AN APPROACH TO THE SYNTHESIS

OF ASPERGILLIC ACID.

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by

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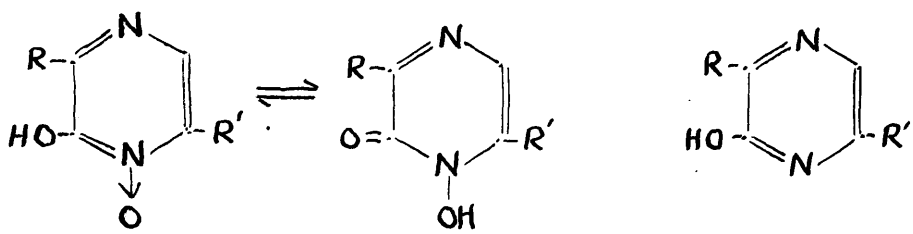
AN APPROACH TO THE SYNTHESIS OF ASPERGILLIC ACID

SUMMARY

Aspergillic acid, the antibiotic isolated from cultures of the mould Aspergillus flavus, was shown by Dutcher and Wintersteiner to have structure (I) or (IA). Later, Dunn, Newbold and Spring identified the side-chains and showed that aspergillic acid must be either (I; R = Bu^s, R' = Bu^i) or (I; R = Bu^i, R' = Bu^s).



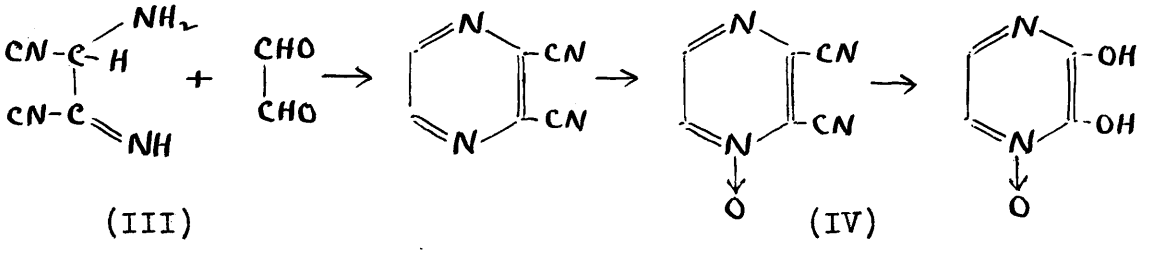
(I) (IA) (II)

The investigations described in this Thesis were directed towards establishing methods for the synthesis of pyrazine cyclic hydroxamic acids with the synthesis of aspergillic acid as the ultimate objective.

Reduction of aspergillic acid gives deoxyaspergillic acid (II), a 2-hydroxy-3:6-disubstituted pyrazine. Thus it appeared possible that oxidation of a suitably substituted pyrazine would lead to a synthesis of aspergillic acid. This route was shown to be

impracticable by Baxter, Newbold and Spring who found that when a 2-ethoxy-3:6-disubstituted pyrazine was oxidised, oxidation occurred at the nitrogen remote from the ethoxy group and hence hydrolysis to give a hydroxyl group in the 2-position gave a compound which was not a hydroxamic acid.

Although the direct oxidation method had proved unsuccessful for a synthesis of aspergillic acid there was a possibility that it could be used to prepare a pyrazine cyclic hydroxamic acid by the following route:-

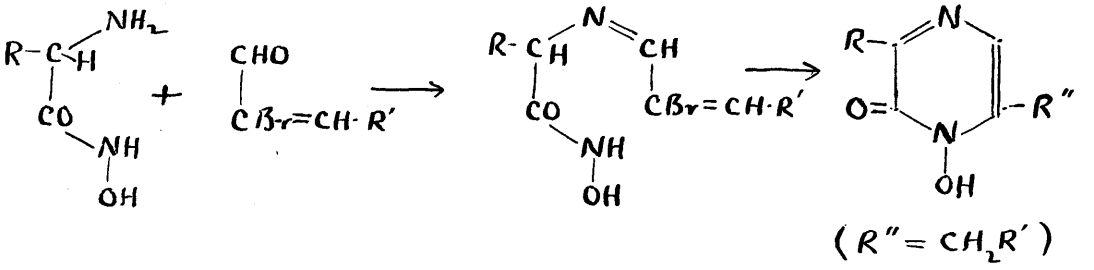


(III) (IV)

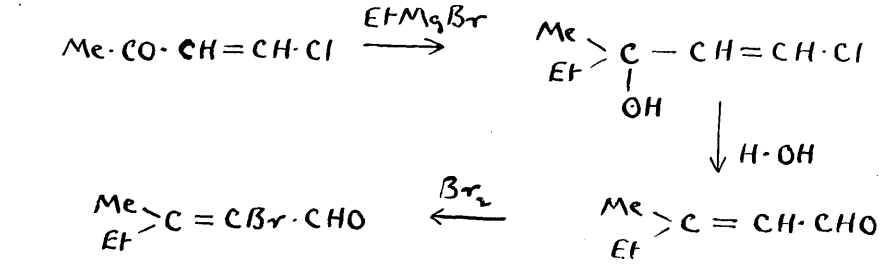
The oxide (IV) was obtained in low yield. The scheme however, had to be abandoned when difficulty was experienced in hydrolysing the nitrile groups of the oxide as a preliminary to the formation of the required hydroxyl groups. An account is given of the experiments with aminoiminosuccinonitrile (III) which extend its application as a synthetic intermediate.

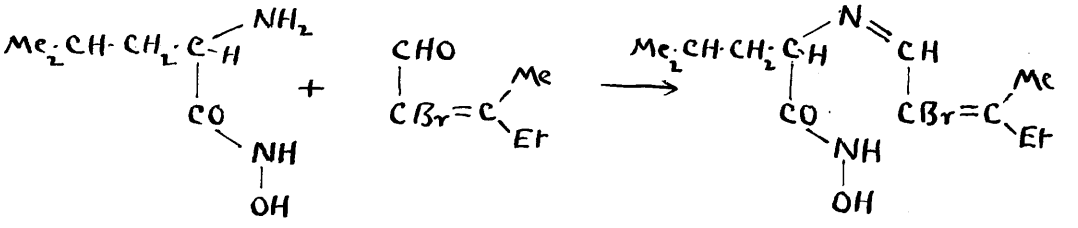
An entirely different approach, namely the formation

of the pyrazine ring by ring-closure of an acyclic hydroxamic acid proved more successful. The following general method for the synthesis of 3:6-disubstituted pyrazine cyclic hydroxamic acids has been developed:-



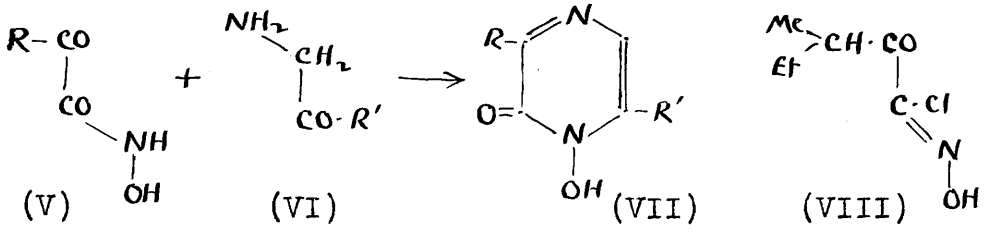
This method was applied successfully to three cases including that of a homologue of aspergillic acid (R = R'' = Et) which was isolated in very low yield. The synthesis of one of the possible structures of aspergillic acid (I; R = Bu^i, R' = Bu^s) was attempted by this route. After a number of methods for the synthesis of $\alpha\beta$-unsaturated aldehydes had been investigated, the required Schiff's base was prepared as follows:-





A satisfactory cyclisation of the Schiff's base to give (I; R = Bu^i, R' = Bu^s) was not achieved. An ultra-violet absorption spectrum showed that the cyclic acid had been formed but only to the extent of 30% of a resinous reaction product isolated in low yield. The difficulties encountered at this ring-closure stage are discussed.

An alternative route to 3:6-disubstituted pyrazine cyclic hydroxamic acids has been developed. An α-keto-hydroxamic acid is condensed with an aminoketone to form the cyclic acid in one step thus:-



In the first instance pyruvchydroxamyl chloride was converted into the bisulphite compound of pyruvohydroxamic acid (V; R = Me). This was condensed with aminoacetone (VI; R' = Me) to give an aspergillic acid homologue (VII; R = R' = Me) in good yield. The structure of the cyclic acid was completely defined when reduction gave 2-hydroxy-3:6-dimethylpyrazine, a known compound.

Application of the above method to the aspergillic acid case (VII; R = Bu^s, R' = Bu^i) proved less successful. A cyclic acid was not formed when the bisulphite compound of the α-keto-hydroxamic acid (V; R = Bu^s) was treated with the aminoketone (VI; R' = Bu^i). An experiment in which the α-keto-hydroxamic acid was formed by hydrolysis of the hydroxamyl chloride (VIII) with sodium acetate in acetic acid showed more promise. The acid was not isolated but treated directly with the aminoketone. A non-crystalline reaction product had an ultra-violet absorption spectrum from which it was evident that it contained a small proportion of the cyclic acid (VII; R = Bu^s, R' = Bu^i).

The author wishes to record his indebtedness to Professor F.S. Spring for his invaluable guidance and constant encouragement. To Dr. J.A. Elvidge the author is indebted for advice, especially on practical techniques.

The author also wishes to thank Dr. G.T. Newbold for his continued interest and in particular for his co-operation on some aspects of this investigation.

C O N T E N T S

HISTORICAL.

ASPERGILLIC ACID.

INTRODUCTION.

The isolation of the antibacterial substance penicillin from culture filtrates of the mould, Penicillium notatum, and its subsequent successful application as a chemotherapeutic agent against systemic bacterial infections naturally stimulated investigation of other mould products (1). It is about one of those products, aspergillic acid, that this work is concerned.

The discovery by White (2) that a strain of Aspergillus flavus, growing in surface culture on a tryptone-salt medium, produced a highly bactericidal filtrate was followed by the isolation by White and Hill (3) of the active material as a crystalline compound which, because of its acidic nature, was named aspergillic acid. It showed antibacterial activity against both Gram positive and Gram negative organisms but possessed a high degree of toxicity. White and Hill apart from a study of antibacterial properties put forward a molecular formula, $C_{12}H_{20}O_2N_2$, for the compound. The same formula was proposed by Menzel, Wintersteiner and Rake (4) who have also reported the isolation of aspergillic acid from Aspergillus flavus. Their preparation had m.p. 93°,

$[\alpha]_D$ 14°.

Isolation of Aspergillic Acid.

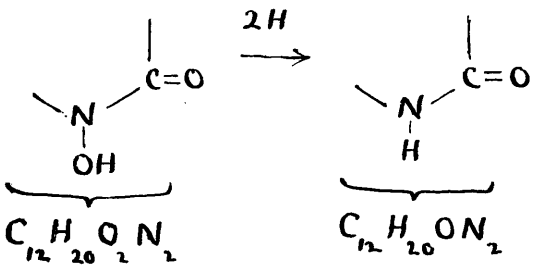
Using a strain of Aspergillus flavus described by Jones, Rake and Hamre (5) with an aqueous solution of the casein hydrolysate "Pronutrin" and sodium chloride as a culture medium Dunn, Gallagher, Newbold and Spring (6) obtained an average yield of 250 mg. of aspergillic acid per litre of culture filtrate. The isolation procedure was based on that described by White and Hill. After removal of mycelium the culture filtrate was acidified to p_H 4 with dilute hydrochloric acid and treated with activated charcoal. The charcoal was air-dried and then ether extracted (Soxhlet). The ethereal solution was extracted with aqueous alkali and the extract acidified with acetic acid. The separated solid was extracted with 2% sodium bicarbonate solution and careful acidification of the extract with acetic acid precipitated material m.p. 80-85°. This was purified by again dissolving it in sodium bicarbonate solution followed by precipitation with acetic acid. Crystallisation from methanol gave aspergillic acid as radial clusters of yellow needles. After a sublimation it had m.p. 97-99°, $[\alpha]_D$ 13.3°.

Structure of Aspergillic Acid

Our knowledge of the structure and general properties of aspergillic acid is largely due to the investigations of Dutcher and Wintersteiner (7). Analysis and molecular weight determination established its molecular formula as $C_{12}H_{20}O_2N_2$. Apart from being a weak acid (pK 5.5) aspergillic acid has basic properties as shown by the formation of a hydrochloride and salt formation with 3:5-dinitrobenzoic acid. The acidity of the compound and the presence of two oxygen atoms suggest a carboxylic acid group but attempts to decarboxylate by dry distillation with copper chromite catalyst gave a neutral product with only one oxygen atom less, $C_{12}H_{20}ON_2$. This compound, which was named deoxyaspergillic acid, has an ultra-violet absorption spectrum very similar to that of aspergillic acid. Such a reaction product cannot be explained by the presence of a carboxylic acid group in the molecule.

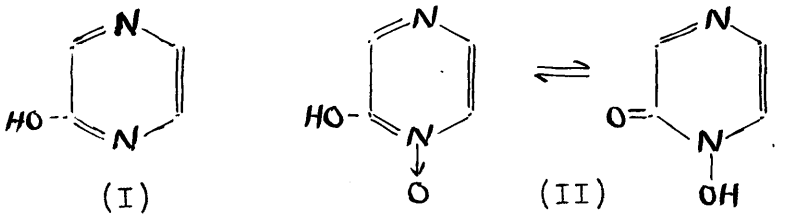
Aspergillic acid forms a green copper salt and in methanolic solution gives a deep red colouration with aqueous ferric chloride. These reactions suggest that the acidity is due to a hydroxamic acid grouping. This was confirmed by a further examination of deoxyaspergillic acid which was produced more efficiently by the action of

either hydrazine or hydriodic acid on aspergillic acid. Both of these reagents were found, in model experiments, to be capable of reducing hydroxamic acids to the corresponding amides. The formation of deoxyaspergillic acid can then be explained as follows:-



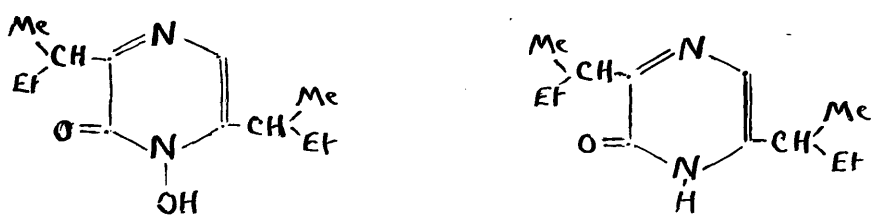
Now aspergillic acid is recovered unchanged after treatment with alkali or acid under reflux and aliphatic hydroxamic acids under such conditions are quickly hydrolysed or rearranged. The hydroxamic acid grouping must therefore be conceived as an integral part of a stable heterocyclic ring system. The second nitrogen atom could not be that of a primary amine in view of the weak basicity and lack of reactivity with nitrous acid. It would appear that it too must be part of a ring system. The close resemblance between the ultra-violet absorption spectrum of deoxyaspergillic acid and that of 2-hydroxy-pyrazine (I) justified the formulation of the aspergillic

acid ring system as (II) a structure which incorporates a hydroxamic acid grouping in a pyrazine ring.



(I) (II)

Treatment of aspergillic acid with bromine water gave bromoaspergillic acid (V) $C_{12}H_{19}O_2N_2Br$, one hydrogen atom having been replaced. This compound had the characteristic properties of a hydroxamic acid and was comparatively stable to alkali thereby indicating nuclear substitution. With this evidence that at least one position of the ring system is available for substitution the following tentative structures were put forward for aspergillic acid (III) and deoxyaspergillic acid (IV) by Dutcher:-



(III) (IV)

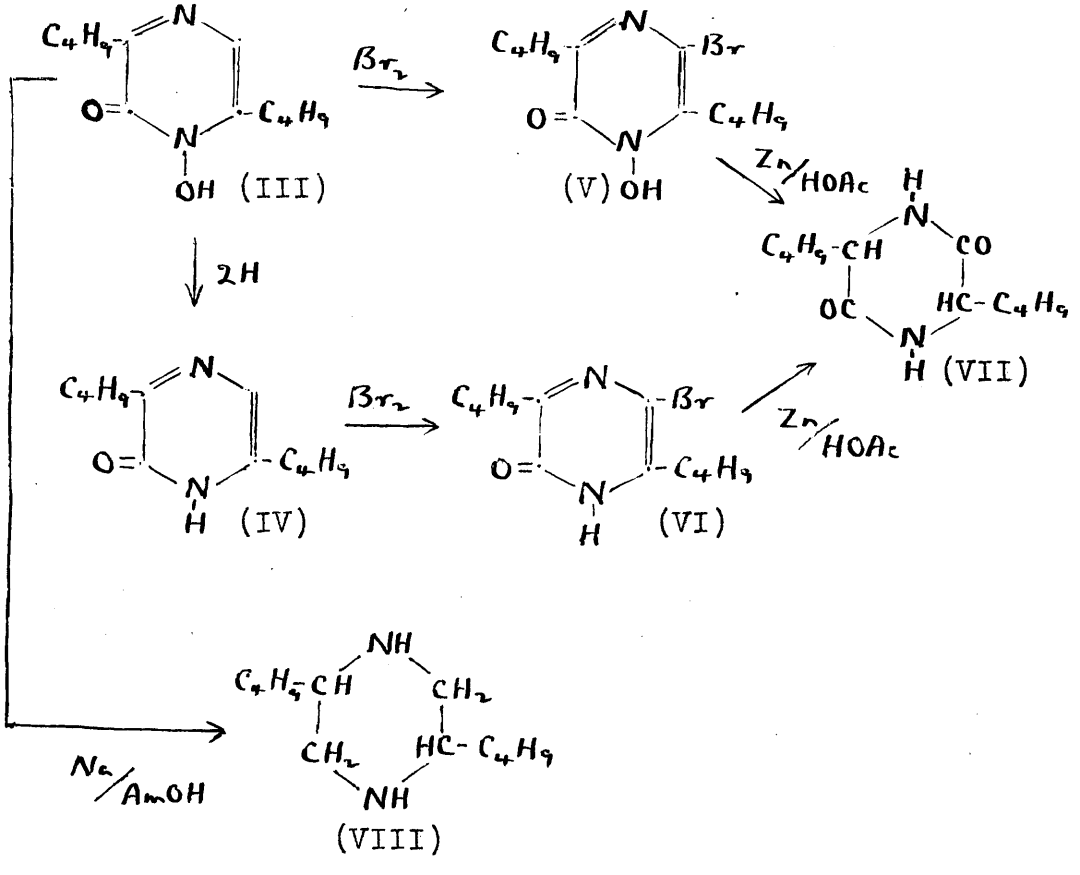
As aspergillic acid is optically active at least one asymmetric carbon atom must occur in the side chains. These as a working hypothesis were both taken to be sec.-butyl groups.

Evidence confirming the fundamental structure (III) proposed will be considered briefly. Like aspergillic acid deoxyaspergillic acid gave a monobromo-compound (VI). Treatment of bromodeoxy-aspergillic acid and bromoaspergillic acid with zinc in glacial acetic acid gave in both cases an optically active neutral compound with the empirical formula $C_{12}H_{22}O_2N_2$. The high melting point and ultra-violet spectrum of this product suggested it was a diketopiperazine and assuming aspergillic acid to have structure (III) it may be formulated as (VII). Because of its optical activity direct comparison with racemic 3:5-di-sec.-butyl-2:5-diketopiperazine (DL-isoleucine anhydride) was not possible but the crystal form and solubilities of the two compounds were in agreement.

Further evidence in support of the proposed structure was obtained by a complete reduction of aspergillic acid with sodium in amyl alcohol. The product, an oxygen free optically active base, $C_{12}H_{26}N_2$, was characterised by derivatives which showed general

similarity to the derivatives of the oxygen-free base obtained by reduction of DL-isoleucine anhydride. The aspergillic acid reduction product was therefore assigned the structure (VIII) of a 2:5-di-sec.-butyl piperazine.

The reactions and products derived from aspergillic acid which have been discussed so far are summarised in the following scheme:-

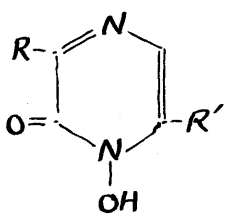


The work of Dutcher outlined above determined the fundamental structure of aspergillic acid. The nature of the alkyl side chains was not, however, completely established, indeed the possibility of there being only one side chain and two unsubstituted nuclear positions in the pyrazine ring cannot be excluded.

The assumption by Dutcher that both alkyl side chains were sec.-butyl groups was proved to be incorrect when with alkali treatment deoxyaspergillic acid gave a racemate which was depressed in melting point when mixed with racemic 3-hydroxy-2:5-di-sec.-butylpyrazine (6, 8). This observation also showed that the compound, $C_{12}H_{22}O_2N_2$ (VII) could not be an isoleucine anhydride as was originally proposed. That the compound was a diketopiperazine seemed highly probable but this had not been definitely established. The required evidence could only be obtained by hydrolysis to the constituent amino-acids followed by their identification. Model experiments showed that the 2:5-diketopiperazine, isoleucine anhydride, was converted into isoleucine in good yield by refluxing with 48% hydrobromic acid. This method was applied to the compound $C_{12}H_{22}O_2N_2$ and from the hydrolysate the amino-acids, DL-leucine and DL-isoleucine, were isolated and identified as

their 3:5-dinitrobenzoyl derivatives. Further, reaction with ninhydrin converted the amino-acids into the corresponding aldehydes, isovaleraldehyde and methylethyl-acetaldehyde, characterised as their 2:4-dinitrophenyl-hydrazones (9).

The compound, $C_{12}H_{22}O_2N_2$, was therefore shown to be leucylisoleucine anhydride 6-isobutyl-3-sec.-butyl-2:5-diketo-piperazine (X) and deoxyaspergillic acid is either 5-isobutyl-2-sec.-butyl-3-hydroxypyrazine (XI) or 2-isobutyl-5-sec.-butyl-3-hydroxypyrazine (XII) from which it follows that aspergillic acid is either (IX; R = Bu^s, R' = Bu^i) or (IX; R = Bu^i, R' = Bu^s).



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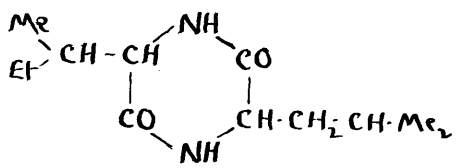

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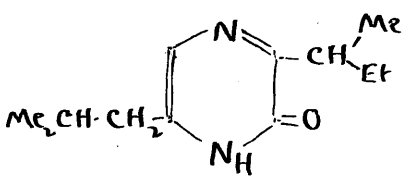

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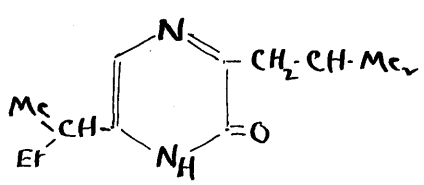


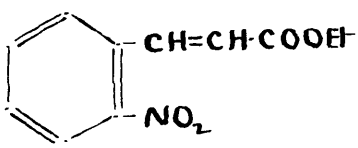
(XII)

Cyclic Hydroxamic Acids

Aspergillic acid has been shown to contain a hydroxamic acid grouping in a pyrazine ring system. A study of the known cyclic hydroxamic acids and their methods of formation is, therefore, a necessary preliminary.

A review of the hydroxamic acids by Yale (10), published in 1943, fails to mention a single case of a cyclic hydroxamic acid but several such compounds had in fact been reported and these will be considered briefly.

Reduction of ethyl o-nitrocinnamate (XIII) gave carbostyril (XIV) with a small amount of 2-hydroxyquinoline 1-oxide (oxycarbostyril) (XV) formed as a by-product (11).



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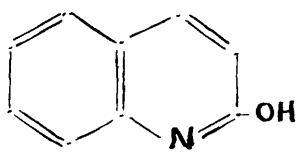

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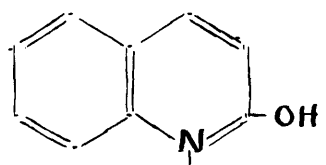
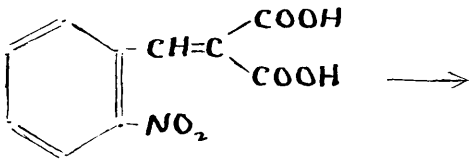


(XV)

The related oxycarbostyril carboxylic acid (XVII) was obtained by reduction of o-nitrobenzylidene malonic acid (XVI) with zinc and acetic acid (12).



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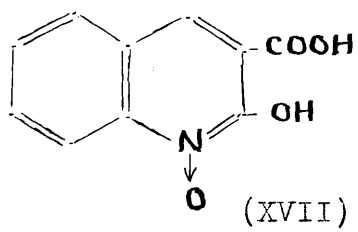
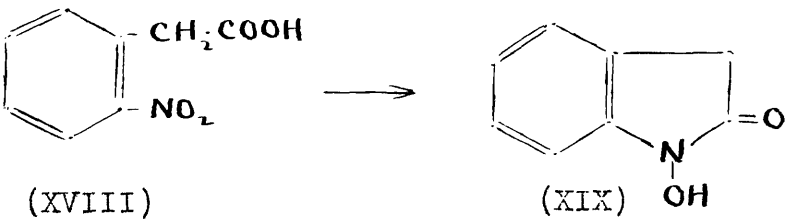


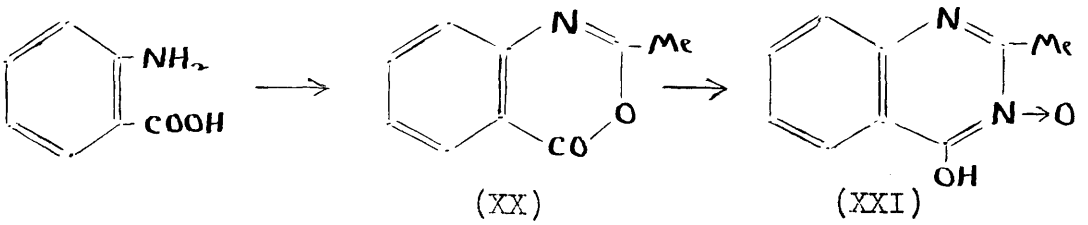
(XVII)

Similarly reduction of o-nitro-phenylacetic acid (XVIII) with zinc and sulphuric acid gave in fair yield, 1:2-dioxindole (XIX) (13).



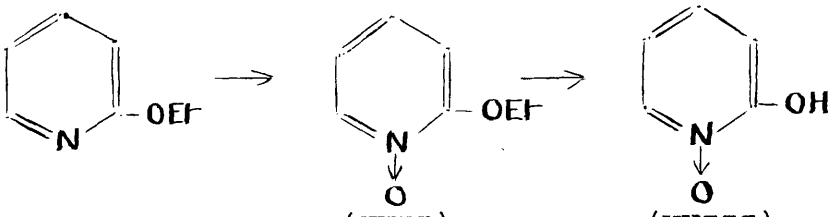
(XVIII) (XIX)

The only other cyclic hydroxamic acid to be reported before this work commenced is 4-hydroxy-2-methylquinazoline 3-oxide (XXI) obtained by treatment of acetylanthranil (XX) with hydroxylamine (14).



(XX) (XXI)

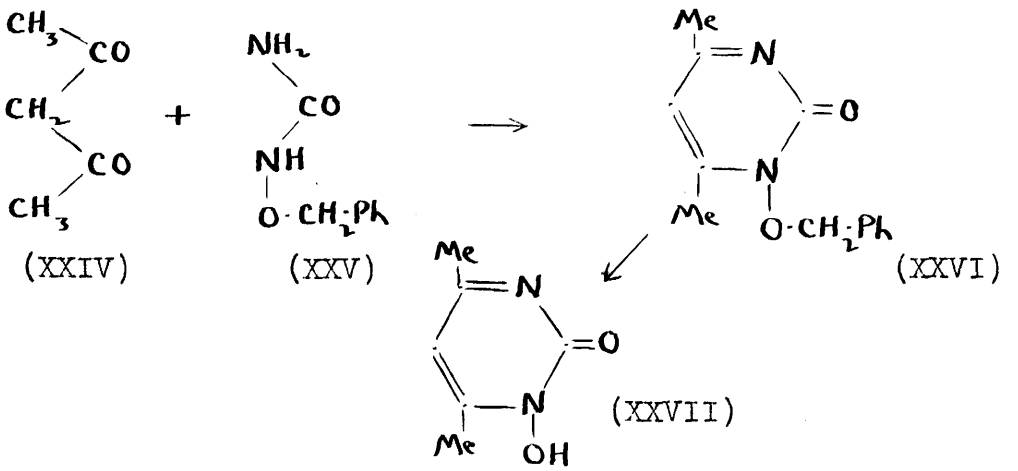
Recently Dr. G.T. Newbold prepared the cyclic hydroxamic acids, 2-hydroxypyridinel-oxide (XXIII) and 2-hydroxy-quinoline 1-oxide (XV) by a general method (15). Treatment of 2-ethoxypyridine with hydrogen peroxide in glacial acetic acid gave 2-ethoxypyridine 1-oxide (XXII). Hydrolysis with dilute hydrochloric acid then gave 2-hydroxypyridine 1-oxide which had the properties expected of a cyclic hydroxamic acid.



