonding is very likely to occur. Furthermore, it is possible that the ortho carboxyl group of the anthranilic acid substituent could participate in the same hydrogen bridged complex. Together with the benzene ring there would then be three six-membered rings, all lying in the same plane. One could write various tautomeric structures, of which two would be of a quinoid type (Figure 8). The best representation of VIII would probably be a resonance structure involving both the nitro and the anthranilic acid substituent.

With compound VI (gluco configuration), on the other hand, it is impossible to obtain, on a model, such a flat three-ring complex since the ring involving the NO_2 and NH groups cannot be brought into one plane. Thus the electronic interplay of the nitro group and anthranilic acid does not develop and the compound remains colorless (Figure 9).

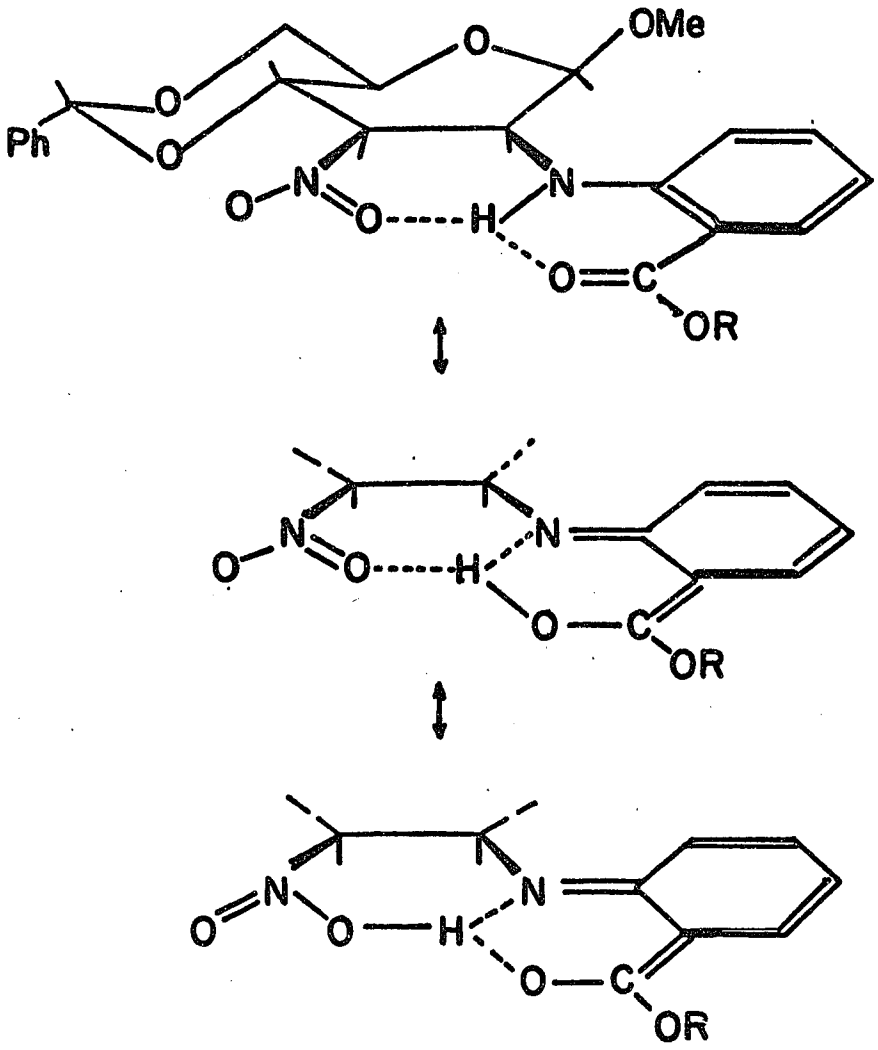
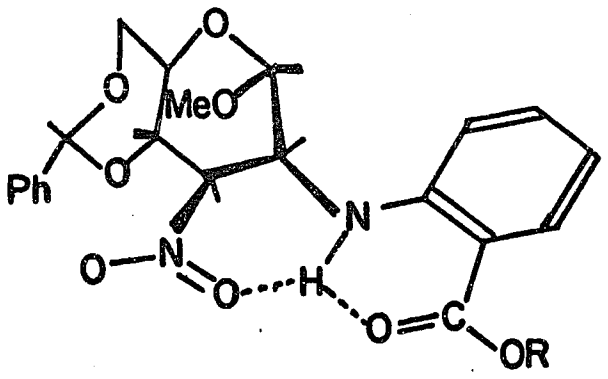


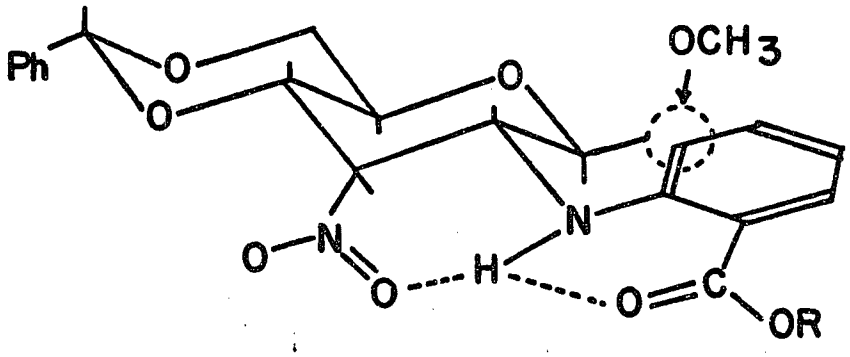
Figure 8

Tautomeric structures of compound VIII.



manno

steric hindrance:



gluco

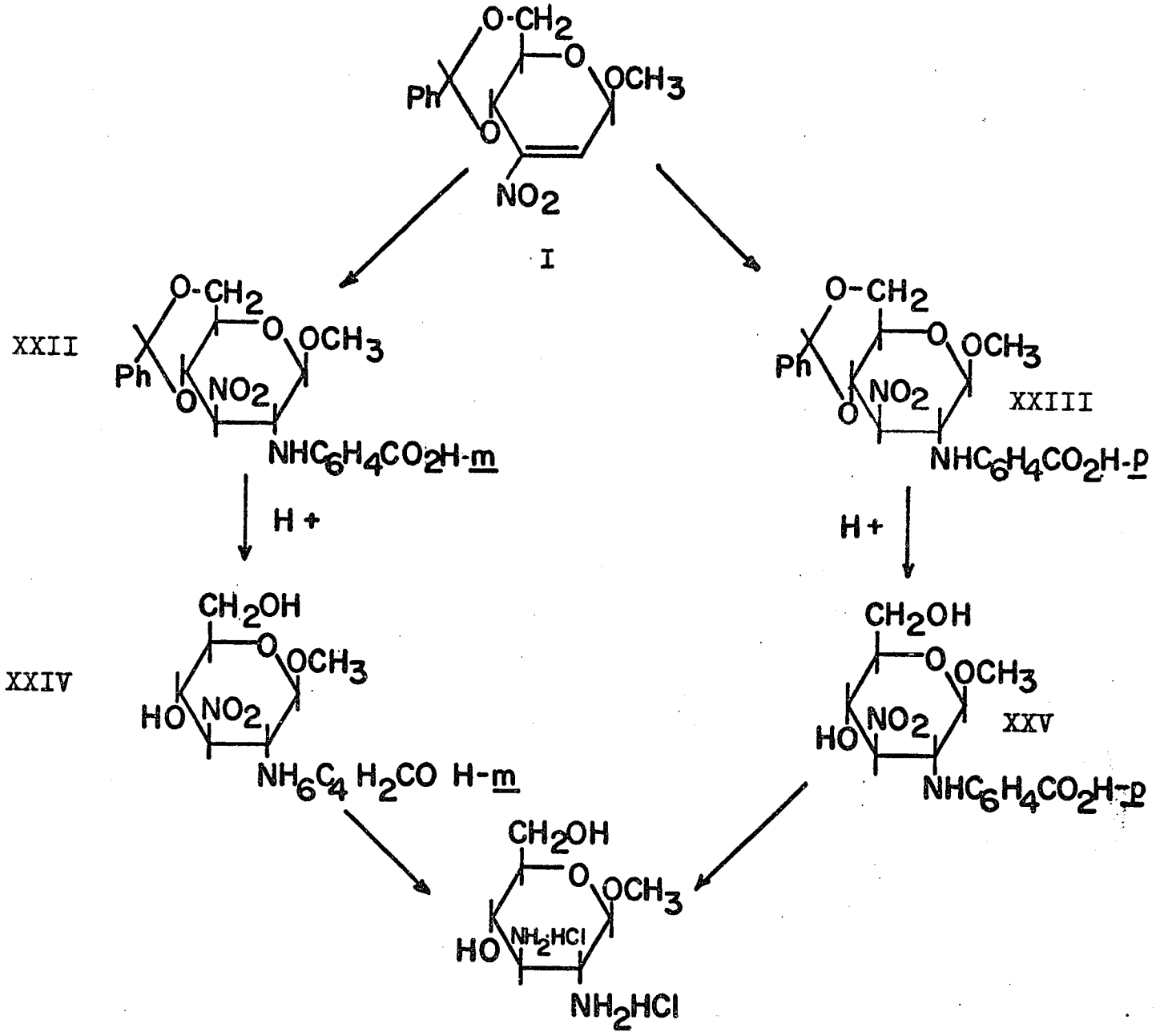
Figure 9

Likely conformation of the manno compound VIII
and
unlikely conformer of the gluco compound VI.

It is emphasized that the foregoing explanation is purely hypothetical. A definite answer, it is felt, can only be given by x-ray crystallography. However, it might be interesting in the meantime to test the hypothesis by devising syntheses of related compounds and predicting the occurrence or absence of similar color phenomena. With this in mind, we proceeded to synthesize analogs containing m- and p-aminobenzoic acid substituents. It was predicted that, even if both gluco and manno derivatives were obtained, none should exhibit color, since the carboxyl group would not be in a position favorable for interactions according to the hypothesis.

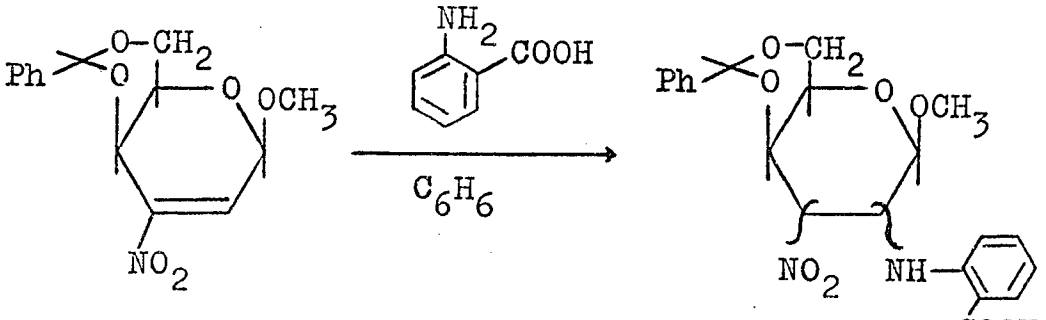
The reaction of olefin I with m- and p-aminobenzoic acid yielded colorless methyl 4,6-O-benzylidene-2,3-dideoxy-2-(3-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (XXII) and its 2-(4-carboxyphenyl)amino isomer (XXIII). In contrast to the addition of o-aminobenzoic acid, no stereoisomers could be isolated, regardless of whether solid potassium hydroxide catalyst was present or not, nor did changes in the ratio of reactants affect the outcome of the reaction. Compounds XXII and XXIII were elucidated in a way similar to that described for the o-amino isomers.

Removal of the benzylidene blocking groups in XXII and XXIII by heating in 70% acetic acid afforded syrupy methyl 2,3-dideoxy-2-(3-carboxyphenyl)amino-3-


O-CH2
Ph
O
OCH3
NO2
I
XXII
NHC6H4CO2H-m
XXIII
NHC6H4CO2H-p
H+
H+
XXIV
CH2OH
HO
NO2
NH6C4 H2CO H-m
XXV
NHC6H4CO2H-p
NH2HCl
XII

nitro-ß-D-glucopyranoside (XXIV) and its crystalline 2-(4-carboxyphenyl)amino isomer (XXV), respectively. Hydrogenation of both XXIV and XXV yielded methyl 2,3-diamino-2,3-dideoxy-ß-D-glucopyranoside dihydrochloride (XII) whose structure and configuration had been established above. Investigation of the mother liquors by paper chromatography showed that in both cases fast moving products were present, presumably incompletely hydrogenated compounds analogous to XIII.

