SYNTHESIS OF AMINO ACIDS

(THROUGH AZLACTONES)

THESIS
SUBMITTED TO
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH
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T1049

ABSTRACT

A general method for the synthesis of N-benzoylamino acid amides involving azlactones as intermediates has been developed. These azlactones were converted, in a one-step operation, into N-benzoylamino acid amides in high yields by subjecting them to hydrogenation under elevated hydrogen pressures (32-55 psi) in ammoniacal ethanol using Raney nickel as catalyst. N-Benzoylamino acid amides were then successfully hydrolysed to the corresponding N-benzoylamino acids and amino acids in sufficiently high yields under different conditions.

The azlactones prepared by the condensation of different aldehydes and ketones with hippuric acid were : 2-phenyl-4-benzal-5-oxazolone (62%), 2-phenyl-4-(p-methoxybenzal)-5-oxazolone (80%), 2-phenyl-4-(3',4'-dimethoxybenzal)-5-oxazolone (71%), 2-phenyl-4-(p-acetoxybenzal)-5-oxazolone (80%), 2-phenyl-4-(3'-methoxy-4'-acetoxybenzal)-5-oxazolone (75%), 2-phenyl-4-cinnamylidene-5-oxazolone (60%), 2-phenyl-4-(2'-furfurylidene)-5-oxazolone (48.3%), 2-phenyl-4-isopropylidene-5-oxazolone (39%),

2-phenyl-4-sec.butylidene-5-oxazolone (32%), 2-phenyl-4-butylidene-5-oxazolone (16%), 2-phenyl-4-isobutylidene-5-oxazolone (30%) and 2-phenyl-4-propylidene-5-oxazolone (42.4%).

N-Benzoylamino acid amides prepared in excellent yields (72-100%) were : DL-N-benzoylphenylalanine amide (95%), DL-N-benzoyl-O-methyltyrosine amide (77.5%), DL-N-benzoyl-3,4'-dimethoxyphenylalanine amide (78%), DL-N-benzoyltyrosine amide (100%), DL-N-benzoyl-3-methoxy-4-hydroxyphenylalanine amide (83.5%), DL-N-benzoyl-δ-phenylnorvaline amide (75%), DL-N-benzoyl-β-furylalanine amide (74%), DL-N-benzoylvaline amide (84%), DL-N-benzoylisoleucine amide (72.6%), DL-N-benzoylnorleucine amide (76%), DL-N-benzoylleucine amide (74%) and DL-N-benzoylnorvaline amide (75%). Of these, DL-N-benzoyl-O-methyltyrosine amide, DL-N-benzoyl-3,4-dimethoxyphenylalanine amide, DL-N-benzoyl-3-methoxy-4-hydroxyphenylalanine amide, DL-N-benzoyl-δ-phenylnorvaline amide, DL-N-benzoyl-β-furylalanine amide and DL-N-benzoylnorvaline amide, are being reported for the first time.

N-Benzoylamino acid amides, on mild hydrolysis with hydrochloric acid (36%), were converted to the corresponding N-benzoylamino acids

in high yields. The N-benzoylamino acids so obtained were : DL-N-benzoylphenylalanine (98.8%), DL-N-benzoyl-O-methyltyrosine (75%), DL-N-benzoyl-3,4-dimethoxyphenylalanine (95%), DL-N-benzoyltyrosine (89%), DL-N-benzoyl-3-methoxy-4-hydroxyphenylalanine (70%), DL-N-benzoyl-δ-phenylnorvaline (90%), DL-N-benzoylvaline (89%), DL-N-benzoylisoleucine (95%), DL-N-benzoylnorleucine (80%), DL-N-benzoylleucine (83%) and DL-N-benzoylnorvaline (85%). DL-N-Benzoyl-β-furylalanine (80%) was obtained from the corresponding amide by hydrolysis with sodium hydroxide (30%). DL-N-Benzoyl-3,4-dimethoxyphenylalanine is being reported for the first time.

Excellent yields (73-100%) of a number of amino acids were also obtained from N-benzoylamino acid amides by hydrolysis with hydrochloric acid (36%) on heating under reflux. The amino acids thus prepared were: DL-phenylalanine (90%), DL-O-methyltyrosine (76%), DL-3,4-dimethoxyphenylalanine (84%), DL-tyrosine (88%), DL-3-methoxy-4-hydroxyphenylalanine (80%), DL-valine (100%), DL-isoleucine (90%), DL-norleucine (85%), DL-leucine (88%) and DL-norvaline (82%). DL-δ-Phenylnorvaline (96%) was obtained by the hydrolysis of the corresponding amide with sodium hydroxide (30%) while barium hydroxide (16%) was used for the preparation of DL-β-furylalanine (73%).

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This is to certify that the work described in this thesis is original and suitable for submission.

(M.H.Khan)

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CONTENTS

α-Amino acids are the constituents of proteins and therefore they are essential for nutrition. In addition they are required for the synthesis of polypeptides. With possible use of essential amino acids as dietary supplements for the enrichment of food and increasing research activities in these fields, synthetic amino acids are increasingly in greater demand.

Although all of the amino acids that occur in proteins can be obtained from protein hydrolysates, it is in many cases convenient to obtain them by synthesis. The choice of the route of synthesis usually depends upon the availability of the starting materials bearing the desired side chains.

There are a number of methods for the synthesis of amino acids with different scope and limitations so that some amino acids can be synthesised better by one particular method in large quantities.

One of these methods employs azlactones as intermediates to obtain a number of naturally occuring α-amino acids. Azlactone method may be considered as the best one for the syntheses of phenylalanine and tyrosine. But the main advantage of this method over others is that it affords acyl derivatives of amino acids as intermediates which are very

useful in resolution of dl-amino acids.

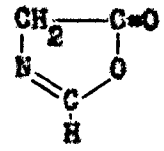
The following is a short description of the various methods used for the syntheses of azlactones and their conversion to α-amino acids.

INTRODUCTION

INTRODUCTION

I. AZLACTONE SYNTHESIS

2-Oxazolin-5-ones(I) have also been referred to as 5-oxazolones and oxazol-5-ones. 2,4-Disubstituted-2-oxazolin-5-ones, whichare regarded as anhydrides ofα-acylamino acids, are commonly known as azlactones. They can conveniently be classified into two groups, saturated and unsaturated, as shown in formulaeII and III.



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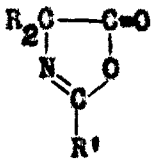

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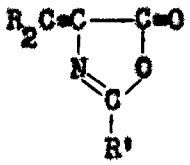


III

PREPARATION OF AZLACTONES:

Several methods available for the preparation of azlactones have been reviewed by Carter[1]. A brief account is given in the following paragraphs:

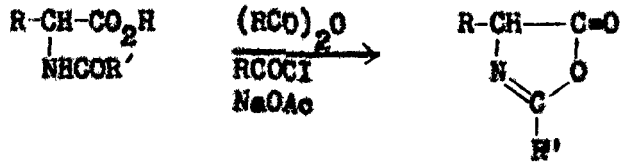
(i) Azlactonization of anα-acylamino acid:

α-Acylamino acids can be converted into azlactones under the following conditions:

(a) Action of an acid anhydride, either alone or in acetic acid as solvent, on an α-acylamino acid (or, occasionally, a free α-amino acid).

(b) Action of an acid anhydride or acid chloride on the sodium salt of an α-acylamino acid (or free α-amino acid) in aqueous solution.

(c) Action of an acid anhydride or chloride on an α-acylamino acid in pyridine solution.



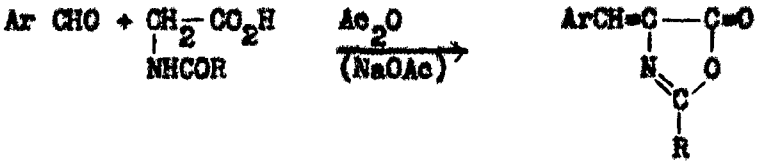
Of these methods the first is the most convenient and the only one generally used.

Since the unsaturated acylamino acids are not readily available, none of these methods is useful for the preparation of unsaturated azlactones.

(ii) <u>Reaction of an aldehyde with an acylglycine in the presence of acetic anhydride</u>:

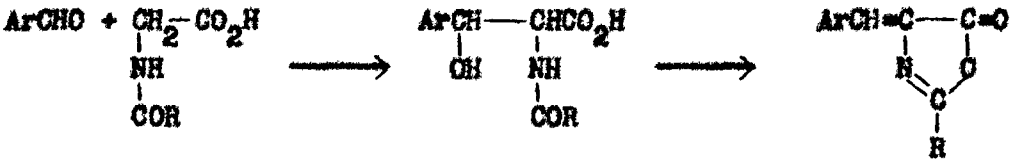
The reaction of an aldehyde with an acylglycine in presence of acetic anhydride (and usually sodium acetate) is referred to as

the Erlenmeyer azlactone synthesis.

