STUDIES ON INTRAMOLECULAR REARRANGEMENTS IN PEPTIDE SYNTHESIS

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in the

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by

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Abstract.

A survey of intramolecular acyl rearrangements involving formation of amide bonds has been made. A short survey of examples where the acyl group is only partially transferred, with cyclol formation, follows. The use of amino-acid N-carboxyanhydrides (NCAs) for specific peptide synthesis has also been reviewed. The second section describes attempts to amino-acylate substrates with NCAs and to then rearrange the derivative to give an amide bond. Thiol derivatives could be acylated but not rearranged. Reaction with a variety of nucleophiles showed that N,N-dialkylhydroxylamines are readily acylated by NCAs and that the ester produced is displaced by amines to give an amide. Bis-N,N-dialkylhydroxylamines have been synthesised but not monoacylated. α-Carbamido-N,N-dialkylhydroxylamines form esters with NCAs that can undergo rearrangement to form an amide link, but only at extreme basic conditions.

Acknowledgements.

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Introduction

The first synthesis of a peptide was by Emil Fischer in 1901[1]. In the paper describing this work Fischer also outlined the steps needed to make a peptide link. These steps involved taking two fragments of the molecule and combining them in an intermolecular fashion with the formation of an amide link.

The intermolecular method of making amides has been employed in nearly all the peptide syntheses that have been described[2]. By contrast the synthesis of proteins by Nature involves, almost certainly, an intramolecular process[3]. This fact is apparent from the specific arrangement of amino-acids that exists in any naturally occuring protein; the order must be predetermined by the surface at which the protein is synthesised.

The method of preparing peptides in an analogous way to Nature has received little attention. It was the purpose of this present work to make a preliminary study of amide bond formation by an intramolecular process and adaption of this to peptide synthesis.

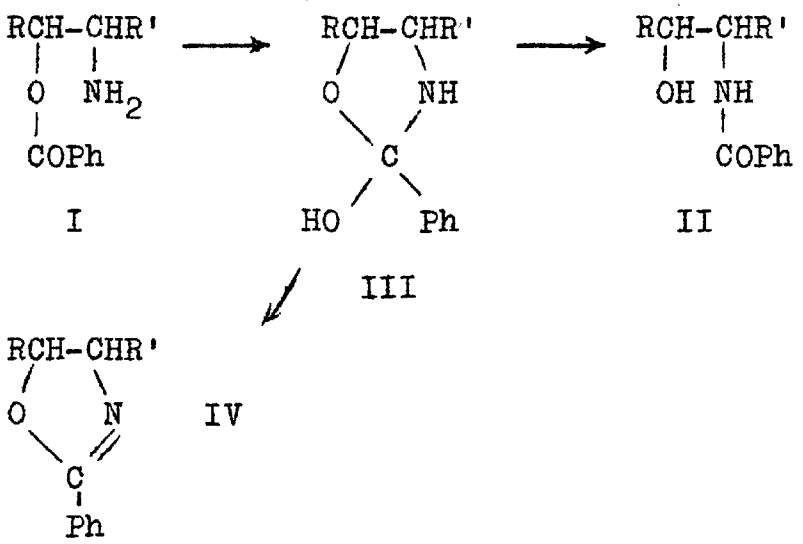
To accomplish this two distinct steps are required, (i) the attachment of an amino-acid residue, via

some reactive derivative, to a site on a suitable substrate and (ii), in a controlled manner, transferring the amino-acyl group from the active site to an adjacent peptide chain, also connected to the substrate.

The reactive amino-acid derivative chosen for most of the work was the N-carboxy anhydride, referred to as amino-acid 'NCA'. Recent chemistry of the amino-acid NCAs is reviewed. It is pertinent, however, to review intramolecular transacylations onto nitrogen first of all.

Transacylations involving amino groups.

In 1890 Gabriel noticed that basification of 1-amino-2-benzoyloxypropane hydrochloride(I;R=CH_3, R'=H) led to the corresponding amide(II;R=CH_3, R'=H)[4]. Since then many examples of this rearrangement have been recognised[18]. The first indication that the transfer occurs internally came with the observation that the benzoate of ethanolamine(I;R,R'=H) rearranges more readily than the benzoate of 1,3-propanolamine[5].



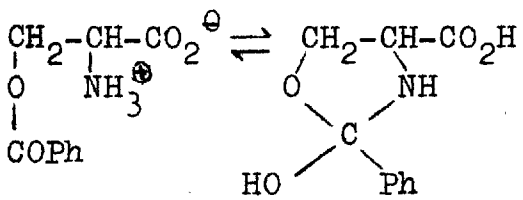
The transfer is reversible. Bergmann found that in base the ester(I;R=$PhCOOCH_2$,R'=H) rearranged to the amide(II;R=$PhCOOCH_2$,R'=H). On treating with strong acid the amide gave back the starting benzoate hydrochloride[6]. Under very vigorous acid

conditions, for example thionyl chloride, the amides rearranged to give large amounts of a different type of compound from the benzoates. These products were thought to be produced by dehydration of the intermediate(III) in the acyl transfer, forming the oxazoline(IV)[7].

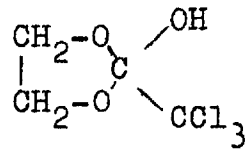
The oxazolines could possibly be intermediates in both the acid and base catalysed rearrangements. However,Fry[8], could not detect any oxazoline in the base catalysed rearrangements, even though oxazolines were stable to the conditions used. Phillips and Baltzy[9] have studied the nature of the reaction. Studying ethanolamine benzoate hydrochloride(I;R,R'=H) basic titration gave only the benzamido ethanol(II;R,R'=H). No intermediate of the type (III) or (IV) could be detected. The similarity of the postulated intermediate (III) to the known and isolated compound (V) was noticed[10]. Since compounds containing a monosubstituted amine also undergo the rearrangement[11] it can be concluded that oxazolines are not intermediates in the O$\longrightarrow$N transfer and, most probably, form only as a side product in the acid catalysed N$\longrightarrow$O transfers.

Derivatives of serine and threonine also give

the rearrangement [12]. Elliott isolated the free threonine-O-benzoyl ester[13]. The compound behaved as a normal organic acid on basic titration, this result requiring the free amino compound(VI) to be in rapid equilibrium with the intermediate(VII).



VI VII

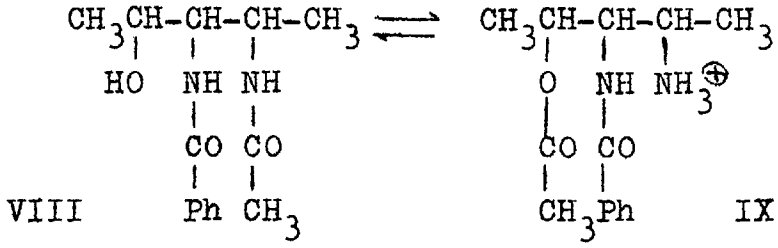


V

Serine and threonine derivatives behave similarly in peptides. Mild acid hydrolysis of peptides cleaves the chain at seryl and threonyl functions initially. This is because the attached peptide chain transfers to the alcohol to give an ester which rapidly hydrolyses[14]. Methods for specifically cleaving peptides by this process have been developed[15].

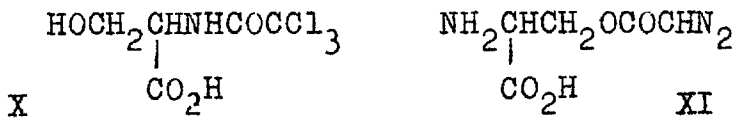
The rearrangement is affected by electronic and steric factors. Serine derivatives give a faster O⟶N rearrangement than those of threonine[12]. Grob and Wagner describe an interesting case in point[16]. The amide(VIII) rearranges under acid catalysis to the ester (IX). This involves a 1,3 shift of the acyl group instead of the 1,2

shift that would be expected. Basification again yields the amide(VIII).



VIII IX

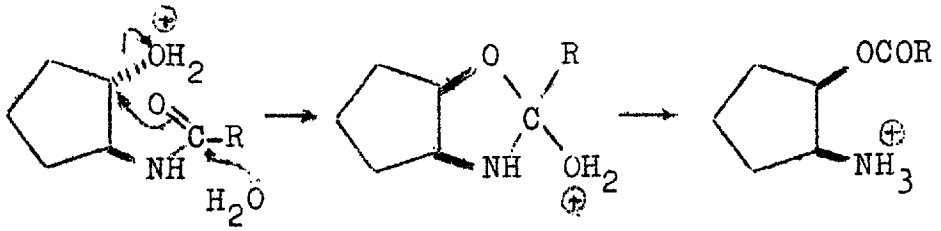
The nature of the acyl group is important. An acyl group which is extremely electron attracting will inhibit the N⟶O exchange. Thus, although mono- and di-chloroacetyl groups will give reversible exchanges in serine derivatives[17,18], the trichloroacetyl derivative (X) is stable to acid[18]. Conversely, the diazoacetyl group of azaserine(XI) only rearranges slowly to the corresponding amide[19].



X XI

The normal mechanism for the acyl transfer can be replaced by an alternative type of exchange[20] when the transition state is strained. The mechanism involves displacement. Trans-2-p-nitrobenzamidocyclopentanol(XII), in acid, gives cis- p-nitro-

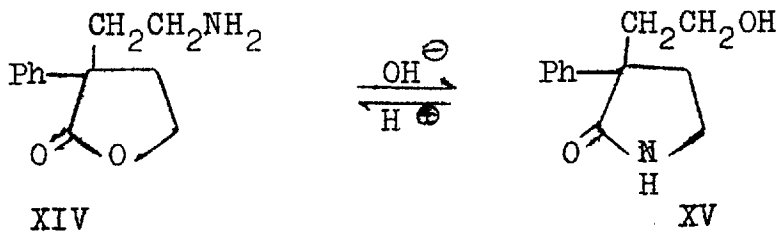
benzoate(XIII) only[21].



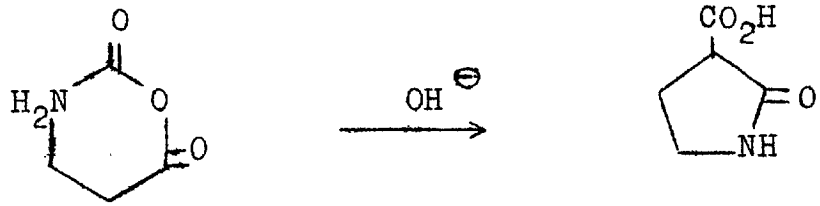
XII R=p-Nitrobenzoyl. XIII

A similar mechanism has been afforded to explain the change in configuration in the erythro-ephedrine series[22].

The rearrangement of acyl groups is not confined to amino-alcohols. As would be expected the lactone, (XIV),readily isomerises to the lactam(XV) on treating with base[23]. The anhydride of glutamic acid(XVI) is converted by base to the lactam (XVII) in a reaction not reversed by acid[24]

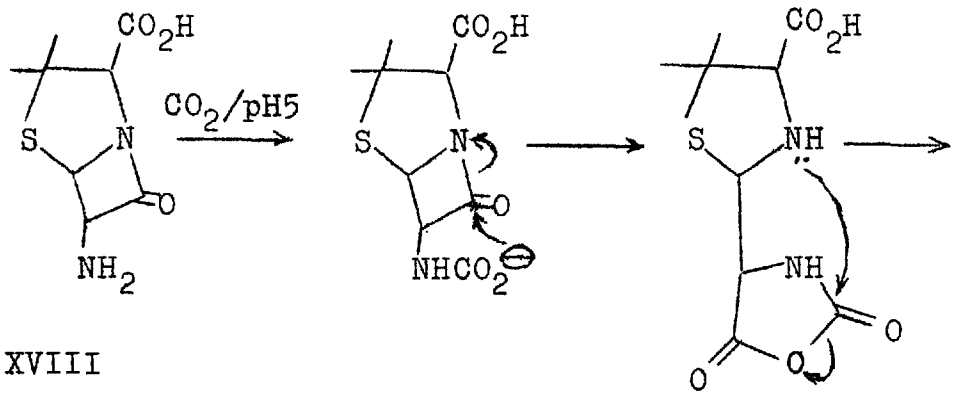


XIV XV

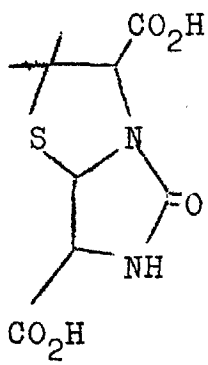


XVI XVII

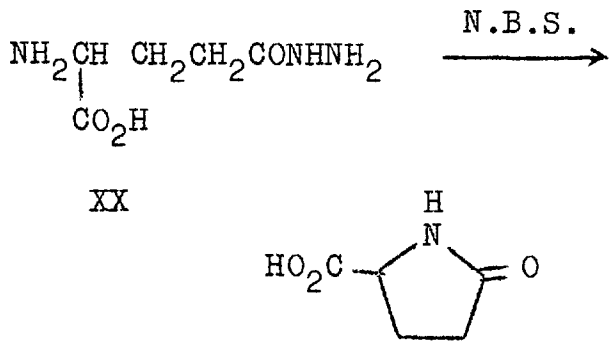
A similar example to the latter is the re-arrangement of 6-aminopenicillanic acid(XVIII) to the ureide(XIX) in the prescence of carbon dioxide. The driving force for this reaction is the opening of the highly strained β-lactam group[25].



XVIII



XLX



XX

XVII

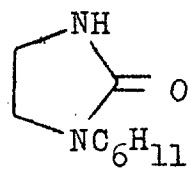
The nitrogen of the amine function can also form an amide bond by internal attack on a carbonyl

group with displacement of another group or fragment. For example the amino-acid, γ-hydrazido-glutamic acid(XX), on oxidation with N-bromo-succinimide, yields the γ-lactam(XVII)[26].

As indicated by the above examples the acyl transfers are not confined to oxygen-nitrogen rearrangements. Several examples of nitrogen to nitrogen acyl shifts have been studied. The observed order and direction of reactivity follows the nucleophilicities of the nitrogen atoms involved, in accord with intermolecular reactions[27]. Stirling found that the N-benzoylethylenediamine hydrobromides(XXI;R=Ph or C_6H_{11}), on liberation of the free base, gave the primary amide(XXII)[28]. The phenyl derivative rearranged rapidly but the cyclohexyl compound was more stable, only rearranging at 150°. The urethane(XXIII) was also found to be stable until heated to 180° when benzyl alcohol was eliminated with formation of the oxo-imidazolidine(XXIV).

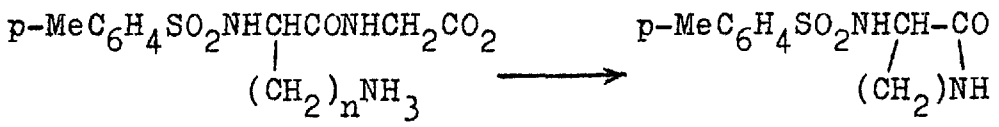
$NH_2CH_2CH_2\underset{\underset{R}{|}}{N}COPh \longrightarrow PhCONHCH_2CH_2NHR$

XXI XXII

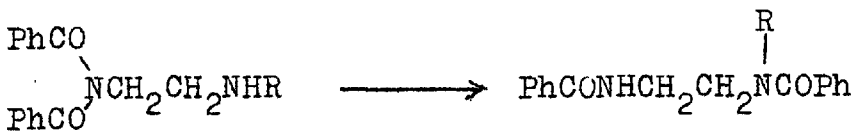
$PhCH_2OCONHCH_2CH_2NHC_6H_{11} \longrightarrow$ 

XXIII XXIV

The possibility of lactam formation via elimination of a molecule of amine was also realised. The γ- alkylaminobutyranilides(XXV;R=$PhCH_2$ or C_6H_{11}) slowly cyclised on heating to give the N-alkyl-pyrrolid-2-ones(XXVI) with elimination of aniline. The benzyl derivative reacted very readily. The latter type of reaction has been observed with peptides containing the diamino-acids, α,γ-di-aminobutyric,ornithine and lysine(XXVII;n=2,3,or 4 respectively). Basification with ammonia produces the lactams(XXVIII)[29]. A low yield was obtained in the case of lysine where a seven membered lactam forms.



XXVII XXVIII

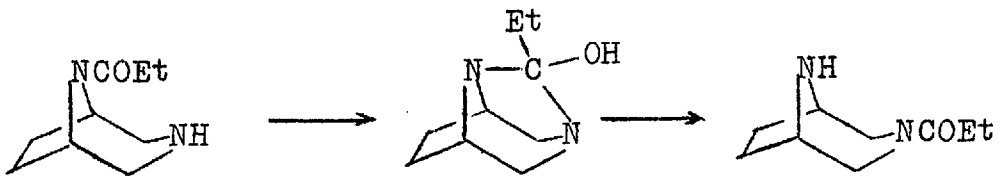


XXIX XXX

Amines attack diacylimides intramolecularly with great ease. The dibenzoylimide hydrobromide(XXIX; R=Ph or C_6H_{11}) rearranges spontaneously on basification to give the N,N'-dibenzoylamide(XXX)[28].

Wieland has made use of diacylimides in a peptide synthesis[30]. Glycylvalylimide(XXXI) gave a mixture of two amides(XXXII) glycylvalylamide preponderating. α-Caprylyl-αγ-diaminobutyric acid rearranges in base to the γ- caprylyl compound. This exchange is reversibly catalysed by dilute acid when the α-amide is favoured[31].

An acid induced irreversible acyl shift occurs with 8-propionyl-3,8-diaza-bicyclo- [3:2:1] - octane(XXXIII), the 3-propionylamide(XXXV) being formed, via the intermediate(XXXIV)[32].



XXXIII XXXIV XXXV

Thiol-esters have long been known to be readily displaced by amines[33]. During an investigation into the reactions of Coenzyme A Wieland and his collaborators studied the properties of thiol esters of the cysteamine moiety[34]. In strong acid the S-acetyl derivative(XXXVI;n=2) was stable. Above pH 5, however, the acetyl group was readily displaced onto the adjacent nitrogen atom. When the carbon chain length was increased the rate of transfer

slowed down eventually reaching the rate characteristic of an intermolecular reaction,(XXXVI;n 5)[35].

$$CH_3COS(CH_2)_nNH_2 \longrightarrow HS(CH_2)_nNHCOCH_3$$

XXXVI

N,N-Dimethylcysteamineacetate, in alkaline solution,hydrolyses at a much faster rate than an alkylthiol ester. This must be due to catalysis of the hydrolysis by the dimethylamino group[34].

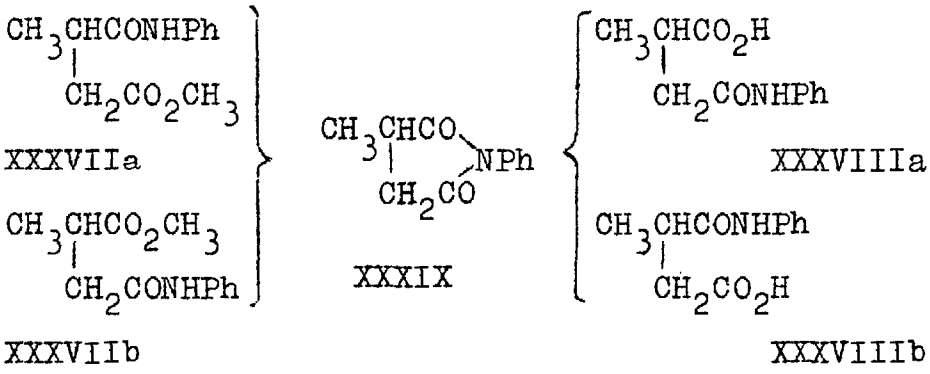
Reinvestigating such examples Stirling also found a very rapid S⟶N shift of acyl groups[36]. Attempts to reverse the S⟶N acylation by dissolving in strong acid leads to hydrolysis. However there is some spectroscopic evidence that the reverse, N⟶S acylation can occur, albeit with difficulty.[37]

Amide participation in transacylations.

Primary and secondary amides can enter into many reactions leading to acyl rearrangements. Extensive studies in the peptide field have only been made since 1950.

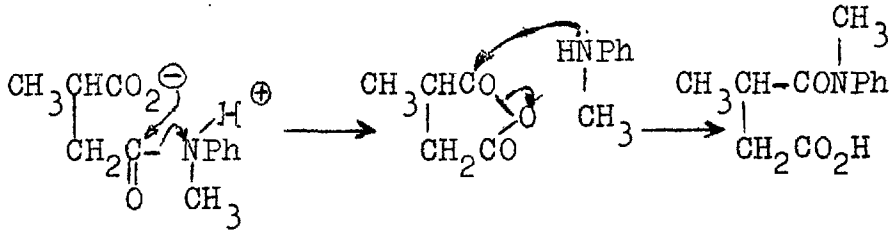
Hancock and Linstead found that the alkaline hydrolysis of the isomeric monomethyl succinic anilides(XXXVIIa,b) afforded a mixture of the isomeric acid anilides(XXXVIIIa,b)[38]. The rearrangement

must proceed through a common intermediate formed during the hydrolysis. This is the imide(XXXIX).



XXXVIIa XXXVIIb XXXIX XXXVIIIa XXXVIIIb

The N-methyl-anilide esters do not rearrange during hydrolysis. However, as had been shown earlier, the N-methyl anilide(XL) rearranges on heating to give small amounts of its isomer(XLI)[39]. In this case the reaction must proceed through the anhydride.



XL XLI

Amido esters of aspartic and glutamic acid behave similarly, for example, N-benzyloxycarbonyl asparagine methyl ester, on mild alkaline hydrolysis, affords N-benzyloxycarbonylasparagine and iso-

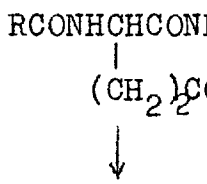
asparagine.[40] In the latter case the intermediate imide was isolated. This is not unexpected since, in 1907, Mouipied and Rule[41] had isolated succinimide from methyl succinamate under similar conditions. The imide from glutamine ester was not isolated from aqueous solution since it is rapidly hydrolysed. When anhydrous conditions were used it could be isolated.

Later work on glutamine and asparagine esters revealed that alkaline hydrolysis gives mainly the γ-glutamide and β-aspartamide derivatives[42]. It was pointed out that during the alkaline hydrolysis of poly- -glutamic ester[43] large amounts of γ-linked material would be formed. This was later proved to be so[44].

In glutamic acid derivatives further reaction can occur. If the α-amino group is acylated it can compete in cyclisation, to the γ-carbonyl group, with the α-carbamoyl group[45]. Thus the derivative (XLII) gives the pyrrolidone(XLIII),rather than the piperidone(XLIV) anticipated, when the acid is treated with thionyl chloride and pyridine.

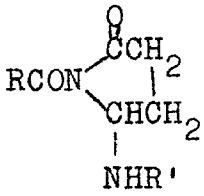
The acyl pyrrolidone(XLIII;R=Ph,R'= $CH_2CONHC_6H_{11}$) can undergo an interesting rearrangement with dilute sodium hydroxide. The amide anion formed

attacks the diacylpyrrolidine function to form the cyclic imide(XLIV) . This can then hydrolyse by

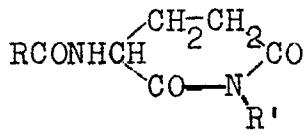


XLII R= $C_7H_7SO_2NHCH_2CONHCH_2$-

R'= $C_6H_{11}NHCOCH(CH_2Ph)$-

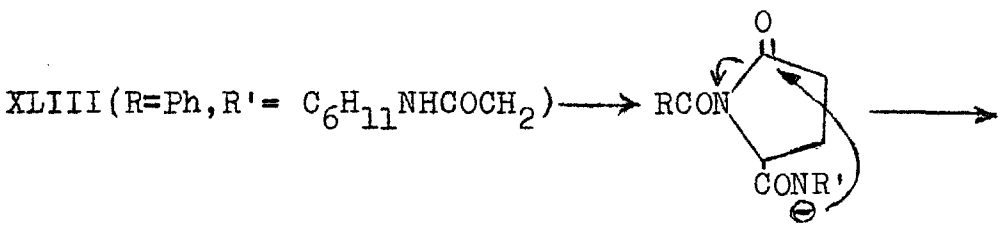


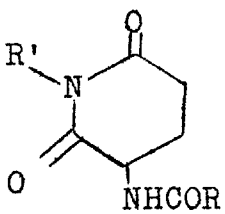
XLIII



XLIV

opening of the ring to give ∝- and (mainly) ɣ- derivatives[46].

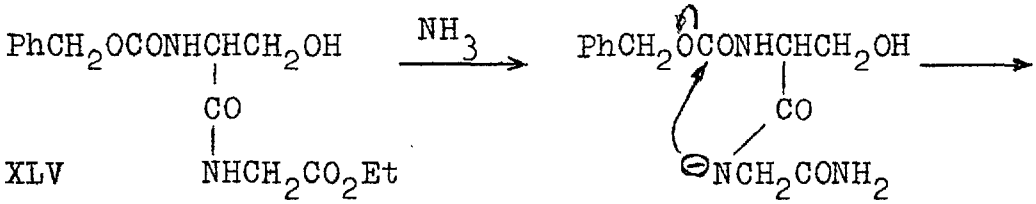




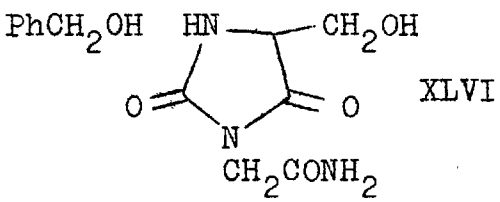
XLIV

Use of urethanes to protect amino groups in peptide chemistry is well known. Nevertheless use of this group can have its disadvantages. When N- benzyl-oxycarbonylamino-acid esters are treated with amines hydantoins can form as side products[47]. For instance

the ester(XLV) on prolonged treatment with ammonia yields the hydantoin(XLVI)[48]. This is produced from the amide originally formed,by attack on the urethane group with loss of benzyl alcohol.



XLV



XLVI

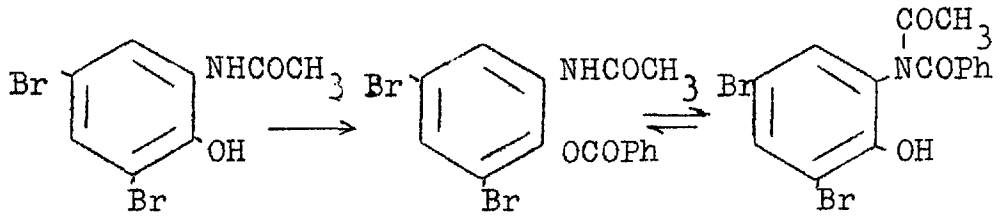
An interesting case of imide formation occurs in the hydrolysis of Bacitracin A. With concentrated hydrochloric acid all of the peptide bonds are opened up except for that of aspartyl-ε-lysine. This is isolated as the imide. Alkaline hydrolysis leads to α- and β-aspartyl-ε-lysine. Bacitracin A with dilute hydrochloric acid is completely hydrolysed.[49]

As yet nothing has been mentioned about the mechanism by which amide groups can attack. Under neutral conditions the amide nitrogen does not, generally, give rise to reactions that lead to rearrangement. In this state the lone pair of

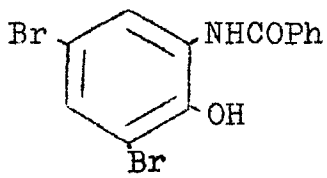
electrons on the nitrogen atom is resonating with the adjacent carbonyl and hence delocalised. The effect of resonance, together with the electronegative character of the nitrogen is sufficient to make the amide hydrogens, in primary and secondary amides, fairly acidic. When a proton is removed by base the nitrogen atom becomes negatively charged and can then attack as a nucleophile. The acidity of the hydrogen atoms on the nitrogen depends on the electronic effects of the substituents and the steric situation of the amide group with respect to surrounding substituents. An example of the effect of neighboring groups is in the aspartyl-seryl series[50]. β-Benzyl esters are rapidly hydrolysed. Again the amide anion is responsible for displacement of the ester group with formation of an intermediate imide. However the presence of the seryl hydroxyl facilitates the abstraction of a proton from the amide to enable the imide formation to occur. The intermediate imide, although itself rapidly hydrolysed, has been isolated. This neighboring group effect is taken up again in a later section(see p.25)

Participation of amides in acyl rearrangements in derivatives of amino-phenols has been thoroughly

investigated, mainly by Raiford[51]. Schotten-Baumann benzoylation on 2-acetamido-4,6-dibromo-phenol(XLVII) gave the N-benzoylated phenol(XLVIII) as product. Presumably the phenol is benzoylated, the product being in equilibrium with the amido-phenol(XLIX) which is rapidly hydrolysed to the benzamide.



XLVII XLIX

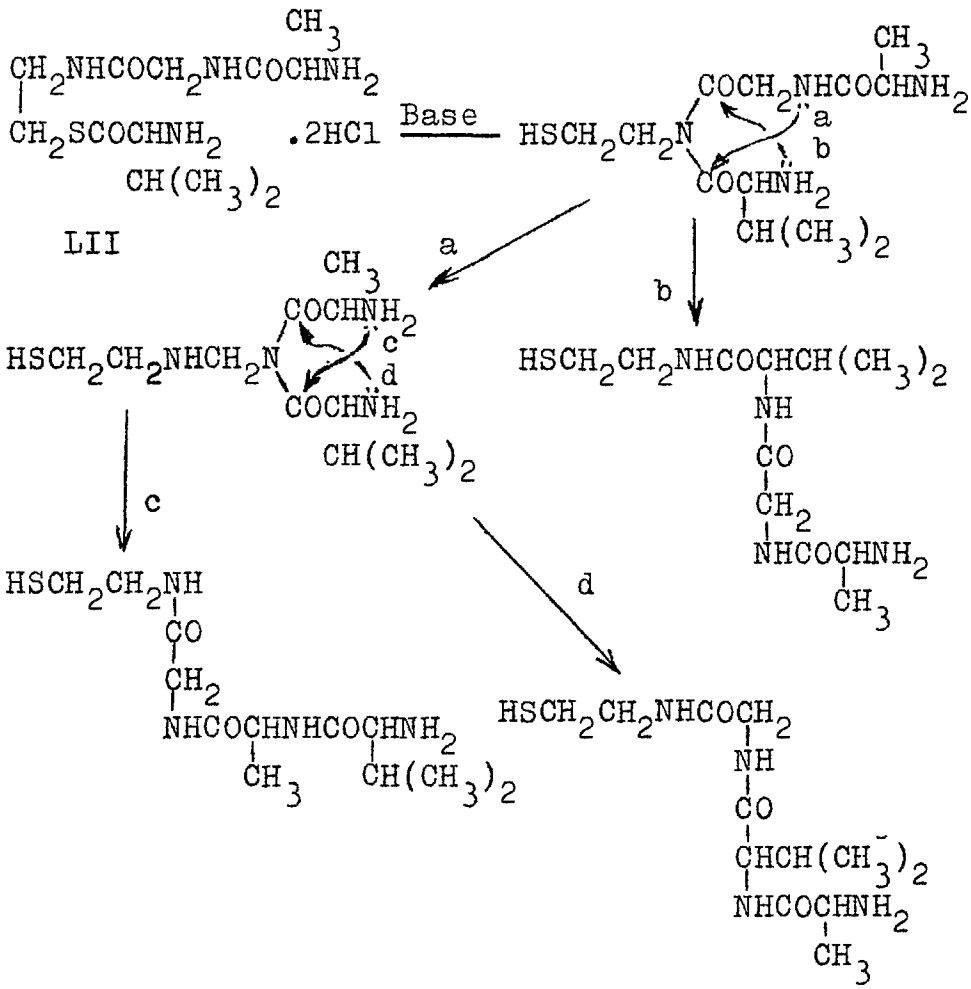


XLVIII

Lankelma and Knauf[52] found a similar behaviour for ortho-amino-thiophenols. Wieland[53] continuing studies on cysteamine made S-amino-acyl derivatives of N-acetylcysteamine.For the valyl ester(L) it was thought the amide would attack to give, in equilibrium, the imido-thiol. The amine of the amino-acyl group can then displace the acetyl group giving rise to a new amide bond(LI). In aqueous sodium bicarbonate solution the hydrochloride,(L) gradually gave a precipitate.

