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THE REACTION OF CARBOHYDRATES WITH AMMONIA

A thesis presented in partial fulfilment

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in Chemistry at

Massey University

David John Giltrap

1969

ABSTRACT

A feature of typical carbohydrate/ammonia reactions is the formation of complex mixtures of imidazoles (among other products). These imidazole mixtures have proved difficult to separate in many cases. A theory for cation exchange chromatography of bases has been developed in this work and applied to the separation of imidazole mixtures. The technique used appears to be capable of separating mixtures of imidazoles more effectively than other previously used.

D-Glucosone (D-arabo-hexosulose) was prepared by the action of benzaldehyde on glucosazone (d-arabo-hexosephenylosazone) and its reaction with ammonia investigated. It was found that the reaction mixture included a number of imidazoles. These imidazoles were separated by the ion exchange technique developed earlier and a total of sixteen compounds giving a positive reaction with the imidazole-specific. Pauly reagent (diazotised sulphanilic acid) were detected. Fifteen of these compounds were isolated and six were identified by mass spectrometry and/or nuclear magnetic resovance spectrometry.

It was also intended to investigate the reaction of 4-O-methyl-D-glucose and ammonia. It was proposed to prepare this compound by methylation of methyl-2,3,4-tri-O-acetyl- -D-glucopyranoside with methyl iodide in the presence of silver oxide. Under these conditions an acetyl migration from the 4-O to 6-O position occurs with the methylation to give methyl-2,3,6-tri-O-acetyl-4-O-methyl- -D-glucopyranoside which may be hydrolysed to give 4-O-methyl-D-glucose. It was intended to prepare the starting material for this reaction (methyl-2,3,4-tri-O-acetyl- -D-glucopyranoside) from D-glucose by the following steps.

(1) Methanolysis of D-glucose catalysed by an H^+ cation exchange resin to give methyl- -D-glucopyrunoside.

(2) Blocking of the 6-O position with triphenylchloromethane.

(3) Acetylation with acetic anbydride to give methyl-6-O-triphenylmethyl-2,3,4-tri-O-acetyl- -D-glucopyranoside.

(4) Removal of the triphenylmethyl blocking group to give the required methyl-2,3,4-tri-O-acetyl- D-glucopyranoside.

In fact at the time of this writing the first three steps had been accomplished but attempts to remove the triphenylmethyl blocking group while leaving the ocetyl groups intact had proved unsuccessful.

ACKNOWLEDGEMENT

The author wishes to thank Dr. E.L. Richards for encouragement and advice.

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Alkaline Degradation of Sugars.

Simple sugars under alkaline conditions, undergo a series of reactions known as the Lobry de Bruyn-Alberda Van Ekenstein reaction[1,2]. The transformation converts aldoses to ketoses and other isomeric aldoses. The general mechanism is as outlined below (fig. 1).

The important features are that the reactions are all reversible and that the assymetry of carbon 2 in the aldose is destroyed in the enediolate form so that the epimeric aldose - that is the aldose with opposite steriochemistry at carbon 2 will also be formed.

In general the transformation can proceed a step further that is the 2-ketose can form a further enediolate anion which will give rise to a further series of products.

However this second step will be slower as the enediolate form has two substituents in a cis position. In fact with hexoses 3-ketoses will not generally be found although inversion of stereochemistry at carbon 3 will occur. This means a total of 6 sugars will exist in equilibrium along with the ions of three enediols (see fig. 1).

For example if R= D-erythro-trihydroxypropyl then the six sugars will be - D-glucose, D-fructose, D-Mannose, D-allose, D-Allulose (D-psicose) and D-altrose.

In general monosaccharises will form groups of this type. Each group will consist of two 2-ketoses and four aldoses. Under alkaline conditions all members of these groups will be in equilibrium.

A number of side reactions will generally occur as well as this transformation.

Aldolisation and Dealdolisation.

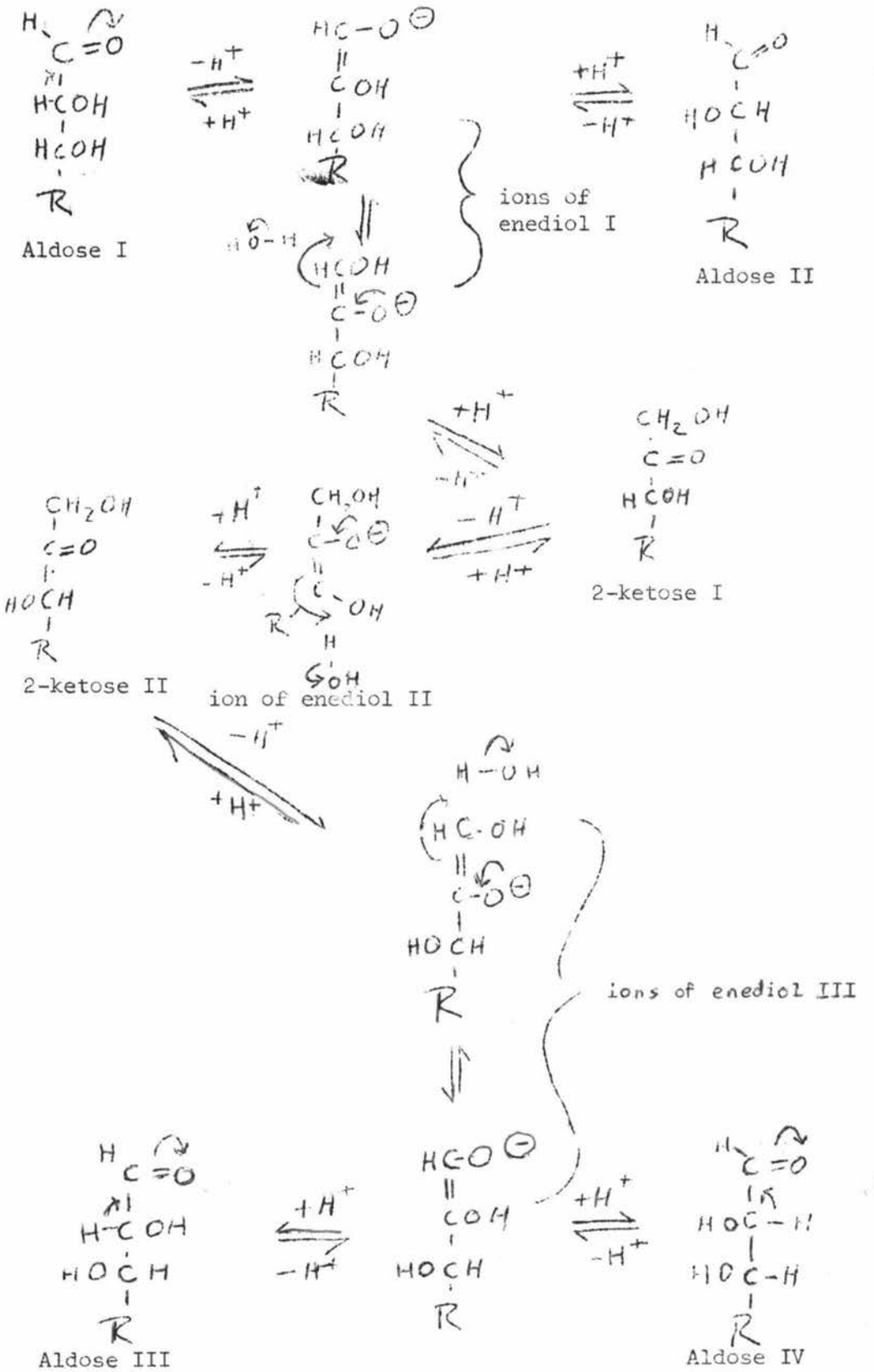
Under basic conditions an evolate ion may add to an aldehyde to yield an aldol.

The aldol formed is characterised by a carbonyl and a secondary (or primary) alcohol 1,3 to one another. The reaction is reversible and hence any compound of this type may be degraded by this mechanism. The forward reaction is termed aldolisation and the reverse dealdolisation. (see fig. 2).

Most monosaccharides are in fact aldols of this type and will undergo dealdolisation.

An aldohexose would be expected to yield a biose and a tetrose while

(fig. 1). Lobry de Bruyn - Alberds Van Ekenstein Reaction.



a 2-ketohexose should yield two trioses.

However, since aldoses and 2-ketoses will be in equilibrium by virtue of the Lobry de Bruyn-Alberden Van Ekenstein reaction, both sets of products will be formed. Similarly pentoses will give trioses and bioses, trioses may eliminate formaldehyde to give a biose, tetroses may give bioses or trioses and formaldehyde. Aldolisation on the other hand will result in the formation of the larger sugars from the smaller.

The aldolisation reaction in general creates two new assymmetric centres and so the combination of the forward and reverse reaction permits further isomerisation of the sugars (see fig. 3).

Formation of α-Desoxyosones

If an ionised enol has an alcohol or other suitable leaving group in the β position then β elimination may occur. If the starting compound was in fact on enediol then the product will be the enol of a desoxyosone[3] (see fig. 4). In general a sugar can give rise to three different types of desoxyosone (see fig. 4). Although for a triose type II desoxyosones cannot be formed while types I and III will be equivalent, and for a tetrose types II and III will be equivalent. Substitution may also block (or activate) the formation of some of these types.

Reactions of the Desoxyosones

(i)Alkaline fission.

1,2 dicarbonyl compounds may undergo a hydrolytic cleavage in alkaline solution to yield a carboxylic acid and an aldehyde (fig. 5).

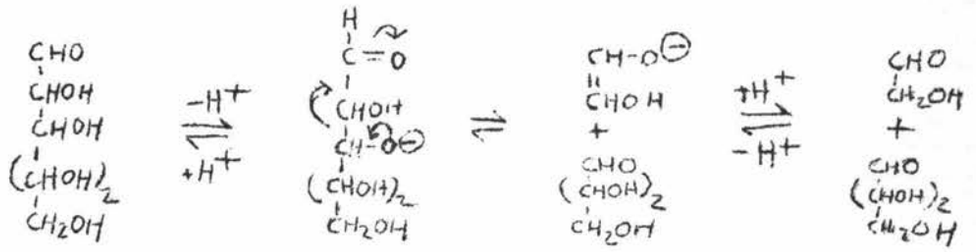
In general an unsymetrical dicarbonyl will be able to undergo fission to give two sets of products depending on which carbonyl forms the carboxyl function.

(ii) Saccharinic Acid Formation

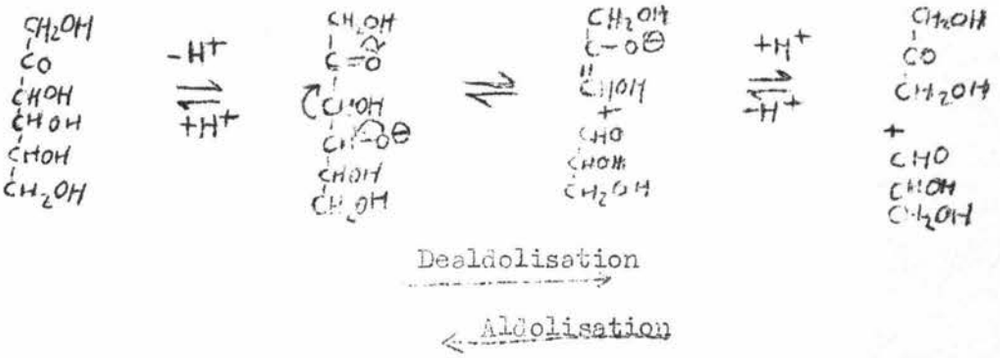
α-Dicarbonyls may undergo a "benzilic acid rearrangement" (fig. 2). The desoxyosones of types I, II and III will undergo this reaction in the presence of Calcium hydroxide to yield metasaccharinates,

(fig. 2.) Mechanism of the aldol Condensation for hexoses.

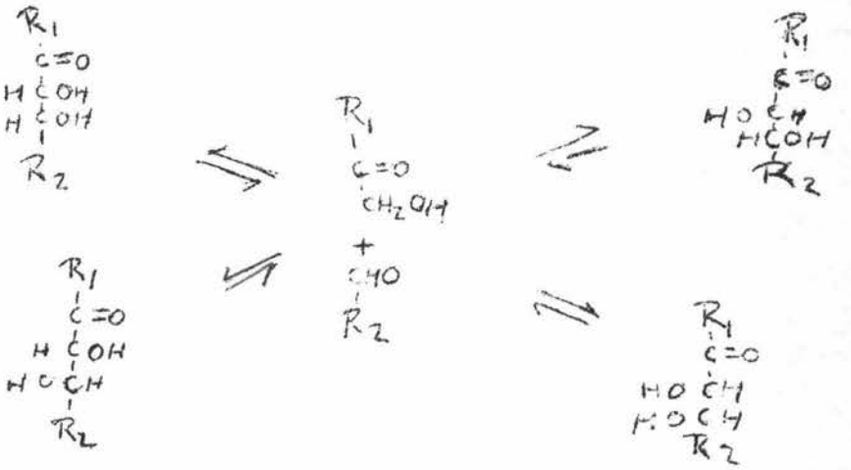
I Aldohexose



II 2-Ketohexose



(Fig. 3) Isomerisation by Aldol Condensation



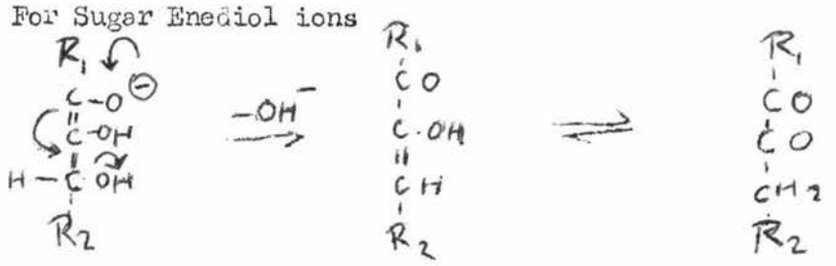
(fig. 4) β-Elimination Mechanism

I. General Reaction

ionised enol

L = OH , OR etc.