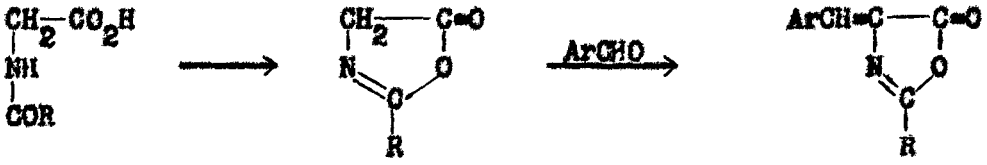


Mechanism of Erlenmeyer azlactone reaction:

According to Erlenmeyer the reaction proceeds in two steps as shown in the equations:



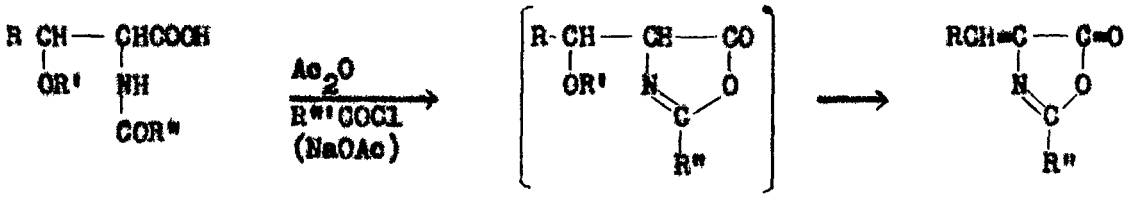
Experimental data indicate that the intermediate involved in this synthesis contains an extremely active methylene group and therefore is the azlactone formed by the action of acetic anhydride on the acylglycine. Thus the actual condensation takes place between the aldehyde and the so formed azlactone rather than the acylglycine.



2-Phenyl-oxazolones usually are prepared by heating a mixture of 1 mole each of aldehyde, hippuric acid, and freshly fused sodium acetate with 3 moles of acetic anhydride on a water bath for varying lengths of time.

(iii) Reaction of an α-acylamino-β-hydroxy acid with an acid anhydride or acid chloride:

The action of acetic anhydride on an α-acylamino-β-hydroxy (alkoxy or acyloxy) acid produces an unsaturated azlactone.

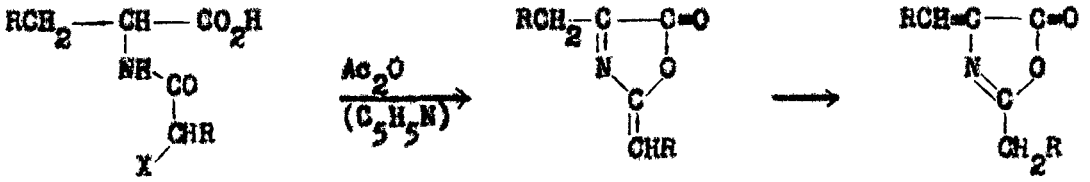


The first step in this transformation is the conversion of the acyl derivative into the corresponding saturated azlactone. This saturated azlactone possesses an extremely active α-hydrogen atom which splits out with the β-substituent under very mild conditions

to form the unsaturated azlactone.

(iv) Reaction of an α-(α'-haloacyl)-amino acid with acetic anhydride:

The conversion of an α-(α'-haloacyl)-amino acid into an unsaturated azlactone has not been studied extensively. A proposed mechanism is shown in the equation below:



Besides the above four methods of preparation of azlactones, a number of other methods have also been developed.

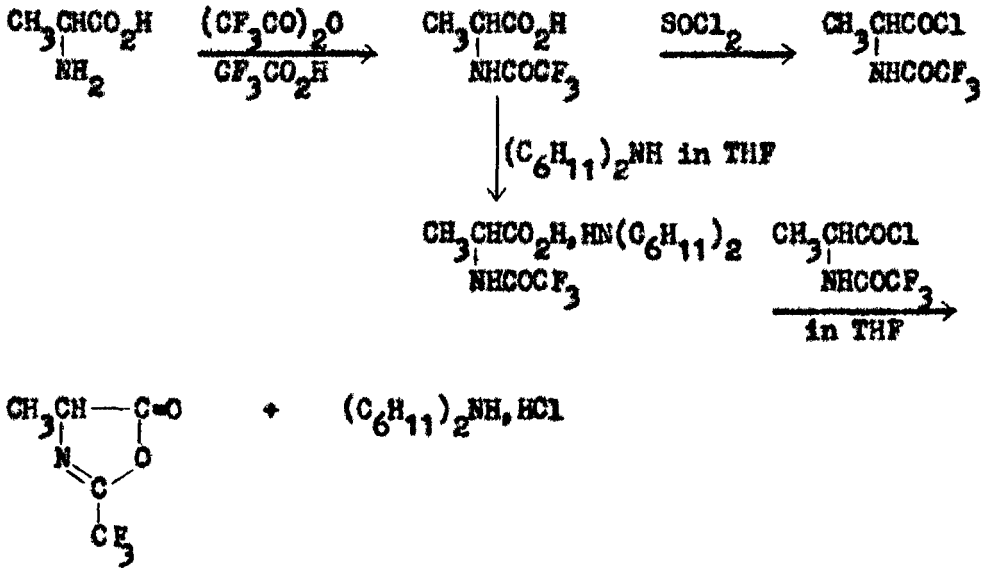
Azlactones are also obtained by the reaction of a dialkyl or diaryl formamidine with acylglycine and acetic anhydride[2].

Potassium carbonate (or bicarbonate) is an excellent catalyst[3]

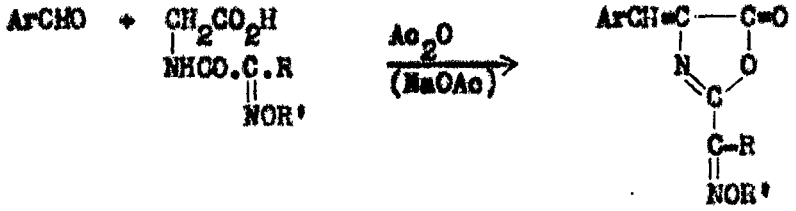
for the condensation of aldehydes with hippuric acid and is, in several respects, superior to sodium acetate used in the standard procedure. In the presence of potassium carbonate the condensation takes place without external heating. It is complete in a short period of time and gives appreciably higher yields than those obtained by standard methods.

Triethylamine has also been employed as a catalyst in the formation of azlactones[4,5].

Weygand et al.[6] have synthesised 2-trifluoromethyl-4-methyl-5-oxazolone using trifluoroacetic anhydride and anhydrous trifluoroacetic acid as shown in the following equations.

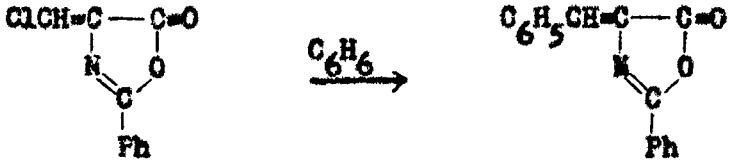


The α-alkyloximino acids combine readily with an arylaldehyde to make available a large number of azlactones[7] using the usual conditions.



Although aliphatic aldehydes do not readily undergo Erlenmeyer reaction, the lower members react readily in the absence of sodium acetate or acetic anhydride[8] with 2-phenyl-5-oxazolones and its derivatives in a type of Perkin-Erlenmeyer reaction. Reaction also readily occurs with aromatic aldehydes and some ketones (loc.cit.).

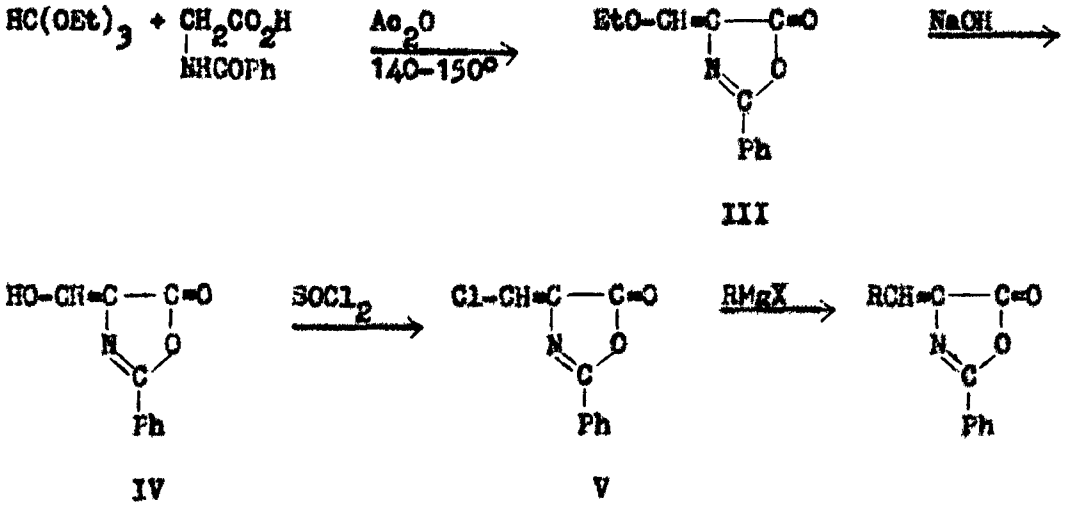
Unsaturated azlactones are prepared from 2-aryl-4-halomethylene-5-oxazolones by reaction with organo-metallic compounds, compounds with an acidic H and compounds which undergo Friedel-Craft's reaction[9].



N-Acylamino acids react in the presence of triethylamine with ethoxycarbonyl chloride (as also with dicyclohexylcarbodiimide) to form azlactones[5].

Synthesis of azlactones has also been effected by cyclodehydration with sulphur trioxide complexes[10]. Condensation of appropriate aldehyde with hippuric acid in the presence of $HCON\cdot Me_2-SO_3$ complex on a steam bath for 15-20 minutes afforded the desired azlactone. The complex containing sulphur trioxide (30%) can be prepared by the addition of liquid sulphur trioxide to $HCON\cdot Me_2$ at 0-5° (loc.cit).