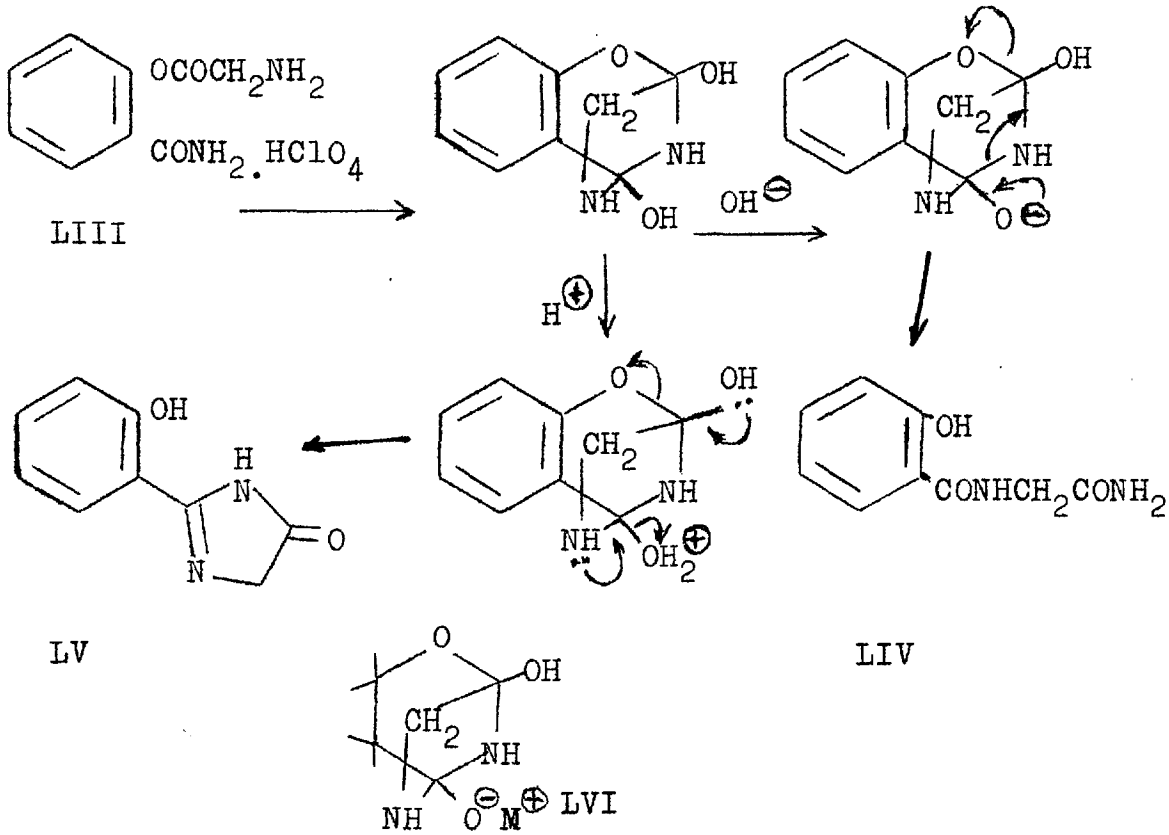
The products isolated were N-acetylcysteamine, N-oligovalylcysteamine and N,S-dioligovalylcysteamine, the term'oligo' representing two or more residues. The expected product, N-acetylvalylcysteamine, was not isolated. Wieland regarded the isolated products as having come from 'internal' and 'external' transacylations. A much more probable explanation is that, in the prescence of bicarbonate, the valyl-N-carboxy anhydride forms(see p.32). That some intramolecular transacylation can occur under the conditions used was proved by using N-acetyl-S-benzoylcysteamine, a mixture of N-acetyl and N-benzoylcysteamine being formed.[54] A series of extended reaction schemes was then envisaged and investigated.[55] Initially S-valyl-N-glycyl cysteamine dihydrochloride as a very dilute solution in aqueous sodium bicarbonate gave a mixture of valylglycyl and glygylvalylcysteamine, glycine, valine and glycylvaline. Finally the investigation was extended to S-valyl-N-alanylglycylcysteamine dihydrochloride(LII). Under similar conditions to that previously used the major product was a mixture of N-tripeptidylcysteamine derivatives. On analysis of the latter fraction no terminal glycyl group was detected. This is as expected from Wieland's

reaction scheme shown below.



O-Aminoacyl derivatives of salicylamide rearrange in base to give amides[56]. O-glycylsalicylamide perchlorate(LIII) in weak base gives salicylglycylamide(LIV) in high yield[57]. In acid the imidazolone (LV) forms. The fundamental requirement for the reaction, in which an amide bond is formed, is a β-hydroxyacid amide derivative. It was thought

that the reaction could be exhibited by hydroxyl or mercapto containing amino-acids such as serine or cysteine. However, for the β-hydroxy butyric acid and seryl derivatives, under the mild basic conditions used to convert (LIII) to (LIV) no reaction occurred. This was because, in the latter cases, free rotation can occur around the carbon-carbon bonds joining the reacting groups and the formation of the -acid skeleton(LVI) is prevented at all but extremely low hydrogen ion concentration. Use of the strong base, potassium t-butoxide did give the required rearrangement.

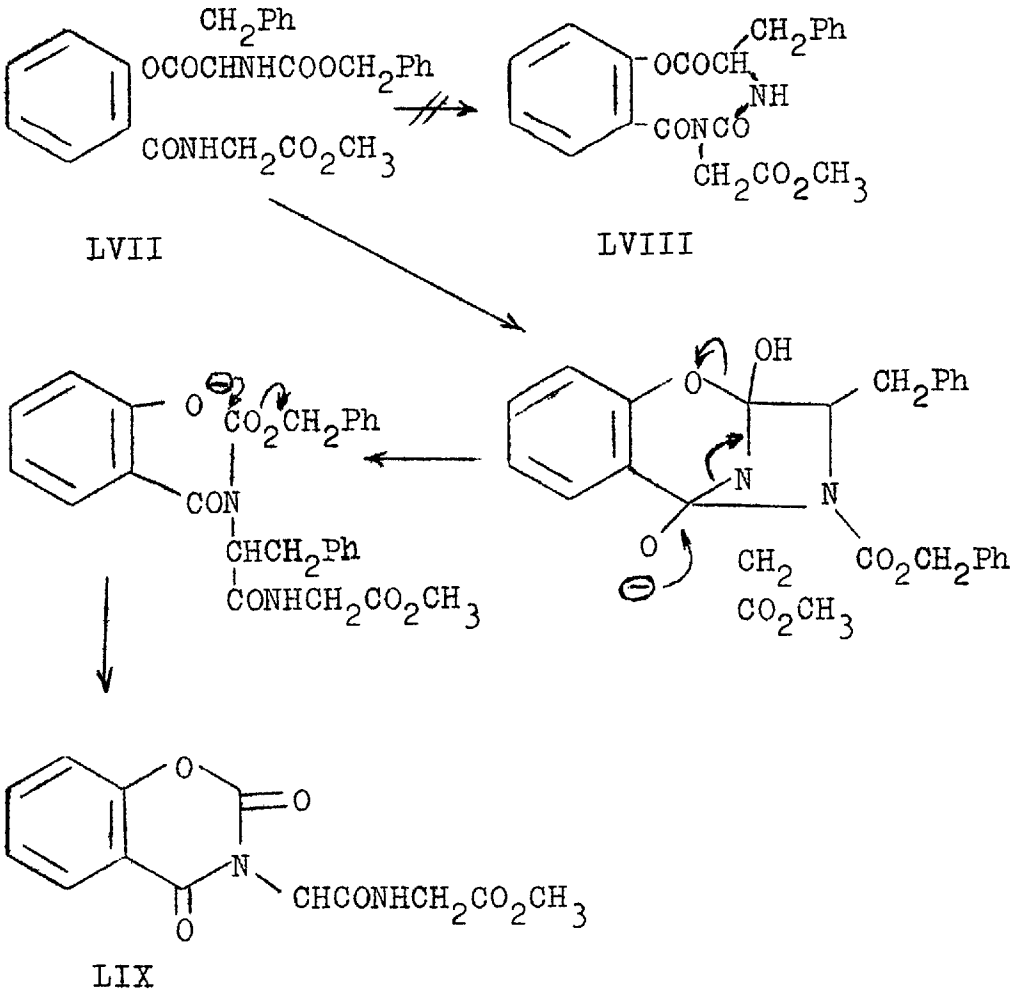


The ease of reaction with the salicyl derivatives is partly due to the benzene nucleus holding the participating groups in a very favourable conformation. An interesting case where the rearrangement goes under very mild alkaline conditions is for N-glycyl-O-(N-benzyloxycarbonyl)-phenylalanylserine. At pH8-8.5 N-(N-benzyloxycarbonyl)-phenylalanylglycylserine forms[58].

The O-L-phenylalanyl derivative of salicylglycyl-methyl ester rearranged with retention of configuration[59].

Brenner postulates the rearrangement as going through the bicyclic intermediate, of the type (LVI), rather than through a diacylimide. When N-benzoyl-N-glycyl-N-methylamine was made no rearrangement apparently occurred, yet O-glycyl-N-methyl-salicylamide did give the rearrangement[60]. O-(N-Benzyloxycarbonyl)-L-phenylalanylsalicyl-glycine methyl ester(LVII) with base gives a product assigned the structure of a urea(LVIII)[61]. This structure requires that the glycyl nitrogen, already known to be fixed near the phenolic ester group, attacks the benzyloxycarbonyl group with displacement of benzyl alcohol and formation of a nine-membered ring. A much more probable structure

for the 'urea' is the amide(LIX) arising by the scheme shown.

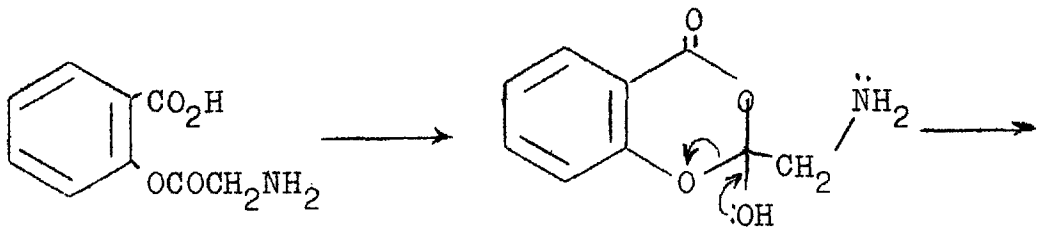


LIX

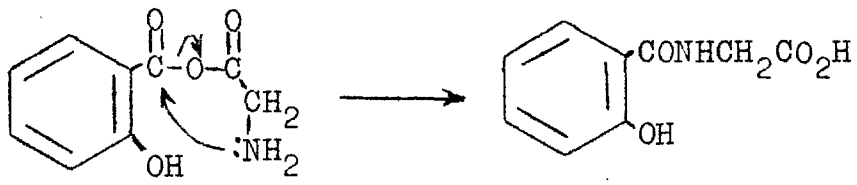
Extending the aminoacyl insertion reaction to amides, Brenner's group found that, in the presence of strong base, phenylalanylglycyl-amide was equilibrated with glycylphenylalanyl-amide - in the same ratio as that obtained from

pure glycylphenylalanylamide.

On attempting to prepare O-glycyl salicylic acid(LX) an acid catalysed rearrangement, analogous to that of the amide, occurred, even under mildly acid conditions such as glacial acetic acid[62]. Presumably the latter rearrangement involves attack by the carboxylic group to give a mixed anhydride intermediate which then rapidly reverts to the amide[63](LXI).



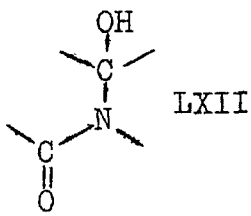
LX



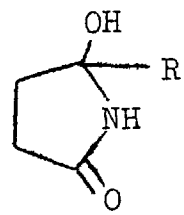
LXI

'Cyclol' formation.

It has been assumed, in the foregoing sections, that amides can attack carbonyl groups to give an intermadiate having a tautomeric structure(LXII). In 1937 Wrinch had outlined a theory in which the amide groups in peptides were assumed to interact to form the tautomeric structures, called 'cyclols'.[64] X-Ray crystallography has since disproved the generality of this hypothesis[65]. However, in certain cases, such an interaction can occur with concommitant cyclol formation. Thus Lukes and Prelog found that the amide of laevulinic acid (LXIII;R=CH_3) would not form a hydrazone derivative[66]. γ-(α-Keto)-glutamine(LXIII;R=CO_2H) could not be easily oxidised with alkaline hydrogen peroxide, a reaction characteristic of α-keto acids[67].



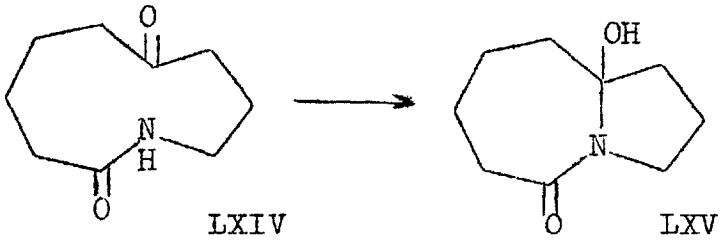
LXII



LXIII

Unambiguous identification of an amide-ketone interaction was first obtained by Cohen and Witkop[68]. Freshly prepared amide(LXIV) rearranged spontaneously in solution to the cyclol(LXV). The reaction was

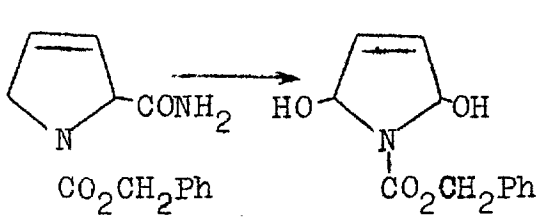
followed by infrared spectroscopy.



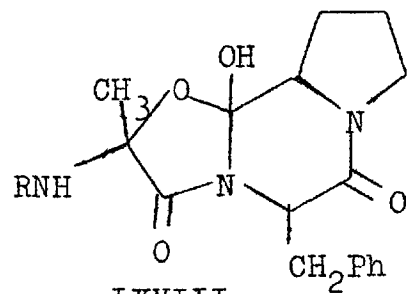
LXIV LXV

When N-benzyloxycarbonyl-Δ^3-dehydroprolylamide(LXVI) was treated with N-bromosuccinimide in base, Hofmann rearrangement and allylic oxidation occorred to yield the interesting compound(LXVII)[69]. The diacetate of the cyclol(LXVII) could be made. Oxidation led to the maleimide derivative. The compound is stabilised by the double bond in the ring.

Amide-ketone interactions have also been recorded in decalin [70] and benzophenone[71] derivatives.



LXVI LXVII

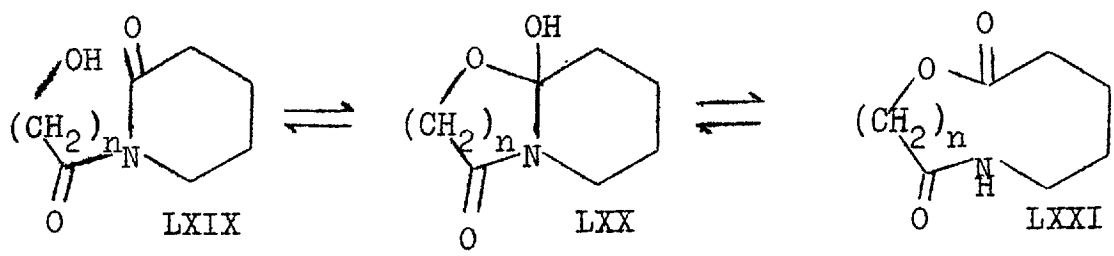


LXVIII

In the peptide side-chain of the ergot alkaloids an amide group interacts with an ester function across a nine membered ring, for instance, ergotamine(LXVIII; R=lysergic acid)[72].

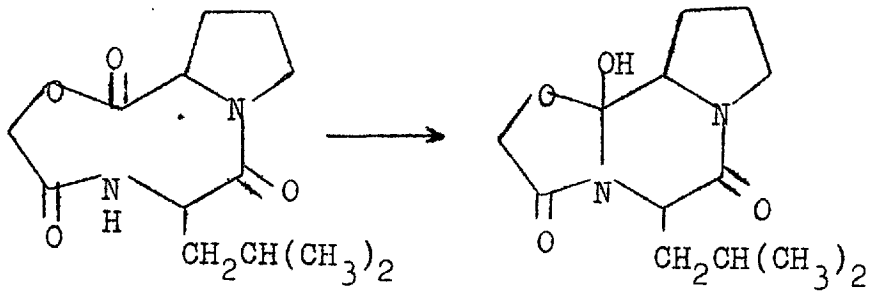
The peptide antibiotic, Bacitracin A, yields on partial hydrolysis phenylalanylisoleucine and phenylalanylhistidine. Since there is only one phenylalanyl residue in the molecule it must be assumed that the isoleucyl amine function is interacting with phenylalanyl- histidine bond to give a cyclol linkage[73].

Some synthetical studies on models of the ergot alkaloid side chain have been made[74]. Glycolyl lactams and glycolyl diketopiperazines were found to be in equilibrium with the cyclol structures. Thus the compound (LXIX;n=1) was in equilibrium with the cyclol(LXX). None of the cyclic ester-amide(LXXI) was observed. However when the homologue(LXLX;n=2) was made[75] it rearranged directly into the ester-amide(LXXI) and no cyclol was isolated. Physical evidence, such as the infrared spectrum, did show that a weak ester-amide interaction was present.

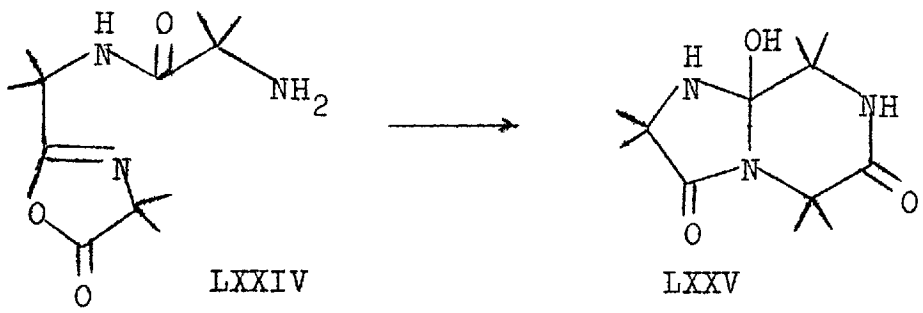


Sheppard found that the related cyclic ester(LXXII) was stable in chloroform but that addition of a trace of acid caused isomerisation to the cyclol (LXXIII)[76].

Cyclo-tri-(α-methyl)-alanyl has recently been prepared from the oxazolone(LXXIV)[77]. The cyclol form(LXXV) predominates because the extra methyl groups, in the α-position of the alanyl moiety, cause considerable steric strain, relieved by collapse to the bicyclic form.



LXXII LXXIII

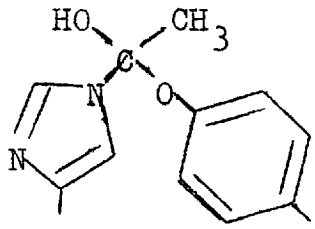


LXXIV LXXV

A cyclol structure has been invoked to explain the behaviour of an interesting cyclic hexapeptide[78].

When L-histidyl-diglycyl-L-tyrosyl-diglycyl was treated with p-nitrophenylacetate, nitrophenol was liberated, as expected, by catalysis due to the imidazole ring of the histidyl moiety. Instead of the imidazole acetyl being rapidly hydrolysed the acetyl group was incorporated into the cyclohexapeptide ring. The explanation advanced suggested that the phenol group of the tyrosyl residue is so placed to be able to stabilise the acetyl-imidazole bond by cyclol formation as in (LXXVI).

LXXVI



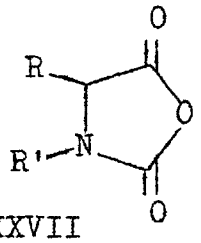
N-Carboxy amino-acid anhydrides in peptide synthesis.

Leuchs discovered N-carboxyglycine anhydride(LXXVII; R,R'=H) in 1906[79]. Since then the corresponding derivative of nearly all the known, naturally occurring amino-avids have been prepared[80]. The major use of 'NCAs' has been in the preparation of synthetic polypeptides[81].

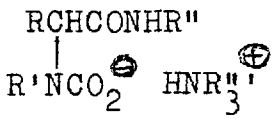
Attempts to use NCAs in a stepwise peptide synthesis have been described. Wesseley, in 1925, made N-phenylglycyl-L-tyrosine ethyl ester from N-phenylglycine NCA and L-tyrosine ethyl ester.[82] The nature of the product from reaction between an amine and an NCA depends on the character of the parent amino-acid and the base used. Highly nucleophilic bases react with NCAs to give, mainly, the substituted amide. When a weak base is used, such as aniline, a competition, for reaction with the anhydride, with the amino-acid amine liberated can occur. The net result in the latter case is polypeptide formation.[83] Alternatively, when the amine function of the NCA is only weakly basic, as in N-phenylglycine, little polymer formation occurs[82].

Bailey has tried to avoid polypeptide formation by reacting NCAs with amines at low temperatures.

Under these conditions ring opening occurs without loss of carbon dioxide, the carbamic acid formed being stabilised as a tertiary amine salt such as (LXXVIII)[84].



LXXVII



LXXVIII

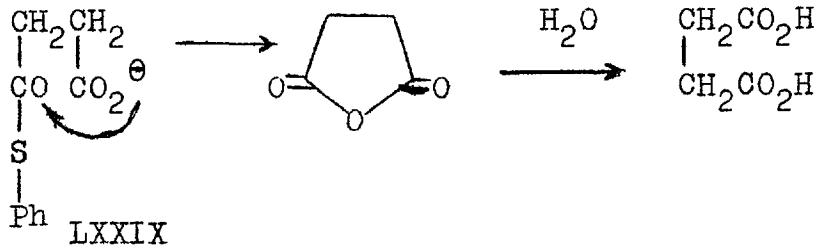
The method has not often been used for peptide synthesis although it shows considerable promise. The advantages of the method were, the abscence of reagent side products, the formation of a product that could be used directly in further synthesis without having to remove a protecting group,and, the claimed, abscence of racemisation. The disadvantages of the process were the possibility of small amounts of polymer formation and contamination of the product by attack at the ureido carbonyl group with formation of a urea[85].

Bailey's method has been used in a preparation of glutathione,using S-benzyl-L-cysteine NCA[86]. Some tripeptides, of use as intermediates in work on oxytocin, have also been made using O-acetyl-tyrosine NCA[87].

The reaction of NCAs with groups other than amines or polymerisation catalysts has been little studied. Bartlett has made a kinetic investigation as to the possibility of using NCAs in a specific peptide synthesis in aqueous solution[88]. In aqueous solution NCAs are hydrolysed and the amine formed rapidly interferes by competitive reaction. Whereas the rate of hydrolysis was found to be too large to allow a specific amide formation an interesting effect was accidentally uncovered. When DL-phenylalanine NCA was reacted with cysteine the thiol concentration rapidly decreased, rising again after a few minutes and eventually reaching its original value. The thiol group had initially reacted with the NCA to give the thioacyl ester. An intramolecular transfer of the phenylalanyl group to the amine then occurred to give the free thiol of the N-acylated cysteine.

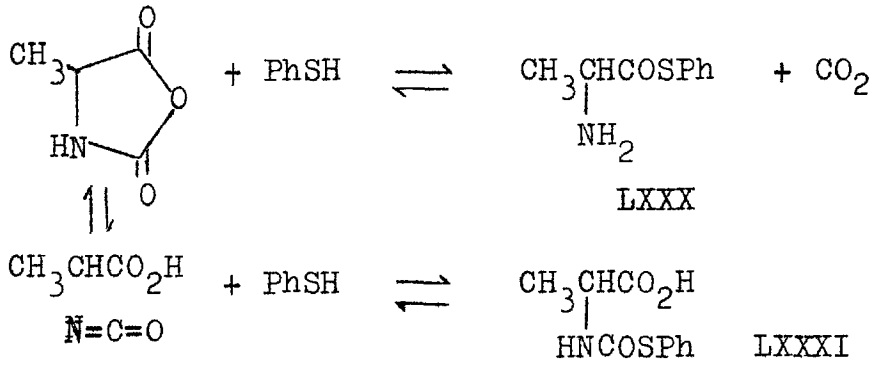
Wieland, in work on cysteamine derivatives, showed that sodium bicarbonate catalyses the hydrolysis of amino-acid thiol esters[89]. The catalysis is due to the formation of the N-carboxy-derivative of the amine. The anion of the carbamic acid then displaces the thiol moiety with formation of an NCA; the NCA is eventually destroyed by

hydrolysis and polymerisation. To check that the effect was real the monothiophenyl ester of succinic acid was made as a model compound(LXXIX). Under the mild basic conditions formerly used, but in the abscence of carbonate, rapid hydrolysis occurred. Succinic anhydride initially formed with liberation of thiophenol[89].



LXXIX

To investigate the reversibility of the reaction thiophenol was reacted with DL-alanine NCA in dioxane[90]. After one hour at 10° mainly the thiophenyl ester(LXXX) was detected. On prolonged standing another compound formed that was presumed to be the phenylthiourethane(LXXXI)

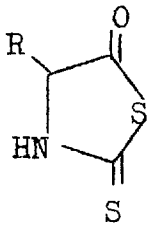


LXXX

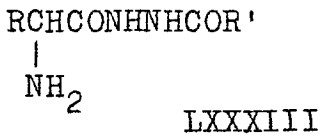
LXXXI

An NCA has been postulated as the intermediate in the reported rearrangement of penicillanic acid to a ureide(see page 8)[25].

Sulphur containing analogues of NCAs have been prepared. The dithio- compounds(LXXXII) were found to react with acid hydrazides in glacial acetic acid[91]. Extending this observation to normal NCAs Brenner found that acid hydrazides are smoothly acylated in glacial acetic acid to give N-acyl-N'-amino-acyl hydrazides(LXXXIII)[92].



LXXXII



LXXXIII

References.

1. Fischer and Fourneau, Ber.,1901,34,2868.

2. Fruton, "Advances in Protein Chemistry",
1949,5,1.(Academic Press,N.Y.)
Elmore, Ann.Reports,1959,56,304 and 1961,58,300.

3. Lipmann,Hülnaus,Hartmann,Boman, and Acs,
J.Cell.Comp.Physiol.,1959,54(Suppl.1),75.
Snellman, Acta Chem.Scand.,1962,16,2194.
Nirenberg, "Scientific American",1963,208,80.
(Scientific American,N.Y.)

4. Gabriel and Heymann, Ber.,1890,23,2493.

5. Gabriel, Ann.,1915,409,326.

6. Bergmann,Brand, and Dreyer, Ber.,1921,54,936.

7. Bergmann,Brand, and Weimmann, Z.physiol.Chem.,
1923,131,1.

8. Fry, J.Org.Chem., 1949,14,887.

9. Phillips and Baltzly, J.A.C.S.,1947,69,200.

10. Meerwein and Sönke, Ber., 1931,64,2375.

11. Immediato and Day, J.Org.Chem.,1940,5,512.

12. Elloitt in "The Chemical Structure of Proteins",
ed. by Wolstenholme and Cameron; Little,Brown,
Boston,1953,p.129.

13. Elliott, J., 1949,589.

14. Desnuelles and Casals, Biochim.Biophys.Acta,
1948,2,64.

15. Schneider, Fed.Proc., 1960,19,334.
and Bailey, Biochem.J.,1953,60.173.
16. Grob and Wagner, Helv.Chim.Acta, 1955,38,1699.
17. Benoitin, J., 1961,763.
18. Levi,Weed,LaFlamme,and Koller, Canad.J.Chem., 1961,39,2491.
19. Fusari,Haskell,Frohardt, and Bartz, J.A.C.S., 1954,76,2881.
20. Winstein and Boschan, J.A.C.S., 1950,72,4669.
21. van Tamelen, J.A.C.S., 1951,73,5773.
22. Welsh, J.A.C.S., 1947,69,128 and 1949,71,3500.
23. Walton and Green, J., 1945,315.
24. Hanby,Waley, and Watson, J., 1950,3239.
25. Johnstone and Hardcastle, J.A.C.S., 1961,83,3534.
26. Wohlman,Gallop,Patchornik,and Berger, J.A.C.S.,1962,84,1889.
27. Smith and Adkins, J.A.C.S., 1938,60,657.
and Arnett,Miller,and Day, J.A.C.S., 1950,72,5635.
28. Stirling, J., 1958,4531.
29. Barrass and Elmore, J., 1957,4830.
30. Wieland and Urbach, Ann., 1958,613,84.
31. Silaev,Katrukha, and Kyz'mina, Zhur.obschei Khim., 1961,31,3111.
32. Cignarella,Testa, and Pasqualucci, Tetrahedron, 1963,19,143.

33. Dalgliesh and Mann, J., 1947,559.
and Schwyzer, Helv.Chim.Acta, 1953,36,414.
34. Wieland and Bokelmann, Ann., 1952,576,20.
35. Wieland and Hornig, Ann., 1956,600,12.
36. Stirling, J., 1958,4524.
37. Martin,Lowey,Elson,and Edsall, J.A.C.S., 1959,81,5089.
38. Hancock and Linstead, J.,1953,3490.
39. Baker,Schaub, and Williams, J.Org.Chem., 1952,17,116.
40. Sondheimer and Holley, J.A.C.S.,1954,76,2467.
41. Mouilpied and Rule, J., 1907,91,176.
42. Battersby and Robinson, J., 1955,259.
43. Bruckner,Kovács,Kovács, and Kótai, Experentia, 1954,10,166.
44. Bruckner,Kajtár,Kovács,Nagy, and Wein, Tetrahedron, 1958,2,211.
45. Clayton,Kenner, and Sheppard, J., 1956,371.
46. Battersby and Robinson, J., 1956,2076.
47. Koenigs and Mylo, Ber., 1908,41,4427.
48. Kienhuis, Ph.D. Thesis, Leiden, 1962, p.16.
49. Swallow and Abraham, Biochem.J.,1958,70,364.
50. Bernhard,Berger,Carter,Katchalski,Sela, and Shalitin, J.A.C.S., 1962,84,2421.
51. Raiford, J.A.C.S., 1919,41,2068.