Finally, the addition of o-aminobenzoic acid to the D-threo isomer (XXVI) of the olefin I was studied. Various reaction conditions yielded only one compound (XXVII) whose structure has not yet been determined. Elemental analysis leaves, however, little doubt that XXVII is an isomer of VI and VIII. Its infrared spectrum exhibits a broad absorption reaching from 3600 - 2500 cm^{-1} which is characteristic of the carboxyl OH, strong peaks at 1675 (CO), 1555 (NO_2), 1225 (broad), and 1045 with a shoulder at 1025 cm^{-1}. Bands of medium intensities at 1608, 1580, 1515, 750, and 705 cm^{-1} are indicative of aromatic structure.



XXVI XXVII

In summarizing the findings reported in this section, the following comments may be made. In every single nucleophilic addition to the nitroolefin I that had been previously studied, 2,3-diequatorial substituent orientation in the adduct had occurred predominantly if not exclusively[a]. In fact, there was only one case, namely the addition of ammonia, where a second isomer, now revealed to possess the manno configuration, had been encountered as a minor product. Addends employed included methyl, ethyl and benzyl alcohol, thiobenzyl alcohol, diethylamine, piperidine, ethyl glycine, ethyl malonate, and a number of nitroalkanes; despite the diversity of these compounds with regard to molecular size and nucleophilicity, only gluco adducts were found. The addition of isopropyl lactate elaborated in Part II. 1 of this thesis followed the same course, and it has now been found that m- and p-aminobenzoic acid, and also methyl o-aminobenzoate, fall into the same pattern. Why, then, does o-aminobenzoic acid behave in an anomalous manner under certain reaction conditions? The formation of the

---

a. In some of the previously reported reactions, I had been employed as such (as it has in the present work), in others it had been generated in situ by ß-elimination of acetic acid from methyl 2-O-acetyl-4,6-O-benzylidene-3-deoxy-3-nitro-ß-D-glucopyranoside, but no variations in the stereochemical course were observed (31,32,79).

manno adduct, isolated in 56% yield when a 1:1 molar ratio of reactants is used in the absence of extraneous catalyst, is as remarkable as is the gradual return to 'normal' behaviour when a moderate excess of the amino acid and, eventually, potassium hydroxide catalyst is provided. This peculiarity has no counterpart in the m- and p-amino isomers or elsewhere, and it would be premature at the present time to attempt a rationalization. Despite this lack in understanding, the unexpected reaction is valuable from the viewpoint of synthesis, for it has made accessible a number of hitherto unknown derivatives of 2,3-diamino-2,3-dideoxy-D-mannose and has culminated in the first preparation of the reducing sugar itself[a].

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a. Some α-glycosides of XX have been described (80).

EXPERIMENTAL

General Remarks.

Melting points were determined in capillaries in an electrically heated aluminium block apparatus equipped with a calibrated thermometer.

Paper chromatography was carried out by the descending technique on Whatman No.1 paper in the system, pyridine - ethyl acetate - water - acetic acid (5:5:3:1) according to Fischer and Dörfel (81). The spots were indicated by ninhydrin spray. R_{GN} = speed relative to glucosamine hydrochloride.

All nmr spectra (60 Mc) were obtained on a Varian HA-60 instrument. Ultraviolet and visible absorptions were recorded on a Perkin-Elmer 202 spectrophotometer using 1-cm cells. Unless otherwise specified, infrared data refer to Nujol mull spectra taken on a Beckman IR-8 infrared spectrophotometer.

Rotations were measured using a Perkin-Elmer 141 polarimeter.

All evaporations were done <u>in vacuo</u> at 35 - 40° (bath temperature) unless otherwise indicated. Petroleum ether refers to the fraction boiling at 30 - 60°.

Part I [a]

3,4,4a,8a-Tetrahydro-3,5-dimethoxy-8a,9-dinitro-1,4-ethenopyrano[4,3-c]pyran-1,7(5H)-dimethanol Diacetate (VIII) and Stereoisomer XXX.

All reactions were performed under exclusion of moisture and with carefully dried solvents and reagents.

A solution of 3.0 g of methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-glucopyranoside (I)(51) in benzene (150 ml) was heated under reflux and magnetically stirred with sodium bicarbonate (15 g) for 8 hr. The cooled reaction mixture was filtered, and the solution was evaporated to give a syrup which was dissolved in about 10 ml of ethyl acetate. Addition of petroleum ether to incipient turbidity caused crystallization of small, colorless prisms which were collected after 3 hr and washed with ethyl acetate - petroleum ether (1:3). The product (850 mg, mp 124°) was recrystallized once from the same solvents, which raised its mp to 128°. According to an nmr spectrum, the product was VIII (free from XXX), but it contained an estimated one-half mole of ethyl acetate of crystallization.

---

a. The most characteristic infrared frequencies of new compounds are compiled in Table II (p.60).

Recrystallization from chloroform - petroleum ether furnished solvent-free VII, mp 130 - 131° dec., $[\alpha]^{23}$ 0° (at 365 - 589 mμ ; c 2, chloroform).

Anal. Calcd for $C_{18}H_{22}N_2O_{12}$ (458.4): C, 47.17; H, 4.84; N, 6.11; O, 41.86. Found: C, 47.18; H, 5.29; N, 6.22; O, 41.50; mol. wt., 448 (by osmometry in benzene).

Crystalline VIII gave a single spot on silica gel G thin layer plates irrigated with butanone - heptane (1:1); the mother liquor gave the same spot and, in addition, contained faster and more slowly travelling material, but did not yield any more crystals.

In an alternative procedure, a solution of I (3.8 g) in benzene (150 ml) and anhydrous potassium carbonate (20 g, finely powdered) were vigorously stirred together for 5 hr at 25 - 30°. Filtration and removal of the solvent gave a syrup which partially crystallized from ethyl acetate - petroleum ether. The crude crystals (1.473 g) melted at 118 - 119°, and according to their nmr spectrum they consisted mainly of VIII but contained 10 - 20% of the stereoisomer XXX. One recrystallization from ethyl acetate - petroleum ether raised the mp to 126°, and the infrared spectrum of the product was identical with that of VIII as obtained by the bicarbonate procedure.

The experiment just described was repeated several times, in some cases with minor modifications regarding the reaction time and temperature. Yields were gener-

ally between 45 and 55%, and the crude products melted in the range 116 - 122°. In one case the crude product (mp 116°) was recrystallized twice from chloroform - petroleum ether, which gave a material melting at 110 - 111° and showing $[\alpha]_D^{23}$ -1.5° (c 1, chloroform). According to an nmr spectrum it was a mixture of VIII and XXX in a ratio of about 2:1.

Attempts to isolate pure XXX by fractional crystallization proved very laborious. A fractionation that involved 15 recrystallizations afforded pure VIII (mp 131°) and a 1:2 mixture of VIII and XXX.

The following procedure furnished pure XXX. A solution of I (1.0 g) in tetrahydrofuran (60 ml) was stirred with anhydrous potassium carbonate (10 g) for 53 hr at -70°. The mixture was then allowed to stand for another 2 days at -5° before it was worked up by filtration and evaporation. Inspection of the resulting syrup by thin layer chromatography revealed that all I had been consumed; a faster-moving main spot accompanied by at least three weak spots due to unknown products was seen. Crystallization from ethyl acetate - petroleum ether gave a product (348 mg) melting at 109 - 110°. Two recrystallizations from the same solvents raised the mp to 113°. An nmr spectrum showed that at this point the product was a 2:1 mixture of VIII and XXX. Two further recrystallizations from chloroform - carbon tetrachloride - cyclohexane finally

gave 67 mg of pure XXX as colorless platelets of mp 115° dec., $[\alpha]_D^{23}$ -4.5° (c 0.5, chloroform).

Anal. Calcd for $C_{18}H_{22}N_2O_{12}$ (458.4): C, 47.17; H, 4.84; N, 6.11. Found: C, 47.33; H, 4.91; N, 5.95.

The D-manno and D-galacto isomers of I (XXVIII and XXIX, respectively (51)) were dehydroacetylated on a 100 mg scale by the use of sodium bicarbonate in refluxing benzene (for XXVIII) or potassium carbonate in benzene at 25 - 30° (for XXIX). The recrystallized products, which were obtained in yields of 12 - 14%, proved identical with VIII from I, showing undepressed mixture melting points and identical infrared spectra.

Catalytic hydrogenation of VIII was attempted using palladium on charcoal, palladium oxyhydrate on barium sulfate, rhodium on charcoal, or platinum dioxide, all in ethyl acetate medium at room temperature and atmospheric pressure. Only starting material could be isolated. A hydrogenation with platinum oxide at 54 psi for 20 hr resulted in a mixture consisting of at least four components, according to thin layer chromatography.

1-Hydroxy-7-nitro-1H-2-benzopyran-3,5-dimethanol 3,5-Diacetate (X).

A. Preliminary Experiments. — Small samples (5 - 10 mg) of VIII and a drop of pyridine were dissolved in 2 - 3 ml of benzene or dioxane and heated on a steam bath for 5 min.

The solutions turned yellow and a strong absorption band at λ_{max} 346 mμ was engendered. Evaporation gave a yellow, partly crystalline solid which was difficult to purify. In another experiment, 70 mg of VIII was kept for 3 min in an oil bath at 133°, whereby the substance melted, turned yellow, and evolved fumes of oxides of nitrogen. Crystallization from ethyl acetate and petroleum ether gave then crystals (8 mg) melting at 144 - 147°. Their infrared and ultraviolet spectra were identical with those of X which were prepared as described under B, and also with those of the previously mentioned (51) yellow by-product, mp 147 - 148°. A sample (3 mg) of pure VIII was dissolved in molten acetamide (100 mg) on a steam bath. After 2 min the mixture showed the characteristic ultraviolet peak at 346 mμ (in methanol). An identical experiment with pure XXX gave the same result.

B. Preparation. — Acetamide (6 g) was fused on a steam bath, and crystalline VIII (1.25 g) was introduced into the liquid in which it dissolved rapidly with evolution of nitrous fumes. After 3 min the yellow liquid was cooled to 25° whereby it solidified. The solid was dissolved by boiling in ethyl acetate (50 ml) from which, upon cooling to 0°, acetamide crystallized out. It was removed and washed with cold benzene - petroleum ether (2:1). The combined filtrate and washing solvents were concentrated to about 15 ml and cooled, thereby yielding another crop of

acetamide which was removed and washed as above. The filtrate, upon evaporation to dryness, gave a yellow crystalline solid which was triturated for some time with 5 ml of ice-water. Part of the solid dissolved and the remainder became gummy and stuck to the wall of the vessel. The aqueous extract was discarded, and the residue was triturated a few more times with small amounts of ice-cold water and then dried by evaporation with several successive portions of benzene. Finally, the residue was dissolved in the minimum amount of hot benzene, and the solution, placed overnight in a refrigerator, deposited 390 mg (42.5%) of X as small, yellow prisms of mp 148°, raised to 150 - 151° by one and to 151 - 152° by a second recrystallization. On thin layer plates of silica gel G the product gave a single spot and moved at lower speed than VIII (with butanone - heptane, 1:1). Ultraviolet bands were at λ_{max} 240 mμ (ϵ,9800) and 346 mμ (ϵ, 11000)(in methanol). Fehling solution was reduced on heating. With 0.01 N alcoholic potassium hydroxide a bright red, but gradually fading, color was produced. The color was more intense and more stable when a drop of aqueous N sodium hydroxide was added to the alcoholic solution.

Anal. Calcd for $C_{15}H_{15}NO_8$ (337.3): C, 53.42; H, 4.49; N, 4.15; O, 37.95. Found: C, 53.58; H, 4.70; N, 4.13; O, 38.05.

Performance of the acetamide fusion using mixtures of VIII and XXX gave the same result; yields up to 50% were obtained.

C. Phenylhydrazone XVI — A solution of phenylhydrazine hydrochloride (250 mg) and sodium acetate (400 mg) in water (3 ml) was clarified by filtration with a small amount of activated charcoal and added to a solution of X (100 mg) in ethanol (40 ml). The mixture was gently refluxed with magnetic stirring for 15 min. After cooling, water (30 ml) was added and the mixture was allowed to stand at 4° for 2 hr. The crystalline product was collected, washed with cold, 50% aqueous ethanol, and dried. The yield was 140 mg of XVI showing mp 155 - 156°. Twice recrystallized from aqueous ethanol, the small needles melted sharply at 161°.