II. For Sugar Enediol ions



enediol ion

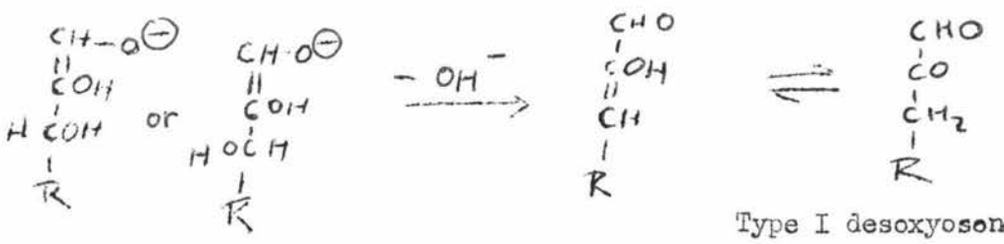
Desoxyosone

III. Different types of ∝ Desoxyosone from Sugars.

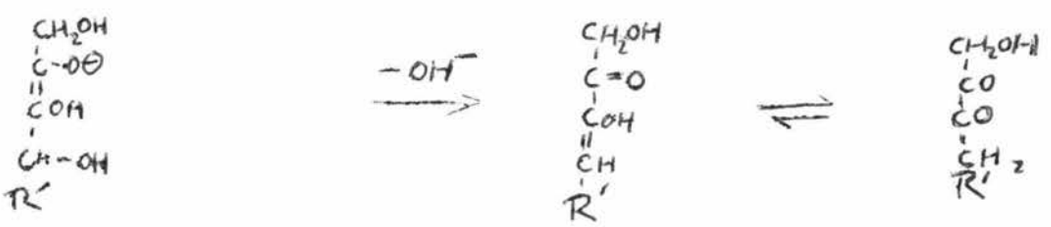
Enediolate ion arising

from Lobry de Bruyn

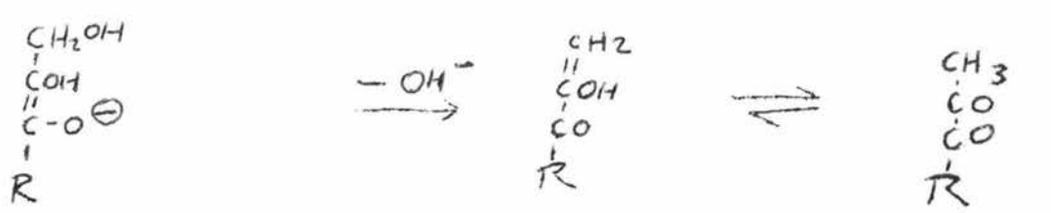
Reaction (see fig.I)



Type I desoxyosone

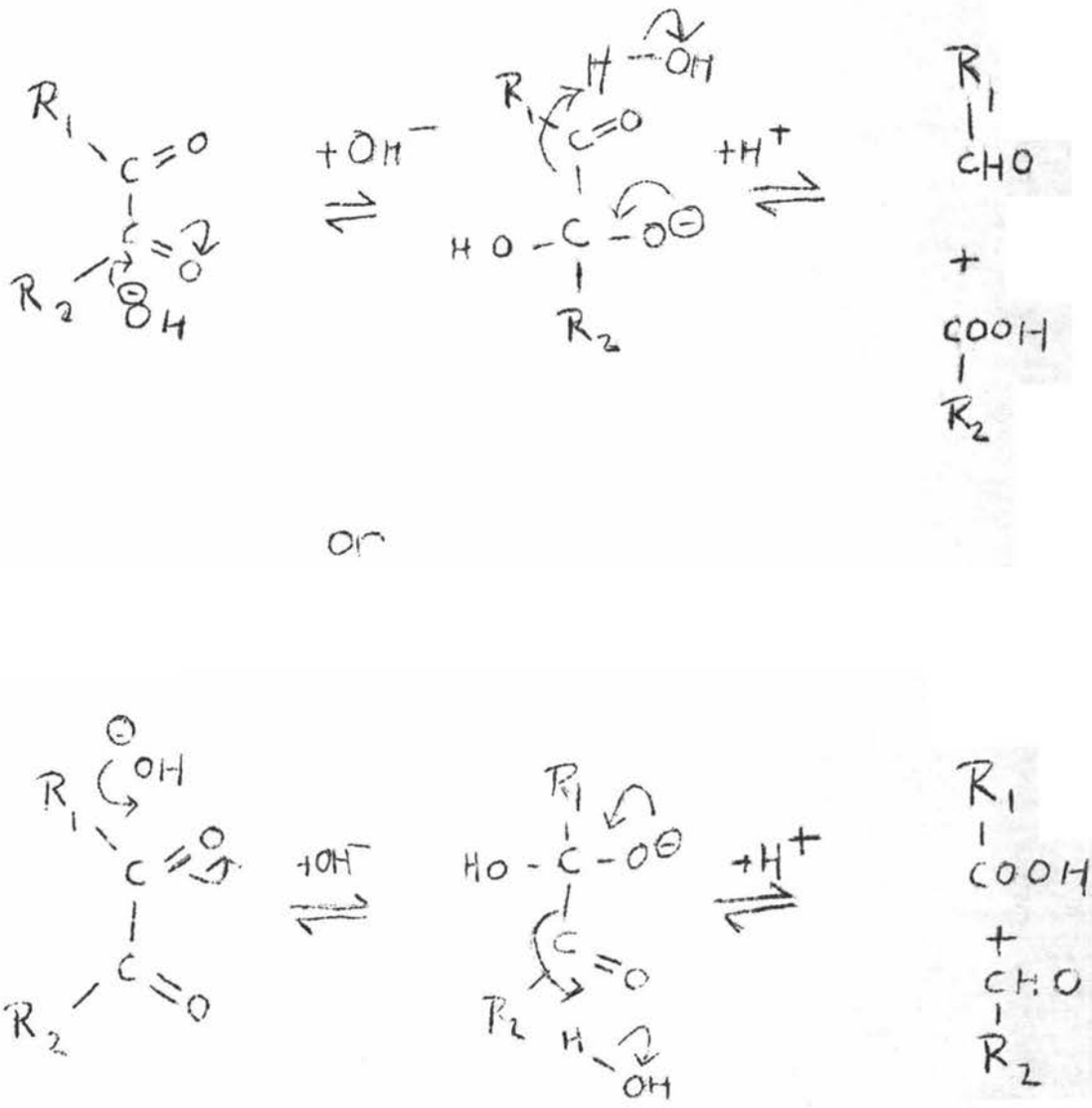


Type II desoxyosone



Type III desoxyosone

(fig. 5) Alkaline Fission of Dicarbonyls



isosaccharinates and saccharinates respectively.

The Effect of Substitution

O-Substituted sugars may be prevented from forming some or all of the desoxyosone types discussed above or alternatively may be activated towards a formation of some particular desoxyosone.

In general substitution of an oxygen will block formation of a carbonyl function on that oxygen but in many cases will activate loss of the substituted oxygen.

For these reasons 1-O or 2-O substituted sugars may also be prevented from undergoing the full range of Lobry de Bruyn-Alberda Van Ekenstein reactions.

The effects of various types of substitution in hexoses is discussed below (see also fig. 7).

(1) Non-reducing hexoses

These sugars lack a free carbonyl function and will therefore be unable to form the enediolate ion which is the intermediate in both the Lobry de Bruyn reactions and formation of α desoxyosones.

They will therefore not give rise to hexose desoxyosones or to any compounds arising from them.

(2) 1-O-substituted 2-ketohexoses

These compounds are blocked from forming aldoses or type I desoxyosones (both require a 1 carbonyl function). Types II or III desoxyosones may be formed but the substitution will favour formation of type III (1 desoxy). For example 1-O-methyl-D-fructose gives D-glucosaccharinic acid when reacted with Calcium hydroxide[4].

(3) 2-O-substituted aldohexoses

All three types of desoxyosones contain a 2 carbonyl function and hence a 2-O-substituted aldohexose should be blocked from forming hexose α desoxyosones.

(4) 3-O-substituted hexoses

These are blocked from forming desoxyosones of types II or III but are activated towards forming type I desoxyosones. Since the full

range of Lobry de Bruyn reactions as discussed previously may occur it is immaterial whether the starting product is a 2-ketose or an aldose. However all the six hexoses normally in equilibrium under these conditions will give the same desoxyosone, e.g. glucose, fructose, mannose, allose, altrose and allulose would all give D-erythro 3 desoxyhexosulose as the type I desoxyosone. In the presence of calcium hydroxide 3-O-substituted hexoses will form metasaccharinates[5].

(5) 4-O-substituted hexoses

A 4-O-substituent will not block any of the normal Lobry de Bruyn transformations or desoxyosone formations discussed above. However the substituted oxygen will be activated as a leaving group in the β -elimination reaction and hence formation of type II desoxyosones should be favoured. In fact it is found that 4-O substituted hexoses form iso-saccharinates with calcium hydroxide[6]

(6) 6-O-substituted hexoses

5-O- and 6-O-substitution would not be expected to exert any direct effect on the formation of hexose α desoxyosones but it is found that 6-O-substitution tends to activate dealdolisation of a hexose to give two trioses. In the presence of calcium hydroxide these trioses will give rise to lactic acid.

FORMATION OF HETEROCYCLIC COMPOUNDS

Imidazoles

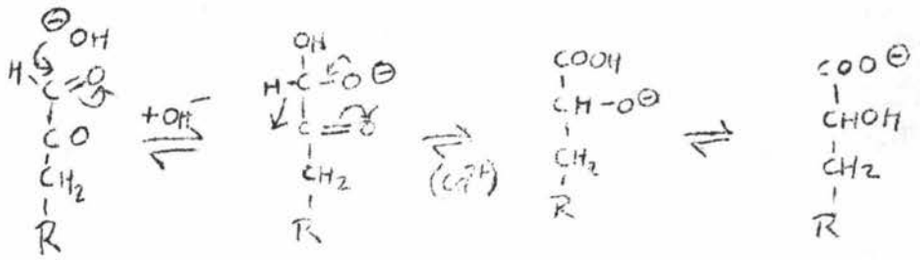
If ammonia is used as the base for degradation of sugars imidazoles are formed. This phenomenon was originally observed by Windaus and Knoop[8] who isolated 4 (5) methylimidazole from a reaction mixture of glucose, szinc hydroxide and ammonia.

The mechanism appears to involve an aldehyde an α-dicarbonyl and two molecules of ammonia (fig. 8).

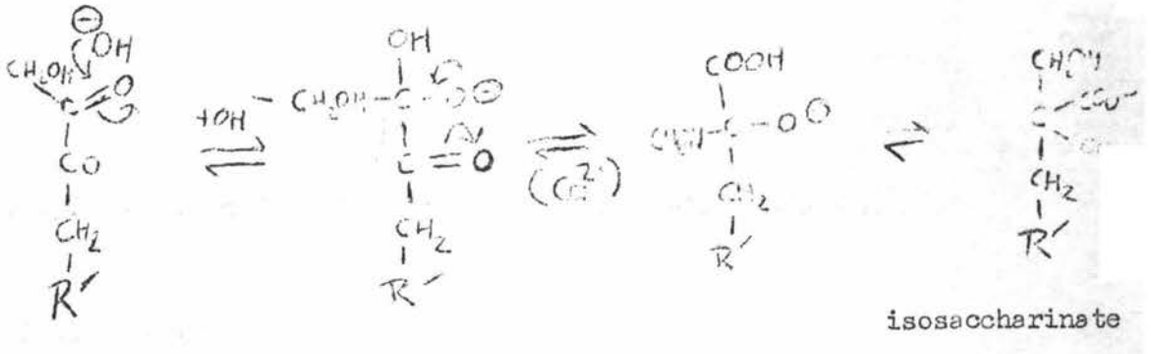
A number of imidazoles have been isolated from sugar/ammonia reactions. A mechanism involving a dicarbonyl compound and an aldehyde should permit the formation of a large number of different imidazoles since a number of aldehydes may be formed (by dealdolisation, possibly

(fig. 6). Saccharinic Acid Formation from -Desoxyosones.