52. Lankelma and Knauf, J.A.C.S., 1931,53,309.

53. Wieland,Bokelmann,Bauer,Lang, and Lau, Ann.,1953,583,129.

54. Wieland and Mohr, Ann., 1956,599,222.

55. Wieland,Lang, and Liebsch, Ann.,1955,597,227.

56. Brenner,Zimmermann,Wehrmüller,Quitt, and Photaki, Experentia,1955,11,397.

57. Brenner,Wehrmüller,Quitt,Hartmann,Schneider, and Beglinger, Helv.Chim.Acta, 1957,40,1497.

58. Botvinik and Koksharova, Zhur,obschei Khim., 1961,31,2078.

59. Brenner and Zimmermann, Helv.Chim.Acta,1958,41,467.

60. Brenner, J.Cell.Comp.Physiol.,1959,54(Suppl.1),221.

61. Brenner, U.S.pat.2850491(1958); cf.C.A., 1959,53,5152g and also Albertson,Org.Reactions,1962,12,157(p.230).

62. Brenner and Wehrmüller, Helv.Chim.Acta, 1957,40,2374.

63. Dahn,Menassé,Rosenthaler, and Brenner, Helv.Chim.Acta, 1959,42,2249.

64. Wrinch, Proc.Roy.Soc.,1937,A160,59.

65. Springall,"The Structural Chemistry of Proteins", Butterworths,London,1954,chapter 3.

66. Lukes and Prelog, Chem.Listy,1930,24,251.

67. Otami amd Meister, J.Biol.Chem., 1957,274,137.

68. Cohen and Witkop, J.A.C.S., 1955,77,6595.

69. Robertson,Francis, and Witkop,J.A.C.S., 1962,84,1704.

70. Meyer and Schnautz, J.Org.Chem., 1962,27,2011. see also Nagata,Kilkawa, and Fujimoto, Chem.Pharm.Bull.Japan,1963,11,226.

71. Graf,Girod,Schmid, and Stoll, Helv.Chim.Acta, 1959, 42,1085.

72. Glenn, Quart.Rev., 1954,8,192.

73. Wrinch, Nature, 1957,179,536.

74. Shemyakin,Antonov,Shkrob,Sheinker, and Senyavina, Tetrahedron Letters,1962,16,701.

75. Antonov,Shkrob, and Shemyakin, Tetrahedron Letters, 1963,7,439.

76. Sheppard, Experentia, 1963,19,125.

77. Jones,Kenner, and Sheppard, Experentia, 1963,19,126.

78. Kopple and Nitecki,J.A.C.S.,1961,83,4103 see also 1962,84,4457.

79. Leuchs, Ber., 1906,39,857.

80. Katchalski and Sela,"Advances in Protein Chemistry",1958,13,243.(Academic Press,N.Y.)

81. Bamford,Elliott, and Hanby,"Synthetic Polypeptides", Academic Press,N.Y.,1956.

82. Wesseley, Z.pysiol.Chem., 1925,146,72.

83. Sigmund and Wesseley, Z.physiol.Chem., 1926,151,91.

84. Bailey, J., 1950,3461.

85. Ballard and Bamford, J., 1958,355.

86. Rudinger and Sorm, Coll.Czech.Chem.Comm., 1951,16,214.

87. Zaoral and Rudinger, Coll.Czech.Chem.Comm., 1955,20,1183.

Honzl and Rudinger, Coll.Czech.Chem.Comm., 1955,20,1190.

88. Bartlett and Dittmer,J.A.C.S.,1957,79,2159.

89. Wieland,Lambert,Lang, and Schramm, Ann., 1955,597,181.

90. Wieland and Euler, Chem.Ber., 1958,91,2305.

91. Hofmann,Lindenmann,Magee, and Khan, J.A.C.S., 1952,74,470.

92. Brenner and Hofer, Helv,Chim.Acta,1961,44,1798.

THEORETICAL SECTION.

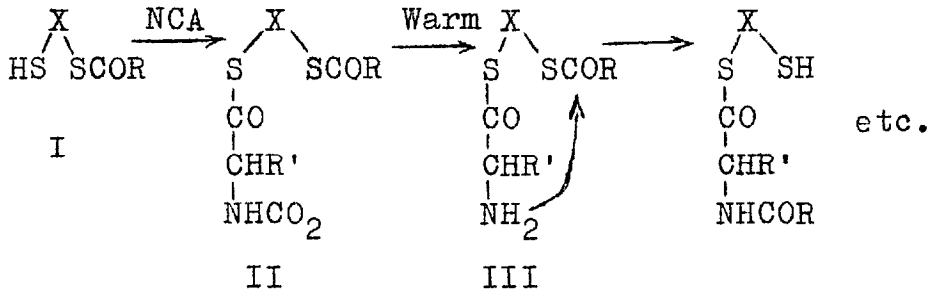
Compounds mentioned have been redesignated with Roman numerals that are referred to in the Experimental Section. 'NCA'refers to N-carboxyamino-acid anhydrides.

Work with thiols.

With the facts outlined in the preceding section in mind the task in hand was to attempt to find a nucleophile that would react specifically, and in high yield, with amino-acid NCAs to give an aminoacyl derivative. The product had to be able to undergo an internal rearrangement with formation of a peptide bond.

At the outset of this work it was considered that a suitable nucleophile would be the mercapto-group. Wieland's work had shown that thiol compounds would react with NCAs.[1] The substrate containing thiol groups that was first considered was an alkaneα,ω-dithiol monoester(I). If the free thiol could react with an NCA under the conditions used by Bailey[2], the product would be the amino-acyl ester in the form of its carbamic acid salt(II). No rearrangement could occur by S $\longrightarrow$ N acyl transfer at this stage because the carbon dioxide would act as a protecting group on the amine function. On warming up to room temperature carbon dioxide would be lost and the free amine could, under suitable steric conditions, attack the adjacent thiol ester group with amide bond formation. The free thiol(III) produced could then

be reacted with another NCA and the process repeated.



As a preliminary, conditions for reaction of thiols with L-∝-alanine NCA were sought. Using benzyl mercaptanno reaction occurred under acidic conditions. Using the conditions Bailey had used for the reaction of amines with NCAs[2], a smooth reaction proceeded. The initial carbamic acid formed was methylated with diazomethane to give N-methoxycarbonyl-L-∝-alanyl-S-benzyl thiolester(IV) in good yield. Attempts to use temperatures above -40°, together with a stream of carbon dioxide to maintain the carbamic acid concentration, failed to give better results. An equivalent of tertiary base was necessary for a smooth reaction.

The thiolester produced was reacted with benzylamine at room temperature when the corresponding amide(V) formed by displacement of benzylthiol.

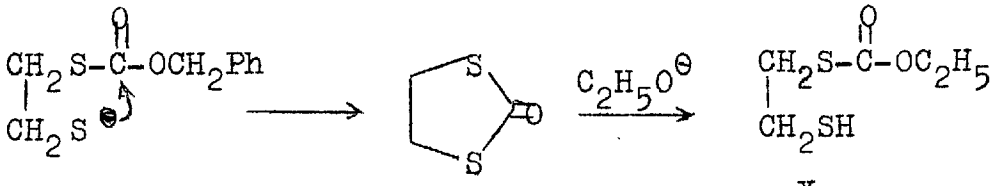
Glycine ester also reacted slowly with the thiol ester to give N-methoxycarbonyl-L-α- alanylglycine ethyl ester(VI).

Extension to a study of the reaction between L-α-alanine NCA and α,ω-dithiolmonoesters was preceded by an attempt to find out whether or not, in the scheme (I) to (III), a suitable amine protecting group could be used. The benzyloxycarbonyl group was chosen for initial study. Benzylthiol and benzylchloroformate were condensed to give O,S-dibenzylmonothiolcarbonate(VII). Reaction of the ester with benzylamine afforded the anticipated N,O-dibenzylurethane with the liberation of benzylthiol.

$$\underset{\text{IV}}{PhCH_2SCO\underset{\displaystyle CH_3}{\underset{|}{C}}HNHCO_2CH_3} \xrightarrow{PhCH_2NH_2} \underset{\text{V}}{PhCH_2NHCO\underset{\displaystyle CH_3}{\underset{|}{C}}HNHCO_2CH_3}$$

The problem of preparing monoacyl derivatives of α,ω-dithiols now presented itself. 1,2-ethane-dithiol was initially chosen for study. Selective monoacylation with benzylchloroformate afforded only the bis-benzyloxycarbonyl derivative(VIII) together with a little ethylenedithiol carbonate(IX). The latter must have formed from the monobenzyl-

oxycarbonate via elimination of benzyl alcohol.



IX X

Since an alcohol will normally displace a thiol under suitable conditions[3] attempts to find conditions to reverse this reaction were sought. When one equivalent of sodium ethoxide was reacted with the carbonate(IX) the ring opened to give mono-ethoxycarbonyl ethane-1,2-dithiol(X). Reaction between the carbonate and benzyl alcohol afforded a mixture.

Since the above reaction was specific for cyclic carbonates and could not be used for any α,ω-dithiols having a longer alkyl chain, a method for preparing α,ω-dithiolmonoacetates(XI) was developed. The corresponding diacetates of a series of α,ω-dithiol alkanes(XII;n=2-6) were prepared by refluxing the mercaptan with acetic anhydride in the prescence of a little anhydrous sodium acetate. Half-hydrolysis, using exactly one equivalent of sodium hydroxide, in aqueous tetra-hydrofuran afforded a mixture of the parent dithiol,

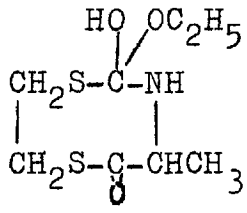
diacetate, and the required monoacetate(XI). Distillation of the mixture was not very successful in purification since, on heating, thermal acylations occur, the net result being the formation of dithiol which was preferentially distilled off. A better method of purification was by chromatographic separation on acid-washed silica-gel. The dithiol moved the fastest, followed by the monoacetate and finally the unchanged diacetate. This order of elution is in accordance with the acidities of the compounds. The mono-acetate fraction was characterised by thiol estimation against standard iodine and by preparation of the corresponding 2,4-dinitro-phenylsulphide derivative,

The next stage was to attempt the reaction between the prepared monoesters and L-α-alanine NCA. Initially the reaction with the monoethoxy-carbonyl ester(X) was tried. Under acid conditions no reaction occurred. With tertiary base[4] a smooth reaction proceeded, which was followed by titration of aliquots against standard iodine. After three hours almost all of the mercaptan had reacted(see graph I, p. 49.). On warming to room temperature carbon dioxide was liberated and

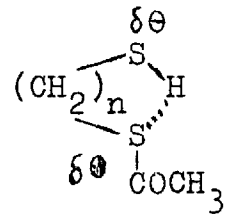
needles of alanine anhydride slowly formed. The latter product must have come from an intermolecular reaction. The result indicates that it is difficult for the amine group to reach the adjacent ester group. Although an eight membered transition state(XIII) is required, molecular models had shown that the prescence of the two sulphur atoms and the two carbonyl groups would decrease transannular interactions thus allowing intramolecular transacylation. No ethoxycarbonylalanine could be found after partial hydrolysis and paper chromatographic examination of the products. This result indicates that the amino group of the amino-acyl residue reacts specifically with the carbonyl group of another amino-acyl group. This is to be expected since the carbonyl group of the amino-acyl residue is slightly activated towards nucleophilic attack by the electronegative α-amino group. Hence on standing the reaction mixture only alanine anhydride forms.

A similar mode of reaction was observed between L-α-alanine NCA and ethylenedithiol monoacetate. However, on using the higher homologues(XI;n=2) the rate of reaction with NCA decreased. For the

pentane and hexane derivatives hardly any reaction with L-α-alanine NCA occurred, large amounts of polymers being formed(see graph I, p.49.). To explain the rapid rate of reaction for the lower members of the series some form of internal catalysis must be invoked. Such a catalysis is not unexpected. 1,2-Diol-monoesters are hydrolysed with intramolecular catalytic assistance.[5] Bruice and Fife have shown that hydrogen bonding to the carbonyl or the ether atom of the ester results in a catalysis of the hydrolysis[6]. The catalytic effect operating in the present instance must be the weakening of the S-H bond by hydrogen bonding, the net result being an increase in negative charge on the sulphur atom of the thiol group(XIV) which can then act as a better nucleophile.



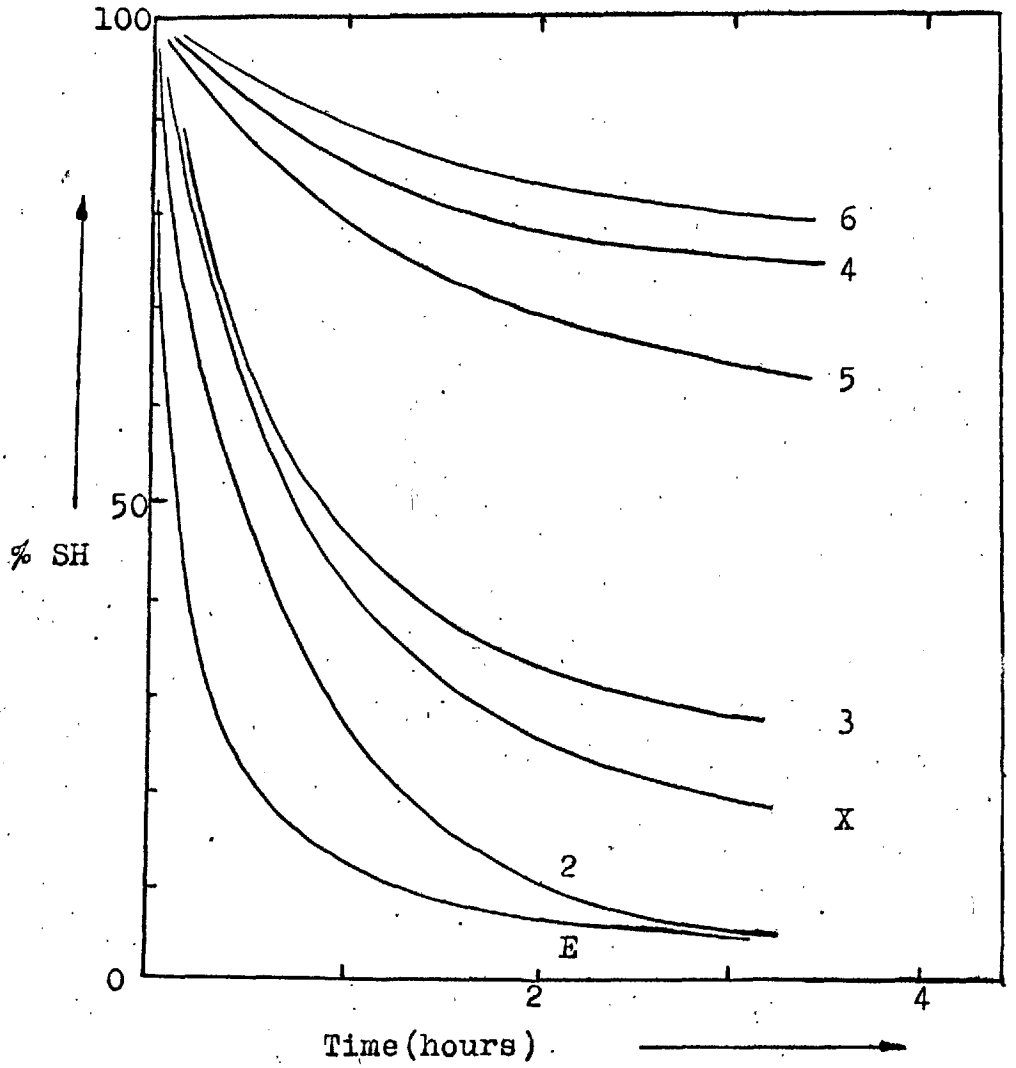
XIII



XIV

The initial choice of benzylthiol as the thiol for model studies had been fortuitous. When iso-propyl thiol was used no reaction occurred.

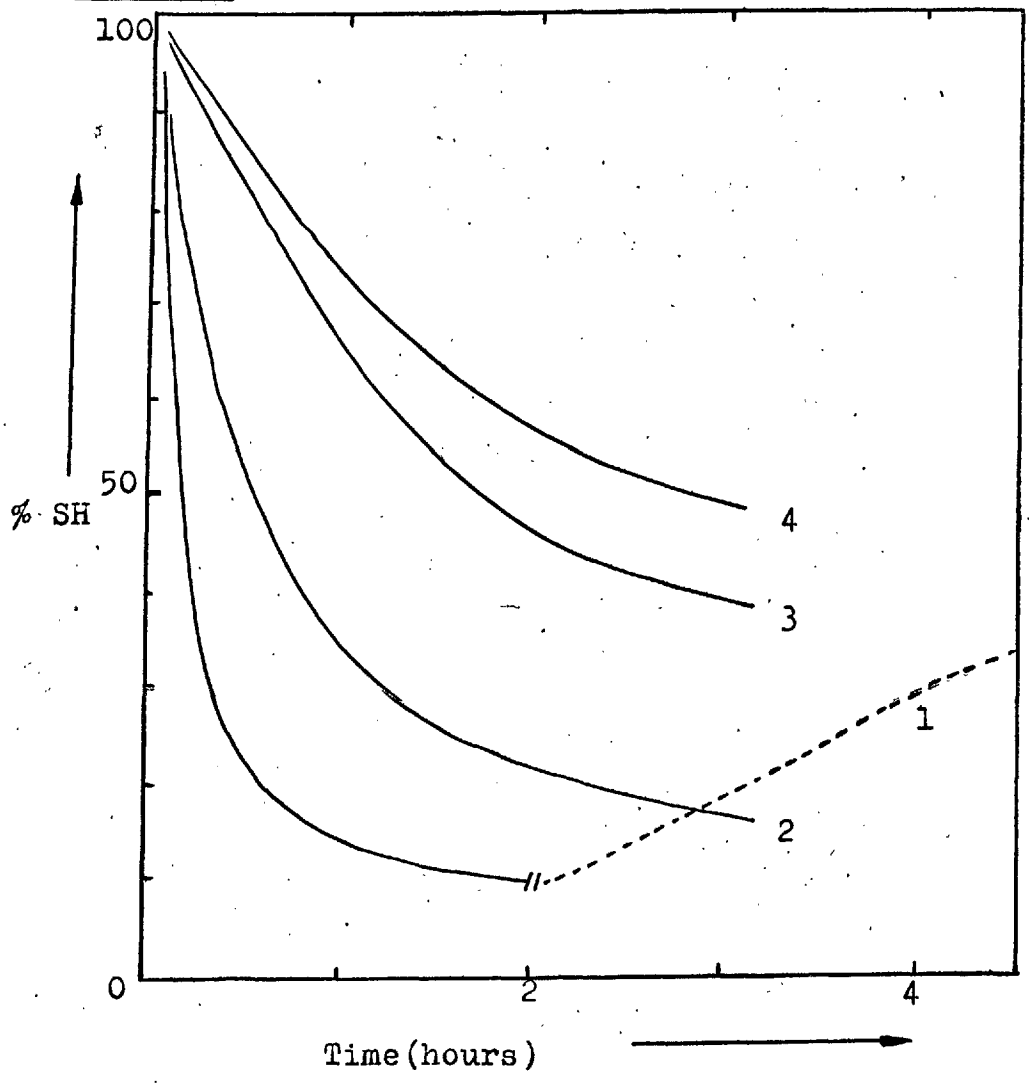
Graph I.



Reaction of L-∝-alanine NCA with dithiolmonoacetates. 0.08N solution in THF; one equivalent of tertiary base at -40°.

2: $HS(CH_2)_nSCOCH_3$, n=2. 3: n=3. 4: n=4. 5: n=5. 6: n=6. X: o-Xylene-∝,∝'-dithiolmonoacetate. E: Monoethoxycarbonylethylene dithiol.

Graph II.



Reaction of L-α-alanine NCA with benzylthiol.

	NCA.	Base.	Temperature.
1:	0.08N	0.08N	-40°
2:	0.04N	0.04N	-20°
3:	0.04N	0.04N	-40°
4:	0.08N	0.02N	-40°

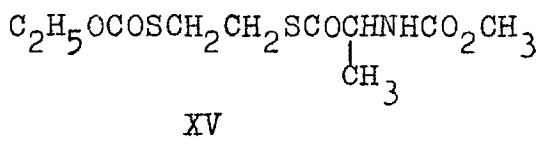
(--- refers to room temperature)

In order to confirm positively that the lower members of the series of esters(XI) had initially reacted with the L-α-alanine NCA used the N-methoxycarbonyl derivative(XV) from reaction with S-monoethoxycarbonylethane-1,2-dithiol was isolated. The amine hydrochloride(XVI) was also prepared by passing a stream of dry hydrogen chloride through the cold reaction product. Basification of the isolated salt, followed by partial hydrolysis, again gave no N-methoxycarbonyl-alanine, in accordance with the result obtained earlier.

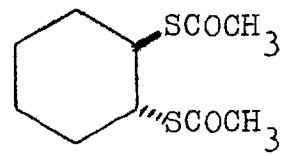
To find out whether intramolecular S⟶N transacylation was not occurring because of free rotation about the carbon-carbon bonds, an attempt to make trans-cyclohexane-1,2-dithiol monoacetate was made. The diacetate(XVII), on attempted half hydrolysis, gave only a mixture of dithiol and diacetate. Presumably catalytic hydrolysis of any monoacetate formed occurs[5,6]. A similar result was observed in the attempted hydrolysis of toluene-3,4-dithiolacetate(XVIII).

Since Einhorn had successfully prepared mono-ethoxycarbonylcatechol from catechol carbonate and ethanol by refluxing,[7] toluene-3,4-dithiol-

carbonate(XIX) was prepared. No monothiolester could be made with ethanol even though many variations of conditions were employed.



XV



XVII

Thiophenol was successfully reacted with L-α-alanine NCA, the product being isolated as its hydrochloride.

One final attempt at finding a suitable substrate containing thiol groups was made. Since benzylthiol had reacted rapidly with the anhydride it was considered that o-xylylene-dithiolmonoacetate(XX) should also react smoothly. Preparation of the dithioldiacetate(XXI) was accomplished directly from o-xylylene-dibromide and potassium thiolacetate. Preparation of the monoacetate was achieved by half hydrolysis. Reaction with L -α-alanine NCA proved to be smooth. However, on working up, no evidence of intramolecular acetyl transfer could be adduced.

Reaction of NCAs with nucleophiles other than amines or thiols.

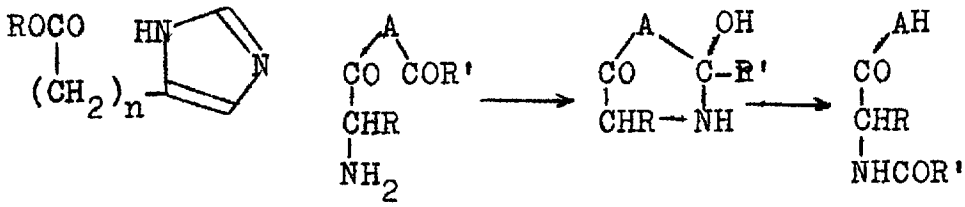
Since it was almost impossible to envisage a useful mercaptan model capable of undergoing the desired reactions the scope of study was widened. A study of the reaction of various nucleophiles with NCAs was initiated. Jencks and Carriuolo have listed the relative nucleophilicity of a variety of reagents to p-nitrophenylacetate[8]. Bender has also given a similar list in a review on reactioms of carboxylic derivatives[9]. The choice of nucleophile, in the present instance, was restricted by the need for reaction to go to completion and that the product, once formed, would be readily attacked by the amino group of an adjacent amino-acyl residue to form an amide and liberate the nucleophile once more. Thus hydroxyl is of no use because the reaction would not go to completion; hydrazines, although excellent nucleophiles, cannot be readily displaced by amines.

Since phenols are more acidic than alcohols, reaction with L-α-alanine NCA was attempted. Polymer formation occurred. The use of mono-ethoxycarbonyl-catechol also gave polymer, no catalysis by the ortho-ester group being observed. Since triethyl-

amine had been used in these reactions an attempt to use a model cotaining the tertiary base internally was made[10]. N,N-Dimethylethanolamine was used but only polymer was obtained.

Catalysis of the reaction with imidazole and triazole was then attempted. Bruice and Pandit have found that imidazole derivatives of the type(XXII) are readily hydrolysed[11]. Since triazole is an excellant nucleophile it was considered that a rapid reaction with NCA would occur. The acyl triazole formed could then react rapidly with a weaker nucleophile. However,under the conditions used, polymer formation took place. A variety of other nucleophiles were also tried with similar results.

On reflection of the initial scheme that had been envisaged for the reactions required, i.e. (XXIII) to (XXIV) it was realised that,for a facile transacylation, the substrate A had to consist of one or two atoms only. The transition



XXII XXIII XXV XXIV

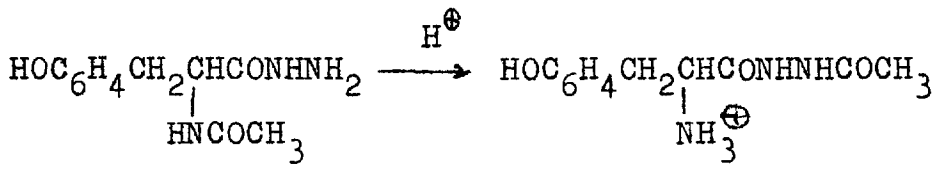
state(XXV) then becomes the favored five or six atoms long. Diacylamino-imides have been prepared and readily[12] rearrange on basification to the amide. The possibility of using N,N'-diacyl-hydrazides(XXVI) then occurred. The electron withdrawing acyl groups are now separated by two nitrogen atoms. Nevertheless some form of inductive activation of the carbonyl groups should be apparent. Attack by amine would be expected to occur displacing one of the acyl groups to form a monoacylhydrazide and an amide. The effect should be accentuated if the amino group and the diacylhydrazine could react intramolecularly. Before attempts to acylate acid hydrazides with NCAs were initiated some preliminary equilibration experiments were attempted. It was considered more important at this stage to find out whether

$$NH_2CHRCONHNHCOR' \rightleftharpoons R'CONHCHRCONHNH_2$$

XXVI XXVII

the postulated rearrangement could occur. N,N'-diacethydrazide was treated with a variety of amines but in every case hardly any amide formation could be detected. At this point of the work Kurtz and

Niemann reported[13] that α-N-acetyl-L-tyrosin-hydrazide(XXVIII) rearranges in aqueous hydrochloric acid to 1-acetyl-2-(L-tyrosyl)-hydrazine(XXIX).



XXVIII XXIX

Hopes of effecting the required rearrangement, (XXVI) to (XXVII), were thus very much diminished. Nevertheless it was decided to try and find out whether conditions for reaction between NCAs and acid hydrazides could be found. Hofmann et al[14] had found that 2-thio-thiazolidones(XXX) reacted with acid hydrazides in glacial acetic acid . Since the thiothiazolidones are sulphur analogues of NCAs an attempt to react alanine NCA with acid hydrazides was made. Before any evidence of the required reaction had accumulated Brenner had published a paper on the same reaction[15]. Using glacial acetic acid as solvent a variety of N,N'-diacylhydrazines were prepared from the corresponding acid hydrazide and NCA. In an extensive investigation conditions for the postulated rearrangement, (XXVI) to (XXVII) had

also been unfolded[16]. These conditions were very mild acid(10% acetic acid in an inert solvent, such as dioxane). In neat acetic acid the reverse rearrangement occurred, in accordance with the result of Kurtz and Niemann[13].

In order to determine the generality of the work of Brenner on the equilibrium, (XXVI) to (XXVII), some intermolecular reactions were tried. In glacial acetic acid N,N'-diacetylhydrazine and benzylamine gave no N-benzylacetamide even after heating at 100° for two days. However on heating in 10% glacial acetic acid in THF overnight a good yield of N-benzylacetamide was obtained.

Work with hydroxamic acids.

Attention was turned to N,O-diacylhydroxylamines in the expectation that reaction with an amine, under the correct conditions, would liberate a hydroxamic acid and form an amide. N,O-Dibenzoylhydroxylamine(XXXI) was prepared and treated with benzylamine at room temperature. A clean reaction occurred with formation of benzohydroxamic acid and N-benzylbenzamide. Very little, if any, Lossen rearrangement occurred under these conditions. With a possible method of circumventing the

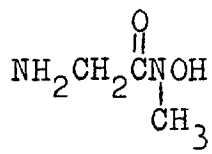
Lossen rearrangement in hand, glycylhydroxamic acid was prepared and acetylated with acetyl chloride. A crude sample of O-acetyl-N-glycylhydroxylamine hydrochloride(XXXII) was obtained but could not be purified. Basification afforded a mixture, the major product being the desired N-acetylglycyl-hydroxamic acid(XXXIII); this was confirmed by paper chromatography, authentic acid(XXXIII) having been prepared from methyl aceturate and hydroxylamine. The observed rearrangement was encouraging. The only comparable reaction described previously[17] involved the potassium salt of lactic hydroxamyl benzoate when Lossen rearrangement occurred.

$$\underset{\text{XXXII}}{CH_3COONHCOCH_2NH_2.HCl} \xrightarrow{\text{Base}} \underset{\text{XXXIII}}{HONHCOCH_2NHCOCH_3}$$

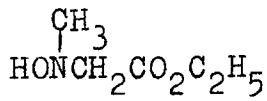
Since N-methylhydroxamic acids should be more stable to base than the unalkylated series several N-methylhydroxamic acids were prepared. It was shown initially that N,O-dianisoyl-N-methyl hydroxylamine(XXXIV) reacted smoothly with benzyl-amine, to give the amide and anisoyl-N-methyl hydroxamic acid, just as the unalkylated di-benzoylhydroxylamine(XXXI) had done.

The reaction of N-methylhydroxylamine with esters

was found to be exceedingly slow compared to that for hydroxylamine. Methyl aceturate had to be left with the alkylhydroxylamine for three days before an appreciable amount of N-aceturyl-N-methylhydroxylamine(XXXV) formed. The product gave a deep red colour with ferric chloride. The benzoyl derivative(XXXVI) was also prepared by using hippuryl chloride and N-methyl-hydroxylamine. Attempts to prepare N-glycyl-N-methyl-hydroxylamine (XXXVII) failed. When methylglycinate was treated with the hydroxylamine only diketopiperazine could be isolated; under similar conditions free hydroxylamine reacts to give the desired hydroxamic acid. Since ammonia reacts with ethyl chloroacetate to give chloroacetamide an attempt to make N- -chloroacetyl-N-methylhydroxylamine was made, the idea being to treat the product with ammonia to give the desired glycine derivative(XXXVII). The product obtained was, however, 2-(N-methyl)-hydroxylaminoacetic ester(XXXVIII).



XXXVII

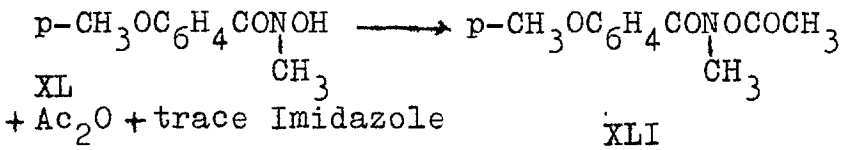


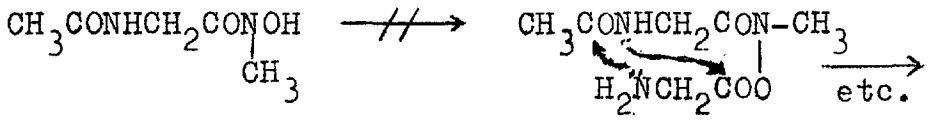
XXXVIII

The next step was to try and react the N-methylhydroxamic acids that had been prepared with glycine NCA. No reaction was obtained. In base only polymer formed; in glacial acetic acid only a very slow carbon dioxide evolution was observed and no clean product was isolated.