Anal. Calcd for $C_{21}H_{21}N_3O_7$ (427.4): C, 59.01; H, 4.96; N, 9.86. Found: C, 59.21; H, 5.05; N, 9.75.

1-Methoxy-7-nitro-1H-2-benzopyran-3,5-dimethanol Diacetate (XIII).

Compound X (65 mg) was dissolved by heating in methanol (25 ml), and N hydrochloric acid (2 ml) was added to the solution after it had cooled to room temperature. The solution was then stored for 8 hr at 0° and subsequently evaporated to dryness, with several additions of fresh methanol. The yellow residue was dissolved in a small amount

of methanol, water was added to incipient turbidity, and the product (XIII), which crystallized at 4° in the course of several days, was washed with water and amounted to 48 mg (70.5%). It melted at 102 - 104° and, after recrystallization from 50% methanol, at 111 - 112°.

Anal. Calcd for $C_{16}H_{17}NO_8$ (351.3): C, 54.70; H, 4.88; N, 3.99. Found: C, 54.49; H, 5.01; N, 4.18.

1-Ethoxy-7-nitro-1H-2-benzopyran-3,5-dimethanol Diacetate (XIV).

Compound X (95 mg) was dissolved in hot ethanol (15 ml), and after cooling, addition of N hydrochloric acid (3 ml) and water (20 ml) the reaction mixture was allowed to stand at 4° for 20 hr. Partial evaporation caused a yellow syrup to separate. The supernatent liquid was discarded and the syrup was washed twice with water. Crystallization from 50% aqueous ethanol gave 60 mg (67%) of XIV; its mp of 115° was raised to 118 - 119° by one recrystallization from the same solvent. Ultraviolet bands were at λ_{max} 240 mμ (ϵ, 1050) and 345 mμ (ϵ, 12500)(in methanol).

Anal. Calcd for $C_{17}H_{19}NO_8$ (365.4): C, 55.89; H, 5.24; N, 3.83. Found: C, 55.29; H, 5.33; N, 4.36.

The infrared spectrum of XIV was identical with that of the previously mentioned (51) yellow by-product, mp 119°.

1-Isopropoxy-7-nitro-1H-2-benzopyran-3,5-dimethanol Diacetate (XV).

Compound X (74 mg) was dissolved with heating in isopropyl alcohol (25 ml). Hydrogen chloride gas was briefly passed into the cooled solution which was then stored at 0° for 7 hr. Evaporation gave a yellow syrup which crystallized from isopropyl alcohol - water, affording XV (44 mg, 53%) as fine yellow needles of mp 103°.

Anal. Calcd for $C_{18}H_{21}NO_8$ (379.4): C, 56.99; H, 5.58; N, 3.69. Found: C, 57.21; H, 5.69; N, 3.76.

7-Nitro-1H-2-benzopyran-1-one-3,5-dimethanol Diacetate (XVII).

Chromium trioxide (250 mg) and pyridine (10 ml) were stirred for 2 hr in a nitrogen atmosphere at room temperature; a solution of X (190 mg) in pyridine (5 ml) was then added and stirring was continued for 6 hr (82). The pyridine was evaporated, the last traces being removed by two evaporations with added toluene. The dark brown residue was extracted with three 30 ml portions of ether, the extract was concentrated, clarified with activated charcoal, and evaporated to a yellow syrup. The syrup was dissolved in hot water containing a small amount of ethanol and, in the course of 24 hr at 4°, furnished 59 mg of XVII as beautiful, pale yellow plates of mp 110 - 111°. The ultraviolet spectrum in methanol showed a peak

of λ_{max} 326 mμ (ε, 13500) with a shoulder at 264 mμ (ε, 7400) and strong end absorption.

Anal. Calcd for $C_{15}H_{13}NO_8$ (335.3): C, 53.78; H, 3.97; N, 4.18. Found: C, 54.01; H, 4.09; N, 4.39.

Nitrobenzene-3,4,5-tricarboxylic Acid (XVIII).

A magnetically stirred suspension of X (400 mg) in a solution of sodium dichromate (1.2 g) in water (10 ml) was slowly heated, and a mixture of conc. sulfuric acid (14 ml) and water (7 ml) was gradually added. After the addition the reaction mixture was gently boiled under reflux with continued stirring for 30 min. The cooled, dark green solution was diluted with water (50 ml) and extracted thrice with 50 ml portions of ether. The combined ether extracts were washed twice with water, dried over anhydrous sodium sulfate and evaporated to give a syrup which was dissolved again in a small amount of ether. Upon addition of benzene and petroleum ether crystallization of XVIII (96 mg, 29%) occurred at 4° in the course of 48 hr. The crude product (mp 196 - 198°) was recrystallized from ether - benzene to give 54 mg of oblique prisms, mp 208 - 210° [(58) mp above 190°], which were easily soluble in water but nearly insoluble in benzene or chloroform. The ultraviolet spectrum in water showed strong end absorption with a shoulder at 274 mμ (ε, 8000).

Anal. Calcd for $C_9H_5NO_8 \cdot H_2O$ (273.2): C, 39.72; H, 2.59; N, 5.16. Found: C, 40.04; H, 2.75; N, 5.45.

A 5 mg sample of XVIII was sublimed once in a high vacuum at 210° (bath temperature) to give crystals of mp 178° [reported (58) for the internal anhydride of XVIII, mp 182 - 183° after two sublimations].

Trimethyl Nitrobenzene-3,4,5-carboxylate (XIX).

An ether solution of XVIII (20 mg) was treated with ethereal diazomethane until a yellow color persisted. After 15 min the solution was evaporated leaving a white solid residue which was dissolved in deuteriochloroform for nmr spectroscopy and thereafter recovered by evaporation. Crystallization from ethanol - water gave fine, pale yellow needles (9 mg) of mp 144° [reported (58) for XIX mp 143 - 144° and after sublimation, 144 - 145°].

Anal. Calcd for $C_{12}H_{11}NO_8$ (297.2): C, 48.50; H, 3.73; OCH_3, 31.32. Found: C, 48.63; H, 3.90; OCH_3 31.08; mol. wt., 297 (by mass spectrometry).

3,5-Bishydroxymethyl-4-(2,3-dihydroxypropyl)-nitrobenzene (XX).

To a solution of X (250 mg) in methanol (50 ml) was added sodium borohydride (500 mg) in water (5 ml), at room temperature. Within five minutes, the 346 mμ absorption band of X disappeared, and a new peak arose at

282 mμ. After 15 min the solution was deionized with 20 ml of Rexyn 101(H^+) and evaporated. The residue was evaporated 8 times with methanol for removal of boric acid. The resulting syrup crystallized upon trituration with ethyl acetate, and recrystallization from that solvent gave 128 mg (67%) of pointed prisms, mp 135 - 136°, λ_{max} 282 mμ (ε, 10500, in methanol).

Anal. Calcd for $C_{11}H_{15}NO_6$ (257.3): C, 51.35; H, 5.88; N, 5.46; O, 37.31. Found: C, 51.02; H, 6.07; N, 5.48; O, 37.41.

3,4-Dihydro-3-hydroxy-7-nitro-1H-2-benzopyran-5-methanol (XXI).

The tetraol XX (100 mg) was dissolved in a solution of sodium metaperiodate (83 mg, 1 molar equiv.) in water (10 ml). The reaction mixture was allowed to stand in the dark at room temperature for 18 hr and was then evaporated to dryness with several additions of absolute ethanol. The organic product was extracted from the inorganic salt by trituration with cold absolute ethanol. The extract was clarified with a small amount of charcoal and evaporated to give a residue which was taken up in a small amount of ethyl acetate. The ethyl acetate solution was separated from some brownish, insoluble material and yielded, upon evaporation, a pale yellow solid (XXI, 78 mg, 89%) that melted at 146 - 147°.

Recrystallized fromacetone - chloroform - petroleum ether, it had mp 149 - 149.5°; λ_{max} 283 mμ (ε, 6100, in ethyl acetate). It reduced hot Fehling solution.

Anal. Calcd for $C_{10}H_{11}NO_5$ (225.2): C, 53.33; H, 4.92; N, 6.22. Found: C, 53.21; H, 4.93; N, 6.38.

2,6-Bishydroxymethyl-4-nitrophenylacetaldehyde Semicarbazone (XXII).

A mixture of compound XXI (30 mg) dissolved in ethanol (1 ml) and of semicarbazide hydrochloride (100 mg) and sodium acetate (150 mg) dissolved in water (1 ml) was heated for 10 min on a steam bath. No crystallization took place on storing the reaction mixture at 4° for a prolonged period of time, but upon allowing it to evaporate slowly in the open air to about half its volume, nearly colorless prisms of XXII were deposited. They were washed with ice-cold water; yield, 19 mg, mp 186° dec.

Anal. Calcd for $C_{11}H_{14}N_4O_5$ (282.3): C, 46.80; H, 5.00; N, 19.86. Found: C, 46.63; H, 5.15; N, 19.86.

4-(2-Hydroxyethyl)-3,5-bishydroxymethyl-nitrobenzene (XXIII).

A mixture of compound XXI (40 mg) in methanol (5 ml) and sodium borohydride (50 mg) in water (2 ml) was allowed to react at room temperature for 2 hr. Deionization with 5 ml of Rexyn 101(H^+) followed by eight co-evaporations

with methanol furnished a crystalline residue which was dissolved in the minimum amount of hot water. The triol XXIII separated as long needles of mp 135° in the course of 3 days at 4°. The yield was 28 mg (69%).

Anal. Calcd for $C_{10}H_{13}NO_5$ (227.2): C, 52.86; H, 5.77; N, 6.17. Found: C, 52.82; H, 5.91; N, 6.38.

Methyl 2,3,4-trideoxy-2,4-diacetamido-3-nitro-hexopyranoside (XXXIII).

Methyl 2,4,6-tri-O-acetyl-3-deoxy-3-nitro-ß-D-glucopyranoside (I) (1 g) was dissolved in liquid ammonia (100 ml) at -70°. After the ammonia had been slowly removed at this low temperature by application of vacuum, which took approximately 4 hr, a yellow solid residue remained. Inspection of a small sample of this solid by ultraviolet spectroscopy revealed that it absorbed strongly at λ_{max} 251 mμ and 306 mμ. When the solid was dissolved in methanol it turned dark brown. Subsequent evaporation, which was difficult due to foaming caused by evolution of a gas, left a syrup which was taken up in absolute ethanol and left at 4° for 3 days. A dark brown solid was deposited during that time. The supernatant dark liquid was decanted and the solid dissolved in warm ethanol. Treatment with activated charcoal did not remove the coloration. The solution was evaporated again and the remaining syrup deposited, after two days of standing in the cold,

some solid material. Trituration of the mixture with 90% ethanol, and another three days of standing at 4°, afforded 42 mg (4.5%) of colorless XXXIII of mp 209 - 210°. Recrystallization from water to which a few drops of ethanol had been added yielded 23 mg of fine needles of XXXIII, mp 218° dec.

Anal. Calcd for $C_{11}H_{19}N_3O_7$ (305.3): C, 43.39; H, 6.27; N, 13.76. Found: C, 43.21; H, 6.34; N, 13.84.

An ultraviolet spectrum of a solution of XXXIII in 0.01 N sodium hydroxide shows a strong absorption at λ_{max} 255 mμ. Heating of this solution for 10 min at 98° did not affect the appearance of the spectrum to a great extent; only a small absorption developed at 300 mμ. The strong peak at 255 mμ seemed unchanged.

It was impossible to isolate more of XXXIII or other products from the mother liquors. Shortening or extending the reaction time did not increase the yield of XXXIII significantly.

Methyl 2,3,4-triacetamido-6-O-acetyl-2,3,4-trideoxy-hexopyranoside (XXXIV).

The diacetamidonitro compound XXXIII (24 mg) and pre-hydrogenated platinum oxide (25 mg) in water (30 ml) were shaken for 16 hr under hydrogen. Evaporation of the filtered solution left a solid residue which was taken up in pyridine (5 ml) and acetic anhydride (2 ml). After 3 hr

of standing at room temperature the mixture, which began to deposit crystals, was evaporated twice with toluene, once with water, and once with ethanol. The remaining solid was suspended in ethyl acetate and filtered off. The filter residue (25 mg) was recrystallized from 2-propanol to give 19 mg of XXXIV as small prisms that slowly turned dark on heating but had no melting point. The compound was easily soluble in water and insoluble in chloroform. It showed $[\alpha]_D^{23}$ +21.6° (c 0.4 in water). A solution in 0.01 N sodium hydroxide exhibited no absorption in the near ultraviolet.