Type I Desoxyosone

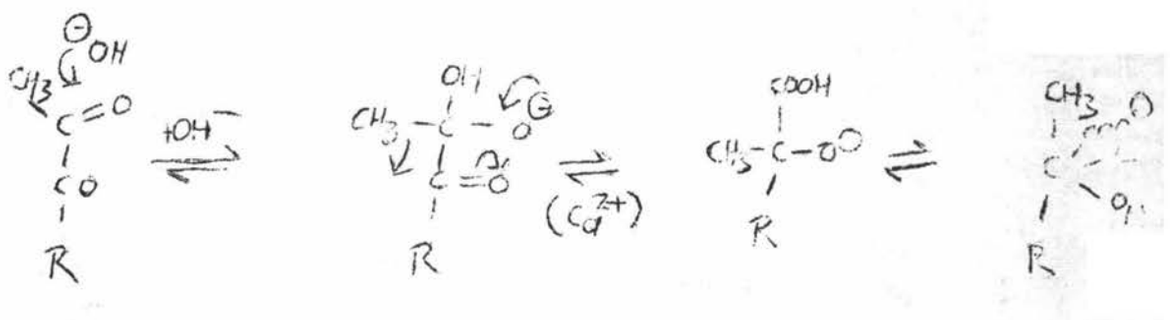


Type II Desoxyosone



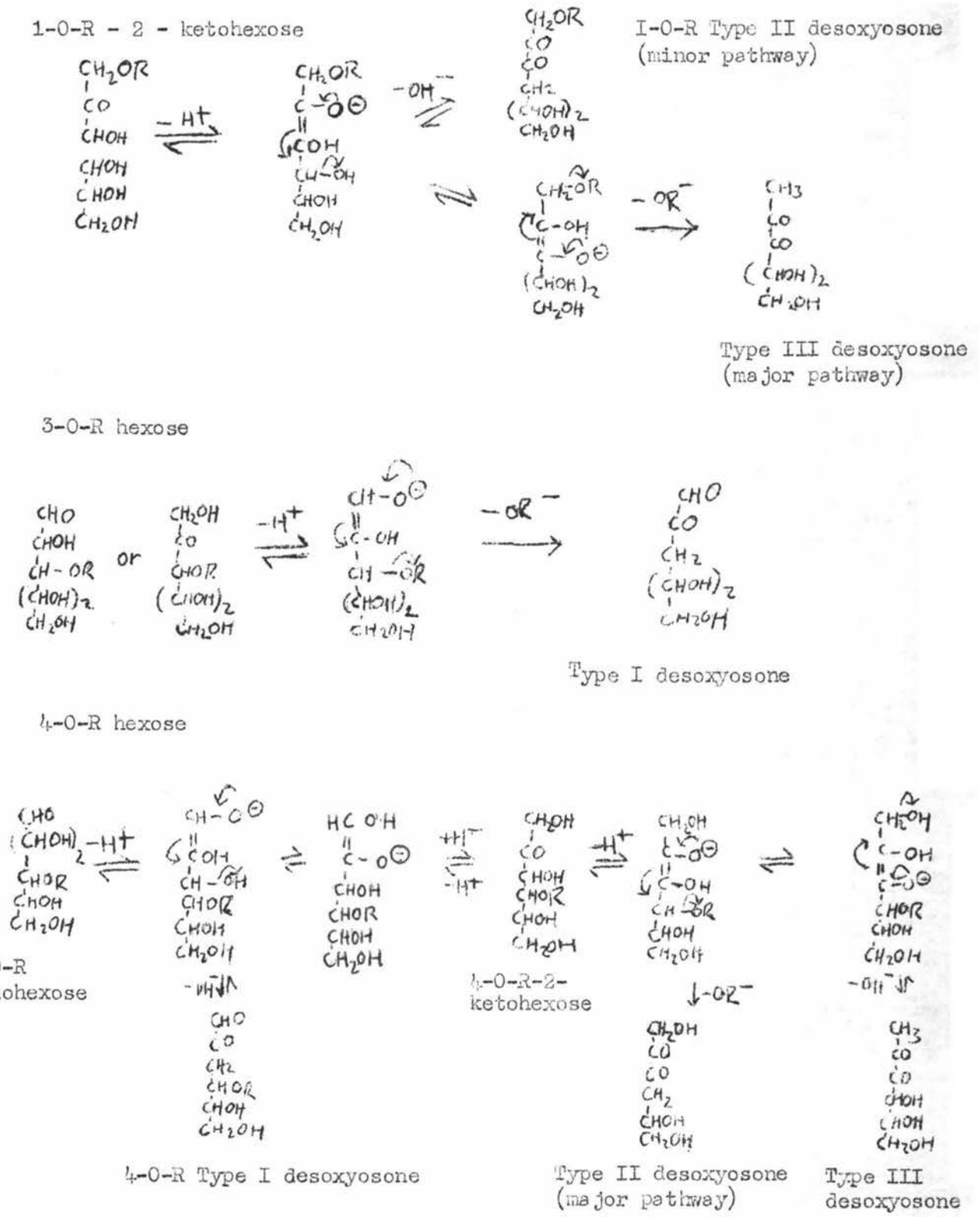
isosaccharinate

Type III Desoxyosone



Saccharinate

(fig. 7) The effect of Substitution on Desoxyosone Formation



followed by epimerisation or by alkaline fission of dicarbonyls), any of the three types of ∝-desoxyosone may be formed for each group of related sugars (i.e. glucose, mannose, fructose, allose altrose and allulose etc.) and by virtue of dealdolisation and aldolisation a considerable number of such groups may be present. In principle any combination of an aldehyde and a dicarbonyl should give a distinct imidazole. In most cases the mixtures of imidazoles formed are complex although substitution of the starting material may reduce the number of imidazoles formed in significant amounts.

3-O-substituted hexoses can give rise only to type I hexose desoxyosones although dealdolisation permits formation of other desoxyosones from the fragments. Formaldehyde is generally the most common aldehyde and hence quite good yields of a particular ismidazole (4) can sometimes be obtained.

e.g. 3-O-methyl D-Glucose[9] gives a good yield of 4(5) (D-erythro-2,3,4-trihydroxybutyl)imidazole as the major basic product.

2-O-methyl aldohexoses will be unable to form hexose desoxyosones and the only imidazoles formed will be derived from dealdolisation fragments.

The complexity of the mixtures formed in most cases, however, has meant that many of the imidazoles formed have not yet been identified.

Separation of the imidazoles from one another and from other bases has been a major problem.

Cellulose and alumina column chromatography have been used with some success but a number of imidazoles, particularly those with longer polyhydroxyalky sidechains, have proved to be slow moving and hence difficult to separate by these means.

The system D-glucose/ammonia was the first investigated and has been examined by a number of workers under a variety of conditions. However in 1965, sixty years after Windaus and Knoop's original discovery of the formation of 4(5)-methylimidazole only nine

imidazoles had been found in the system [10]. Of these only two (2-hydroxymethyl-4(5)-methylimidazole and 2,4 (2,5) dimethlimidazole) were substituted in the 2 position and these were also the only disubstituted imidazoles found. Only two compounds (4(5)(2,3,4-trihyroxybutyl) imidazole and 4(5) (D-arabo-tetrahydroxybutyl) imidazole) of six carbons or larger were conclusively identified. It seems highly probably that a considerable number of the imidazoles formed in this system have yet to be isolated.

Separation of imidazoles by ion exchange chromatography has been employed in this work. Using this technique it appears possible to separate complex mistures of imidazoles (including polyhydroxyalkyl imidazoles). This technique was applied to the reaction mixture of D-glucosone (D-arabino-hexosulose and ammonia. Fifteen imidazoles were isolated from this system and five of these were identified by mass spectrometry and nuclear magnetic resonance spectrometry.

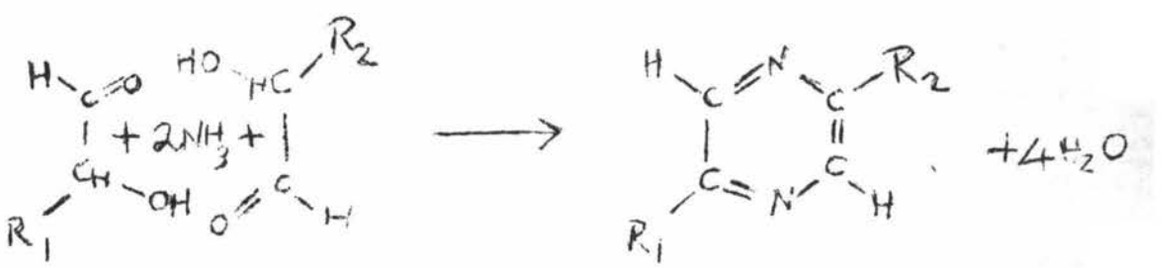
OTHER HETEROCYCLIC COMPOUNDS

In addition to imidazoles hyrazines [11,12] and pyrazines [12] have been found in sugar/ammonia reaction mixtures. The probable mechanism of pyrazine formation involves the actual sugars as starting materials (fig 8).

Imidazoles

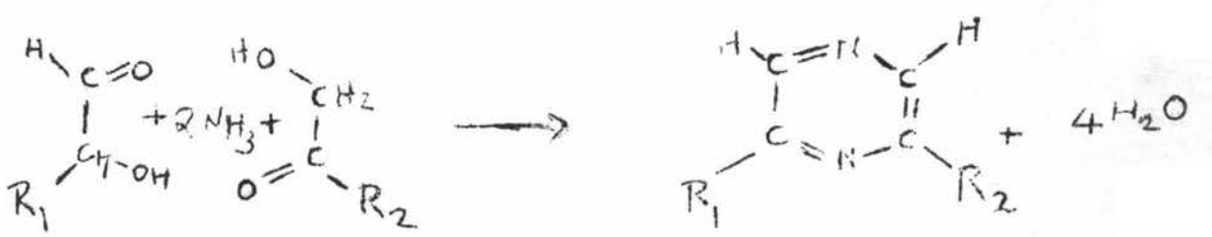
-Dicarbonyl aldehyde imidazole

Pyrazines



pyrazine

OR



Pyrazine

SEPARATION OF IMIDAZOLES BY CATION EXCHANGE CHROMATOGRAPHY

The reaction of reducing sugars with ammonia forms a complex mixture which includes, as well as neutral and acidic compounds, imidazoles and, to a lesser extent, pyrazines and pyridines.

The bases may be separated from the neutral and acidic compounds on a cation exchange resin in the H form. The bases will form positively charged conjugate acids which will be retained on the resin while the neutrals and acids may be washed off. The bases may then be removed from the resin by eluting with ammonia.

The individual bases have previously been separated from this mixture by cellulose or alumina column chromatography with various solvents. These methods, and also most paper and thin layer systems, all appear to depend largely on the overall polar or non-polar character of the whole molecule and a strong correlation appears among the R_f values in all these systems. In particular a number of imidazoles arising from sugar/ammonia reactions have low R_f values in all these systems and hence are difficult to separate. Further, since the R_f values depend largely on the same molecular properties for all the above systems, the use of two such methods in succession rarely gives a much better separation than that obtained with a single system.

Ion exchange chromatography was considered as a means of providing a more general basis of separation. In principle the rate of elution of an imidazole in this system should depend principally on the basicity of the imidazole nucleus, and for any base, it should be possible to devise a set of conditions to give any desired rate of elution. The rate of elution of a base in any given set of conditions would be characteristic of that base.

Further since ion-exchange chromatography depends on different molecular properties to the techniques mentioned earlier it should be possible to combine it with one of them (e.g. thick paper chromatography or T.L.C.) to form an effective two dimensional system.

However, in ion-exchange work the rates of elution will depend on a number of parameters which will vary over a wide range, so that it is highly desirable to be able to predict the elution rate of any compound under any set of conditions.

The following simple theory appears to give good qualitative agreement with experiment and appears to be adequate for the prediction of the ability of a given system to separate a given mixture of bases.

THEORY OF ION-EXCHANGE SEPARATION OF BASES

Consider a solution in equilibrium with a cation-exchnage resin (single function) and let the cation exchange capacity of the resin be C_R milliequivalents per ml. of solution in equilibrium with the resin.

Let the solution contain n cationic species X_i $(i=1,\cdots,n)$ and m anionic species X_{-i} $(i=1,\cdots,m)$

let Z_i = the charge of X_i

C_i moles/litre = the concentration of X_i in the solution.

let f_i = the fraction of the resin capacity utilised by X_i $(i<0 \Rightarrow f_i=0)$

let $C_i^R = f_i C_R$

$$\sum_{i=1}^{n} f_i = 1 \quad \text{(by definition } f_i\text{)} \qquad (1)$$

$$\sum_{i=1}^{n} Z_i C_i + \sum_{i=1}^{m} Z_{-i} C_{-i} = 0 \quad \text{(since solution is electrically neutral)} \qquad (2)$$

$$\text{let } C_S = \sum_{i=1}^{n} Z_i C_i = -\sum_{i=1}^{m} Z_{-i} C_{-i} \qquad (3)$$

If X_i is a positively charged Brønsted acid let B_i be its conjugate base and let C'_i moles/litre = concentration of B_i in the solution. If X_i is not a positively charged Brønsted acid let $C'_i = 0$.

let $X_1 = H_3O^+$

and $\forall i : 2 \le i \le n$

$$\text{let } K_i = \frac{C'_i C_1}{C_i} \qquad (4)$$

(i.e. K_i = acid dissociation constant of X_i)

$$\text{let } C^S_i = C'_i + C_i \qquad (5)$$

then

$$C^S_i = \frac{C_i}{C_1}(K_i + C_1) \quad \text{(from (4) \& (5))} \qquad (6)$$

Consider the special case where

$$Z_i = 1 \quad \forall i : 1 \le i \le n$$

and assume that in this case

$$\frac{C_i}{C_1} = \alpha_i \frac{f_i}{f_1} \quad \forall i : 1 \le i \le n \qquad (7)$$

where α_i is a constant $\forall i$

then $C_i^S = \alpha_i \frac{f_i}{f_1}(K_i + C_1)$ (from (7) & (6))

and $C_i^R = C_R f_i$ (definition)

let $p_i = \frac{C_i^S}{C_i^R}$

then $p_i = \frac{\alpha_i}{f_1}\frac{(K_i + C_1)}{C_R}$ (8)

also from (7)

$$\sum_{i=1}^{n} \alpha_i f_i = \frac{f_1}{C_1} \sum_{i=1}^{n} C_1$$

let $\sum_{i=1}^{n} \alpha_i f_i = \bar{\alpha}$

then $\bar{\alpha} = \frac{f_1 C_S}{C_1}$

and from (8)

$$p_i = \frac{\alpha_i C_S (K_i + C_1)}{C_1 \bar{\alpha} C_R} \qquad (9)$$

The function p_i determines the elution rate of X_i on an ion-exchange column. The only functions of X_i on which p_i depends are α_i and K_i. In fact α_i will not differ very greatly for related compounds and the separation depends largely on K_i. But $p_i \propto (K_i + C_1)$ and therefore if $C_1 >> K_i$, p_i is virtually independent of K_i, and p_i is fully sensitive to changes in K_i only if $K_i >> C_1$

Assume that α_i is constant for $i = 2, \ldots, n$ and assume further that for these values

$$\alpha_n = \alpha_{n-1} = \cdots = \alpha_2 = \frac{K_R + 1}{K_R}$$

where K_R is the acid dissociation constant of the cation exchange functional group. (e.g. for a polystyrenesulphonic acid resin such as Dowex 50 W $K_R \doteq 0.2$

$$\therefore \alpha_n = \cdots = \alpha_2 \doteq 6$$

let $\alpha = \frac{\alpha_2}{\bar{\alpha}} = \cdots = \frac{\alpha_n}{\bar{\alpha}} = \frac{K_R + 1}{\bar{\alpha} K_R}$

but $\bar{\alpha} = \sum_{i=1}^{n} f_i \alpha_i = f_1 + \sum_{i=2}^{n} f_i \cdot \frac{K_R + 1}{K_R}$

$$= \frac{K_R f_1 + (K_R + 1)(1 - f_1)}{K_R}$$

$$= \frac{K_R + 1 - f_1}{K_R}$$

hence $$\alpha = \frac{K_R + 1}{K_R + 1 - f_1} \qquad (10)$$

and from (9)

$$\forall i : 2 \le i \le n \quad p_i = \alpha \frac{C_s(K_i + C_1)}{C_R C_1} \qquad (11)$$

also if $K_R >> 1$ or if $f_1 << 1$

$$\alpha \doteq 1$$

and then $\forall i : 2 \le i \le n$

$$p_i = \frac{C_s(K_i + C_1)}{C_R C_1} \qquad (12)$$

If the solution is being run down a column of ion exchange resin then if the peak of the compound X_i has run a volume V_i' while a total volume V has been run through the resin.

then $\frac{dv_i}{dV} = \frac{p_i}{p_{i+1}}$ where p_i is calculated for the conditions actually occuring at Volume V_i'. The sharpness of the peak may be enhanced by an increasing gradient in f_1 running down the column. However this will only occur if the actual values of f_1 are reasonably high.