Consideration of previously applied arguments led to the view that addition of imidazole to the reaction mixture should catalyse the reaction. Imidazole, being a weak base, should react with glycine NCA in very much the same way that an acid hydrazide does[15]. The N-acylated imidazole formed should be a very powerful acylating agent[18] and should acylate the hydroxamic acid present. To test this hypothesis N-acetyl-imidazole was reacted with N-aceturyl-N-methyl-hydroxylamine(XXXV) in glacial acetic acid. A good yield of the required O-acetyl derivative(XXXIX) was obtained. The strength of the acylating conditions was readily confirmed. Phenol, with N-acetylimidazole, in glacial acetic acid, gave a high yield of phenyl acetate after only ten minutes at room temperature. To prove that imidazole can act catalytically in the acylation N-anisoyl-N-methylhydroxylamine(XL) was treated

with acetic anhydride in glacial acetic acid and in the prescence of a small amount of imidazole. A high yield of the O-acetyl derivative(XLI) was obtained.



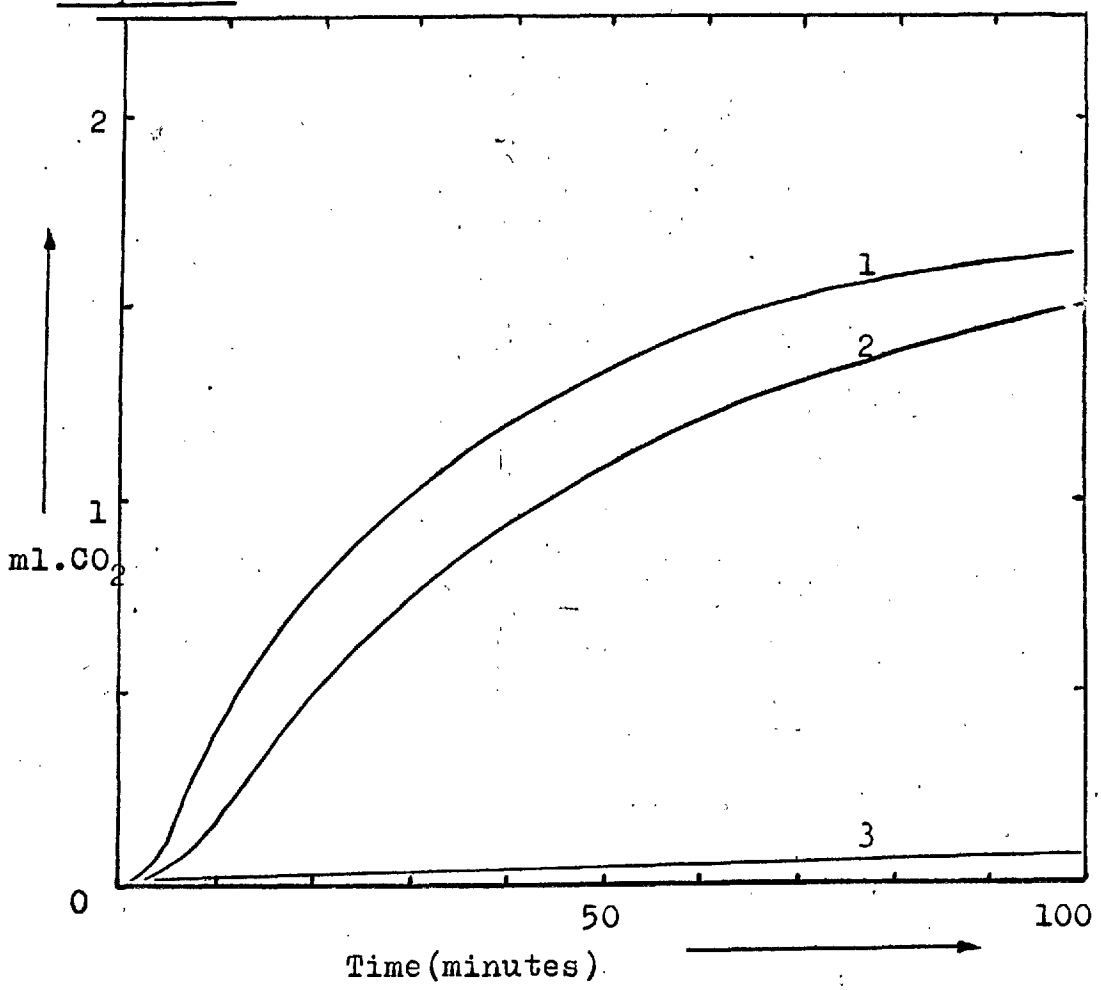


XXXV

On reacting glycine NCA with the aceturyl compound(XXXV) in the prescence of imidazole a rapid carbon dioxide evolution was observed. It was hoped that the reaction would proceed via the O-glycyl derivative to the amide by internal rearrangement on basification. On working up no rearranged product could be found, the starting hydroxamic acid being recovered.

A series of reactions following the decomposition of glycine NCA in glacial acetic acid by a volumetric method were then carried out. The results are summarised graphically(see graph III, p.62). N-Anisoyl-N-methylhydroxylamine(XL) and imidazole were used. Imidazole catalyses the

Graph III.



Reaction between glycine NCA and Anisoyl-N-methyl-hydroxamic acid.

Temperature: 27°; pressure: 762 mm Hg.

1: 1equivalent NCA, 1 equivalent hydroxamic acid, and 1 equivalent of imidazole.

2: 1 equivalent NCA, 1 equivalent imidazole.

3: 1 equivalent NCA, 1 equivalent hydroxamic acid.

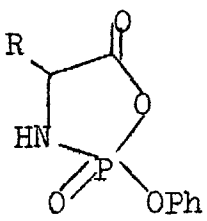
Solution 0.066N in glacial acetic acid.

decomposition but the rate of decomposition is increased in the prescence of hydroxamic acid These results indicate that the released amino groups, from the glycine NCA after loss of carbon dioxide, are successfully competing with the hydroxamic acid for the remaining NCA or its imidazole derivative with the formation of polymers. Weygand and Steglich have demonstrated that amino groups can act as nucleophiles in glacial acetic acid[19].

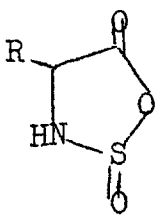
Attempted inner anhydride formation.

Little work has been done on amino-acid inner anhydrides. N-Carboxyanhydrides and the mono and dithio analogues[2,20] have been made and also a series of inner anhydrides(XLII) with phosphoric acid[21].

Some exploratory efforts to make further examples were made. Michaelis[22] showed that thionyl chloride behaved similarly to phosgene in some of its reactions. With glycine no anhydride(XLIII)

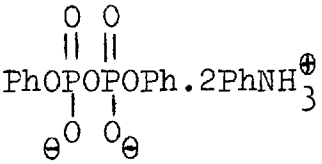


XLII

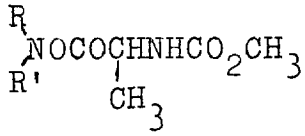


XLIII

formed. When ethyl glycinate was used an unstable product formed that decomposed at room temperature, with elimination of sulphur dioxide. Use of phosphorus oxychlorides gave unexpected results. Cramer and Winter[23] have shown that dimethyl-formamide catalyses reactions of phosphorus halides. When phenyl phosphorodichloridate was tretaed with glycine and dimethylformamide a reaction occurred and the glycine went into solution. Attempts to isolate pure products were void. Addition of amines afforded crystalline derivatives, proved to be the amine salt of the corresponding pyrophosphate. Thus aniline gave the salt(XLIV), Use of phenyldichlorophosphoric oxide gave a similar result. Presumably the amino-acid reacts with the dimethyl-formamide adduct of the phosphorus compound to give a mixed anhydride which then breaks down to polypeptide and pyrophosphate. The mechanism of this reaction was not investigated and attempts to make cyclic anhydrides of amino-acids were discontinued.



XLIV



XLV

Use of N,N-dialkylhydroxylamines

N,N-Dialkylhydroxylamines are good nucleophiles, especially to carbonyl groups[8], and were therefore invetigated. Reaction of N,N-dimethylhydroxylamine with L-α-alanine NCA at low temperature, in the prescence of triethylamine, and isolation of the product by methylation with diazomethane afforded the required ester(XLV;R,R'=CH_3) in low yield. When N-hydroxy piperidine was used a better yield of ester(XLV;R,R'= $(CH_2)_5$) was obtained.

It was next necessary to determine whether O-acyl-dialkylhydroxylamines could react with amines with formation of amides. Gambarian had found that O-benzoyl-N-benzylhydroxylamine(XLVI) reacted with benzylamine to give the amide and the hydroxylamine[24]. Carpino found that O-benzoylhydroxylamine rearranged on standing to give benzohydroxamic acid, an O ⟶ N transacylation.

With the acetyl derivative of N-hydroxypiperidine(XLVII), benzylamine,glycine ester, and aniline reacted at room temperature to give the correspomding amide. When the benzoyl ester(XLVIII) was used the reaction was much slower and with aniline little reaction occurred. The rate of reaction was followed by the rate of disappearance

of the hydroxylamine ester band in the infrared spectrum.

Since dialkylhydroxylamines appeared to have the properties being sought, namely, good nucleophilic powers and displacement by amines from esters, a suitable method of preparation had to be found. Mainly oxidation of the corresponding secondary amine has been used. Gambarian used diacylperoxides[26] and lately oxidation of the acrylonitrile adduct of the secondary amine with perphthalic acid has been used[27].

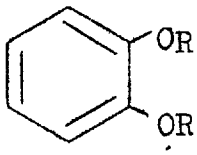
In the present case oxidation was not convenient for preparation of the desired hydroxylamines. Utzinger and Regenass have alkylated momoalkylhydroxylamines with alkyl halides to give the corresponding N,N'-dialkyl compounds[28].

Alkylation of N-methylhydroxylamine with n-hexyl-tosylate was attempted and gave a good yield of the N-hexyl-N-methylhydroxylamine(XLIX). Cyclohexyl-tosylate did not react under similar conditions.

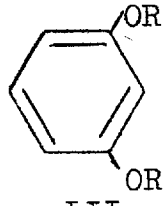
The preparation of bis-hydroxylamines was then attempted. Molecular models indicated that the compounds (L) to (LII), as their monoacyl-monoaminoacyl derivatives could possibly undergo intra-

molecular O⟶N transacylation. The aryl ring holds the two alkyl substituents in a configuration suitable for the necessary transfer to occur.

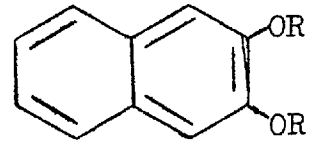
The general scheme of preparation was simple. The parent, aromatic diol was alkylated with ethyl chloroacetate to the ester(LIII). Reduction with lithium aluminium hydride, followed by tosylation afforded the ditosylate(LIV). Reaction with N-methylhydroxylamine gave the bis-hydroxylamine.



L



LII



LI

R=$CH_2CH_2\underset{\displaystyle CH_3}{\overset{\displaystyle |}{N}}OH$

Route:

$$R'OH \longrightarrow \underset{\text{LIII}}{R'OCH_2CO_2C_2H_5} \longrightarrow \underset{\text{LV}}{R'OCH_2CH_2OH} \longrightarrow$$

$$\underset{\text{LIV}}{R'OCH_2CH_2OTos} \longrightarrow R'OCH_2CH_2\underset{\displaystyle CH_3}{\overset{\displaystyle |}{N}}OH \qquad R' = \text{Arylresidue.}$$

The ortho-diphenols reacted with ethyl chloroformate to give large amounts of the cyclic esters(LVI) as side product. When the ditosylate(LIV)

from the catechol series was reacted with N-methyl-hydroxylamine in pyridine reaction with benzoyl chloride gave, not the expected bis-hydroxyl-amine benzoate, but the N,O-dibenzoyl-N-methyl-hydroxylamine(LVII). Presumably the tosylate had been quaternised by the pyridine before reaction with the N-methylhydroxylamine. Use of triethylamine in THF was therefore employed when the required bis-hydroxylamine(L) was obtained.

Although the bis-hydroxylamines are stable solids acylation with acetic anhydride or benzoyl chloride yielded the corresponding esters a viscous oils. Chromatography through silica gel or alumina did not give the analytically pure esters. Attempted vacuum distillation gave decomposition. Only one ester, the dibenzoate of the naphthalene derivative(LI), could be induced to crystallise, the solid having low melting point(48^{o}). Attempts to specifically hydrolyse the dibenzoate to the monoester gave rise to complex mixtures that vould not be separated. Attempted half-aminolysis met with the same difficulty.

Amido-hydroxylamines.

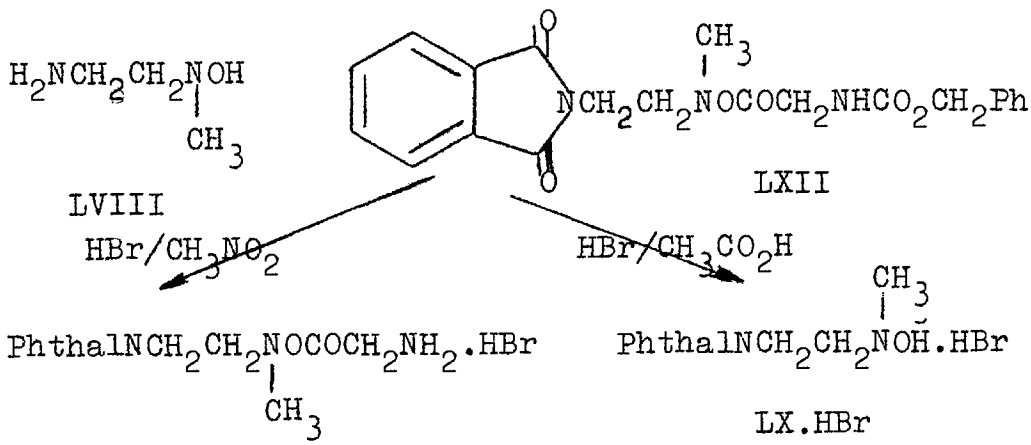
Since Wieland[29] and Brenner[30] had found that amides could react with esters of amino-acids, resulting in rearrangement and incorporation of the amino-acid into a peptide chain, the possibility of using hydroxylamino-amides was studied.

To begin with the synthesis of 2-aminoethyl-methylhydroxylamine(LVIII) was attempted.

Ethanolamine was condensed with phthalic anhydride and the alcohol then tosylated. Reaction of the tosylate(LIX) with N-methylhydroxylamine in dimethylformamide gave the required 2-phthalimido-ethyl hydroxylamine(LX). Attempts to effect the latter step in triethylamine and tetrahydrofuran failed.

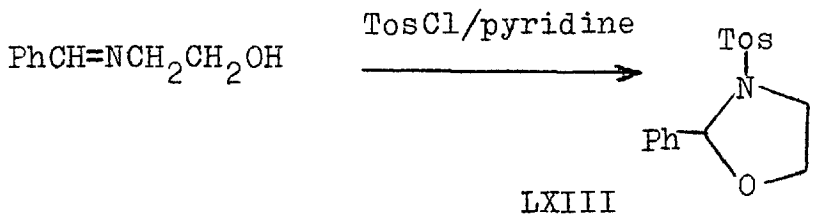
The protected hydroxylamine(LX) proved to be a useful intermediate. Reaction with glycine NCA was tried. On methylating the product with diazomethane the N-methoxycarbonyl-glycylester(LXI) formed in good yield. The hydroxylamine was also coupled with benzyloxycarbonylglycine using dicyclohexylcarbodiimide as reagent. The corresponding ester(LXII) was isolated. The latter ester was made to find out whether benzyloxycarbonyl

groups could be selectively removed without affecting the hydroxylamino-ester function. Treatment with anhydrous hydrogen bromide in glacial acetic acid[31] yielded, unexpectedly, only the free hydroxylamine(LX) as its hydrobromide salt. Use of anhydrous hydrogen bromide in nitromethane as solvent[32] prevented this hydrolysis and the ester was obtained as the amine hydrobromide.



Liberation of the aminoethylhydroxylamine(LVIII) from the phthalimido derivative was achieved by use of hydrazine hydrate. The hydroxylamine was isolated as its bis-hydrochloride salt. In an attempt to devise an alternative route to the aminoethylhydroxylamine(LVIII) it was considered of interest to ascertain whether the benzylidene group could be used as a protecting agent for the

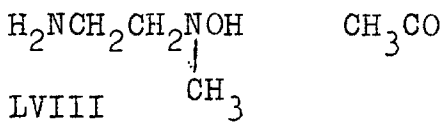
amino group of ethanolamine during tosylation. Benzylidene-ethanolamine is kmown to exist as the free alcohol and not as the cyclic form. During reaction with tosyl chloride in pyridine a facile ring closure occurred. The nitrogen of the Schiff's base had initially reacted and was followed by addition of the alcohol group to the aldehydic carbon to form the N-tosyl oxazolidine(LXIII). Speculation that this reaction might be of significance in peptide chemistry has been confirmed by the report that arylidene derivatives of amino-acids are cyclised under the influence of acylating agents to N-acyloxazolidones[33].



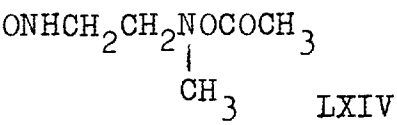
LXIII

The bis-hydrochloride(LVIII) was converted to the N,O-diacetyl derivative(LXIV) by acetic anhydride in aqueous sodium bicarbonate. Removal of the ester acetyl group was achieved by reaction at room temperature with benzylamine. The acetamido-ethylhydroxylamine so formed was isolated as its acid oxalate(LXV).

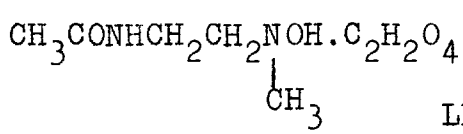
Although successful up to the present stage the formation of an amino-acyl ester had to be attempted. Coupling of N-benzyloxycarbonylglycine to the hydroxylamine gave a viscous oil, similar in properties to the esters that had been prepared from bishydroxylamines. The product could not be obtained in an analytically pur state. Attempts to remove the benzyloxycarbonyl group from the crude ester by use of hydrogen bromide in nitromethane failed to give a crystalline product.



LVIII



LXIV

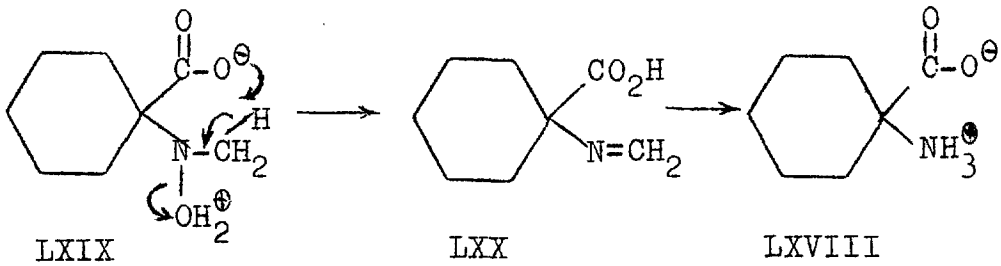


LXV

Reluctantly new model systems were considered. α-Hydroxylamino-nitriles have been prepared by modified Strecker syntheses[34]. The use of N-methylhydroxylamine was found to give the α- N-methylhydroxylamino-nitrile. Thus on treating cyclohexanone with sodium cyanide and N-methylhydroxylamine hydrochloride a good yield of α-(N-methyl) hydroxylamino-nitrile(LXVI) was obtained. The

N-methyl nitrone is initially formed followed by hydrogen cyanide addition. Attempts to hydrolyse the nitrile group with alkaline hydrogen peroxide[35] gave mixtures, including oxidation products. After trying many reagents the desired amide(LXVII) was obtained by dissolving the nitrile in concentrated sulphuric acid and leaving at room temperature overnight.

Hydrolysis of the amide with hot hydrochloric acid gave a mixture of products. After ion exchange chromatography two compounds were isolated. The first proved to be 1-aminocyclohexylcarboxylic acid(LXVIII) and the second impure 1-(N-methyl)-hydroxylaminocyclohexylcarboxylic acid(LXIX). The amino-acid is probably derived from the hydroxylamine by loss of water and hydrolysis of the Schiff's base(LXX) formed.

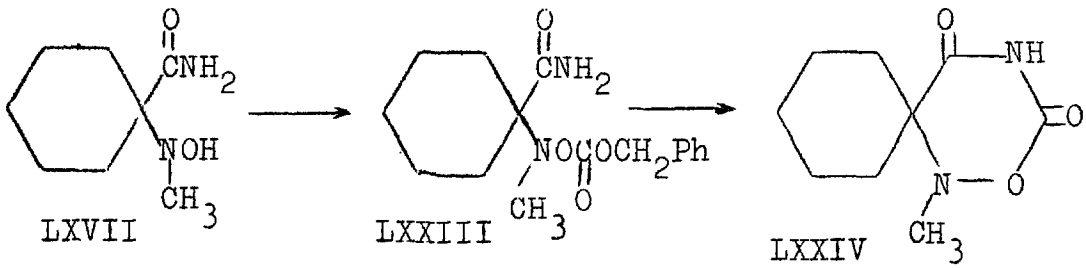


LXIX LXX LXVIII

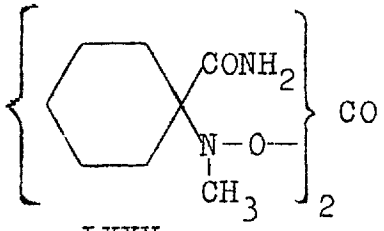
The hydroxylamino-acid could not be obtained pure. On paper chromatography a ninhydrin positive

streak. A similar behaviour has been observed for unsubstituted α-hydroxylamino-acids[36].

Several preliminary experiments were carried out with the amide(LXVII) in order to determine whether the amide group could react with the carbonyl group of the adjacent hydroxylamine ester derivatives. The acetate(LXXII) was made and the infrared spectrum compared to that of the acetate obtained from the corresponding nitrile(LXXI). No difference in the carbonyl absorption frequencies was observed, indicating no large amide-ester interaction. The benzyloxycarbonyl ester(LXXIII) of the amide was next prepared using benzyl chloroformate. Treatment of this ester with triethylamine gave no change, but when a stronger base, potassium t-butoxide, was used, a new compound was isolated. Microanalysis and the infrared spectrum confirmed that this was the cyclic ester(LXXIV) formed by attack of the amide anion on the carbonate group with loss of benzyl alcohol. This reaction is similar to that caused



by base on ethoxycarbonyl salcylamide[37], when ethanol is lost and a cyclic imide formed.



LXXV

An attempt to prepare the spiro-compound directly, by the action of phosgene on the amide (LXVII), gave instead the bis-amido carbonate(LXXV).

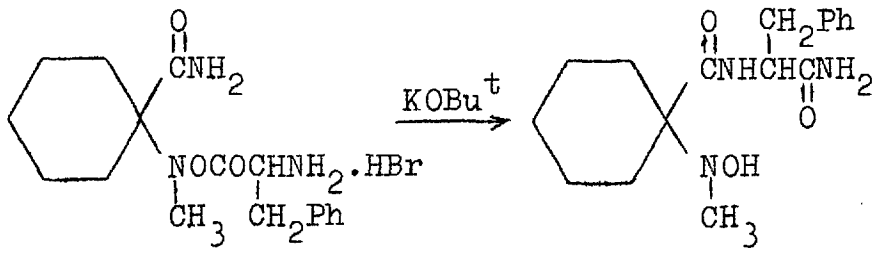
Coupling of the amide(LXVII) with benzyloxy-carbonylglycine by means of dicyclohexylcarbo-diimide yielded a crystalline ester(LXXVI). The action of hydrogen bromide in nitromethane gave an oil that could not be purified. Treatment with base yielded a mixture of products, as indicated by thin-film chromatography on silica gel and using neutral silver nitrate as developer.

Instead of trying to separate the mixture, the ester from the amide(LXVII) and benzyloxy-carbonylphenylalanine was made in the hope that this would give a crystalline hydrobromide instead of an oil as was obtained with the glycine derivative. This was found to be so, a crystalline hydrobromide salt(LXXVII) being obtained when the ester(LXXVIII) was treated with hydrogen bromide in nitromethane.

On treatment of the hydrobromide(LXXVII) with

triethylamine in anhydrous tetrahydrofuran for two weeks, the time taken for the ester function to disappear from the infrared spectrum, only crude DL-phenylalanine anhydride and starting amide(LXVII) were obtained. Use of potassium t-butoxide on the hydrobromide, however, rapidly afforded a mixture of products, the major one crystallising out during work-up.

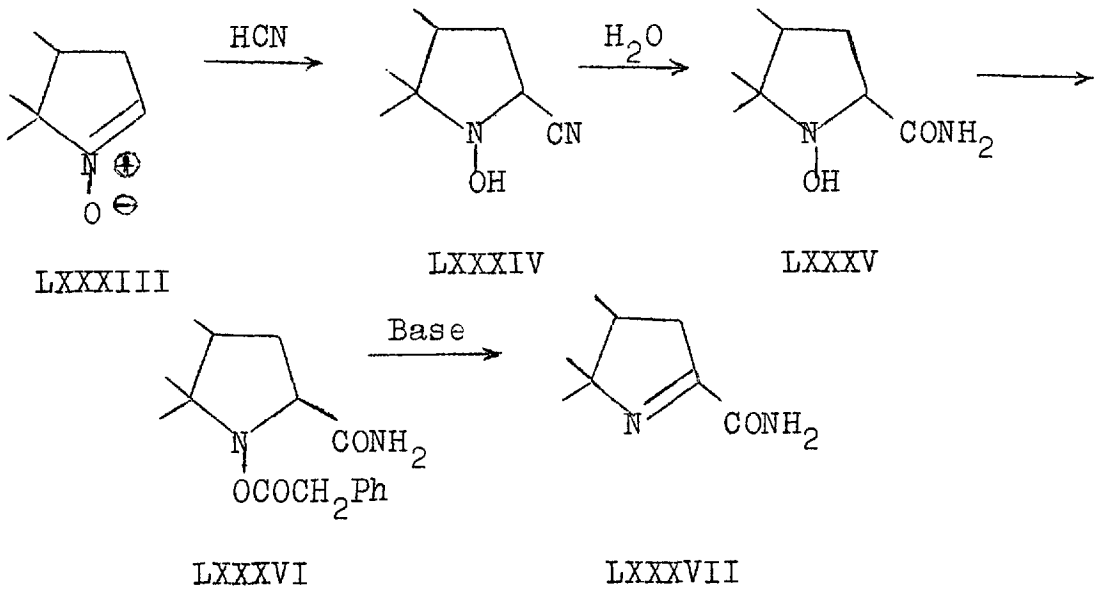
The isolated compound gave a correct micro-analysis and infrared spectrum for the required rearrangement product(LXXIX). The structure of the product was not confirmed by an alternative synthesis but hydrolysis gave phenylalanine and an unstable product behaving as the hydroxylamino-acid(LXIX). Reduction of the rearranged product gave the corresponding secondary amine(LXXX). Reduction of the starting amide(LXVII) under similar conditions, namely using 10% palladium on charcoal as catalyst, had previously given the corresponding 1-N-methylaminocyclohexylcarbamide(LXXXI).



LXXVII LXXIX

When the optically active L-phenyalanyl ester (LXXXII) was made, rearrangement,under the conditions used above, caused complete racemisation. Both the rearranged amide and the phenylalanine obtained from it by acid hydrolysis were optically inactive. Reaction between the amide(LXVII) and DL-phenylalanine NCA followed by treatment with strong base also afforded the rearranged amide(LXXIX).

Owing to the very vigorous conditions necessary to effect the rearrangement and insertion with the cyclohexyl derivatives, new model substrates were prepared. The known nitrone(LXXXIII) was made and hydrogen cyanide addition gave the known nitrile(LXXXIV)[38]. Hydrolysis by concentrated sulphuric acid gave the amide(LXXXV). The amide



LXXXIII LXXXIV LXXXV

LXXXVI LXXXVII

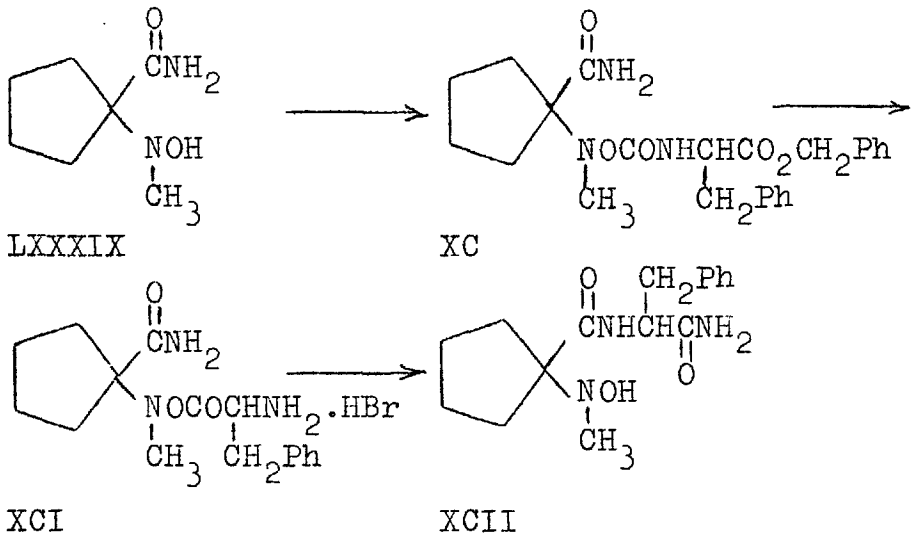
was successfully coupled with N-benzyloxycarbonyl-DL-phenylalanine with dicyclohexylcarbodiimide to the corresponding ester(LXXXVIII). Before rearrangements were tried with this compound the benzylcarbonate(LXXXVI) was prepared. Treatment with triethylamine had no effect but when potassium t-butoxide was used reaction occurred. Instead of the expected cyclic carbonate a product of elimination formed. The infrared and N.M.R. spectra, coupled with the microanalytical data proved that the product was the α-imino amide(LXXXVII). Elimination, across the α-carbon and nitrogen atoms, of the benzylcarbonate moiety had occurred.

To prevent such an elimination from interfering from the rearrangement an α-alkyl group must be present. Since the corresponding nitrones carrying an α-alkyl group do not readily add hydrogen cyanide[38] this route was discontinued.

The generality of the rearrangement of aminoacyl esters of α-(N-alkyl)hydroxylaminocarbamides had to be demonstrated. One further substrate madel was therefore prepared, that from cyclopentanone. Addition of hydrogen cyanide and N-methylhydroxyl-amine to cyclopentanone afforded an unstable product that could not be isolated in a pure state; this was in

accord with the known behaviour of cyclopentanones to additions[39]. Whem the crude adduct was hydrolysed by the concentrated sulphuric acid method the required carbamido derivative(LXXXIX) was prepared.

Coupling of this compound(LXXXIX) with N-benzyloxycarbonyl-DL-phenylalanine was again achieved with diimide in good yield. Removal of the protecting groups by hydrogen bromide in nitromethane followed by rearrangement by base afforded the rearranged amide(XCII).



LXXXIX XC

XCI XCII

References.

1. Wieland and Euler, Chem.Ber., 1958,91,2305.

2. Bailey, J., 1950,3461.

3, Wieland,Merz, and Pfleiderer, Chem.Ber., 1960,93,1816.