(XXII) (XXIII)

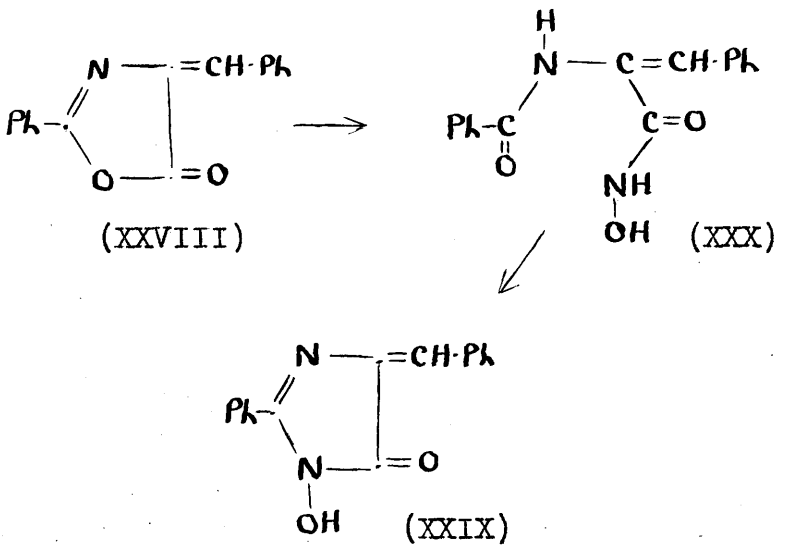
2-hydroxyquinoline 1-oxide identical with the product described by Friedlander and Ostermaier (11) was prepared by a similar series of reactions starting from 2-ethoxyquinoline.

Mention must also be made of the work of Lott and Shaw (16, 17) on analogues of aspergillic acid. Firstly, they prepared the pyridine hydroxamic acid (XXIII) which has already been described by a similar scheme, but using perbenzoic acid as the oxidising agent. Secondly, a cyclic hydroxamic acid in the pyrimidine series was obtained by condensing acetylacetone (XXIV) with benzyloxyurea (XXV) to give an N-benzyloxypyrimidone (XXVI). Catalytic hydrogenation then gave the acid, 1-hydroxy-4:6-dimethyl-2-pyrimidone (XXVII).



(XXIV) (XXV) (XXVI) (XXVII)

Finally, Mc Dowell and Shaw (18) synthesised a 5-ring cyclic hydroxamic acid starting from the azlactone, 4-benzal-2-phenyl-oxazol-5-one (XXVIII). Treatment with methanolic hydroxylamine gave the cyclic acid, 4-benzal-N-hydroxy-2-phenyl-imidazol-5-one (XXIX) directly in poor yield. With different conditions the intermediate (XXX) could be isolated and this was cyclised by treatment with boiling dilute hydrochloric acid.

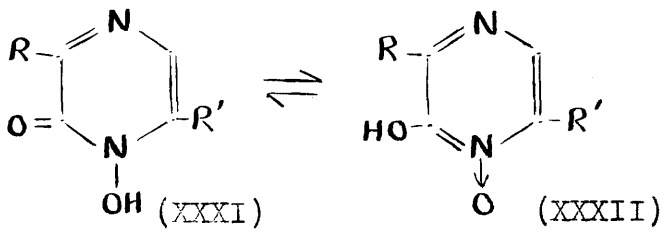


(XXVIII) (XXX) (XXIX)

THEORETICAL.

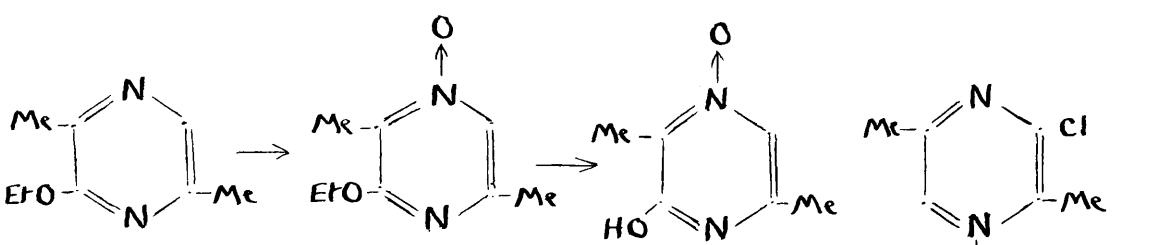
THEORETICAL

In the Introduction aspergillic acid has been shown to have structure (XXXI) of which (XXXII) is a tautomeric form.



(XXXI) (XXXII)

It can be reduced to give deoxyaspergillic acid, a 2-hydroxy-3:6-disubstituted pyrazine, so that a possible synthesis would be by oxidation of the appropriate hydroxypyrazine. This route was investigated by Baxter, Newbold and Spring (19) who found with model compounds that oxidation occurred at the nitrogen remote from the hydroxyl group. Starting with 2-ethoxy-3:6-dimethylpyrazine (XXXIII), treatment with hydrogen peroxide in glacial acetic acid gave (XXXIV), the structure of which was established when hydrolysis yielded the compound (XXXV) which had none of the properties of a hydroxamic acid. This

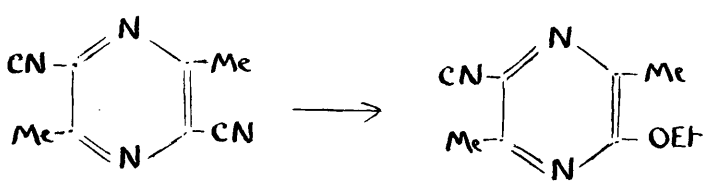


(XXXIII) (XXXIV) (XXXV) (XXXVI)

4-mono-oxide resisted further oxidation at the 1-position. Likewise, peroxidation of 2-chloro-3:6-dimethylpyrazine gave 2-chloro-3:6-dimethylpyrazine-4-oxide (XXXVI) and not the required 1-oxide. The synthesis of aspergillic acid by direct oxidation of a 2-substituted pyrazine thus appeared to be impracticable.

As deoxyaspergillic acid is not an antibacterial agent the activity of aspergillic acid must be attributed to the hydroxamic acid grouping. It was therefore considered worthwhile to investigate all possible routes to pyrazine cyclic hydroxamic acids which need not have the same substitution as aspergillic acid.

During a study of 2:5-disubstituted 3:6-dicyanopyrazines, Golombok and Spring (20) prepared 3-cyano-6-ethoxy-2:5-dimethylpyrazine (XXXVIII) by treatment of 3:6-dicyano-2:5-dimethylpyrazine (XXXVII) with sodium ethoxide. The compound was treated with hydrogen peroxide in glacial

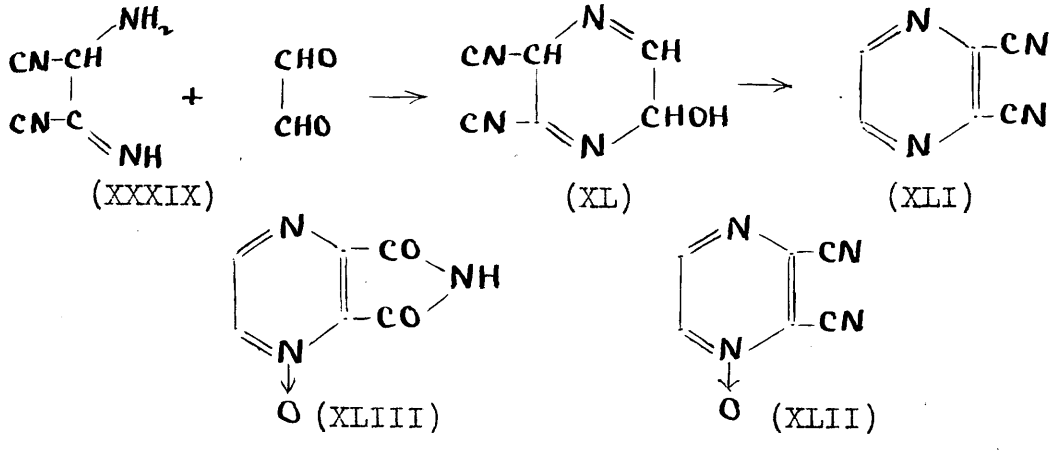


(XXXVII) (XXXVIII)

acetic acid since, if oxidation at either the 1- or 4-positions occurred, the product by suitable treatment

could be converted into a cyclic hydroxamic acid. A mono-oxide was obtained which did not give a hydroxamic acid on hydrolysis as treatment with either acid or alkali resulted in decomposition with evolution of hydrocyanic acid.

The symmetry of 2:3-dicyanopyrazine (XLI) is such that only one mono-oxide (XLII) is possible. By a series of reactions ($-CN \rightarrow -CONH_2 \rightarrow -NH_2 \rightarrow -OH$) the nitrile groups might then be replaced by hydroxyls thus ensuring the synthesis of a pyrazine cyclic hydroxamic acid.



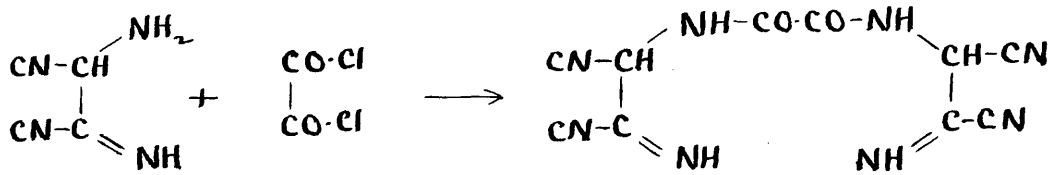
(XXXIX) (XL) (XLI)

(XLIII) (XLII)

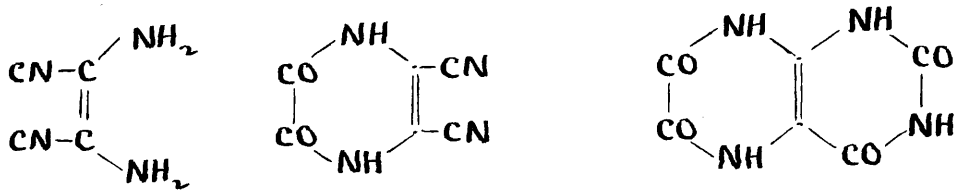
2:3-Dicyanopyrazine (XLI) was prepared by the method of Hinkel, Richards and Thomas from aminoiminosuccinonitrile (XXXIX) and glyoxal (21, 22). The intermediate (XL) is isolated but it readily loses the elements of water to form 2:3-dicyanopyrazine. Oxidation with hydrogen peroxide in glacial acetic acid gave the required mono-oxide

(XLII) m.p. 220° (decomp.) in 13% yield. Hydrolysis of this 2:3-dicyanopyrazine 1-oxide (XLII) to the corresponding diamide was attempted with sulphuric acid as the hydrolysing agent. Considerable decomposition resulted and no crystalline material could be isolated. With hydrochloric acid a product m.p. 200° (decomp.) was obtained. There was insufficient material for a complete analysis, but from a nitrogen determination and taking account of its acidity, it is suggested that the compound was the imide $C_6H_3O_3N_3$ (XLIII). As the yield at the oxidation stage could not be improved, and the acid hydrolysis of the oxide was not promising, this route was abandoned.

In view of the considerable effort which had been put into preparing aminoiminosuccinonitrile for the above work, the possibility of using it in a wider field of synthesis was investigated. Firstly, the reaction with oxalyl chloride was examined. The possible product (XLIV) would be a useful intermediate for the synthesis of the pterin (XLV). The product (XLVII) obtained, however, was, on analytical evidence, derived from 2 mols. of aminoiminosuccinonitrile and 1 mole of oxalyl chloride.



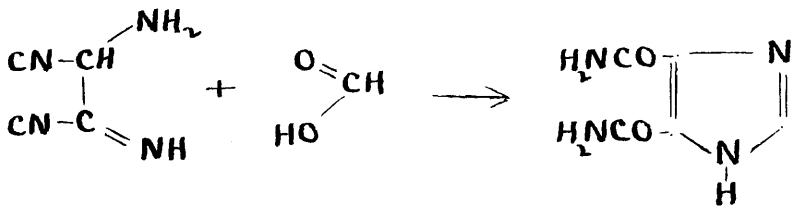
(XXXIX) (XLVII)



(XLVI) (XLIV) (XLV)

The acid chloride had reacted preferentially with two amino groups rather than with the much less reactive imino group. This reaction serves to confirm the opinion of Hinkel (22) that the tetramer of hydrocyanic acid exists as aminoiminosuccinonitrile (XXXIX) and not as the form, diaminomaleinitrile (XLVI). He observed that it forms a mono-hydrochloride and not a di-hydrochloride produced by true diamines. Further, acetylation under the usual conditions gave a mono-acetate and only with vigorous treatment was a di-acetate produced.

Secondly, the reaction with formic acid was studied. The product, from its properties and by analysis, appeared to be imidazole-4:5-dicarboxyamide (XLVIII). This compound was first prepared by Baxter and Spring (23) from methyl imidazole-4:5-dicarboxylate and a specimen prepared by this route was available for comparison. A mixed melting point was not conclusive as the compound does not melt sharply but decomposes over a range of temperature above 300°. Identification was confirmed instead by measuring the ultra-violet spectrum of both specimens in N/10 sodium hydroxide. The hydrolysis of the nitrile groups was not unexpected as formic acid is a specified reagent for the conversion of nitriles to acid amides.

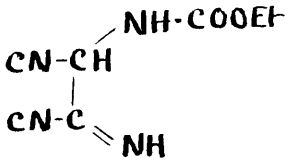


(XXXIX) (XLVIII)

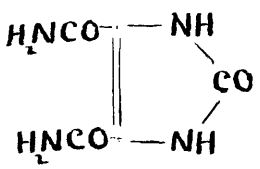
With glacial acetic acid there was no reaction under similar conditions.

Thirdly, with chloroformic ester aminoiminosuccinonitrile was converted smoothly into the N-carbethoxy derivative (XLIX). With a view to achieving ring-closure, this

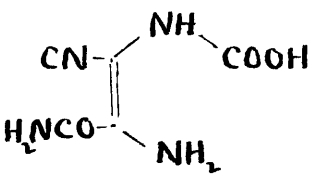
product was treated with alkali under conditions described by Cook, Davis, Heilbron and Thomas (24)



(XLIX)

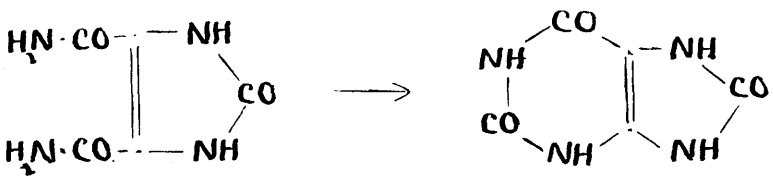


(L)



(LI)

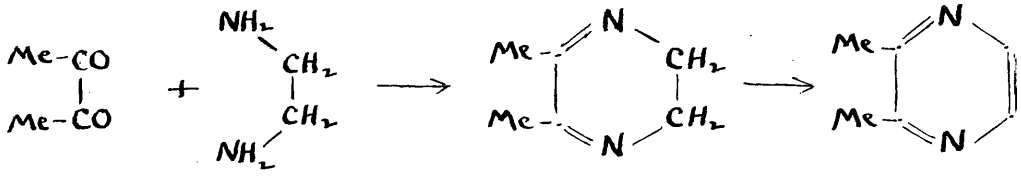
for a similar type of cyclisation. The product, which had a very high decomposition point (sintering at 320°), analysed for $C_5H_6O_3N_4$. It may be formulated as either (L) or (LI). The latter structure, which contains a carboxylic acid grouping, was excluded when the compound gave no effervescence with a saturated solution of ammonium hydrogen carbonate. By treatment with alkaline potassium hypobromite, Baxter and Spring converted imidazole-4:5-dicarboxyamide into xanthine (23). This new compound, 2-imidazolone-4:5-dicarboxyamide (L) is therefore a potential intermediate for a new synthesis of uric acid (LII) thus:-



(L) (LII)

To conclude this part of the work and as an

introduction to what follows, reference must be made to an investigation of the reaction between diacetyl and ethylene diamine. The purpose here was twofold. Firstly, there was a strong case for obtaining confirmation of the report by Jorre (25) that oxidation of a dihydro intermediate (LIII) gave 2:3-dimethylpyrazine (LIV).

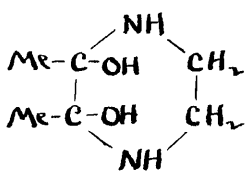


(LIII) (LIV)

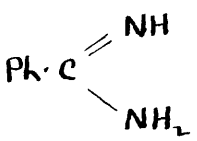
The original report (Thesis) was not available, and the Beilstein abstract lacked essential experimental detail. Secondly, this reaction between a 1:2-diamine and a 1:2-dicarbonyl compound was not unlike the type of condensation which was under consideration for an aspergillic acid synthesis, and which is dealt with later.

All attempts to prepare 2:3-dimethylpyrazine, initially following Jorre's conditions, and subsequently with many variations, were unsuccessful. A product m.p. 56°, which was at first taken to be the intermediate (LIII), was obtained from most experiments. It proved to be

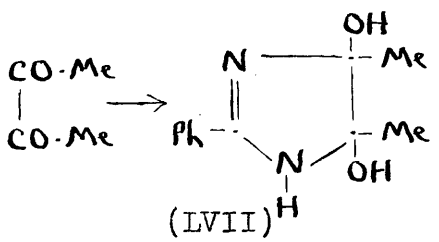
unstable and decomposed rapidly on exposure; a specimen could only be kept by sealing it in vacuo. The compound analysed for $C_6H_{14}O_2N_2$ and was a strong base. Treatment with oxidising agents in a number of experiments broke down the melecule and gave back ethylene diamine which was characterised by its picrate. A likely structure for the compound is (LV) and some support for this supposition comes from the work of Cornforth and Huang (26) who treated the amidine (LVI) with diacetyl and isolated a product, the analysis and properties of which fit the structure (LVII).



(LV)



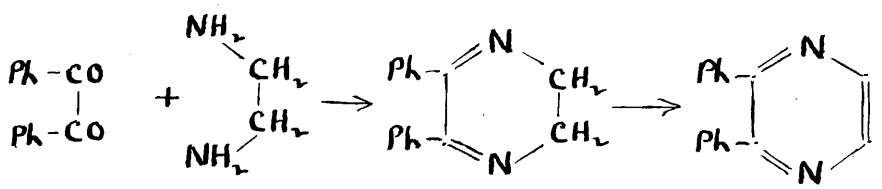
(LVI)



(LVII)

Apart from the reaction of the potential diamine, aminoiminosuccinonitrile, with glyoxal which has already been described in detail there has been only one other report of a reaction of this type with aliphatic substituents. It concerns the reaction of methyl propyl diketone with ethylene diamine (27) and being a Thesis reference lacks experimental detail. With aromatic

substituents Mason (28) successfully condensed 1:2-diamines with 1:2-dicarbonyl compounds to give pyrazine derivatives. For example, benzil and ethylene diamine reacted to give a dihydro compound (LVIII) which on distillation oxidised spontaneously to yield 2:3-diphenylpyrazine (LIX).



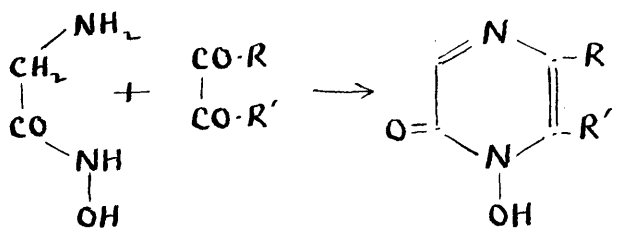
(LVIII) (LIX)

The above investigation has shown that the reaction of 1:2-diamines with 1:2-dicarbonyl compounds to give pyrazine derivatives is not so general as claimed by Krems and Spoerri in their review of the pyrazines (29) and in particular the reaction of diacetyl with ethylene diamine does not give 2:3-dimethylpyrazine as has been reported.

The principal structural features of the aspergillic acid molecule are the pyrazine ring and the hydroxamic acid grouping. It has been shown that all attempts to synthesis such a pyrazine cyclic hydroxamic acid

starting from a suitably substituted pyrazine derivative were unsuccessful. Clearly, the other approach must now be considered and that is the formation of the pyrazine ring by ring-closure starting with an acyclic hydroxamic acid. It seemed that either an α-keto or an α-amino-hydroxamic acid would be required. Because of the indirect methods of formation, the α-keto-hydroxamic acids were not considered at this stage; of the α-amino-hydroxamic acids only glycine hydroxamic acid had been described when this work commenced. In order to carry out a series of exploratory experiments glycine hydroxamic acid was prepared by a modification of the method of Jones and Sneed (30) from glycine ethyl ester and hydroxylamine.

Firstly, the following condensation was attempted:-

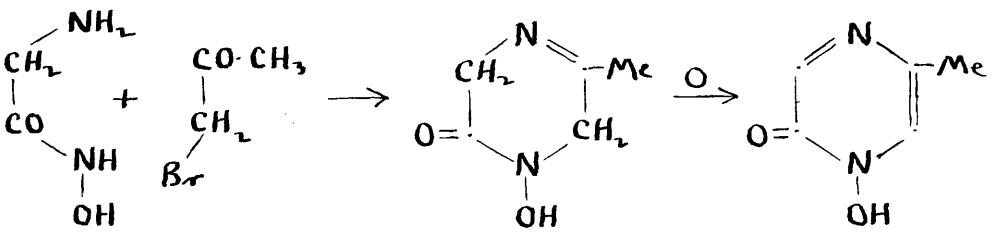


Glycine hydroxamic acid was treated with a molecular proportion of glyoxal in aqueous solution at 60-70°. The reaction mixture darkened quickly and the material

isolated was invariably resinous except in one experiment when a crystalline product $C_4H_6O_2N_2$ m.p. 310° was obtained. It proved to be glycine anhydride.

With diacetyl as the dicarbonyl component there was a similar lack of success.

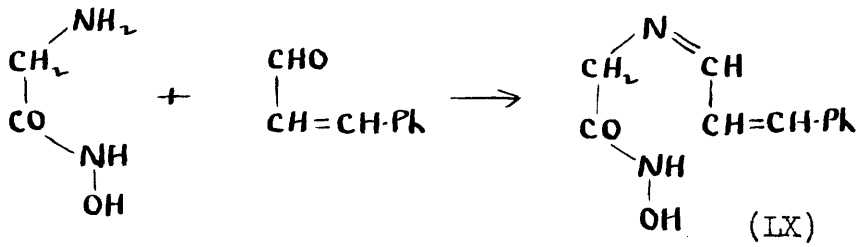
Secondly, the reaction of bromacetone with glycine hydroxamic acid was studied with a view to achieving ring-closure thus:-



Even under mild conditions a vigorous reaction was observed but once again a crystalline product could not be isolated.

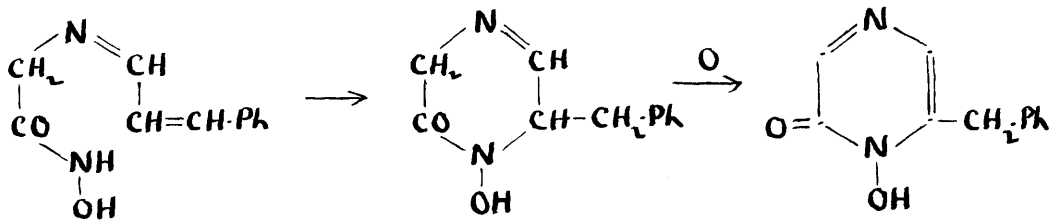
The experiments designed to form the pyrazine ring in one stage having proved unsuccessful, a route was sought by which the cyclisation might be effected through an intermediate. Now glycine hydroxamic acid is strongly basic, the acidity of the hydroxamic acid grouping being weak compared with the basicity of the amino-group. There was therefore a possibility that

condensation with an aldehyde would give a Schiff's base. For a preliminary experiment cinnamaldehyde was selected because the unsaturation might well force Schiff's base formation in order to give a stable conjugated system of bonds.



(LX)

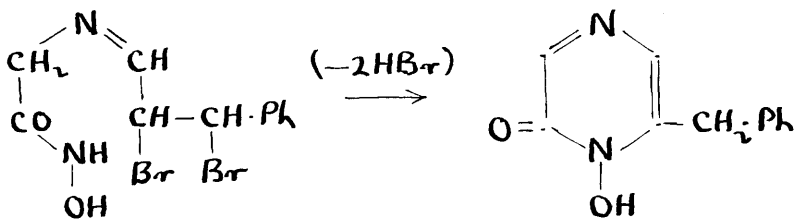
When glycine hydroxamic acid was treated with cinnamaldehyde a crystalline product $C_{11}H_{12}O_2N_2$ m.p. 204° (d.) was obtained in fair yield. That this product was indeed the Schiff's base (LX) was shown by the ease with which it was hydrolysed by mineral acids. The structure was proved conclusively when the compound was treated with Brady's reagent and the 2:4-dinitrophenylhydrazone of cinnamaldehyde was formed rapidly. It was just possible that cyclisation of the Schiff's base could be enforced to give the compound (LXI), which on oxidation would give the pyrazine cyclic hydroxamic acid (LXII).



(LX) (LXI) (LXII)

Heat treatment in high boiling inert solvents such as xylene failed to effect cyclisation. The Schiff's base tended to polymerise even in refluxing ethanol.

An attempt was made to prepare the compound (LXIII) by the addition of bromine. The required cyclic hydroxamic acid might then be formed thus:-

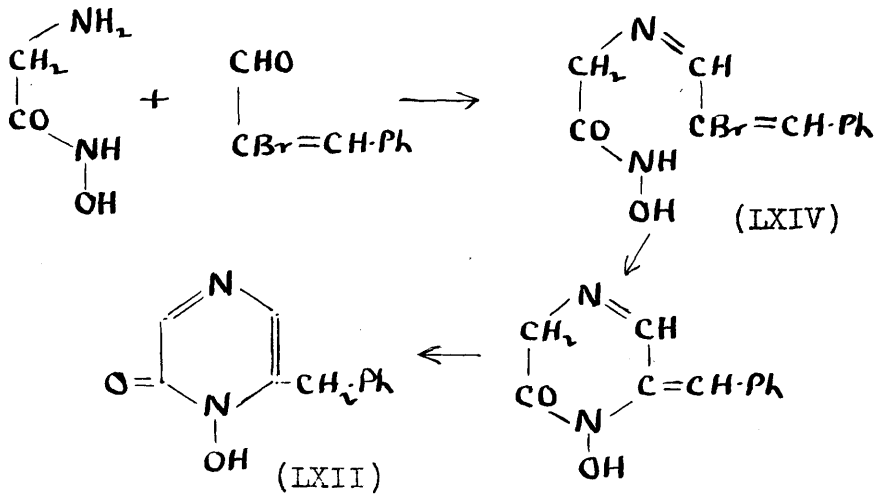


(LXIII) (LXII)

The Schiff's base took up bromine but a product m.p. 180-200° (d.) which was isolated could not be crystallised to constant melting point as decomposition occurred when it was dissolved in hot solvent.

The next step was to form the Schiff's base (LXIV) from 2-bromocinnamaldehyde. This compound appeared to be stabilised by the presence of the bromine atom

as there was no tendency to polymerise and it had a much better crystalline form than (LX). Further,



loss of the elements of hydrogen bromide, followed by a bond rearrangement as shown would give the pyrazine cyclic hydroxamic acid directly. Both aqueous and ethanolic alkali proved ineffective for this purpose as on warming the reaction mixture darkened rapidly, the hydroxamic acid group was destroyed, and no crystalline product could be isolated. This result was not unexpected as, although the Schiff's base bond is stable to alkali, the hydroxamic acid group is likely to undergo the well-known Lossen rearrangement thus:-

$$R\cdot CO\cdot NHOH \longrightarrow R\cdot NCO \longrightarrow R\cdot NH_2$$

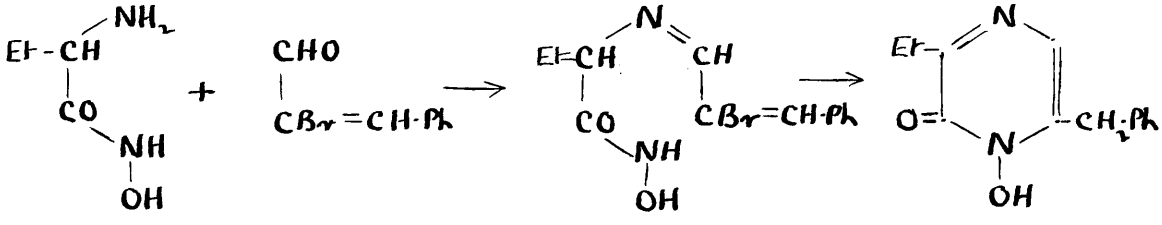
N-Methylmorpholine, a representative tertiary base, failed to remove the hydrogen bromide, the Schiff's

base being recovered unchanged. With sodium in methanol there was no reaction but when the Schiff's base was treated with sodium in dry ethanol under reflux the required pyrazine cyclic hydroxamic acid $C_{11}H_{10}O_2N_2$ (LXII) was obtained in 40% yield. A proportion of the Schiff's base was recovered unchanged.

This represented the first synthesis of a pyrazine cyclic hydroxamic acid. The acid had the same characteristic properties as aspergillic acid. It dissolved in aqueous sodium hydrogen carbonate with effervescence, and gave the intense red coloration with ferric chloride solution which is characteristic of hydroxamic acids. Further, it formed a green copper salt crystallisable from dioxan. The acid had an ultra-violet absorption spectrum very similar to that of aspergillic acid.