Anal. Calcd for $C_{15}H_{25}N_3O_7$ (359.4): C, 50.13; H, 7.01; N, 11.69. Found: C, 50.01; H, 6.80; N, 11.61.

Part II. 1

Methyl 4,6-O-Benzylidene-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-ß-D-glucopyranoside (II) and Methyl 4,6-O-Benzylidene-3-deoxy-2-O-[D-1-(isopropoxycarbonyl)ethyl]-3-nitro-ß-D-glucopyranoside (III).

A. Addition of Isopropyl DL-Lactate to Nitro Olefin I.

To a solution of methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-erythro-hex-2-enopyranoside (I)(18) (1 g) in dry benzene (30 ml) was added isopropyl DL-lactate (83) (1 ml) and a small chip (about 5 mg) of potassium hydroxide. The mixture was gently boiled under reflux for 20 min, after which time thin layer chromatography (solvent, ethyl acetate - petroleum ether (3:1)) indicated a complete conversion of I into a faster-moving product. The yellowish solution was cooled, stirred briefly at 0° with 5 g of dry cation exchange resin, Rexyn 101(H^+), and was then treated with activated charcoal and evaporated to give a slightly yellowish syrup. Crystallization from ethyl acetate - petroleum ether in the course of several hours at 0° gave needles (550 mg) of the L-adduct II, mp 124°. Upon recrystallization from aqueous ethanol it showed mp 126° and $[\alpha]_D^{23}$ -69.7° (c 0.85 in ethanol).

Anal. Calcd for $C_{20}H_{27}NO_9$ (425.4): C, 56.40; H, 6.35; N, 3.29. Found: C, 56.68; H, 6.43; N, 3.30.

The mother liquor from the first crop of II was partially evaporated whereby a crystalline mixture (410 mg, mp 85 - 89°) of II and III was obtained. Recrystallization from aqueous ethanol yielded 200 mg of II (mp 124°), thus raising the total yield of II to 52%. From the mother liquor of this recrystallization a material melting at 80 - 81° was isolated which was recrystallized three more times from aqueous ethanol to show mp 96 - 97°. It was the D-adduct III, $[\alpha]_D^{23}$ -40.0° (c 1 in ethanol). The yield of pure III was 53 mg (3.6%). In a similar experiment, 1.50 g of I gave 1.223 g (56%) of II and 0.120 g (5.5%) of III.

Anal. Calcd for $C_{20}H_{27}NO_9$ (425.4): C, 56.40; H, 6.35; N, 3.29. Found: C, 56.52; H, 6.23; N, 3.50.

A mixture melting point of II and III was strongly depressed, and the infrared spectra showed small but distinct differences in the region of 1100 - 800 cm^{-1}. Both epimers had their ester carbonyl bands at 1750 cm^{-1}; the asymmetrical nitro vibration was at 1560 in II and at 1565 cm^{-1} in III.

B. Addition of Isopropyl L-Lactate to Nitro Olefin I.

Isopropyl L-lactate was prepared (83) from L(+)-lactic acid (84) and had $[\alpha]_D^{23}$ -9.1° (c 2.5 in methanol). Its addition to nitro olefin I was performed as in A,

610 mg of I and 0.6 ml of ester being used. There was obtained 730 mg (82.5%) of crude II with mp 116 - 118° which was raised by recrystallization to 119° and was not depressed upon admixture of II from A, $[\alpha]_D^{23}$ -63.5° (c 1 in ethanol). The identity of the samples was supported by the infrared spectra and by the debenzylidenations (see below) that led to identical products.

C. Addition of Isopropyl D-Lactate to Nitro Olefin I.

For the preparation of isopropyl D-lactate, 5 g of the calcium salt (tetrahydrate) of D(-)-lactic acid (85) and 10 g of Rexyn 101(H^+) were suspended in dry benzene (60 ml) and 2-propanol (15 ml), and the mixture was refluxed with magnetic stirring in an apparatus equipped with an automatic water separator. Refluxing was continued until no more water was collected in the separator. The filtered solution was then evaporated, and the ester distilled at 71 - 76° (22 - 24 mm), yield 2.5 g, $[\alpha]_D^{23}$ +9.2° (c 2.5 in methanol).

Using the procedure of A, nitro olefin I (1.0 g) and isopropyl D-lactate (1 ml) were allowed to interact and gave 1.2 g (83%) of III, mp 91 - 92°. Admixture of III from A caused no depression, and the infrared spectra were identical.

D. Reaction of Isopropyl L-Lactate with Methyl 2-O-Acetyl-4,6-O-benzylidene-3-deoxy-3-nitro-ß-D-glucopyranoside.

The acetyl derivative (350 mg) was added in

small portions to isopropyl L-lactate (2 ml) containing sodium isopropoxide (250 mg) and 2-propanol (0.2 ml). Omission of the 2-propanol resulted in the recovery of unchanged starting material and failure to produce II. The crystals dissolved on swirling, gentle warming being required towards the end of the operation. The solution was kept at room temperature for 30 min and then mixed under ice cooling with ethanol (5 ml) containing acetic acid (2 ml). Dropwise addition of water gave a crystalline precipitate of crude II which was washed with ethanol - water; 380 mg, mp ca. 110°. Pure II (needles of mp 120 - 121°) was obtained only after three recrystallizations which reduced the yield to 105 mg and so offset partially the advantages of this simpler procedure over method A.

Methyl 3-Deoxy-2-O-[L-1-(isopropoxycarbonyl) ethyl]-3-nitro-ß-D-glucopyranoside (IV).

The benzylidene compound II (400 mg) was hydrolyzed by heating on a steam bath for 25 min, with 15 ml of 75% acetic acid. Complete debenzylidenation was revealed by thin layer chromatography (solvent, ethyl acetate - petroleum ether (3:1)) which showed the disappearance of fast-moving II and the sole presence of a slow-moving product. The solvent was removed, and water was added twice to the residue and evaporated. The residue

was then recrystallized from hot water. The product IV (216 mg, 67%) had mp 148 - 149°, raised to 151° by another recrystallization, $[\alpha]_D^{23}$ -27° (c 1 in ethanol). Infrared bands for the hydroxyl, ester carbonyl, and nitro groups were at 3480—3260, 1750, and 1555 cm^{-1}, respectively.

Anal. Calcd for $C_{13}H_{23}NO_9$ (337.3): C, 46.35; H, 6.83. Found: C, 46.36; H, 6.92.

Methyl 3-Deoxy-2-O-[D-1-(isopropoxycarbonyl) ethyl] -3-nitro-ß-D-glucopyranoside (XV).

Compound XV was obtained from 100 mg of the benzylidene derivative III by the procedure described in this thesis for IV. However, the crude product was syrupy at first and crystallized, as long needles, from an ethanol solution by slow evaporation in the air. Upon recrystallization from water it melted at 102 - 103°; yield, 46 mg (58%). Infrared bands for the hydroxyl, ester carbonyl, and nitro groups were at 3450 — 3250, 1753, and 1563 cm^{-1}, respectively.

Anal. Calcd for $C_{13}H_{23}NO_9$ (337.3): C, 46.35; H, 6.83. Found: C, 46.51; H, 7.06.

Hydrogenation of IV. - A. In 2-Propanol.

The nitro glycoside IV (400 mg) and platinum dioxide (100 mg) in 2-propanol (50 ml) were shaken for

4 days under hydrogen at 70 psi. Evaporation of the filtered solution gave a syrup that was dissolved in a small amount of ethyl acetate and kept at 4° for crystallization. After 3 days 105 mg (28.8%) of prisms of methyl 3-amino-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (VII) had been deposited, mp 183°, unchanged upon recrystallization from 2-propanol - ethyl acetate, $[\alpha]_D^{23}$ -43.5° (c 1 in water), R_{GN} 1.9.

The compound showed strong infrared absorption at 3450 (OH, NH) and 1745 (ester carbonyl), and a medium peak at 1585 cm^{-1} (NH bending).

Anal. Calcd for $C_{13}H_{25}NO_7$ (307.3): C, 50.80; H, 8.21; N, 4.56. Found: C, 50.65; H, 8.13; N, 4.51.

The mother liquor from VII was concentrated a little, and petroleum ether was added to incipient turbidity. After several hours at 0° a by-product (44 mg) was collected, mp 134 - 137°. Recrystallization from the same solvents gave 32 mg of crystals melting at 150 - 151° (air dry), or at 165 - 166° with slight prior sintering (dried in vacuo). A mixture melting point with IV was depressed, and a ninhydrin test was negative. The analytical data (Found: C, 47.36; H, 7.13; N, 5.18) of this by-product lay between those expected for methyl 3-amino-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside (Calcd for $C_{10}H_{19}NO_7$: C, 45.28; H, 7.22; N, 5.28.) and for its lactam (VIII) (Calcd for $C_{10}H_{17}NO_6$: C, 48.65; H, 6.94;

H, 5.67.). The infrared spectrum showed the same general features as that of VIII, but in addition there was a strong peak at 1710 cm^{-1} and weak absorption in the region of 2700 - 2400 cm^{-1}, which could be attributed to a free carboxyl group. A sample of the by-product (36 mg) was treated with acetic anhydride (0.25 ml) and pyridine (0.5 ml) for 7 hr at 23°. After two evaporations with excess toluene and two more with ethanol a white solid remained. It gave 17 mg of thin prisms, mp 193°, on recrystallization from absolute ethanol and petroleum ether. The infrared spectrum and analytical values (Found: C, 51.10; H, 6.70.) were similar to those of the lactam diacetate(XI).

- B. In 2-Propanol - Water.

The nitro glycoside IV (600 mg) and platinum dioxide (200 mg) in a mixture of water (25 ml) and 2-propanol (25 ml) were hydrogenated at 60 psi for two days. Work-up as described under A yielded 80 mg of amino glycoside VII, mp 178 - 180°. The mother liquor was found to contain mainly starting material. It was brought to dryness, and the hydrogenation was repeated (three days) using 100 mg of fresh catalyst and a pressure of 70 psi. Evaporation followed by crystallization of the residue from ethyl acetate furnished 53 mg of a colorless substance which had mp 138 - 140° (raised to 151 - 152° by recrystallization from ethyl acetate - petroleum ether) and proved identical with the by-product described under A. The

ethyl acetate solution from which the by-product had separated was concentrated, and addition of petroleum ether gave crystals (170 mg) melting at 142 - 146°. Recrystallization of this crop from ethyl acetate - petroleum ether afforded 100 mg of prisms, mp 166°, showing an infrared spectrum different from those of IV, VII, and the by-product. There was no peak attributable to ester or carboxyl carbonyl but a strong peak at 1640 cm^{-1} that was assigned to an amide grouping (see discussion p. 71). The compound was the lactam (VIII) of methyl 3-amino-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside, $[\alpha]_D^{23}$ -73.5° (c 1.3 in methanol).

Anal. Calcd for $C_{10}H_{17}NO_6$ (247.3): C, 48.65; H, 6.94; N, 5.67. Found: C, 48.72; H, 7.06; N, 5.62.

A sample (16 mg) of the amino ester VII in water (5 ml) was heated on a steam bath for 3 hr, with the addition of a small drop of acetic acid. Thin layer chromatography (solvent, ethyl acetate - ethanol - petroleum ether (2:1:1)) indicated a nearly complete conversion of the slow-moving VII into the last-moving lactam VIII.

Lactam Diacetate (XI).

A sample (40 mg) of the lactam VIII was acetylated overnight at 23° with acetic anhydride (0.25 ml) and pyridine (0.5 ml). The reaction mixture was evaporated thrice with excess toluene and twice with ethanol. The

colorless crystalline residue was dried *in vacuo* and had mp 191°. The yield was 53 mg (quantitative). Recrystallization from absolute ethanol gave needles (44 mg), and the melting point was unchanged. The product was the lactam (XI) of methyl 4,6-di— O-acetyl-3-amino-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside. Strong infrared bands at 1735 and 1670 cm^{-1} indicated ester and amide carbonyl groups.

Anal. Calcd for $C_{14}H_{21}NO_8$ (331.3): C, 50.80; H, 6.36; N, 4.23. Found: C, 50.69; H, 6.41; N, 4.17.

Benzylidene Lactam (XIII).