For imidazoles K_i is typically 10^{-6} to 10^{-8} hence for reasonable separation pH cannot be less than about 6 and it can be this low only for the more acidic imidazoles. ($C_1 >> K_i \Rightarrow$ poor separation). Assume $C_1 = 10^{-6}$ but from (7)

$$f_1 = C_1 \sum_{i=1}^{n} \frac{\alpha_i f_i}{C_s}$$

if Dowex 50W used as resin then $\alpha_1 = 1, \alpha_2 = \cdots = \alpha_n \doteq 6$

$$1 \le \sum_{i=1}^{n} \alpha_i f_i \le 6$$

hence $$f_1 C_s < 6 \times 10^{-6} \qquad (13)$$

also $K_i \le 10^{-6}$ for typical imidazoles

hence $\frac{K_i + C_1}{C_1} \le 2$

$$p_i \le 2\alpha \frac{C_s}{C_R}$$

where $\alpha = \frac{1 \cdot 2}{1 \cdot 2 - f_1}$

$C_R \simeq 0.7$ for Dowex 50W

$$C_s \ge \frac{p_i \times 0.7}{2\alpha}$$

assume $f_1 << 1 \cdot 2$

then $\alpha = 1$

$$C_S \geq \tfrac{1}{3} P_i$$

but for satisfactory rate we may require $p_i > 10^{-2}$

then $C_S > \frac{1}{3} \times 10^{-2}$

$f_1 < 1{\cdot}8 \times 10^{-3}$ (from (13))

this justifies assumption that $f_1 << 1{\cdot}2$ also since f_1 is small at all times when elution is occuring at a reasonable rate there will be no point in starting with $f_1 = 1$ and so developing a gradient in f_1 For separation of imidazoles on a Dowex 50 resin time may be saved by starting with the resin more or less in equilibrium with the resin. Further under these conditions $f_1 << 1 + K_R \therefore \alpha \fallingdotseq 1$ and equation (12) holds.

CRITERIA FOR SEPARATION

In general for a set of eluting conditions the peak of any compound X_i will be eluted from a column of ion-exchange resin after a total volume V_i ml. has been run. This value V_i will be characteristic of the compound for any given conditions. V_i will depend on K_i. Two compounds X_i and X_j will be separated if $|V_i - V_j| \geq l$ where l is some arbitrary limit depending on the degree of separation required, the rate at which the column is run, the size of fractions collected etc. V_i is a monotone decreasing function of K_i (that is if $K_j \geq K_i$ then $V_i \geq V_j$ i.e. or the more acidic compound is always eluted first). If we require

$$|V_i - V_j| \geq l$$

then $$|V_i(K_i) - V_j(K_j)| \geq l$$

We may require that separation to the extent of $|V_i - V_j| \geq l$ occurs whenever $|K_i - K_j| \geq d$

in particular if $K_i - K_j = d$

$$V_j - V_i \geq l$$

This is satisfied if $-\left(\frac{dV_i}{dK_i}\right) \geq \frac{l}{d}$

then $$\alpha \geq -\frac{\ell}{\left(\frac{dV_i}{dK_i}\right)}$$

and the quantity $$-\frac{\ell}{\left(\frac{dV_i}{dK_i}\right)}$$

is the lower limit of the difference in K_i for separation.

now V_i and K_i are related by the equation

$$\int_0^{V_i} \frac{p_i}{1+p_i}\, dV = V_R \qquad (14)$$

where V_R = volume of the column

but from (12)

$$p_i = \frac{C_s(K_i + C_1)}{C_R C_1}$$

if $$\gamma_s = \frac{C_s}{C_R C_1}$$

and $$p_o = \frac{C_s}{C_R}$$

then $$p_i = \gamma_s K_i + p_o$$

$$\frac{p_i}{1+p_i} = \frac{\gamma_s K_i + p_o}{1+\gamma_s K_i + p_o}$$

but in practice $p_o \ll 1$ and therefore either $\gamma_s K_i \ll 1$

or $p_o \ll \gamma_s K_i$

$$\frac{p_i}{1+p_i} = \frac{\gamma_s K_i}{1+\gamma_s K_i} + p_o \qquad (15)$$

then substituting in (14)

$$\int_0^{V_i} \frac{\gamma_s K_i}{1+\gamma_s K_i}\, dV + \int_0^{V_i} p_o\, dV = V_R$$

or $$\int_0^{V_i} \frac{\gamma_s K_i}{1+\gamma_s K_i}\, dV = V_R - \int_0^{V_i} p_i\, dV \qquad (16)$$

differentiating (16) w.r.t. K_i

$$\int_0^{V_i} \frac{\gamma_s\, dV}{(1+\gamma_s K_i)^2} + \frac{dV_i}{dK_i}\left(\frac{\gamma_s K_i}{1+\gamma_s K_i} + p_o\right) = 0$$

but $p_o \ll 1$ $\therefore$ either $\gamma_s K_i \ll 1$ or $p_o \ll \frac{\gamma_s K_i}{1+\gamma_s K_i}$

$$\therefore \int_0^{V_i} \frac{\gamma_s \, dV}{(1+\gamma_s K_i)^2} + \frac{dV_i}{dK_i}\left(\frac{\gamma_s K_i + p_o}{1+\gamma_s K_i}\right) = 0 \qquad (17)$$

As an index of separation take

$$\sigma = \frac{K_i}{\ell}\frac{dV_i}{dK_i}$$

that is $\sigma = \frac{K_i}{d'}$ where d' is the least difference in K_i for satisfactory separation

then $$\sigma = \frac{\int_0^{V_i} \frac{\gamma_s K_i \, dV}{(1+\gamma_s K_i)^2}}{\ell\left(\frac{\gamma_s(V_i) K_i + p_o(V_i)}{1+\gamma_s(V_i) K_i}\right)}$$

$$\sigma \geq \sigma' = \frac{\int_0^{V_i} \frac{\gamma_s K_i \, dV}{(1+\gamma_s K_i)(1+\gamma_s(V_i) K_i)}}{\ell\left(\frac{\gamma_s(V_i) K_i + p_o(V_i)}{1+\gamma_s(V_i) K_i}\right)}$$

(If γ_s is a monotone increasing function of V)

then $$\sigma' = \frac{\int_0^{V_i} \frac{\gamma_s K_i \, dV}{(1+\gamma_s K_i)}}{\ell\left(\gamma_s(V_i) K_i + p_o(V_i)\right)}$$

$$\sigma' = \frac{V_R - \int_0^{V_i} p_o \, dV}{\ell\left(\gamma_s(V_i) K_i + p_o(V_i)\right)} \qquad (18)$$

where $$\int_0^{V_i}\left(\frac{\gamma_s K_i}{1+\gamma_s K_i} + p_o\right) dV = V_R$$

Provided that the column is run at a sufficiently low loading the peaks will be narrow and two compounds will be resolved by a minimum volume ℓ if the proportional difference in K_i for these compounds is greater than $\frac{1}{\sigma'}$

In the special case where γ_s, p_o are constant,

then $$V_i\left(\frac{\gamma_s K_i}{1+\gamma_s K_i} + p_o\right) = V_R$$

or when $p_o \ll 1$

$$V_i\left(\frac{\gamma_s K_i + p_o}{1+\gamma_s K_i}\right) = V_R$$

$$\therefore V_i \gamma_s K_i + p_o V_i = V_R + \gamma_s K_i V_R$$

$$\gamma_s K_i (V_i - V_R) = V_R - p_o V_i$$

$$\int_0^{V_\alpha} \frac{\gamma_s(V)\,dV}{\gamma_s(V_\alpha)+\gamma_s(V)} > V_R$$

The upper limit of volume for resolution occurs by virtue of the $V_R - \int_0^{V_\alpha} P_0 dV$ term in σ' and in all cases this may be expressed by the condition.

$$aV_R > \int_0^{V_\alpha} P_0 dV \qquad \text{where } 0 < a < 1$$

Then in general it will be adequate to require

$$\frac{1}{a}\int_0^{V_\alpha} P_0 dV < V_R < \int_0^{V_\alpha} \frac{\gamma_s(V)\,dV}{\gamma_s(V_\alpha)+\gamma_s(V)} \qquad (21)$$

to ensure reasonable separation of compounds of reasonably different K_i values. However if it is desired to calculate conditions for a specific minimum degree of separation equation (18) should be used.

SEPARATION OF AN IMIDAZOLE MIXTURE

The ion-exchange technique described above was tested on a mixture of imidazoles made up for this purpose. The mixture nominally contained four components but the purity of some of these was highly questionable and in fact ten products were separated.

The mixture was separated on an NH_4^+ form polystyrenesulphonate resin eluting with ammonium bicarbonate buffers with γ_s ranging from 10^6 to 1.6×10^9 and pH ranging from 8.0 to 9.2

Fractions were collected and examined by paper chromatography. A total of ten compounds were detected by this procedure and these compounds were largely separated by the ion exchange procedure.

THE REACTION OF D-GLUCOSONE AND AMMONIA

D-Glucosone was prepared from D-glucose in two steps. Glucose was first converted to the phenylosazone by the action of phenylhydrazine and the phenylosazone was then converted to D-glucose with benzaldehyde 13 (see fig. 9).

The glucosone prepared was then reacted with ammonia and the bases from the reaction mixture were investigated by a combination of ion-exchange chromatography and paper chromatography.

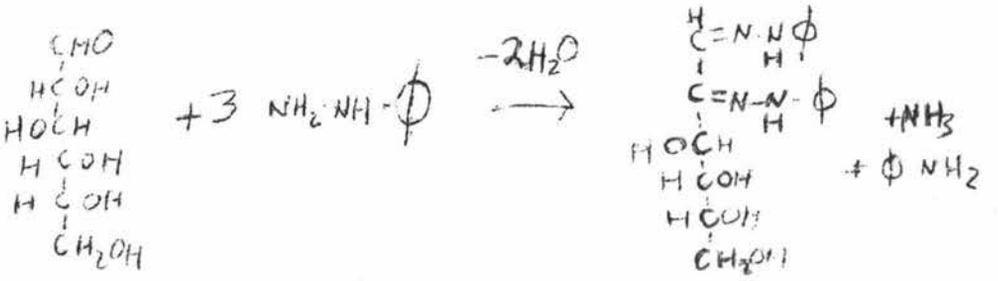
Glucosone is a 1,2 dicarbonyl compound and is rather reactive, so that it might be expected to form a range of products with ammonia readily. Further, there are no blocking groups on the molecule and so glucosone would be expected to undergo the full range of Lobry-de-Bruyn transformations and the various side reactions discussed in the introduction. It would be expected that a number of the products formed would be imidazoles since a number of 1,2 dicarbonyls (including the starting material) would be expected to occur in the reaction mixture.

A complex mixture of imidazoles was in fact found in this reaction mixture. A total of fifteen basic compounds which gave positive tests with the imidazole-specific-diazotised sulphanilic acid reagent were isolated and five of these compounds which were identified all proved to be imidazoles.

fig. 9

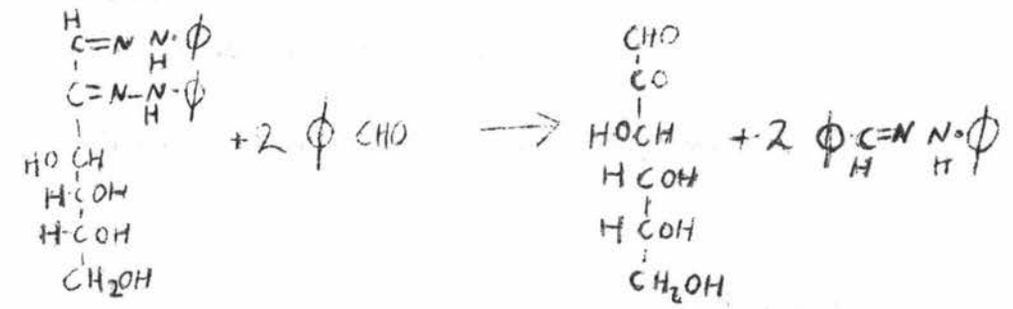
Preparation of D-Glucosone

I. Preparation of D-Arabo-Hexosephenylosazone



D-glucose phenylhydrazine D-arabo-hexosephenylosazone

II. Preparation of D-Glucosone



D-arabo-hexosephenylosazone D-glucosone

Chromatography

Solvents

Solvent A

n-Butanol (400 ml.) was mixed with glacial acetic acid (100 ml.) and water (100 ml.).

Solvent B

Ethyl acetate (300 ml.) was shaken with glacial acetic acid (100 ml) and water (300 ml.) in a 1 litre separating funnel. Two phases separated on standing. The lower phase was rejected and the upper phase used as the solvent.

Spray Reagents

Ammonial Silver Nitrate

Silver nitrate (10 gr.) was dissolved in water (c. 70 ml.), aqueous ammonia was added until the precipitate originally formed was completely dissolved and the solution was made up to 100 ml. The reagent was sprayed onto the dried paper chromatogram which was then heated. Polyhydroxyalkyl compounds (or other suitable reducing agents) gave dark spots.