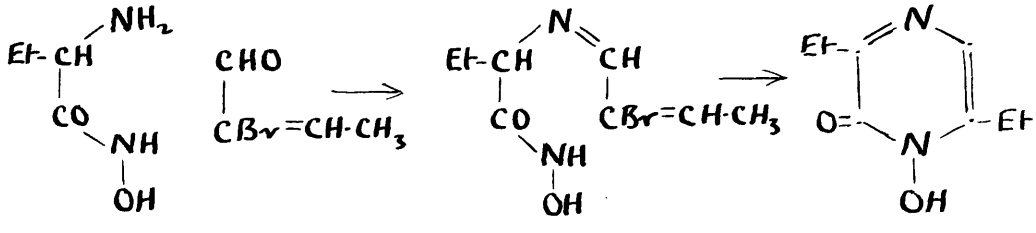
It was now necessary to extend the synthesis to pyrazine cyclic hydroxamic acids substituted in both the 3- and 6- positions with a view to the ultimate synthesis of aspergillic acid. The new α-amino-hydroxamic acid, α-amino-n-butyrohydroxamic acid (LXV) was prepared by the action of free hydroxylamine in methanol on the methyl ester of DL-α-amino-n-butyric acid. With 2-bromocinnamaldehyde the hydroxamic acid

formed the Schiff's base (LXVI) in high yield. Treatment with sodium in dry ethanol in this case failed to effect cyclisation. This was eventually achieved in small yield by using potassium in tert.-butanol under reflux. The cyclic acid (LXVII) had the characteristic properties and ultra-violet spectrum expected.



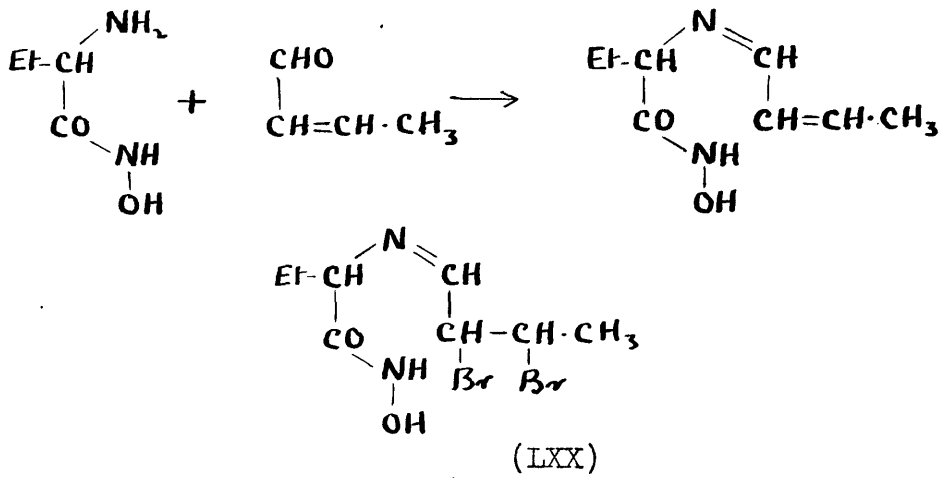
(LXV) (LXVI) (LXVII)

The next step was to synthesis a true homologue of aspergillic acid with aliphatic substituents in both the 3- and 6- positions. 2-Bromocrotonaldehyde with α-amino-n-butyrohydroxamic acid gave in 51% yield the Schiff's base (LXVIII). Cyclisation in this case



(LXVIII) (LXIX)

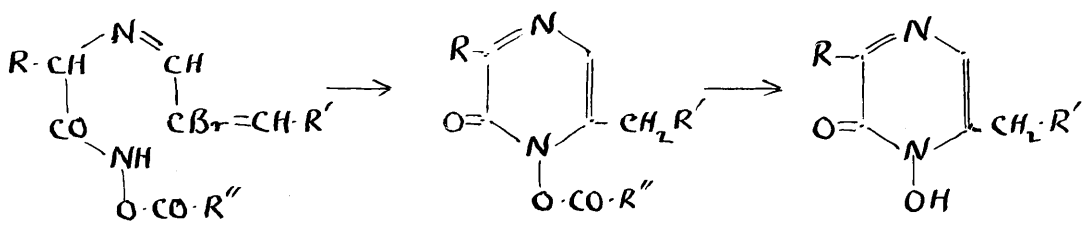
proved extremely difficult. Initially with potassium in tert.-butanol no crystalline product could be isolated other than a high proportion of unchanged starting material. This difficulty led to a renewed search for a more efficient cyclising agent. The Schiff's base was, for instance, powdered in a mortar with copper-bronze powder and the mixture heated in a sublimation unit to the melting point of the Schiff's base. A non-crystalline sublimate was crystallised and identified by mixed melting point as unchanged starting material. In another experiment α-amino-n-butyrohydroxamic acid was treated with crotonaldehyde and a non-crystalline product obtained. This was treated with bromine in chloroform solution with a view to preparing the compound (LXX).
A crystalline solid was isolated which proved to be the hydrobromide of α-amino-n-butyrohydroxamic acid. Its formation can only be explained by the ready loss of hydrogen bromide from (LXX) and subsequent hydrolysis of the Schiff's base bond.



(LXX)

Experiments using potassium in tert.-butanol remained the most promising as potassium bromide always separated during the reaction. From one such experiment a product was obtained as a clear resin which could not be crystallised but which showed ultra-violet absorption approximating to that expected of a cyclic acid. The resin was treated with copper acetate in the minimum of water and the precipitated salt crystallised from dioxan. From analytical evidence it could only be the copper salt of the required cyclic hydroxamic acid. The salt was decomposed by hydrogen sulphide and the pale yellow crystalline product thus obtained in poor yield was shown to be the cyclic acid (LXIX) by analysis, ultra-violet absorption and characteristic reactions.

In considering all possible means of improving the yield of the cyclisation stage before proceeding to the case of aspergillic acid by this route, the acyl derivatives of a Schiff's base were examined. If an O-acylated Schiff's base (LXXI) could be prepared,



(LXXI) (LXXII)

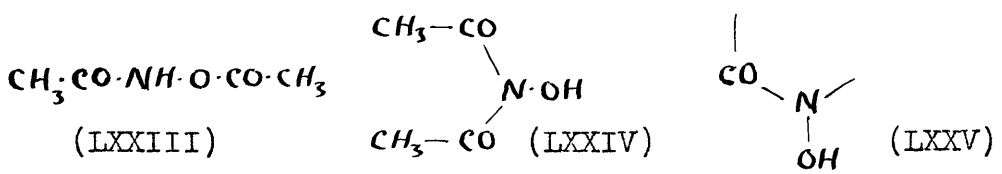
it might prove to be more readily cyclised than the unsubstituted compound. Removal of the acyl group from a cyclic product (LXXII) would not be difficult as experience with aspergillic acid has shown that pyrazine cyclic hydroxamic acids are exceptionally stable to both acid and alkali. Before describing the products obtained by acylation of a Schiff's base, it is relevant to consider the question of order of substitution of the hydroxylamine hydrogen atoms. The accepted order of substitution (10) is as follows:-

(1) $R \cdot CO \cdot Cl + NH_2OH \longrightarrow R \cdot CO \cdot NHOH$

(2) $R' \cdot CO \cdot Cl + R \cdot CO \cdot NHOH \longrightarrow R \cdot CO \cdot NH \cdot O \cdot CO \cdot R'$

(3) $R'' \cdot CO \cdot Cl + R \cdot CO \cdot NH \cdot O \cdot CO \cdot R' \longrightarrow R \cdot CO \cdot \underset{\displaystyle O \cdot CO \cdot R'}{\overset{\displaystyle |}{N}} \cdot CO \cdot R''$

The first stage gives a hydroxamic acid and this is a recognised preparative method. The second stage gives a product in which the hydrogen attached to the oxygen atom has been replaced. Evidence in support of this structure is considerable. For example, when hydroxylamine is treated with acetic anhydride, a disubstituted product is obtained which gives no coloration with ferric chloride solution (31). The two possible structures are (LXXIII) and (LXXIV) and the latter can be excluded because it contains the grouping (LXXV) for which a red coloration with ferric chloride solution is characteristic.



Similarly, the monoacetate of benzhydroxamic acid gives no coloration with ferric chloride (32). In order to have this compound available for comparative tests it was prepared by the following method. Treatment of hydroxylamine with benzoyl chloride after Jones (33) gave benzhydroxamic acid in good yield. The acid was allowed to react with acetic anhydride in ether at room temperature for 3 hours, and the product

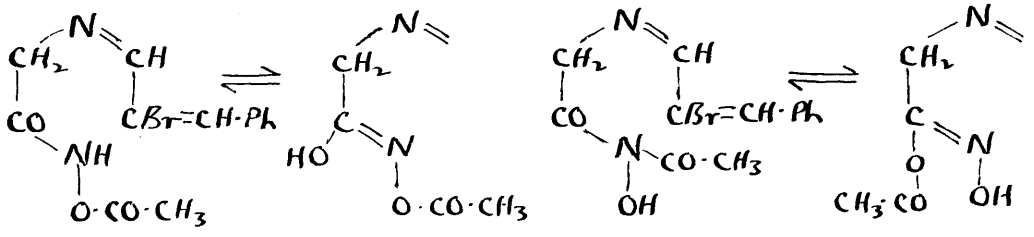
isolated was shown to be the monoacetate by analysis.

Turning now to the case of the Schiff's base (LXIV), it was found that with acetic anhydride at the temperature of the steambath a diacetate m.p. 145° was formed. At room temperature the diacetate and a monoacetate m.p. 188° were obtained in about equal yield from treatment with a molecular proportion of acetic anhydride. The diacetate in ethanol gave no coloration with aqueous ferric chloride solution. It dissolved slowly in 3N-sodium hydroxide solution at room temperature; acidification and extraction yielded a product which was shown by mixed melting point to be the monoacetate.

The monoacetate dissolved in ethanol at room temperature gave a red coloration with aqueous ferric chloride solution. In contrast, the monoacetate of benzhydroxamic acid under identical test conditions gave no coloration. As the ferric test is characteristic of the grouping (LXXV) it may be argued that the monoacetate has structure (LXXVIII) with the hydrogen attached to nitrogen substituted whereas as has already been shown a monoacylated hydroxamic acid is generally accepted as being an O-acyl derivative such as (LXXVI) and there is a negative ferric test. The problem, however,

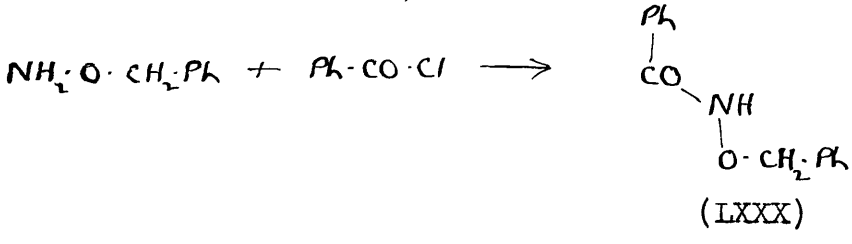
is complicated by tautomerism and further evidence is required.

The four possible structures for the monoacetate are:-



(LXXVI) (LXXVII) (LXXVIII) (LXXIX)

Structure (LXXVI) may be excluded because monoacetates described to date give a negative ferric test and more definitely because the compound (LXXX) also gives a negative ferric test (34). This ether was prepared

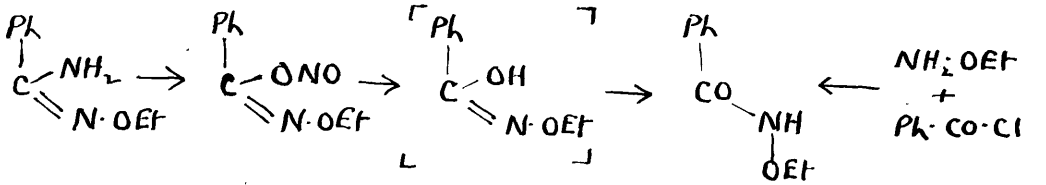


(LXXX)

by the treatment of α-benzylhydroxylamine with benzoyl chloride and hence its structure is completely defined.

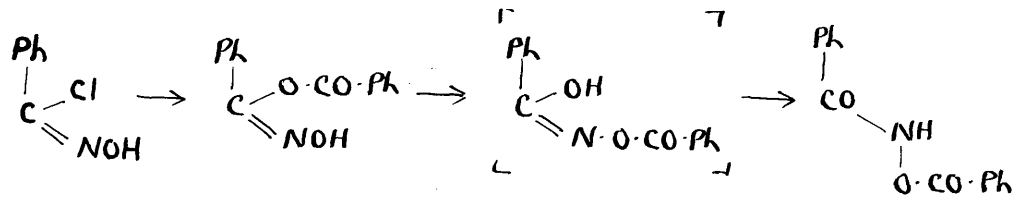
The tautomeric form (LXXVII) contains an enolic group and so might give a coloration with ferric chloride. Compounds of this type are not known to exist in a

stable state. For instance, the ether (LXXXI) has been prepared by the action of benzoyl chloride on α-ethylhydroxylamine and its structure thereby completely defined (35). On the other hand, treatment of the amidoxime ether (LXXXII) with sodium nitrite and dilute mineral acid followed by hydrolysis of the intermediate (LXXXIII) gave, not a new compound (LXXXIV) but a product identical with (LXXXI) (36).



(LXXXII) (LXXXIII) (LXXXIV) (LXXXI)

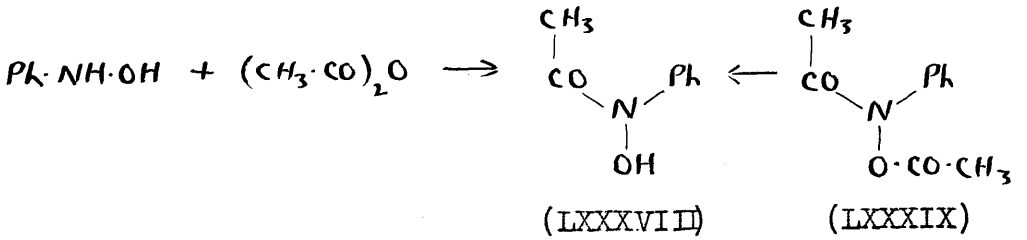
Compounds with structures such as (LXXIX) are not normally stable. To take one example, the hydroxamyl chloride (LXXXV) with silver benzoate gave a benzoate (LXXXVI) m.p. 95°, which on standing rearranged to give the benzoate (LXXXVII) m.p. 161° (37).



(LXXXV) (LXXXVI) (LXXXVII)

This leaves the structure (LXXVIII) and there is ample evidence that compounds of this type give a red coloration with ferric chloride solution. The known

cyclic acids with the grouping (LXXV) do so and so do the acylated β-hydroxylamines such as (LXXXVIII) which were prepared by Bamberger (38) thus:-

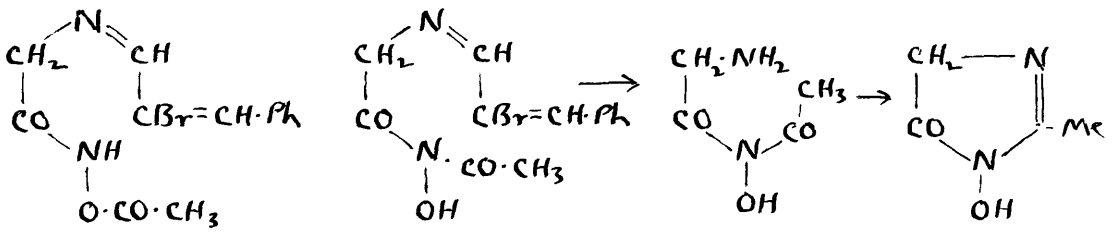


(LXXXVIII) (LXXXIX)

Excess acetic anhydride produced (LXXXIX) which gave no coloration with ferric chloride. This disubstituted compound was hydrolysed by alkali to the mono-acyl derivative (LXXXVIII). Now it has already been shown that the diacetate of the Schiff's base gave the mono-acetate on alkaline hydrolysis. These observations agree with the generally accepted rule that O-acyl groups are more readily hydrolysed than N-acyl groups. They also complete the analogy between the Schiff's base acyl derivatives and those of a β-substituted hydroxylamine.

Although it seemed certain that the monoacetate must have structure (LXXVIII), more direct evidence was sought to give complete confirmation. If the compound could be hydrolysed at the Schiff's base double bond without affecting the rest of the molecule, then the

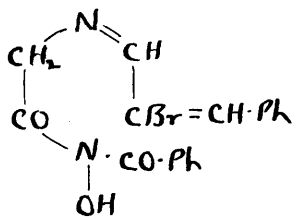
formation of a five-membered ring analogue of aspergillic acid (XC) by the following scheme would provide the necessary proof, as with the alternative structure (LXXVI) such a cyclisation would be impossible:-



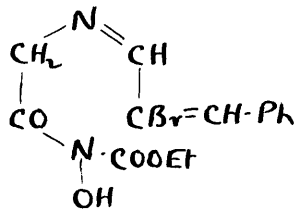
(LXXVI) (LXXVIII) (XC)

It was found that acid hydrolysis of the Schiff's base could not be accomplished except in conditions which were sufficient to hydrolyse the hydroxamic acid grouping as well. Products isolated from one experiment were 2-bromocinnamaldehyde and its oxime. The hydroxylamine for this oxime could only have come from hydrolysis of the hydroxamic acid grouping.

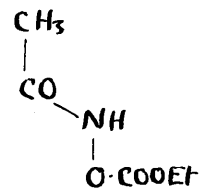
Treatment of the Schiff's base with benzoyl chloride gave a mixture of monobenzoate and dibenzoate. The mono-derivative in ethanol gave a red coloration with aqueous ferric chloride solution and hence the most likely structure is (XCI). The dibenzoate, unlike the diacetate, was insoluble in 3N-sodium hydroxide solution at room temperature. With chloroformic ester the Schiff's



(XCI)



(XCII)



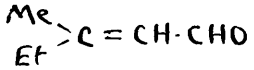
(XCIII)

base yielded a mono-substituted derivative which in ethanol gave a red coloration with aqueous ferric chloride solution and hence the most probable structure is (XCII). Now acetohydroxamic acid with chloroformic ester gave a mono-substituted derivative to which Jones (39) assigned the structure (XCIII). He observed that when this compound was suspended in water and then treated with aqueous ferric chloride solution a red coloration slowly developed. According to Jones this indicated the gradual hydrolysis of the derivative but clearly it could also be due to the slow dissolution of the compound in water.

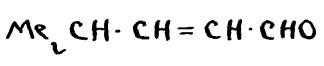
In conclusion, it seems that the properties of three different derivatives which have been prepared leave little doubt that with the Schiff's base hydroxamic acid the hydrogen attached to nitrogen is substituted first. The reactivity is therefore like that of the

β-substituted hydroxylamines and unlike that of other acyclic hydroxamic acids. The original intention of forming an O-acyl derivative of a Schiff's base for cyclising experiments could not be carried out but the attempts to prepare such a compound have contributed information on the controversial question of the order of substitution of hydroxylamine derivatives.

To extend the Schiff's base route to a synthesis of aspergillic acid it was necessary to consider first of all the synthesis of the isomeric αβ-unsaturated aldehydes (XCIV) and (XCV) which have not been described in the literature to date.

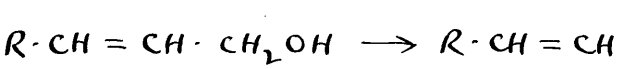


(XCIV)



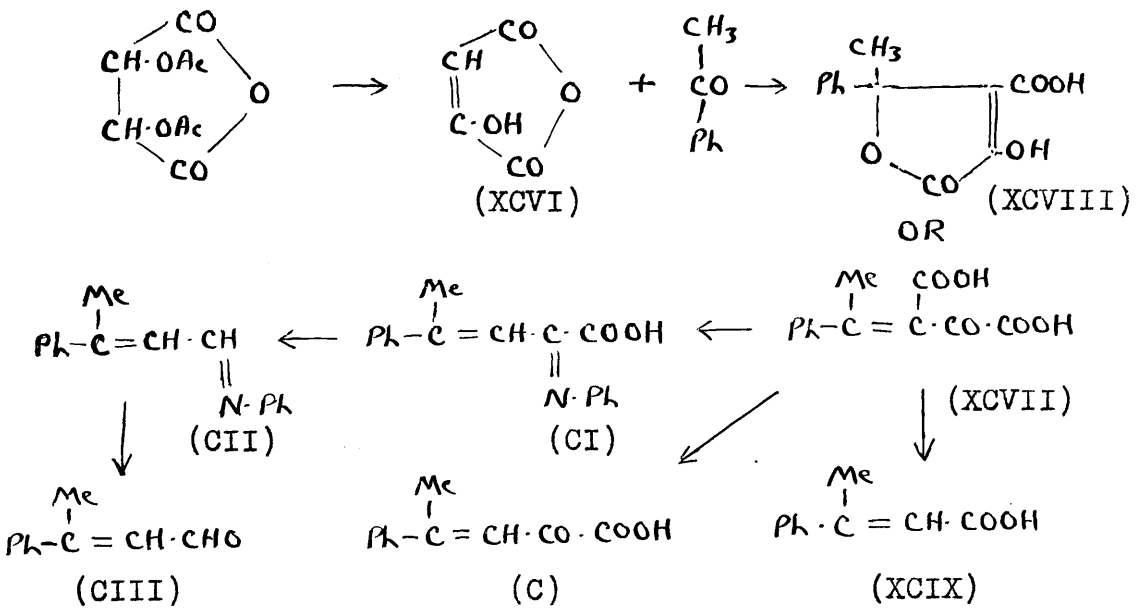
(XCV)

αβ-Unsaturated aldehydes have been synthesised by a number of methods principally following the work of Delaby (40) on the oxidation of the corresponding primary alcohols thus:-



For the required aldehydes the primary alcohols were also not known and their preparation presented some difficulty.

A new route has been described by van Dorp and Arens (41) and as this seemed to offer a possible approach it was investigated. Dorp and Arens prepared $\alpha\beta$-unsaturated aldehydes by the following series of reactions:-

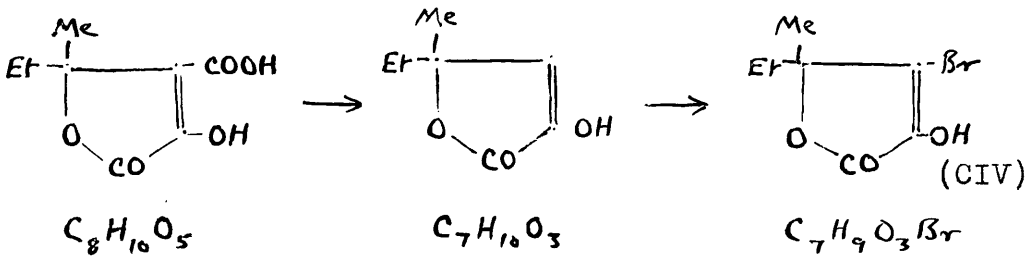


By the action of acetic anhydride on tartaric acid diacetyl tartaric anhydride was prepared in good yield. With pyridine it was converted into the pyridine salt of hydroxymaleic anhydride (XCVI). This was condensed with acetophenone to give the acid (XCVII). That the product was indeed (XCVII) and not the lactone (XCVIII)

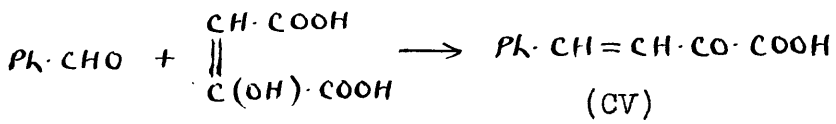
was shown by the absence of a colour reaction with ferric chloride solution. A lactone containing an enolic group would give a red coloration with this reagent. Further proof was provided when the acid was shown to be dibasic, andalso when treatment with hydrogen peroxide gave the known compound β-methylcinnamic acid. (XCIX). Dorp and Arens found that the acid (XCVII) was easily decarboxylated by heating in quinoline to give (C). More important, however, was the observation that the α-keto-acid reacted with aniline to give the anil or Schiff's base (CI). The Schiff's base was decarboxylated in quinoline to give (CII) which on acid treatment was hydrolysed to the αβ-unsaturated aldehyde (CIII) in poor yield.

It seemed that (XCIV) might be prepared by this route so for the initial reaction ethyl methyl ketone was used and the conditions described by van Dorp and Arens followed exactly. A compound $C_8H_{10}O_5$ was obtained in reasonable yield. Quinoline decarboxylation proceeded smoothly to give a product $C_7H_{10}O_3$. Treatment with bromine then gave a bromo- derivative $C_7H_9O_3Br$. When testing with ferric chloride solution it was found that this compound gave a red coloration, similarly with the compounds $C_8H_{10}O_5$ and $C_7H_{10}O_3$.

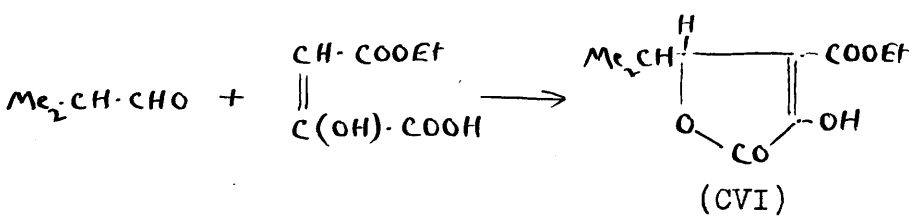
We must therefore formulate the products as lactones:-



That this type of reaction can proceed in either of two ways has already been shown. Schopf and Thierfelder (42) condensed free oxalacetic acid with benzaldehyde at p_H 7 in aqueous solution and obtained benzal pyruvic acid (CV).

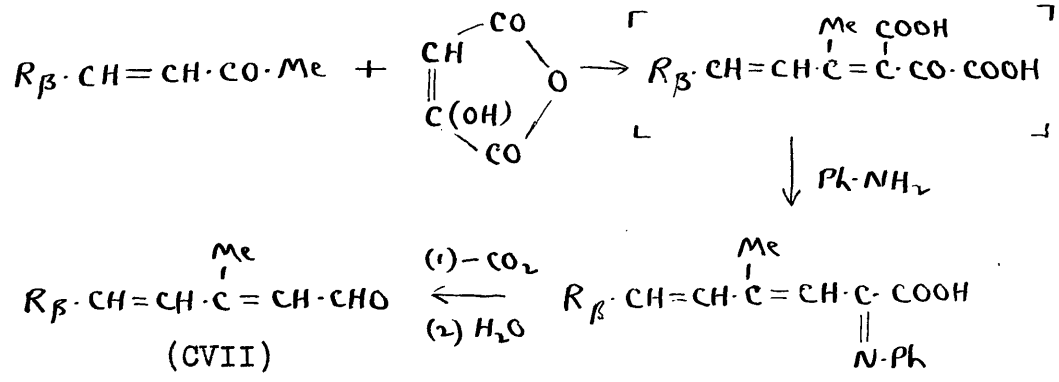


whereas Nield (43) reacted isobutyraldehyde with the sodium salt of ethyl oxalacetate in ethanol and obtained β-carbethoxy-α-keto-γ-isopropylbutyrolactone (CVI).

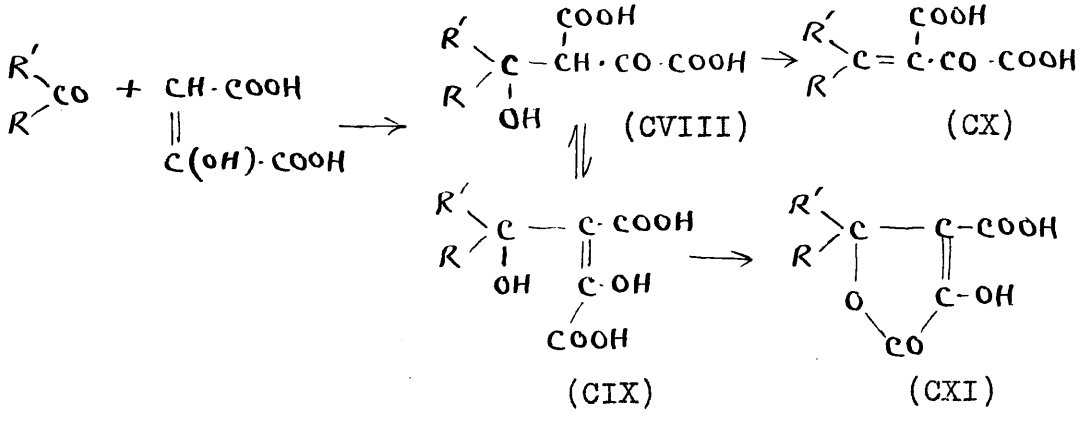


Mention must also be made of the application of the method by Dorp and Arens to the synthesis of the

vitamin-A intermediate β-ionylidene-acetaldehyde (CVII) (41):-



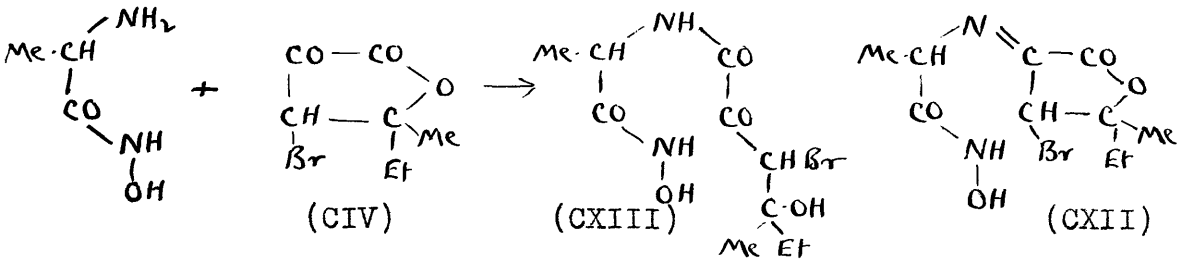
In general, the reaction proceeds through an intermediate which is not isolated and which has the tautomeric forms (CVIII) and (CIX):-



A keto-acid (CX) is formed when (CVIII) loses the elements of water. On the other hand a lactone (CXI) is obtained when the enolic form (CIX) is dehydrated. The available

evidence shows conclusively that when double bonds are suitably placed the potential conjugation provides a driving force for the reaction product to be an α-keto-acid. When R and R' are aliphatic and fully saturated, there is no such driving force and the reaction proceeds preferentially by lactonisation.