A sample (70 mg) of amino ester VII, anhydrous zinc chloride (200 mg), and benzaldehyde (2 ml) were stirred together for 4 hr at 23°. The reaction mixture was agitated thoroughly with water (5 ml) and petroleum ether (20 ml) in an ice - water bath. After 10 min, the white precipitate was filtered off, washed with cold water and with petroleum ether, and then immediatly recrystallized from ethanol-water (7:3). The yield was 32 mg (42%) of the lactam (XIII) of methyl 3-amino-4,6-O-benzylidene-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside; thin platelets of mp 257° remained unchanged on further recrystallization. The amide-I band was at 1663 cm^{-1}; there was no amide-II band.

Anal. Calcd for $C_{17}H_{21}NO_6$ (335.4): C, 60.95; H, 6.31; N, 4.18. Found: C, 60.79; H, 6.23; N, 4.28.

Methyl 3-Acetamido-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (IX).

The amino ester VII (85 mg) was dissolved in water (2 ml) and methanol (1 ml). Acetic anhydride (0.1 ml) was added with ice cooling, and the mixture was then stirred for 90 min at ambient temperature. Evaporation gave a white solid (IX) which was recrystallized from 2-propanol, mp 189 - 191°, raised to 192 - 193° by a second recrystallization. The yield was 63 mg (65%). The infrared spectrum had several sharp bands in the region 3500 - 3150 (OH, NH), a band at 1745 (ester carbonyl), and amide-I and -II bands at 1660 and 1570 cm^{-1}.

Anal. Calcd for $C_{15}H_{27}NO_8$ (349.4): C, 51.55; H, 7.79; N, 4.01. Found: C, 51.47; H, 7.79; N, 4.10.

Methyl 3-Acetamido-4,6-di-O-acetyl-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (X).

The amino ester VII (7o mg) was acetylated, at 23° for 18 hr, with acetic anhydride (0.5 ml) and pyridine (1 ml). The reaction mixture was worked up using the common chloroform extraction procedure. The triacetyl derivative X was obtained as a white microcrystalline powder, mp 148 - 149°, from ethyl acetate - petroleum ether, in

a yield of 66 mg (67%). Infrared bands were at 3320 (NH stretching), 1745 (ester carbonyl), 1660 (amide-I), and 1560 cm^{-1} (amide-II).

Anal. Calcd for $C_{19}H_{31}NO_{10}$ (433.5): C, 52.75; H, 7.22; N, 3.24. Found: C, 52.96; H, 7.36; N, 3.42.

Methyl 3-Acetamido-4,6-O-benzylidene-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (XIV) from IX.

The acetamido ester IX (6o mg), anhydrous zinc chloride (200 mg), and benzaldehyde (1.5 ml) were stirred together for 5hr at 23°. Work-up as described for XIII yielded 43 mg (57%) of long needles (from 90% ethanol), mp 226 - 227°, $[\alpha]_D^{23}$ -61° (c 0.5 in ethanol). Infrared bands were at 3320 (NH stretching), 1748 (ester carbonyl), 1650 (amide-I, and 1560 cm^{-1} (amide-II).

Anal. Calcd for $C_{22}H_{31}NO_8$ (437.5): C, 60.40; H, 7.14; N, 3.20. Found: C, 60.34; H, 6.91; N, 3.02.

3-Amino-2-O-(L-1-carboxyethyl)-3-deoxy-D-glucose Hydrochloride (XII).

The amino glycoside VII (150 mg) was hydrolyzed with 3 N hydrochloric acid (10 ml) at 100° for 5 hr. Most of the acid was removed by distillation in vacuo, and the remaining nearly colorless syrup was dissolved in water, and brought to pH 5.2 by treatment with Dowex 1-X2(OH^-),

Evaporation, ultimately with two additions of ethanol, left a white solid which was washed with ether. The yield was 103 mg (73.5%). The material reduced Fehling solution, showed $[\alpha]_D^{23}$ +23° (c 1.5 in water) and R_{GN} 0.8. It gradually decomposed on heating above 100°. A combustion residue revealed the presence of some inorganic impurity.

Anal. Calcd for $C_9H_{18}ClNO_7$ (287.7): C, 37.65; H, 6.31; N, 4.88. Found: C, 38.01; H, 6.91; N, 4.34.

The lactam VIII (2 mg) in 5 N hydrochloric acid (0.3 ml) was heated in a sealed tube at 110° for 5 hr. Subsequent paper chromatography gave a strong ninhydrin-positive spot of R_{GN} 0.80.

Hydrogenation of Nitro Glycoside XV. - A. In 2-Propanol.

The nitro glycoside XV (600 mg) was hydrogenated as described for IV. A syrup was obtained which could not be crystallized from a variety of solvents. Paper chromatography revealed the presence of one ninhydrin-positive compound, R_{GN} 2.0, presumed to be methyl 3-amino-2-O-[D-1-(isopropoxycarbonyl)ethyl]-3-deoxy-ß-D-glucopyranoside (XVI). When an approximately 1% solution of XVI in water containing a little acetic acid was heated on a steam bath for 2.5 hr, lactam XVII was produced although the lactamization was rather incomplete and other products

were indicated by thin layer chromatography (solvent, ethyl acetate - ethanol - petroleum ether (2:1:1)) to be present. Nevertheless, crystalline XVII was isolated in 7% yield and identified by its infrared spectrum with XVII obtained under B.

- B. In 2-Propanol - Water.

The nitro glycoside XV (320 mg) and platinum dioxide (100 mg) in a mixture of water (25 ml) and 2-propanol (25 ml) were hydrogenated at 54 psi for 3 days. The solution was treated with activated charcoal and evaporated to give a colorless syrup that crystallized in large prisms after standing at 25°. The material was triturated with ethyl acetate - 2-propanol (3:1) at 0°, and it was then isolated after several hours and washed with chilled ethyl acetate, yielding 75 mg (45%) of the lactam (XVII) of methyl 3-amino-2-O-(D-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside, mp 204 - 205° dec, $[\alpha]_D^{23}$ -40.0° (c 0.6 in methanol). Infrared absorption was in the region of 3600 - 3200 (OH, several bands) and at 1645 cm^{-1} (amide carbonyl). There was no amide-II band. In the fingerprint region the spectrum was generally similar to that of VIII, though small differences were observed.

Anal. Calcd for $C_{10}H_{17}NO_6$ (247.3): C, 48.65; H, 6.94; N, 5.67. Found: C, 48.50; H, 7.04; N, 5.60.

The mother liquor appeared to contain mainly starting material, according to thin layer chromatography.

A ninhydrin test was negative.

A sample (53 mg) of lactam XVII was acetylated as described for the preparation of XI from VIII. Recrystallization from absolute ethanol of the product gave 41 mg of oblong platelets, mp 167 - 168°, the lactam (XVIII) of methyl 4,6-di-O-acetyl-3-amino-2-O-(D-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside. Infrared bands were at 1735 and 1675 cm^{-1}.

Anal. Calcd for $C_{14}H_{21}NO_8$ (331.3): C, 50.80; H, 6.36; N, 4.23. Found: C, 50.59; H, 6.33; N, 4.03.

Hydrolyses of XVI and XVII with 5 N hydrochloric acid in sealed tubes at 110° for 5 hr resulted in the formation of 3-amino-2-O-(D-1-carboxyethyl)-3-deoxy-D-glucose hydrochloride (XIX), as indicated by paper chromatography in which both XVI and XVII gave the same pattern, R_{GN} 1.3 (strong) and 0.8 (weak).

Methyl 3-Acetamido-4,6-O-benzylidene-3-deoxy-ß-D-glucopyranoside (XXI).

Methyl 3-acetamido-3-deoxy-ß-D-glucopyranoside (XX)(14)(410 mg) was stirred with benzaldehyde (5 ml) and anhydrous zinc chloride (1 g) for 20 hr. The mixture was stirred vigorously, in an ice-bath, with 10 ml of water and 50 ml of petroleum ether. The precipitate was washed with the same solvents and immediatly recrystallized from 90% ethanol, from which XXI crystal-

lized as a monohydrate, mp 294°, $[\alpha]_D^{23}$ -78° (c 0.8 in pyridine). The yield was 410 mg (69%). The infrared spectrum showed broad hydroxyl absorption (3600 - 3200 cm^{-1}) and a water peak (1615 cm^{-1}) that persisted during the drying for analysis (6 hr at 78° in vacuo). The amide-I and -II bands were at 1645 and 1555 cm^{-1}.

Anal. Calcd for $C_{16}H_{21}NO_6 \cdot H_2O$ (341.4): C, 56.55; H, 6.56; N, 4.37. Found: C, 56.40; H, 6.66; N, 4.21.

An anhydrous product was obtained by dissolution of the hydrate in absolute ethanol, evaporation of the solution, and several repetitions of this procedure. Recrystallized from dioxane - petroleum ether, it then melted at 296° dec and no longer showed the peak at 1615 cm^{-1}. Three sharp peaks were now at 3570, 3300, and 3120 cm^{-1}.

Methyl 3-Acetamido-4,6-O-benzylidene-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside (XXII).

Sodium hydride (550 mg) was added to a solution of anhydrous XXI (650 mg) in dry dioxane (100 ml). The mixture was gently refluxed for 1 hr and was then allowed to cool to 65°. DL-2-Chloropropionic acid (1.2 ml) and, after 1 hr, another portion (2 g) of sodium hydride were added, and the mixture was magnetically stirred for 19 hr at 65°. Upon cooling, 70 ml of water was cautiously introduced, and the solution was concentrated in vacuo to

a small volume. The yellowish solution obtained on diluting the concentrate with water was extracted once with chloroform (which was discarded), and then it was acidified, under ice cooling, with 30 ml of chilled 6 N hydrochloric acid. Without delay the acidic solution was extracted four times with chloroform, and the combined extracts were immediatly washed with four portions of cold water, dried briefly with sodium sulfate, and evaporated to give a solid residue. The residue was triturated with a few milliliters of hot methanol, and, after cooling, 262 mg (38%) of XXII, mp 282 - 283°, was collected. Recrystallization from methanol, with addition of a little petroleum ether, furnished fine needles, mp 287°, $[\alpha]_D^{23}$ -48° (c 0.9 in methanol). Infrared absorption was at 3300 (sharp, NH), 2700 - 2500 (weak, OH), 1705 (strong, COOH), 1655 (amide-I), and 1560 cm^{-1} (amide-II).

Anal. Calcd for $C_{19}H_{25}NO_8$ (395.4): C, 57.71; H, 6.37; N, 3.54. Found: C, 57.88; H, 6.58; N, 3.30.

From the mother liquor were isolated small fractions of crystals (26 and 85 mg) which appeared to be impure XXII and starting material XXI. The remainder was a brown syrup that may have contained the D-1-carboxyethyl isomer.

Methyl 3-Acetamido-4,6-O-benzylidene-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (XIV) from XXII.

One hundred milligrams of XXII was dissolved in 1 ml of thionyl chloride. After 5 min the excess chloride was quickly evaporated and the treatment was repeated. The solid residue was immediatly dissolved in 2-propanol (5 ml), and the mixture was twice evaporated after addition of fresh 2-propanol. The isopropyl ester XIV (47 mg, mp 218 - 219°) crystallized from 50% ethanol, and upon recrystallization from 90% ethanol it showed mp 220 - 221° and $[\alpha]_D^{23}$ -56° (c 0.5 in ethanol). A mixture melting point with XIV obtained from IX, was 220 - 221°, and the infrared spectra of both compounds were identical.

Methyl 3-Acetamido-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside (XXIII).

A suspension of benzylidene compound XXII (70 mg) in water (15 ml) was heated on the steam bath for 2 hr, during which complete dissolution occurred. The solution was evaporated and the residue was once more heated, for 1 hr, in 10 ml of water. A smell of benzaldehyde was noticed. Evaporation gave a colorless syrup which, on two evaporations with ethanol and trituration with a small amount of ether, yielded fine needles (32 mg, 59%) of crude XXIII, mp 172 - 175°. Three recrystallizations from

ether containing 1 drop of methanol gave 21 mg of pure XXIII, mp 190 - 191°, $[\alpha]_D^{23}$ -4.8° (c 0.5 in methanol). Infrared absorption was in the 3600 - 3200- and 2700 - 2500 cm^{-1} regions (OH, NH), and at 1720 (COOH), 1635 (amide-I), and 1565 cm^{-1} (amide-II).

Anal. Calcd for $C_{12}H_{21}NO_8$ (307.3): C, 46.90; H, 6.89; N, 4.56. Found: C, 47.00; H, 7.02; N, 4.64.