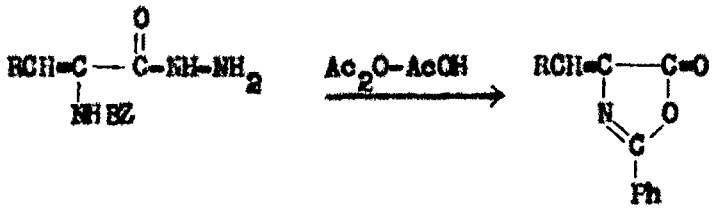
Behringer et al.[11] carried out azlactone synthesis by refluxing a mixture of hippuric acid and ethylorthoformate in acetic anhydride at 140-150° for one hour.



Hydrolysis of (III) with N NaOH at room temperature afforded (IV) which when warmed slightly with $SOCl_2$ gave (V). Different azlactones were prepared from (V) by reaction with different Grignard reagents.

The cyclization[12] of α-haloacylamino acids to give azlactones have been accomplished by treating with benzoic anhydride and sodium benzoate at 100° and immediately distilling the product in vacuo(method A), or treating with $POCl_3$ and lutidine in CH_2Cl_2 at -15° (method B).

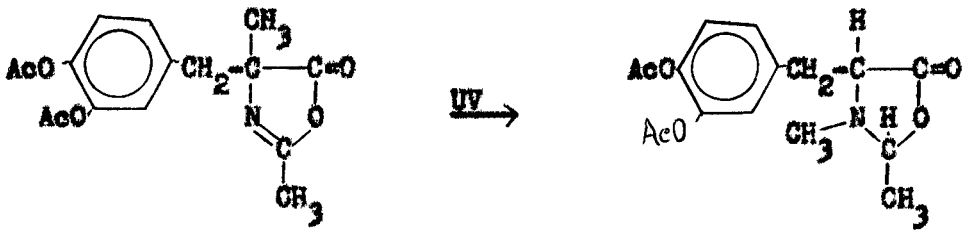
Bodea et al.[13] obtained the azlactones by the cyclization of α-acylamino acid hydrazides in presence of acetic acid-acetic anhydride mixture.



Woodward et al.[14] have reported that the reaction of hippuric acid with isoxazolium salts to give enol esters was accompanied by azlactone formation.

Harry et al.[15] have been successful in converting one

azlactone into another by exposure to ultraviolet light.



A solution of D(+)-2,4-dimethyl-4-(3',4'-diacetoxybenzyl)-5-oxazolone in dioxane was irradiated for 6 days in a quartz flask with a high pressure mercury arc ultraviolet lamp. The solvent was removed in vacuo to yield DL-2,3-dimethyl-4-(3',4'-diacetoxybenzyl)-5-oxazolone. D(+)-2,4-Dimethyl-4-(3',4'-dimethoxybenzyl)-5-oxazolone was similarly converted to its DL-analogue.

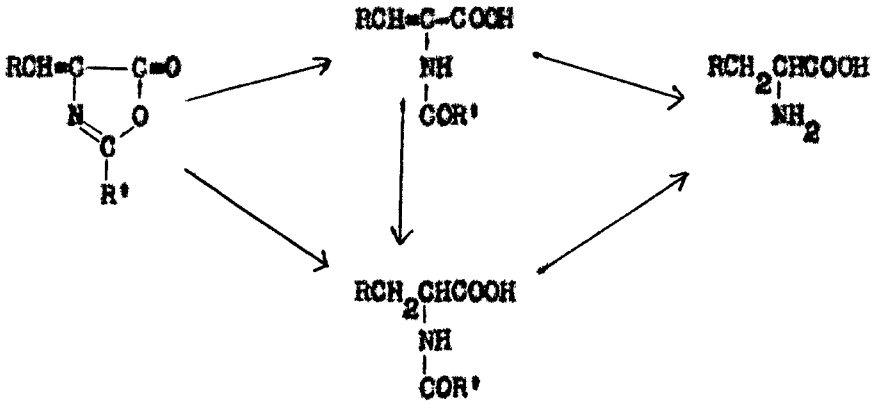
II. AZLACTONES IN α-AMINO ACID SYNTHESIS

Azlactones have been employed as intermediates in the synthesis of α-amino acids[16-19]. There are three general methods of reduction and hydrolysis by which the unsaturated azlactones and acylaminoacrylic acids can be converted into α-aminoacids. These are as under:

1. Sodium or sodium amalgam and water or ethanol.

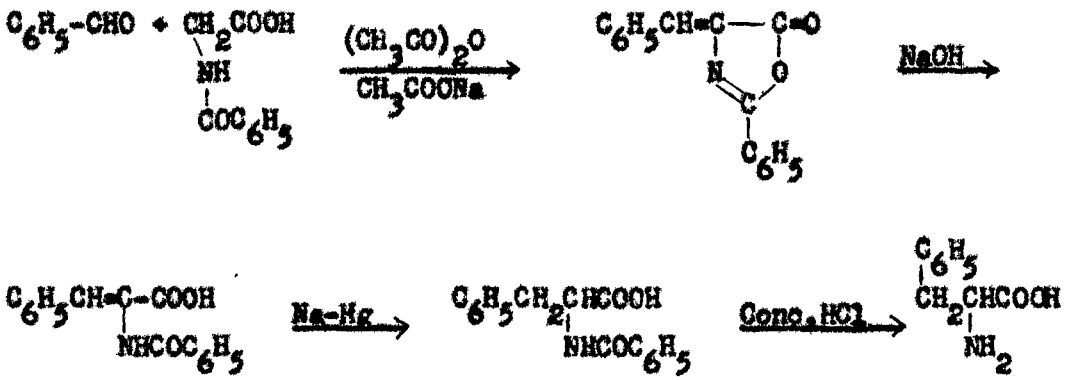
2. Hydriodic acid, red phosphorus in acetic acid or acetic anhydride.

3. Catalytic hydrogenation.



1. The reduction of α-benzoylaminoacrylic acids with an equivalent amount of sodium amalgam (3%) has originally been

described by Erlenmeyer[17]. Thus phenylalanine was prepared according to the following scheme:

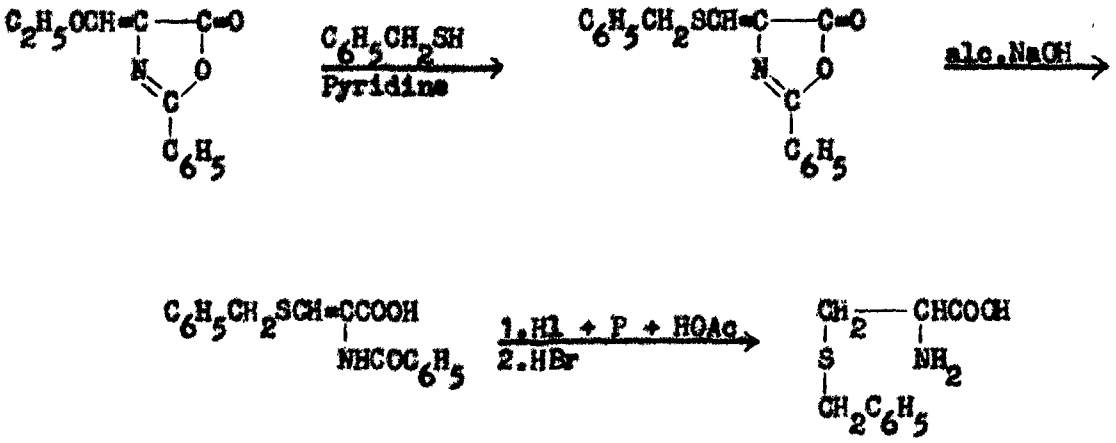


This method has been improved in several ways[20,21]. In an improved procedure[21], α-benzoylaminopropionic acids are obtained in 62-80% yields by treating aqueous solutions of the sodium salts of α-benzoylaminoacrylic acids with a large excess of sodium amalgam. However, reduction with sodium amalgam is not always satisfactory, as 2-phenyl-4-(3',4',5'-trimethoxybensal)-5-oxazolone is not reduced[22,23] while α-benzoylamino-β-(4-methoxy-1-naphthyl)-acrylic acid gives only a 10% yield[24]. α-Benzoylamino-β-indolacrylic acids[25-27] and α-benzoylamino-β-pyrroleacrylic acids[28] are also not reduced satisfactorily by sodium amalgam. Reductions have been carried out by

sodium and ethanol,[26,27,29,30] which hydrolyses a considerable proportion of the reduction product to the free amino acid as well. Tryptophane has been synthesised by this procedure[26]. Phenylacetyl-phenylalanine was obtained in 90-95% yield by the reduction of α-phenylacetaminocinnamic acid by sodium amalgam[18,31].

2. Harington and Barger[32] reported for the first time the use of a mixture of hydriodic acid and red phosphorus as a reducing agent for benzoylaminoacrylic acids and accomplished the synthesis of thyroxine, in which an alkaline agent could not be employed. Lamb and Robson[33] and Harington and McCarthey[34] have improved the yields by the addition of acetic acid and acetic anhydride to the reaction mixture. Free amino acid is produced directly with acetic anhydride containing reagent and alkylphenyl ether linkages are cleaved at the same time. Azlactones can be used satisfactorily but the best results have been obtained from acrylic acids or esters. Hydroxybenzal oxazolones, which are destroyed by alkalies, are smoothly reduced by hydriodic acid and red phosphorus in acetic anhydride. This reagent has been applied successfully to obtain a number of amino acids in good yields[35-38]. In 1955 Baltazzi and

Davis[39] synthesised S-benzylcysteine starting with 2-phenyl-4-ethoxymethylene-5-oxazolone according to the following scheme:

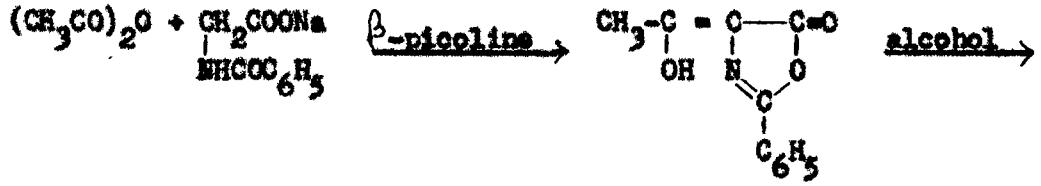


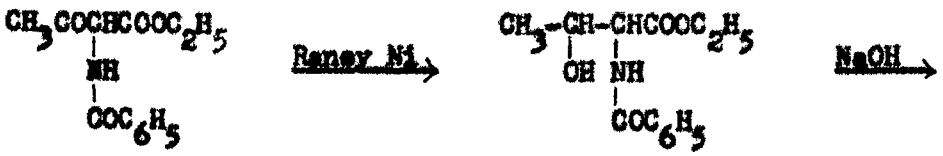
A mixture of equal amounts of constant boiling hydriodic acid and acetic anhydride is an excellent solvent, and, by its use, the reaction could be made to proceed smoothly and to yield a clean product which could be worked up without difficulty[34]. The reaction, i.e., the reduction and the simultaneous hydrolytic removal of the benzoyl group, was complete after boiling for 1.5 hour.