4. Iwakura and Okada, Canad.J.Chem., 196o,38,2418.

5. Kupchan,Slade, and Young, Tetrahedron Letters, 1960,24,22.

6. Bruice and Fife, Tetrahedron Letters,1961,8,263.

7. Einhorm, Ann., 1898,300,135.

8. Jencks and Carriuolo, J.A.C.S., 1960,82,1778.

9. Bender, Chem.Rev., 1960,60,53.

10. Bruice and Benkovic, J.A.C.S., 1963,85,1.

11. Pandit and Bruice, J.A.C.S.,1960,82,3386.

12. Wieland and Urbach, Ann., 1958,613,84.

13. Kurtz and Niemann, J.A.C.S., 1961,83,3309.

14. Hofmann,Lindenmann,Magee, and Khan, J.A.C.S., 1952,74,470.

15. Brenner and Hofer, Helv.Chim.Acta, 1961,44,1798.

16. Brenner and Hofer, Helv,Chim.Acta, 1961,44,1794.

17. Hurd and Fan, J.A.C.S.,1951,73,110.

18. Anderson and Paul, J.A.C.S.,1958,80,4423.

19. Weygand and Steglich, CHem.Ber., 1960,93,2983.

20. Khorana, Chem. and Ind.,1951,129.

21. Keller,Netter, and Niemann, Z.physiol.Chem., 1958,313,244.

22. Michaelis and Bottinger, Ber., 1878,11,1407.

23. Cramer and Winter, Chem.Ber., 1961,94,989.

24. Gambarian,Cialtician, and Babajan, Bull.inst. sci.RSSd'Armenia,1931,265 cf. C.A.,1933,27,1332.

25. Carpino,Giza, and Carpino, J.A.C.S.,1959,81,955.

26. Gambarian and Kazarian, J.Gen.Chem. USSR, 1933,3,222.

27. Rogers, J., 1955,769.

28. Utzinger and Regenass, Helv.Chim.Acta, 1954,37,1888.

29. Wieland,Bökelmann,Bauer,Lang, and Lau, Ann., 1953,583,129.

30. Brenner,Zimmermann,Wehrmüller,Quitt,and Photaki, Experentia, 1955,11,397.

31. Ben Ishai and Berger, J.Org.Chem., 1952,17,1564.

32. Albertson and Mckay, J.A.C.S., 1953,75,5323.

33. Hiskey and Jung, J.A.C.S., 1963,85,578.

34. Miller and Plöchl,Ber., 1892,25,2020.

35. Wiberg, J.A.C.S., 1955,77,2519.

36. Spenser and Ahmad, Proc.Chem.Soc.,1961,375.

37. Einhorn and Mettler, Ber., 1902,35,3647.

38. Bonnett,Brown,Clark,Sutherland, and Todd, J., 1959,2094.

39. Brown,Brewster, and Schechter, J.A.C.S., 1954,76,467.

EXPERIMENTAL SECTION

Melting points, uncorrected, were determined on a Kofler block except where stated. Infrared spectra were recorded on either a Perkin Elmer Infracord 137 or a Unicam SP200 spectrophotometer. The bracketed numerals, with the cipher E in front, refer to experiment numbers as recorded in the original notebook. The designation of compounds with Roman numerals follows that used in the preceding pages. THF refers to tetrahydrofuran; petroleum ether refers to the fraction boiling 40-60°; N-carboxy-amino-acid anhydrides are denoted by the suffix 'NCA'.

A.Preparation of dithiol-monoesters.

O,S-Dibenzylmonothiolcarbonate.(VII)(E25)

Benzylchloroformate(3.5ml.,2N soln. in toluene) and benzyl thiol(0.89g.) were shaken with 1N sodium hydroxide solution(7.5ml) for one hour. Extraction with ether(50ml.), washing with water(4x5ml.), followed by drying(Na_2SO_4), filtration,and evaporation afforded a crystallising oil.Recrystallisation from chloroform,petroleum ether(1:1) at 0° afforded the carbonate(VII) as colorless prisms,m.p.27°(0.94g.,51%). A sample was recrystallised twice more for analysis (Found: C,69.7;H,5.55;S,12.8. $C_{15}H_{14}O_2S$ requires C,69.7;H,5.5;S,12.4%).

S,S'-Dibenzyloxycarbonylethane-1,2-dithiol(VIII) (E27).

Benzylchloroformate(3.5ml.;2N soln. in toluene) and ethylene dithiol(0.63g.) were added to THF(3ml.) cooled in an ice-water bath. Pyridine(0.9ml.) was added and the mixture left for five minutes before ether extracting the product. Working up the extract afforded a pale yellow oil. After drying at 100° and 0.2mm.Hg for two hours colorless needles of the diester(VIII) formed. Recrystallisation from ethanol afforded needles of m.p.89°, ν_{max}(Nujol) 1702cm^{-1}(ester)(Found: C,59.4;H,5.2; S,17.3. $C_{18}H_{18}O_4S_2$ requires C,59.6;H,5.0;S,17.7%).

Ethylenedithiolcarbonate[1](E54).

Ethylenedithiol(13.3ml.) was added to ethyl acetate containing phosgene(100ml.,1.5 equivalents) at 0°. Pyridine(26ml.) was added,whilst stirring, over thirty minutes. After a further one hour the mixture was poured into water(50ml.), the organic layer separated off and washed with water(4x50ml.) before drying(Na_2SO_4),filtering and distilling off the organic solvent. The residual oil was dried at 100° and 0.1mm. Hg before adding ether(10ml.) and cooling to -20°. The needles of ethylenedithiol carbonate that formed were collected and recrystallised from ether(30ml.) at -20°,m.p.25-30°(lit.m.p. 34°)(13.3g.,70%).

Action of sodium ethoxide on ethylene dithiol-carbonate (E50).

The carbonate(1.43g.) was shaken with sodium ethoxide(ex 0.29g. sodium) in ethanol(12.5ml.) at 0° for ten minutes before pouring the solution into glacial acetic acid(3.5 ml.). To the cooled solution was added water(20ml.) before extracting with ether(15ml.), washing with water(3x20ml.), back-washing with more ether(20ml.). The dried (Na_2SO_4) organic extract was filtered and evaporated to small bulk. The residual oil was fractionally

distilled, the portion boiling at 150-165°, 762mm.Hg being collected (1.14g., 58%). Redistillation gave, as a colorless oil, S-monoethoxycarbonylethane-1,2-dithiol(X), b.p.165-7° at 762mm. Hg, $n_D^{21.5}$ 1.5235, ν_{max}(film) 2560(thiol) and 1695(thiol-ester)cm^{-1}. (Found: C,36.01;H,5.9;S,38.4. $C_5H_{10}O_2S_2$ requires C,36.1;H,6.1;S,38.6%).

The 2,4-dinitrophenylsulphide of the dithiol-monoester was prepared, as described below, affording pale yellow needles from ethanol, m.p.52°.(Found: C,39.6;H,3.75;,N,8.25;S,18.9. $C_{11}H_{12}N_2O_6S_2$ requires C,39.8;H,3.6;N,8.4;S,19.3%).

Method of preparation of 2,4-dinitrophenylsulphides.*

The thiol(1 mmole.) was added to dry pyridine (5ml.) containing 2,4-dinitrochlorobenzene(1 mmole.) and the solution warmed on the steam bath for fifteen minutes. The dark red solution was cooled to room temperature, poured into water(50ml.)

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*N.B. This method was adopted in the preparation of all the 2,4-dinitrophenylsulphides described in the following pages. On occasions the oily precipitate from water crystallised spontaneously, when it was collected and recrystallised for analysis directly.

and extracted with ethyl acetate(25ml.). The extract was washed with 1N hydrochloric acid(20ml.) and water(10ml.) before drying(Na_2SO_4), filtering, and evaporating to dryness when the residue crystallised. The product was collected and recrystallised to constant melting point for analysis.

Preparation of α,ω-dithiolalkane diacetates (See Table I).

The α,ω-dithiolalkane(0.2mole) was dissolved in a solution of anhydrous sodium acetate(5g.) in acetic anhydride(50ml.). The solution was heated to a gentle reflux under an atmosphere of pure nitrogen for five to six hours. The solution was cooled and water(50ml.) slowly added. The mixture was left for one hour before extracting with ether (50ml.), washing with water(4x50ml.), saturated sodium bicarbonate solution(50ml.), and backwashing the aqueous extracts with more ether(50ml.). The ether extract was dried(Na_2SO_4), filtered. and distilled in vacuo to afford the corresponding , -dithiolacetyl alkane.

Preparation of α,ω-dithiolalkane monoacetates (See Table II).

The α,ω-dithiolalkane diacetate(0.1mole) was dissolved in THF(75ml) in the prescence of

sodium hydroxide(4.0g.) in water(75ml.). The mixture was stirred rapidly whilst gently refluxing under a pure nitrogen atmosphere for two to three hours. The mixture was then poured into water(50ml.) and ether extracted(2x75ml),washing with water(3x50ml.). The dried(Na_2SO_4) extract was filtered and evaporated to small bulk before purifying either by fractional distillation through a Wiley spinning band column or by passing through an acid washed silica-gel column, elutimg with benzene. On chromatographic treatment dithiol was initially eluted, then the monoacetate and finally unchanged diacetate. The purity of the product was estimated by titration of the thiol with standard iodine solution. The productwas characterised as the 2,4-dinitrophenylsulphide derivative (see Table III).

Table I

Preparation of α,ω-dithiolalkane diacetates

$(CH_3COS(CH_2)_nSCOCH_3)$.

n	Yield,%	b.p.$^{mm}_{Hg}$	n_D^T	m.p.	Run no.	Reference.
2	91	-	-	69	E73	3
3	95	-	1.5204^{24}	-	E83	4
4	99	-	-	24-6	E81	5
5	57[b]	89/0.1	1.5082^{22}	-	E104	New compound[a]
6	93	-	-	28	E105	New compound[c]

a) Analysis, found: C,48.9;H,7.0;S,29.6.
$C_9H_{16}O_2S_2$ requires C,49.1;H,7.3;S,29.1%.

b) Starting dithiol contaminated with hydroxy compounds.

c) Analysis, found: C,50.95;H,7.85;S,27.7.
$C_{10}H_{18}O_2S_2$ requires C,51.25;H,7.7;S,27.4%.

Table II

α,ω-Dithiolalkane monoacetates. $(CH_3COS(CH_2)_nSH)$.

n	Yield,%	% SH.	b.p.$^{mm.}_{Hg}$	n_D^T	Run no.
2	24.8	102.5	72/14	1.5354^{23}	E75
3	18.0	98.6	70-3/2	1.5255^{22}	E85
4	28.8	101.3	125-6/15	1.5186^{25}	E84
5	39.7	102.7	108-112/0.8	1.5142^{23}	E108
6	39.2	101.7	-	1.5083^{24}	E117

Table III.[a,b]

2,4-Dinitrophenylsulphides of α,ω-dithiolalkane-monoacetates. ($CH_3COS(CH_2)_nSC_6H_3N_2O_4$).

n	Yield,%	m.p.°C	Found				Required			
			C	H	N	S	C	H	N	S
2	76	98	39.84	3.41	9.38	20.99	39.72	3.34	9.27	21.23
3	63	77	41.79	3.85	8.80	19.72	41.79	3.83	8.86	20.28
4	85	102	43.86	4.38	8.39	19.02	43.69	4.27	8.48	19.44
5	93	67	45.02	4.99	8.11	18.21	45.32	4.68	8.14	18.62
6	79	77	46.92	5.05	7.82	17.90	46.86	5.19	7.96	17.53

a. See p.85 for preparative details

b. The sodium hydroxide method of preparing the 2,4-dinitrophenylsulphides afforded mainly bis-dinitrophenylsulphides in the present cases[6].

o-Xylene-α,α'-dithiolacetate.(XXI)(E103).

α,α'-Dibromo-o-xylene(19.8g.) and potassium thiolacetate(17.14g.) in ethanol(180ml.) were heated to reflux, with stirring under a nitrogen atmosphere, for four hours. After distilling off some of the solvent(100ml.) the mixture was poured into water(50ml.) and ether extracted(2x50ml.) and washed with water(3x50ml.). The combined organic extract was dried(Na_2SO_4), filtered, and the solvent removed to yield a colorless oil. Distillation afforded pure o-xylene-α,α'-dithiolacetate(XXI), b.p. 117-8° at 0.8mm.Hg.(16.4g.,84%). n_D^{22}1.5870;$\nu_{max.}$(film): 3040(aromatic C-H) and 1680(thiol ester) cm^{-1}. (Found: C,56.8;H,5.3; S,25.2. $C_{12}H_{14}O_2S_2$ requires C,56.7;H,5.55;S,25.25%).

o-Xylene-α,α'-dithiolmonoacetate(XX)(E106).

The diacetate(XXI)(16g.) was dissolved in THF(50ml.) in the prescence of sodium hydroxide (2.52g.) in water(100ml.). The mixture was heated to a gentle reflux under a nitrogen atmosphere for three hours before cooling and adding ether(50ml.). The organic phase was collected, washed with water (4x50ml.), dried(Na_2SO_4), filtered and the solvent removed in vacuo. Theeresidual oil was chromatographed through acid washed silica gel(200g.) using benzene

as solvent. The recovered monothiolmonoacetate fraction was distilled under reduced pressure to afford, as a colorless oil, o-xylene-α,α'-dithiol monoacetate(XX), b.p. 126-8°at 0.9mm. Hg.; n_D^{21} 1.6032; iodine estimation,105% thiol.(2.35g.,35%). Elution of the silica gel column with chloroform-benzene(1:9) afforded starting material. The initial fractions from the column gave needles of o-xylene-α,α'-dithiol(0.71g.) on evaporation[7].

The monoacetate gave yellow prisms of the 2,4-dinitrophenylsulphide,m.p. 124.5°(Found: C,50.55;H,3.95;N,7.1;S,17.1. $C_{16}H_{14}N_2O_5S_2$ requires C,50.75;H,3.7;N,7.4;S,17.0%).

Toluene-3,4-dithiolacetate[8](XVIII)(E122).

Toluene-3,4-dithiol(5.0g.) was added to acetic anhydride(20ml.) containing anhydrous sodium acetate (0.2g.) under a nitrogen atmosphere and the solution left to stand at room temperature overnight. The clear solution was poured into water(50ml.) and after standing for a further hour extracted with ethyl acetate(50ml.), washing with water(3x50ml.) and sodium bicarbonate solution(50ml). The aqueous fractions were backwashed with ethyl acetate(25ml.) before combining the organic extracts and drying(Na_2SO_4), filtering, and concentrating

in vacuo. The residual oil crystallised on standing. The needles were harvested, washing with petroleum ether. Air drying gave the diacetate(XVIII), m.p.48°(6.48g.,83%).

Toluene-3,4-dithiolcarbonate(XIX)(E217).

A solution of 3,4-dithioltoluene(5.0g.) and phosgene(4.0g.) in ethyl acetate(50ml.) was treated with pyridine(10ml.) dropwise, with stirring, at -20°. After addition(20 minutes) the mixture was left to warm to ambient temperature for fourty minutes. The mixture was poured into dilute sulphuric acid(50ml.,1.5N) and separated. The organic solution was washed with water(3x50ml) and saturated sodium bicarbonate solution(50ml.), the aqueous extracts being backwashed with more ethyl acetate(25ml.), before drying(Na_2SO_4) in the prescence of a little animal charcoal. The mixture was filtered and the solvent removed in vacuo. The crystalline product was recrystallised from ethyl acetate-ether(1:1) at 0° to afford needles of the carbonate(XIX)(3.4g.,58%),m.p. 56°,ν_{max} 1720 (broad, carbonate) cm^{-1}.(Found: C,52.5;H,3.2; S,3..75. $C_8H_6OS_2$ requires C,52.7;H,3.3;S,35.7%).

Attempted reactions with toluene-3,4-dithiol-carbonate[9](E217b).

i) In ethanol,no catalyst.

The carbonate(XIX)(2.3g.) in ethanol(25ml.) was heated to reflux under nitrogen for twenty hours. A sample gave a negative thiol test with aqueous lead acetate solution. Evaporation of the solvent afforded the carbonate(2.2g.)

ii) Extended ethanol treatment.

The carbonate(2.0g.) in ethanol(25ml.) was left at room temperature for seven days. The solution again gave a negative thiol test; working up gave the carbonate(1.9g.)

iii) In ethanol, with triethylamine.

The carbonate(1.8g.) in ethanol(15ml.) was heated to reflux with triethylamine(1ml.). After sixteen hours no thiol could be detected and again carbonate was recovered(1.6g.).

Attempted half-hydrolysis of toluene-3,4-dithiol-acetate(E200).

The diacetate(XVIII)(6.0g.) was dissolved in aqueous THF(1:1,50ml.) containing sodium hydroxide (1.0g,) and the mixture stirred under a nitrogen atmosphere at room temperature for four hours. The

mixture was ether extracted(50ml.) and washed with water before drying(Na_2SO_4), filtering, and evaporating to small bulk. The dark yellow residue was distilled under reduced pressure to afford a crude sample of the monoacetate,b.p. 125-130°. (0.68g,). The product was contaminated with a lot of impurities and was not used further.

Acetylation of cyclohexane-1,2-trans-dithiol(E196)

Cyclohexane-1,2-trans-dithiol[10] (5.5g.) and some anhydrous sodium acetate were dissolved in acetic anhydride(15ml.) before heating to a gentle reflux under a nitrogen atmosphere for three hours. The solution was cooled and poured into a mixture of water(25ml.) and ether(25ml.). The organic phase was separated, washed with water(4x25ml.) and backwashing with ether(25ml.) and dried(Na_2SO_4). The solution was filtered and worked up to give, as a colorless oil, cyclohexane-1,2-trans-dithiol-acetate(XVII); b.p. 126-128°at 1.8mm. Hg.(7.1g.,82%). n_D^{25} 1.5373, ν_{max}(film) at 1685cm^{-1}(thiolester). (Found: C,51.9 ;H,7.35;S,27.1. $C_{10}H_{16}O_2S_2$ requires C,51.7;H,6.9;S,27.6%).

Attempted hydrolysis of the acetate(XVII)

The diacetate(XVII)(6.43g.) in THF(20ml.) was heated to a gentle reflux whilst stirring with

sodium hydroxide solution(27.6ml.,1N) under a nitrogen atmosphere. After two hours the mixture was ether extracted(2x25ml.) and washed with water before drying(Na_2SO_4), filtering, and evaporation in vacuo to afford a pale yellow oil. Chromatography on acid-washed silica-gel(200g.) and eluting with benzene-chloroform(1:1) gave a crude oil(1.24g,) that would not give a pure 2,4-dinitrophenyl-sulphide derivative. The material was not examined or used further.

B. Reaction of thiols with NCAs.

L-α-Alanine NCA[11](E7).

Finely ground L-α-alanine(0.51g.) was suspended in anhydrous THF(12.5ml.) whilst passing a stream of phosgene gas with stirring and heating to 35-40°. After three hours the solution was decanted from the trace of residual solid, excess phosgene removed by pumping at the water pump via a calcium chloride trap and then evaporated in vacuo to small bulk. The colorless oil was dissolved in THF-petroleum ether(1:1,5ml.) before leaving at 0° overnight. The colorless needles that formed were collected and dried under reduced pressure over phosporus pentoxide, yielding the pure

NCA(0.41g.,63%), m.p. 90-91°(decomp.).

The product could be sublimed at 55° and 10^{-5}mm.Hg. in 77% yield, m.p.91-91.5°(decomp.); $\nu_{max.}$(Nujol) 3350(amide),1820 and 1765(anhydride)cm^{-1}.

Optical stability of L-α-alanine NCA to N HCl.[12]

L-α-Alanine NCA(47.8mg.) was dissolved in N-hydrochloric acid(2ml.). After two hours the optical rotation was measured.

Found: $[\alpha]_D^{25}$ +13.7°

Reference: $[\alpha]_D^{25}$ +13.6°(ex starting amino-acid).

Reaction of benzylthiol with L-α-alanine NCA.

i) At 0°,no triethylamine(E11).

Benzylthiol(228mg.) in anhydrous THF(5.7ml) was added to a solution of L-α-alanine NCA(208mg.) in THF(5.0ml.) at 0° whilst passing a stream of dry carbon dioxide. After four hours an excess of diazomethane in ether(20ml.) at 0° was added and the mixture left at 0° overnight. The cloudy mixture was filtered, washed with water, dried (Na_2SO_4), and re-filtered before evaporating to small bulk. The pale yellow oil crystallised on standing. Recrystallisation from chloroform-petroleum ether(1:4) eventually afforded colorless needles of N-methoxycarbonyl-L-α-alanine-S-benzylthiol ester(IV)(18mg.), m.p. 57-57.5°,

$[\alpha]_D^{23}$ -42.1°($CHCl_3$,c.2.4); $\nu_{max.}$ 3340(N-H), 1680(urethane), and 1675(thiolester)cm^{-1}(in Nujol). (Found: C,57.5;H,6.1;N,5.5;S,12.25. $C_{12}H_{15}NO_3S$ requires C,57.0;H,6.0;N,5.5;S,12.65%).

ii) At -10°, with triethylamine(E28).

Benzylthiol(260mg.) and triethylamine(210mg.) were added to a solution of L-α-alanine NCA(242mg.) cooled to -10°. After two hours working up as in (i) afforded the urethane(342mg.,64%),m.p. 56-7°.

Reaction of N-methoxycarbonyl-L-α-alanine - S-benzyl ester with benzylamine.(E20).

N-Methoxycarbonyl-L-α-alanine-S-benzyl ester(106mg.) and benzylamine(100mg.) were dissolved in anhydrous THF(5ml) at room temperature, reaction being followed by optical rotation changes. After thirty hours, when the reaction was essentially complete, the solution was evaporated under reduced pressure and the product dissolved in ethyl acetate-petroleum ether(1:2,2ml.) before leaving at 0° overnight. The fine needles of N-methoxycarbonyl-L-α-alanyl-(N-benzyl)amide(V) were collected and dried in vacuo, m.p. 98°;$[\alpha]_D^{26}$ -26.3° ($CHCl_3$,c.2.0); $\nu_{max.}$(Nujol) 3300(N-H), 1680(shoulder, urethane) and 1670(amide) cm^{-1}.(Found: C,60.6;H,6.8;N,11.45.

$C_{12}H_{16}N_2O_3$ requires C,61.0;H,6.8;N,11.8%).

Reaction of O,S-dibenzylmonothiolcarbonate(VII) with benzylamine.

The carbonate(VII)(0.71g,) and benzylamine(0.32g.) were dissolved in THF(5ml.) and the solution left at ambient temperature for seven days. The clear solution was then concentrated in vacuo to an oil which crystallised on trituration with petroleum ether. The needles of N,O-dibenzylurethane were collected and air dried (0.47g.,71%). They had m.p. 62-63°(lit. m.p. 64°)[13].

Reaction of S-momoethoxycarbonyl-ethane-1,2-dithiol(X) with L-α-alanine NCA(E74).

Thiolester(X)(1.26g.) was added to L-α-alanine NCA(0.79g.) dissolved in THF(25ml.). The solution was cooled to -40° before adding redistilled N,N-dimethylcyclohexylamine(0.87g.) in more THF(5ml.). The solution was left at -40° for three hours before dividing into two equal parts.

i) Preparation of the N-methoxycarbonyl derivative.

To the cloudy solution was added an excess of diazomethane in ether at -30°. The solution was allowed to warm to 0° before leaving at this temperature overnight. The mixture was filtered and worked up in the usual manner to give a

colorless oil(0.596g.,88%). A portion was molecularly distilled at 90° and 10^{-5}mm. Hg.to give 1-S-ethoxycarbonyl-2-S-(N-methoxycarbonyl)-L-α-alanyl-1,2-dimercaptoethane(XV),$[\alpha]_D^{25}$ -30.73°, ($CHCl_3$, c,1.1); $\nu_{max.}$(film) 1710(urethane), 1700(thiolcarbonate) and 1675(thiolester) cm^{-1}. (Found: C,40.7;H,5.6;N,4.9;S,21.6. $C_{10}H_{17}NO_5S_2$ requires C,40.95;H,5.80;N,4.75;S,21.7%).

ii) Preparation of hydrochloride

Dry hydrogen chloride gas was bubbled through the remaining portion of the reaction product until just acidic to wet litmus. Concentration of the solution to dryness gave a white solid which was dissolved in hot ethyl acetate(10ml.). The solution was slowly cooled to room temperature and the mixture filtered. Concentration of the mother liquors afforded a colorless solid. After a further four treatments with ethyl acetate, following the same procedure, and with a final recrystallisation from ethyl acetate, colorless needles of 1-S-ethoxycarbonyl-2-S-alanyl-1,2-dimercaptoethane hydrochloride(XVI) were obtained; m.p. 128°;$[\alpha]_D^{22}$ +15.9° (H_2O, c.0.5); $\nu_{max.}$2900-2400(complex,NH_3),1705(thiolcarbonate) and 1680(thiolester) cm^{-1}(in Nujol). (Found: C,35.3;

H,6.1;Cl,13.1;N,5.3;S,23.5. $C_8H_{16}ClNO_3S_2$ requires C,35.15;H,5.9;Cl,13.0;N,5.1;S,23.5%).

Action of thiophenol on L-α-alanine NCA(E124).

L-α-Alanine NCA(0.125g.) in anhydrous THF(15ml) was treated with thiophenol(0.121g.) and N,N-dimethylcyclohexylamine(0.133g.) at -40°, the volume of solution being increased by the addition of more THF(10ml.) The clear solution was kept at -40° for three hours before passing dry hydrogen chloride gas until just acidic. Removal of the solvent afforded a colorless solid. The product was dissolved in THF(8ml.) and slowly cooled when needles of S-thiophenyl-L-α-alanine hydrochloride[14] formed; m.p. 140-142°(0.146g.,61%).

Attempted reaction of L-α-alanine NCA with isopropyl mercaptan.

Isopropylthiol(0.153g.) and NCA(0.226g.) in THF(20ml.) were cooled to -40° before adding N,N-dimethylcyclohexylamine(0.262g) in THF(5ml.). Aliquots were taken at intervals and the thiol concentration estimated, by use of an iodimetric titration. No reaction occurred other than formation of a cloudy precipitate of poly-alanine.

Kinetic studies on the reaction of L-α-alanine NCA with α,ω-dithiolmonoacetates.

i) Benzylthiol under various conditions.(E68,E112).

Benzylthiol in THF was treated with L-α-alanine NCA(1 equivalent) and N,N-dimethylcyclohexylamine (1 equivalent) in a stoppered,graduated flask, cooled in an acetone-drikold bath to the appropriate temperature. Aliquots(1 ml. portions) were withdrawn at intervals and the amount of free thiol present estimated by immediately quenching in hydrochloric acid(4ml.,3N), adding excess standard iodine solution and back-titrating the excess with standard sodium thiosulphate solution. Starch-solution was used as indicator. The results are presented graphically(see graph II, p.50).

ii) α,ω-Dithiolmonoacetates.

The method employed was essentially the same as described above. The results are presented graphically(see graph I, p.49).

Attempted rearrangement of thiolesters. (E56)(E77)

i) 1-S-Ethoxycarbonyl-ethane-1,2-dithiol.

Ester(0.22g.) was reacted with L-α-alanine NCA (0.15g.) at -10° in anhydrous THF(10ml.) in the prescence of triethylamine(0.13g.). The solution

was left at -10° for thirty minutes before warming to room temperature and leaving for a further three hours. Ethanol(3ml.) was added, the solution warmed and then left at 0° for several hours. On concentration fine needles, together with some amorphous material, precipitated from the solution. On centrifugation the mixture afforded 62.7mg. of precipitate. The supernatant liquor was dried in vacuo to give starting mono-thiol ester(0.187g.,86%)

ii) 1-S-Acetyl-ethane-1,2-dithiol.

Reaction of the ester(0.242g.) was carried out with NCA at -40° using N,N-dimethylcyclohexylamine as the tertiary base. After three hours the reaction mixture was warmed to room temperature and left for a further two hours. Evaporation to small bulk, under slightly reduced pressure, afforded an amorphous solid(85mg.), m.p.290-293°(closed tube). The mother liquor, after drying, gave an infrared spectrum identical to starting ester(0.158g.).

Chromatographic examination of hydrolysate from reaction of α,ω-dithiolmonoacetates with L-Ψ-alanine NCA(E134).

Preparation of hydrolysates.

A sample containing ca. 0.01mmole. of product

form the reaction between α,ω-dithiolmonoacetyl alkanes(butane,pentane,and hexane series) and L-α-alanine NCA(see graph I, p.49.) was suspended in barium hydroxide solution(2ml.,0.1N) and heated to reflux for one hour. The solution was cooled and neutralised with sulphuric acid(0.1N), filtered and concentrated in vacuo to ca. 0.5ml. bulk.

Chromatographic procedure.

Samples(0.1ml.) of the hydrolysates were chromatographed on Whatman no. 1 paper by descending front development, being irrigated with an equilibrated mixture of ammonia(3ml.,concentrated), water(17ml.), and ethanol(80ml.). Authentic N-acetyl-L-α-alanine was used as reference[15]. The papers were air-dried and developed with an ethanolic solution of bromocresol green(100mg.per ml.)[16]. No spots were observed at R_f0.55, where authentic N-acetylalanine gave a blue spot on the yellow background.

C. Attempted reactions with L-α-alanine NCA.

Use of monoethoxycarbonylcatechol[9](E140)

Monoethoxycarbonylcatechol(0.31g.) and N,N-dimethylcyclohexylamine(0.22g.) in THF(5ml.) at -40° were treated with L-α-alanine NCA(0.20g.) in THF(5ml.). After fifteen minutes precipitation of an amorphous solid commenced. After two hours the mixture was centrifuged to give polyalanine (0.12g.).

Use of phenol(E139).

Under similar conditions to the above only polyalanine was recovered.

Equilibration of amines and N,N'-diacetylhydrazine[17]. E183,E393.

i) Aniline

N,N'-Diacethydrazide(0.23g.) and aniline(0.18g.) in THF(20ml.) were refluxed for two days before cooling and evaporating in vacuo to small bulk. N,N'-diacetylhydrazine(0.22g.,95%) was recovered.

ii) Benzylamine,100% acetic acid.

A solution of benzylamine(0.52g.) and N,N'-diacethydrazide(0.58g.) in glacial acetic acid(5ml.) was left at room temperature for sixty hours. The clear solution was poured into water(20ml.) and ether extracted(20ml.). No material was found in the extract.

iii) Benzylamine, 5% acetic acid.

A solution of benzylamine(0.62g.) and N,N'-diacethydrazide(0.60g.) in 5% glacial acetic acid in THF(5ml.) was heated to a gentle reflux for twenty hours. On cooling the solution was poured into water(10ml.) and ether extracted(2x10ml.). The combined organic layers were washed with water (2x10ml.) before drying(Na_2SO_4), filtering and evaporating off the solvent. The oily residue crystallised on standing as needles of N-benzyl-acetamide, m.p. 57-9°(0.59g.,76%).

Use of benzhydrazide[18,19](E184).

Benzhydrazide(0.28g.) in glacial acetic acid(2ml.) was treated with L-α-alanine NCA(0.20g.) at room temperature. Carbon dioxide was evolved. After three hours the solvent was evaporated off under reduced pressure to leave a clear oil. Ethanol was added and then removed *in vacuo* and this repeated twice more to remove traces of acetic acid. Hydrochloric acid(1ml., conc.) was added and the solution left at 0°. No solid hydrochloride formed.

Use of cyclohexylcarboxylic hydrazide[20](E189).