Part II. 2

Methyl 4,6-O-Benzylidene-2-(2-carboxyphenyl)-amino-2,3-dideoxy-3-nitro-ß-D-glucopyranoside (VI).

A. In the Presence of Potassium Hydroxide and a Twofold Excess of Anthranilic Acid.

To a solution of methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-erythro-hex-2-enopyranoside (I)(18) (500 mg, 0.0017 moles) in dry benzene (50 ml) was added anthranilic acid (500 mg, 0.0037 mole) and a small chip (about 20 mg) of potassium hydroxide. The mixture was then gently boiled under reflux. The progress of the reaction was followed by thin layer chromatography [solvent, ethyl acetate - petroleum ether (3:1)]. The fast-moving starting material was slowly replaced by a non-moving compound which became the only product after 5 hr. Filtration and evaporation left a solid, yellowish white residue which was dissolved in warm ethanol. Addition of some water to the warm solution and allowing the mixture to cool down to room temperature (slowly, to avoid gel formation) furnished 610 mg (83%) of fine, colorless needles of VI, mp 210 - 211°. One recrystallization from aqueous ethanol gave 500 mg, mp 220 - 221°, $[\alpha]_D^{23}$ -25.5° (c 1.2 in acetone), λ_{max} 255 mμ(ε, 10100) and 340 mμ(ε, 5000).

The infrared spectrum of VI has been discussed (p.83) and is depicted (Figure 6) on page 84.

Anal. Calcd for $C_{21}H_{22}N_2O_8$ (430.4): C, 58.50; H, 5.14; N, 6.50. Found: C, 58.46; H, 5.20; N, 6.50.

No other product was isolated from this reaction.

B. In the Absence of Potassium Hydroxide but with a Two-fold Excess of Anthranilic Acid.

The olefin I (300 mg) and anthranilic acid (300 mg) were gently refluxed in dry benzene (50 ml). Work-up as described under A yielded 507 mg of a compound melting at 215 - 218°. Three recrystallizations, from 95% ethanol, hot benzene, and ethyl acetate - petroleum ether, respectively, afforded 96 mg (22%) of yellow needles (VIII), mp 222° dec, $[\alpha]_D^{23}$ -173° (c 0.6 in acetone), λ_{max} 254 mμ (ϵ, 11100) and 343 mμ (ϵ, 5300). A mixture melting point with VI was strongly depressed. The infrared spectrum of methyl 4,6-O-benzylidene-2-(2-carboxyphenyl)amino-2,3-dideoxy-3-nitro-ß-D-mannopyranoside (VIII) has been discussed (p.83) and is depicted (Figure 7) on page 85.

Anal. Calcd for $C_{21}H_{22}N_2O_8$ (430.4): C, 58.50; H, 5.14; N, 6.50. Found: C, 58.72; H, 5.29; N, 6.35; mol. wt., 430 (mass spectrometry).

After addition of some water to the mother liquor, 180 mg (41%) of VI, mp 214 - 215°, could be isolated. Recrystallization from aqueous ethanol raised the mp to

218 - 219°. An infrared spectrum was identical with that of VI obtained above.

Methyl 4,6-O-Benzylidene-2-(2-carboxyphenyl)-amino-2,3-dideoxy-3-nitro-ß-D-mannopyranoside (VIII).

The olefin (I)(1 g, 0.00342 mole) and anthranilic acid (470 mg, 0.00342 mole) were gently refluxed in dry benzene (100 ml) for 5 hr. Work-up as described for VI (under A) yielded 570 mg of yellow crystals of mp 218 - 219° whose infrared spectrum was identical with that of VIII obtained above. Another 252 mg of the same compound could be isolated from the mother liquor upon evaporation of part of the solvent, thus increasing the total yield of VIII to 56%.

No other compound was isolated from the reaction.

Dihydrate (XV) of VIII

The yellow compound (VIII)(100 mg) was dissolved in 20 ml of a mixture of chloroform - dimethyl sulfoxide (1:1). Subsequent addition of petroleum ether to incipient turbidity afforded, after several hours of standing at 4°, 95 mg of colorless XV. Recrystallization from ethyl acetate - petroleum ether gave 86 mg of parallelogram-shaped plates of mp 216 - 217°, $[\alpha]_D^{23}$ -150° (c 0.7 in ethanol), $[\alpha]_D^{23}$ -158.5° (c 0.6 in acetone). The melting point of XV was dependent upon the introduction temperature (in

brackets), 205° (160°), 209° (195°), 216 - 217° (210°). Infrared absorptions were at 3500 - 2500 (broad), 1680 (strong, CO), and a strong doublet centered at 1554 cm^{-1} (splitting, 8 cm^{-1}). Other strong bands were at 1255, 1100, and 990 cm^{-1} (broad). Bands of medium intensity were at 1605, 1589, 1518, 1302, 760, and 708 cm^{-1}.

Anal. Calcd for $C_{21}H_{22}N_2O_8 \cdot 2H_2O$ (466.4): C, 54.02; H, 5.58; N, 6.01. Found: C, 54.00; H, 5.71; N, 5.70; mol. wt., 430 (mass spectrometry).

The same compound (XV) could be obtained directly from olefin I (480 mg) and anthranilic acid (250 mg) when refluxed (5 hr) in benzene (50 ml) to which dimethyl sulfoxide (2 ml) had been added. Evaporation left a syrup which yielded 405 mg of colorless crystals from ethyl acetate - petroleum ether. Recrystallization from the same solvents gave 360 mg of XV, mp 198 - 199° (150°). An infrared spectrum was identical with that of XV obtained above.

Regeneration of VIII from the Dihydrate (XV).

The colorless dihydrate (60 mg) was dissolved in warm ethanol (8 ml). Cooling to room temperature and addition of water (2 ml) yielded 52 mg of yellow VIII, mp 217 - 218°. An infrared spectrum was identical with that of VIII obtained before.

The acid VIII could also be regenerated from XV

by dissolution of XV in refluxing chloroform. Cooling and addition of petroleum ether furnished, after several hours of standing at 4°, in near-quantitative yield the yellow acid (VIII).

Methyl Ester (VII) of VI.

A. From VI.

The colorless acid (VI)(100 mg) was dissolved in ethyl acetate (10 ml), and ethereal diazomethane was added under ice cooling until a yellow color persisted. Evaporation left a white solid which was once more dissolved in ethyl acetate and treated with an ether solution of diazomethane. After evaporation and dissolution of the residue in ethyl acetate, addition of petroleum ether, and standing over night at 5°, 74 mg of VII crystallized as needles, mp 225°, $[\alpha]_D^{23}$ -22.0° (c 0.8 in acetone), λ_{max} 255 mμ and 345 mμ. Infrared bands were at 3280 (NH), 1710 (ester carbonyl), and 1555 cm^{-1} (NO_2); other strong bands were at 1252, 1100, and 975 cm^{-1} (broad); medium intensity absorptions occurred at 1608, 1590, 1525, 760, and 702 cm^{-1}.

Anal. Calcd for $C_{22}H_{24}N_2O_8$ (444.5): C, 59.45; H, 5.44; N, 6.30. Found: C, 59.34; H, 5.27; N, 6.40.

B. By Addition of Methyl Anthranilate to Nitro Olefin I.

The olefin I (95 mg) was refluxed together with methyl anthranilate (49 mg, 1 molar equiv.) in dry benzene (25 ml) for 5 hr. Evaporation, followed by two co-evaporations with toluene, left a syrupy residue which afforded 89 mg of VII from ethyl acetate - petroleum ether. Melting point (224 - 225°) and an infrared spectrum ascertained the identity of this product with VII obtained above.

Methyl Ester (IX) of VIII.

An ethyl acetate solution of the acid VIII (700 mg) was treated with an ether solution of diazomethane as described for the preparation of VII (A). From ethyl acetate - petroleum ether crystallized 579 mg of big, yellow prisms (IX), mp 167 - 168°, $[\alpha]_D^{23}$ -188° (c 0.7 in acetone). Recrystallization did not change the melting point. Infrared bands were at 3335 (NH), 1690 (ester carbonyl), and at 1560 cm^{-1} (NO_2). Two strong absorptions occurred at 1090 (broad) and 1008 cm^{-1}, respectively. Bands of medium intensities were at 1610, 1590, 1528, 1265, 1185, 1140, 1050, 750, and 705 cm^{-1}.

Anal. Calcd for $C_{22}H_{24}N_2O_8$ (444.5): C, 59.45; H, 5.44; N, 6.30. Found: C, 59.47; H, 5.31; N, 6.49.

The same ester was obtained by treatment of a solution of the dihydrate (XV) in ethyl acetate with ethereal diazomethane.

Methyl 2,3-Dideoxy-2-(2-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (X).

The benzylidene compound VI (200 mg) was hydrolyzed by heating on a steam bath for 40 min with 10 ml of 60% acetic acid. The solvent was removed and water was added twice to the residue and evaporated. The residue was then crystallized from water - ethanol (6:1). The product : (112 mg) was obtained as fine needles, mp 192° (160° introduction), 204° (195°), $[\alpha]_D^{23}$ -3.0° (c 1 in ethanol), λ_{max} 254 mμ and 347 mμ (in methanol). Recrystallization did not affect the melting point. The infrared spectrum showed a broad absorption reaching from 3600 to 2500, a strong peak at 1680 (carbonyl), and bands of medium intensity at 1585, 1560, 1550, and 1525 cm^{-1}.

Anal. Calcd for $C_{14}H_{18}N_2O_8$ (343.3): C, 49.05; H, 5.28; N, 8.18. Found: C, 48.94; H, 5.50; N, 8.22.

Methyl Ester (XI) of X.

Methyl 2,3-dideoxy-2-(2-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (X)(100 mg) was methylated as described for the preparation of VII (A). There was obtained 91 mg of XI (needles), mp 161 -162°, $[\alpha]_D^{23}$ +3.1° (c 0.6 in ethanol). Infrared bands were at 3500 - 3100 (broad), 1695, 1555, 1260,1240, and 1085 cm^{-1} (all strong). Medium intensity peaks occurred at 1608, 1590, 1525, 1155,1125, 1090, 1040, 750, and 705 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}N_2O_8$ (356.4): C, 50.56; H, 5.66; N, 7.86. Found: C, 50.43; H, 5.56; N, 7.74.

Methyl 2,3-Diamino-2,3-dideoxy-ß-D-glucopyranoside Dihydrochloride (XII) and Methyl 3-Amino-2-(2-carboxyphenyl)amino-2,3-dideoxy-ß-D-glucopyranoside Dihydrochloride (XIII).

A suspension of X (500 mg) in water (30 ml) and N hydrochloric acid (3 ml) was added to 500 mg of palladium oxyhydrate - barium sulfate catalyst (75) that had been prehydrogenated in water, and the mixture was shaken for 24 hr under hydrogen. The solution was filtered through celite and evaporated to give a slightly brownish syrup which was twice co-evaporated with water. The coloration of the material was removed by a treatment with activated charcoal in water. Final evaporation, with two additions of ethanol at the end, yielded a colorless syrup. Crystallization took place from absolute ethanol and gave nice platelets (191 mg) of XII. Upon concentration of the mother liquor another 22 mg of XII was isolated. Crystalline XII showed no melting point but decomposed slowly above 240°; $[\alpha]_D^{23}$ -41° (c 0.7 in water), R_{GN} 1.17. Infrared bands were at 3480, 3380, 3100 - 2500 (broad), 1610 and 1585 (medium intensity), and 1090 - 1030 cm^{-1} (broad, consisting of several bands).

Anal. Calcd for $C_7H_{18}Cl_2N_2O_3$ (249.2): C, 33.75; H, 7.28; N, 11.25. Found: C, 33.75; H, 7.14; N, 11.41.

The mother liquor was evaporated, the residue dissolved in water, and the solution was allowed to flow slowly through a column (1 × 15 cm) of Dowex 1-X2 (OH^-). The eluate was evaporated after addition of N hydrochloric acid (3 ml). Two evaporations with water and two with ethanol furnished another 12 mg of XII.