Having prepared the bromo-lactone (CIV), an attempt was made to prepare a Schiff's base (CXII). The lactone and DL-alanine hydroxamic acid were reacted under the conditions used for Schiff's base formation and a



product $C_{10}H_{17}O_5N_2Br$ obtained which on analytical evidence must have structure (CXIII). The lactone ring has been opened by the primary amino- group in a manner completely analogous with the action of ammonia on a lactone to give the acid amide of a hydroxy-acid.

In an attempt to prepare the other unsaturated aldehyde (XCV) hydroxymaleic anhydride as its pyridine salt was treated with isobutyraldehyde. There was no reaction product; the only crystalline material which could be

isolated proved to be hydroxy maleic acid.

It has been shown that the first route to $\alpha\beta$-unsaturated aldehydes to be examined is not completely general and in particular is not effective for the preparation of the required aldehydes (XCIV) and (XCV).

Yet another method for preparing $\alpha\beta$-unsaturated aldehydes was described by van Dorp and Arens somewhat later (44).

$$Et\cdot Mg\cdot Br + CH\equiv C\cdot OEt \longrightarrow Mg\cdot Br\cdot C\equiv C\cdot OEt$$
(CXIV) (CXV)

$$\begin{matrix} R' \\ R \end{matrix}\!\!>\!CO + Mg\cdot Br\cdot C\equiv C\cdot OEt \longrightarrow \begin{matrix} R' \\ R \end{matrix}\!\!>\!\underset{\underset{OH}{|}}{C} - C\equiv C\cdot OEt$$

$$\downarrow H_2 \quad (CXVI)$$

$$\begin{matrix} R' \\ R \end{matrix}\!\!>\!C = CH\cdot CHO \longleftarrow \begin{matrix} R' \\ R \end{matrix}\!\!>\!\underset{\underset{OH}{|}}{C} - CH = CH\cdot OEt$$
(CXVIII) (CXVII)

Treatment of ethoxyacetylene (CXIV) with ethyl magnesium bromide gives the Grignard reagent (CXV) which is condensed with a ketone to form the ethynylic carbinol (CXVI). Semi-hydrogenation then gives the vinyl carbinol (CXVII). This is hydrolysed with dilute mineral acid and after spontaneous anionotropic rearrangement the final product is the $\alpha\beta$-unsaturated aldehyde (CXVIII).

Ethoxyacetylene (CXIV) was prepared after Jacobs, Cramer, and Hanson (45) by treatment of bromethoxyethylene (CXIX) with powdered potassium hydroxide. For the

preparation of one of the required $\alpha\beta$-unsaturated

$$BrCH{\cdot}CH(OEt)_2 \xrightarrow[EtOH]{Zn} Br{\cdot}CH{=}CH{\cdot}OEt \xrightarrow{KOH} CH{\equiv}C{\cdot}OEt$$
(CXIX) (CXIV)

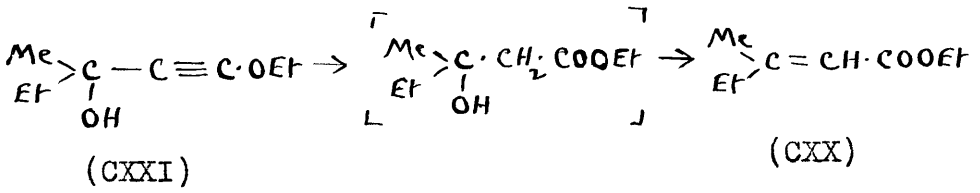
aldehydes (XCIV) ethyl methyl ketone was used in the Grignard reaction and the ethynylic carbinol $C_8H_{14}O_2$ (CXVI; R = Et, R' = Me) was isolated in an overall yield of 19% based on bromethoxyethylene.

The ethynylic carbinol was also obtained in comparable yield but by much less tedious operations when bromethoxy-ethylene was treated with sodium in liquid ammonia and ethyl methyl ketone run into the reaction mixture.

The hydrogenation was carried out in the presence of Adams catalyst and the absorption of hydrogen stopped when the calculated amount for reduction to a double bond had been absorbed. The hydrogenation product distilled over a range of temperature but was used for the next stage without further purification. It was treated with dilute hydrochloric acid under reflux and the reaction mixture steam distilled. The $\alpha\beta$-unsaturated aldehyde $C_6H_{10}O$ (CXVIII; R = Et, R' = Me) was recovered from the distillate as its semicarbazone. The yield of semicarbazone (25%) was low and ether extraction of the distillate after the semicarbazone formation led to

the isolation of a sweet-smelling liquid b.p. 33-35°/15 mm. in a yield indicating that it was derived from a substantial proportion of the starting material.

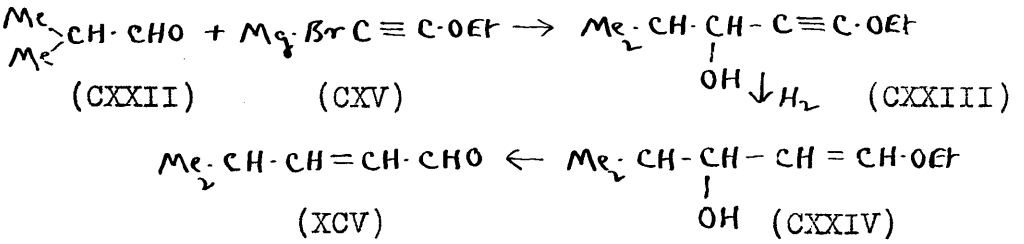
The constitution of this liquid by-product was investigated. Analysis and a molecular-weight determination indicated a molecular formula of $C_8H_{14}O_2$. The compound did not contain a carboxylic or a carbonyl group. It gave a positive test for an ester when it was treated with hydroxylamine and the resulting hydroxamic acid detected by the deep red colour of its ferric salt. The ester was hydrolysed with mineral acid and the carboxylic acid component isolated from the reaction mixture as an S-benzylthiouronium salt. The salt analysed for $C_{14}H_{21}O_2N_2S$ giving the acid a molecular formula $C_6H_{10}O_2$. The compound $C_8H_{14}O_2$ is therefore its ethyl ester. The ethyl ester of 3-methylpent-2-enoic acid (CXX) has been described (46) and the recorded b.p. and n_d are identical with those of the compound $C_8H_{14}O_2$. The formation of this ester as a by-product can only be explained by incomplete hydrogenation and the presence of unchanged ethynylic carbinol (CXXI) in the hydrogenation product which, it will be recalled, did not have a constant boiling point. The carbinol, on treatment with dilute mineral acid, would give the ester (CXX) by hydration of the triple bond thus:-



(CXXI) (CXX)

Confirmation was obtained when the carbinol was treated directly with dilute mineral acid and the ester (CXX) obtained as a product and identified by hydrolysis and formation of the known S-benzyl-thiouronium salt.

An attempt was made to prepare the isomeric $\alpha\beta$-unsaturated aldehyde (XCV) by the same route. The Grignard reagent (CXV) from ethoxyacetylene was treated with isobutyraldehyde (CXXII). A colourless liquid



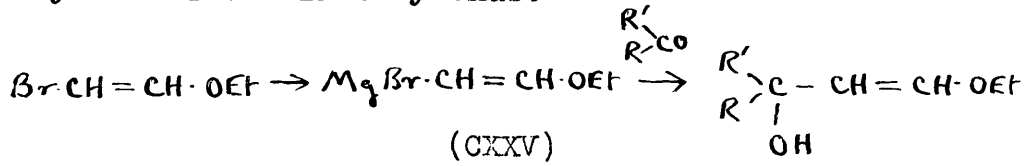
(CXXII) (CXV) (CXXIII) (XCV) (CXXIV)

b.p. 63-65°/10^{-2}mm. was isolated in reasonable yield. It coloured slowly on standing, even in a sealed tube; a constant analysis could not be obtained. Assuming the product to be (CXXIII), it was hydrogenated immediately after its formation. Treatment of the hydrogenated material with p-nitrobenzoyl chloride gave

a compound $C_{14}H_8O_7N_2$ m.p. 185-187° which proved to be the anhydride of p-nitrobenzoic acid and not a carbinol derivative. Treatment with Brady's reagent gave a 2:4-dinitrophenylhydrazone. If the hydrogenation had given the vinyl carbinol (CXXIV) this 2:4-dinitrophenylhydrazone must be the derivative of the required aldehyde (XCV). This was disproved by analysis and no alternative formula could be derived from the analysis figures for the 2:4-dinitrophenylhydrazone.

As similar results were obtained on repeating the work, it was decided to abandon efforts to prepare the aldehyde (XCV) and concentrate on the preparation of (XCIV) on a larger scale.

The overall yield of (XCIV) was, at this point, so low that subsequent stages of the aspergillic acid synthesis could not be attempted. Now if the inefficient hydrogenation stage could be eliminated, a more practicable overall yield might be achieved. Van Dorp and Arens (47) have claimed that bromethoxyethylene forms a Grignard reagent (CXXV) which makes it possible to prepare the vinyl carbinol directly thus:-



This reaction was investigated but attempts to form the Grignard reagent were unsuccessful.

The preparation of isobutenyl-lithium and its condensation with aldehydes and ketones to form vinyl carbinols has recently been reported (48). Bromethoxy-ethylene with lithium metal appeared to form the lithium compound (CXXVI). Reaction with ethyl methyl ketone ought to have given the required vinyl carbinol (CXXVII)

$$CH_2Br{=}CH{\cdot}OEt + Li \longrightarrow LiCH{=}CH{\cdot}OEt \xrightarrow{Me\,Et>CO} \underset{OH}{Me\,Et\,C}{-}CH{=}CH{\cdot}OEt$$

(CXXVI) (CXXVII)

but the reaction product consisted mainly of a compound $C_8H_{14}O$ produced by the self-condensation of ethyl methyl ketone. It was characterised by the formation of a 2:4-dinitrophenylhydrazone $C_{14}H_{18}O_4N_4$ and a semicarbazone $C_9H_{17}ON_3$ m.p. 163°. One of the possible self-condensation products from ethyl methyl ketone described by Abbott, Kon and Satchell (49) had a semicarbazone m.p. 163°. A higher-boiling fraction was isolated from the reaction mixture in small yield. This probably contained some vinyl carbinol as treatment with mineral acid gave a product which, with Schiff's reagent, gave a positive test for aldehyde. This reaction did not seem promising and was not followed up when other workers

reported a similar lack of success (50).

Another route to $\alpha\beta$-unsaturated aldehydes which has had some application in the vitamin A field (51) was now investigated. β-Chlorovinyl methyl ketone (CXXVIII) was prepared by a Friedel-Craft reaction from acetyl chloride and vinyl chloride. A Grignard reaction with ethyl magnesium bromide then gave the carbinol (CXXIX) in 49% yield. The carbinol was steam distilled with 1% sulphuric acid and the required aldehyde (XCIV) isolated as its semicarbazone in 14% yield.

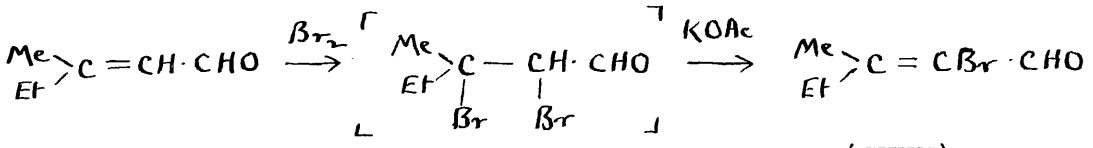
$$CH_3 \cdot CO \cdot Cl + CH_2 = CH \cdot Cl \longrightarrow CH_3 \cdot CO \cdot CH = CH \cdot Cl$$

(CXXVIII)

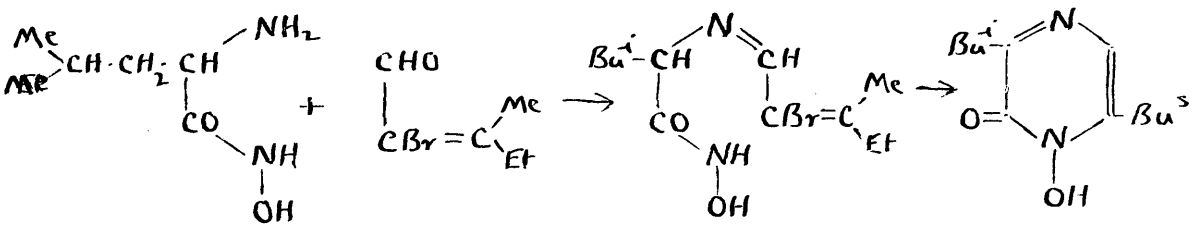
$$CH_3 \cdot CO \cdot CH = CH \cdot Cl \xrightarrow{EtMgBr} \underset{Et}{\overset{Me}{>}}C(OH) - CH = CH \cdot Cl \longrightarrow \underset{Et}{\overset{Me}{>}}C = CH \cdot CHO$$

(CXXIX) (XCIV)

The free aldehyde (XCIV) b.p. 65-67°/20 mm. was obtained by hydrolysing the recrystallised semicarbazone with oxalic acid. It was treated with bromine and potassium acetate in glacial acetic acid and the α-bromaldehyde (CXXX) isolated by steam distillation. For the next stage the aldehyde was used without further purification



(CXXX)



(CXXXI) (CXXXII) (CXXXIII)

as distillation even at 0.5 mm. resulted in considerable decomposition. Characterisation was obtained by the formation of a 2:4-dinitrophenylhydrazone $C_{12}H_{13}O_4N_4Br$.

DL-leucine hydroxamic acid (CXXXI), prepared by the standard method from the amino-acid ester and hydroxylamine, reacted smoothly with the α-bromaldehyde (CXXX) to give the required Schiff's base (CXXXII) in 87% yield.

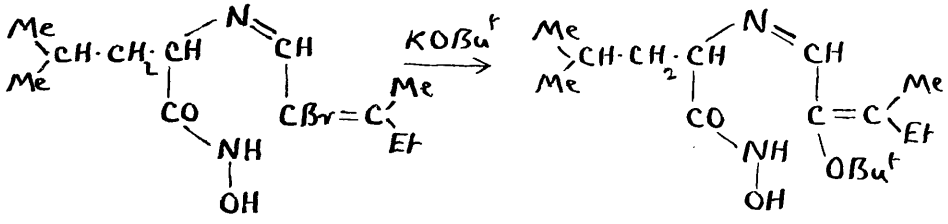
As L-leucine is more readily available than DL-leucine L-leucine hydroxamic acid was prepared from L-leucine methyl ester in good yield. It was not, however, used for subsequent work.

A cyclisation experiment in which the Schiff's base

(CXXXII) was treated with potassium in tert.-butanol under reflux did not yield an analysable product. From the reaction mixture a clear resin was obtained which had some of the characteristics of a cyclic acid. With a saturated solution of copper acetate, however, a test portion formed a copper salt largely insoluble in dioxan. This insolubility in dioxan showed that a high proportion of the resin must be acyclic hydroxamic acid. The crude material was sublimed in order to separate it into cyclic and acyclic fractions. A non-crystalline sublimate obtained in very low yield had the characteristic properties of a cyclic hydroxamic acid, namely solution with effervescence in sodium hydrogen carbonate and a deep red coloration with ferric chloride solution. From the ultra-violet spectrum of this material it is estimated that it contained the required cyclic acid (CXXXIII) to the extent of 30%.

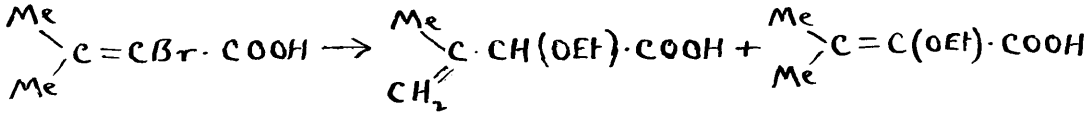
There is some evidence to support the theory that there is a reaction competing with the cyclisation. A much greater weight of potassium bromide was filtered from the reaction mixture than is accounted for by the yield of cyclic acid. Further, a gum was isolated in fair yield which had the properties of an acyclic

hydroxamic acid and was too ether soluble to be unchanged Schiff's base. It is suggested that the other reaction product is the ether (CXXXIV) formed thus:-



(CXXXII) (CXXXIV)

Similar reactions have recently been described by Owen (52). To take one example, α-bromo-ββ-dimethylacrylic acid (CXXXV) refluxed with ethanolic sodium ethoxide gives a mixture of α-ethoxy-β-methylenebutyric acid (CXXXVI) and α-ethoxy-ββ-dimethylacrylic acid (CXXXVII).



(CXXXV) (CXXXVI) (CXXXVII)

In conclusion, to summarise the progress towards aspergillic acid by the Schiff's base route it can be

said that a method for the synthesis of one of the Schiff's bases required has been developed. A preliminary cyclisation experiment has shown that the required acid is formed in very small yield and that there is a considerable loss of material due to a competing reaction. This would indicate that further work must be directed towards obtaining a more efficient cyclising agent.

Before giving an account of another type of synthesis of 3:6-disubstituted pyrazine cyclic hydroxamic acids, it is relevant, at this stage, to present and discuss the results of some anti-bacterial tests. The comparative in vitro activities of representative compounds prepared during this investigation are given in the following table:-

Minimal Inhibitory Concentration in Mg. per 100 c.c.

Culture Medium.

Compound.	Medium:	Strep. haem. Blood	Broth	Staph. aureus. Broth	B.coli. Broth
1. 6-Benzyl-1-hydroxy-2-keto-1:2-dihydro pyrazine.		100	5	100	50
2. Glycine hydroxamic acid.			20	50	200
3. α-Amino-n-butyro-hydroxamic acid.			500	500	500
4. DL-Valine hydroxamic acid.			> 10	> 10	> 10
5. Cinnamylidene glycine hydroxamic acid.			> 2	> 2	> 2
6. 2-Bromocinnamylidene-glycine hydroxamic acid.		> 2	0.5	> 1	0.5
7. α-(2-Bromocrotonylideneamino) -n-butyrohydroxamic acid.			200	300	500
8. Diacetate of (6).			> 100	> 100	> 100

Mouse toxicity tests.

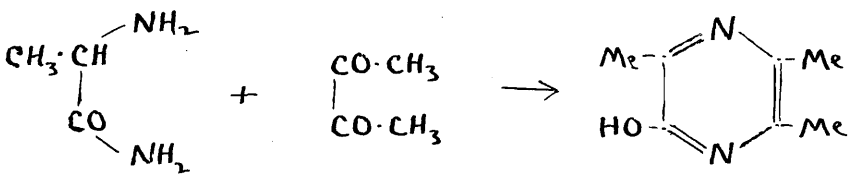
Route of administration:- intra-peritoneal.

Weight of animals:- 18 - 22 g.

	Max. tolerated dose.	Min. lethal dose
2-Bromocinnamylidene glycine hydroxamic acid.	10 mg.	20 mg.

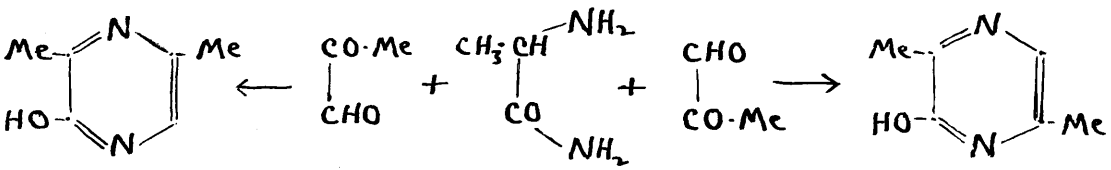
Compound (1), a pyrazine cyclic hydroxamic acid, is not particularly active. Compounds (2) - (4) are acyclic hydroxamic acids which have measurable activity. Compounds (5) - (8) are Schiff's bases, of which (5) and (6) are the most active. When the hydroxamic acid grouping is protected by acylation as in (8), the activity becomes negligible. The compounds are highly toxic and as both the activity and toxicity appear to be due to the hydroxamic acid grouping these effects cannot be separated.

Attempts to form a pyrazine cyclic hydroxamic acid from an α-amino-hydroxamic acid and a 1:2-dicarbonyl compound have already been described. The experiments were unsuccessful and the conditions necessary to enforce such a cyclisation were never found. Interest in this approach, however, was renewed when Jones reported the successful condensation of α-amino-acid amides with 1:2-dicarbonyl compounds to give hydroxypyrazines the reactions being carried out at very low temperatures and in aqueous alkali (53). For example, DL-alanine



(CXXXVIII) (CXXXIX)

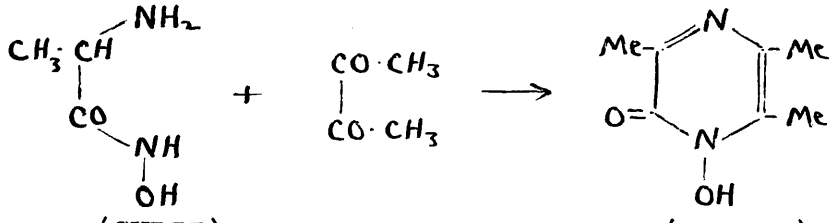
amide (CXXXVIII) with diacetyl reacted smoothly to give 2-hydroxy-3:5:6-trimethylpyrazine (CXXXIX) in good yield. When DL-alanine amide is condensed with methylglyoxal there are two possibilities. The product may be either 2-hydroxy-3:5-dimethylpyrazine (CXL) or 2-hydroxy-3:6- dimethylpyrazine (CXLI) thus:-



(CXL) (CXLI)

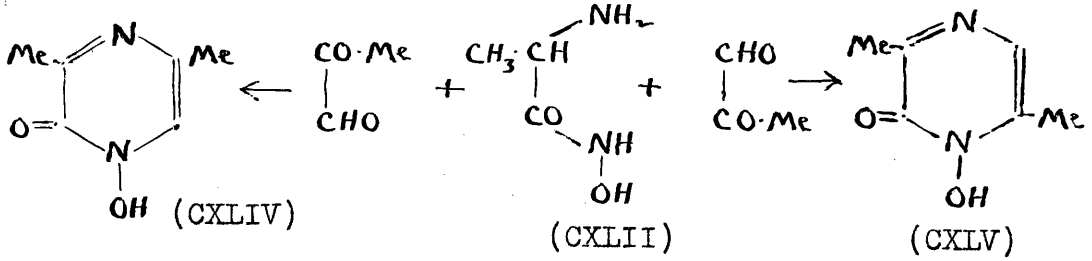
Jones found that the reaction went completely in one direction and gave 2-hydroxy-3:5-dimethylpyrazine exclusively. In all cases of this reaction which he describes the 2-hydroxy-3:5-disubstituted pyrazine was obtained with no trace of the 3:6-disubstituted isomeride.

It is obvious that if an α-amino-hydroxamic acid is used in place of an α-amino-acid amide the product will be a pyrazine cyclic hydroxamic acid thus:-



(CXLII) (CXLIII)

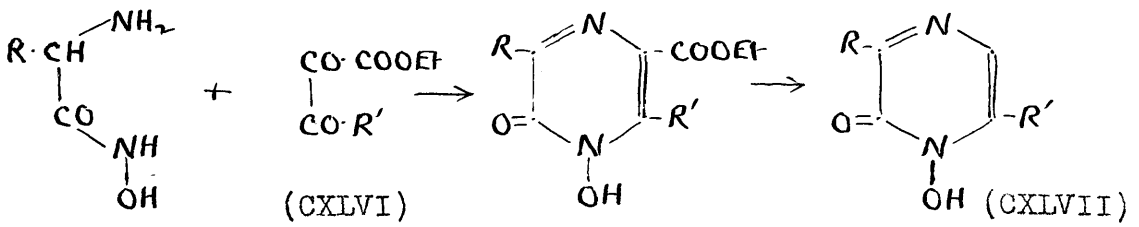
Using the conditions described by Jones (53) such cyclisations were effected by Dr. G.T. Newbold and the authors colleagues Messrs. G. Dunn and W. Sweeny. For example, DL-alanine hydroxamic acid (CXLII) condensed with diacetyl to give the acid 1-hydroxy-2-keto-3:5:6-trimethyl-1:2-dihydropyrazine (CXLIII). When DL-alanine hydroxamic acid was condensed with methylglyoxal the 3:5-disubstituted acid (CXLIV) was isolated in fair yield, there being no trace of the 3:6-disubstituted isomeride (CXLV).



(CXLIV) (CXLII) (CXLV)

The cyclisations were carried out in aqueous alkali; at p_H 7 the yield was reduced but even with glacial

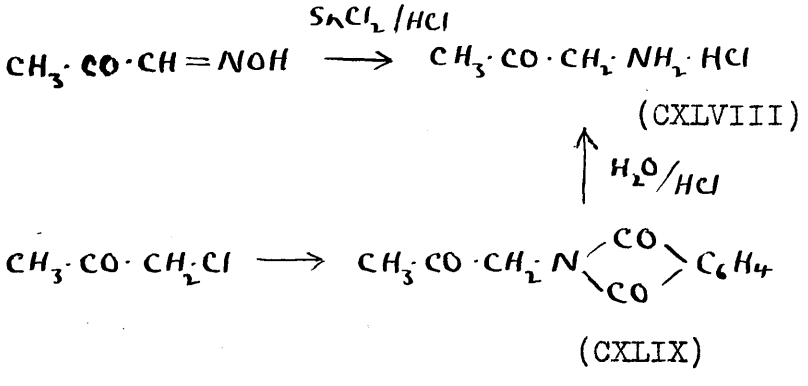
acetic acid as the reaction solvent some of the cyclic product could be isolated. It was thought that with variations in pH partial formation of the 3:6-disubstituted isomeride might be induced but this was not the case. It is of interest at this point to note that the reaction of glycine hydroxamic acid with 1:2-dicarbonyl compounds did not proceed smoothly. Only in one case, that with phenyl-glyoxal, was a small yield of pyrazine cyclic hydroxamic acid obtained. The failure of the early experiments on this type of condensation may therefore be attributed to some extent to the choice of glycine hydroxamic acid for the α-amino-hydroxamic acid.



The reaction between an α-amino-hydroxamic acid and a diketo-carboxylic ester (CXLVI) was examined by W. Sweeny as there was the possibility that the carbethoxy-group would induce the condensation to proceed in the direction shown above. Removal of the carbethoxy-group to give the required acid (CXLVII) would not present any difficulty. This scheme, however, proved unsuccessful.

The synthesis which has just been described does not provide a route to aspergillic acid as it could not be adapted to give a 3:6-disubstituted pyrazine cyclic hydroxamic acid. To get such a product unambiguously the reaction between an α-keto-hydroxamic acid and an aminoketone was examined.

Aminoketones as such are not known but they have been isolated as hydrochlorides. To start with, the simplest case was taken. Aminoacetone hydrochloride (CXLVIII) was prepared after Gabriel and Pinkus (54) from isonitrosoacetone by reduction with stannous chloride.



It was also prepared by the method of Gabriel and Colman (55) from chloracetone via the phthalimido-ketone (CXLIX).