The hydrazide(0.29g.) in methylene chloride(10ml.) was added to L-α-alanine NCA(0.22g,) and triethylamine(0.19g.) in THF(5ml.) at -40°. A

precipitate slowly formed and, after two hours was collected as before as polyalanine(0.125g.).

Use of N-acetylthiourea(E194).

N-Acetylthiourea[21](0.12g.) in acetone(5ml.) at -40° gave, with NCA(0.12g.) in acetone(10ml.) and triethylamine(0.11g.) a precipitate of polyalanine(81mg.).

Use of N,N-dimethylethanolamine[22](E219).

L-α-Alanine NCA(0.58g.) was treated with N,N-dimethylethanolamine[23] in anhydrous THF at -40°. Polypeptide began to precipitate out very quickly and was collected after thirty minutes as polyalanine(0.34g.).

Reaction of phenol with L-α-alanine NCA using triazole as catalyst(E272).

Phenol(99mg.) and L-α-alanine NCA(120mg.) in anhudrous THF(5ml.) at -40° were treated with triazole(70mg.) and triethylamine(100mg.). After two hours the clear solution was warmed to room temperature when a cloudy precipitate of polyalanine formed, being collected in the usual way(64mg.).

D. Reactions with hydroxamic acids.

N,O-Dianisoyl-N-methylhydroxylamine(XXXIV)(E264).

N-Methylhydroxylamine(ex. 3.3g. hydrochloride) in triethylamine(10ml.) was added to a solution of anisoyl chloride(3.4g.) in pyridine(20ml.) at room temperature. After two hours the solution was poured into water(100ml.) and ethyl acetate extracted(2x50ml.). After washing with hydrochloric acid(50ml.,1N) and saturated sodium bicarbonate solution(50ml.), the organic layer was dried(Na_2SO_4), filtered, and evaporated to dryness. On trituration with petroleum ether the oily product crystallised. Recrystallisatio from ethyl acetate-petroleum ether (1:1) produced prisms of N,O-dianisoyl-N-methyl hydroxylamine(XXXIV), m.p. 81°; $\nu_{max.}$(Nujol) at 1755 and 1660cm^{-1}(diacylhydroxylamine).(2.05g., 65%).(Found: C,64.55;H,5.3;N,4.7. $C_{17}H_{17}NO_5$ requires C,64.8;H,5.4;N,4.45%).

Action of benzylamine on N,O-dianisoyl-N-methyl hydroxylamine(XXXIV)(E270).

The ester(0.31g.) was treated with benzylamine (0.11g,) in THF(10ml.) at room temperature overnight. The product was poured into water(20ml.) and extracted with chloroform(10ml.). The aqueous phase afforded needles of N-anisoyl-N-methyl-

hydroxylamine[25] on evaporation(0.15g.,83%), m.p. 96-102°(lit. m.p.102-103°). The organic phase afforded colorless needles of N-benzyl-anisoylamide(0.20g.,83%), m.p. 129-130°(lit. m.p. 131°)[26].

Action of benzylamine on N,O-dibenzoyl-N-methyl-hydroxylamine(LVII)(E261).

The ester(LVII)(0.23g.) was treated with benzylamine(0.11g.) at room temperature in chloroform(5ml.) for fifteen hours. Working up as above afforded from the aqueous phase N-benzoyl-N-methylhydroxylamine[25] as a gum(0.12g.,90%). From the organic phase was obtained N-benzylbenzamide, m.p. and mixed m.p. 107-108°(0.17g.,89%).

Action of benzylamine on N,O-dibenzoylhydroxyl-amine[27](XXXI)(E299).

N,O-Dibenzoylhydroxylamine(0.23g.) was treated with benzylamine(0.11g.) under the conditions described above. From the chloroform extract was obtained, after twenty hours reaction, N-benzyl-benzamide(0.20g.,96%), m.p. 101-3°; mixed m.p. with authentic material, 102-4°. The aqueous phase gave benzohydroxamic acid(0.10g.,74%),m.p. 117-122°; identical infrared spectrum(Nujol) to authentic material.

Attempted reaction between L-α-alanine NCA and N-methyl hydroxamic acids.(E257).

N-Methylbenzohydroxamic acid(0.15g.) and NCA(0.13g.) were dissolved in glacial acetic acid (3ml.) at room temperature. A slow stream of carbon dioxide was liberated. After three hours the solution was evaporated *in vacuo*. Hydrochloric acid(1ml.,2N) was added and the solution evaporated; addition of ethanol yielded a white precipitate(23.9mg.),m.p.195-205°(subl.,closed tube). The infrared spectrum was identical to alanine anhydride.

A similar result was obtained with N-methyl-anisohydroxamic acid.

N-Acetylglycylhydroxamic acid(XXXIII)(E295).

Methyl aceturate(1.31g.) in ethanol(5ml.) was treated with hydroxylamine(*ex*. 0.70g. hydrochloride) in methanol(5ml.) for twenty hours at room temperature. The mixture was filtered and the solvent removed *in vacuo*. The gum produced was dissolved in water(10ml.) and continuously extracted with ethyl acetate for three days. Removal of the organic solvent followed by addition of acetone afforded prisms of N-acetyl-glycylhydroxamic acid(XXXIII)(0.85g.),m.p.132-

134°(decomp.); $\nu_{max.}$(Nujol) 3380(N-H),3180(hydroxyl), 1660(amide),and 1640(hydroxamic acid)cm^{-1}. Positive(cherry red) colour with ferric chloride. (Found: C,36.6;H,6.0;N,20.95. $C_4H_8N_2O_3$ requires C,36.4;H,6.1;N,21.2%).

Acetylation of glycine-hydroxamic acid(E285).

Glycine-hydroxamic acid[28](0.18g.) was dissolved in glacial acetic acid(2ml.) and acetyl chloride (1ml.) added. After shaking overnight the crystalline precipitate was collected and recrystallised(at less than 80°) from glacial acetic acid. Plates of O-acetyl-N-glycylhydroxylamine hydrochloride(XXXII) were obtained(0.13g.), m.p. 163-164°(decomp.). The crystals could not be obtained in an analytically pure state.

Rearrangement of O-acetyl-N-glycylhydroxylamine hydrochloride(XXXII)(E288).

The hydrochloride(0.34g,) was ground to a fine powder before suspending in ethanol(5ml.) and shaking with sodium methoxide(1 equivalent) in methanol(5ml). After several hours the mixture was filtered and the solution evaporated to dryness in vacuo. The viscous oil that formed refused to crystallise(0.32g.). The infrared spectrum(film) was very similar to that of an

authentic specimen of N-acetylglycylhydroxamic acid(XXXIII). The product gave a positive ferric chloride test. Paper chromatography, using Whatman no. 1 paper and amyl alcohol, acetic acid, water(4:1:5) as irrigant[29] gave a major spot at R_f 0.2(identical to the acid(XXXIII)) and material that remained near the starting point.

N-(N-Benzoyl)-glycyl-N-methylhydroxylamine(XXXVI)(E307).

Hippuryl chloride(3.0g.) was added, in small portions, to a solution of N-methylhydroxylamine hydrochloride(1.5g.) in saturated sodium bicarbonate solution(100ml.). During the addition, taking fourtyfive minutes, solid sodium bicarbonate was added to maintain alkalinity. After addition the mixture was stirred for a further fifteen minutes before continuously extracting with ethyl acetate overnight. Removal of solvent afforded prisms which were recrystallised from water to give needles of the hydroxamic acid(XXXVI). M.p.164-166°(decomp.); ν_{max}(Nujol) 3400(N-H),3190(hydroxyl), 1675(amide) and 1620(hydroxamic acid)cm^{-1}.(1.63g., 52%).(Found: C,58.05;H,5.8;N,13.4. $C_{10}H_{12}N_2O_3$ requires C,57.7;H,5.8;N,13.5%).

Attempted reaction of the product with L- -alanine NCA in acetic acid gave no reaction.

N-(N-Acetyl)-glycyl-N-methylhydroxylamine(XXXV)(E312).

Methyl aceturate(13.1g.) and N-methylhydroxylamine(ex. 8.4g. of the hydrochloride) were dissolved in anhydrous THF(70ml.) in the prescence of triethylamine(10ml.). The clear solution was left at room temperature for three days before evaporating down to small bulk and collecting the solid that formed. The needles of the hydroxamic acid(XXXV) were washed with a little ethyl acetate before drying in vacuo, (6.5g.,44%); m.p.145-7°; $\nu_{max.}$ 3300(N-H), 3100(hydroxyl),1640(broad,amide and hydroxamic acid)cm^{-1}.(Found: C,41.3;H,6.95;N,19.2. $C_5H_{10}N_2O_3$ requires C,41.1;H,6.95;N,19.2%).

Acetylation of aceturylhydroxamic acid(XXXV).

i) N-Acetylimidazole(E315).

Aceturylhydroxamic acid(XXXV)(0.44g.) was added to a solution of N-acetylimidazole[30] (1 equivalent) in glacial acetic acid(3ml.). The solution was left for thirty minutes at room temperature before evaporating to dryness in vacuo. Ethyl acetate(5ml.) was added and the mixture evaporated down to dryness again. The latter process was repeated twice more to

remove acetic acid and yielding a crystallising oil. Needles of N-(N-acetyl)-glycyl-N-methyl-O-acetylhydroxylamine(XXXIX)(0.53g.,93%) were collected, giving, after sublimation at 60° and 10^{-4}mm. Hg., a m.p.110-110.5°; $\nu_{max.}$ 3350(N-H), 1793 and 1680(diacyl hydroxylamine) and 1650(amide)cm^{-1}. (Found: C,44.7;H,6.4;N,15.0. $C_7H_{14}N_2O_4$ requires C,44.65;H,6.4;N,14.9%).

ii) Using acetic anhydride(E334).

The hydroxamic acid(XXXV)(0.23g.) in glacial acetic acid(5ml.) was treated with acetic anhydride (0.26g.) and imidazole(75mg.) for two hours at room temperature. Working up as above afforded needles of the O-acetyl derivative(XXXIX)(0.26g., 89%),m.p. 109-110°.

Attempted reaction between glycine NCA and anisoyl-N-methylhydroxylamine(E328).

To glycine NCA(0.10g.) and anisoyl-N-methyl-hydroxamic acid(0.18g.) in glacial acetic acid(10ml.) was added imidazole(37mg.). After carbon dioxide had been evolved the solution was left for a further thirty minutes and then acetic anhydride(0.2ml.) added. After a further five hours at room temperature the solvent was removed in vacuo and water(3ml.) added. The mixture was extracted with

ethyl acetate(3x10ml). The organic extract was washed with sodium bicarbonate solution(5ml.), before separating, drying(Na_2SO_4), and filtering. Evaporation of solvent left a pale yellow oil which was dried to constant weight(0.165g.)*in vacuo*. A sample was distilled at 70° and 10^{-2}mm.Hg. to give, as a colorless, viscous oil, *N-anisoyl-N-methyl-O-acetylhydroxylamine*(XLI). The infrared spectrum had $\nu_{max.}$(film) 1785 and 1660(diacylhydroxylamine)cm^{-1}.(Found: C,59.4;H,6.05;N,6.1. $C_{11}H_{13}NO_4$ requires C,59.2;H,5.9;N,6.0%).

Acetylation of phenol(E333).

Phenol(0.19g.) and N-acetylimidazole(0.23g.) were dissolved in glacial acetic acid(3ml.) at room temperature for ten minutes before evaporating down under reduced pressure. The residual oil was dissolved in benzene(10ml.), washed with water(3x5ml.) before drying(Na_2SO_4),and filtering. Evaporation of solvent left a colorless oil, which, on drying showed n_D^{23} 1.5025(lit. n_D^{20} 1.503)[31]; (0.14g.,51%).

Ethyl 2-N-(N-methyl)-hydroxylaminoacetate(XXXVIII) (E356).

Ethyl chloroacetate(3.1g.) and N-methylhydroxylamine hydrochloride(2.07g.) in anhydrous THF(25ml.) were treated with triethylamine(10ml.).

The suspension was shaken thoroughly for twenty minutes before leaving to stand at room temperature overnight. The mixture was filtered and the solvent removed *in vacuo* to give a pale yellow oil(2.9g.). Distillatio afforded a fraction, b.p. 64-66° at 0.2mm. Hg. of *ethyl 2-N-(N-methyl)-hydroxyl-aminoacetate*(XXXVIII)(1.54g.,46%). The oil crystallised to needles on standing at room temperature, m.p.53°.(Found: C,45.0;H,8.35;N,11.1; $C_5H_{11}NO_3$ requires C,45.1;H,8.3;N,10.5%). The compound turned dark brown on standing in air. $\nu_{max.}$(film) at 3450,3300(broad,hydroxyl), and 1740(ester)cm^{-1}.

2-N-(N-Methyl)-hydroxylaminoacetamide(E363).

Chloroacetamide[32] (1.87g.) and N-methyl-hydroxylamine hydrochloride(1.7g.) in THF(20ml.) were treated with triethylamine(4ml.). The suspension was shaken for thirty minutes before filtering and leaving at room temperature overnight. The colorless prisms that had formed were collected, dissolved in ethanol(8ml.) and ammonia in chloroform(ca.2%,10ml.) added. The ammonium chloride that formed was removed by filtration and the liquors evaporated *in vacuo* to yield a crystalline mass. The product was

immediately dissolved in hot ethanol(4ml.) and ether(10ml.) added. The prisms that formed were collected,dried to give 2-N-(N-methyl)-hydroxyl-aminoacetamide(0.92g.,44%), m.p. 138-140°; $\nu_{max.}$(Nujol) at 3480,3350,3240(amide and hydroxyl), 1663(amide)cm^{-1}.(Found: C,34.7;H,7.8;N,26.8. $C_3H_8N_2O_2$ requires C,34.6;H,7.75;N,26.9%).

Reaction of glycine NCA with anisoyl-N-methyl-hydroxamic acid(E327).

Method.

Standard solutions(0.2N) of glycine NCA, imidazole, and anisoyl-N-methylhydroxamic acid in glacial acetic acid were prepared. Samples were taken as required and the reaction followed volumetrically, by measurement of the carbon dioxide evolved. The reactions were carried out in a micro-hydrogenation apparatus. Glycine NCA solutions were added via a side arm on the micro-hydrogenator flask. The volume of the reaction solution was made up to 1.5ml. with glacial acetic acid when necessary. The results are expressed graphically(see graph III, p.62).

E. Attempts to prepare amino-acid inner anhydrides.

Reaction of ethyl glycinate with thionyl chloride(E157).

Ethyl glycinate hydrochloride(1.9g.) was suspended in chloroform(15ml.) in the prescence of thionyl chloride(1.5ml.) and anhydrous sodium carbonate(1.7g.). The suspension was heated to a gentle reflux for three hours before cooling and quickly filtering. The solvent and excess thionyl chloride were removed by evaporation under reduced pressure at less than 30°. A red mobile oil remained that crystallised to a colorless solid at 0°. The product(0.4g.) decomposed on warming to room temperature, with evolution of sulphur dioxide.

Reaction between DL-α-alanine and phenyl phosporodichloridate(E170).

Phenyl phosphorodichloridate[33](1.06g.) was added to a suspension of alanine(0.45g.) in acetonitrile(3ml.). Dimethylformamide(36mg.) was added and the mixture shaken in a stoppered flask for two hours. The solution was cooled to 10° and triethylamine(1.11g.) added in THF(3ml.). After cooling the mixture at 0° for two hours

filtration and evaporation of the solution gave a colorless gum(1.24g.). A portion(0.29g.) was treated with aniline(0.5ml.) and then acetone(5ml.) when a crystalline precipitate formed. The product was collected and recrystallised from ethanol(6ml.) to give plates of dianilinium O,O'-diphenylpyrophosphate(XLIV),m.p. 166°; (0.44g.)(Found: C,55.95;H,5.1;N,5.6;P,12.0. $C_{24}H_{26}N_2O_7P_2$ requires C,55.85;H,5.1;N,5.4;P,12.0%).

From the mother liquors was obtained an amorphous solid showing a broad amide absorption band in the infrared spectrum at 1600-1675cm^{-1}.

Use of phenylphosphorusoxydichloride(E163)

DL-α-Alanine(0.45g,) in acetonitrile(3ml.) was treated with dimethylformamide(0.39g.) and phenylphosphorusoxydichloride(0.94g,). On shaking the amino-acid quickly dissolved and after fifteen minutes triethylamine(1.0g.) in THF(5ml.) was added. After shaking at room temperature for one hour the mixture was filtered, washing with THF, and the liquors evaporated to small bulk to yield an amorphous solid(1.3g.)that gave a negative hydroxamic acid test[34]. The product was divided into two parts

i) The material was treated with benzylamine(2ml.)

to give an immediate precipitate. Recrystallisation from ethanol afforded colorlass plates of di-(N-benzyl)-ammonium-P,P'-diphenylpyrophosphinate (0.59g.),m.p. 205-207°. (Found: C,60.8;H,6.05; N,5.8;P,12.1. $C_{26}H_{30}N_2O_5P_2$ requires C,60.9;H,5.9; N,5.5;P,12.1%).

ii) The product was treated with aniline(2ml.). After warming at 100° for one hour the mixture was cooled and the crystalline product collected. Recrystallisation from ethanol afforded needles of dianilinium P,P'-diphenylpyrophosphinate(0.57g.), m.p. 176°. (Found: C,59.7;H,5.55;N,5.9;P,13.1. $C_{24}H_{26}N_2O_5P_2$ requires C,59.1;H,5.4;N,5.8;P,13.2%).

F. Preparation and reactions of O-acyl-N,N-dialkyl-hydroxylamines.

O-Benzoyl-N-hydroxypiperidine[35](XLVIII)(E221).

Benzoylperoxide(7.26g.) in ether(100ml.) at -10° was stirred whilst adding piperidine(5.1g.) dropwise over fifteen minutes. The reaction mixture was stirred for a further one hour at -10° before pouring into saturated sodium bicarbonate solution(100ml.). The ether solution was separated and washed with water(50ml.), hydrochloric acid (25ml.,1N), and water(50ml.). The dried(Na_2SO_4)

ether solution was filtered and evaporated to small bulk. The oil crystallised at 0° overnight. Recrystallisation from ether-petroleumether(1:2) gave colorless plates of O-benzoyl-N-hydroxy-piperidine(XLVIII)(1.6g.,26%), m.p.58-61°; $\nu_{max.}$ 1765(ester)cm^{-1}.

Reaction of benzoate(XLVIII) with amines(E222,E223).

i) Benzylamine.

Benzoate(0.52g.) and benzylamine(0.25g.) were dissolved in ether(5ml.). The clear solution was kept at room temperature for one day, samples being taken at intervals and the disappearance of the ester band at 1765cm^{-1} followed in the infrared spectrum. After one day no ester could be detected. Crystals had deposited in this time; these were collected,washed with petroleum ether and dried in vacuo. A second crop was obtained from the mother liquors and combined with the main crop(0.42g.,79%),m.p. and mixed m.p. with authentic N-benzylbenzamide, 108-109°.

ii) Aniline.

Aniline(0.11g.) and the ester(0.21g.) in ether(3ml.) were left at room temperature for ten days. An extremely slow reaction occurred and no amide was isolated.

iii) Ethyl glycinate.

Ethyl glycinate hydrochloride(0.15g.) was treated with ammonia in chloroform(ca.2%,10ml.), the mixture filtered andthe excess chloroform and ammonia removed by evaporation in vacuo. The residual solution was added to the ester(0.2g.) in ether(3ml.) at room temperature and left for seventeen days. The solution was washed with water(2x5ml.) before working up to yield, as a waxy solid, N-benzoylglycyl ethyl ester(96mg., 47%), m.p. 63-67°(lit. m.p. 67.5°)[36].

N-Hydroxypiperidine(E226).

To a stirred quantity of piperidine(8.5g.), cooled to -10°, was added hydrogen peroxide(30g., 14%w/v) dropwise over fifteen minutes, a vigorous reaction occurring. The pale yellow solution was left stirring for a further thirty minutes at 0° before neutralising with cold, concentrated hydrochloric acid. The mixture was evaporated in vacuo to yield a pale brown oil that slowly crystallised. Ethanol(5ml.) was added and the solution re-evaporated and this procedure repeated before leaving at 0°..The product was recrystallised from acetone(5ml) to give needles of N-hydroxy-piperidine hydrochloride(0.9g.,7%), m.p. 138-

140°(lit. m.p. 140°)[37].

O-Acetyl-N-hydroxypiperidine(XLVII)(E229).[37]

N-Hydroxypiperidine hydrochloride(1.37g.) and acetyl chloride(5ml.) were heated to a gentle reflux. After five minutes, when all the hydrochloride had dissolved, the solution was cooled and the excess acetyl chloride removed *in vacuo*. Methanol was added and the solution re-evaporated, this being repeated. Ethyl acetate(10ml.) was added and the solution washed with saturated sodium bicarbonate solution(10ml.) and water(5ml.). The solution was dried(Na_2SO_4) and filtered. Evaporation afforded O-acetyl-N-hydroxypiperidine, purified by distillation at 42-43°and 0.5mm. Hg.(0.65g., 45%); n_D^{25} 1.4553; $\nu_{max.}$ 1770(ester)cm^{-1}(film).

Reaction of acetate(XLVII) with amines(E230).

i) Aniline.

Aniline(97mg.) and ester(130mg.) were left in ether(5ml.) at room temperature for six days, the reaction being followed by disappearance of the ester peak from the infrared spectrum. Ether(5ml.) was added and the solution washed with hydrochloric acid(10ml.,1N) and water(5ml.). After drying (Na_2SO_4) and filtering, evaporation afforded granular crystals of acetanilide(46.2mg.,44%),m.p.114°.

ii) Ethyl glycinate.

Ethyl glycinate(ex.0.14g. hydrochloride) and ester(0.13g.) were dissolved in ether(5ml.). After six days at room temperature the reaction was worked up as above to yield needles of ethyl N-acetylglycinate(91mg.,87%), m.p.38-40°.

iii) Benzylamine.

Benzylamine(91mg.) and ester(123mg.) were dissolved in ether(5ml.) at room temperature for ten hours before working up as above. Needles of N-benzylacetamide(99mg.,98%) were obtained, m.p.57-59°.

O-(N-Methoxycarbonyl)-L-α-alanyl-N,N-dimethylhydroxylamine(XLV,R,R'=CH_3)(E212).

N,N-Dimethylhydroxylamine hydrochloride(0.23g.) was shaken with ammonia in chloroform(ca.2%,10ml.) for twenty hours before filtering and evaporating to small bulk(ca.3ml.) under reduced pressure. The solution was added to L-α-alanine NCA(0.28g.) in anhydrous THF(3ml.) at -40°. Triethylamine(0.23g.) was added and the solution left for three hours. Excess diazomethane in ether was added and the mixture left at 0° overnight. The solution was evaporated down to yield a crystalline mass. Recrystallisation from THF-ether(1:1,1ml.) gave

needles of the hydroxylamine ester(XLV)(201mg., 45%), m.p.69°. $[\alpha]_D^{25}$ -5.91°($CHCl_3$,c.2); $\nu_{max.}$(Nujol) 3270(N-H),1753(ester),and 1720(urethane)cm^{-1}. (Found: C,44.5;H,7.2;N,14.5. $C_7H_{14}N_2O_4$ requires C,44.2;H,7.4;N,14.7%).

O-(N-Methoxycarbonyl)-L-α-alanyl-N-hydroxypiperidine (XLV,R,R'=$(CH_2)_5$)(E236).

L-α-Alanine NCA(0.23g.) and N-hydroxypiperidine(ex. 0.28g. hydrochloride) were dissolved in THF at -40° and after three hours worked up as above. Crystallisation from chloroform-petroleum ether(1:3) gave needles of the hydroxylamine ester(XLV)(0.28g.,61%), m.p.128-130°. $[\alpha]_D^{23}$ -10.83°($CHCl_3$,c.2); $\nu_{max.}$(Nujol) 3300(N-H), 1775(ester) and 1720(urethane)cm^{-1}. (Found: C,52.2;H,7.8;N,12.1. $C_{10}H_{18}N_2O_4$ requires C,52.1; H,7.9;N,12.2%).

G. Preparation of bis-hydroxylamines.

N-Methyl-N-hexylhydroxylamine(XLIX)(E269).

n-Hexyltosylate(2.56g.) and N-methylhyroxylamine hydrochloride(0.84g.) were dissolved in triethylamine(15ml.) and left at room temperature for three days. Excess triethylamine was distilled off and the residue extracted with ether(25ml.), washing with water(10ml.). The dried(Na_2SO_4) extract was filtered and the solvent removed to leave a volatile solid,m.p.34°(1.1g.). A sample (0.5g.) was dissoved in ether(5ml.) and added to a solution of anhydrous oxalic acid(0.35g.) in ether(20ml.). An immediate precipitate formed, which was collected and recrystallised from acetone to yield prisms of N-n-hexyl-N-methylhydroxylamine acid oxalate(0.72g.),m.p.120°. (Found: C,48.8;H,8.5;N,6.1. $C_9H_{19}NO_5$ requires C,48.8; H,8.7;N,6.3%).

N-n-Hexyl-N-methyl-O-benzoylhydroxylamine(E269).

The benzoate was prepared from the above hydroxylamine(ex.2.56g. tosylate) by treatment with benzoyl chloride(1.6g.) in pyridine(10ml.). Working up after three hours at room temperature afforded N-n-hexyl-N-methyl-O-benzoylhydroxylamine (1.54g.) as a colorless oil,b.p. 116° at

0.1mm Hg.; $\nu_{max.}$ 1740(ester)cm^{-1}(film).(Found: C,70.8;H,8.7;N,5.8. $C_{14}H_{21}NO_2$ requires C,71.5; H,9.0;N,5.95%).

Attempted reaction of cyclohexyltosylate with N-methylhydroxylamine(E259).[38]

Cyclohexyltosylate(2.5g.) and N-methylhydroxylamine hydrochloride(0.9g.) in pyridine(10ml.) was left at room temperature for two days. The solution was poured into water(200ml.), the oil precipitated crystallising after several hours. The solid collected was recovered cyclohexyltosylate(2.0g.).

Catechol-O,O'-diacetic ester(LIII)(E251).

Sodium ethoxide(ex.9.2g. sodium) in ethanol(300ml.) was added to a solution of catechol(22g,) in ethanol (100ml.). The mixture was added, under nitrogen, to a solution of ethyl chloroacetate(50g.) in alcohol(100ml.) whilst stirring and heating to reflux. After two hours the mixture was concentrated by distilling off ethanol(200ml.) and then poured into water(250ml.). The mixture was extracted with ether(2x100ml.) and washed with sodium hydroxide (2x100ml.,2N) and water(3x100ml.). The combined extracts were dried(Na_2SO_4), filtered,solvent removed and the residue distilled. The fraction, boiling at 160-163° at 0.8mm.Hg., corresponding

to catechol-O,O'-diacetic ester(LIII) was collected (20.5g.,36%)[39].

O,O'-Di-(2-hydroxy)-ethylcatechol(LV)(E252).

Lithium aluminium hydride(4.2g.) was crushed to a fine powder under anhydrous ether and the slurry(75ml.) stored under nitrogen. To the slurry was added a solution of catechol-O,O'-diacetic ester(27g.) in ether(75ml.) over twentyfive minutes, whilst stirring. A vigorous reaction occurred. After addition the mixture was heated to reflux with stirring for a further one hour before cooling, and carefully adding water(5ml.) to decompose the excess hydride. Sulphuric acid (100ml.,15N) and ether(100ml.) were added and the organic layer separated, the aqueous layer being re-extracted with ethyl acetate(200ml.). The organic layers were washed with saturated ammonium sulphate solution(100ml.) before combining,drying, and filtering. Removal of solvent afforded a crystallising oil.Recrystallisation from ethyl acetate gave colorless needles of O,O'-di-(2-hydroxy)-ethylcatechol(LV)(17.8g., 93%), m.p. 86°; $\nu_{max.}$(Nujol) at 3550,3400(hydroxyl), 1600(aromatic C=C)cm^{-1}. (Found: C,60.7;H,7.2. $C_{10}H_{14}O_4$ requires C,60.6;H,7.1%).

O,O'-Di-(2-hydroxy)-ethylcatechol ditosylate(LIV) (E253)

The diol(LV)(17.0g.) was dissolved in dry pyridine(80ml.) at +5 to 10° with stirring. Tosyl chloride(38g.) was added in portions over thirty minutes. After a total of three hours the mixture was poured into cold water(500ml.) and left at 0° for an hour before filtering, washing with water and collecting the needles formed. After drying the crystals of O,O'-di-(2-hydroxy)-ethylcatecholditosylate(LIV)(38.3g.,77%) were passed to the next stage. A sample was recrystallised from ethyl acetate for analysis, m.p.96°; $\nu_{max.}$(Nujol) at 1602(aromatic C=C),1170 and1360 (tosyl)cm^{-1}. (Found: C,57.3;H,5.4;S,12.9. $C_{24}H_{26}O_8S_2$ requires C,57.0;H,5.2;S,12.7%).

Attempted reaction of ditosylate with N-methyl-hydroxylamine in pyridine(E254).

Ditosylate(LIV)(25.00g.) was dissolved in pyridine(75ml) at room temperature. N-Methyl-hydroxylamine hydrochloride[40] (10g.) was added and the mixture stored under nitrogen, with occasional shaking, for three days. Benzoyl chloride (14.8g.) was added dropwise at 0° to the stirred reaction mixture before allowing to stand at room temperature overnight. The product was poured into

water(500ml.) and extracted with ethyl acetate (2x150ml.). The organic extract was washed with water(100ml.), hydrochloric acid(100ml.),and then more water(100ml.) before drying(Na_2SO_4), filtering, and evaporating off the solvent in vacuo. The pale yellow oil crystallised after leaving at 0° for several days. The product was recrystallised from ether-petroleum ether(1:1,80ml.) to yield colorless prisms of N,O-dibenzoyl-N-methyl hydroxylamine(LVII)(12.5g.),m.p. 56°; $\nu_{max.}$(Nujol) at 1760 and 1680cm^{-1}(diacylhydroxylamine). (Found: C,70.6;H,5.1;N,5.5. $C_{15}H_{14}NO_3$ requires C,70.5;H,5.15;N,5.5%).

O,O'-Di-2-(N-hydroxy-N-methyl)-aminoethylcatechol (L)(E267).

O,O'-Di-(2-hydroxy)-ethylcatecholditosylate(LIV) (5.06g.) and N-methylhydroxylamine hydrochloride (2g.) were treated with triethylamine(25ml) for four days,with occasional shaking, at room temperature. THF(25ml.) was added , the mixture filtered and the filtrate evaporated to dryness in vacuo. The residual oil was dissolved in ethyl acetate(25ml.) and washed with saturated ammonium sulphate solution(2x25ml.), backwashing with more ethyl acetate(25ml.). The combined organic layers

were washed with hydrochloric acid(45ml.,2N) and water(25ml.). The aqueous acid extracts were basified with sodium hydroxide(50ml.,2N) before saturating with ammonium sulphate and extracting with ethyl acetate. On working up as usual needles of O,O'-di-2-(N-hydroxy-N-methyl)-aminoethylcatechol(L) were obtained from THF-petroleum ether(1:1)(1.48g.,58%),m.p.118°; $\nu_{max.}$(Nujol) at 3270(broad,hydroxyl) and 1600 (aromatic C=C)cm^{-1}. (Found: C,56.3;H,7.8;N,11.1. $C_{12}H_{20}N_2O_4$ requires C,56.25;H,7.9;N,10.9%).