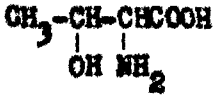
3. Catalytic reduction has been used to a limited extent only[40-43]. Reduction, using a catalyst, is the most satisfactory reaction, except where other reducible or catalyst-poisoning groups

are present. Thus catalytic hydrogenation could not be used in the synthesis of thyroxine[34] or for the reduction of pyrrole azlactones[44]. Carter et al.[41] have reduced benzoylaminocrotonic acid azlactone smoothly over platinum catalyst in glacial acetic acid containing 1 mole of water. It has been suggested[41] that the saturated azlactone formed first hydrolyses immediately since it is much more reactive than the original compound.

In 1948 Attenburrow et al.[45] used Raney nickel catalyst to obtain threonine according to the following scheme:



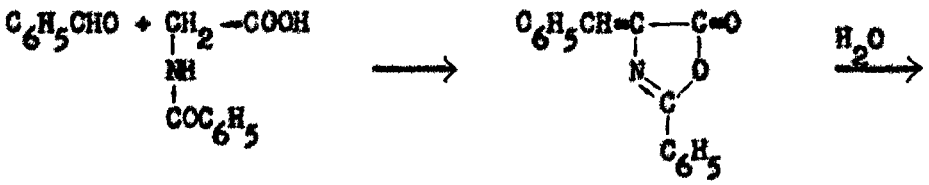


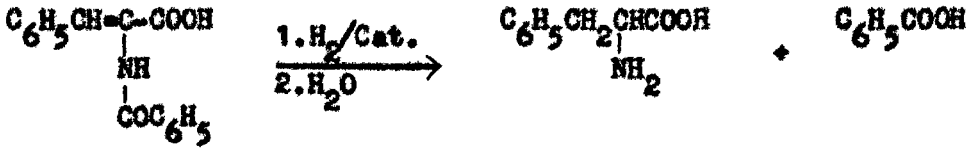


A number of naturally occuring α-amino acids can be prepared by the reduction and hydrolysis of azlactones. Azlactone method is the best method for the syntheses of phenylalanine and tyrosine. The main advantage of this method over others is that it affords benzoylamino acids as intermediates, which could be used for the resolution of the amino acids and for other synthetic purposes.

III. CATALYTIC REDUCTION IN AMINO ACID SYNTHESIS

Erlenmeyer in 1892[31,46,47] suggested for the first time the use of reductive amination in the synthesis of amino acids. He obtained the azlactone of α-benzoylaminocinnamic acid by the condensation of benzaldehyde with benzoylglycine. Hydrolysis of the azlactone yielded α-benzoylaminocinnamic acid, which on catalytic reduction followed by hydrolysis gave phenylalanine.



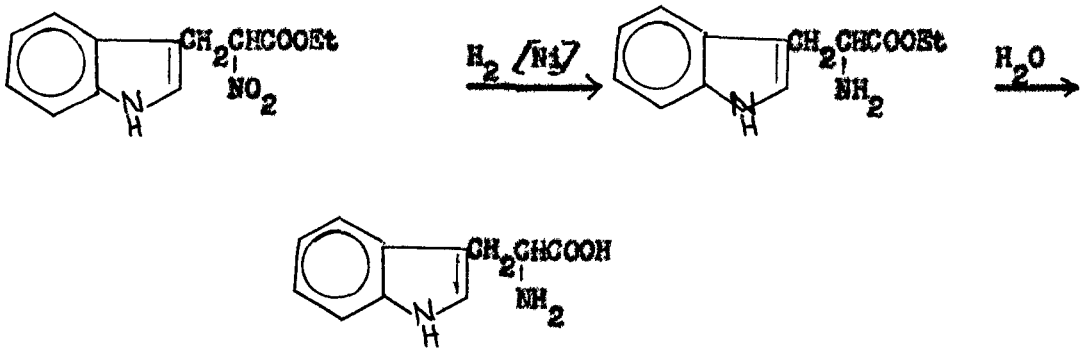


α-Acylaminocinnamic acids have also been reduced by sodium amalgam[16,48] but over all yield was only 3 percent in case of tyrosine.

Raney Nickel Catalyst:

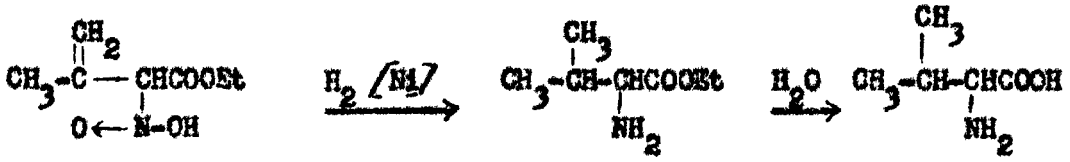
Catalytic reduction of α-acylaminocinnamic acid has also been carried out by hydrogenation at 80 atmospheres of pressure and at room temperature in the presence of Raney nickel[49].

Weisblat and Lyttle[50,51] hydrogenated ethyl α-nitro-β-(3-indole) propionate in the presence of Raney nickel catalyst to ethyltryptophanate which yielded tryptophane after hydrolysis.

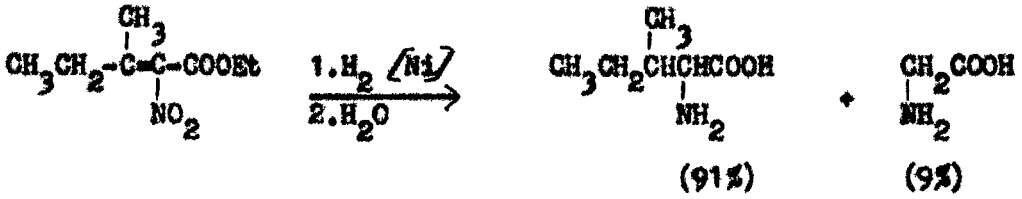


They have also obtained leucine and glutamic acid by reduction and hydrolysis of ethyl α-nitro-β-hydroxyisocaproate and α-nitroglutarate respectively using Raney nickel as catalyst.

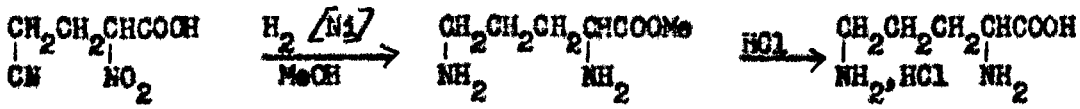
Sus[52] prepared valine by Raney nickel catalysed reduction of the β-form of α-nitro-β,β-dimethylacrylic ester in 47 percent yield.



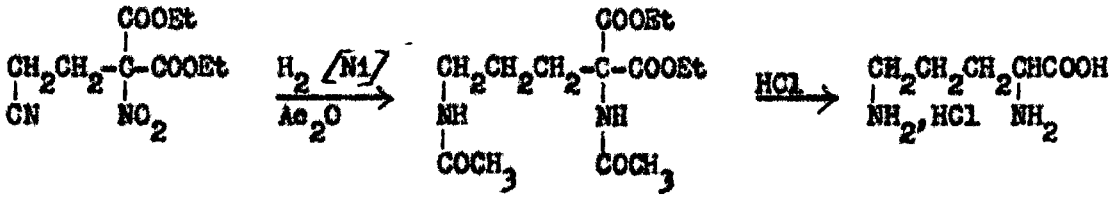
Mori et al.[53] prepared isoleucine and alloisoleucine from ethyl α-nitro-β-methyl-β-ethylacrylate which on Raney nickel catalysed reduction and subsequent hydrolysis yielded a mixture of isoleucine and alloisoleucine in 91 percent yield, but glycine (9%) was also obtained as a side product showing that to certain extent hydrogenolysis also occured.



Akabori et al.[54] prepared dl-ornithine by Raney nickel catalysed reduction of γ-carbethoxy-γ-nitrobutyronitrile in methanol at 100° and 75 atmospheres of pressure. Acid hydrolysis of the ester gave ornithine monohydrochloride in 51 percent yield.

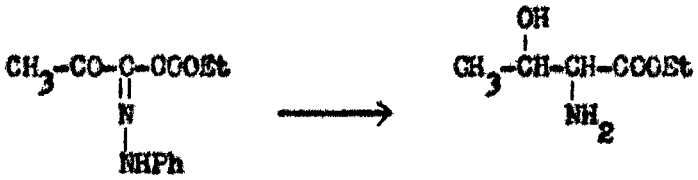


An alternative scheme by the same workers was the Raney nickel catalysed reduction of γ,γ-dicarbethoxy-γ-nitrobutyronitrile in acetic anhydride at 100° and 80 atmospheres of pressure, whereby ethyl α-carbethoxy-α,δ-diacetamidovalerate was obtained. This on acid hydrolysis yielded ornithine monohydrochloride in 99 percent yield.