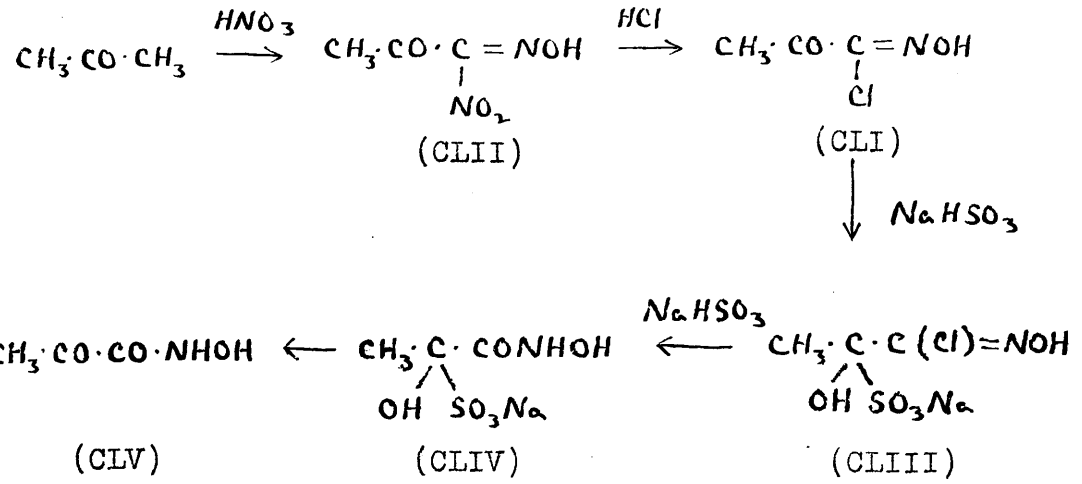
The α-keto-hydroxamic acid presented greater difficulty. Hydroxamic acids are normally prepared by

the action of hydroxylamine on an acid ester or acid chloride. The action of hydroxylamine on an α-keto-carboxylic ester leads to oxime formation (56) and the acid chlorides of α-keto-acids have a doubtful existence (57). Only a few α-keto-hydroxamic acids have been described in the literature and those were obtained by rather novel routes. The first route is limited in that it applies to aromatic acids only (58). For example, phenyl-glyoxal and benzene sulphonic hydroxamic acid react to give the α-keto-hydroxamic acid (CL) thus:-

$$Ph \cdot CO \cdot CHO + Ph \cdot SO_2 NHOH \longrightarrow Ph \cdot CO \cdot CO \cdot NHOH$$

(CL)

Another method which is due to Gastaldi is more general in that it has been applied to both aromatic and aliphatic cases (59). It is illustrated by the preparation of pyruvohydroxamic acid (CLV) by the following scheme:-



Pyruvohydroxamyl chloride (CLI) or α-chloro-α-isonitroso-acetone was prepared after Ponzio (60) by the successive action of nitric acid and hydrochloric acid on acetone. The intermediate nitrolic acid (CLII) was not isolated. The compound, m.p. 107°, was obtained more easily and in better yield by following the conditions described by Ponzio exactly than by the preparation given in Beilstein (61) although the method is the same in both cases. Hydrolysis of the hydroxamyl chloride to pyruvohydroxamic acid as described by Gastaldi could not be reproduced exactly. To avoid confusion a brief account of Gastaldi's work will be given first.

Treatment of pyruvohydroxamyl chloride with a saturated solution of sodium hydrogen sulphite gave

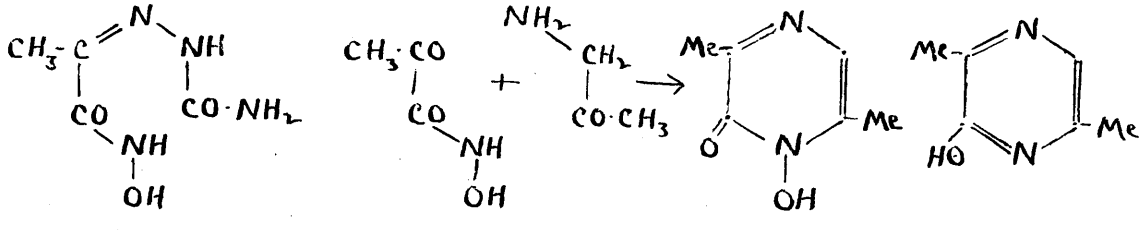
initially the bisulphite compound (CLIII) (59). This was hydrolysed with excess of the reagent to (CLIV), the bisulphite compound of pyruvohydroxamic acid which was precipitated by adding a large volume of alcohol to the aqueous reaction mixture. The precipitated oil solidified on standing and crystallisation from aqueous alcohol gave the bisulphite compound as a mono-hydrate, m.p. 65°. The bisulphite compound was treated with a saturated solution of copper acetate followed by alkali and the insoluble complex copper salt, $Cu(C_3H_3O_3N)Na_2$ 4.5 H_2O collected. Decomposition of this salt with sulphuric acid followed by ether extraction gave a mixture of pyruvohydroxamic acid, m.p. 106° (d.) and its dimer, m.p. 137° (d.). Gastaldi does not record the yields of these products (59).

Repeating the above work, the preparation reached the stage where the bisulphite compound is precipitated and then the solid which separated could not be crystallised. From the yield obtained the compound was obviously contaminated with sodium hydrogen sulphite. The mixture gave an intense red coloration with ferric chloride solution and an aqueous solution when treated with copper acetate and alkali deposited a copper salt.

The salt did not separate until the alkali was added. Decomposition of the salt by acid followed by ether extraction gave material m.p. 135° (d.) in very small yield. This melting point is close to that obtained by Gastaldi for the free pyruvohydroxamic acid dimer.

Ponzio has claimed that treatment of pyruvohydroxamyl chloride with sodium acetate in acetic acid gives pyruvohydroxamic acid but experimental details are not given (62). An attempt to prepare the free acid by this method gave a resin which could not be crystallised.

As pyruvohydroxamic acid was unobtainable and the formation of its semicarbazone (CLVI) from the bisulphite compound has been described (63), the reaction with aminoacetone was attempted under the same conditions.



(CLVI) (CXLV) (CXLI)

The bisulphite compound, as it was first prepared, contained a high proportion of sodium hydrogen sulphite but was used without further purification. The product

isolated in small yield from the first successful experiment was identified by mixed melting point and analysis as 2-hydroxy-3:6-dimethylpyrazine (CXLI). This compound could only have been formed by reduction of the required acid 1-hydroxy-2-keto-3:6-dimethyl-1:2-dihydropyrazine (CXLV). The reduction was not surprising considering the excess of sodium hydrogen sulphite present in the reaction mixture. The mother liquors from the crystallisation of the hydroxypyrazine gave a red coloration with ferric chloride solution. Evaporation and crystallisation of the residue failed to yield a product of constant melting point but the solid obtained gave a red coloration with ferric chloride solution and was soluble with effervescence in sodium hydrogen carbonate solution. It had, therefore, the characteristics of an impure specimen of the required acid.

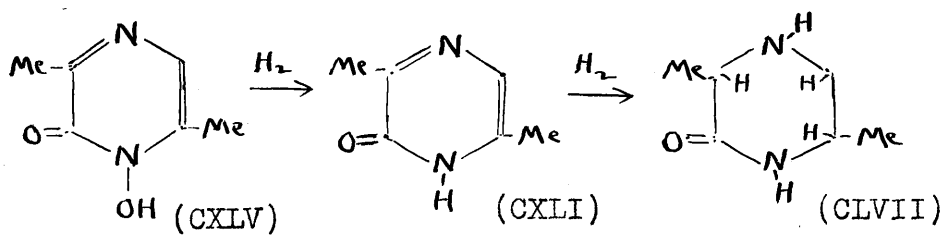
The obvious step was to free the bisulphite compound from sodium hydrogen sulphite. This was achieved by precipitation of the inorganic salt with ethanol from an aqueous solution of the mixture, the sodium hydrogen sulphite being less soluble in ethanol than the bisulphite compound. Repetition of this treatment eventually gave the compound as a pale yellow amorphous powder m.p. $> 300^{\circ}$.

This was reacted under the same conditions as before with aminoacetone, namely at 60° in aqueous acetic acid for 8 hours. The required acid (CXLV) separated in good yield after standing overnight. It gave the reactions expected of a homologue of aspergillic acid, a red coloration with aqueous ferric chloride and solution with effervescence in sodium hydrogen carbonate solution. Also with copper acetate solution it gave a green copper salt which crystallised from dioxan. The ultra-violet absorption spectrum of the acid had two well defined maxima which coincided with those shown by aspergillic acid and differed only in intensity. The acid had one peculiarity in that analysis showed it to be a monohydrate $C_6H_8O_2N_2,H_2O$. The molecule of water must be attached more firmly than normal water of crystallisation as it was not removed during a crystallisation from acetone followed by sublimation at 10^{-3} mm. The free acid was obtained by overnight drying at 10^{-2} mm/20° and was analysed immediately. That the H_2O was not constitutional was shown conclusively when the copper salt of the acid analysed for $Cu(C_6H_7O_2N_2)_2$ and not $Cu(C_6H_9O_3N_2)_2$.

It has been shown that aspergillic acid is reduced with hydrazine to give a 2-hydroxy-3:6-disubstituted

pyrazine and hence reduction of the homologue ought to give 2-hydroxy-3:6-dimethylpyrazine, a known compound. The formation of this hydroxypyrazine in the initial experiment described above provided some evidence but reduction of the acid itself was necessary to give a conclusive proof of its structure.

In the first reduction experiment a large excess of hydrazine was used and a product m.p. 92-94° was obtained which analysed for $C_6H_{12}ON_2$. A suggested structure is (CLVII) where the pyrazine ring has been partly reduced but the product $C_6H_{12}ON_2$ showed ultra-violet absorption and (CLVII) with no conjugation would not absorb. The reduction was repeated with less hydrazine and a compound $C_6H_8ON_2$ m.p. 208-210° obtained. This melting point was not depressed on admixture with

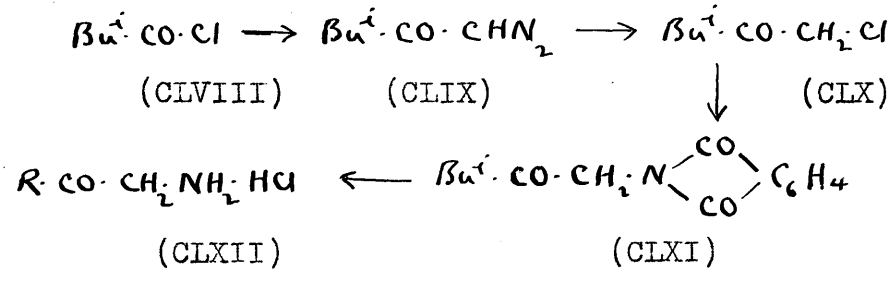


a specimen of 2-hydroxy-3:6-dimethylpyrazine (CXLI) prepared via another route by Baxter, Newbold and Spring (64). The reduction to a known hydroxypyrazine completely defines the structure of the cyclic acid as (CXLV) and

it is a true homologue of aspergillic acid.

As work was proceeding simultaneously on the Schiff's base approach to give one aspergillic acid isomer, it was decided to attempt a synthesis of the other by the route outlined above.

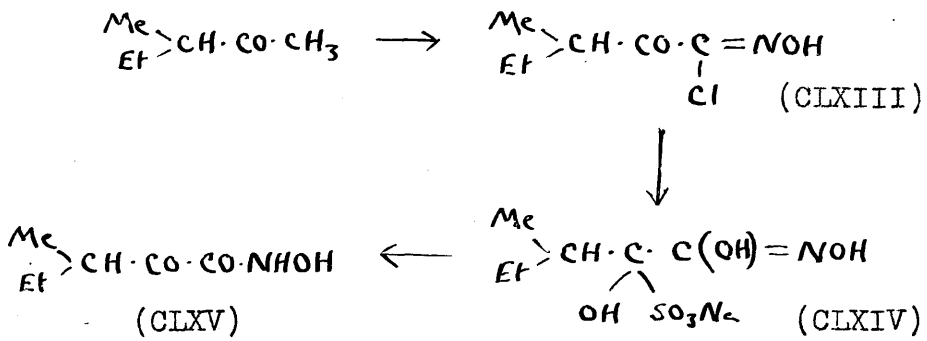
The preparation of the required aminoketone (CLXII; R = Bu^i) will be considered first. The isomeric aminoketone (CLXII; R = Bu^s) had been prepared by Newbold and Spring (8) so that a method was available. It involved the following series of reactions:-



Isovaleric acid was converted into the acid chloride (CLVIII) in 70% yield using the benzoyl chloride method first described by Brown (65). Addition of the acid chloride to excess diazomethane in ether gave the diazoketone (CLIX) which was not isolated but treated with hydrogen chloride to give 1-chloro-4-methylpentan-2-one (CLX) in good yield. This was converted into 1-phthalimido-4-methylpentan-2-one (CLXI) by heating with potassium phthalimide in xylene. This phthalimido-

intermediate was isolated in 48% yield. Hydrolysis with hydrochloric acid then gave the required aminoketone, 1-amino-4-methylpentan-2-one (CLXII; R = Bu^i) as its hydrochloride m.p. 176 - 178° in 81% yield.

The preparation of the required α-keto-hydroxamic acid (CLXV) presented greater difficulty. As pyruvohydroxamic acid was not isolated but used successfully in the form of its bisulphite derivative, attention was first of all directed towards the synthesis of the bisulphite compound (CLXIV).



Sec.-butyl methyl ketone was prepared by the acetoacetic ester ketone synthesis. Its conversion into a hydroxamyl chloride (CLXIII) through an intermediate nitrolic acid was not practicable. Use was made of the method described by Rheinboldt and Schmitz-Dumont (66). By treating a ketone with nitrosyl chloride the hydroxamyl chloride is prepared in one step thus:-

$$R\cdot CO\cdot CH_3 \xrightarrow{NOCl} R\cdot CO\cdot C(Cl)=NOH$$

(CLXVI)

In a preliminary experiment starting with acetone the author obtained pyruvohydroxamyl chloride (CLI) in good yield. Treatment of sec.-butyl methyl ketone with nitrosyl chloride gave a compound which distilled at 10^{-3} mm./bath temperature 120° and then separated from light-petroleum as transparent plates m.p. 59 - 61°. It analysed for $C_6H_{10}O_2NCl$, gave a positive Beilstein test for halogen, and formed a 2:4-dinitrophenyl-hydrazone. The compound dissolved rapidly in aqueous alkali and when the solution was acidified it gave with aqueous ferric chloride the deep red coloration characteristic of hydroxamic acids. These observations confirm that the product is 1-keto-2-methyl-n-valerohydroxamyl chloride (CLXIII).

The isomeric hydroxamyl chloride (CLXVI; R = Bu^i) was also prepared starting from the more readily available isobutyl methyl ketone. When the ketone was treated with nitrosyl chloride and the reaction product distilled at 10^{-3} mm. two distinct fractions were obtained. A fraction collected at bath temperature 90° did not solidify and decomposed on standing. A fraction collected at bath temperature 130° in small yield solidified on standing. It was crystallised from light-petroleum to give the

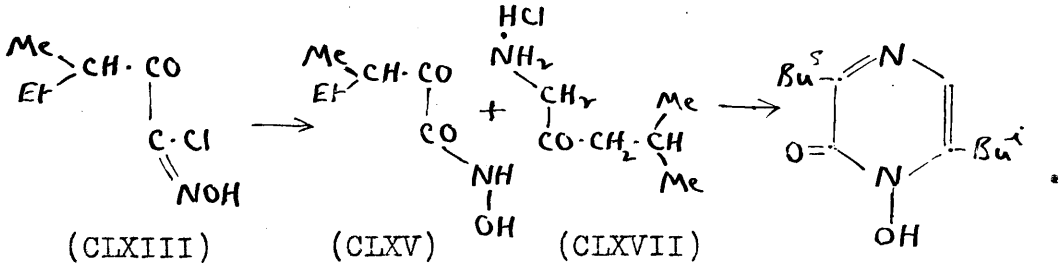
hydroxamyl chloride (CLXVI; R = Bu^i) as plates m.p. 64 - 66°. The 2;4-dinitrophenylhydrazone of this compound was formed by treatment with Brady's reagent under standard conditions.

In order to develop the next stage of the synthesis, namely the formation of the bisulphite compound (CLXIV), the hydroxamyl chloride (CLXIII) was prepared on a larger scale. As there was a considerable drop in yield after crystallisation from light-petroleum it was distilled once only and the semi-solid distillate used for the following experiments. After treatment with a saturated solution of sodium bisulphite at room temperature followed by 12 hours stirring, the hydroxamyl chloride was mainly unchanged forming a top layer of the reaction mixture. At 30 - 40° the reaction went more quickly, about 60% of the hydroxamyl chloride reacting. The aqueous phase was then taken to dryness under vacuum at room temperature and the excess sodium hydrogen sulphite removed from the residue by precipitation with ethanol. The concentration of the bisulphite compound was followed by the intensity of the coloration with ferric chloride solution. The final product was still contaminated with sodium hydrogen sulphite and no satisfactory method for the complete elimination of inorganic material could be devised.

A number of cyclisation experiments in which the crude bisulphite compound (CLXIV) was treated with the aminoketone (CLXII; R = Bu^i) proved unsuccessful, there being no trace of cyclic acid. A reaction product, isolated in low yield, formed a 2:4-dinitro-phenylhydrazone which was analysed. No reasonable formula could be found to fit the analysis results.

It was now necessary to consider other means of converting the hydroxamyl chloride (CLXIII) into the α-keto-hydroxamic acid (CLXV). The known methods for hydrolysis of hydroxamyl chlorides are limited. Aqueous alkali has been shown to give products other than the α-keto-hydroxamic acid (67). Apart from the bisulphite compound route there remains the method of Ponzio (62) which has already been discussed and which makes use of sodium acetate as the hydrolysing agent.

In a final cyclisation experiment the hydroxamyl chloride (CLXIII) and the aminoketone hydrochloride (CLXVII) were reacted in the presence of sufficient



(CLXIII) (CLXV) (CLXVII)

sodium acetate to hydrolyse both the hydroxamyl chloride to the α-keto-hydroxamic acid (CLXV) and the aminoketone hydrochloride to the free aminoketone. From this experiment a reaction product was obtained in very small yield which had the properties of a cyclic hydroxamic acid and had the expected ultra-violet absorption spectrum. The intensities of the absorption bands were low and the material could not be crystallised. Apart from the trace of cyclic acid there was also a ketonic reaction product. This was a liquid which distilled at bath temperature $80^{\circ}/10^{-3}$ mm.; it dissolved with effervescence in a solution of sodium hydrogen carbonate and gave a deep red coloration with ferric chloride solution. The properties of this compound suggest a ketonic hydroxamic acid such as (CLXV). The 2:4-dinitrophenylhydrazone of the α-keto-hydroxamic acid would be $C_{12}H_{15}O_6N_5$ requiring C, 44.3; H, 4.6%. A 2:4-dinitrophenyl-hydrazone from the reaction product analysed C, 45.0; H, 4.0% which is not near enough to give the required confirmation.

To summarise the progress towards a synthesis of aspergillic acid by the above approach it can be said that the exact method which gave the homologue (CXLV) in good yield fails in the aspergillic acid case.

The failure can be ascribed, to a large extent to lack of reactivity of the α-keto-hydroxamic acid with sodium bisulphite compared with that of pyruvohydroxamic acid. An alternative method of hydrolysing the hydroxamyl chloride to the α-keto-hydroxamic acid was attempted and from the subsequent cyclisation a trace of the required cyclic acid was obtained. This type of reaction does show some promise and with correct conditions might lead to an aspergillic acid synthesis.

EXPERIMENTAL.

EXPERIMENTAL

Aminoiminosuccinonitrile (HCN Tetramer)

(cf. Linstead, Noble and Wright, J., 1937, 911)

Liquid hydrocyanic acid (350 g.) was poured into a stout bottle (500 c.c.), potassium cyanide (2 g.) added, and the mixture left corked in the open air for 1 month. The last trace of liquid was then allowed to evaporate and the residual solid (240 g.) broken up and powdered. Soxhlet extraction with ether for 48 hours, and evaporation of the ether, yielded crystalline material (35 g.). Repeated crystallisation from water (charcoal) gave aminoiminosuccinonitrile (23 g.) as pale red needles, m.p. 178-179° (decomp.).

Found:	C, 44.0;	H, 3.7;	N, 52.1%
Calc. for $C_4H_4N_4$:	C, 44.4;	H, 3.7;	N, 51.9%

2:3-Dicyanopyrazine

(Hinkel, Richards and Thomas, J., 1937, 1432)

Glyoxal (1.2 g.) in warm water (20 c.c.) was added to a solution of aminoiminosuccinonitrile (2 g.) in hot water (50 c.c.) and the reaction mixture warmed on the water bath for 5 minutes. The orange-red precipitate which separated was collected and crystallised from ethanol to give 5:6-dicyano-2-hydroxy-2:5-dihydropyrazine

as a red amorphous powder, m.p. 240° (decomp.) (1.5 g.; 55%).

5:6-dicyano-2-hydroxy-2:5-dihydropyrazine (1.5 g.) was dissolved in the minimum of boiling water and a trace of oxalic acid added. On standing, pale yellow needles, m.p. 128° separated. Sublimation at 60°/10^{-4} mm. followed by crystallisation from water gave 2:3-dicyanopyrazine as needles, m.p. 131° (0.9 g.; 60%).

Found: C, 55.6; H, 1.8%

Calc. for $C_6H_2N_4$: C, 55.4; H, 1.6%

2:3-Dicyanopyrazine 1-oxide

2:3-Dicyanopyrazine (0.5 g.) was dissolved in glacial acetic acid (5.0 c.c.) and treated with hydrogen peroxide (2.0 c.c.; 100 vol.). The solution was kept at 56° for 16 hours and then at room temperature overnight. The crystalline solid (80 mg.) was collected and the mother liquors concentrated under reduced pressure to yield a second crop (20 mg.) Sublimation at 150°/10^{-4} mm. gave 2:3-dicyanopyrazine 1-oxide as small needles, m.p. 220° (decomp.) (80 mg.; 14%).

Found: C, 49.5; H, 1.3%

$C_6H_2ON_4$ requires C, 49.3; H, 1.4%

Hydrolysis of 2:3-dicyanopyrazine 1-oxide

The compound (40 mg.) was treated with hydrochloric

acid (2.0 c.c.; d, 1.2) and glacial acetic acid added until solution was complete. After standing overnight the solution was evaporated to dryness under reduced pressure. The residue was sublimed at $140^{\circ}/10^{-4}$ mm. to give a colourless sublimate (20 mg.) which in water gave a solution acid to litmus. The product, after further purification by sublimation, was obtained as small needles with constant m.p. 200° (decomp.).

Found:		N, 26.0%
$C_6H_3O_3N_3$	requires	N, 25.5%
$C_6H_6O_3N_4$		N, 30.8%
$C_6H_5O_4N_3$		N, 23.0%

N N'-di(1:2-dicyano-2-iminoethyl)oxamide

(a) Aminoiminosuccinonitrile (2 g.) in dry dioxan (50 c.c.) cooled to 0° was treated with oxalyl chloride (Staudinger, Ber., 1908, 41, 3563) (3.8 g.) added dropwise with stirring. The reaction mixture was allowed to stand at room temperature overnight, the pale yellow solid collected, washed with dioxan and dried in vacuo (2.8 g.). The product was insoluble in water and the common organic solvents; it did not melt below 300°.

(b) The experiment was repeated starting with materials of analysis purity. After all the oxalyl chloride had been added the reaction mixture was allowed to stand 1 hour and then the separated solid collected,

washed with dioxan, acetone and methanol. After drying in vacuo the compound was obtained as a white amorphous powder which decomposed over a range of temperature above 300°.

Found:	C, 45.0, 44.7;	H, 2.2, 2.4%
$C_{10}H_6O_2N_8$ requires	C, 44.4;	H, 2.2%

A pure specimen of the product was found to be soluble on warming in glycol monomethyl ether, and in tetrahydrofuran but could not be induced to crystallise from either of these solvents.

Glyoxaline-4:5-dicarboxyamide.

Aminoiminosuccinonitrile (1.0 g.) was dissolved in formic acid (10 c.c.; 90%) and the solution refluxed for 5 hours. The precipitated solid was collected, washed with hot water and dried (1.5 g.). It was insoluble in the common organic solvents but soluble in glacial acetic acid and sparingly in water. The material was dissolved in the minimum of hot glacial acetic acid and the solution filtered after treatment with charcoal. Evaporation of the clear filtrate to dryness under reduced pressure left as a residue a colourless solid which did not melt below 300°. Crystallisation from water (1,000 c.c.) gave glyoxaline-4:5-dicarboxyamide as small prisms, m.p. 300° (1.0 g.)

Found: C, 39.45; H, 3.6; N, 35.8%

Calc. for $C_5H_6O_2N_4$ C, 39.0; H, 3.9; N, 36.4%

Light absorption in N/10 sodium hydroxide: Maximum at 2802A ε = 13,000. A specimen prepared by Baxter and Spring (J., 1945. 232) had a maximum at 2802A. ε = 10,500.

Attempted Reaction with Acetic acid.

Aminoiminosuccinonitrile (1.0 g.) was dissolved in dioxan (5.0 c.c.) and glacial acetic acid (1.5 g.) added. The solution was refluxed for 5 hours and then diluted with ethanol (50 c.c.). Treatment with charcoal followed by filtration (Hiflo) gave a clear filtrate which was concentrated to small bulk under reduced pressure. The precipitated solid was collected and crystallised from ethanol to yield unchanged starting material (0.6 g.), m.p. 179° (decomp.) either alone or when mixed with a pure specimen of aminoiminosuccinonitrile.

N-Carbethoxyaminoiminosuccinonitrile.

A solution of aminoiminosuccinonitrile (2.6 g.) and sodium bicarbonate (2.5 g.) in aqueous dioxan (80 c.c.) was stirred at room temperature and treated with ethyl chloroformate (3.0 g.) added dropwise over a period of 30 minutes. Stirring was continued for 4 hours and then the solution treated with charcoal, filtered (Hiflo), and the filtrate concentrated under reduced pressure

to 100 c.c.. On standing overnight long needles separated (2.5 g.). After further concentration a second crop was obtained (1.0 g.). Crystallisation from water yielded N-carbethoxyaminoiminosuccinonitrile (2.6 g.) as fine needles, m.p. 128°.

Found: C, 46.9; H, 4.7%

$C_7H_8O_2N_4$ requires C, 46.7; H, 4.5%

Cyclisation of N-Carbethoxyaminoiminosuccinonitrile.

The compound (0.8 g.) was treated with 7.5% sodium hydroxide (15 c.c.) and the resulting solution refluxed for 5 minutes. The hot solution was acidified to p_H 4 with glacial acetic acid. On cooling the precipitate (0.6 g.) was collected, washed with methanol followed by ether and dried at 80°. The solid proved to be insoluble in all the common organic solvents and in water. Repeated solution in dilute ammonia followed by precipitation with hydrochloric acid gave as a lemon-yellow powder sintering at 320°, 2-imidazolone-4:5-dicarboxyamide.

Found: C, 34.9; H, 3.8; N, 32.9%

$C_5H_6O_3N_4$ requires C, 35.3; H, 3.5; N, 32.8%

The compound sometimes separated as fine needles. It gave no effervescence with a saturated solution of ammonium bicarbonate but was soluble in alkali. Light absorption in N/10 sodium hydroxide: Maximum at 3600A.

ε = 5,800. This maximum was only observed when the measurement was done quickly; in a short time it rapidly decreased to negligible intensity.

Reaction of Diacetyl with Ethylene Diamine

(a) To a solution of ethylene diamine (0.7 g.; anhydrous) in dry ether (20 c.c.) cooled to 0° diacetyl (1.0 g.) was added dropwise with shaking. The precipitated solid was collected quickly, transferred to a flask and distilled. At bath temp. 100°/20 mm. a colourless distillate was collected which solidified on cooling. The solid (1.0 g.) had m.p. 55 - 60° and on exposure rapidly decomposed to a red oil. The experiment was repeated and after distillation the material quickly transferred to a sublimation apparatus. At 60°/10^{-3} mm. a colourless sublimate was obtained which had m.p. 56° and was analysed immediately.

Found:	C, 49.5;	H, 10.2%
$C_6H_{14}O_2N_2$ requires	C, 49.3;	H, 9.7%

The compound with picric acid gave a picrate which separated from ethanol as needles m.p. 236°, either alone or when mixed with an authentic specimen of the picrate of ethylene diamine.

(b) Ethylene diamine hydrate (2.0 g.) in ethanol (20 c.c.) was treated with a solution of diacetyl (2 g.)

in ethanol (20 c.c.) and the mixture refluxed gently for 30 minutes, then with charcoal for 10 minutes. The solution was filtered and the filtrate concentrated under reduced pressure to 20 c.c. On standing large cubic crystals, m.p. 56° separated. Even when crystalline, this compound decomposed rapidly on exposure but a specimen sealed in vacuo kept indefinitely.

Glycine hydroxamic acid

(cf. Jones and Sneed, J.A.C.S., 1917, 39, 673)

A solution of freshly distilled glycine ethyl ester (70 g.; prepared after Fischer, Ber., 1901, 34, 436) in dry methanol (200 c.c.) was cooled to 0° and treated with a similarly cooled methanolic solution of hydroxylamine, prepared from hydroxylamine hydrochloride (230 g.) in methanol (330 c.c.) and sodium methoxide (164 g.) in methanol (1,300 c.c.). After 3 days at 0° the precipitated glycine hydroxamic acid (41 g.; m.p. 136°) was collected. The mother liquors after standing 1 week at 0° deposited a second crop (15 g.).

Glycine hydroxamic acid prepared thus was pure enough to be used in subsequent reactions. It was insoluble in ethanol and the common organic solvents but crystallised from water as prisms m.p. 138° (decomp.).

Found: C, 27.1; H, 6.85%

Calc. for $C_2H_6O_2N_2$: C, 26.7; H, 6.7%

Reaction of Glycine hydroxamic acid with Glyoxal

Glycine hydroxamic acid (5.0 g.) in hot water (50 c.c.) was treated with glyoxal (6.5 g. 50% W/W) in water (20 c.c.). The reaction solution, which darkened quickly, was warmed with charcoal for 20 minutes and then filtered. Concentration of the filtrate under reduced pressure yielded a small amount of solid m.p. 150 - 160°, which was not worked up. After further concentration a high-melting solid (2.0 g.) separated which, when crystallised from ethanol, afforded glycine anhydride as small needles, m.p. 310° (sintering at 280°) (1.5 g.).