Resorcinol-O,O'-diacetic ester[39](LIII)(E255).

The preparation(ex.22g. resorcinol) closely followed the procedure described for the corresponding catechol derivative(see p. 126) giving the ester(23.5g.,42%), b.p. 169-170° at 0.8mm. Hg.

O,O'-Di-(2-hydroxy)-ethylresorcinol(LV)(E262).[41]

Resorcinol-O,O'-diacetic ester(22g.) in ether(50ml.) was added dropwise to a suspension of lithium aluminium hydride(4g.) in ether(75ml.), whilst stirring. The mixture was stirred for a further one hour after addition(thirty minutes) whilst heating to a gentle reflux. The mixture was cooled, water(5ml.) carefully added,followed by

sulphuric acid(100ml.,15N) and then extracted with ethyl acetate(2x150ml.) after saturating with ammonium sulphate. The organic extract was washed with more saturated ammonium sulphate solution (3x100ml.) before drying(Na_2SO_4), filtering and working up. The product(14g.) was directly converted to the ditosylate.

O,O'-Di-(2-hydroxy)-ethylresorcinolditosylate(LIV)(E263).

O,O'-Di-(2-hydroxy)-ethylresorcinol(14g.) in pyridine(70ml.) at +5° was stirred whilst adding tosyl chloride(30g.) over thirty minutes. After a further two hours stirring, with cooling, the solution was poured into water(1l.) and extracted with ethyl acetate(2x100ml.) Working up the organic extract in the usual way gave, from methanol, needles of O,O'-di-(2-hydroxy)-ethyl resorcinolditosylate(LIV)(25.6g.,56%),m.p.99°; $\nu_{max.}$(Nujol) at 1601(aromatic C=C),1360 and 1180(tosylate)cm^{-1}. (Found: C,56.8;H,5.0;S,12.7, $C_{24}H_{26}O_8S_2$ requires C,57.0;H,5.2;S,12.7%).

O,O'-Di-2-(N-hydroxy-N-methyl)-aminoethylresorcinol (LII)(E275).

N-Methylhydroxylamine hydrochloride(3.5g.) was stirred with triethylamine(50ml.) for five minutes before filtering. The clear solution was added to

O,O'-di-(2-hydroxy)-ethylresorcinolditosylate (LIV)(5.1g.) in anhydrous THF(100ml.) and the mixture left at ambient temperature for five days. The solution was washed with saturated ammonium sulphate solution(100ml.) before adding ethyl acetate(200ml.) and extracting with hydrochloric acid(2x25ml.,1N). The acid extract was basified with sodium hydroxide(60ml.,1N) before re-extracting with ethyl acetate(2x150ml.). The organic extract was washed with saturated ammonium sulphate solution(2x50ml.) before drying(Na_2SO_4), filtering,and evaporating to dryness under reduced pressure. Small needles of O,O'-di-2-(N-hydroxy-N-methyl)-aminoethylresorcinol(LII) (0.54g.), m.p. 105°; $\nu_{max.}$(Nujol) at 3400(shoulder) and 3250(hydroxyl) and 1600(aromatic C=C)cm^{-1}. (Found: C,56.4;H,7.7;N,10.8. $C_{12}H_{20}N_2O_4$ requires C,56.25;H,7.9;N,10.9%).

2,3-Dioxy-O,O'-di-(1'-ethoxycarbonyl)-methyl-naphthalene(LIII)(E323).

2,3-Dihydroxynaphthalene(24g.) in absolute ethanol(50ml.) was added to a solution of sodium ethoxide(ex.6.9g. sodium) in ethanol(100ml.). The pale red solution was poured slowly into ethyl chloroacetate(25g.) in ethanol(50ml.) whilst

stirring vigorously under nitrogen. After addition(twenty minutes) the mixture was heated to a gentle refluf for two hours and then ethanol (150ml.) distilled off. The residue was poured into water(200ml.) and extracted with ethyl acetate(2x200ml.), the insoluble material being removed by filtration. The organic extract was washed with water(100ml.), hydrochloric acid (50ml.,1N), sodium bicarbonate solution(50ml.) and water(50ml.). The dried(Na_2SO_4) extract was filtered and after distlling off the solvent afforded a crystalline solid. Recrystallisation from ethanol(30ml.) gave colorless plates of 2,3-dioxy-O,O'-di-(ethoxycarbonyl)-methyl-naphthalene(LIII)(12.5g.,25%), m.p.74°; $\nu_{max.}$ at (Nujol) 1755(ester)cm^{-1}.(Found: C,65.0;H,6.2. $C_{18}H_{20}O_6$ requires C,65.0;H,6.1%).

2,3-Dioxy-O,O'-di-(2'-hydroxy)-ethyl-naphthalene(LV)(E325).

The diester(LIII) (12.5g.) in THF(100ml.) was added, with stirring, to a suspension of lithium aluminium hydride(1.9g.) in ether(50ml.). The mixture was stirred,whilst gently refluxing, for three hours before cooling. Water(5ml.) was carefully added, followed by sulphuric acid(50ml.,15N).

THF(200ml.) and solid ammonium sulphate were added before separating the organic phase,washing with ammonium sulphate solution(2x100ml.),drying (Na_2SO_4) and working up in the usual way. Recrystallisation afforded the naphthalene diol compound(LV) as clorless plates from THF(7.4g., 80%), m.p.146-147°; $\nu_{max.}$(Nujol) at 3350(broad, hydroxyl)cm^{-1}.(Found: C,67.7;H,6.5. $C_{14}H_{16}O_4$ requires C,67.9;H,6.8%).

2,3-Dioxy-0,0'-di-(2'-hydroxy)-ethylnaphthalene ditosylate(LIV)(E326).

Diol(LV)(11g.) in pyridine(60ml.) at 5° was stirred whilst adding portions of tosyl chloride (24g.) over thirty minutes. The mixture was stirred for a further two hours at 0 to 5° before pouring into water(1 l.). Extraction with ethyl acetate (2x100ml.), washing with hydrochloric acid(N), water, sodium bicarbonate solution and water(100ml. portions), was followed by drying(Na_2SO_4), filtering, and removal of solvent. The gummy residue was chromatographed through alumina(200g.) in benzene, eluting with ethyl acetate-benzene(1:3). On evaporation colorless needles of the ditosylate(LIV) formed(21.5g.,86%), m.p.110°; $\nu_{max.}$(Nujol) 1378 and 1180(tosylate)cm^{-1}.

(Found: C,60.4;H,5.2;S,11.6. $C_{28}H_{28}O_8S_2$ requires C,60.4;H,5.1;S,11.5%).

2,3-Dioxy-O,O'-di-(N-hydroxy-N-methyl)-amino-ethylnaphthalene(LI)(E330).

Ditosylate(19.0g.) and N-methylhydroxylamine (ex.8.0g.hydrochloride) in anhydrous THF(150ml.) were treated with triethylamine(60ml.) under ni nitrogen at room temperature for six days. The crystals that formed were collected and washed with ether(50ml.). The combined mother liquors were concentrated to small bulk, dissolved in ethyl acetate(2x100ml.) and extracted with hydrochloric acid(100ml.,2N). On basification with sodium hydroxide(60ml.,4N) and cooling at 0° a further crop of crystals formed. The combined material was recrystallised from THF(200ml) and petroleum ether(100ml.) to give needles of the bis-hydroxylamine(LI)(2.64g.,25%),m.p. 184°; $\nu_{max.}$(Nujol) 3270(broad,hydroxyl),1630 and 1603(aromatic C=C)cm^{-1}.(Found: N,9.42 and 9.31. $C_{16}H_{22}N_2O_4$ requires N,9.15%). A repeated reaction afforded the bis-hydroxylamine in 61.7% yield.

Dibenzoylation of naphthalene derived bishydroxylamine(E338)

Benzoyl chloride(1.8g.) in benzene(15ml.) was added to the bishydroxylamine(LI)(1.7g.) suspended in benzene(20ml.) and saturated sodium bicarbonate solution(20ml.) whilst stirring vigorously under nitrogen. The reaction was stirred for one hour, solid sodium bicarbonate being added as required to maintain alkalinity. The benzene layer was separated and washed with water(3x10ml.),dried (Na_2SO_4), filtered, and finally chromatographed through alumina(25g.) eluting with ether-benzene (1:9). The major fraction was dissolved in ether (10ml.) and petroleum ether added until the solution became cloudy. On cooling to 0^o fine needles of dibenzoyl 2,3-dioxy-O,O'-di-2'-(N-hydroxy-N-methyl)-aminoethylnaphthalene were formed(2.3g.,79%), m.p. 45-46°; $\nu_{max.}$ 1740(ester) and1630,1603 and 1590(aromatic C=C),(in Nujol).(Found: C,69.6; H,6.3;N,5.3. $C_{30}H_{30}N_2O_6$ requires C,7o.0;H,5.9; N,5.45%).

Attempted half aminolysis of dibenzoate(E339).

Dibenzoate(1.54g.) was left with benzylamine (0.32g.) in benzene(20ml.) for two days at room temperature. A strong amide band had appeared in

the infrared spectrum by this time. The solution was reduced to small bulk and chromatographed through alumina(75g.,Brockmann grade V) in benzene. Elution with benzene gave N-benzylbenzamide (0.52g.,82%),m.p. and mixed m.p. 108-109°. Ethyl acetate- benzene(1:9) afforded dibenzoate (0.23g.) followed by a pale brown fraction (0.31g.) that appeared to be a mixture. This latter fraction could not be purified. A large amount of material could not be eluted from the column.

H. Preparation and reactions of amido-dialkyl-hydroxylamines.

N-(2-Phthalimido)-ethyl-N-methylhydroxylamine(LX) (E357).

N-(2-Hydroxy)-ethylphthalimide tosylate[42] (34g.) was dissolved in dimethylformamide(200ml.) and N-methylhydroxylamine hydrochloride(12.5g.) and anhydrous potassium acetate(26.6g.) added. The mixture was thoroughly mixed before heating at 100° for twenty hours. The product was poured into water(1 l.), extracted with ethyl acetate (2x250ml.), washed with water(100ml.), dried(Na_2SO_4), filtered and evaporated to small bulk. The oily product was extracted with hydrochloric acid (200ml.,N),separated from the organic phase and

the aqueous extract basified with sodium carbonate(200ml.,2N). Extraction with ethyl acetate afforded a pale yellow solid which recrystallised from ethanol as colorless needles of the hydroxylamine(LX)(6.37g.,30%),m.p.156°; $\nu_{max.}$(Nujol) at 3200(hydroxyl),1765 and 1708 (phthalimide)cm^{-1}.(Found: C,60.1;H,5.25;N,12.3. $C_{11}H_{12}N_2O_3$ requires C,60.0;H,5.5;N,12.7%).

N-(2-Amino)-ethyl-N-methylhydroxylamine bishydrochloride(LVIII)(E366).

Hydrazine hydrate(1.8g.) was added to a stirred solution of hydroxylamine(LX)(7.15g.) in acetonitrile (40ml.) at room temperature. The mixture was heated to a gentle reflux for one hour before cooling and filtering. Hydrochloric acid(80ml., 3N) was added and the solution evaporated to small bulk under reduced pressure, re-filtered and evaporated to dryness. The residual oil crystallised at 0° and was recrystallised from ethanol to give colorless plates of the bishydrochloride(LVIII)(1.56g.,30%), m.p. 138°. (Found: C,21.6;H,7.3. $C_3H_{10}N_2O.2HCl$ requires C,22.1;H,7.4%).

N,O-Diacetyl-N-(2-amino)-ethyl-N-methylhydroxylamine(LXIV)(E370).

N-(2-Amino)-ethyl-N-methylhydroxylamine bis. hydrochloride(1.02g.) was added to saturated sodium bicarbonate solution(25ml.) containing acetic anhydride(2.8ml.) at room temperature. Whilst stirring solid sodium bicarbonate was added in portions to maintain alkalinity. After thirty minutes the solution was saturated with ammonium sulphate and extracted with ethyl acetate(4x15ml.). On working up in the usual manner the diacetate(LXIV) was obtained as a colorless oil(0.8g.), b.p. 80^{o} at $2x10^{-3}$mm. Hg. $\nu_{max.}$(film) at 3380(N-H), 1760(ester) and 1665(amide)cm^{-1}. (Found: C,48.3; H,8.1;N,16.4. $C_7H_{14}N_2O_3$ requires C,48.3;H,8.1; N,16.1%).

N-(2-Acetamido)-ethyl-N-methylhydroxylamine acid oxalate(LXV)(E371).

Diacetate(LXIV)(0.54g.) and benzylamine(0.34g.) were dissolved in chlorform(5ml.) at room temperature for one day. The clear solution was poured into a solution of anhydrous oxalic acid(0.34g.) in ethyl acetate(10ml.). The white precipitate that formed was collected and recrystallised from ethanol(10ml.) to give fine needles of N-(2-acetamido)-

ethyl-N-methylhydroxylamine acid oxalate(LXV) (0.57g.,84%),m.p. 137°. (Found: C,38.0;H,6.3; N,12.65. $C_7H_{14}N_2O_6$ requires C,37.9;H,6.35;N,12.6%).

N-(2-Phthalimido)-ethyl-N-methyl-O-(N-methoxycarbonyl)-glycylhydroxylamine(LXI)(E372).

N-(2-Phthalimido)-ethyl-N-methylhydroxylamine (216mg.) was dissolved in THF at -40° before adding glycine NCA(113mg.) in THF(5ml.) and triethylamine(120mg.). After three hours the solution was warmed to 0° and excess diazomethane in ether added. After leaving at 0° overnight the solvent was removed by evaporation and the product treated with ethyl acetate, centrifuged, and the supernatant liquor concentrated *in vacuo* to give a crystallising oil. Recrystallisation from ethyl acetate-ether(1:2) at 0° gave colorless needles of the N-methoxyglycyl ester(LXI)(221mg., 67%), m.p. 113-115°. $\nu_{max.}$(Nujol) 3320(N-H), 1760 and 1715(ester and imide)cm^{-1}.(Found: C,53.8; H,5.3;N,12.3. $C_{15}H_{17}N_3O_6$ requires C,53.75;H,5.1; N,12.5%).

N-(2-Phthalimido)-ethyl-N-methyl-O-(N-benzyloxycarbonyl)-glycylhydroxylamine(LXII)(E377).

Hydroxylamine(LX)(0.22g.) and N-benzyloxycarbonylglycine(0.22g.) were dissolved in ethyl

acetate(15ml.) at room temperature before adding dicyclohexylcarbodiimide[43](0.32g.). After three hours glacial acetic acid(0.5ml.) was added and the mixture left for a further fifteen minutes before filtering and washing the solution with water, hydrochloric acid(1N), and sodium bicarbonate solution(saturated)(5ml. portions). After drying(Na_2SO_4) the solution was filtered and evaporated down to a crystallising oil. Recrystallisation from ethyl acetate-ether(1:2) gave needles of N-(2-phthalimido)-ethyl-N-methyl-O-(N-benzyloxycarbonyl)glycylhydroxylamine(LXII) (0.32g.,78%), m.p. 116-117°. $\nu_{max.}$(Nujol) at 3360(N-H), 1765(ester and imide),1718(urethane) and 1705(imide) cm^{-1}. (Found: C,61.1;H,4.9;N,10.0. $C_{21}H_{21}N_3O_6$ requires C,61.3;H,5.1;N,10.2%).

Reaction with benzylamine.

The ester(0.14g.) with benzylamine(0.051g.) in THF(5ml.) at room temperature for twenty hours gave, on working up, N-benzyloxycarbonylglycyl-(N-benzyl)-amide(0.10g.,98%) as needles, m.p.114-115°(lit. m.p. 116-117°)[44]. The hydroxylamine (LX)(49mg.,63%) was also obtained,m.p. 156°. Both products had identical infrared spectra to authentic samples.

Attempted preparation of N-(2-phthalimido)-ethyl-N-methyl-O-glycylhydroxylamine hydrobromide(E387).

Ester(LXII)(0.22g.) was dissolved in nitromethane (4ml.) and hydrogen bromide in glacial acetic acid (0.25ml.,45%w/v) added. After fourty hours at room temperature the needles that formed were collected and washed with ether(50ml.). The hydrobromide was not obtained analytically pure. Recrystallisation from acetic acid caused decomposition. The product(86mg.) had m.p. 126-128°; $\nu_{max.}$(Nujol) at 1765(ester),1760(shoulder,imide) and 1703(imide) cm^{-1}.

1-Tosyl-2-phenyloxazolidine(LXIII)(E360).

To a stirred solution of 2-aminoethanol-benzylidine[45](10.0g.) in pyridine(30ml.) at 0 to +5° was added, portionwise, tosyl chloride(15g.) over one hour. The mixture was stirred for a further thirty minutes before pouring into water. The precipitated solid was collected and recrystallised from ethyl acetate as prisms of 1-tosyl-2-phenyloxazolidine(LXIII)(16.8g.,83%), m.p. 143°; $\nu_{max.}$(Nujol) at 1600(aromatic C=C), 1350 and 1160(tosyl)cm^{-1}. N.M.R.(in $CDCl_3$)spectrum had τ 7.54(singlet,aryl methyl),6.7-6.0(complex, methylene envelope) and 3.69(singlet,ArC<u>H</u> $\overset{N}{O}$).

(Found: C,63.9;H,5.8;N,4.7;S,10.9. $C_{16}H_{17}NO_3S$ requires C,63.4;H,5.65;N,4.6;S,10.6%).

1-Cyano-1-(N-methyl)-hydroxylaminocyclohexane(LXVI) (E384).

Cyclohexanone(0.25g.) in ethanol(5ml.) was added to a suspension of sodium cyanide and N-methylhydroxylamine hydrochloride(0.24g.) in aqueous ethanol(2ml. H_2O in 10ml.). After stirring overnight at room temperature the mixture was distilled to small bulk *in vacuo* , THF(5ml.) added and filtered. Evaporation of the solution afforded a crystallising oil. The product was collected, washed with water and dried over sodium hydroxide to give prisms of the nitrile (LXVI)(0.33g.,84%),m.p. 73-76°. A sample was sublimed at 65° and2×10^{-3}mm. Hg.,m.p.77°. (Found: C,62.2;H,9.1;N,18.05. $C_8H_{14}N_2O$ requires C,62.3;H,9.15;N,18.2%). $\nu_{max.}$(Nujol) at 3300 (hydroxyl),2265(nitrile)cm^{-1}.

1-Carbamoyl-1-(N-methyl)-hydroxylaminocyclohexane (LXVII)(E407).

The nitrile(LXVI)(1.50g.) was added, in portions, to cold, concentrated sulphuric acid (10ml.), with shaking and cooling. After four hours at room temperature the solution was poured into crushed ice(150g.). Whilst cooling,ammonia

(15ml.,conc.) was added until the mixture was alkaline. The solution was extracted with ethyl acetate (2x50ml.) and working up of the extract in the usual way gave prisms of 1-carbamoyl-1-(N-methyl)-hydroxylaminocyclohexane(LXVII) (0.79g.,47%), m.p. 126-129°, which on further recrystallisation from ethyl acetate rose to 139°. $\nu_{max.}$(Nujol) at 3460 and 3200(amideN-H), 3300(hydroxyl) and 1663(amide)cm^{-1}. (Found: C,56.2;H,9.3;N,16.25. $C_8H_{16}N_2O_2$ requires C,55.8; H,9.4;N,16.3%).

1-Carbamoyl-1-(N-methyl)-aminocyclohexane(LXXXII) (E423).

Hydroxylamino-amide(LXVII)(49mg.) in ethanol(3ml.) was hydrogenated at room temperature and atmospheric pressure in the prescence of palladium on charcoal(19mg.,10%) and two drops of hydrochloric acid(conc.)[46]. After thirtyfive minutes, when hydrogen uptake had ceased(5.5ml.), solid sodium bicarbonate(ca. 0.2g.) was added and the mixture shaken for a further ten minutes. The mixture was filtered after adding ethyl acetate(3ml.). Evaporation gave colorless needles of the amine (LXXXII)(24mg.,59%),m.p. 134°. $\nu_{max.}$(Nujol) at 3430,3330,3180(N-H), and 1665(amide)cm^{-1}. (Found: C,61.5;H,10.2;N,18.1. $C_8H_{16}N_2O$ requires

C,61.5;H,10.1;N,17.9%).

1-Cyano-1-(N-methyl-O-acetyl)-hydroxylamino-cyclohexane(LXXI)(E431).

The cyclohexylnitrile(LXVI)(0.42g.) in ethyl acetate(10ml.) was stirred whilst adding saturated sodium bicarbonate solution(10ml.) and acetic anhydride(1.0ml.). Solid sodium bicarbonate(ca.0.5g.) was added in portions over thirty minutes to maintain alkalinity. The organic layer was separated, re-extracting the aqueous layer with more ethyl acetate(2x10ml.). The combined extracts were dried (Na_2SO_4), filtered,and evaporated down to leave a colorless oil. Trituration with petroleum ether gave needles of the acetate(LXXI)(0.52g., 91%), m.p. 52°.A sample was sublimed at 60° and 10^{-3}mm. Hg. to give $\nu_{max.}$($CHCl_3$) 2260(nitrile), 1765(ester) cm^{-1}. (Found: C,61.3;H,8.2;N,14.2. $C_{10}H_{16}N_2O_2$ requires C,61.2;H,8.2;N,14.3%).

1-Carbamoyl-1-(N-methyl-O-acetyl)-hydroxyl-aminocyclohexane(LXXII)(E429).

The cyclohexylcarbamide(LXVII)(0.50g.) was acetylated with acetic anhydride(1.0ml.) by the above procedure. Working up gave a crystalline product, which gave,after recrystallisation from ethyl acetate-petroleum ether(1:1), needles

of 1-carbamoyl-1-(N-methyl-O-acetyl)-hydroxylamino-cyclohexane(LXXII)(0.49g.,88%), m.p. 108°. $\nu_{max.}$(Nujol) at 3500,3300(N-H), and 1760(ester) and 1690(amide)cm^{-1}. (Found: C,55.8;H,8.3;N,12.8. $C_{10}H_{18}N_2O_3$ requires C,56.1;H,8.5;N,13.1%).

1-Carbamoyl-1-(N-methyl-O-benzyloxycarbonyl)-hydroxylaminocyclohexane(LXXIII)(E428).

Benzylchloroformate in toluene(2ml.,2N) was added dropwise to a stirred mixture of the cyclohexyl-amide(LXVII) in ethyl acetate(5ml.) and saturated sodium bicarbonate solution(10ml.). After addition(five minutes) sodium bicarbonate (0.3g.) was added and the mixture then left at room temperature for thirty minutes. The product was extracted in the usual way. Recrystallisation from THF-petroleum ether(1:2) gave needles of the benzyloxycarbonate(LXXIII), m.p. 91-2° (0.38g.,65%). $\nu_{max.}$(Nujol) at 3500,3200(N-H), 1760(carbonate), and 1673(amide) cm^{-1}.

Action of potassium t-butoxide on the benzyl-carbonate(LXXIII)(E430).

Benzylcarbonate(LXXIII)(0.23g.) in anhydrous THF(5ml.) was treated with potassium t-butoxide (0.8ml.,1.25N) at room temperature for one hour. Glacial acetic acid(0.2ml.) was added,

followed by ethyl acetate(10ml.). The mixture was washed with water(5ml.),saturated sodium bicarbonate solution(5ml.) and finally water(5ml.). After drying(Na_2SO_4) the organic extract was filtered and evaporated to dryness in vacuo. The product was recrystallised from ether-petroleum ether(2:3) to yield colorless needles of 1,4-diaza-2-oxa-1-methyl-spiro[5,5]undecane-3,5-dione(LXXIV)[47](96mg.,65%), m.p. 128°. $\nu_{max.}$(Nujol) at 3250,3150(N-H), and 1750,1710 (imide) cm^{-1}.(Found: C,54.6;H,7.1;N,14.2. $C_9H_{14}N_2O_3$ requires C;54.5;H,7.12;N,14.1%).

Attempted reaction of the benzylcarbonate(LXXIII) with triethylamine(E430).

A solution of the benzylcarbonate(LXXIII)(87mg.) and triethylamine(0.4ml.) in chloroform(10ml.) showed no change in the infrared spectrum after three days at room temperature.

O,O'-Di-[N-methyl-N-(1-carbamoyl)-cyclohexyl]-hydroxylaminocarbonate(LXXV)(E437).

Cyclohexylcarbamide(LXVII)(0.3g.) was stirred in ethyl acetate(10ml.) and saturated sodium bicarbonate solution(10ml.). Phosgene in toluene (5ml.,15%w/v.) was added and the mixture stirred with sodium bicarbonate(0.5g.) for fourty minutes

before working up in the normal manner, extra ethyl acetate(25ml) having been used in the extraction. Recrystallisation from ethyl acetate afforded prisms of O,O'-di-[N-methyl-N-(1-carbamoyl)-cyclohexyl]-hydroxylamino carbonate (LXXV)(0.29g.,88%), m.p.153°. $\nu_{max.}$(Nujol) at 3460,3255(N-H),1785(carbonate), and 1682(amide)cm^{-1}. (Found: C,55.2;H,8.1;N,15.0. $C_{17}H_{30}N_4O_5$ requires C,55.2;H,8.2;N,15.1%).

1-Carbamoyl-1-[N-methyl-O-(N-benzyloxycarbonyl)-glycyl]-hydroxylaminocyclohexane(LXXXVI)(E411).

The cyclohexylcarbamide(LXVII)(0.176g.) and N-benzyloxycarbonylglycine(0.217g.) in ethyl acetate (10ml.) at -10°were treated with dicyclohexylcarbodiimide(0.225g.). The mixture was left to warm to 0° over three hours before adding glacial acetic acid(0.2ml.) and,after a further ten minutes, filtering off the urea precipitate. The solution was washed with sodium bicarbonate solution(5ml.) and water(2x5ml.) before drying(Na_2SO_4), filtering and evaporating to dryness. The produuct crystallised after trituration with petroleum ether and leaving at 0° over several days. Recrystallisation from THF-petroleum ether(3:2) gave needles of the glycyl ester(LXXVI)(0.30g.,

84%), m.p. 106°. $\nu_{max.}$(Nujol) at 3630, 3500, 3250 (N-H), 1765(ester), 1735(urethane), and 1670 (amide)cm^{-1}. (Found: C,59.65;H,7.0;N,11.2. $C_{18}H_{25}N_3O_5$ requires C,59.5;H,7.0;N,11.6%).

Fission and rearrangement of the glycyl ester (LXXVI)(E420).

The ester(0.77g.) was dissolved in nitromethane (4.5ml.) before adding hydrogen bromide in acetic acid(1.1ml.,45%w/v). After leaving at room temperature overnight anhydrous ether(10ml.) was added and the solvent removed from the precipitate by decantation. After washing with more ether(4x10ml.) the precipitate was dissolved in dimethylformamide (4ml.) and poured into THF(200ml.) containing triethylamine(4ml.). The mixture was left at ambient temperature for twenty hours before filtering and evaporatimg to small bulk in vacuo. Ethyl acetate(10ml.) was added and the solution washed with water(10ml.) and dried(Na_2SO_4). The oil obtained from the solution on working up showed a broad absorption at 1650-1690cm^{-1} in the infrared spectrum. Thin film chromatography on silica gel G(acetone-petroleum ether) showed two main spots sensitive to silver nitrate(R_f0.12 and 0.35). The oil was chromatographed through

silica gel gave a fraction(eluted by acetone) that gave glycine as one of its acid hydrolysis products, as indicated by paper chromatography.[16]

1-Carbamoyl-1-[N-methyl-O-(N-benzyloxycarbonyl)-DL-phenylalanyl]-hydroxylaminocyclohexane(LXXVIII)(E425).

The cyclohexylamide(LXVII)(0.80g.) was coupled to N-benzyloxycarbonyl-DL-phenylalanine(1.39g,) with dicyclohexylcarbodiimide(1.1g.) in ethyl acetate(10ml.) at room temperature. Working up in th usual manner gave, from benzene-ether(1:2) needles of the phenylalanylester(LXXVIII) (1.01g.,48%), m.p.138°. $\nu_{max.}$(Nujol) at 3520, 3470(N-H), 1755(ester), 1720(urethane), 1680 (amide) cm^{-1}. (Found: C,66.0;H,7.0;N,9.1. $C_{25}H_{31}N_3O_5$ requires C,66.2;H,6.9;N,9.3%).

Fission of ester(LXXVIII) with hydrogen bromide(E426).

The ester(0.71g.) in nitromethane(8ml.) at room temperature was treated with hydrogen bromide in acetic acid(1.2ml.,45%w/v). After four hours anhydrous ether(50ml.) was added. The solvent was decanted from the amorphous precipitate, which was washed with further quantities of ether(4x50ml.). The precipitate was dissolved in acetone. After leaving at 0° overnight needles of 1-carbamoyl-1-(N-methyl-O-phenylalanyl)-hydroxylaminocyclo-

hexane hydrobromide(LXXVII)(0.217g.,35%) formed. The needles were washed with acetone and dried *in vacuo* to give m.p. 132-134°(decomp.). $\nu_{max.}$(Nujol) at 1760(ester) and 1684(amide) cm^{-1}. (Found: Br,19.7. $C_{17}H_{26}N_3O_3Br$ requires Br,19.9%).

Rearrangement of the phenylalanyl ester(LXXVII)(E442).

i) Triethylamine.

The hydrobromide(LXXVII)(0.136g.) was shaken with triethylamine(0.4g.) and chloroform (9.6ml.) at room temperature. The reaction was followed by scanning the infrared spectrum at intervals. After fourteen days, the time taken for the ester peak to disappear, the mixture was filtered and extracted with hydrochloric acid(5ml,,2N). Work-up of the organic phase gave a gummy solid(25mg.), of similar infrared properties to phenylalanine anhydride. The acid extract was basified with sodium bicarbonate and re-extracted with ethyl acetate to yield, on working up, a pale yellow gum(27mg.) that proved to be a mixture of starting ester and 1-carbamoyl-1-(N-methyl)-hydroxylaminocyclohexane(LXVII) from its infrared spectrum. Thin-film chromatography of the latter fraction afforded two main spots, after elution with acetone-petroleum ether and development with aqueous silver nitrate, at

R_f 0.1(weak) and 0.30(strong), the latter being identical to authentic amide(LXVII).

ii) Potassium t-butoxide.

To a suspension of the hydrobromide(0.30g.) in THF(8ml.) at room temperature was added potassium t-butoxide in t-butanol(1.5ml.,1N). After two hours glacial acetic acid(0.15ml.) was added and the mixture poured into ethyl acetate(15ml.). The mixture was washed with saturated sodium bicarbonate solution(2x5ml.) and water(5ml.) before drying (Na_2SO_4), filtering,and evaporating down to a gum which crystallised on standing. Recrystallisation from ethyl acetate gave needles of 1-(N-methyl)-hydroxylaminocyclohexylcarbonylphenylalanylamide (LXXIX)(79mg.,33%), m.p. 169°. $\nu_{max.}$(Nujol) at 3510,3400(N-H),3220(hydroxyl),1680 and 1660 (amides) cm^{-1}. (Found: C,63.9;H,8.0;N,13.0. $C_{17}H_{25}N_3O_3$ requires C,63.9;H,7.9;N,13.2%). Thin-film chromatography on silica gel G with acetone-petroleum ether(1:2) showed a spot at R_f 0.16 to aqueous silver nitrate.

Reduction of the rearranged amide(LXXIX)(E443).