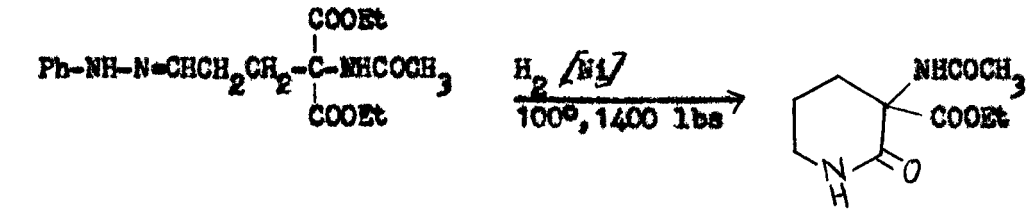


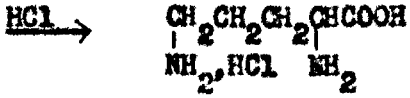
α-Oximino-β-phenylpyruvic acid was reduced to α-amino-β-phenylpropionic acid by Gaudry and Melvor[55] using Raney nickel catalyst.

Raney nickel has been used to reduce α-phenylazoacetoacetate. A large amount of Raney nickel in methanol was used to reduce the α-phenylhydrazone of acetoacetic ester yielding ethyl threoninate in 63 percent yield[56,57]. A more facile reduction leading to ethyl threoninate was also described in which the compound reduced was ethyl α-acetyl-α-phenylazoacetate.



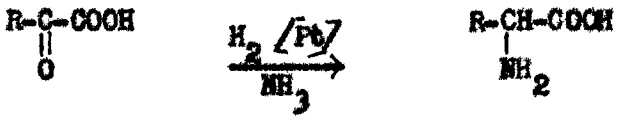
Warner and Moe[58] prepared ornithine by catalytic hydrogenation of α-phenylhydrazone of δ-acetamido-δ,δ-dicarbethoxy-n-butyraldehyde in presence of Raney nickel.





Platinum and Palladium Catalysts:

Knoop and Oesterlin[59] in 1925 for the first time carried out catalytic hydrogenation of a number of α-keto acids to amino acids using platinum or palladium on carbon as catalyst in the presence of ammonia.



Using this method they synthesised the following amino acids in about 65 percent yields excepting the last three which were obtained in very low yields.

1. α-Aminobutyric acid
2. α-Amino-β-trimethylpropionic acid
3. Aspartic acid
4. Glutamic acid
5. α-Amino-α-phenylacetic acid
6. Phenylalanine
7. α-Aminophenylbutyric acid
8. β-Amino-β-phenylpropionic acid
9. γ-Amino-γ-phenylbutyric acid
10. β-Aminobutyric acid

Aubel and Bourguel[60] developed a similar procedure and obtained alanine by shaking the ammonium salt of pyruvic acid with

aqueous ammonia in an atmosphere of hydrogen using colloidal palladium. The colloidal palladium was stabilised with starch.

Desmuelle and Fromageot[61] also employed colloidal palladium for the preparation of glycine by reducing ammonium salt of glyoxylic acid in ammonia in an atmosphere of hydrogen. The amino acid was obtained in only 8 percent yield.

Schoenheimer and Ratner[62] slightly modified the Knoop and Oesterlin method. They prepared a number of amino acids by carrying out the reduction of keto acids in 50 percent alcohol with palladium in presence of ammonia. The yields, even when calculated on the basis of the keto acids, were good except in the case of alanine and aspartic acid.

Amino acid	% yield
Alanine	68
Phenylalanine	84
Tyrosine	82.8
Norleucine	73
Glutamic acid	84.2
Aspartic acid	44

Palladised charcoal has been employed[63] for the preparation of N-methylphenylalanine in about 80 percent yield. A mixture of

phenylpyruvic acid and an aqueous solution of methylamine in 70 percent ethanol was hydrogenated at 3 atmospheres at 25° in presence of palladised charcoal. After the removal of the catalyst N-methylphenylalanine was obtained from the filtrate.

$$C_6H_5CH_2\text{-}\underset{\underset{O}{\|}}{C}\text{-}COOH + H_2N\text{-}CH_3 \xrightarrow[3atm.,\ 25^{\circ}]{H_2\ /Pd/C/} C_6H_5CH_2\underset{\underset{NH\text{-}CH_3}{|}}{CH}COOH$$

Isumiya et al.[64] have prepared the N-alkylamino esters by reducing amino acid esters in presence of aldehydes.

$$CH_3\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOEt + CH_2O \xrightarrow{H_2\ /Pd/C/} CH_3\text{-}\underset{\underset{NH\text{-}CH_3}{|}}{CH}\text{-}COOEt$$

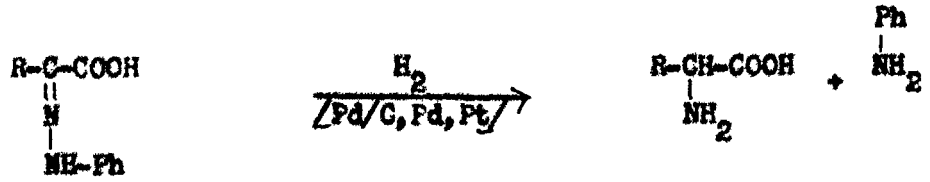
Hartung et al.[65-67] obtained satisfactory yields of amino acids or their esters by reducing α-oximino acids or their esters using palladium on carbon in ethanolic hydrogen chloride at room temperature and at a hydrogen pressure of 10 atmospheres.

$$R\text{-}\underset{\underset{NOH}{\|}}{C}\text{-}COOH \xrightarrow[H_2]{/Pd/C/} R\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH$$

They successfully prepared the following amino acids from the corresponding oximino acids or their esters.

Amino acid	Catalyst $Pd/C, PdCl_2$ in HCl	% yield
Alanine	"	75
α-Aminobutyric acid	"	78
Norvaline	"	83
Isoleucine	"	80
Norleucine	"	85
Aspartic acid	"	69
Glutamic acid	"	74
Phenylalanine	"	89
O-Methyltyrosine	"	85
Tyrosine	"	96

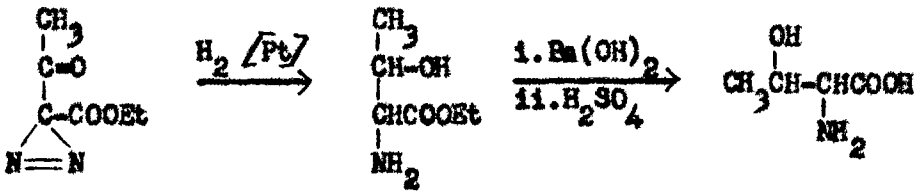
Naseem[68] prepared successfully a number of amino acids by reducing aqueous suspensions of phenylhydrazones of α-keto acids with palladium on carbon (or palladium or platinum) at room temperature and pressure.



The following amino acids were synthesised by this method using palladium on carbon (5% Pd).

Amino acid	% yield
Glycine	96
Alanine	92
Valine	85
Norleucine	94
Leucine	87
Isoleucine	83
γ-Aminovaleric acid	93
Phenylalanine	88
O-Methyltyrosine	95

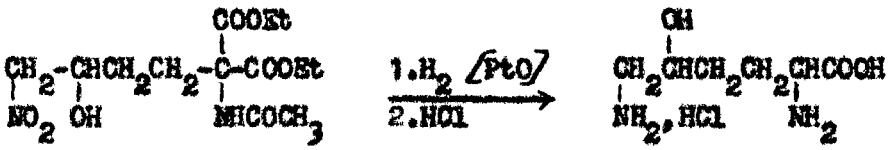
Birkofer[69] prepared dl-threonine by the reduction of ethyl diazoacetoacetate in the presence of platinum as catalyst. The amino acid ester obtained was hydrolysed with barium hydroxide to give the amino acid in 39 percent yield.



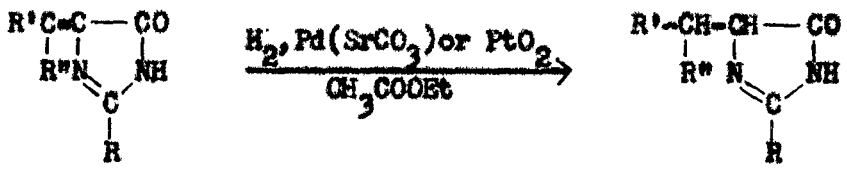
Alanine was prepared by palladium catalysed hydrogenation of pyruvic acid phenylhydrazone by Anker[70].

Vanzyl et al.[71] reduced diethyl γ-hydroxy-δ-nitrobutyl-acetamidomalonate with platinum oxide and hydrogen in the presence of a little hydrochloric acid. The reduction product on hydrolysis with

hydrochloric acid and on subsequent decarboxylation yielded δ-hydroxylysine monohydrochloride in 20 percent yield.



Kidwai and Devasia[72] developed a method for the synthesis of α-amino acids, involving 2,4-disubstituted-5(4H)-imidazolones as intermediates. The unsaturated 2,4-disubstituted-5(4H)-imidazolones were reduced to saturated imidazolones in high yields by catalytic hydrogenation. The saturated imidazolones were then successfully hydrolysed to the corresponding acylamino acid amides, acylamino acids and amino acids under different conditions.