The column was then thoroughly washed with water (discarded) and eluted with 0.01 N hydrochloric acid (250 ml). The eluate was evaporated with ultimate additions of two portions of water followed by two portions of ethanol. The turbid residue was taken up in ethanol and the solution was clarified with activated charcoal and brought to dryness again. A greyish, glassy material (XIII, 35 mg) resulted. Crystallization could not be induced although a second treatment with charcoal was performed. However, the material gave only one spot (R_{GN} 2.30) on a paper chromatogram, and a nitrogen determination agreed with formula XIII. The substance showed $[\alpha]_D^{23}$ -52.5° (c 0.4 in water). Infrared absorptions were at 3600 - 2500 (broad), 1670 (strong), 1610, 1585, and 1525 (all of medium intensity), 1210, 1150, and 1050 cm^{-1} (all broad and strong).

Anal. Calcd for $C_{14}H_{22}Cl_2N_2O_6$ (385.3): N, 7.27. Found: N, 7.09.

Methyl 2,3-Diacetamido-2,3-dideoxy-ß-D-glucopyranoside (XIV).

The diamino glycoside XII (120 mg) was dissolved in water (10 ml) and methanol (5 ml). Acetic anhydride (0.3 ml) and Dowex 1-X2(OH^-)(10 ml) were added and the mixture was stirred for 9o min at ambient temperature. Filtration and evaporation afforded a partly crystalline solid (XIV) which was recrystallized from acetone. Fine, colorless needles (85 mg), mp 256 - 258°, were obtained. Recrystallization from the same solvent raised mp to 270 -271° dec.; $[\alpha]_D^{23}$ -103.5° (c 1 in water). The compound was found to be identical with methyl 2,3-diacetamido-2,3-dideoxy-ß-D-glucopyranoside (XIV) obtained by Baer and Neilson (32) with respect to its mp, infrared spectrum, and optical rotation [reported (32), mp 262 - 263° (depending on introduction temperature), $[\alpha]_D^{23}$ -105.2°]. A mixture melting point with an authentic sample remained undepressed.

Methyl 2,3-Dideoxy-2-(2-methoxycarbonylphenyl)-amino-3-nitro-ß-D-mannopyranoside Monohydrate (XVI).

The methyl ester IX (570 mg) was hydrolyzed by heating on a steam bath for 40 min in 15 ml of 70% acetic acid as described for the preparation of X. A pale yellow residue was recrystallized from aqueous ethanol giving 405 mg (84%) of XVI, mp 82 - 83°. Another recrystallization

from ethylacetate - petroleum ether furnished big, yellow prisms of mp 90 - 91°, $[\alpha]_D^{23}$ -129° (c 0.65 in ethanol). Strong infrared absorptions were at 3600 - 3200 (broad, several bands), 1680 (with a shoulder at 1650), 1558, 1245, and 1050 cm^{-1} (broad). Bands of medium intensity occurred at 1605, 1585, 1520, 1330, 1175, 755, and 705 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}N_2O_8 \cdot H_2O$ (374.4): C, 48.15; H, 5.89; N, 7.46. Found: C, 48.45; H, 5.92; N, 7.21; mol. wt., 356 (mass spectrometry).

A mass spectrum of XVI at low ionization voltage exhibited as the peak of highest intensity one with m/e 18.

Methyl 2,3-Diamino-2,3-dideoxy-ß-D-mannopyranoside Dihydrochloride (XVII).

A. From VIII.

The benzylidene compound VIII (200 mg) was hydrolyzed as described for the preparation of X. The syrupy residue obtained after evaporation of the solvents was shaken in water (40 ml) containing a few drops of ethanol and N hydrochloric acid (3 ml), together with pre-hydrogenated (in water) palladium oxyhydrate - barium sulfate catalyst (75), for 24 hr. Evaporation after filtration left a nearly colorless syrup which failed to crystallize from various solvents. Paper chromatography revealed that two ninhydrin-positive compounds were present (R_{GN} 1.20 and R_{GN} 2.16). The syrup was therefore dissolved in water and

the solution was allowed to flow through a Dowex 1-X2(OH^-) column. Evaporation of the eluate after addition of N hydrochloric acid (4 ml), followed by two evaporations with water and two with 2-propanol, afforded a white powder (XVII) which was suspended in acetone and filtered off. The substance (84 mg) was uniform on a paper chromatogram (R_{GN} 1.18), had an unsharp melting point (about 100 - 108°, sintering[a]), and $[\alpha]_D^{23}$ -45.5° (c 0.5 in water). After dissolution in ethanol, clarification with activated charcoal, evaporation and reprecipitation with 2-propanol - acetone, the rotation was $[\alpha]_D^{23}$ -46.5 (c 0.7 in water). Infrared spectra were of poor quality; they showed a broad absorption at 3600 - 2500, a strong, broad band at 1075, bands of medium intensities at 1590, 1515, 1220, 1120, and 1015 cm^{-1}. Nmr spectra of XVII (discussed on p. 94) suggested the presence of either 2-propanol or acetone of crystallization.

Anal. Calcd for $C_7H_{18}Cl_2N_2O_3 \cdot 1C_3H_8O$ (309.2): N, 9.06; Cl, 22.93. Found: N, 8.90; Cl, 23.19.

Inspection of an eluate of the column (with N hydrochloric acid) by paper chromatography revealed the presence of some fast-moving material (XVIII), R_{GN} 2.20,

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a. When heated very slowly the substance turns brownish and melts around 180° under foaming.

and of some slow-moving impurities. No attempt was made to isolate this compound.

B. From XVI.

The methyl ester XVI (255 mg) was hydrogenated as described for the preparation of XII. The syrup obtained after evaporation of the solvents was twice evaporated with 2-propanol to give a white solid which was suspended in acetone. A white powder (195 mg) was filtered off. It was shown by paper chromatography to consist of one main compound (R_{GN} 1.18) accompanied by faster-moving impurities. Dissolution in methanol, clarification with charcoal, and two evaporations with 2-propanol, gave chromatographically pure XVII, $[\alpha]_D^{23}$ -46.7° (c 1 in water). Comparison of its infrared spectrum with that of XVII obtained above proved the compounds to be identical.

Methyl 2,3-Diacetamido-2,3-dideoxy-ß-D-manno-pyranoside (XIX).

The diamino glycoside XVII (210 mg) was N-acetylated as described for the preparation of XIV, giving a syrup which failed to crystallize. Inspection by thin layer chromatography [solvent, methanol - ethanol - acetone (1:1:2)] revealed the presence of at least three minor components besides one major product. Separation was achieved on a column (22 × 1 cm) containing silica gel (10 g), with benzene - methanol (10:1) as eluant.

The 10 ml fractions were inspected by thin layer chromatography. The fractions containing the pure major product were combined and evaporated leaving a solid residue which could not be recrystallized. After suspension in petroleum ether - acetone (3:1) and short boiling under reflux, the mixture was left standing at 4° for 24 hr. Filtration afforded 57 mg of XIX which was pure on thin layer chromatography where it moved a little, but distinctively, slower than XIV. It melted over a range from 116 - 135°, and showed $[\alpha]_D^{23}$ -108.0° (c 0.6 in water). Infrared bands were at 3550 - 3200 (broad), 1655 (amide-I), 1550 (amide-II), and 1075 cm^{-1} (broad).

Anal. Calcd for $C_{11}H_{20}N_2O_6$ (276.3): C, 47.82; H, 7.30; N, 10.14. Found: C, 47.61; H, 7.36; N, 9.96.

2,3-Diamino-2,3-dideoxy-D-mannose Dihydrochloride (XX).

The diacetamido compound XIX (40 mg) was hydrolyzed with 5 N hydrochloric acid (10 ml) at 100° for 2 hr. Evaporation, followed by two co-evaporations with water and clarification with activated charcoal, furnished a colorless syrup which failed to crystallize from ethanol. Evaporation with a mixture of ethanol - ethyl acetate (1:1) gave white, solid XX (30 mg). The material reduced Fehling solution, showed $[\alpha]_D^{23}$ +1° (2 min)→ -3.0° (1 hr)(c 0.6 in water, and R_{GN} 0.91 (brownish color). Infrared bands: 3600

- 2500 (broad), 1590, and 1510 (medium peaks), 1150, 1105, 1070, and 1035 cm^{-1} (increasing intensity towards smaller wavenumbers). A combustion residue revealed the presence of some inorganic impurity.

Anal. Calcd for $C_6H_{16}Cl_2N_2O_4$ (251.1): C, 28.70; H, 6.42. Found: C, 28.55; H, 7.00.

Determination of Optical Rotation of VI and VIII in Alkaline Solution.

The benzylidene compound VIII (60 mg) was dissolved in 0.01 N sodium hydroxide (12 ml). The solution showed $[\alpha]_D^{23}$ -243°. Deionization after 5 min with Rexyn 101(H^+)(15 ml, suspended in 100 ml of methanol) and evaporation furnished a yellow residue. Filtration after addition of water gave 54 mg of VIII (mp 212 - 213°) whose infrared spectrum was identical with that of the starting material.

In another experiment, the solution was not deionized but left standing at room temperature for 20 days. The optical rotation changed as follows: -243° (5 min), -242.5° (20 hr), -208° (3 days), -182° (5 days), -157° (7 days), -120° (11 days), -100° (20 days). Inspection of the solution by ultraviolet spectroscopy at this point showed two strong absorptions at λ_{max} 252 mμ and 302 mμ, respectively. A strong smell of benzaldehyde was noticed. Deionization and evaporation yielded a dark brown syrup

that failed to crystallize.

The gluco isomer VI showed in alkaline solution $[\alpha]_D^{23}$ -13.4° (c 0.9 in 0.01 N sodium hydroxide).

Methyl 4,6-O-Benzylidene-2,3-dideoxy-2-(3-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (XXII).

The nitro olefin I (200 mg), m-aminobenzoic acid (200 mg), and potassium hydroxide (5 mg) were refluxed together in dry benzene (25 ml) for 5 hr. Work-up as described (for VI) afforded after recrystallization from 50% ethanol, 87 mg of XXII, mp 215° dec., $[\alpha]_D^{23}$ -75.0° (c 0.9 in acetone). Concentration of the mother liquor gave an additional 52 mg of XXII, mp 214° dec. Infrared bands: 3400 (sharp), 3320 (broad), 1725 (strong), 1555, 1090, and 1010 (all strong), 1612, 1592, 1518, 1300, 1255, 1200 (broad), 1040, 755, and 705 cm^{-1} (all medium intense).

Anal. Calcd for $C_{21}H_{22}N_2O_8$ (430.4): C, 58.50; H, 5.14. Found: C, 58.84; H, 5.33.

The same compound (XXII) was obtained in 94.5% yield when m-aminobenzoic acid was added to olefin I following the procedure described for the synthesis of VIII.

Methyl 4,6-O-Benzylidene-2,3-dideoxy-2-(4-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (XXIII).

Addition of p-aminobenzoic acid to olefin I (200 mg) as described for the preparation of XXII afforded 163 mg

of XXIII, mp 252° dec., $[\alpha]_D^{23}$ -95.5° (c 0.7 in acetone). Recrystallization did not affect the melting point. Infrared bands were at 3500 - 2600 (broad, with a sharp band at 3380), 1685, 1560, 1095, and 990 cm^{-1} (all strong). Medium peaks were at 1610, 1190, 1155, 1135, 755, and 705 cm^{-1}.

Anal. Calcd for $C_{21}H_{22}N_2O_8$ (430.4): C, 58.50; H, 5.14. Found: C, 59.25; H, 5.32.

Methyl 2,3-Dideoxy-2-(4-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (XXV).

The benzylidene compound XXIII (110 mg) was hydrolyzed with 70% acetic acid as described for the preparation of X. Crystallization from aqueous ethanol gave 26 mg of XXV in the form of needles, mp 195° dec., $[\alpha]_D^{23}$ -41.0° (c 0.5 in ethanol). From the mother liquor an additional 12 mg of XXV could be isolated. The infrared spectrum had a broad band at 3550 - 2600 cm^{-1}, strong bands at 1690, 1610, 1552, 1125 (broad), and 1075 cm^{-1} (broad).

Anal. Calcd for $C_{14}H_{18}N_2O_8$ (343.3): C, 49.05; H, 5.28; N, 8.18. Found: C, 49.12; H, 5.47; N, 8.30.

Hydrogenation of XXV: Methyl 2,3-Diamino-2,3-dideoxy-ß-D-glucopyranoside Dihydrochloride (XII).

The acid XXV (60 mg) was hydrogenated as described for the preparation of XII (from X). The crystalline

crystallized from ethanol giving 28 mg of XII. The identity of this compound with XII obtained before was established by infrared spectroscopy, optical rotation $[[\alpha]_D^{23}$ -41.5° (c 0.8 in water)], and paper chromatography (R_{GN} 1.17). Investigation of the mother liquor by chromatography revealed that it contained more of XII and a faster-moving material (R_{GN} 1.92).