Diazotised Sulphanilic Acid

Sulphanilic acid solution (9 g.p.l. in 1NHCl, 20 ml.) was mixed with sodium nitrate solution (25 g.p.l., 20 ml.) 10% sodium carbonate solution (40 ml.) was then added. The reagent was stable for only a short time (c. 10 minutes). Imidazoles gave a red, orange or yellow spot with this reagent.

General Conditions

Descending paper chromatography was used throughout this work. Unless otherwise stated Whatmans No. 1 chromatography paper and one of the solvents above were used.

PREPARATION OF D-GLUCOSONE

Reagents

Urea Phosphate Spray Reagent 14

Orthophosphoric acid (S.G. 1.75, 6 ml.) was added to water-saturated n-Butanol (100 ml.). A solution of urea (3 g.) in ethanol (5 ml.) was added. The reagent was sprayed onto the dried chromatogram which was then warmed. Ketoses gave a blue spot.

Nickel Hydroxylamine Spray Reagent 15,

Solution A

Hydroxylamine hydrochloride (50 g.) and sodium acetate (50 g.) were dissolved in water (100 ml.) and the solution was filtered. The clear filtrate constituted the reagent.

Solution B

Nickel nitrate (5 g.) was dissolved in water (100 ml.)

The dried chromatogram was sprayed with solution A, dried at 110°C for 5 minutes and then sprayed with solution B. An α-dicarbonyl compound gave a red or orange spot.

Reactions

D-Arabo-Hexosephenylosazone

D (+) Glucose (200 g.) was dissolved in water (1.5 l) in a 3 necked 3 litre flask equipped with a mechanical stirrer and a reflux condenser. Phenylhydrazine (450 ml.) and glacial acetic acid (250 ml.) were added. The mixture was then refluxed gently for 30 minutes and allowed to cool overnight.

The reaction mixture was filtered and the precipitate washed with 10% acetic acid (2x500 ml.) and water (4x1l). The precipitate was then recrystallised from 50% aqueous pyridine (3 l) the recrystallised product washed with absolute ethanol (3x250 ml.) and diethyl ether (2x250 ml.) and finally dried in a vacuum oven to give D-Arabo-hexosephenylosazone (250 g.) with a melting point of 199-201°C.

A further recrystallisation from absolute ethonol lowered the yield to 150 g. and raised the m.p. to 204-205°C.

D-Arabo-hexosulose (D-Glucosone)

D-Arabo-hexosephenylosazone (m.p. 204-5°C, 20 g.) suspended in absolute ethanol (600 ml.) was poured into water (1 l.) in a 3 litre 3 necked flask fitted with a stirrer and reflux condenser. Benzaldehyde (32 ml.) and glacial acetic acid (12 ml.) were added and the mixture refluxed for 4 hours. The condenser was then reversed and condensate (c. 600 ml.) was distilled off the reaction mixture over about 1 hour with simultaneous addition of water (1 l.) The mixture was then siphoned into a 3 litre beaker and allowed to cool overnight.

The mixture was filtered, the filtrate concentrated to about 400 ml. at 40-45°C and then extracted with diethyl ether (6 x 75 ml.) The aqueous phase was warmed to 60°C to remove dissolved ether and then shaken for 10 minutes with activated charcoal (5 g.). The mixture was filtered and the filtrate concentrated, at 35-40°C, to a thick syrup which was dissolved in 95% ethanol (210 ml.). The solution was filtered and the filtrate concentrated, at 35-40°C, to a thin syrup which was dissolved in water (200 ml.) and then shaken with Amberlite IR 120 H^+ cation exchange resin (5 g.) and IR4B OH^- anion exchange resin (2 g.). The mixture was filtered and the filtrate concentrated to a syrup (3 g.) which was dried by repeated evaporation from methanol (5 X).

Paper chromatography with solvent A revealed two spots. The first occured at R_f 0.20 and gave a dark spot with silver nitrate, a brown spot with urea phosphate, an orange-red spot with nickel/ hydroxylamine and a yellow spot with p-Anisidine hydrochloride. The second occured at R_f 0.5 and gave a dark spot with silver nitrate but appeared to give no reaction with the other reagents.

The final product from three runs as above was combined and dissolved in 75% aqueous ethanol. A solid crystallised out. The mixture was filtered and the filtrate evaporated, at 35-40°C, to a thick syrup (4 g.) which was dried by repeated evaporation from

methanol (5 X).

This product gave only a single spot on paper chromatography. This was the compound running at R_f 0.20 and gave the same colour reactions as before. This product was therefore taken to be pure D-Glucosone.

SEPARATION OF IMIDAZOLES

A mixture of histamine dihydrochloride (75 mg.) 4(5)-(D-erythro-2,3,4 trihydroxybutylimidazole (40 mg. doubtful purity), 4 (5) hydroxymethylimidazole (15 mg.) and 2-methylimidazole (10 mg.) was dissolved in a small volume (c. 10 ml.) of water.

AG 50 W/X2 H^+ cation exchange resin (70 g. wet weight) was washed with 3N ammonia (100 ml.), distilled water (6 x 500 ml.) and .007M pH8.0 ammonium carbonate buffer (200 ml.). The resin was slurried with .007M pH 8.0 ammonium carbonate buffer (200 ml.) and poured into a column (20 x 250 mm.).

The imidazole solution was diluted to 100 ml. and a solution of carbon dioxide in acetone was added to adjust the pH to 6.5. The pH was further adjusted to 6.0 with 10% acetic acid. This solution was run through the column and followed by ammonium carbonate buffer solutions of :- 0.007 pH 8.0 (800 ml.), 0.007M pH 9.0 (400 ml.), 0.03 M pH 9.0 (400 ml.), 0.3M pH 9.2 (400 ml.) and 0.7 M pH 9.2 (400 ml.).

10 ml. fractions were collected and examined by paper chromatography using solvent A. The imidazoles were located by spraying with diazotised sulphanilic acid. A total of ten compounds were located (see table I) and in most cases the ion exchange procedure provided complete separation.

TABLE I

RESULTS OF SEPARATION OF IMIDAZOLE MIXTURE

Compound No.	fractions located in	Rf (Solvent A)	Colour of Spot (Diazotised Sulph. Acid)	Probable Identification
1	7-14	0.12	Yellow	
2	26-35	0.32	Orange	4(5)hydroxymethyl imidazole
3	42-56	0.20	Red	
4	80-118	0.15	Red	
5	116-135	0.02	Yellow	
6	145-155	0.12	Red	
7	140-165	0.06	Red	
8	180-200	0.53	Yellow	2-Methylimidazole
9	220-250	0.21	Red	Histamine
10	220-250	0.07	Red	

DIAZOTISED SULPHANILIC ACID REAGENT FOR IMIDAZOLE DETERMINATION

Sulphanilic acid solution (9g.p.l. in IN HC, 0.75 ml.) was mixed with aqueous sodium nitrate (25 g.p.l. 0.75 ml.) at 0°C for 5 minutes in a 25 ml. volumetric flask.

A further quantity of the sodium nitrate solution (3 ml.) was added and the mixture allowed to stand for 5 minutes. The solution was made up to volume with cold distilled water and allowed to stand in an ice bath for at least 20 minutes before use. A fresh batch of the reagent was made up every day.

KINETIC STUDY OF THE REACTION

D-Glucosone (70 mg.) was dissolved in 25% aqueous ammonia (7 ml.) and placed in a water bath at 37°C. From time to time samples (0.1 ml.) were taken and the imidazole level determined as below.

IMIDAZOLE DETERMINATION

Diazotised sulphanilic acid reagent (0.8 ml.) was mixed for 30 seconds with 10% aqueous sodium carbonate (2.0 ml.) in a stoppered test tube. Distilled water (0.3 ml.) and the sample (0.1 ml.) were added and the optical density at 480 nm. in a 1 cm. glass cell, was determined using a Unicam SP500 Speetrophotometer. A blank, using distilled water, was freshly prepared for each determination.

RESULTS (See Fig. 10)

From the graph it can be seen that the imidazole level is more or less constant at a maximum level between 20 hours and 70 hours. Hence the reaction time of 24 hours used in the main reaction should ensure maximum imidazole formation.

PREPARATIVE REACTION

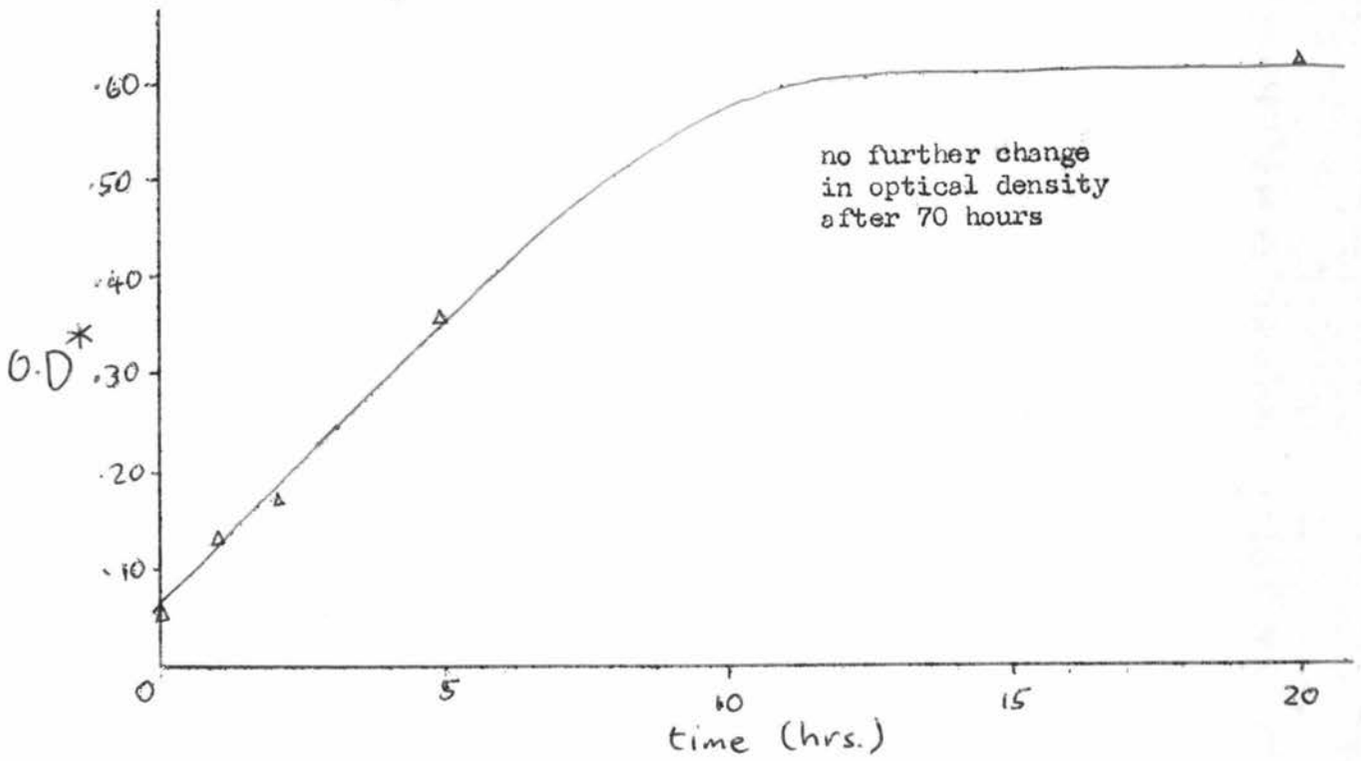
D-Glucosone (4 g.) was dissolved in 25% aqueous ammonia (400 ml.) in a 1 litre stoppered flask. The flask was kept in a water bath at 37°C for 24 hours. The mixture was then evaporated to dryness. Distilled water (3x400 ml.) was added with evaporation to dryness after each addition. After the final evaporation the mixture was dissolved in distilled water (200 ml.) and passed through a column of Amberlite

IR 120 H^+ cation exchange resin. The column was washed with water (2.5 l.) until the washings were colourless. The combined washings were evaporated to dryness to give the neutral and acidic non-volatile reaction products (2.5 g.).

The column was then eluted with 25% aqueous ammonia (4 l.) until the effluent was colourless and gave no reaction with diazotised sulphanilic acid. The eluate was evaporated to dryness and distilled water (3x400 ml.) added with evaporation to dryness after each addition to give the non-volatile basic compounds (1.0 g.)

Samples of the total reaction mixture and the two fractions obtained above were examined by paper chromatography with solvent A. Ammoniacal silver nitrate and diazotised sulphanilic acid were used as locating reagents. A total of seven spots were observed (see table II).

fig. 10 Development of Imidazole Concentration with time in Glucosone/Ammonia Reaction



*Optical density of imidazole/diazotised sulphanilic acid dye (see p. 32)

TABLE II

Spots detected in Glucosone/Ammonia Reaction Mixture by Paper Chromatography (p. 33)

P of Compound	fraction found in	AgNO3 Reaction	Diazotised Sulph. Acid Reaction
0.06	Basic	Black Spot	Red Spot
0.14	Basic	Black Spot	Red Spot
0.18	Non-Basic	Black Spot	Nil
0.24	Basic	Black Spot	Red Spot
0.35	Basic	Nil	Faint red spot
0.40	Non-Basic	Black spot	Nil
0.53	Basic	Nil	Faint red spot

ION EXCHANGE SEPARATION OF BASES

A column (20x250 mm.) of Bio-Rad AG50W/X2 NH_4^+ cation exchange resin was prepared as previously (p. 30). A saturated solution of carbon dioxide in acetone (50 ml.) was added to a solution of the bases in water (150 ml.)

The acetone and excess carbon dioxide were removed by distillation at 20°C. The pH was lowered to 6.5 with a small further quantity of the carbon dioxide in acetone and to 5.9 with 10% acetic acid. The total volume was made up to 200 ml. with distilled water and an aliquot (80 ml.) was run through the column. The column was the eluted with ammonium carbonate buffers of :- 0.007M,pH 7.5 (450 ml.); 0.007 M, pH8.3 (600 ml.); 0.007 M,pH 9.0 (500 ml.); 0.02 M, pH 9.0 (500 ml.), 0.06M, pH 9.0 (400 ml.); 0.02 M, pH9.0 (350 ml.) and 1.0M, pH 9.0 (400 ml.). 10 ml. fractions were collected.