Found: C, 42.0; H, 5.3%

Calc. for $C_4H_6O_2N_2$ C, 42.1; H, 5.3%

Reaction of Glycine hydroxamic acid with Cinnamaldehyde

Glycine hydroxamic acid (2.0 g.) was treated with a solution of cinnamaldehyde (3.0 g.) in ethanol (25 c.c.), and the mixture heated under reflux for 30 minutes, dissolution being then complete. On cooling a solid separated which was crystallised from aqueous ethanol to give cinnamylideneglycine hydroxamic acid (1.3 g.) as small pale orange prisms, m.p. 204° (decomp.). This acid

is insoluble in water but soluble in 3N-sodium hydroxide.

Found:	C, 65.4; H, 6.0%
$C_{11}H_{12}O_2N_2$ requires	C, 64.7; H, 5.9%

Attempted cyclisation of Cinnamylideneglycine hydroxamic acid

Cinnamylideneglycine hydroxamic acid (2.0 g.) in glacial acetic acid (20 c.c.) was treated with bromine (1.6 g.) added dropwise with stirring. The solid which separated was collected (1.75 g.) and crystallised from aqueous ethanol to give small orange prisms (1.0 g.), m.p. 180 - 190° (decomp.). Recrystallisation failed to yield a product of constant melting point; there was considerable loss of material due to resin formation at each crystallisation.

Hydrolysis of Cinnamylideneglycine Hydroxamic acid

A saturated ethanolic solution of the acid was treated with a slight excess of Brady's reagent. The red precipitate which separated rapidly on standing was collected and crystallised from glacial acetic acid to give the 2:4-dinitrophenylhydrazone of cinnamaldehyde as bright red needles, m.p. 245°, undepressed when mixed with a specimen obtained directly from the aldehyde.

2-Bromocinnamaldehyde

(Zincke and Hagen, Ber., 1884, 17, 1815)

A solution of freshly distilled cinnamic aldehyde

(30 g.) in glacial acetic acid (120 c.c.) was treated with bromine (36 g.) added dropwise and then with potassium carbonate (15 g.). The reaction mixture was heated under reflux for 45 minutes, the precipitated potassium bromide filtered off, and the filtrate diluted with water. The solid which separated was collected and crystallised from ether to give 2-bromocinnamaldehyde as prisms, m.p. 72° (40 g., 62%).

2-Bromocinnamylideneglycine Hydroxamic Acid

Glycine hydroxamic acid (5.0 g.) was treated with 2-bromocinnamaldehyde (11.7 g.) in ethanol (900 c.c.), and the mixture heated under reflux for 30 minutes. The crystalline solid (11.0 g.) which separated on cooling was collected and crystallised from ethanol; 2-bromocinnamylideneglycine hydroxamic acid separated as needles m.p. 157 - 158° (decomp.).

Found: C, 46.6; H, 3.9; N, 9.4%

$C_{11}H_{11}O_2N_2Br$ requires C, 46.65; H, 3.9; N, 9.9%

Light absorption in ethanol: Maximum at 2525A., ε = 6,800.

The acid is insoluble in water but soluble in 3N-sodium hydroxide.

Attempted Cyclisation of 2-Bromocinnamylideneglycine Hydroxamic Acid

(a) A refluxing solution of 2-bromocinnamylideneglycine

hydroxamic acid (2.0 g.) in ethanol (200 c.c.) was treated with a solution of potassium hydroxide (0.4 g.) in ethanol (20 c.c.). After 15 minutes the solution was concentrated to 50 c.c. under reduced pressure and acidified with dilute hydrochloric acid. Evaporation to dryness was followed by extraction of the residue with hot benzene (3 x 30 c.c.). The dried extract (Na_2SO_4) was evaporated to yield an oil which solidified on treatment with water. The solid (0.7 g.) after crystallisation from methanol yielded 2-bromocinnamaldehyde, m.p. and mixed m.p. 71 - 72°.

(b) 2-Bromocinnamylideneglycine hydroxamic acid (1.0 g.) in dioxan (50 c.c.) was treated with N-Methylmorpholine (0.4 g.). The solution was heated under reflux for 1½ hours and then the dioxan removed under reduced pressure. The residue (0.8 g.) had m.p. 130-140°. Crystallisation from ethanol gave 2-bromocinnamylideneglycine hydroxamic acid as needles m.p. 158° (decomp.).

(c) A refluxing solution of 2-bromocinnamylideneglycine hydroxamic acid (2.0 g.) in dry methanol (400 c.c.) was treated with a solution of sodium (0.15 g.) in dry methanol (20 c.c.) and after 15 minutes was concentrated to 50 c.c. under reduced pressure. The solution was acidified with dilute hydrochloric acid and then taken to dryness. Extraction with hot chloroform (2 x 50 c.c.) and evaporation of the dried extract (Na_2SO_4) yielded an oil which

solidified on treatment with dilute hydrochloric acid. The solid was sublimed at $60^{o}/10^{-3}$ mm. and crystallisation of the sublimate from methanol gave 2-bromocinnamaldehyde (0.5 g.), m.p. and mixed m.p. 71 - 72°.

The chloroform insoluble residue (1.0 g.) after one crystallisation from ethanol had m.p. 158° (decomp.) undepressed when mixed with a specimen of starting material.

(d) 2-Bromocinnamylideneglycine hydroxamic acid (4.5 g.) was treated with a solution of sodium (0.4 g.) in ethanol (500 c.c.), and the mixture heated under reflux for 30 minutes. The solution was concentrated to small volume under reducedpressure, acidified with dilute hydrochloric acid, and then taken to complete dryness. The residue was extracted with hot benzene (4 x 50 c.c.), and evaporation of the dried extract (Na_2SO_4) left a crystalline solid (2.75 g.). Sublimation at $100^{o}/10^{-3}$ mm., followed by crystallisation from benzene afforded 2-bromocinnamaldehyde oxime as transparent plates, m.p. 142° undepressed when mixed with a specimen prepared directly from the aldehyde.

Found:	C, 47.3;	H, 3.4%
Calc. for C_9H_8ONBr	C, 47.8;	H, 3.6%

6-Benzyl-1-hydroxy-2-keto-1:2-dihydropyrazine

A refluxing solution of 2-bromocinnamylideneglycine hydroxamic acid (4.0 g.) in dry ethanol (500 c.c.) was treated with a solution of sodium (0.33 g.) in dry ethanol (20 c.c.). After 30 minutes the mixture was filtered and the filtrate concentrated under reduced pressure to 50 c.c. The solid (2.0 g.) separating on dilution of the solution with water was collected (solution A) dried, and extracted with hot chloroform (2 x 100 c.c.). The chloroform insoluble solid (1.2 g.) was crystallised from ethanol. It proved to be 2-bromocinnamylideneglycine hydroxamic acid m.p. 158° (decomp.).

The chloroform extract was shaken with saturated ammonium hydrogen carbonate solution (2 x 50 c.c.). Acidification of the extract with dilute hydrochloric acid precipitated a solid (0.3 g.) m.p. 70 - 71° either alone or when mixed with a specimen of 2-bromocinnamaldehyde.

Acidification of the solution A precipitated a crystalline solid (1.2 g.) m.p. 140 - 160° which after crystallisation from benzene, sublimation at 100°/10^{-3} mm, and a final crystallisation from benzene gave 6-benzyl-1-hydroxy-2-keto-1:2-dihydropyrazine as small needles, m.p. 171°.

Found: C, 65.7; H, 5.4; N, 13.9%; equiv.,200.

$C_{11}H_{10}O_2N_2$ requires C, 65.3; H, 5.0; N, 13.9%; equiv.,202.

The acid is soluble with effervescence in sodium hydrogen carbonate solution, and gives an intense red colour with aqueous ferric chloride. Light absorption in ethanol: Maxima at 2350 A., ε = 13,200, and 3330 A., ε = 9800.

α-Amino-n-butyric acid methyl ester

(c.f. Zelinsky, Ber., 1908, 41, 2061)

A solution of potassium cyanide (60 g.) in water (150 c.c.) was added dropwise with cooling and constant stirring to a mixture of propionaldehyde (50 g.), ammonium chloride (52 g.) and ether (600 c.c.). The reaction mixture was shaken at room temperature for 4 hours. The ethereal layer was separated and the aqueous layer extracted with ether (2 x 50 c.c.). The combined ether extracts were dried over calcium chloride and the dry solution treated with a stream of dry hydrogen chloride. The precipitated nitrile hydrochloride was filtered off quickly and dissolved in several times its volume of concentrated hydrochloric acid. After standing overnight the solution was refluxed for 3 hours and then evaporated to dryness under reduced pressure. The residue was extracted with boiling ethanol (1,000 c.c.) to give solution A.

The aqueous layer from the initial reaction was treated with an equal volume of concentrated hydrochloric acid and the mixture refluxed for 1 hour. It was then taken to dryness under reduced pressure. The residue was extracted with boiling ethanol (600 c.c.) and the extract combined with solution A. The combined extracts were evaporated to dryness to yield α-amino-n-butyric acid (47 g.; 53%). The acid was contaminated with inorganic material but was used without further purification.

α-Amino-n-butyric acid (40 g.) was suspended in dry methanol (700 c.c.) and refluxed for 6 hours while a stream of dry hydrogen chloride was passed into the mixture. The methanol was then removed under reduced pressure and the residue treated with a mixture of water (50 c.c.) and ether (100 c.c.). A 30% solution of sodium hydroxide (10 g.) was added slowly with cooling and the aqueous layer made into a paste by adding potassium carbonate. The ethereal layer was decanted off and the residue extracted with ether (2 x 50 c.c.). The ether extracts were combined, dried (Na_2SO_4), and the ether evaporated. The residue was distilled at 38 - 40°/12 mm. to yield α-amino-n-butyric acid methyl ester (12 g.; 25%).

α-Amino-n-butyrohydroxamic Acid

To a solution of hydroxylamine (15 g.) in methanol (200 c.c.) (Org. Synth., 1940, 20, 74) was added α-amino-n-butyric acid methyl ester (11.5 g.) and after 3 days at 0° the crystalline solid (5 g.) was collected. From hot water α-amino-n-butyrohydroxamic acid formed small prisms, M.p. 166 - 167°.

Found:	C, 41.0;	H, 8.6%
$C_4H_{10}O_2N_2$ requires	C, 40.7;	H, 8.5%

α-(2-Bromocinnamylideneamino)-n-butyrohydroxamic acid

α-amino-n-butyrohydroxamic acid (3 g.) was heated under reflux with 2-bromocinnamaldehyde (5.4 g.) in ethanol (250 c.c.) until dissolution was complete (30 minutes). On cooling, a crystalline solid (4.2 g.) separated, which after crystallisation from ethanol gave α-(2-bromocinnamylideneamino)-n-butyrohydroxamic acid as transparent plates, m.p. 166° (decomp.)

Found:	C, 50.3;	H, 5.1;	N, 9.1%
$C_{13}H_{15}O_2N_2Br$ requires	C, 50.2;	H, 4.8;	N, 9.0%

Reaction of α-(2-Bromocinnamylideneamino)-n-butyrohydroxamic Acid with Sodium Ethoxide

A refluxing solution of α-(2-bromocinnamylideneamino)-n-butyrohydroxamic acid (3 g.) in dry ethanol (250 c.c.) was treated with a solution of sodium (0.22 g.)

in dry ethanol (20 c.c.) and after 30 minutes charcoal was added. After continued refluxing for 15 minutes, the mixture was filtered and the filtrate concentrated under reduced pressure to 20 c.c. The solution was acidified with dilute hydrochloric acid and the precipitated solid (2.2 g.) collected. Extraction with hot benzene (2 x 30 c.c.) left a residue (0.8 g.) which crystallised from ethanol in transparent plates, m.p. 166° (decomp.) undepressed on admixture with a specimen of α-(2-bromocinnamylideneamino)-n-butyrohydroxamic acid. The dried benzene extract (Na_2SO_4) was evaporated under reduced pressure and the residue (1.3 g.) separated from methanol as large plates, m.p. 70 - 72° either alone or when mixed with a specimen of 2-bromocinnamaldehyde.

6-Benzyl-3-ethyl-1-hydroxy-2-keto-1:2-dihydropyrazine

A refluxing solution of α-(2-bromocinnamylideneamino)-n-butyrohydroxamic acid (3.0 g.) in dry tert.-butanol (220 c.c.) was treated with a solution of potassium (0.38 g.) in dry tert.-butanol (20 c.c.), and the mixture heated under reflux for 5 hours. Potassium bromide (0.4 g.) was filtered off and the filtrate concentrated to small bulk under reduced pressure. After acidification with dilute hydrochloric acid the mixture was evaporated

to dryness, and the residue extracted with hot benzene (500 c.c.). The extract (charcoal) was evaporated to dryness, the residue washed with a little ethanol, and solid (80 mg.) collected. Sublimation at $100^{o}/10^{-3}$ mm. and crystallisation from benzene gave 6-benzyl-3-ethyl-1-hydroxy-2-keto-1:2-dihydropyrazine as clusters of small pale yellow prisms, m.p. 137 - 138°.

Found: C, 68.1; H, 6.3; N, 11.9%

$C_{13}H_{14}O_2N_2$ requires C, 67.8; H, 6.1; N, 12.2%

The acid is soluble with effervescence in sodium hydrogen carbonate solution and gives a deep red colour with aqueous ferric chloride. Light absorption in ethanol: Maxima at 2350A., ε = 13,900, and 3290A. ε = 10,600.

2-Bromocrotonaldehyde

(Claisen, Ber, 1911, 44, 1164)

To freshly distilled crotonaldehyde (20 g.) in a 500 c.c. flask bromine (14.8 c.c.) was added dropwise with cooling. The reaction mixture at room temperature was treated with finely divided potassium acetate (34 g.) added in small portions with frequent shaking. During this process the temperature of the reaction mixture was kept below 30° by occasional cooling. After one hour the 2-bromocrotonaldehyde was distilled off with steam. The oil was separated from the water, and dissolved

acetic acid removed by shaking with 5% sodium carbonate solution. After drying over calcium chloride it was distilled and the fraction b.p. 60° - 64°/15 mm. collected. (12 g.; 28%).

α-(2-Bromocrotonylideneamino)-n-butyrohydroxamic acid

α-Amino-n-butyrohydroxamic acid (6.0 g.) was treated with 2-bromocrotonaldehyde (9.0 g.) in ethanol (300 c.c.) under reflux until dissolution was complete (30 minutes). On cooling a crystalline solid (7.0 g.) separated. When crystallised from ethanol it gave α-(2-bromocrotonylideneamino)-n-butyrohydroxamic acid (6.5 g; 51% yield) as transparent plates m.p. 135 - 137° (decomp.).

Found: C, 38.5; H, 5.0; N, 11.4%

$C_8H_{13}O_2N_2Br$ requires C, 38.6; H, 5.3; N, 11.2%

Attempted cyclisation of α-(2-bromocrotonylideneamino)-n-butyrohydroxamic acid

(a) With sodium ethoxide

(i) α-(2-Bromocrotonylideneamino)-n-butyrohydroxamic acid (3.3 g.) in dry ethanol (150 c.c.) was treated under reflux with a solution of sodium (0.3 g.) in dry ethanol (20 c.c.). After 10 hours the reaction mixture was treated with dilute hydrochloric acid to ph. 5 and then evaporated to dryness under reduced pressure. The residue was extracted with boiling

chloroform (3 x 50 c.c.). Evaporation of the dried extract (Na_2SO_4) to dryness gave no residue.

The reaction residue was then extracted with dry ethanol under reflux (3 x 50 c.c.). This extract on evaporation yielded a crystalline solid (2.5 g.), m.p. 180°. Recrystallisation from ethanol gave α-amino-n-butyrohydroxamic acid hydrochloride as plates m.p. 195 - 196°.

Found:	C, 31.2;	H, 7.1%
$C_4H_{11}O_2N_2Cl$ requires	C, 31.1;	H, 7.2%

The compound gave a red coloration with ferric chloride solution, dissolved with effervescence in a saturated solution of sodium hydrogen carbonate, and gave a positive Beilstein test for halogen.

(ii) α-(2-Bromocrotonylideneamino)-n-butyrohydroxamic acid (1 g.) in dry ethanol (30 c.c.) was treated with sodium (0.1 g.) in dry ethanol (10 c.c.) and the reaction mixture kept at 120 - 125° in an autoclave for 2 hours. The solution was concentrated to small volume and acidified to ph.3 with dilute hydrochloric acid. The acid solution was extracted with benzene (3 x 50 c.c.); the dried extract (Na_2SO_4) was evaporated and left a gum which gave a red coloration with ferric chloride solution but did not sublime.

Evaporation of the acid solution to dryness under

reduced pressure left a residue which was extracted with dry ethanol (2 x 50 c.c.) under reflux. This extract, on evaporation, left a resin which at 120°/10^{-4} mm. sublimed to give a semi-solid sublimate. The sublimed material gave a red coloration with ferric chloride solution, dissolved in a saturated solution of sodium bicarbonate with effervescence, and gave a negative Beilstein test for halogen. Dried on a porous plate it had m.p. 80 - 90°.

(b) With Copper Powder

The acid (100 mg.) was ground with copper-bronze powder (an equal volume) in an agate mortar. The mixture was heated in a sublimation apparatus to a bath temperature of 130° at a pressure of 10^{-3} mm. for several hours. The sublimate (30 mg.) was collected and crystallised from acetone to yield small plates, m.p. 135 - 136° (decomp.). A mixed melt with a specimen of the starting material gave no depression.

(c) With Aqueous Alkali

(i) The acid was dissolved in 3N-sodium hydroxide solution in the cold. The addition of dilute hydrochloric acid to ph.7 precipitated the unchanged acid quantitatively.

(ii) The acid (1.0 g.) was suspended in a mixture of

water (30 c.c.) and methanol (30 c.c.) cooled to -60°, and sodium hydroxide (0.5 g.) in water (5.0 c.c.) added dropwise with stirring, the temperature being kept below -30°. The mixture was gradually heated to -10° over 1 hour and then left overnight at 0°. Acidification to ph.2 precipitated the acid (0.8 g.) m.p. 135 - 136° (decomp.), unchanged.

Reaction of α-amino-n-butyrohydroxamic acid with crotonaldehyde

The acid (0.8 g.) was suspended in ethanol (25 c.c.) and treated with crotonaldehyde (0.6 g.; 1 mol.). Dissolution of the acid was complete after 10 minutes reflux; the resulting solution on evaporation under reduced pressure left an oil which would not solidify. The oil was dissolved in chloroform (10 c.c.) and a solution of bromine (1.0 g.) in chloroform added dropwise with shaking. Decoloration after each addition indicated that the bromine was being absorbed. The solvent was evaporated under reduced pressure and the residual oil dissolved in the minimum of acetone. Ether precipitated a solid (0.5 g.) which was recrystallised from ethanol to give the hydrobromide of α-amino-n-butyrohydroxamic acid as small rods, m.p. 187 - 188°.

Found: C, 24.5; H, 5.6%

$C_4H_{11}O_2N_2Br$ requires C, 24.1; H, 5.5%

The compound gave a red coloration with ferric chloride solution, dissolved with effervescence in a saturated solution of sodium hydrogen carbonate, and gave a positive test for ionic halogen with silver nitrate solution.

Cyclisation of α-(2-bromocrotonylideneamino)-n-butyrohydroxamic acid

The acid (9 g.) in tert.-butanol (200 c.c.) was treated with potassium (1.42 g.) in tert.-butanol (20 c.c.) and the reaction mixture refluxed for 8 hours. Potassium bromide (2.5 g.) was collected and the filtrate concentrated to 100 c.c. under reduced pressure. Dilute hydrochloric acid wasadded to ph. 6.4. On standing unchanged acid (3 g.) was collected and the filtrate evaporated to dryness under reduced pressure. The residue was extracted with ether (3 x 200 c.c.) under reflux and the dried extract (Na_2SO_4) evaporated. The residue was triturated with a little ether and unchanged acid (0.6 g.) m.p. 139° (decomp.) filtered off. Evaporation of the filtrate left a gum (1.7 g.) which was ether soluble and gave a red coloration with ethanolic ferric chloride. Sublimation of a portion of this gum gave at $60°/10^{-3}$ mm. a semi-solid sublimate m.p. 60 - 80°, which gave a red coloration with ferric

chloride solution.

The ether soluble gum was redissolved in ether (2 x 50 c.c.) and shaken with 2% sodium hydrogen carbonate solution (3 x 20 c.c.). This extract was acidified with dilute acetic acid. There was no precipitate. Extraction with ether (4 x 20 c.c.) and evaporation of the dried extract (Na_2SO_4) left a clear gum. Absorption in ethanol; maxima at 2310A. ϵ = 3,950 and at 3200A. ϵ = 2,470.

The gum, even after charcoal treatment, refused to solidify. It was dissolved in the minimum of hot water (leaving a dark insoluble residue) and treatment of the aqueous solution with an excess of saturated copper acetate solution precipitated a copper salt (85 mg.) which was washed with water, ethanol and ether. The salt was soluble in dioxan and slightly soluble in ether. Crystallisation from dioxan gave the copper salt of 3:6-diethyl-1-hydroxy-2-keto-1:2-dihydropyrazine as light green transparent plates, m.p. 237 - 239° (decomp.)

Found: C, 48.5; H, 5.6; N, 14.4%

$Cu(C_8H_{11}O_2N_2)_2$ requires C, 48.3; H, 5.6; N, 14.1%

3:6-Diethyl-1-hydroxy-2-keto-1:2-dihydropyrazine

The above copper salt (210 mg.) in dioxan (20 c.c.)

diluted with water (50 c.c.) was treated with hydrogen sulphide for 4 hours. The mixture was evaporated to dryness under reduced pressure and the residue extracted with boiling ethanol (2 x 100 c.c.). The filtered extract (Hiflo) was evaporated and the residue sublimed at $70°/10^{-4}$ mm. The non-crystalline sublimate was dissolved in the minimum of hot acetone, and the pale yellow rosettes of needles which separated on cooling were collected (25 mg.). Sublimation at $50°/10^{-3}$ mm. gave 3:6-diethyl-1-hydroxy-2-keto-1:2-dihydropyrazine, m.p. 95 - 96°.

Found: C, 56.5; H, 7.3; N, 16.5%

$C_8H_{12}O_2N_2$ requires C, 57.1; H, 7.2; N, 16.7%

Absorption in ethanol: Maxima at 2335A. ε = 9,250 and at 3265A ε = 7,860.

Benzhydroxamic Acid

(Jones and Hurd, J.A.C.S., 1921, 43, 2447)

With vigorous stirring an intimate mixture of anhydrous sodium carbonate (4.2 g.) and hydroxylamine hydrochloride (2.8 g.) suspended in ether (100 c.c.) was treated with benzoyl chloride (5.6 g.) added dropwise. Stirring was continued and water (7.0 c.c.) was added slowly to the reaction mixture. After 30 minutes, the reaction being complete, the mixture was filtered and the ethereal solution evaporated to

dryness. The crystalline residue was recrystallised from toluene to yield benzhydroxamic acid m.p. 124° (4.0 g.; 72%).

Acetylation of Benzhydroxamic Acid

(cf. Werner, Ber., 1892, 25, 27)

The acid (1.0 g.) in ether (100 c.c.) was treated with acetic anhydride (0.75 g.) and the reaction mixture left at room temperature for 2 hours. The solution was then evaporated to dryness and the residue crystallised from ethanol to give the monoacetate as needles m.p. 128° (0.8 g.).

Found:	C, 60.7;	H, 5.0;	N, 7.8%
Calc. for $C_9H_9O_3N$	C, 60.5;	H, 5.0;	N, 7.9%

The acetate was soluble in 3N-sodium hydroxide solution and an ethanolic solution gave no coloration with aqueous ferric chloride.

Derivatives of 2-bromocinnamylideneglycine hydroxamic acid

(a) By Acetylation

(i) 2-Bromocinnamylideneglycine hydroxamic acid (2.0 g.) was treated with acetic anhydride (2.0 g.) on the steam-bath for 1 hour. The mixture was cooled and diluted with ether. The separated solid (2.2 g.) was collected and crystallised from ethanol to give the diacetyl derivative as small prisms m.p. 145°.

Found: C, 48.9; H, 4.4; N, 7.8%

$C_{15}H_{15}O_4N_2Br$ requires C, 49.1; H, 4.1; N, 7.6%

(ii) A suspension of 2-bromocinnamylideneglycine hydroxamic acid (1.0 g.) in ethanol (15 c.c.) was treated with acetic anhydride (0.5 g.) and the mixture shaken at room temperature for 3 hours. The solid (0.5 g.) was collected and crystallised from ethanol to yield the diacetyl derivative m.p. 145° (0.5 g.).

The mother liquors were evaporated to dryness under reduced pressure and the residue crystallised from ethanol to give the monoacetate (0.4 g.) as large prisms m.p. 188 - 189° (d.).

Found: C, 47.9; H, 3.9%

$C_{13}H_{13}O_3N_2Br$ requires C, 48.0; H, 4.0%

An ethanolic solution of the monoacetate gave a deep red coloration with aqueous ferric chloride; the diacetate gave no coloration.

(iii) Hydrolysis of the Diacetate

The diacetate (2.5 g.) was added to 3N-sodium hydroxide (50 c.c.) and after 5 minutes the resulting solution was acidified with dilute hydrochloric acid and extracted with chloroform (3 x 50 c.c.). The extract was washed with water and dried (Na_2SO_4). Evaporation and crystallisation of the residue from

ethanol yielded the monoacetate (1.0 g.) m.p. 188 - 189° (d) undepressed when mixed with an authentic specimen prepared directly from the acid.

(iv) Hydrolysis of the Monoacetate

The monoacetate (1.0 g.) was treated with 3N-hydrochloric acid (50 c.c.) under reflux for 10 mins. An insoluble gum was separated by decantation, washed with ether (3 x 50 c.c.) and the residue crystallised from ethanol to yield unchanged acetate (0.3 g.) m.p. 188° (d.). Evaporation of the dried ether extract (Na_2SO_4) gave 2-bromocinnamaldehyde (0.2 g.) m.p. 72° undepressed when mixed with an authentic specimen. Evaporation of the decanted solution under reduced pressure left a residue which was crystallised from ethanol to give the oxime of 2-bromo-cinnamaldehyde (0.2 g.) as transparent plates m.p. 142° undepressed when mixed with an authentic specimen.

Found:	N, 6.0%
Calc. for C_9H_8ONBr	N, 6.2%

(b) By Benzoylation

A solution of 2-bromocinnamylideneglycine hydroxamic acid (0.5 g.) in 3N-sodium hydroxide (0.6 c.c.) was treated dropwise with benzoyl chloride (0.23 g.). The solid was collected, washed with hot light-petroleum (b.p. 60 - 80°) and crystallised from ethanol to give the

dibenzoyl derivative (0.2 g.) as colourless needles m.p. 185°.

Found: C, 61.0; H, 3.6; N, 5.5%

$C_{25}H_{19}O_4N_2Br$ requires C, 61.1; H, 3.9; N, 5.7%

Reduced pressure evaporation of the ethanolic mother liquors from the dibenzoyl derivative gave a residue which was crystallised from ethanol to give the monobenzoyl derivative (50 mg.) as colourless needles m.p. 225° (d.).

Found: C, 56.1; H, 4.0%

$C_{18}H_{15}O_3N_2Br$ requires C, 55.8; H, 3.9%

The monobenzoyl derivative is soluble in 3N-sodium hydroxide; the dibenzoyl derivative is insoluble.

(c) By reaction with Chloroformic Ester

2-Bromocinnamylideneglycine hydroxamic acid (2.2 g.) was dissolved in water (30 c.c.) containing sodium hydroxide (0.31 g.). The solution was cooled to 0° and treated with chloroformic ester (0.84 g.) added dropwise with stirring. Stirring was continued for 2 hours and then the separated solid (2.0 g.) collected. The material was ether extracted (2 x 50 c.c.) and the insoluble residue (0.5 g.) crystallised from ethanol to yield unchanged acid m.p. 158° (d.). The dried ether extract (Na_2SO_4) was evaporated and the

residue (1.3 g.) crystallised from ether and finally from benzene/light-petroleum (b.p. 60 - 80°) to give the N-carbethoxy-derivative of the acid as transparent plates m.p. 160 - 161°.

Found:	C, 47.2;	H, 4.5%
$C_{14}H_{15}O_4N_2Br$ requires	C, 47.3;	H, 4.3%

The derivative is soluble in 3N-sodium hydroxide and when dissolved in the minimum of ethanol gives a deep red coloration with aqueous ferric chloride.

Diacetyl Tartaric Anhydride

(Wohl and Oesterlin, Ber., 1901, 34, 1144)

Finely powdered tartaric acid (100 g.) was treated with a mixture of acetic anhydride (220 c.c.) and sulphuric acid (3.0 c.c.). The reaction mixture was shaken at room temperature until the evolution of heat had subsided; it was then refluxed for 30 minutes to complete the reaction. After cooling, the crystalline product was collected, washed with benzene (100 c.c.) to free it of acetic anhydride and then recrystallised from benzene to yield diacetyl tartaric anhydride as needles m.p. 135° (120 g.; 80%).

Pyridine Salt of Hydroxymaleic Anhydride

(Dorp and Arens, Rec. Trav. chim., 1948, 67, 459)

Diacetyl tartaric anhydride (240 g.) was dissolved

in dry acetone (500 c.c.) under gentle heating. The solution was cooled in ice to 10° and dry pyridine (180 c.c.) added slowly with continued cooling. After a few minutes the colour of the mixture changed to light-green; glacial acetic acid (144 c.c.) was then added, the temperature rising to 18°. The reaction mixture was cooled to 0° and left overnight in the refrigerator. The pyridine salt, which separated as long needles, was filtered from the red reaction mixture, washed with dry ether and then twice with cold dry acetone (100 c.c.) and dried in vacuo. The salt was obtained as pale-brown needles m.p. 108 - 109° (100 g.; 46%).