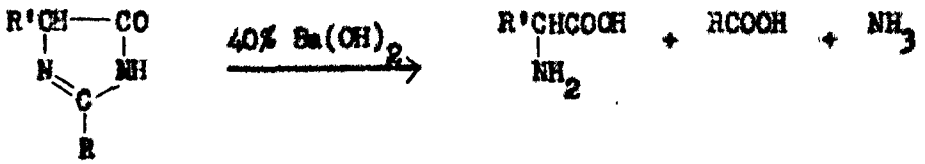


The following saturated imidazolones were prepared by this method using palladium on strontium carbonate as catalyst:

Imidazolone	% yield
2-phenyl-4-benzyl-5(4H)-imidazolone	63.5
2-Phenyl-4-anisyl-5(4H)-imidazolone	82.4
2-Phenyl-4-sec.butyl-5(4H)-imidazolone	71.9
2-Phenyl-4-isopropyl-5(4H)-imidazolone	74.9
2,4-Dibenzyl-5(4H)-imidazolone	59.5
2-Benzyl-4-anisyl-5(4H)-imidazolone	64.6

Platinum oxide catalyst was also employed for the reduction of unsaturated imidazolones to saturated ones at room temperature and under atmospheric pressure. Only 2-phenyl-4-benzylidene-5(4H)-imidazolone and 2-phenyl-4-(p-hydroxybenzylidene)-5(4H)-imidazolone were hydrogenated by this method.

Saturated 2,4-disubstituted-5(4H)-imidazolones were hydrolysed with barium hydroxide to amino acids. Phenylalanine, O-methyltyrosine and valine were prepared by this method in 67.9, 58.9 and 31.9 percent yields respectively.



D I S C U S S I O N

DISCUSSION

PREPARATION OF AZLACTONES

Azlactones obtained from aromatic aldehydes have been prepared by heating a mixture of 1 mole each of aldehyde, hippuric acid, and freshly fused sodium acetate with 3 moles of acetic anhydride (6moles in case of saturated aliphatic aldehydes) either on an electric hot plate and then transferring to a steam bath or directly on a boiling water bath for varying lengths of time. The length of heating varied from ten minutes to two hours. In several preparations good yields have been obtained with a shorter reaction time (ten to thirty minutes). p-Hydroxybenzaldehyde and p-methoxybenzaldehyde gave 80% yield of the azlactone by heating the reaction mixture for fifteen and thirty minutes respectively.

Saturated aliphatic ketones required even longer time (two to six hours) of heating at reflux temperature to complete the reaction. The yields obtained were much lower (32-39%) than those in the case of aromatic aldehydes.

Saturated aliphatic aldehydes generally have given low yields

in the azlactone synthesis ranging from 16 to 42 percent. As was the case with saturated aliphatic ketones, saturated aliphatic aldehydes also required heating at reflux temperature but with a shorter reaction time (twenty minutes). Anhydrous lead acetate has been used in place of fused sodium acetate in the preparation of azlactones from saturated aliphatic aldehydes.

(a) Isolation:

The azlactones have usually been isolated either by cooling the reaction mixture and removing the azlactone by filtration or by pouring the cold reaction mixture into water, allowing the excess acetic anhydride to decompose, and collecting the azlactone. In some cases (saturated aliphatic aldehydes), after decomposition of the excess acetic anhydride by water, the azlactones have been extracted with boiling light petroleum ether (bp 40-60°) which on evaporation yielded the azlactone in crude form. Azlactones from propionaldehyde, butyraldehyde and isobutyraldehyde have been obtained in this manner. Azlactone from ethylmethyl ketone has been obtained by the distillation of the ethereal extract under reduced pressure.

(b) Purification:

The azlactones have been purified by recrystallisation from a suitable solvent. Thus azlactones prepared from benzaldehyde, 3,4-dimethoxybenzaldehyde and furfuraldehyde were recrystallised from benzene. Methyl alcohol was employed for the recrystallisation of azlactones obtained from propionaldehyde, butyraldehyde and isobutyraldehyde. Azlactone from vanillin was purified by recrystallisation from glacial acetic acid. Certain solvent mixtures have also been used, e.g., ethyl acetate-ethanol and chloroform-ethanol for the recrystallisation of azlactones prepared from p-methoxybenzaldehyde and cinnamaldehyde respectively. Ethanol was used for the recrystallisation of the azlactones prepared from p-hydroxybenzaldehyde and acetone. Table I shows the results obtained.

T A B L E - I

AZLACTONES

S.No.	Carbonyl Component	Reaction Time	M.P/B.P.*°C	% Yield	Ref.
1.	Benzaldehyde	2 hr	165-66	62	35
2.	p-Methoxybenzaldehyde	30 min	157-58	80	73
3.	3,4-Dimethoxybenzaldehyde	2 hr	150-51	71	76
4.	p-Hydroxybenzaldehyde	15 min	171-72	80	16
5.	3-Methoxy-4-hydroxybenzaldehyde	15 min	188-89	75	34
6.	Cinnamaldehyde	10 min	151-52	60	78
7.	Furfural	10 min	170-71	48.3	79
8.	Acetone	6 hr	99-100	39	74
9.	Ethylmethyl ketone	2 hr	170-190 at 10mm/Hg*	32	75
10.	Butyraldehyde	20 min	56-57	16	77
11.	Isobutyraldehyde	20 min	86-87	30	77
12.	Propionaldehyde	20 min	83-84	42.4	77

REDUCTION OF AZLACTONES

Unsaturated azlactones and acylaminoacrylic acids have been converted to α-amino acids by reduction and hydrolysis. Reduction can be accomplished in three general ways:

(i) Chemical reduction with sodium or sodium amalgam in water or ethanol.

(ii) Hydriodic acid in presence of red phosphorus and acetic acid (or acetic anhydride).

(iii) Catalytic hydrogenation over Pt or Pd.

(i) Chemical reduction is not always satisfactory as some azlactones are not reduced at all while in other cases the yields are often very low.

(ii) Reduction and hydrolysis by treatment with hydriodic acid and red phosphorus in acetic acid or acetic anhydride was first employed in the synthesis of thyroxine and with the exception of tryptophane where this method is inapplicable, most of the amino acids have been synthesised by this method.

(iii) Catalytic hydrogenation followed by hydrolysis has been used to a limited extent only. Pt and Pd are the catalysts so far employed for this purpose. The use of Raney nickel catalyst has been studied to a very limited extent only.

Azlactones as such do not undergo catalytic reduction at low pressures of hydrogen (45-65 lbs/sq inch), but acylaminoacrylic acids and their amides can be reduced smoothly.

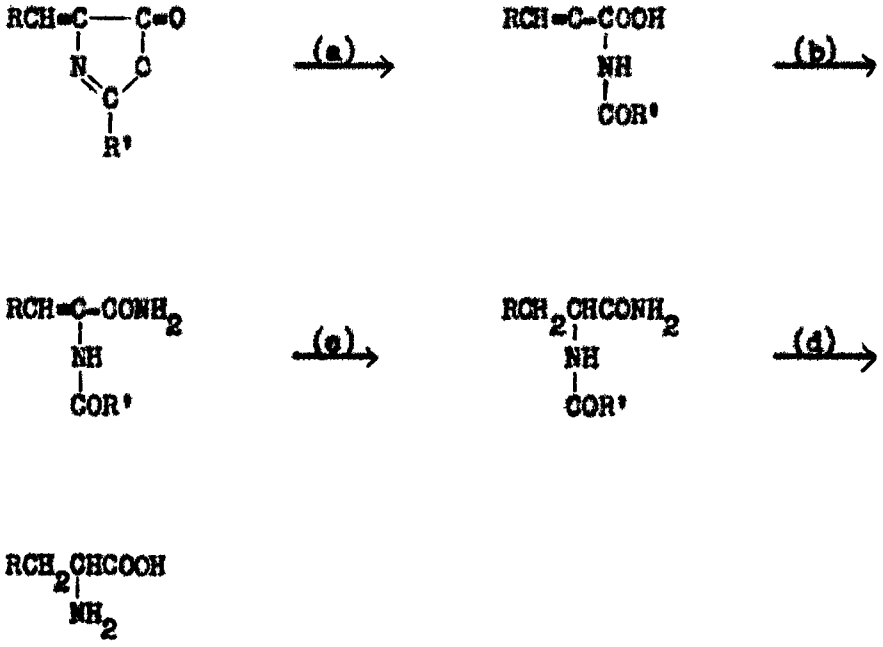
The sequence of reactions leading to amino acids from azlactones generally involves four steps, i.e.,

(a) hydrolysis of the azlactone to acylaminoacrylic acid

(b) conversion to unsaturated acylamino acid amide[78,99-102]

(c) reduction to saturated acylamino acid amide

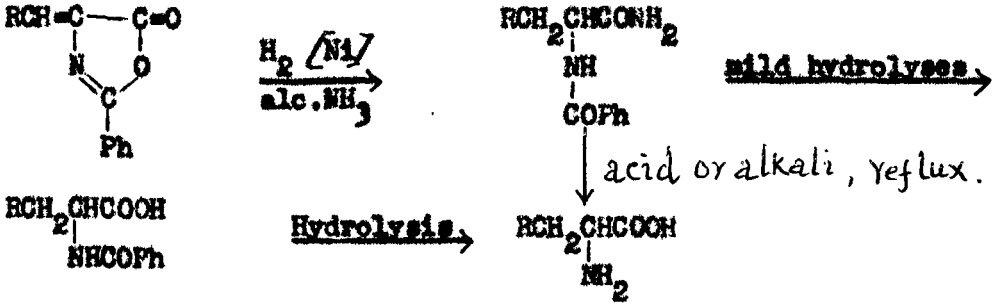
(d) hydrolysis to amino acid



The isolation of intermediates at each step results in lowering the yield of the amino acid. Another drawback of this method is that Pt and Pd are very sensitive to impurities. Moreover, they are very costly, thus making this method uneconomical for large scale preparation of amino acids.