The column was then regenerated by washing with 4N ammonium acetate (500 ml.) and distilled water (2 l.).

A sample (c. 1 ml.) of every second (or fifth after the first 50 fractions) fraction was concentrated (c 10X) and examined by paper chromatography with solvent A using diazotised sulphanilic acid as a locating reagent. A total of sixteen imidazolic compounds were detected (see table III).

The following groups of fractions were then combined:- 41-89, 90-120, 121-130, 131-139,140-160, 161-174, 175-188, 189-200. 201-210, 211-250 and 251-300. Each of the combined groups were concentrated to a small volume (c. 5 ml.).

The 41-89 group was dissolved in water (50 ml.) and carbon dioxide in acetone (30 ml.). The acetone and excess carbon dioxide were removed by evaporation at 20°C. The pH was lowered to 8.0 with carbon dioxide in acetone and 5.9 with 10% acetic acid. The solution was run through the column and followed by ammonium carbonate buffers of:- 0.007M, pH7.7 (500 ml.); 0.007, pH 8.0 (500 ml.); 0.007 M.pH 8.3 (500 ml.); 0.007M, pH 9.0 (450 ml.). 10 ml. fractions were collected

and examined by paper chromatography as before.

Compounds I,II,III and IV were detected in fractions 20-65, 40-55, 55-65 and 55-70 respectively.

The groups of fractions 16-50, 51-60 and 61-70 were recombined and concentrated to c. 5ml. each.

The thirteen concentrated solutions S_1, (fractions 16-50 from 2nd run), S_2 (51-60 from 2nd run), S_3 (fractions 61-70 from 2nd run), S_4 (fractions 90-120 from 1st run), S_5 (fractions 121-130 from 1st run), S_6 (fractions 131-139 from 1st run), S_7 (fractions 140-160 from 1st run), S_8 (fractions 161-174 from 1st run), S_9 (fractions 175-188 from 1st run) S_{10}(fractions 189-200 from 1st run), S_{11} (fractions 201-210 from 1st run) S_{12}(fractions 211-250 from 1st run), and S_{13} (fractions 251-300 from 1st run) were each separated by descending thick paper chromatography on Whatamns No. 3MM papers with solvent A. Marker spots were run on either side of the band containing the mixture and these were sprayed with diazotised sulphanilic acid to locate the compounds. The strips containing each compound were then cut out and eluted with water. The isolated compounds were examined by paper chromatography with solvents A and B. Fifteen of the sixteen compounds were isolated by this means (see table IV).

Compounds I, IV, VI, XI, XII, XIII and XV were examined by mass spectrometry and I, IV and VI by nuclear magnetic resonance spectrometry. The results obtained were as given below.

TABLE III:- COMPOUNDS DETECTED IN FRACTIONS FROM ION-EXCHANGE CHROMATOGRAPHY

Compound No.	fractions detected in	R_{Im} * solvent A	Colour with Diazotised Sulphanilic Acid
I	40-90	0.16	red
II	50-85	0.60	red
III	70-90	0.72	red
IV	50-130	0.34	red
V	90-105	1.15	red
VI	100-130	0.68	red
VII	125-135	1.00	red
VIII	135-140	1.30	red
IX	140-155	0.51	red
X	140-160	1.40	red
XI	140-165	0.75	red
XII	160-190	1.01	red
XIII	170-195	1.60	red
XIV	190-220	0.20	orange yellow
XV	215-245	1.50	red
XVI	240-260	0.29	red

* see overleaf

TABLE IV:- COMPOUNDS ISOLATED FROM GLUCOSONE/AMMONIA REACTION

Compound No.	Concentrated solutions isolated from.	Total Yield (mg.	R_{Im}* Solvent A	R_{Im}* Solvent B	Colour with Diazotised Sulphanilic Acid
I	$S, S_2, S_3,$	20	0.13	0.00	red
II	$S_1, S_2,$	6	0.62	0.37	red
III	$S_2, S_3,$	3	0.72	0.52	red
IV	$S_2, S_3, S_4, S_5,$	25	0.26	0.10	red
V	S_4	21	0.97	0.85	red
VI	$S_4, S_5,$	22	0.87	0.60	red
VII	$S_5, S_6,$	6	0.86	0.78	red
VIII	S_6	2	1.17	0.95	red
IX	S_7	6	0.56	0.33	red
XI	$S_6, S_7, S_8,$	13	0.76	0.54	red
XII	$S_8, S_9, S_{10},$	14	1.02	0.91	red
XIII	$S_8, S_9, S_{10},$	10	1.44	1.28	red
XIV	$S_{10}, S_{11},$	5	0.11	0.05	orange
XV	$S_{12},$	9	1.36	1.29	red
XVI	S_{12}, S_{13}	7	0.22	0.15	red

* R_{Im} = the ratio of the distance run by a compound to the distance run by imidazole under the same conditions.

RESULTS

Compound I

Mass Spectrometry

Peaks at	272	$C_{11} H_{16} N_2 O_6$	242 (?) 272 → 199
	213	$C_9 H_{13} N_2 O_4$	242, 272 → 213
	199	$C_8 H_{11} N_2 O_4$	181, 199(S), 213? → 139
	181	$C_8 H_9 N_2 O_3$	170, 181, 199, 213(S) → 153
	153	$C_7 H_9 N_2 O_2$	
	139	$C_6 H_7 N_2 O_2$	
	97	$C_4 H_5 N_2 O$	

Metastable peaks were found for

272 → 213, 272 → 199, 242 → 213, 213 → 195, 213 → 153, 181 → 153, 181 → 139.

NMR indicated a ratio of tertiary to secondary protons of 3:2.

Compound IV

The mass spectrum gave two series of peaks.

Series I		Series II	
200	$C_8 H_{12} N_2 O_4$	170	$C_7 H_{10} N_2 O_3$
191	$C_6 H_9 N_2 O_2$	97	$C_4 H_5 N_2 O$
127	$C_5 H_7 N_2 O_2$	69	$C_3 H_5 N_2$
111	$C_5 H_7 N_2 O$		
109	$C_5 H_5 N_2 O$		

Metastable Peaks were also found for

170 → 97, 97 → 69 200 → 127, 127 → 109

The NMR spectrum was inconclusive and gave a probable ratio of secondary to tertiary protons of 2:1.

Compound VI

The Mass Spectrum gave peaks at:-

170	$C_7 H_{10} N_2 O_3$
111	$C_5 H_7 N_2 O$
97	$C_4 H_5 N_2 O$

Compound VI (con'td.)

82	$C_4 H_6 N_2$
81	$C_4 H_5 N_2$
69	$C_3 H_5 N_2$

Metastable peaks were found for the transitions

170 → 97, 97 → 69, 111 → 82, 82 → 81.

No meaningful data could be obtained from NMR spectrum.

Compound XI

The mass spectrum gave peaks at:-

142	$C_6 H_{10} N_2 O_2$	and
124	$C_6 H_8 N_2 O$	142, 111 → 82
112	$C_5 H_8 N_2 O$	142 (?), 124, 112 → 95
111	$C_5 H_7 N_2 O$	
95	$C_5 H_7 N_2$	
82	$C_4 H_6 N_2$	
81	$C_4 H_5 N_2$	

Metastable peaks were found for 124 → 95 and 112 → 95

Compound XII

The mass spectrum gave peaks at

112	$C_5 H_8 N_2 O$	112 → 111
111	$C_5 H_7 N_2 O$	112, 82 → 81
82	$C_4 H_6 N_2$	112, 82 → 81
81	$C_4 H_5 N_2$	

The ratio of the intensities of 112 : 82 was about 1 : 3.

Compound XIII

The mass spectrum gave the peaks for

112	$C_5 H_8 N_2 O$
111	$C_5 H_7 N_2 O$
82	$C_4 H_6 N_2$
81	$C_4 H_5 N_2$

the ratio of intensities of 112 : 82 was about 1 : 0.9

Compound XV

Molecular ion at 82 = $C_4 H_6 N_2$

and spectrum identified to a known spectrum for methyl imidazole.

IDENTIFICATION OF PRODUCTS

Compound I 2,4(2,5)bis(tetrahydroxybutyl) imidazole

The formula $C_{11}H_{16}N_2O_6$ contains five double bond equivalents. An imidazole ring contains three double bond equivalents and would also contain both Ns and 3 C's. This leaves sidechains containing in total 8 C's, 6 O's and 2 double bond equivalents. This would probably mean that the sidechains were originally saturated and contained 8 O's (i.e. fully hydroxylated) but had undergone a double dehydration to give the peak found. (This behavious is normal for imidazoles with reasonably large polyhydroxyalkyl sidechains and indeed molecular ions are rarely found with such compounds or with carbohydrates in general.).

Losses of up to 3 - carbon fragments in single steps (272 → 199, 213 → 139) but no losses of fragments larger than this were observed.

This probably indicates that no sidechain was longer than 4 carbons.

Also the NMR spectrum indicated a ratio of secondary : tertiary protons of 2: 3. If there were a total of n sidechains then there would be n $-CH_2CH$ groups and 8- n $\backslash CHOH$ groups giving 2 n secondary protons and 8-n tertiary protons. The ratio

2n: 8-n = 2:3 implied n = 2

and hence there were two sidechains. But since neither sidechains was longer than 4 carbons and since the two sidechains totalled 8 carbons both sidechains must have contained 4 carbons and since there was a 1:1 ratio between sidechains carbons and oxygens each must in fact have been a tetrahydroxybutyl group. Hence compound I was a bis (tetrahydroxybutyl) imidazole. Of the two possible compounds 2,4(2,5)-bis (tetrahydroxybutyl)imidazole and 4,5-bis (tetrahydroxybutyl) imidazole, the latter would require a 10 carbon precursor and so may be assumed to be highly unlikely to have been formed. Hence I was

assumed to be 2,4(2,5)-bis (tetrahydroxybutyl) imidazole.

The detailed mass spectrum was explained as in fig. 11.

Compound IV

This was probably a mixture of two compounds as the two series of peaks appeared to be unrelated. As a result, with the small quantities of compound available it was not possible to satisfactorily determine the structure.

Compound VI 4(5) - (tetrahydroxybutyl) imidazole

The mass spectrum indicated an imidazole of formula $C_7 H_{12} N_2 O_4$ which would give rise to the peak at 170 by dehydration.

Single step loss of fragments of up to 3 carbons in length occured without appearing to break down the imidazole nucleus. This indicated that the imidazole contained a 4 carbon sidechain and hence there was only one sidechain which would be a tetrahydroxybutyl group. Compound VI was therefore identified as 4(5) tetrahydroxybutylimidazole.

The detailed mass spectrum was explained as in fig. 11.

Compound XI 4(5)-(2,3-dihydroxypropyl) imidazole.

The mass spectrum peak at 142 ($C_6 H_{10} N_2 O_2$) corresponds to an imidazole with saturated sidechains totalling 3 carbons and 2 oxygens.

The single step loss of 2 carbons and 2 oxygens (142 → 82) implied that the compound had a 3 - carbon sidechain and that the 2 oxygens were located on the 2 carbons furthest from the imidazole nucleus.

Compound XI was therefore identified as 4(5) - (2,3-dihydroxypropyl) imidazole. The detailed mass spectrum was explained as in fig. 11.

Compounds XII 4(5)-(2-hydroxyethyl) imidazole and XIII 2-hydroxymethyl - 4(5)-methylimidazole.

Both these compounds gave molecular ions at 112 ($C_5 H_8 N_2 O$) and similar fragmentation patterns. However compound XII gave a much higher ratio of peak intensities at 82:112 than did XIII indicating that the loss of formaldehyde (112 - 82) occured much more readily for XII than XIII.

The formula $C_5H_8N_2O$ could represent a (1-hydroxyethyl) imidazole, a (2-hydroxyethyl) imidazole or a hydroxymethyl-methylimidazole. However, a (1-hydroxyethyl) imidazole could not lose formaldehyde while a (2-hydroxyethyl) imidazole would be expected to lose formaldehyde more readily than a hydroxymethyl-methylimidazole.

In fact the chromatographic data would indicate that XII was probably 4(5)-(2 hydroxyethyl) imidazole (expected R_{Im}'s in solvents A & B 0.99 & 0.80, found 1.02 and 0.91) while XIII was probably 2-hydroxymethyl-4(5) methylimidazole (expected R_{Im}'s 1.37 & 1.11, found 1.44 and 1.28).

The mass spectra were explained as in fig. 11.