γ-Ethyl-α-keto-γ-methylbutyrolactone-β-carboxylic acid

Dry hydrochloric acid was passed into a mixture of glacial acetic acid (250 c.c.) and acetic anhydride (12.5 c.c.) for 5 minutes and the mixture then refluxed for 15 minutes. The completely anhydrous glacial acetic acid thus obtained was cooled in ice and dry hydrochloric acid (23 g.) passed into it under anhydrous conditions. Ethyl methyl ketone (46 g.) and dry pyridine salt of hydroxymaleic anhydride (124 g.) were added. The salt dissolved quickly and the light-green reaction mixture was kept in a stoppered flask at

room temperature for 8 days. It was then poured into water (1.5l) and twice extracted with ether. The extract was washed with water (250 c.c.) and then several times, while cooling, with 2N-sodium hydroxide solution until the extract was colourless. The combined alkaline extracts were extracted with ether. Crushed ice was added to the alkaline extract and afterwards hydrochloric acid to p_H 3. Renewed extraction with ether (2 x 100 c.c.) was carried out, the extract washed with water to remove acetic acid, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The residue was crystallised from benzene to yield γ-ethyl-α-keto-γ-methylbutyrolactone-β-carboxylic acid (27.5 g.; 24%) as small plates m.p. 130° (with decarboxylation).

Found:	C, 51.4;	H, 5.4%
$C_8H_{10}O_5$ requires	C, 51.6;	H, 5.4%

The compound gives an intense red coloration with ferric chloride solution and dissolves with effervescence in a saturated solution of sodium hydrogen carbonate.

γ-Ethyl-α-keto-γ-methylbutyrolactone

(cf. Dorp and Arens, Rec. Trav. chim., 1948, 67, 459)

A mixture of γ-ethyl-α-keto-γ-methylbutyrolactone-β-carboxylic acid (2.0 g.) and freshly distilled quinoline

(4.0 c.c.) was heated in a test-tube suspended in an oil bath at 70 - 80°. The test-tube was fitted with a gas outlet tube through a rubber stopper. The evolution of carbon dioxide, which was bubbled through liquid paraffin, was finished after 10 minutes. While it was being heated the reaction mixture was shaken at frequent intervals and as soon as the reaction had finished the tube was cooled and the solution diluted with ether. The ethereal solution was washed with excess 2N-hydrochloric acid followed by water; after drying (Na_2SO_4) the ether was evaporated leaving a pale-yellow oil which could not be induced to crystallise. Distillation at bath temperature 100°/10^{-3} mm. gave a colourless oil which solidified on standing to give γ-ethyl-α-keto-γ-methylbutyrolactone (0.5 g.; 33%) m.p. 42 - 44°.

Found:	C, 58.9;	H, 7.0%
$C_7H_{10}O_3$ requires	C, 59.1;	H, 7.1%

<u>3:5-Dinitrobenzoate of γ-Ethyl-α-keto-γ-methylbutyrolactone</u>

γ-Ethyl-α-keto-γ-methylbutyrolactone (0.9 g.) in benzene (60 c.c.) was treated with pyridine (2.0 c.c.) and 3:5-dinitrobenzoyl chloride (1.5 g.) in benzene (50 c.c.) was added slowly over a period of 1 hour with stirring at room temperature. After standing overnight the reaction mixture was diluted with ether (200 c.c.), washed twice

with dilute hydrochloric acid, once with water and then dried (Na_2SO_4). Evaporation to dryness left a residue which was crystallised from benzene/light-petroleum (b.p. 60 - 80°) to give the 3:5-dinitrobenzoate (1.5 g.) as prisms m.p. 116 - 119°.

Found: C, 49.8; H, 3.6; N, 8.35%

$C_{14}H_{12}O_8N_2$ requires C, 50.0; H, 3.6; N, 8.3%

<u>β-Bromo-γ-ethyl-α-keto-γ-methylbutyrolactone</u>

(a) γ-Ethyl-α-keto-γ-methylbutyrolactone (0.75 g.) in a mixture of ethanol (5.0 c.c.) and water (2.0 c.c.) was cooled in ice and bromine (0.85 g.) added dropwise. The reaction mixture was taken to dryness under reduced pressure and the residue dissolved in the minimum of hot light-petroleum (b.p. 60 - 80°). After cooling the crystalline solid was collected. Recrystallisation from the same solvent gave β-bromo-γ-ethyl-α-keto-γ-methylbutyrolactone (1.0 g.; 83%) as long needles m.p. 79 - 80°.

Found: C, 37.7; H, 4.1%

$C_7H_9O_3Br$ requires C, 38.0; H, 4.1%

The compound gives a red coloration with ferric chloride solution.

(b) γ-Ethyl-α-keto-γ-methylbutyrolactone-β-carboxylic acid (2.2 g.) in ethanol (10 c.c.) and water (3.0 c.c.) was

cooled in ice and bromine (1.9 g.) added dropwise with stirring. Evaporation of the solvent under reduced pressure left an oily residue which was treated with hot light-petroleum (b.p. 60 - 80°) (40 c.c.). The solvent was decanted from a black tar and allowed to cool. The long needles of β-bromo-γ-ethyl-α-keto-γ-methylbutyrolactone (1.3 g.; 50%) m.p. 79 - 80°, which separated, were collected.

DL-Alanine Hydroxamic Acid

(Cunningham, Newbold, Spring and Stark, J., 1949, 2091)

Hydroxylamine from the hydrochloride (15 g.) and DL-alanine methyl ester (6.0 g.) gave the acid m.p. 163 - 164° (4.0 g.; 66%).

Reaction of DL-alanine hydroxamic acid with β-Bromo-γ-ethyl-α-keto-γ-methylbutyrolactone

DL-Alanine hydroxamic acid (0.25 g.) was suspended in a refluxing solution of β-bromo-γ-ethyl-α-keto-γ-methylbutyrolactone (0.5 g.) in ethanol (50 c.c.). After 3 hours the acid had completely dissolved. Concentration of the solution under reduced pressure precipitated a crystalline solid (0.45 g.). Crystallisation from ethanol gave 1-(2'-bromo-3'-hydroxy-1'-keto-3'-methylcapramido)-propionhydroxamic acid as small blades m.p. 142 - 143°.

Found: C, 37.3; H, 5.2; N, 8.9%

$C_{10}H_{17}O_5N_2Br$ requires C, 36.9; H, 5.3; N, 8.6%

The compound gave a red coloration with ferric chloride solution, a positive Beilstein test for halogen, and dissolved slowly in a saturated solution of sodium hydrogen carbonate.

Attempted Condensation of Isobutyraldehyde with Hydroxymaleic Anhydride

Dry hydrochloric acid was passed into a mixture of glacial acetic acid (120 c.c.) and acetic anhydride (6.0 c.c.) for 5 minutes and the mixture then refluxed for 15 minutes. The anhydrous glacial acetic acid thus obtained was cooled in ice and dry hydrochloric acid (11 g.) passed into it. Isobutyraldehyde (22 g.) and dry pyridine salt of hydroxymaleic anhydride (57 g.) were added and the reaction mixture kept in a stoppered flask at room temperature for 8 days. The separated solid (27 g.) was collected; all attempts at crystallisation failed. The solid (10 g.) in water (100 c.c.) was extracted with ether (3 x 50 c.c.). The dried extract (Na_2SO_4) was evaporated and the residue (2.0 g.) crystallised from dioxan to give prisms m.p. 128 - 130° (with loss of carbon dioxide). This material gave a red coloration with aqueous ferric

chloride, effervescence with a saturated solution of sodium hydrogen carbonate, and a negative Beilstein test for halogen.

After several crystallisations from dioxan hydroxymaleic acid separated as prisms m.p. 150° (with loss of CO_2).

Found:	C, 36.7;	H, 3.3%
Calc. for $C_4H_4O_5$	C, 36.4;	H, 3.0%

Synthesis of 3-Methylpent-2-enal Dibromacetal

(cf. Beyerstedt and McElvain, J.A.C.S., 1937, 59, 2267)

Freshly distilled paraldehyde (308 g.; equivalent to acetaldehyde, 7 moles) was diluted with carbon tetrachloride (200 c.c.) and treated in a three-necked flask fitted with a dropping funnel, stirrer and reflux condenser and cooled in acetone /CO_2, with bromine (2,240 g.; 14 moles) added dropwise with caution at first. The bromine was added over 3 hours with ultra-violet irradiation. After standing 3 - 4 hours at 0 - 5° the reaction mixture was treated with dry ethanol (2 litres; precooled to -10°) which was added cautiously in the first place with acetone/CO_2 cooling. After standing overnight the solution was poured into a mixture of crushed ice (2 kg,) and water 2l.). The lower layer was separated,

washed with sodium carbonate solution, water and the dried (Na_2SO_4). The aqueous phase was neutralised with sodium carbonate and then extracted with ether (2l.) in portions. The extract was washed with sodium carbonate solution, water and then dried (Na_2SO_4). The ether extract was evaporated and the residue combined with the dried organic layer and distilled. The fraction b.p. 95 - 110° was collected (980 g.; 40%).

Bromethoxyethylene

(cf. Jacobs, Cramer and Hanson, J.A.C.S., 1942, 64, 223)

To dibromacetal (414 g.; 1.5 mole) in ethanol (500 c.c.; 95%) heated under reflux with stirring in a 2l. three-necked flask was added in portions zinc dust (195 g.) (which had been activated by treating with cold 3N-hydrochloric acid, washing well with water, ethanol, filtering and drying at 80°) added at such a rate to keep reflux without heating (45 minutes). The reaction mixture was then refluxed 45 minutes, cooled and filtered from unreacted zinc using Hiflo and a large funnel. The filtrate was poured into N-ammonium chloride solution (2.5l.) and the lower oily layer separated, washed with N-ammonium chloride solution, water, and dried (Na_2SO_4). The aqueous phase was extracted with ether and the extract similarly washed

and dried. The ether extract was evaporated and the residue combined with the dried organic layer. Distillation gave the following fractions:-

(1) b.p. to 37°/15 mm. 15 g.

(2) b.p. 37 - 45°/15 mm. 70 g.

Redistillation of the residue gave

b.p. 39 - 45°/15 mm. 20 g.

The higher boiling fractions were combined and retreated with zinc dust. Distillation after working up as above then gave

b.p. 39 - 47°/15 mm. 30 g.

The overall yield of bromethoxyethylene was 120 g.; 54% of the theoretical.

Ethoxyacetylene

(Jacobs, Cramer and Hanson, loc. cit.)

Bromethoxyethylene (100 g.) was mixed with twice its weight of powdered potassium hydroxide in a distilling flask and the mixture heated on an oil-bath. The distillation apparatus was connected to a running water-pump on an open circuit. At bath temperature 50° a vigorous reaction commenced and heating was continued slowly to bath temperature 90 - 100°. The ethoxy-acetylene distilled over quickly and was collected in a trap immersed in acetone/CO_2. The product was dried (Na_2SO_4) and distilled under nitrogen up a short column

and under slightly reduced pressure. The yield b.p. 27 - 29°/300 mm. was 12 g.; 30% of theoretical.

1-Ethoxy-3-methylpent-1-yn-3-ol

(a) A solution of ethoxyacetylene (13 g.) in dry ether (15 c.c.) was added dropwise over 15 minutes with stirring at -5° to the Grignard reagent prepared from ethyl iodide (29.0 g.) and magnesium (4.46 g.) in dry ether (80 c.c.). After a further 15 minutes stirring at -5° a solution of ethyl methyl ketone (14.2 g.) in dry ether (40 c.c.) was added dropwise over 30 minutes. The cooling bath was removed and the reaction mixture stirred for a further 30 minutes and then poured into a solution of ammonium chloride (150 c.c.; 10% W/W) containing ice (200 g.). The ethereal solution was separated, washed with ammonium chloride solution, water and dried (Na_2SO_4). Removal of the ether gave a yellow oil which distilled almost completely at 55 - 57°/0.7 mm. to give 1-ethoxy-3-methylpent-1-yn-3-ol as a colourless oil (18.0 g.) n_D^{18} 1.4484.

Found:	C, 67.9; H, 10.2%
$C_8H_{14}O_2$ requires	C, 67.6; H, 9.8%

(b) A suspension of sodamide in liquid ammonia was prepared according to Vaughn, Vogt and Nieuwland

(J.A.C.S., 1934, 56, 2120) from sodium (9.5 g.) and liquid ammonia (300 c.c.). To the well stirred mixture bromethoxyethylene (31 g.) was added over 10 minutes. After 2 hours stirring ethyl methyl ketone (14.4 g.) was added over 10 minutes and the reaction mixture stirred for 3 hours. Ammonium chloride (11.0 g.) was then added and the ammonia allowed to evaporate overnight. The residue was treated with water (200 c.c.) and extracted with ether (3 x 100 c.c.). The ethereal solution was thrice washed with water and dried (Na_2SO_4). Removal of the ether gave a brown oil which distilled at 52 - 55°/1.5 mm. to give 1-ethoxy-3-methylpent-1-yn-3-ol (5.0 g.) n_D^{18} 1.4520.

1-Ethoxy-3-methylpent-1-en-3-ol

1-Ethoxy-3-methylpent-1-yn-3-ol (18 g.) was dissolved in redistilled ethyl acetate (50 c.c.) and shaken at atmospheric pressure with hydrogen in the presence of Adams catalyst (300 mg.) until the calculated volume (1 mole; 2800 c.c. at N.T.P.) had been absorbed. The catalyst was filtered off and the solvent removed under reduced pressure, The residual oil distilled between 67 and 80°/11 mm. (15.9 g.) n_D^{18} 1.4400 and was used for the next stage without further purification.

3-Methylpent-2-enal semicarbazone

The vinyl carbinol as prepared above (50 g.) was refluxed with a mixture of hydrochloric acid (100 c.c.; 0.9N), water (100 c.c.) and methanol (50 c.c.) for 1 hour. The cooled mixture was exactly neutralised with potassium hydroxide solution and treated with a solution of semicarbazide from the hydrochloride (30 g.) and anhydrous sodium acetate (20 g.) in water (150 c.c.). The semicarbazone which rapidly separated was collected and crystallised from aqueous methanol to give prismatic needles m.p. 196 - 198° (decomp.) (13.0 g.)

Found: C, 54.6; H, 8.1; N, 26.6%

$C_7H_{13}ON_3$ requires C, 54.2; H, 8.4; N, 27.1%

Light absorption in ethanol: Maximum at 2710A., ε = 30,500 cf. Evans and Gillam, J., 1943, 563, who give for β-methylcrotonaldehyde (3-methylbut-2-enal) maximum at 2730A., ε = 33,460.

3-Methylpent-2-enal 2:4-dinitrophenylhydrazone

1-Ethoxy-3-methylpent-1-en-3-ol (0.5 g.) in methanol (2.0 c.c.) was treated with a saturated solution of 2:4-dinitrophenylhydrazine in N-hydrochloric acid (75 c.c.) and the red precipitate collected after 18 hours at room temperature. The 2:4-dinitrophenylhydrazone separated from benzene/light-petroleum (b.p. 60 - 80°) as red

needles m.p. 176 - 178°.

Found: C, 51.4; H, 5.1; N, 19.9%

$C_{12}H_{14}O_4N_4$ requires C, 51.8; H, 5.0; N, 20.1%

Light absorption in ethanol: Maxima at 2550A., ε = 14,000, 2860A., ε = 8,000 and 3800A., ε = 28,000 (c.f. Braude and Jones, J., 1945, 498; $\beta\beta$-dimethylacraldehyde 2:4-dinitrophenylhydrazone has maxima at 2560A., ε = 18,500, 2810A., ε = 11,500 and 3810A., ε = 28,500).

Identification of a By-product from the above Semicarbazone Formation

The mother liquors from which the semicarbazone had separated were extracted with ether (2 x 100 c.c.). Evaporation of the dried extract (Na_2SO_4) gave a sweet smelling oil (35 g.) which distilled almost completely at 33 - 35°/15 mm. The compound gave a negative test with Brady's reagent, no coloration with ferric chloride solution and a slow effervescence with sodium metal. A positive test for an ester grouping was obtained when ferric chloride solution gave a claret coloration after the compound had been treated with hydroxylamine hydrochloride and alkali. A specimen which was fractionated and then redistilled for analysis had b.p. 33.5-34°/12 mm. and n_D^{18} 1.4370

Found:	C, 67.7; H, 10.6%
Calc. for $C_8H_{14}O_2$	C, 67.6; H, 9.9%
Found:	Molecular weight, 148.
$C_8H_{14}O_2$ requires:	Molecular weight, 142.

Light absorption in ethanol: Maximum at 2150A., ε = 13,300.

Hydrolysis of the above Ester

The ester (5.0 g.) was refluxed with sodium hydroxide solution (30 c.c.; 20%) until the top layer had disappeared (5 hours). The solution was ether extracted to remove any unchanged ester and then acidified to p_H 2 with dilute hydrochloric acid. The oil which separated was extracted into ether (50 c.c.). The ethereal solution was shaken with 2% sodium hydrogen carbonate solution (2 x 50 c.c.) and the combined extracts acidified with dilute hydrochloric acid. Renewed extraction with ether (2 x 50 c.c.) and evaporation of the dried (Na_2SO_4) extract gave a colourless oil (2.0 g.) which would not solidify. The oil in ethanol (20 c.c.) was exactly neutralised with 5% alcoholic potassium hydroxide and the solution concentrated to 5 c.c. under reduced pressure. It was then treated with a saturated solution of S-benzylthiourea hydrochloride in ethanol (20 c.c.) and the mixture, after being warmed for a few minutes left at room temperature.

The solid (2.0 g.) which separated was crystallised from ethanol to give the S-benzylthiouronium salt of 3-methylpent-2-enoic acid as transparent plates m.p. 148 - 149°.

Found: C, 60.1; H, 7.5; N, 9.9%

$C_{14}H_{21}O_2N_2S$ requires C, 59.75; H, 7.5; N, 10.0%

Hydration of 1-Ethoxy-3-methylpent-1-yn-3-ol

The compound (1.0 g.; prepared by the sodamide method) was shaken with sulphuric acid (20 c.c.; 10%) at room temperature for 1 hour. The reaction mixture was then extracted with ether (2 x 50 c.c.) and the extract dried (Na_2SO_4). Evaporation left an oily residue which was treated with 2N-sodium hydroxide to pH 7 followed by a saturated solution of S-benzylthiourea hydrochloride in ethanol (10 c.c.). The solid (0.5 g.) which separated immediately was collected and crystallised from ethanol to give the S-benzylthiouronium salt of 3-methylpent-2-enoic acid m.p. 148° undepressed on admixture with an authentic specimen.

Reaction of Isobutyroaldehyde with Ethoxyacetylene

To an ethereal solution of ethyl magnesium bromide (prepared from 2.25 g. of magnesium, 10.2 g. of ethyl bromide and 50 c.c. ether) ethoxyacetylene (6.5 g.) was added in 10 minutes with stirring and ice-cooling.

The evolution of ethane was observed at this stage. After stirring for a further 15 minutes isobutyraldehyde (6.6 g.) in ether (50 c.c.) was added in 1 hour with ice-cooling. The reaction mixture was decomposed by pouring into an ammonium chloride solution (100 c.c.; 10%) and crushed ice. After filtration, the ethereal layer was washed with ammonium chloride solution and several times with water, dried (Na_2SO_4) and evaporated. Distillation of the residue gave a clear liquid b.p. 63 - 65°/ 10^{-2} mm. (7.1 g.) n_D^{18} 1.4575. A redistilled specimen did not give a constant analysis; it was observed to develop a yellow colour on standing.

Hydrogenation of the above Compound

The liquid (7.1 g.) was dissolved in dry ethyl acetate (100 c.c.) and hydrogenated with Adams catalyst (100 mg.). The volume of hydrogen absorbed was 1108 c.c.. The catalyst was filtered off and the solvent evaporated. The residual oil was not distilled (5.6 g.).

Derivatives of the Hydrogenation Product

(a) The compound (0.2 g.) and p-nitrobenzoyl chloride (0.2 g.) were warmed until solution was complete. The solid which separated on cooling was treated with aqueous sodium carbonate. The insoluble material was collected, dried and then crystallised from benzene to

give the derivative as plates m.p. 185 - 187°.

Found: C, 53.2; H, 3.2; N, 8.6%

Calc. for $C_{14}H_8O_7N_2$ C, 53.2; H, 2.6; N, 8.9%

(b) The compound (0.5 g.) in methanol (2. 0 c.c.) was treated with excess of Brady's reagent and the precipitate collected after standing overnight. The 2:4-dinitrophenylhydrazone separated from methanol as orange prisms, m.p. 138 - 140°.

Found: C, 60.3; H, 7.0%

Light absorption in ethanol: maxima at 2200A., ϵ = 13,700, 2530A., ϵ = 11,600, 2750A., ϵ = 7,750, and 3700A., ϵ = 20,000.

<u>The Reaction of Bromethoxyethylene with Lithium and the attempted condensation of the Lithium Compound with a Ketone</u>

Lithium wire (1.2 g.; 2.2 mole) was cut into small pieces under dry ether and then with stirring and under reflux in ether was treated with bromethoxyethylene (12 g.). After 6 hours all the metal appeared to have reacted and the reaction mixture had assumed an orange colour. Heating was discontinued and ethyl methyl ketone (6.0 g.) in ether (50 c.c.) was added dropwise at a rate sufficient to maintain reflux over a period of 1 hour. The reaction mixture was then stirred at room temperature for 2 hours and left standing overnight. It was poured into water

and the decomposition of excess lithium was observed. The ethereal layer was separated and the aqueous phase extracted with ether (100 c.c.). The ethereal layer and extract combined were dried (Na_2SO_4) and evaporation of the ether left a pale yellow oil which was fractionated using a short column.

Fraction (1) b.p. 55 - 65°/15 mm. 3.0 g. n_D^{18} 1.4510

Fraction (2) b.p. 55 - 65°/2 mm. 1.0 g. n_D^{18} 1.4417

The first fraction (1.2 g.) in methanol (5.0 c.c.) was treated with an excess of Brady's reagent and the red precipitate collected after standing overnight. The 2:4-dinitrophenyl-hydrazone separated from ethanol as bright red prisms m.p. 86°.

Found: C, 54.5; H, 5.9; N, 18.3%

$C_{14}H_{18}O_4N_4$ requires C, 54.9; H, 5.9; N, 18.7%

Light absorption in ethanol: main maximum at 3720A., ε = 20,000.

The first fraction (0.5 g.) in methanol (2.0 c.c.) was treated with a solution of semicarbazide from the hydrochloride (1.0 g.) and anhydrous sodium acetate (0.6 g.) in water (3.0 c.c.). The semicarbazone did not form immediately but separated when the reaction mixture was warmed for 20 minutes. The solid was crystallised several times from aqueous methanol to give

the semicarbazone as nacreous plates m.p. 164°.

Found:	C, 59.1;	H, 9.1%
Calc. for $C_9H_{17}ON_3$	C, 59.0;	H, 9.3%

The second (higher-boiling) fraction was redistilled to give a colourless liquid b.p. 38 - 40°/0.5 mm. (0.8 g.) n_D^{18} 1.4407. This was refluxed for 1 hour with a mixture of N-hydrochloric acid (1.0 c.c.), water (1.0 c.c.) and methanol (2.0 c.c.). The reaction mixture was ether extracted and the dried extract (Na_2SO_4) evaporated. The residual liquid was insufficient for identification but gave a positive test for aldehyde with Schiff's reagent.

β-Chlorovinyl methyl ketone

(private communication; Dr. B.C.L. Weedon)

A 1 litre flask (wide-neck) was fitted with a mercury sealed stirrer, a gas inlet reaching to the bottom of the flask, and a short wide tube for the additionof solid reagent. Redistilled acetyl chloride (234 g.; 3 moles) was placed in the flask, the stirring started, and the flask cooled to -10°. Finely powdered aluminium chloride (268 g.; 2 moles) was then slowly added through the wide tube which was afterwards fitted with a calcium chloride tube. The aluminium chloride dissolved initially in the

acetyl chloride but towards the end of the addition the contents of the flask turned to a thick dark yellow paste. (More acetyl chloride was added at this point if stirring became too difficult). The cooling bath was then removed and replaced by a water bath at 15 - 20° and vinyl chloride, purified by bubbling through sulphuric acid, was passed into the mixture at a fairly rapid rate. After 1 hour the mixture turned to a dark yellow liquid and after 3 hours it became very viscous again. The reaction mixture was then poured on to crushed ice (2 kg.) with good stirring; the heavy dark oil was separated and the aqueous layer extracted with carbon tetrachloride (6 x 200 c.c.). The organic material was placed in a 2l. distilling flask, hot water (500 c.c.) added, and steam passed through the mixture. The carbon tetrachloride distilled first with a little water after which a reaction occurred in the flask with evolution of heat. The ketone steam-distilled and a small tarry residue was left with the water in the flask. The organic layer in the distillate was separated and the aqueous layer extracted twice with carbon tetrachloride. The extracts and organic phase were combined, washed with water and dried overnight (Na_2SO_4) in the presence of a trace of magnesium oxide and

hydroquinone. The solution was then evaporated under reduced pressure (100 mm.) through a short column in nitrogen; when all the carbon tetrachloride had been removed the column was dispensed with and the ketone distilled rapidly into a receiver cooled in ice. Fractionation of the crude product gave β-chlorovinyl methyl ketone as a colourless liquid b.p. 55 - 58°/40 mm. (95 g.; 45%). The ketone was kept in a refrigerator whereupon it partially solidified. It was used within a few hours for the following preparation.

1-Chloro-3-methylpent-1-en-3-ol

(cf. Jones and Weedon, J., 1946, 937)

Freshly prepared β-chlorovinyl methyl ketone (95 g.) in ether (200 c.c.) was added dropwise during 1 hour to ethyl magnesium bromide (from 27 g. magnesium and 120 g. ethyl bromide) in ether (800 c.c.) and after 30 minutes stirring at room temperature the complex, which had separated as an orange coloured solid, was decomposed by pouring into a mixture of crushed ice and a solution of ammonium chloride (250 g.) in water. The ethereal layer was washed with ammonium chloride solution and then several times with water. After drying overnight (Na_2SO_4) the ether was removed and the residue fractionated to give the compound (59 g.; 49%)

b.p. 46 - 47°/3 mm. as a colourless liquid n_D^{18} 1.4550. A redistilled specimen did not give constant analyses.

3-Methylpent-2-enal semicarbazone

The above compound (56 g.) was divided into two portions; each portion was treated with dilute sulphuric acid (2l.; 1% w/w) and the mixture steam distilled. The distillates were combined and ether extracted (3 x 200 c.c.). The extract was dried (Na_2SO_4) and the ether removed. The residual yellow oil (35 g.) was dissolved in methanol (50 c.c.) and treated with a solution of semicarbazide from the hydrochloride (50 g.) and anhydrous sodium acetate (33 g.) in water (250 c.c.). The semicarbazone which separated quickly was collected after standing overnight and crystallised from aqueous methanol to give small needles m.p. 192 - 195° (decomp.) (16 g.). The melting point was undepressed on admixture with a specimen from the preparation already described.

3-Methylpent-2-enal

The semicarbazone (11.0 g.) was suspended in water (500 c.c.) containing oxalic acid (55 g.) and steam passed through the boiling solution until 2.5l of distillate had been collected. The distillate was saturated with salt and then extracted with ether in a continuous extractor. The ethereal extract was dried (Na_2SO_4) and the ether

removed (short column). Distillation of the residual oil gave a main fraction (almost all that distilled) b.p. 64 - 67°/22 mm. (3.45 g.; 59%) n_D^{18} 1.4570.

2-Bromo-3-methylpent-2-enal

The aldehyde above (2.0 g.) in glacial acetic acid (5.0 c.c.) was treated dropwise while cooling in ice with a solution of bromine (3.3 g.) in glacial acetic acid (5.0 c.c.). After standing for 10 minutes at room temperature an amber solution was obtained. This was treated with fused potassium acetate (3.0 g.) and the mixture, from which potassium bromide separated, heated on the water bath for 30 minutes. The cooled mixture was filtered from potassium bromide (2.7 g.) and the latter washed with glacial acetic acid (2.0 c.c.). The combined filtrate and washings were diluted with warm water (20 c.c.) and steam passed into the solution rapidly. 100 c.c. of the distillate were collected and extracted with ether (3 x 50 c.c.). The ethereal solution was washed with water, sodium hydrogen carbonate, water and dried (Na_2SO_4) then evaporated. The residual oil (3.0 g.) was not distilled; with Brady's reagent it gave the 2:4-dinitrophenylhydrazone of 2-bromo-3-methylpent-2-enal which separated from ethanol as red plates m.p. 154 - 156° (decomp.).

Found: C, 40.7; H, 3.9%

$C_{12}H_{13}O_4N_4Br$ requires C, 40.35; H, 3.7%

DL-Leucine hydroxamic acid

DL-Leucine methyl ester (11.6 g.) was added to a solution of hydroxylamine (16.5 g.) in methanol (600 c.c.) at 0°; after 2 days at 0° the separated solid was collected, washed with water and dried. DL-Leucine hydroxamic acid separates from water as small prisms m.p. 192 - 194° (decomp.) (6.2 g.).