Debenzylidenation and Hydrogenation of XXII.

The benzylidene compound XXII (374 mg) was hydrolyzed with acetic acid in the usual manner (see e.g. the debenzylidenation of VI). After evaporation of the solvents a syrup was obtained which was presumed to contain methyl 2,3-dideoxy-2-(3-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (XXIV) but failed to crystallize. It was, nevertheless, dissolved in water and hydrogenated in the presence of palladium oxyhydrate - barium sulfate catalyst as described for the synthesis of XII. Pure XII (69 mg, 32%) crystallized from absolute ethanol and had $[\alpha]_D^{23}$ -41.5° (c 0.7 in water), and R_{GN} 1.16. An infrared spectrum was identical with that of XII obtained before. Paper chromatography of the mother liquor showed that besides XII another main component (R_{GN} 2.10) was present. There were additional fainter spots at R_{GN} 0.70, 0.88, 1.30, and 1.75.

Addition of Anthranilic Acid to Nitro Olefin XXVI.

The olefin XXVI (100 mg) and anthranilic acid (100 mg) were gently refluxed in dry benzene (25 ml) for 2 hr. Inspection by thin layer chromatography [solvent, ethyl acetate - petroleum ether (1:1)] showed the absence of fast-moving starting material. Evaporation left a partly crystalline residue which furnished 85 mg of XXVII (mp 135 - 136°) from 50% ethanol. Recrystallization from ethyl acetate - petroleum ether gave pale yellow needles of mp 137 - 138°, $[\alpha]_D^{23}$ -60.0 (c 0.6 in acetone). Its infrared spectrum has been discussed on page 108.

Anal. Calcd for $C_{21}H_{22}N_2O_8$ (430.4): C, 58.50; H, 5.14; N, 6.50. Found: C, 58.40; H, 5.19; N, 6.40.

The same compound XXVII (175 mg) was obtained from olefin XXVI (180 mg) and anthranilic acid (90 mg) when refluxed together in dry benzene (30 ml) for 2 hr.

Methyl 2-Acetamido-4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-mannopyranoside (XXI).

A small amount of this compound had been obtained by Baer and Neilson (32) who recorded its melting point (223 - 224°) but did not give data of elemental analysis and optical rotation, and who did not make an assignment of configuration.[a] The compound was now pre-

a. The compound is referred to as 'isomer 6' in the article cited.

pared in larger quantity.

From the previous work (32) were available a number of preparations of methyl 2-amino-4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-hexopyranosides. Several of the samples were rich in what is now known to be the manno isomer, although they contained varying amounts of the gluco isomer. The samples were pooled and N-acetylated in methanolic solution as described (32). From 1.843 g of material was obtained: (a) a fraction (330 mg) of sparingly soluble gluco N-acetate, mp 308 - 310° dec. (reported, mp 310 - 311° dec.); (b) a fraction (727 mg) of methanol-soluble manno N-acetate, mp 223 - 225° dec., $[\alpha]_D^{23}$ -78° (c 1 in DMF); (c) an additional fraction (925 mg) of manno N-acetate, mp 217 - 218° dec., $[\alpha]_D^{23}$ -92.5° (c 0.9 in DMF). Recrystallization of fractions b and c from methanol - ether gave prisms of mp 224 - 225° dec. and $[\alpha]_D^{23}$ -94.7° (c 1.03 in DMF).

Anal. Calcd for $C_{16}H_{20}N_2O_7$ (352.3): C, 54.54; H, 5.72; N, 7.95. Found: C, 54.44; H, 5.86; N, 7.84.

The infrared spectrum of the product was identical with that of a sample previously obtained (32).

Methyl 2-Acetamido-2,3-dideoxy-3-nitro-ß-D-mannopyranoside (XXVIII).

The benzylidene derivative XXI (500 mg) was heated on the steam bath for 30 min in 50 ml of 70% acetic

acid. The solution was then evaporated with consecutive additions of toluene, water, and ethanol. A nearly colorless syrup was obtained which crystallized in part from ethanol - ethyl acetate - ether at 5°. The large prismatic needles (145 mg, mp 90 - 91°) were recrystallized from the same solvents, mp 90°, $[\alpha]_D^{23}$ -48.5° (c 0.8 in water). The infrared spectrum showed a strong, broad absorption reaching from 3500 to 3100 cm^{-1} (OH, NH), strong bands at 1660 (amide-I), 1550 (broad, NO_2 and amide-II), and 1065 cm^{-1} (broad, C-O-C). Peaks of medium intensity were at 1300, 1225, 1180, 1125, 1020, 980, 875, and 720 cm^{-1}.

Anal. Calcd for $C_9H_{16}N_2O_7$ (264.2): C, 40.91; H, 6.10; N, 10.60. Found: C, 40.69; H, 6.23; N, 10.49.

The mother liquor of XXVIII was clarified with activated charcoal and evaporated. A solution of the remaining syrup in methanol was then shaken together with platinum catalyst [platinum oxide, 200 mg, pre-hydrogenated in 10 ml of methanol - acetic acid (10:1)] for 22 hr under hydrogen after acetic anhydride (0.5 ml) had been added. Filtration and evaporation left a syrupy residue which according to thin layer chromatography [solvent, methanol - ethanol - acetone (1:1:2)] still contained a large amount of starting material. Dissolution of the syrup in methanol (30 ml) and, after addition of new catalyst (200 mg), acetic acid (1 ml), and acetic anhydride (0.5 ml), repeating the hydrogenation for 24 hr, furnished

a syrup which still contained some unreduced material (thin layer) but was rich in a slower-moving compound. Separation on a silica gel column [10 g silica gel, column 25 x 1 cm] as described for the preparation of XIX, yielded 31 mg of the pure, slower-moving product, mp 115 - 119°, $[\alpha]_D^{23}$ -107.5° (c 0.65 in water). Its identity with XIX was established by infrared spectroscopy and by thin layer chromatography . A mixture melting point with XIX remained undepressed.

CLAIMS TO ORIGINAL RESEARCH

Part I

1. The dehydroacetylation of methyl 3-deoxy-3-nitro-hexopyranose triacetates was investigated. Tricyclic Diels - Alder compounds resulting from self-addition of an intermediate nitrodiene were found to be the unexpected products. Their structures were established by spectroscopic methods and by their conversion to derivatives of isochromene.

2. In the course of these investigations the following new compounds were isolated in crystalline condition:

a. 3,4,4a,8a-Tetrahydro-3,5-dimethoxy-8a,9-dinitro-1,4-ethenopyrano [4,3-c] pyran-1,7(5H)-dimethanol diacetate (VIII)

b. A stereoisomer of VIII

c. 1-Hydroxy-7-nitro-1H-2-benzopyran-3,5-dimethanol 3,5-diacetate (X)

d. Phenylhydrazone of X

e. 1-Methoxy-7-nitro-1H-2-benzopyran-3,5-dimethanol diacetate

f. 1-Ethoxy-7-nitro-1H-2-benzopyran-3,5-dimethanol diacetate

g. 1-Isopropoxy-7-nitro-1H-2-benzopyran-3,5-dimethanol diacetate

h. 7-Nitro-1H-2-benzopyran-1-one-3,5-dimethanol diacetate

i. Nitrobenzene-3,4,5-tricarboxylic acid monohydrate

j. 3,5-Bishydroxymethyl-4-(2,3-dihydroxypropyl)-nitrobenzene

k. 3,4-Dihydro-3-hydroxy-7-nitro-1H-2-benzopyran 5-methanol

l. 2,6-Bishydroxymethyl-4-nitrophenylacetaldehyde semicarbazone

m. 4-(2-Hydroxyethyl)-3,5-bishydroxymethyl-nitrobenzene

n. Methyl 2,3,4-trideoxy-2,4-diacetamido-3-nitro-hexopyranoside

o. Methyl 2,3,4-triacetamido-6-O-acetyl-2,3,4-trideoxy-hexopyranoside

Part II

3. Nucleophilic additions to methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-erythro-hex-2-enopyranoside and to its ß-D-threo isomer were studied.

In Part II.1, the addition of isopropyl lactate was investigated and found to lead exclusively to 2,3-diequatorial substituent orientation. Using this approach the synthesis of positional isomers of muramic acid was achieved.

In Part II.2, o-, m-, and p-aminobenzoic acid and o-aminobenzoic acid methyl ester additions were found

to give also 2,3-diequatorially substituted products, with the exception of o-aminobenzoic acid where variations in the reaction conditions resulted in an axial substitution at C-2. This unexpected behaviour led to the synthesis of 2,3-diamino-2,3-dideoxy-D-mannose.

The structures of new compounds synthesized in Part II, 1 and 2, were established by spectroscopic and chemical means.

4. The following new compounds were isolated in crystalline condition,

in Part II.1:

a. Methyl 4,6-O-benzylidene-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-ß-D-glucopyranoside (II)

b. The D-1-(isopropoxycarbonyl)ethyl isomer of II

c. Methyl 3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-3-nitro-ß-D-glucopyranoside (IV)

d. The D-1-(isopropoxycarbonyl)ethyl isomer of IV

e. Methyl 3-amino-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside

f. Lactam of methyl 3-amino-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside (VIII)

g. The diacetate of lactam VIII

h. The 4,6-O-benzylidene derivative of lactam VIII

i. The D-1-carboxyethyl isomer (XVII) of lactam VIII

j. The diacetate of lactam XVII

k. Methyl 3-acetamido-3-deoxy-2-O-[L-1-(isopropoxycarbonyl)ethyl]-ß-D-glucopyranoside (IX)

l. The 4,6-di-O-acetyl derivative of IX

m. The 4,6-O-benzylidene derivative of IX

n. 3-Amino-2-O-(L-1-carboxyethyl)-3-deoxy-D-glucose hydrochloride

o. Methyl 3-acetamido-4,6-O-benzylidene-3-deoxy-ß-D-glucopyranoside

p. Methyl 3-acetamido-4,6-O-benzylidene-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside

q. Methyl 3-acetamido-2-O-(L-1-carboxyethyl)-3-deoxy-ß-D-glucopyranoside

in Part II.2:

a. Methyl 4,6-O-benzylidene-2-(2-carboxyphenyl)amino-2,3-dideoxy-3-nitro-ß-D-glucopyranoside (VI)

b. The 2-(3-carboxyphenyl)amino isomer of VI

c. The 2-(4-carboxyphenyl)amino isomer of VI

d. Methyl 4,6-O-benzylidene-2-(2-carboxyphenyl)amino-2,3-dideoxy-3-nitro-ß-D-mannopyranoside (VIII)

e. The dihydrate of VIII

f. The methyl ester of VI

g. The methyl ester of VIII

h. Methyl 2,3-dideoxy-2-(2-carboxyphenyl)amino-3-nitro-ß-D-glucopyranoside (X)

i. The methyl ester of X

j. The 2-(4-carboxyphenyl)amino isomer of X

k. Methyl 2,3-diamino-2,3-dideoxy-ß-D-glucopyranoside dihydrochloride

l. Methyl 3-amino-2-(2-carboxyphenyl)amino-2,3-dideoxy-ß-D-glucopyranoside dihydrochloride

m. Methyl 2,3-dideoxy-2-(2-methoxycarbonylphenyl)amino-3-nitro-ß-D-mannopyranoside monohydrate

n. Methyl 2,3-diamino-2,3-dideoxy-ß-D-mannopyranoside dihydrochloride (XVII)

o. The diacetamido derivative of XVII

p. 2,3-Diamino-2,3-dideoxy-D-mannose dihydrochloride

q. Addition product of o-aminobenzoic acid to methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-threo-hex-2-enopyranoside

5. A theory is proposed which would account for the yellow color of methyl 4,6-O-benzylidene-2-(2-carboxyphenyl)-amino-2,3-dideoxy-3-nitro-ß-D-mannopyranoside and some of its derivatives.

6. A by-product obtained by Baer and Neilson (32) in the addition of ammonia to methyl 4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-erythro-hex-2-enopyranoside was found to possess manno configuration by converting it to methyl 2,3-diacetamido-2,3-dideoxy-ß-D-mannopyranoside, a compound whose configuration had been established during these studies.

Two new compounds were thereby obtained:

a. Methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-3-nitro-ß-D-mannopyranoside

and

b. Methyl 2-acetamido-2,3-dideoxy-3-nitro-ß-D-mannopyranoside.

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