The present study with nickel catalyst as a reducing agent in alcoholic ammonia was undertaken with a view to find a general method which could combine the three steps metnioned above. Nickel catalyst besides being cheaper could also be safely employed for large scale preparations. Moreover, it is easy to prepare and is less sensitive to impurities.

We have observed that reduction of azlactones with nickel catalyst in the presence of alcoholic ammonia at slightly elevated hydrogen pressure and room temperature afforded acylamino acid amides in high yields and in a one step operation.



Hydrolysis of the amides with concentrated hydrochloric acid during 12-18 hours at room temperature resulted in the formation of acylamino acids in nearly quantitative yields, whereas hydrolysis with concentrated hydrochloric acid under reflux temperature gave amino acids in very good yields.

In cases where acidic conditions were undesirable, alkaline hydrolysis with sodium hydroxide or barium hydroxide, choosing appropriate conditions, was used. In most cases excellent yields of amino acids were obtained.

(1) N-Benzoylamino acid amides:

Azlactones as such do not undergo catalytic hydrogenation at room temperature. However, it has been found that the reduction of the azlactones can be made to proceed smoothly and rapidly in alcoholic ammonia in the presence of freshly prepared Raney nickel catalyst under slightly elevated (2 to 3 atmospheres) hydrogen pressures and at room temperature to form benzoylamino acid amides in sufficiently high yields.

In actual practice the azlactone (3-6g) was suspended in about 100 ml ethanol (95%). An excess (about 10 ml) of concentrated ammonia (sp gr 0.888) was then added alongwith freshly prepared Raney nickel catalyst (usually 2-4g). The reduction was carried out in a Parr catalytic pressure hydrogenation apparatus under different hydrogen pressures (32-55 lbs/sq inch) and varying lengths of time (1 to 16 hr). In all cases reduction was stopped when there was no more hydrogen absorption. The completion of the reduction was also indicated by the change in colour of the reaction mixture (i.e., it becomes colourless). After disconnecting the flask the contents were heated on a boiling water bath in order to dissolve the precipitated benzoylamino acid amide and filtered hot. The catalyst was washed thoroughly with boiling ethanol to free it from any adhering benzoylamino acid amide. The filtrate and washings were taken to dryness under reduced pressure and the residue so obtained was crystallised from a suitable solvent to give benzoylamino acid amide in sufficiently pure form and high yield.

Nickel catalyst used was freshly prepared from a (50-50) nickel-aluminium alloy (BDH) using the standard method. Suspension

of the powdered azlactone in ammoniacal ethanol serves the purpose well and it is not at all necessary to have a clear solution of the azlactone before subjecting it to hydrogenation. Ammonia was taken in excess to suffice the amide formation. In most cases, e.g., benzoylphenylalanine amide, benzoyl-3,4-dimethoxyphenylalanine amide, benzoyl-O-methyltyrosine amide, benzoyltyrosine amide and benzoyl-3-methoxy-4-hydroxyphenylalanine amide, the benzoylamino acid amide was obtained in the form of a white precipitate which necessitated the heating of the reaction mixture on a water bath in order to dissolve it. In case of aliphatic benzoylamino acid amides, benzoyl-δ-phenylnorvaline amide and benzoyl-β-furylalanine amide, the benzoylamino acid amides remained in solution. Even then these were heated to boiling and were filtered hot. The catalyst was always washed thoroughly with boiling ethanol to free it from any adhering benzoylamino acid amide.

Aliphatic benzoylamino acid amides were formed in less time (1 to 9 hr) while the time taken by aromatic benzoylamino acid amides was more (3 to 16 hr). The yields were uniformly high (72-84%) and in some cases they were even almost quantitative

(benzoylphenylalanine amide, 95%; benzoyltyrosine amide, nearly 100%).

Most amides were purified by recrystallisation from 95% ethanol. Benzoyl-O-methyltyrosine amide was recrystallised from glacial acetic acid while some aliphatic benzoylamino acid amides were recrystallised from dilute ethanol (30% to 80%). Table II shows the results obtained.

T A B L E -II

DL-N-BENZOYLAMINO ACID AMIDES

S.No.	DL-N-Benzoylamino acid amides	Hydrogenation time,hr	Hydrogen pressure	M.P., °C	% Yield	Ref.
1.	DL-N-Benzoylphenylalanine amide	9	50 psi	197-98	95	80,81
2.	DL-N-Benzoyl-O-methyltyrosine amide	15	37.5 "	215-16*	77.5	
3.	DL-N-Benzoyl-3,4-dimethoxyphenylalanine amide	3	42 "	195-96*	78	
4.	DL-N-Benzoyltyrosine amide	16	40 "	238-39	100	81
5.	DL-N-Benzoyl-3-methoxy-4-hydroxy-phenylalanine amide	8	55 "	209-10*	83.5	
6.	DL-N-Benzoyl-δ-phenylnorvaline amide	5	52 "	160-61*	75	
7.	DL-N-Benzoyl-β-furylalanine amide	5	45 "	198-99*	74	
8.	DL-N-Benzoylvaline amide	9	38 "	220-21	84	72
9.	DL-N-Benzoylisoleucine amide	4.5	53 "	215-16	72.6	72
10.	DL-N-Benzoylnorleucine amide	2	37 "	143-44	76	82
11.	DL-N-Benzoylleucine amide	1	32 "	171-72	74	83
12.	DL-N-Benzoylnorvaline amide	3	41.5"	180-81*	75	

* Compounds reported for the first time

HYDROLYSIS OF THE BENZOYLAMINO ACID AMIDES

Acid amides on treatment with an acid or alkali are converted to carboxylic acids. Thus N-benzoylamino acid amides, obtained from azlactones in a one step operation by reduction in alcoholic ammonia over Raney nickel catalyst, were hydrolysed with an acid or alkali to the corresponding N-benzoylamino acids under mild conditions. Hydrolysis of the amides under more drastic conditions gave amino acids directly in excellent yields. In most cases the hydrolysing agent used was 36% hydrochloric acid while in some cases sodium hydroxide or barium hydroxide were employed.

(a) N-Benzoylamino acids:

Conversion of an N-benzoylamino acid amide into the corresponding N-benzoylamino acid was usually carried out by warming the former with hydrochloric acid (36%) either on a boiling water bath or a sand bath until a clear solution was obtained and then leaving the reaction mixture at room temperature for varying lengths of time (12-18 hr). Hydrolysis of N-benzoyl-β-furylalanine amide was accomplished by warming it with sodium hydroxide solution (30%) on a sand bath till the evolution of ammonia ceased. It was then

acidified by dropwise addition of hydrochloric acid (36%) with cooling and vigorous shaking.

N-Benzoylamino acids were crystallised by diluting the reaction mixture with water. They were further purified by recrystallisation from dilute ethanol (20-80%). N-Benzoyl-β-furylalanine was recrystallised from water. N-Benzoylvaline, N-benzoylnorleucine, N-benzoylleucine and N-benzoylnorvaline were obtained in a well crystalline form from the reaction mixtures and needed no further purification. The yields were generally very high, i.e., 80 to 98.8% except that of N-benzoyl-O-methyltyrosine which gave a 75% yield. Table III gives the results obtained.

T A B L E -III

DL-N-BENZOYLAMINO ACIDS

S.No.	DL-N-Benzoylamino acids	Time, hr	Hydrolysing agent	M.P., °C	% Yield	Refs
1.	DL-N-Benzoylphenylalanine	18	A	184-85	98.8	84,16
2.	DL-N-Benzoyl-O-methyltyrosine	16	A	175-76	75	85,73
3.	DL-N-Benzoyl-3,4-dimethoxyphenylalanine	16	A	180-81	95*	
4.	DL-N-Benzoyltyrosine	16	A	194-95	89	20,16
5.	DL-N-Benzoyl-3-methoxy-4-hydroxy-phenylalanine	14	A	162-63	70	89
6.	DL-N-Benzoyl-δ-phenylnorvaline	12	A	191-92	90	98
7.	DL-N-Benzoyl-β-furylalanine		B	162-63	80	79
8.	DL-N-Benzoylvaline	15	A	147-48	89	86
9.	Dl-N-Benzoylisoleucine	16	A	135-36	95	87,88
10.	DL-N-Benzoylnorleucine	12	A	135-36	80	90
11.	DL-N-Benzoylleucine	16	A	138-39	83	90
12.	DL-N-Benzoylnorvaline	12	A	151-52	85	86

A = Hydrochloric acid (36%)

B = Sodium hydroxide (30%)

* Compound reported for the first time

(b) Amino acids:

Amino acids were obtained directly from N-benzoylamino acid amides by heating them at reflux temperature with hydrochloric acid (36%) for varying lengths of time (1.5-6 hr). The amino acid hydrochlorides so obtained were treated with silver oxide which made the isolation of free amino acids easy. The precipitated silver chloride was removed from the reaction mixture by filtration. The traces of silver ions left in the solution were removed completely by passing water-washed hydrogen sulphide gas through it. Thus amino acids were isolated in almost pure form.

Alkaline hydrolysis was carried out where acidic conditions were undesirable. Thus dl-δ-phenylnorvaline was obtained from the corresponding N-benzoylamino acid amide by refluxing it with sodium hydroxide solution (30%) for 24 hr and dilute hydrochloric acid was carefully added in order to adjust the pH to the isoelectric point of the amino acid. Similarly dl-β-furylalanine amide was heated under reflux with 16% barium hydroxide solution for 24 hr to yield the corresponding amino acid.