Found: C, 49.7; H, 10.4; N, 18.6%

$C_6H_{14}O_2N_2$ requires C, 49.3; H, 9.8; N, 19.1%

L-Leucine methyl ester

(Abderhalden and Spinner, Z. Physiol. Chem., 1919, 107, 1)

L-Leucine (20 g.) was suspended in dry methanol (200 c.c.) and treated with hydrochloric acid under reflux until solution was complete (30 minutes). After standing overnight the methanol was removed under reduced pressure and the residue dissolved in water (50 c.c.). The aqueous solution was covered with ether (100 c.c.) and treated at 0° with a solution of sodium hydroxide (19 c.c.; 33%). The ethereal layer was separated, the aqueous phase extracted with ether (2 x 100 c.c.), the extract combined with the ethereal layer and dried (Na_2SO_4). Evaporation of the ether left the ester (17.5 g.; 79%)

which was used for the next preparation without further purification.

L-Leucine hydroxamic acid

L-Leucine methyl ester (17.5 g.) was treated at 0° with a solution of hydroxylamine from the hydrochloride (52 g.) and sodium (17 g.) in methanol (500 c.c.). After 5 days at 0° the separated solid was collected, washed with water and dried. Crystallisation from water gave L-leucine hydroxamic acid (15 g.) as small prisms m.p. 184 - 186°.

Found:	C, 48.9;	H, 9.4%
$C_6H_{14}O_2N_2$ requires	C, 49.3;	H, 9.8%

2-Bromo-3-methylpent-2-enylidene-DL-leucine hydroxamic acid

2-Bromo-3-methylpent-2-enal (3.0 g.; undistilled) in ethanol (60 c.c.) was refluxed with DL-leucine hydroxamic acid (2.5 g.) for 1 hour. Unchanged acid (0.35g.) was then removed and the reaction mixture concentrated under reduced pressure almost to dryness. The solid which separated was collected, washed with a little cold ethanol and dried (4.5 g.) m.p. 122° (decomp.). 2-Bromo-3-methylpent-2-enylidene-DL-leucine hydroxamic acid separates from ethanol as prisms m.p. 126 - 127° (decomp.) darkening at ca. 118°.

Found: C, 47.4; H, 6.8: N, 9.3%

$C_{12}H_{21}O_2N_2Br$ requires C, 47.2; H, 6.9; N, 9.2%

An ethanolic solution of the compound gives a claret coloration with ferric chloride solution.

Attempted Cyclisation of the above Compound

A refluxing solution of the compound (5.1 g.) in tert.-butanol (200 c.c.) was treated with a solution of potassium (0.65 g.) in tert.-butanol (50 c.c.). After 8 hours potassium bromide (1.2 g.) was collected and the filtered solution concentrated under reduced pressure to 100 c.c. Dilute hydrochloric acid was added to p_H 4 and as there was no separation of solid the solution was concentrated until all the tert.-butanol had been removed. It was then extracted with ether (3 x 100 c.c.) and the ether extract (A) shaken with 2% sodium hydrogen carbonate solution (3 x 50 c.c.). The bicarbonate extract was acidified to p_H 3 with hydrochloric acid and ether extracted (2 x 50 c.c.). Evaporation of the dried extract (Na_2SO_4) left a non-crystalline residue (0.5 g.). After purification by a further bicarbonate extract the material (0.3 g.) could not be crystallised. A test portion with copper acetate solution gave a copper salt largely insoluble in dioxan. Sublimation of the bulk of the material at $100^o/10^{-3}$mm. gave a

resinous sublimate (10 mg.) which was soluble in a saturated sodium hydrogen carbonate solution with effervescence and in the minimum of methanol gave an intense red coloration when treated with aqueous ferric chloride. Light absorption in ethanol: Maxima at 2300A., ε = 4,700 and at 3320A. ε = 1,850. (cf. Dunn, Gallagher, Newbold and Spring, J., 1949, 126S; aspergillic acid has maxima at 2340A., ε = 6,500 and at 3280A., ε = 8,300).

Extraction of the ethereal solution (A) with 5N-sodium hydroxide solution followed by acidification of the extract to p_H 3 and renewed ether extraction, gave, on evaporation of the dried ether extract (Na_2SO_4), a residue (2.0 g.) which could not be crystallised. This material gave a deep red coloration with ferric chloride solution.

Aminoacetone hydrochloride

(a) Preparation from Isonitrosoacetone

(Gabriel and Pinkus, Ber., 1893, 26, 2197)

Isonitrosoacetone (60 g.) was reduced by addition in portions to a solution of stannous chloride (310 g.) in concentrated hydrochloric acid (450 c.c.; d, 1.19) with stirring and cooling, the temperature being kept below 30°. The solution was then heated on the steam bath with granulated tin (104 g.), decanted, and treated

with hydrogen sulphide until no further precipitate was produced. The filtered solution was evaporated as far as possible under reduced pressure and the viscous residue taken up in water (21.), treated with hydrogen sulphide, and the filtrate re-evaporated. The viscous syrup remaining was crystallised from ethanol-ether. The crystalline aminoacetone hydrochloride m.p. 120° (24 g.; 32%) was kept in a desiccator over phosphorus pentoxide.

(b) Preparation from Chloracetone

(Gabriel and Colman, Ber., 1902, 35, 3805)

Chloracetone (19 g.) and phthalimide (30.0 g.) were placed in a 500 c.c. flask fitted with a stirrer and reflux condenser. Sufficient xylene was added to make stirring possible and then the mixture was refluxed gently for 3 hours. The crude phthalimidoacetone was filtered off and crystallised from benzene/light petroleum (b.p. 60 - 80°). Yield 16 g.; 38%.
The phthalimidoacetone (16 g.) was refluxed for 4 hours with hydrochloric acid (120 c.c.; 66%). A small amount of charcoal was then added and the reflux continued for a further 30 minutes. The reaction mixture was filtered and the filtrate collected after two washings with water (10 c.c.). Evaporation to dryness under reduced pressure

left a residue which was dissolved in the minimum of ethanol and the solution once again taken to dryness. Finally the residue was taken up in the minimum of dry ethanol and left to crystallise. The aminoacetone hydrochloride was quickly collected and placed in a desiccator. Yield 5.0 g.; 58%

Pyruvohydroxamyl chloride

(cf. Ponzio, Gazzetta, 1907, 37, ii, 68)

A mixture of acetone (21 c.c.) and nitric acid (20 c.c.; d, 1.37) in a 250 c.c. conical flask was treated with 9 - 10 drops of fuming nitric acid. The flask, with a cork stopper lightly wired on, was kept three days immersed in water at room temperature. The reaction mixture was then poured into water (20 c.c.) and extracted with ether (4 x 50 c.c.). Five portions of acetone were treated thus simultaneously and the ether extracts combined and dried (Na_2SO_4). Evaporation of the ether left a residual oil. This was not purified but cooled in ice and treated with an equal volume of concentrated hydrochloric acid, previously saturated at 0° with dry hydrochloric acid. The mixture was left at 0° for 24 hours, the crystalline solid collected, and the filtrate ether extracted. The dried extract (Na_2SO_4) was evaporated to give a further

yield of solid. Crystallisation from benzene afforded pyruvohydroxamyl chloride as plates m.p. 107° (20 g.).

Attempted preparation of Pyruvohydroxamic acid

Pyruvohydroxamyl chloride (4 g.) was treated with a solution of anhydrous sodium acetate (60 c.c.; 40%) in acetic acid (50%). After standing overnight the reaction mixture was treated with a saturated solution of copper acetate and there was no precipitate. Slow addition of 10% sodium hydroxide solution, however, precipitated an insoluble complex salt. The salt (5 g.) was collected, washed with ethanol and air dried. It was suspended in methanol (200 c.c.) and hydrogen sulphide passed for 4 hours. The sulphide was filtered off (Hiflo) and the filtrate after treatment with charcoal was evaporated to dryness under reduced pressure. The residual gum (1.8 g.) gave a red coloration with ferric chloride solution. It was extracted with ether under reflux (5 x 100 c.c.). Evaporation of the dried extract (Na_2SO_4) left a solid (0.2 g.) m.p. 135° (decomp.) which did not crystallise.

Bisulphite Compound of Pyruvohydroxamic acid

(cf. Gastaldi, Gazzetta, 1923, 53, 638)

Pyruvohydroxamyl chloride (12 g.) was treated with a 40% solution of sodium bisulphite (sodium metabisulphite,

27.6 g., in 48 c.c. of water) saturated at room temperature with sulphur dioxide. After 12 hours the bisulphite compound was precipitated by the addition of ethanol (450 c.c.). It separated at first as a yellow oil but solidified after standing overnight at 0°. The solid was collected, washed with ethanol and crystallised from aqueous methanol. The compound (5.0 g.) separated as small prisms. It was insoluble in ethanol but freely soluble in water; with aqueous ferric chloride it gave an intense red coloration.

2-Hydroxy-3:6-dimethylpyrazine

The bisulphite compound of pyruvohydroxamic acid (9.0 g.; prepared as above) was dissolved in water (50 c.c.) and treated with aminoacetone hydrochloride (4.0 g.) and anhydrous sodium acetate (3.0 g.) in acetic acid (30 c.c.; 25%). The reaction mixture was kept at 50° for 6 hours and then left at room temperature overnight. The reaction solution (p_H 4) was evaporated to dryness under reduced pressure. The residue was extracted with chloroform under reflux (5 x 50 c.c.) and the dried extract (Na_2SO_4) evaporated to dryness. The residue (2.0 g.) which gave an intense red coloration with ferric chloride solution could not be crystallised; at $130°/10^{-3}$mm.

there was partial sublimation. The sublimate (200 mg.) had m.p. 115 - 120° and gave a red coloration with ferric chloride solution. Crystallisation from acetone (1.0 c.c.) yielded small needles (25 mg.) m.p. 204°, and this material gave no coloration with ferric chloride solution. Crystallisation from benzene/light-petroleum (b.p. 60 - 80°) followed by sublimation gave 2-hydroxy-3:6-dimethylpyrazine m.p. 210°, undepressed when mixed with a specimen prepared via 2-amino-3:6-dimethylpyrazine (Baxter, Newbold and Spring, J., 1947,370).

Found:	C, 58.3;	H, 6.3%
Calc. for $C_6H_8ON_2$	C, 58.1;	H, 6.45%

The filtrate from the crystallisation was evaporated to dryness and the residue at 120°/10^{-3}mm. gave a sublimate (85 mg.). This had m.p. 130 - 150°, gave a red coloration with ferric chloride solution and an effervescence with saturated sodium hydrogen carbonate solution. There was insufficient material for crystallisation to constant melting point.

1-Hydroxy-2-keto-3:6-dimethyl-1:2-dihydropyrazine

Pyruvohydroxamyl chloride (15 g.) was dissolved in a solution of sodium bisulphite (60 c.c.; 40%) saturated with sulphur dioxide at room temperature. After 16 hours the crude bisulphite compound was

precipitated on the addition of ethanol (500 c.c.). It separated as a semi-solid. After standing at 0° overnight the supernatant ethanol was decanted off and methanol (200 c.c.) added. The crude bisulphite compound (19 g.) was then collected and dissolved in water (100 c.c.). The addition of ethanol (80 c.c.) followed by standing at 0° for 1 hour precipitated sodium bisulphite (5 g.). The filtrate was treated with more ethanol and the process repeated until the precipitate started to give a pronounced red coloration with aqueous ferric chloride. Evaporation of the filtrate under reduced pressure left the bisulphite compound of pyruvohydroxamic acid (10 g.) as an amorphous powder which did not melt below 300°.

The bisulphite compound (10 g.) in the minimum of water (30 c.c.) was treated with aminoacetone hydrochloride (4.0 g.) and anhydrous sodium acetate (3.0 g.) in acetic acid (20 c.c.; 50%). After being heated at 60 - 70° for 11 hours the reaction mixture was filtered and left overnight at room temperature. The solid (0.75 g. m.p. 180 - 185°) was combined with a second crop (0.75 g.) which separated on concentration of the mother liquors. Sublimation at $100°/10^{-3}$mm. and crystallisation from acetone gave 1-hydroxy-2-keto-3:6-dimethyl-1:2-dihydropyrazine

as pale yellow prisms m.p. 194 - 195° (sintering at 160°)

Found: C, 45.6; H, 6.3; N, 17.7%

$C_6H_8O_2N_2H_2O$ requires C, 45.6; H, 6.3; N, 17.75%

Light absorption in ethanol: Maxima at 2340A., ε = 8,100 and 3265A., ε = 7,000.

The acid is soluble in sodium hydrogen carbonate solution with effervescence and gives a deep red coloration with aqueous ferric chloride.

Drying over phosphorus pentoxide at 20°/10^{-2}mm. for 18 hours gave an anhydrous specimen of the acid which was analysed immediately.

Found: N, 20.1, 20.5:

$C_6H_8O_2N_2$ requires N, 20.0%

The acid (100 mg.) in water (10 c.c.) was treated with a slight excess of a saturated copper acetate solution. The precipitate (70 mg.) was crystallised from dioxan to give the copper salt as light green transparent plates m.p. 280° (decomp.).

Found: C, 41.7; H, 4.1%

$Cu(C_6H_7O_2N_2)_2$ requires C, 42.2; H, 4.1%

Reduction of 1-Hydroxy-2-keto-3:6-dimethyl-1:2-dihydropyrazine

(a) A solution of the acid (200 mg.) in ethanol (20 c.c.) was treated with hydrazine hydrate (3.0 c.c.;

90%) and heated at 170° for 9 hours. Removal of the solvent under reduced pressure left a residue which gave no coloration with ferric chloride solution. Sublimation at 60°/10^{-3} mm. yielded solid material (80 mg.) m.p. 60 - 70°. Crystallisation from benzene/light-petroleum (b.p. 60 - 80°) gave clusters of needles m.p. 92 - 94°.

Found: C, 56.2; H, 9.0%

$C_6H_{12}ON_2$ requires C, 56.2; H, 9.5%

Light absorption in ethanol: Maxima at 2280A., ε = 9,000 and at 3220A., ε = 1,000.

(b) A solution of the acid (170 mg.) in methanol (15 c.c.) was treated with hydrazine hydrate (0.5 g.; 90%) and heated at 180° for 4 hours. Removal of the methanol under reduced pressure left a solid residue which gave no coloration with ferric chloride solution. Sublimation at 80°/10^{-3} mm. yielded material (90 mg.) m.p. 198 - 203°. Crystallisation from benzene/light-petroleum (b.p. 60 - 80°) gave 2-hydroxy-3:6-dimethylpyrazine as felted needles m.p. 208 - 210°, undepressed when mixed with a specimen prepared via 2-amino-3:6-dimethylpyrazine (Baxter, Newbold and Spring, J., 1947, 370).

Found: C, 58.1; H, 6.2%

Calc. for $C_6H_8ON_2$ C, 58.1; H, 6.45%

Light absorption in ethanol: Maxima at 2260A., ε = 8,350 and at 3210A., ε = 7,800.

Synthesis of 1-Amino-4-methylpentan-2-one

1-Chloro-4-methylpentan-2-one

Isovaleric acid was converted into its acid chloride using the method described by Brown (J.A.C.S., 1938, 60, 1325). The acid chloride had b.p. 114° (yield 70%).

Isovaleryl chloride (65 g.) in dry ether (200 c.c.) was added slowly to a dry solution of diazomethane in ether (1,100 c.c.), (prepared from 130 g. of nitrosomethylurea) with shaking and ice-cooling. The reaction vessel was fitted with a soda-lime tube and the solution left for 15 hours. The solution was then cooled in ice and treated with dry hydrogen chloride for 3 hours. The reaction mixture was left overnight and washed, first with saturated sodium carbonate solution until the ether layer was neutral and then with water, and dried (Na_2SO_4). Evaporation of the ether and distillation of the residual oil gave a main fraction b.p. 53 - 56°/15 mm.. Redistillation gave 1-chloro-4-methylpentan-2-one as a colourless liquid, b.p. 53 - 55°/15 mm. (45 g.) n_D^{18} 1.4338.

Found: C, 53.9; H, 8.3%

$C_6H_{11}OCl$ requires C, 53.6; H, 8.2%

1-Phthalimido-4-methylpentan-2-one

A mixture of 1-chloro-4-methylpentan-2-one (20 g.) and potassium phthalimide (30 g.) in dry xylene (35 c.c.) was heated at 140 - 150° (bath temp.) with stirring for 4 hours. After cooling the mixture was diluted with hot benzene (500 c.c.) and filtered. The filtrate was evaporated under reduced pressure to small volume and then treated with light-petroleum (b.p. 40 - 60°). The crystalline material which separated on cooling (17.5 g.; m.p. 115°) was collected and recrystallised from light-petroleum (b.p. 60 - 80°) to give 1-phthalimido-4-methylpentan-2-one as long fine needles m.p. 117 - 118°.

Found: C, 68.7; H, 6.4; N, 5.9%

$C_{14}H_{15}O_3N$ requires C, 68.6; H, 6.1; N, 5.7%

1-Amino-4-methylpentan-2-one hydrochloride

The phthalimido-ketone (26 g.) was heated under reflux with hydrochloric acid (100 c.c.; 30%) for 24 hours. The solution was allowed to cool and the phthalic acid filtered off and washed with a little cold water. The combined filtrate and washings were evaporated to dryness under reduced pressure. Cold water (50 c.c.) was added and the mixture filtered and

taken to dryness. The residue was dissolved in the minimum of warm methanol and the solution treated with an equal volume of ether. On cooling to 0° the hydrochloride separated as plates. Evaporation of the mother liquors and treatment with ether yielded further crops (yield 13.0 g.; 81%).

Recrystallisation from methanol-ether gave 1-amino-4-methyl-pentan-2-one hydrochloride as plates m.p. 176 - 178°.

Found:	C, 47.5;	H, 9.0;	N, 9.5%
$C_6H_{14}ONCl$ requires	C, 47.5;	H, 9.3;	N, 9.2%

Nitrosyl Chloride

(cf. Tilden, J., 1874, 630)

A mixture of nitric acid (50 c.c.; d, 1.42) and hydrochloric acid (200 c.c.; d, 1.2) was heated to 80° and the gases evolved passed down a calcium chloride drying tower, and into sulphuric acid (200 c.c.). The yellow sulphuric acid solution was added dropwise to dry sodium chloride (50 g.) and the mixture heated slowly to 100°. The nitrosyl chloride evolved was collected in a receiver cooled to -60°. By allowing the receiver to warm slowly to 0° the nitrosyl chloride was distilled over phosphorus pentoxide and collected at -60° as a dark red liquid (20 g.).

Pyruvohydroxamyl chloride

(Rheinboldt and Schmitz-Dumont, Ann., 1925, 444, 117).

A solution of nitrosyl chloride (16 g.; 2 mols.) in carbon tetrachloride (200 c.c.) was cooled to 0° and acetone (7.0 g.) added dropwise. Cooling was maintained for 2 hours while hydrochloric acid was evolved. After standing overnight at room temperature the solvent was decanted from the semi-crystalline solid which had separated. The solid was extracted with hot benzene (3 x 50 c.c.) and evaporation of the dried extract (Na_2SO_4) left pyruvohydroxamyl chloride (5.5 g.; 40%) m.p. 105 - 107°.

After several crystallisations from benzene, pyruvohydroxamyl chloride separated as plates m.p. 112 - 113°.

Found:	N, 11.6%
Calc. for $C_3H_4O_2NCl$	N, 11.6%

When the compound was dissolved in 3N- sodium hydroxide and the solution acidified, a red coloration was obtained on treatment with aqueous ferric chloride.

Pyruvohydroxamyl chloride 2:4-dinitrophenylhydrazone

Pyruvohydroxamyl chloride (1.0 g.) in methanol (4.0 c.c.) was treated with an excess of Brady's reagent and the orange precipitate collected after standing overnight. The 2:4-dinitrophenylhydrazone separated

from benzene as small prisms m.p. 211 - 212° (0.7 g.).

Found: C, 35.9; H, 2.7; N, 23.5%

$C_9H_8O_5N_5Cl$ requires C, 35.8; H, 2.7; N, 23.2%

When dried for analysis at 56°/10^{-1} mm. the colour of the specimen changed from light orange to a darker shade without change in melting point.

Sec.-butyl methyl ketone synthesis

Ethyl-α-ethyl-acetoacetate

Dry redistilled ethyl iodide (546 g.) was added dropwise over 2 hours to a stirred gently refluxing solution of sodium (69 g.) in dry ethanol (1.5l.) containing redistilled ethyl acetoacetate (390 g.). The solution was then refluxed for 6 hours. After standing overnight as much ethanol as possible was removed under reduced pressure, sodium iodide separating out at the end. Water (2l.) was added to the residue, the ester layer separated, washed with a saturated solution of calcium chloride, and dried over calcium chloride. The aqueous layer was ether extracted, and the dried (Na_2SO_4) ether extracts combined with the ester layer. The ether was evaporated and the residual oil distilled to give ethyl-α-ethyl-acetoacetate b.p. 80°/15 mm. (320 g.) as a colourless pleasant smelling oil.

Ethyl-α-methyl-α-ethyl-acetoacetate

Dry redistilled methyl iodide (426 g.) was added dropwise over 2 hours to a stirred solution ofsodium (46 g.) in dry ethanol (1,000 c.c.) containing ethyl-α-ethyl-acetoacetate (320 g.) at 40 - 50°. The solution was then refluxed gently for 3 hours and allowed to stand overnight. As much ethanol as possible was removed under reduced pressure on the steam bath and the residue diluted with water (21.). The ester layer was separated, washed with a saturated solution of calcium chloride, and dried over calcium chloride. The aqueous layer was extracted with ether and the dried (Na_2SO_4) extracts combined with the ester layer. Removal of the ether and distillation of the residual oil gave ethyl-α-methyl-α-ethyl-acetoacetate b.p. 78 - 79°/ 10 mm. as a colourless oil (240 g.).

Sec.-butyl methyl ketone

Ethyl-α-methyl-α-ethyl-acetoacetate (240 g.) was refluxed with a solution of potassium hydroxide (1,800 c.c.; 10%) for 5 hours. The reaction mixture was cooled and the upper ketonic layer separated and dried (Na_2SO_4). The aqueous layer was extracted with ether and the dried extract (Na_2SO_4) combined with the dried ketonic layer. Removal of the ether and distillation

of the residue at 760 mm. gave a main fraction b.p. 110 - 120°. Redistillation up a short column gave sec.-butyl methyl ketone as a colourless liquid smelling of peppermint, b.p. 115 - 118° (70 g.).

1-Keto-2-methyl-n-valerohydroxamyl chloride

A solution of nitrosyl chloride (14 g.; 5 mols.) in carbon tetrachloride (100 c.c.) was cooled to 0° and sec.-butyl methyl ketone (4.0 g.) added dropwise. After 2 hours at 0° the reaction mixture was kept at room temperature for 3 days. The dark red colour of the solution had by then changed to bright green and a viscous oil had been deposited on the sides of the flask. The solution was decanted from the oil and evaporated under reduced pressure. The residual pale yellow oil did not solidify even after prolonged cooling. Distillation at bath temperature 130°/10^{-3} mm. yielded a colourless oil (1.0 g.) which solidified on standing. Recrystallisation from light-petroleum (b.p. 40 - 60°) gave 1-keto-2-methyl-n-valerohydroxamyl chloride as transparent plates m.p. 59 - 61°.

Found:	C, 43.7;	H, 6.4%
$C_6H_{10}O_2NCl$ requires	C, 44.0;	H, 6.1%

Action of Nitrosyl chloride upon Isobutyl methyl ketone

The ketone (6.0 g.) in carbon tetrachloride (100 c.c.)

was treated at 0° with a solution of freshly distilled nitrosyl chloride (40 g.) in carbon tetrachloride (200 c.c.). After 2 hours at 0° the reaction mixture was left at room temperature for 4 days. The solution was then decanted from a clear viscous oil which was adhering to the sides of the flask, and the solvent removed under reduced pressure. The residue was distilled at 10^{-3} mm. and the following fractions collected:

(1) A pale yellow oil (2.0 g.) which distilled at bath temperature 90°.

(2) A colourless oil (0.5 g.) distilling at bath temperature 130° and which rapidly solidified.

The first fraction darkened on standing and was not worked up. The solid product was crystallised from light-petroleum (b.p. 40 - 60°). After a number of crystallisations from this solvent 1-keto-3-methyl-n-valerohydroxamyl chloride was obtained as transparent plates m.p. 64 - 66°.

Found:	C, 44.2;	H, 6.2;	N, 8.2%
$C_6H_{10}O_2NCl$ requires	C, 44.0;	H, 6.2;	N, 8.6%

When the compound was dissolved in 3N- sodium hydroxide and the solution acidified, a red coloration was obtained on treatment with aqueous ferric chloride.

An analysis specimen gave a positive test for halogen (Beilstein).

The 2:4-dinitrophenylhydrazone of 1-keto-3-methyl-n-valerohydroxamyl chloride separated from benzene as orange needles m.p. 173 - 175°.

Found:	C, 42.2;	H, 4.2%
$C_{12}H_{14}O_5N_5Cl$ requires	C, 41.9;	H, 4.1%

Attempted condensation of 1-amino-4-methylpentan-2-one with 1-keto-2-methyl-n-valerohydroxamic acid

(a) 1-Keto-2-methyl-n-valerohydroxamyl chloride (20 g.) was treated with a solution of sodium hydrogen sulphite (80 c.c.; 40%) saturated with sulphur dioxide at room temperature. The reaction mixture was stirred and heated to 30 - 40° for 5 hours; it was then kept at room temperature overnight with continued stirring. An organic top layer (8 g.) was rejected and the aqueous phase treated with methanol (60 c.c.). After standing overnight at 0° the separated sodium hydrogen sulphite (25 g.) was collected. The filtrate was concentrated under reduced pressure at room temperature to 50 c.c. and treated with methanol (50 c.c.). The separated sodium hydrogen sulphite (5 g.) was removed and the filtrate concentrated under reduced pressure at room temperature to 40 c.c.

The solution of the bisulphite compound of 1-keto-2-methyl-n-valerohydroxamic acid as prepared above was treated with 1-amino-4-methylpentan-2-one from the hydrochloride (4.2 g.) and anhydrous sodium acetate (2.3 g.) in acetic acid (30 c.c.; 50%). The reaction mixture was kept at 65° for 11 hours and after standing at room temperature overnight was extracted with ether (2 x 100 c.c.). The ethereal solution (A) was extracted with 2% sodium hydrogen carbonate solution (3 x 50 c.c.) and the extract acidified to pH 3 with hydrochloric acid. Ether extraction (2 x 50 c.c.) followed by evaporation of the dried extract (Na_2SO_4) left only a trace of material.

The ethereal solution (A) after the carbonate extraction was evaporated to dryness and the residue dissolved in the minimum of methanol and treated with an excess of Brady's reagent.

A 2:4-dinitrophenylhydrazone was collected and crystallised from ethanol to give yellow needles m.p. 168 - 169°.

Found: C, 52.6; H, 5.8; N, 16.0%

Found: Molecular Weight 345.

(b) A solution of 1-keto-2-methyl-n-valerohydroxamyl chloride (11.0 g.) in methanol (50 c.c.) was treated with anhydrous sodium acetate (20 g.) in acetic acid

(50 c.c.; 50%) and heated to 30 - 40° for 1 hour. 1-Amino-4-methylpentan-2-one hydrochloride (5 g.) in methanol (50 c.c.) was then added and the temperature raised to 60 - 65° for 3 hours. The reaction mixture was diluted with water (300 c.c.) and concentrated to 100 c.c. under reduced pressure. 2N- Sodium hydroxide was added to p_H 8 and the solution extracted with ether (3 x 50 c.c.). This extract was rejected. The p_H was then adjusted to 3 with hydrochloric acid, the solution again extracted with ether (3 x 50 c.c.), and the extract shaken with 2% sodium hydrogen carbonate (3 x 50 c.c.). The bicarbonate extract was acidified to p_H 4 with hydrochloric acid and extracted with ether (2 x 50 c.c.). Evaporation of the dried extract (Na_2SO_4) left a residual oil (0.75 g.).

The oil (0.75 g.) was distilled at 80°/10^{-3}mm. to give a colourless liquid (0.2 g.) which dissolved in a saturated sodium hydrogen carbonate solution with effervescence and in methanol gave a deep red coloration when treated with aqueous ferric chloride.

The liquid (0.1 g.) in methanol (1.0 c.c.) was treated with an excess of Brady's reagent and the yellow precipitate collected after standing overnight. Crystallisation from benzene gave a 2:4-dinitro-

phenylhydrazone as yellow needles m.p. 172°.

Found: C, 45.0, 45.0; H, 4.0, 3.9%

$C_{12}H_{15}O_6N_5$ requires C, 44.3; H, 4.6%

The residue after distillation was sublimed at 100°/10^{-3} mm. to give a resinous sublimate (10 mg.). This material in methanol gave a deep red coloration when treated with aqueous ferric chloride; it also dissolved in a saturated solution of sodium hydrogen carbonate with effervescence. Light absorption in ethanol: maxima at 2430A., ε - 2,700 and 3200A., ε = 1,300 (cf. Dunn, Gallagher, Newbold and Spring, J., 1949, 126S; Aspergillic acid has maxima at 2340A., ε = 6,500 and 3280A., ε = 8,300).

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