The hydroxylamine(65.9mg.) in ethanol(3ml.) containing hydrochloric acid(0.25ml.,1N) was hydrogenated at atmospheric pressure over palladium

on charcoal(25mg.,10%,prereduced). After one hour the reduction was stopped(4.8ml.uptake),the suspension filtered and water(5ml.) added. The solution was washed with ethyl acetate(5ml.) before basifying with sodium bicarbonate. The solution was extracted with ethyl acetate(2x5ml.),dried (Na_2SO_4), filtered, and evaporated to dryness. On trituration with petroleum ether colorless crystals formed(44mg.,71%). Recrystallisation from ethyl acetate gave needles of 1-(N-methyl)-aminocyclohexylcarbonylphenylalanylamide(LXXX), m.p. 160°. $\nu_{max.}$(Nujol) at 3350,3200(N-H),1690 and 1640(amides) cm^{-1}.(Found: C,67.0;H,8.35; N,14.1. $C_{17}H_{25}N_3O_2$ requires C,67.3;H,8.3;N,13.9%).

Attempted rearrangements of the ester(LXXVII)(E445).

Attempts to produce rearrangement in aqueous sodium bicarbonate or carbonate failed. With sodium hydroxide(0.05N) some rearranged product formed. The reactions were followed by thin-film chromatography under the conditions described above.

1-Hydroxyl-2-carbamoyl-4,5,5-trimethylpyrrolidine (LXXXV)(E446).

1-Hydroxy-2-cyano-4,5,5-trimethylpyrrolidine[49] (LXXXIV)(2.7g.) was added to cold, concentrated sulphuric acid(10ml.) with shaking. The solution

was left at room temperature for one hour and then heated to 60° for thirty minutes. The solution was cooled to 0° before pouring into water(20ml.). Concentrated ammonia solution(20ml.,'0.88') was added with cooling before extracting with ethyl acetate and working up the normal way. The amide (LXXXV)(1.85g.,62%) was obtained as prisms from ethyl acetate, m.p.163°. $\nu_{max.}$(Nujol) at 3400, 3230(N-H and hydroxyl) and 1690(amide)cm^{-1}.

1-(O-Benzyloxycarbonyl)-hydroxy-2-carbamoyl-4,5,5-trimethylpyrrolidine(LXXXVI)(E450).

The amide(LXXXV)(0.697g.) was suspended in ethyl acetate(10ml.) and saturated sodium bicarbonate solution(15ml.) and benzylchloroformate in toluene(2.5ml.,2N) added with stirring at room temperature. Sodium bicarbonate(0.5g.) was added in portions tomaintain alkalinity. After thirty minutes the solution was worked up in the usual way to give prisms of the benzylcarbonate(LXXXVI) (1.02g.,82%), m.p. 121° from ethyl acetate.

$\nu_{max.}$(Nujol) at 3450,3200(N-H), 1755(carbonate), and 1680(amide) cm^{-1}.(Found: C,63.0;H,7.1;N,9.1. $C_{16}H_{22}N_2O_4$ requires C,62.7;H,7.2;N,9.1%).

Action of base on the benzylcarbonate(LXXXVI)(E452).

To a solution of the benzylcarbonate(LXXXVI) (0.31g.) in THF(10ml.) was added potassium t-butoxide(0.55ml., 2N) in t-butanol. After fifteen minutes at room temperature glacial acetic acid (0.2ml.) was added and the mixture poured into water(10ml.) before working up in the usual way by ethyl acetate extraction. The isolated crystallising oil gave, after sublimation at 65° and 10^{-4}mm. Hg., fine needles of 2-carbamoyl-4,5,5-trimethyl-Δ^1-pyrroline(LXXXVII)(0.123g., 80%), m.p. 123-124°. $\nu_{max.}$(Nujol) at 3350,3200 (N-H), 1695 and 1660(amide) and 1620(imine)cm^{-1}. N.M.R.($CDCl_3$) gave τ9.05 and 8.91(doublet,secondary methyl,J=7c.p.s.), 8.94 and 8.70(singlets, tertiary methyls),and 2.75 and 3.5(broad, primary amide). (Found: C,62.4;H,9.1;N,18.5. $C_8H_{14}N_2O$ requires C,62.3;H,9.1;N,18.2%).

1- O-(N-Benzyloxycarbonyl)-DL-phenylalanyl-hydroxy-2-carbamoyl-4,5,5-trimethylpyrrolidine (LXXXVIII)(E453).

Amide(LXXXV)(1.28g.) and N-benzyloxycarbonyl-DL-phenylalanine(2.21g.) were dissolved in THF(40ml.) and cooled to 0° before adding dicyclohexylcarbodiimide(1.56g.). After twenty hours at 0° glacial

acetic acid(0.2ml.) was added and the mixture left at room temperature for a further thirty minutes before filtering. The solution was poured into ethyl acetate(50ml.) and washed with water(50ml.) and saturated sodium bicarbonate solution(25ml.). after drying(Na_2SO_4) and filtering, evaporation afforded a crystallising gum. Trituration with petroleum ether followed by recrystallisation from ethyl acetate-petroleum ether(1:1) gave needles of the ester(LXXXVIII), m.p.137^0(1.67g.,50%). $\nu_{max.}$(Nujol) at 3400,3140(N-H), 1750(ester), 1705(urethane), and 1680(amide)cm^{-1}. (Found: C,66.6; H,6.9;N,9.55. $C_{25}H_{31}N_3O_5$ requires C,66.2;H,6.9; N,9.3%).

1-Carbamoyl-(N-methyl)-hydroxylaminocyclopentane (LXXXIX)(E459).

Cyclopentanone(8.4g.) was added to a solution of N-methylhydroxylamine hydrochloride(10.0g.) and sodium cyanide(5.0g.) in aqueous ethanol(25ml., 1:1) at 0^o and the solution left to warm to room temperature overnight. The solution was extracted with ethyl acetate(50ml.) and worked up in the usual way to give an oil(2.5g.) that could not be distilled. The product was dissolved in cold, concentrated sulphuric acid(10ml.) and left at

ambient temperature overnight. The solution was poured slowly into ice-water(30g.), with cooling, before adding ammonia solution(30ml.,conc.). The solution was saturated with ammonium sulphate before extracting with ethyl acetate(4x50ml.). The combined extracts were dried(Na_2SO_4), filtered, and evaporated to afford needles. Recrystallisation from ethyl acetate gave prisms of 1-carbamoyl-(N-methyl)-hydroxylaminocyclopentane(LXXXIX)(0.43g.), m.p. 115°. $\nu_{max.}$(Nujol) at 3430,3200(N-H), 3330 (broad,hydroxyl), and 1660(amide)cm^{-1}. (Found: C,53.2;H,8.65;N,17.9. $C_7H_{14}N_2O_2$ requires C,53.1; H,8.9;N,17.7%).

Coupling of the amide(LXXXIX) with N-benzyloxy-carbonyl-DL-phenylalanine(E461).

To a solution of the amide(LXXXIX)(0.71g.) and N-benzyloxycarbonylphenylalanine(1.5g.) in ethyl acetate(15ml.) was added dicyclohexylcarbodiimide (1.1g.) in ethyl acetate(5ml.) at room temperature. After four hours glacial acetic acid(0.2ml.) was added and the mixture left for a further thirty minutes before filtering and working up in the usual manner. Recrystallisation from ethyl acetate-petroleum ether gave needles of 1-carbamoyl-[N-methyl-O-(N-benzyl-oxycarbonyl)-phenylalanyl]-hydroxylaminocyclopentane

(XC)(1.52g.,77%), m.p.107°. $\nu_{max.}$(Nujol) at 3430,3310,3210(N-H), 1765(ester), 1715(urethane), and 1675(amide)cm^{-1}. (Found: C,65.6;H,6.8;N,9.9. $C_{24}H_{29}N_3O_5$ requires C,65.6;H,6.7;N,9.6%).

1-(N-Methyl)-hydroxylaminocyclopentylcarbonyl-DL-phenylalanylamide(XCII)(E464).

The ester(XC)(0.56g.) was dissolved in nitromethane(4ml.) and hydrogen bromide in glacial acetic acid(0.6ml.,45%w/v.) added at 0°. On leaving a granular solid precipitated out. After two hours the solvent was decanted off and the solid washed with ether(3x15ml.). The crystals were hygroscopic. The product(0.32g.) was dissolved in anhydrous THF (10ml.) and potassium t-butoxide in t-butamol(5ml., 1N) added. After two hours at room temperature glacial acetic acid(0.2ml.) was added and the mixture poured into ethyl acetate(25ml.) before washing with saturated sodium bicarbonate solution(10ml.) and working up in the usual way. The product was a gum. Chromatography through silica gel G(20g.,'Woelm') using acetone-petroleum ether(1:3,200ml.) as eluant gave, from the latter fractions, prisms of the cylcopentylamide derivative(XCII)(68mg.,17%), m.p. 182-184°. Recrystallisation from ethyl acetate gave m.p. 185°, $\nu_{max.}$(Nujol) at 3450,3340(N-H),

3250(broad,hydroxyl), 1700,and 1680(amides)cm^{-1}. (Found: N, 13.6. $C_{16}H_{23}N_3O_3$ requires N,13.8%). The product gave a single spot at R_f0.19 by thin-film chromatography on silica gel G using acetone-petroleum ether as solvent(1:2).

Reaction of DL-phenylalanine NCA with 1-carbamoyl-1-(N-methyl)-hydroxylaminocyclohexane(LXVII)(E463).

Phenylalanine NCA[48] (0.12g.) was added to a solution of amide(0.117g.) in THF(5ml.) at -30°. To the solution was added triethylamine(0.5ml.) before warming to-20° and leaving for six hours. The solution was warmed to room temperature for thirty minutes before adding potassium t-butoxide in t-butanol(2ml.,1N) and leaving for a further hour at room temperature. Glacial acetic acid(0.2ml.) was added before pouring into ethyl acetate(15ml.) and working up in the usual manner. The colorless gum obtained was dissolved in ethyl acetate(3ml.) and slowly evaporated to give gummy prisms. Washing with petroleum ether gave colorless prisms of 1-(N-methyl)-hydroxylaminocyclohexylphenylalanyl amide(LXXIX)(42.3mg.,21%), m.p. 158-162°.

1-Carbamoyl- N-methyl-O-(n-benzyloxycarbonyl)-L-phenylalanyl -hydroxylaminocyclohexane(E460).

The amide(LXVII)(0.56g.) was coupled to

N-benzyloxycarbonyl-L-phenylalanine[50] under the conditions described for the racemic form. The product could not be crystallised(1.31g.,89%), $[\alpha]_D^{18}$ 6.05°($CHCl_3$, c.2).

Cleavage and rearrangement of optically active ester(E466). - The ester(1.3g.) was cleaved with hydrogen bromide in nitromethane by the method described for the racemic form. The ether precipitate was used directly in the rearrangement. Working up of the potassium t-butoxide rearranged material afforded a gum. Chromatography through silica gel G (20g.) using acetone-petroleum ether(1:2) gave, on working up, prisms of rearranged amide(0.174g., 18.4%), m.p. 155-162°. The product was twice recrystallised from ethyl acetae but gave no optical rotation.

Hydrolysis of the rearranged product from the L-ester. (E467).

The amide(80.2mg.) was heated with hydrochloric acid(3ml.,8N) at 100° for twenty hours. Chromatography through a cation exchange resin (40g.,Amberlite IR 120(H)), using water and then 0.5N ammonium hydroxide and collecting 5ml.fractions, gave, as indicated by paper chromatography, phenylalanine. The product was recrystallised from aqueous acetone as small plates(23mg.), optically inactive.

References.

1. Mecke,Mecke, and Luttringhaus, Chem.Ber., 1957,90,975.

2. Bost,Turner, and Norton, J.A.C.S.,1932,54,1985.

3. Ray and Mitra, J.Indian Chem.Soc.,1929,6,865.

4. Chapman and Owen, J.,1950,579.

5. Whittaker, Biochem.J.,1947,41,56.

6. Bost,Turner, and Conn, J.A.C.S.,1933,55,4957.

7. Autenrieth and Hennings, Ber.,1901,34,1772.

8. Clark, Analyst,1957,82,182.

9. Einhorn, Ann.,1898,300,135.

10. Culvenor,Davies, and Pausacker, J.,1946,1050.

11. Iqbal and Owen, J.,1960,1030.

12. Bailey, J.,1950,3461.

13. Ben Ishai and Berger, J.Org.Chem.,1952,17,1564.

14. Wieland and Koppe, Ann.,1953,581,1.

15. Karrer,Escher, and Widmer, Helv.Chim.Acta, 1926,9,301.

16. Block,Durrum, and Zweig,"Paper Chromatography and Paper Electrophoresis",Academic Press,N.Y. 1958, chapter 5,pp 110-169.

17. Turner, J.A.C.S.,1947,69,875.

18. Curtius and Franzen, Ber.,1902,35,3241.

19. Hofmann,Lindenmann,Magee, and Khan, J.A.C.S., 1952,74,470.

20. Olsen and Erlenmeyer, Chem.Ber.,1948,81,359.

21. Dixon and Taylor, J.,1920,720.

22. Jencks and Carriuolo, J.A.C.S.,1960,82,1778.

23. Bruice and Benkovic, J.A.C.S.,1963,85,1.

24. Brenner and Hofer, Helv,Chim.Acta,1961,44,1794.

25. Plapinger, J.Org.Chem.,1959,24,802.

26. Beckmann, Ber.,1904,37,4136.

27. Hurd and Fan, J.A.C.S.,1951,73,110.

28. Cunningham,Newbold,Spring, and Stork, J.,1949,2091.

29. Thompson, Aust.J.Sci.Research,1951,B4,180.

30. Wieland and Schneider,Ann.,1953,580,159.

31. 'Handbook of Chem. and Phys.',Chem.Rubber Co., 40th.Edn.,1959,p.1151.

32. Jacobs and Heidelberger, Org.Syn.,Coll. Vol. I, p.153.

33. Keller,Netter, and Niemann,Z.physiol.Chem., 1958,313,244.

34. Lipmann and Tuttle, J.Biol.Chem.,1945,161,415.

35. Gambarjan, Ber.,1925,58,1775.

36. Fischer, Ber.,1905,38,605.

37. Wolffenstein, Ber.,1892,25,2777.

38. Utzinger and Regenass, Helv.Chim.Acta,1954,37,1888.

39. Bischoff and Fröhlich, Ber.,1907,40,2779.

40. Kjellin, Ber.,1893,26,2377.

41. Rindfusz,Gunnings, and Harnack, J.A.C.S.,

...

1920,42,157.

42. Peacock and Dutting, J.,1934,1303.

43. Sheehan and Hess, J.A.C.S., 1955,77,1067.

44. Sheehan and Frank, J.A.C.S.,1950,72,1312.

45. Bergmann,Zimkin, and Pinchas, Rec.Trav.chim., 1952,71,161.

46. Utzinger and Regenass,Helv.Chim.Acta,1954,37,1885.

47. Einhorn and Mettler, Ber.,1902,35,3647.

48. Leuchs and Geiger, Ber.,1908,41,1721.

49. Bonnett,Brown,Clark,Sutherland, and Todd, J.,1959,2094.

50. Bergmann,Zervas,Reiche, and Schleich, Z.physiol.Chem.,1934,224,36.

List of new compounds.

O,S-Dibenzylmonothiolcarbonate(Found: C,69.7;H,5.55; S,12.8. $C_{15}H_{14}O_2S$ requires C,69.7;H,5.5;S,12.4%).

S,S'-Dibenzyloxycarbonyl-1,2-dithiolethane(Found: C,59.4;H,5.2;S,17.3. $C_{18}H_8O_4S_2$ requires C,59.6;H, 5.0;S,17.7%).

S-Monoethoxycarbonyl-1,2-dithiolethane(Found: C,36.0; H,5.9;S,38.4. $C_5H_{10}O_2S_2$ requires C,36.1;H,6.1;S,38.6%).

2,4-Dinitrophenyl sulphide of latter.(Found: C,39.6; H,3.75;N,8.25;S,18.9. $C_{11}H_{12}N_2O_6S_2$ requires C,39.8; H,3.6;N,8.4;S,19.3%).

n-Pentane-1,5-dithioldiacetate(Found:C,48.9;H,7.0; S,29.6. $C_9H_{16}O_2S_2$ requires C,49.1;H,7.3;S,29.1%).

n-Hexane-1,6-dithioldiacetate(Found: C,50.95;H,7.85; S,27.7. $C_{10}H_{18}O_2S_2$ requires C,51.25;H,7.7;S,27.4%).

2,4-Dinitrophenylsulphide of α,ω-dithiolmonoacetyl-alkanes: (alkane cited),

Ethane(Found: C,39.8;H,3.4;N,9.4;S,21.0.
Required: C,39.7;H,3.3;N,9.3;S,21.2%).

Propane(Found: C,41.8;H,3.85;N,8.8;S,19.7.
Required: C,41.8;H,3.8;N,8.9;S,20.3%).

Butane(Found: C,43.9;H,4.4;N,8.4;S,19.0.
Required: C,43.7;H,4.3;N,8.5;S,19.4%).

Pentame(Found: C,45.0;H,5.0;N,8.1;S,18.2.
Required: C,45.3;H,4.7;N,8.1;S,18.6%).

Hexane(Found: C,46.9;H,5.05;N,7.8;S,17.9.

Required: C,46.9;H,5.2;N,8.0;S,17.5%)

α,α'Dithiol-o-xylylenediacetate(Found: C,56.8;H,5.3; S,25.2. $C_{12}H_{14}O_2S_2$ requires C,56.7;H,5.55;S,25.25%).

2,4-Dinitrophenylsulphide of monoacetate of α,α'-dithiol-o-xylylene(Found: C,50.55;H,3.95;N,7.1;S,17.1. $C_{16}H_{14}N_2O_5S_2$ requires C,50.75;H,3.7;N,7.4;S,17.0%).

Toluene-3,4-dithiolcarbonate(Found: C,52.5;H,3.2;S,35.75. $C_8H_6OS_2$ requires C,52.5;H,3.3;S,35.7%)

Cyclohexane-trans-1,2-dithiolacetate(Found: C,51.9; H,7.4;S,27.1. $C_{10}H_{16}O_2S_2$ requires C,51.7;H,6.9;S,27.6%).

N-Methoxycarbonyl-L-α-alanine-S-benzylthiolester(Found: C,57.5;H,6.1;N,5.5;S,12.3. $C_{12}H_{15}NO_3S$ requires C,57.0; H,6.0;N,5.5;S,12.65%).

N-Methoxycarbonyl-L-α-alanyl(N-benzyl)-amide(Found: C,60.6;H,6.8;N,11.5. $C_{12}H_{16}N_2O_3$ requires C,61.0;H,6.8; N,11.8%).

L-S-Ethoxycarbonyl-2-S-(N-methoxycarbonyl)-L-α-alanyl-1,2-dithiolethane(Found: C,40.7;H,5.6;N,4.9;S,21.6. $C_{10}H_{17}NO_5S$ requires C,40.95;H,5.8;N,4.75;S,21.7%).

1-S-Ethoxycarbonyl-2-S-L-α-alanyl-1,2-dithiolethane hydrochloride(Found: C,35.3;H,6.1;N,5.3;S,23.5;Cl,13.1. $C_8H_{16}ClNO_3S_2$ requires C,35.15;H,5.9;N,5.1;S,23.5;Cl,13.0%).

N,O-Dianisoyl-N-methylhydroxylamine(Found: C,64.55; H,5.3;N,4.7. $C_{17}H_{17}NO_5$ requires C,64.8;H,5.4;N,4,45%).

N-Acetylglycylhydroxamic acid(Found: C,36.6;H,6.0; N,20.95. $C_4H_8N_2O_3$ requires C,36.4;H,6.1;N,21.2%).

N-(N-Benzoyl)-glycyl-N-methylhydroxylamine(Found: C,58.0; H,5.8;N,13.4. $C_{10}H_{12}N_2O_3$ requires C,57.7;H,5.8;N,13.5%).

N-(N-Acetyl)-glycyl-N-methylhydroxylamine(Found:C,41.3; H,6.95;N,19.2. $C_5H_{10}N_2O_3$ requires C,41.4,H,6.95;N,19.2%).

N-(N-acetyl)-glycyl-N-methyl-O-acetylhydroxylamine (found: C,44.6;H,6.4;N,14.8. $C_7H_{12}N_2O_4$ requires C,44.65; H,6.4;N,14.9%).

N-Anisoyl-N-methyl-O-acetylhydroxylamine(Found: C,59.4; H,6.05;N,6.1. $C_{11}H_{13}NO_4$ requires C,59.2;H,5.9;N,6.0%).

Ethyl 2-(N-methyl)-hydroxylaminoacetate(Found: C,45.0; H,8.35;N,11.1. $C_5H_{11}NO_3$ requires C,45.1;H,8.3;N,10.5%).

2-(N-Methyl)-hydroxylaminoacetamide(Found: C,34.7; H,7.8;N,26.8. $C_3H_8N_2O_2$ requires C,34.6;H,7.75;N,26.9%).

Dianilinium O,O'-diphenylpyrophosphate(Found: C,55.95; H,5.1;N,5.6;P,12.0.$C_{24}H_{26}N_2O_7P_2$ requires C,55.85;H,5.1; N,5.4;P,12.0%).

Di-(N-benzyl)-ammonium-P,P'-diphenylpyrophosphinate (Found: C,60.8;H,6.05;N,5.8;P,12.1. $C_{26}H_{30}N_2O_5P_2$ requires C,60.9;H,5.9;N,5.5;P,12.1%).

Dianilinium-P,P'-diphenylpyrophosphinate(Found: C,59.7; H,5.55;N,5.9;P,13.1. $C_{24}H_{26}N_2O_5P_2$ requires C,59.5; H,5.4,N,5.8;P,13.2%).

O-(N-Methoxycarbonyl)-L-α-alanyl-N,N-dimethylhydroxylamine(Found: C,44.5;H,7.2;N,14.5. $C_7H_{14}N_2O_4$ requires C,44.2;H,7.4;N,14.7%).

O-(N-Methoxycarbonyl)-L-α-alanyl-N-hydroxypiperidine (Found: C,52.2;H,7.8;N,12.1. $C_{10}H_{18}N_2O_4$ requires C,52.1;H,7.9;N,12.2%).

N-Methyl-N-hexylhydroxylamine acid oxalate(Found: C,48.8; H,8.5;N,6.1. $C_9H_{19}NO_5$ requires C,48.0; H,8.7;N,6.3%).

O,O'-Di-(2-hydroxy)-ethylcatechol(Found: C,60.7;H,7.2. $C_{10}H_{14}O_2$ requires C,60.6;H,7.1%).

O,O'-Di-(2-hydroxy)-ethylcatechol ditosylate(Found: C,57.3;H,5.4;S,12.9. $C_{24}H_{26}O_8S_2$ requires C,57.0; H,5.2;S,12.7%).

N,O-Dibenzoyl-N-methylhydroxylamine(Found: C,70.6; H,5.1;N,5.5. $C_{15}H_{14}NO_3$ requires C,70.5,H,5.15;N,5.5%).

O,O'-Di-2-(N-methyl-N-hydroxy)-aminoethylcatechol(Found: C,56.3;H,7.8;N,11.1. $C_{12}H_{20}N_2O_4$ requires C,56.25; H,7.9;N,10.9%).

O,O'-Di-2-(N-hydroxy-N-methyl)-aminoethylresorcinol (Found: C,56.4;H,7.7;N,10.8. $C_{12}H_{20}N_2O_4$ requires C,56.25;H,7.9;N,10.9%).

O,O'-Di-(2-hydroxy)-ethylresorcinol ditosylate(Found: C,56.8;H,5.0;S,12.7. $C_{24}H_{26}O_8S_2$ requires C,57.0; H,5.2;S,12.7%).

2,3-Dioxy-O,O'-di-(1'-ethoxycarbonyl)-methylnaphthalene

(Found: C,65.0;H,5.2. $C_{18}H_{20}O_6$ requires C,65.0;H,6.1%).

2,3-Dioxy-O,O'-di-(2-hydroxy)-ethylnaphthalene(Found: C,67.7;H,6.5. $C_{14}H_{16}O_4$ requires C,67.9;H,6.8%).

2,3-Dioxy-O,O'-di-(2-hydroxy)-ethylnaphthalene ditosylate (Found: C,60.4;H,5.2;S,11.6. $C_{28}H_{28}O_8S_2$ requires C,60.4;H,5.1;S,

2,3-Dioxy-O,O'-di-2-(N-hydroxy-N-methyl)-aminoethyl-naphthalene(Found: N, 9.4. $C_{16}H_{22}N_2O_4$ requires N,9.2%).

Dibenzoyl 2,3-dioxy-O,O'-di-2-(N-hydroxy-N-methyl)-aminoethylnaphthalene(Found: C,69.6;H,6.3;N,5.3. $C_{30}H_{30}N_2O_6$ requires C,70.0;H,5.9;N,5.45%).

N-(2-Phthalimido)-ethyl-N-methylhydroxylamine(Found: C,60.1;H,5.25;N,12.3. $C_{11}H_{12}N_2O_3$ requires C,60.0; H,5.5;N,12.7%).

N-(2-Amino)-ethyl-N-methylhydroxylamine bishydrochloride (Found: C,21.6;H,7.3. $C_3H_{10}N_2O.2HCl$ requires C,22.1; H,7.4%).

N,O-Diacetyl-N-(2-amino)-ethyl-N-methylhydroxylamine (Found: C,48.3;H,8.1;N,16.4. $C_7H_{14}N_2O_3$ requires C,48.3;H,8.1;N,16.1%).

N-(2-Acetamido)-ethyl-N-methylhydroxylamine acid oxalate(Found: C,38.0;H,6.3;N,12.65. $C_7H_{14}N_2O_6$ requires C,37.9;H,6.35;N,12.6%).

N-(2-Phthalimido)-ethyl-N-methyl-O-(N-methoxycarbonyl)-glycylhydroxylamine(Found: C,53.8;H,5.3;N,12.3.

$C_{15}H_{17}N_3O_6$ requires C,53.75;H,5.1;N,12.5%).

N-(2-Phthalimido)-ethyl-N-methyl-O-(N-benzyloxycarbonyl)-glycylhydroxylamine(Found: C,61.1;H,4.9; N,10.0. $C_{21}H_{21}N_3O_6$ requires C,61.3;H,5.1;N,10.2%).

1-Tosyl-2-phenyloxazolidine(Found: C,63.9;H,5.8;N,4.7; S,10.9. $C_{16}H_{17}NO_3S$ requires C,63.4;H,5.65;N,4.6;S,10.6%).

1-Cyano-1-(N-methyl)-hydroxylaminocyclohexane(Found: C,62.2;H,9.1;N,18.05. $C_8H_{14}N_2O$ requires C,62.3; H,9.15;N,18.2%).

1-Carbamoyl-1-(N-methyl)-hydroxylaminocyclohexane (Found: C,56.2;H,9.3;N,16.25. $C_8H_{16}N_2O_2$ requires C,55.8;H,9.4;N,16.3%).

1-Carbamoyl-1-(N-methyl)-aminocyclohexane(Found: C,61.5;H,10.2;N,18.1. $C_8H_{16}N_2O$ requires C,61.5;H,10.1; N,17.9%).

1-Cyano-1-(N-methyl-O-acetyl)-hydroxylaminocyclohexane(Found: C,61.3;H,8.2;N,14.2. $C_{10}H_{16}N_2O_2$ requires C,61.2;H,8.2;N,14.3%).

1-Carbamoyl-1-(N-methyl-O-acetyl)-hydroxylaminocyclohexane(Found: C,55.8;H,8.3;N,12.8. $C_{10}H_{18}N_2O_3$ requires C,56.1;H,8.5;N,13.1%).

1-Carbamoyl-1-(N-methyl-O-benzyloxycarbonyl)-hydroxylaminocyclohexane(Found: C,62.8;H,7.1;N,9.1. $C_{16}H_{22}N_2O_4$ requires C,62.75;H,7.2;N,9.1%).

1,4-Diaza-2-oxa-1-methyl-spiro[5,5]undecane-3,5-dione

(Found: C,54.6;H,7.1;N,14.2. $C_9H_{14}N_2O_3$ requires C,54.5;H,7.1;N,14.1%).

O,O'-Di-[N-methyl-N-(1-carbamoyl)-cyclohexyl]-hydroxylaminocarbonate(Found: C,55.2;H,8.1;N,15.0. $C_{17}H_{30}N_4O_5$ requires C,55.2;H,8.2;N,15.1%).

1-Carbamoyl-1-[N-methyl-O-(N-benzyloxycarbonyl)-glycyl] - hydroxylaminocyclohexane(Found: C,59.65;H,7.0; N,11.2. $C_{18}H_{25}N_3O_5$ requires C,59.5;H,7.0;N,11.6%).

1-Carbamoyl-1-[N-methyl-O-(N-benzyloxycarbonyl)-DL-phenylalanyl] -hydroxylaminocyclohexane(Found: C,66.0; H,7.0;N,9.1. $C_{25}H_{31}N_3O_5$ requires C,66.2;H,6.9;N,9.3%).

1-Carbamoyl-1-(N-methyl-O-phenylalanyl)-hydroxylaminocyclohexane hydrobromide(Found: Br,19.7%. $C_{17}H_{26}N_3O_3Br$ requires Br,19.9%).

1-(N-Methyl)-hydroxylaminocyclohexylcarbonylphenylalanylamide(Found: C,63.9;H,8.0;N,13.0. $C_{17}H_{25}N_3O_3$ requires C,63.9;H,7.9;N,13.2%).

1-(N-Methyl)-aminocyclohexylcarbonylphenylalanylamide (Found: C,67.0;H,8.35;N,14.1. $C_{17}H_{25}N_3O_2$ requires C,67.3;H,8.3;N,13.9%).

1-Hydroxyl-2-carbamoyl-4,5,5-trimethylpyrrolidine (Found: C,55.9;H,9.3;H,16.5. $C_8H_{16}N_2O_2$ requires C,55.8;H,9.4;N,16.3%).

1-(O-Benzyloxycarbonyl)-hydroxy-2-carbamoyl-4,5,5-trimethylpyrrolidine(Found: C,63.0;H,7.1;N,9.1.

$C_{16}H_{22}N_2O_4$ requires C,62.7;H,7.2;N,9.1%).

2-Carbamoyl-4.5.5-trimethyl-Δ^1-pyrroline(Found: C,62.4; H,9.1;N,18.5. $C_8H_{14}N_2O$ requires C,62.3;H,9.15;N,18.2%).

1-[O-(N-Benzyloxycarbonyl)-DL-phenylalanyl]-hydroxy-2-carbamoyl-4,5,5-trimethylpyrrolidine(C,66.6; H,6.9;N,9.55. $C_{25}H_{31}N_3O_5$ requires C,66.2;H,6.9;N,9.3%).

1-Carbamoyl-(N-methyl)-hydroxylaminocyclopentane (Found: C,53.2;H,8.65;N,17.9. $C_7H_{14}N_2O_2$ requires C,53.1;H,8.9;N,17.7%).

1-Carbamoyl-[N-methyl-O-(benzyloxycarbonyl)-phenylalanyl]hydroxylaminocyclopentane(Found:C,65.6;H,6.8; N,9.9. $C_{24}H_{29}N_3O_5$ requires C,65.6;H,6.7;N,9.6%).

1-(N-Methyl)-hydroxylaminocyclopentylcarbonyl-DL-phenylalanylamide(Found: N,13.55.$C_{16}H_{23}N_3O_3$ requires N,13.7%).