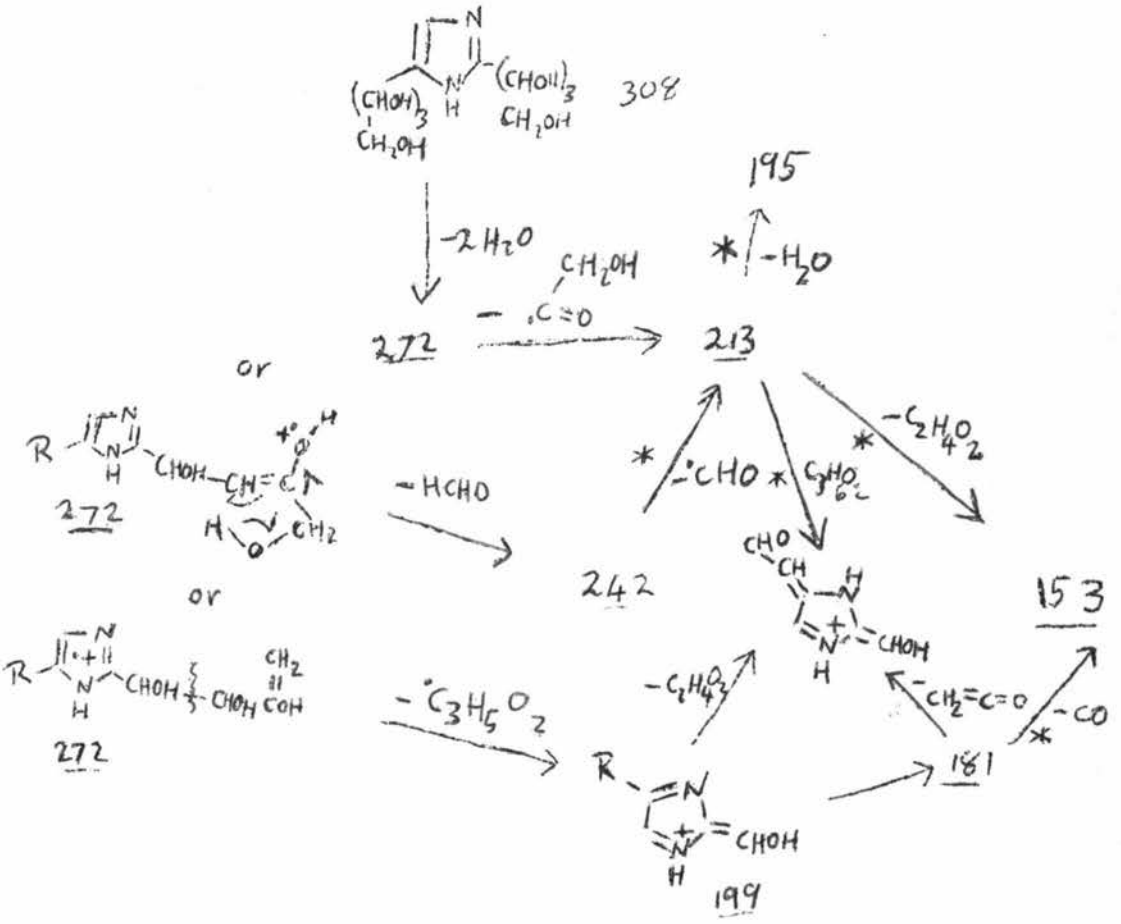
Compound XV 4(5)-methylimidazole

The molecular ion at 82 $C_4H_6N_2$ must have been a methylimidazole Of the two possible methylimidazoles 2-methylimidazole gives a yellow spot with diazotised sulphanilic acid at R_{Im}'s of 1.44 and 1.11 with Solvents A and B respectively while 4(5)-methylimidazole gives a red spot at R_{Im}'s of 1.35 and 1.12.

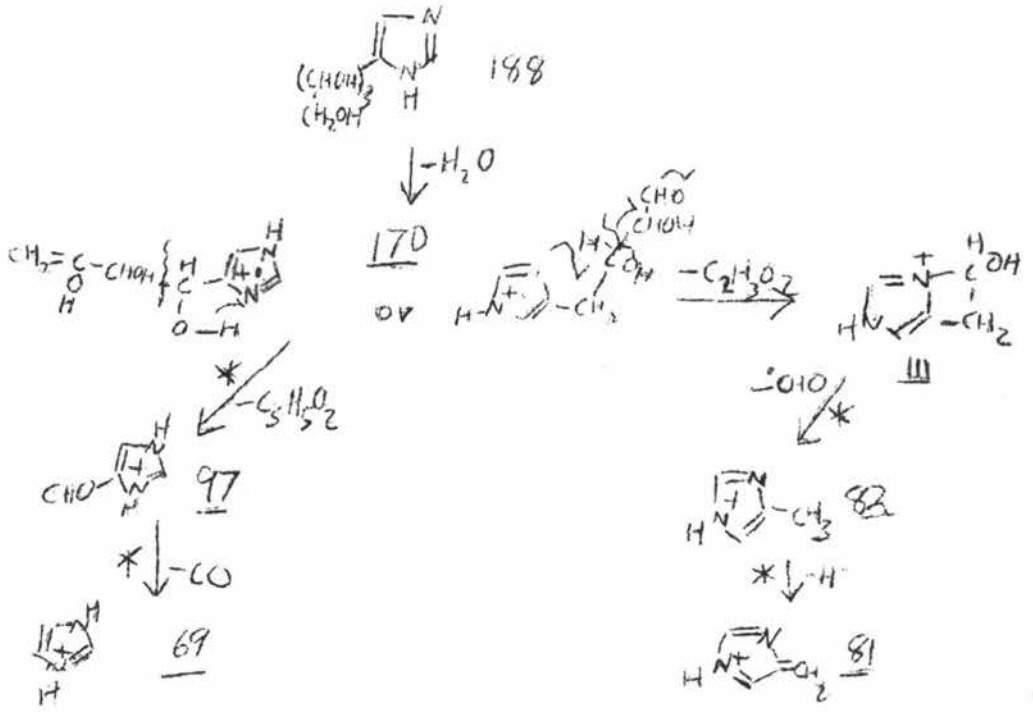
Compound XV gave a red spot at R_{Im}'s of 1.36 and 1.29 and so was identified as 4(5)-methylimidazole.

fig 11 Mass Spectra of Identified Imidazoles

I 2,4(2,5)-bis (tetrahydroxybutyl) imidazole



VI 4(5)-tetrahydroxybutylimidazole

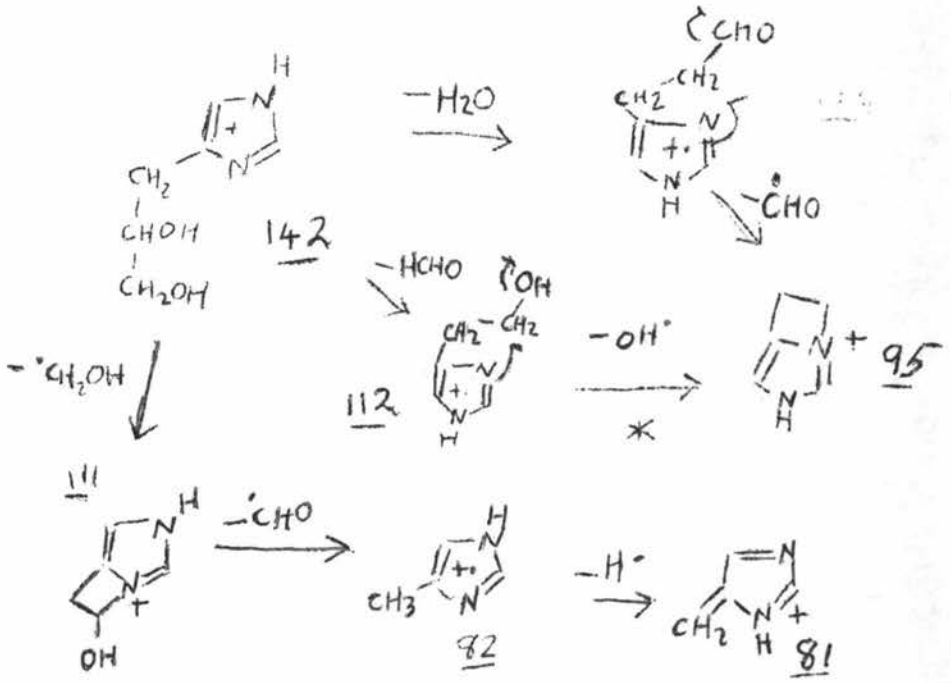


* indicates that a metastable peak was found for the transition

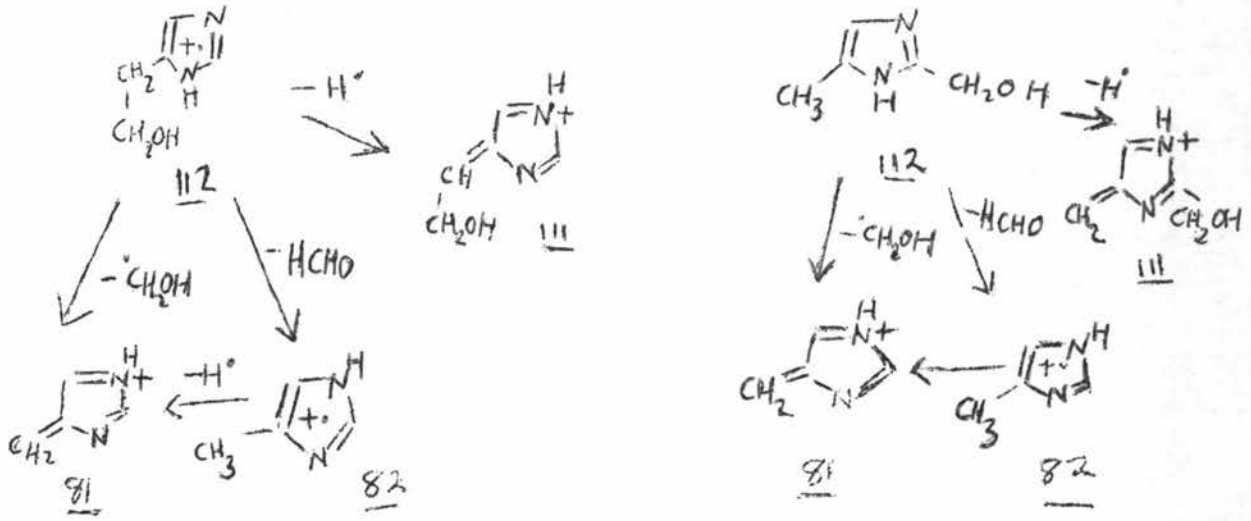
P.T.O.

fig. 11 continued

XI 4(5)-(2,3-dihydroxypropyl) imidazole



XII 4(5)-(2-hydroxyethyl)imidazole XIII 2-hydroxymethyl-4(5)-methylimidazole



*indicates that a metastable peak was found for the transition

D-GLUCOSONE/AMMONIA REACTION

Ths six compounds identified from the reaction mixture are shown in fig. 12 along with their probable precursors in the reaction mixture.

The most common aldehyde appears to be formaldehyde and this would arise from a variety of reactions including alkaline fission of D-Glucosone itself and of any other α-keto-aldehydes formed in the reaction mixture. Arabinose could arise from alkaline fission of D-Glucosone and this is a precursor of compound I. Glycolaldehyde (precursor of compound XIII) may be formed by dealdolisation of arabinose or by alkaline clearage of hydroxypyruraldehyde which would be formed by dealdolisation of glucosone.

The dicarbonyl precursors of the imidazoles identified include glucosone itself (compounds I and VI) 3-desoxypentosulose (compound XI), 3-desoxytetrosulose (compound XII) and pyruvaldehyde (compounds XIII and XV). The 3-desoxyosones could arise by the β- elimination mechanism (see introduction) on pentoses, tetroces and trioses respectively these sugars being formed by aldolisation/dealdolisation reactions. The full mechanism for imidazole formation in the glucosone/ammonia reaction would be expected to include the pathways shown in fig. 13.

fig. 12

Precursors of Imidazoles Identified in D-Glucosone/Ammonia System.

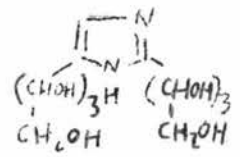
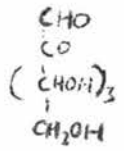
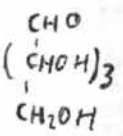
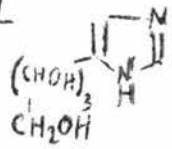
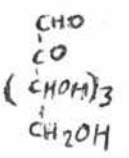
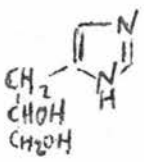
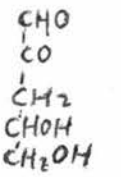
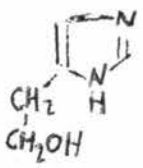
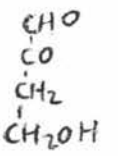
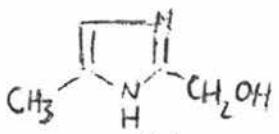
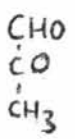
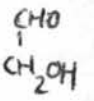
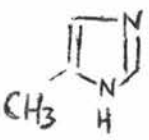
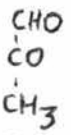
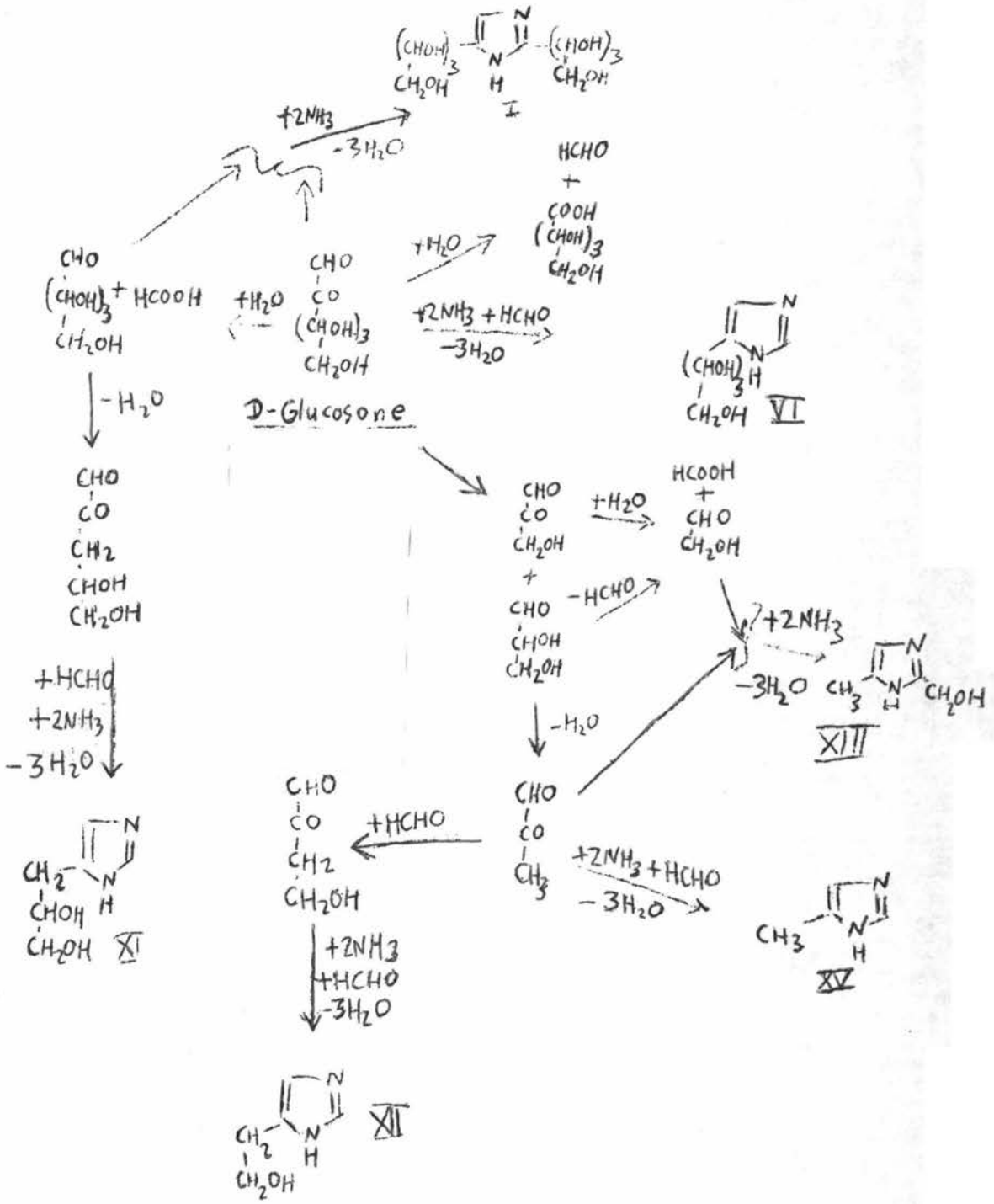
Imidazole		Precursors	
I 	←		+ 
I 2,4(2,5)bis (tetrahydroxybutyl imidazole		D-glucosone	D-Arabinose
VI 	←		+ HCHO
4(5)-(tetrahydroxybutyl)imidazole		D-Glucosone	formaldehyde
XI 	←		+ HCHO
4(5)-(2,3-dihydroxypropyl) imidazole		3-Desoxypentosulose	formaldehyde
XII 	←		+ HCHO
4(5)-(2-hydroxyethyl)imidazole		3-Desoxytetrosulose	formaldehyde
XIII 	←		+ 
2-hydroxymethyl-4(5)-methylimidazole		pyruraldehyde	glycolaldehyde
XV 	←		HCHO
4(5)-methylimidazole		pyruvaldehyde	formaldehyde

fig. 13

Formation of Imidazoles from D-Glucosone
(Partial reaction scheme)



I= 2,4(2,5)-bis(tetrahydroxybutylimidazole VI=4(5)-(tetrahydroxybutyl) imidazole

XI= 4(5)-(2,3-dihydroxypropyl)imidazole XII= 4(5)-(2-hydroxyethyl) imidazole

XIII= 2-hydroxymethyl-4(5)-methylimidazole XV= 4(5)-methylimidazole

The compounds identified here have all been found in glucose/ ammonia systems with or without aeration of the mixture[10]. In fact although D-Glucosone may undergo some reactions more readily than D-Glucose and other sugars there is no real reason to suppose that the majority of the fifteen compounds isolated in this work would not also be formed in significant quantities in such systems as D-Glucose/ ammonia. It may well be, therefore, that the application of the ion exchange technique used here to the reaction mistures from such systems could yield considerable new information about the composition of these mixtures.

No attempt was made in this work to investigate the non-imidazolic products in the reaction mixture. However the total yields of the isolated imidazoles is less than half the weight of the basic fraction which was investigated. (The total weight of bases was 1 g. and in the aliquot which was investigated the weight was 400 mg. The total yield of isolated imidazoles was 150 mg.). While the recovery of these compounds may not have been absolutely quantitative, losses in recovery could account for only a small portion of this difference in weights. The bulk of this difference must then represent bases which were not detected by the diazotised sulphanilic acid reagent. In fact a considerable quantity of dark colcured material which gave no reaction with diazotised sulphanilic acid was eluted in the first 40 fractions during the ion exchange separation of the base mixture. These were presumably non-imidazolic bases less basic than imidazoles. While no attempt was made to investigate this fraction it was noted that it had been effectively separated from the imidazoles. Investigation of the non-imidazolic bases formed in this and in other systems would appear to be a further possibility for future work.

SECTION II

4 - O - METHYL-D-GLUCOSE/AMMONIA

It has been observed that substitution affects the mode of alkaline degradation of carbohydrates. 3 - O - substituted glucoses have been observed to give quite high yields of 4(5)-(D-erythro-2,3,4-trihydroxybutyl)imidazole[9]. This was consistent with the fact that 3-O-substituted sugars give metasaccharinates with calcium hydroxide since in both cases the 3-desoxy-2-ketoaldehyde would be required as an intermediate (see fig. 14).

It was proposed to study the reaction of ammonia and 4-O-methyl-D-glucose prepared by the method of Bouveng, Lindbergh and Theander [16]. This method utilises the migration of an acetyl group from the 4-O- to 6-O - position when methyl-2,3,4 - tri-O-acetyl- - D-glucopyranoside is methylated with methyl iodide and silver oxide in dimethylformamide (see fig. 15). The product from this reaction (- methyl-2,3,6-tri-O-acetyl-4-O-methyl-β-D-glucopyranoside) yields 4-O-methyl-D-glucose on hydrolysis.

It was proposed to prepare the starting material (-methyl-2,3,4-tri-O-acetyl-β-D-glucopyranoside) from D-glucose, forming the β-methyl-glucoside by acid catalysed methanolysis, blocking the 6-O-position with trityl chloride acetylating and removing the trityl group. In fact all steps up to the detritylation were successfully carried out. However attempts to detritylate the -methyl-2,3,4-tri-O-acetyl-6-O-trityl-β-D-glucopyranoside with hydrogen bromide in acetic acid appeared to cause deacetylation as well as detritylation. Because of this the products were generally water soluble and when isolated and subjected to an O-acetyl anaylsis (by saponification) proved to have only about 10-20% of the expected O-acetyl value for -methyl-2,3,6-tri-O-acetyl-β-D-glucopyranoside.

fig. 14

Degradation of 3-O-Methyl-D-Glucose

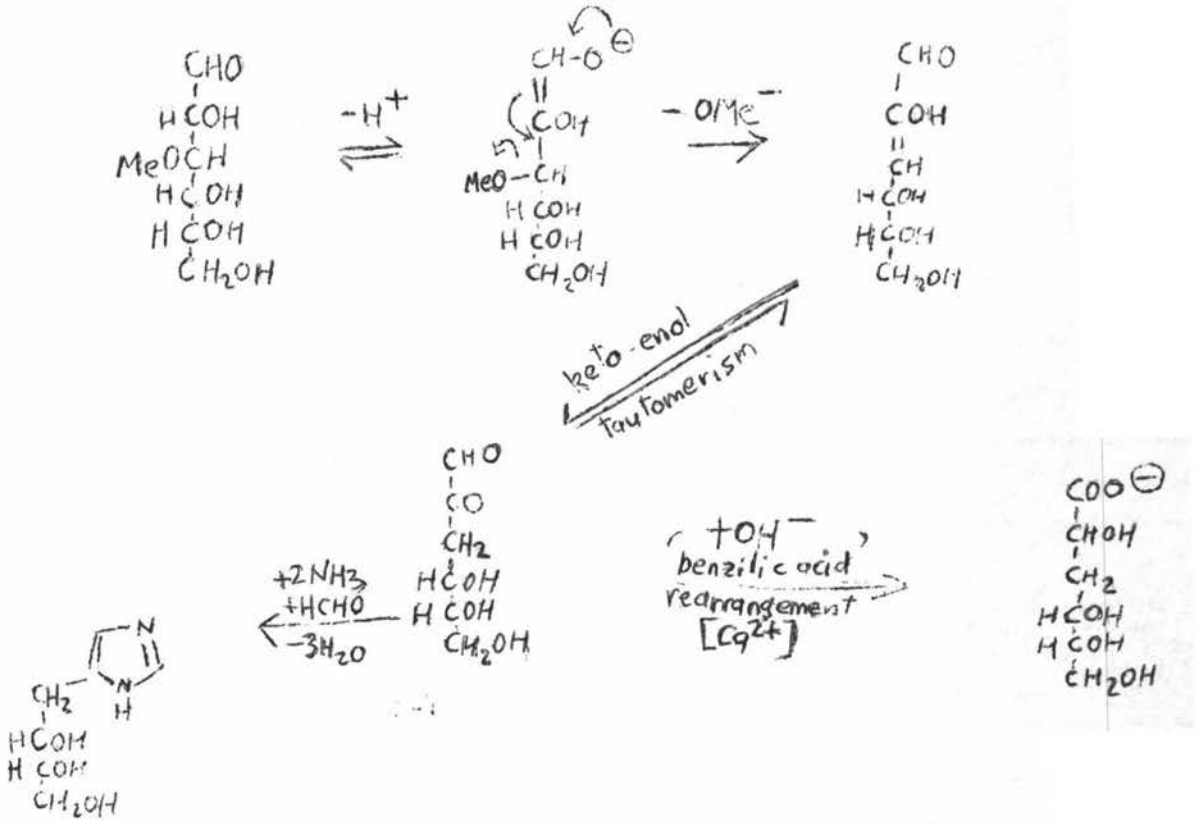
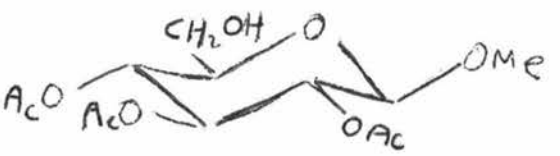


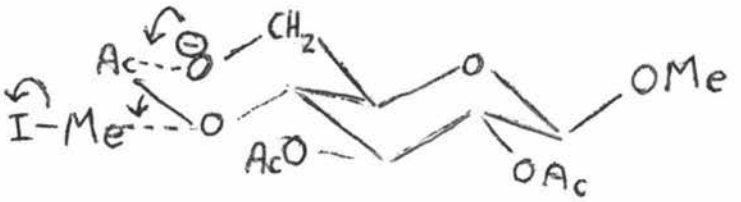
fig. 15

4-O-Methylation of Methyl-2,3,4-tri-O-acetyl- β -D-glucopranoside

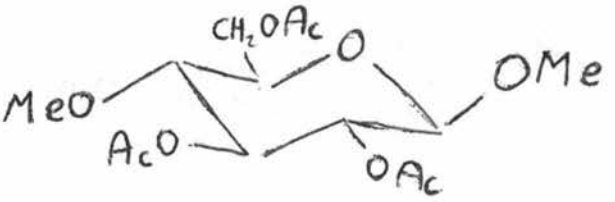


Methyl-2,3,4-tri-O-acetyl- β -D-glucopyranoside

$(Ag_2O) \downarrow -H^+$



$\downarrow$ +MeI, $-I^-$



Methyl-2,3,6-tri-O-Acetyl-4-O-methyl- β -D-glucopyranoside

β-METHYL-D-GLUCOPYRANOSIDE

D(+) Glucose (500 g.), Dowex 50W H^+ cation exchange resin (120 g., methanol washed) and methanol (1200 ml.) were placed in a 2 litre flask equipped with a reflux condenser and stirrer. The mixture was refluxed for 24 hours. The reaction mixture was filtered to remove the resin which was washed with methanol (3x100 ml.). The combined filtrate and washings were concentrated to c. 750 ml., allowed to cool overnight and then filtered. The residue (400 g.) which consisted of a mixture of α and β methyl glucosides was slurried in methanol (60 ml.) at 10°C, and then filtered to give α-methyl-D-glucoside (95 g.) as the residue. This was recrystallised from ethanol (1000 ml.). The recrystallised product had a melting point of 165-7°C and $[\alpha]_{20^\circ}^{D}$ of + 151°.

The combined mother liquors were then concentrated to a thick syrup (c 200 ml.). This was dissolved in hot ethanol (500 ml.) and a solution of potassium acetate (200 g.) in hot ethanol (1000 ml.) was added. The mixture was cooled overnight in a refrigerator. The β-methyl-D-glucoside/potassium acetate complex (195 g.) crystallised out and was removed by filtration and washed with ethanol and acetone.

The complex was dissolved in hot methanol (600 ml.) and a solution of D-tartaric acid (110 g.) in hot ethanol (600 ml.) was added. After 1 hour the mixture was filtered through celite 501 to remove the precipitated potassium acid tartrate and the filtrate was concentrated to a thin syrup (c. 150 ml.) and cooled. β-methyl-D-glucopyranoside (60 g.) crystallised out and this was recrystallised from ethanol to give a product (40 g.) of m.p. 108-111°C and $[\alpha]_{20^\circ}^{D}$ = - 34.8.

β-METHYL-6.O-TRIPHENYLMETHYL-2,3,4-TRI-O-ACETYL-D-GLUCOPYRANOSIDE

β-Methylglucopyranoside (35 g.) was dissolved in pyridine (200 ml. distilled over P_2O_5) and triphenylchloromethane (53 g.) was added. The mixture was allowed to stand for 48 hours and then dry pyridine (300 ml.) and acetic anbydride (300 ml.) were added.

After a further 72 hours the mixture was poured onto a mixture of ice and water (7000 ml.) and allowed to attain room temperature. The aqueous phase was removed and washed with diethyl ether (6x750 ml.) The combined ethereal phases were added to the solid material from the reaction mixture which dissolved. The ethereal solution was washed with saturated aqueous sodium bisulphate (3x20 ml.), saturated aqueous sodium bicarbonate (4x20 ml.) and water (4x20 ml.) and then dried over anhydrous sodium sulphate before being evaporated to a syrup which was taken up in methanol (200 ml.).

A precipitate (100 g.) formed which was removed and washed with a small amount of methanol. The solid material was recrystallised from absolute ethanol to give needles (45 g.) of the product m.p. 134-8°C $[\alpha]_D^{20^\circ} = +24.5^\circ$ (in chloroform).

A sample (0.50 g.) of the material was dissolved in ethanol (20 ml.) and 1.0 M sodium hydroxide (5 ml.).

The solution was heated to boiling point, cooled and titrated with c. 0.1 M hydrochloric acid. A blank was treated similarly. The blank took 43.2 ml. of acid while the sample required 20.4 ml. thence the sample contained 5.3 mE of acetyl groups per g. compared with a theoretical value of 5.4mE/g.

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