Nearly all the amino acids were crystallised from dilute ethanol (40-85%) except dl-O-methyltyrosine which was crystallised from boiling water. The yields of the amino acids so obtained were generally very high and in some cases nearly quantitative. Table IV shows the results obtained.

TABLE-IV

DL-AMINO ACIDS

S.No.	DL-Amino acids	Reflux time,hr	Hydrolysing agent	M.P., °C	% Yield	Ref.
1.	DL-Phenylalanine	5	A	274-75(d)	90	16
2.	DL-O-Methyltyrosine	4	A	264-65(d)	76	91
3.	DL-3,4-Dimethoxyphenylalanine	6	A	241-42(d)	84	94
4.	DL-Tyrosine	5	A	306-7 (d)	88	16
5.	DL-3-Methoxy-4-hydroxyphenylalanine	6	A	245-46(d)	80	94
6.	DL-δ-Phenylnorvaline	24	B	239-40(d)	96	96
7.	DL-β-Furylalanine	24	C	256-57(d)	73	97
8.	DL-Valine	1.5	A	291-92(d)	100	92
9.	DL-Isoleucine	2	A	270-71	90	93
10.	DL-Norleucine	4	A	284-85(d)	85	95
11.	DL-Leucine	3	A	286-87(d)	88	90
12.	DL-Norvaline	2	A	284-85	82	86

A = Hydrochloric acid (36%)

B = Sodium hydroxide (30%)

C = Barium hydroxide (16%)

E X P E R I M E N T A L

EXPERIMENTAL *

I. SYNTHESES OF AZLACTONES

1. 2-Phenyl-4-benzal-5-oxazolone:

A mixture of benzaldehyde, 10.6g (0.1 mol), hippuric acid, 17.9g (0.1 mol), fused sodium acetate, 8.2g (0.1 mol) and acetic anhydride, 28.3 ml (0.3 mol) was heated on an electric hot plate till it melted completely. The flask was then transferred to a steam bath and heated for two hours. At the end of this period 40 ml ethanol (95%) was added slowly with cooling under cold running water and thorough shaking. The mixture was then allowed to stand overnight. The crystalline product thus separated was filtered on a Buchner under suction, washed with two 20-ml portions of ice-cold ethanol and finally with two 20-ml portions of boiling water. The product on drying weighed 15.6g (62%), mp 165-66°. It was recrystallised from benzene, mp. 167-68°. (lit.[35] mp. 167-68°).

2. 2-Phenyl-4-(p-methoxybenzal)-5-oxazolone:

A mixture of anisaldehyde, 13.6g (0.1 mol), powdered

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* Melting points are uncorrected.

hippuric acid, 17.9g (0.1 mol), powdered freshly fused sodium acetate, 8.2g (0.1 mol), and acetic anhydride, 28.3 ml (0.3 mol) was warmed on a water bath for 30 minutes. Yellow crystals soon began to form and the whole liquid mass became solid. Water was added slowly to decompose acetic anhydride. The crystalline material was filtered, washed with 80% ethanol and dried. This was recrystallised from ethyl acetate-ethanol mixture (20:1). The golden yellow needles thus obtained were filtered, dried and weighed 22.3g (80%), mp. 157-58° (lit.[73] mp. 158°).

3. 2-Phenyl-4-isopropylidene-5-oxazolone:

A mixture of hippuric acid, 43.3g (0.27 mol), acetone, 116 g (2.0 mol), acetic anhydride, 84.9 ml (0.9 mol) and freshly fused sodium acetate, 24.6g (0.3 mol) was heated under reflux at 110° for 6 hours. In the early stages of the reaction a pasty solid separated, which slowly dissolved yielding a pink solution. The solid, which separated when the cooled solution was poured into a large volume of water, was collected, washed with aqueous sodium bicarbonate to remove benzoic acid and recrystallised from alcohol. The oxazolone was obtained as yellow needles, mp 99-100° (lit.[74] mp 99-100°) which

weighed 21.1g (39%).

4. 2-Phenyl-4-sec.butylidene-5-oxazolone[36]

Acetic anhydride, 56.6 ml (0.6 mol) was added dropwise over a period of 10 min to a stirred suspension of hippuric acid, 17.9g (0.1 mol) and anhydrous sodium acetate, 8.2g (0.1 mol) in 270 ml of ethylmethyl ketone and the mixture was then refluxed under anhydrous conditions for 2 hr. About 230 ml of the ketone (bp 80-84°) was distilled; the oily residue was diluted with 150 ml of water and solid sodium bicarbonate was added until effervescence ceased. The azlactone formed was extracted with ether and distilled (bp 170-190° at 10 mm/Hg) to yield a pale yellow oil, which partly solidified and turned pink on keeping for some time. The yield was 8g (32%).

5. 2-Phenyl-4-(3',4'-dimethoxybenzal)-5-oxazolone:

A mixture of veratraldehyde, 16.6g (0.1 mol), hippuric acid, 17.9g (0.1 mol), fused sodium acetate, 8.2g (0.1 mol) and acetic anhydride, 28.3 ml (0.3 mol) was heated on an electric hot plate.

As soon as the mixture liquified completely, the flask was transferred to a steam bath and heated for 2 hr. At the end of this period, 50 ml of alcohol was added slowly while cooling the flask. After allowing the mixture to stand overnight, the crystalline product was filtered and washed on the Buchner with two 20-ml portions of ice-cold alcohol and finally with two 20-ml portions of boiling water. On drying, the product weighed 21g (71%) and melted at 147-48°. After recrystallisation from benzene, the oxazolone melted at 150-51°(lit.[76] mp 151-52°).

6. 2-Phenyl-4-(p-acetoxybenzal)-5-oxazolone:

p-Hydroxybenzaldehyde, 12.2g (0.1 mol), hippuric acid, 17.9g (0.1 mol), and anhydrous sodium acetate, 8.2g (0.1 mol) were finely powdered and mixed with 28.3 ml (0.3 mol) of acetic anhydride. The mixture was heated on a boiling water bath for 15 minutes. On cooling the azlactone formed a solid cake. This was powdered, washed first with hot water and then with dilute alcohol. The crude azlactone was dissolved in chloroform and precipitated by the addition of light petroleum ether (bp 40-60°). It was recrystallised from dilute alcohol

to give yellow needles weighing 24.5g (80%) and melting at 171-72° (lit.[16] mp 172-73°).

7. 2-Phenyl-4-(3'-methoxy-4'-acetoxybenzal)-5-oxazolone:

An intimate mixture of vanillin, 15.2g (0.1 mol), hippuric acid, 17.9g (0.1 mol), and freshly fused sodium acetate, 16.4g (0.2 mol) was treated with 28.3 ml (0.3 mol) acetic anhydride and heated on a water bath for 15 minutes. The reaction mixture was then ground up with water, filtered, and the precipitate washed several times with water. The crude product was crystallised from glacial acetic acid and formed yellow needles. The azlactone thus obtained melted at 188-89°(lit.[34] mp 188-89°) and weighed 22.1g (75%).

8. 2-Phenyl-4-butylidene-5-oxazolone:

A mixture of n-butyraldehyde, 17.3g (0.24 mol), acetic anhydride, 56.6 ml (0.6 mol), hippuric acid, 35.8g (0.2 mol), and anhydrous lead acetate, 32.5g (0.1 mol) was heated under reflux for 20 min and the clear orange solution was then poured into water (600 ml). After few hours the gummy product was extracted with five 80-ml portions

of boiling light petroleum ether (bp 40-60°). The petroleum ether extract on evaporation gave a yellow solid (7.2g; 16%) which melted at 54-55°. Recrystallisation from methanol gave colourless plates, mp 56-57° (lit.[77] mp 57°).

9. 2-Phenyl-4-isobutylidene-5-oxazolone:

A mixture of isobutyraldehyde, 17.3g (0.24 mol), hippuric acid, 35.8g (0.2 mol), acetic anhydride, 56.6 ml (0.6 mol), and anhydrous lead acetate, 32.5g (0.1 mol) was heated under reflux for 20 min and the clear orange solution was then poured into water (600 ml). After some hours the gummy product was extracted with five 80-ml portions of boiling light petroleum ether (bp 40-60°). The petroleum ether extract on evaporation gave 13.6 g (30%) of a red solid, melting at 86-87°. Recrystallisation from methanol raised the melting point to 87-88° (lit.[77] mp 87°).

10. 2-Phenyl-4-propylidene-5-oxazolone:

A mixture of propionaldehyde, 15.5g (0.25 mol), hippuric acid, 35.8g (0.2 mol), acetic anhydride, 56.6 ml (0.6 mol), and

anhydrous lead acetate, 32.5g (0.1 mol) was heated under reflux for 20 min and the clear orange solution was poured into 600 ml of water. After some hours the gummy product was extracted with five 80-ml portions of boiling light petroleum ether (bp 40-60°). The petroleum ether extract on evaporation gave 18g (42.4%) of an orange solid, mp 81-82°. Recrystallisation from methanol raised the melting point to 83-84° (lit.[77] mp 84°).

11. 2-Phenyl-4-cinnamylidene-5-oxazolone:

A mixture of cinnamaldehyde, 26.4g (0.2 mol), hippuric acid, 35.8g (0.2 mol), anhydrous sodium acetate, 16.4g (0.2 mol), and acetic anhydride, 56.6 ml (0.6 mol) was heated on a boiling water bath under dry conditions. After few minutes of heating an intense yellow colour developed which changed to orange and after 10 min a clear solution was obtained. The flask was removed from the water bath and kept at room temperature. On cooling a mass of orange coloured crystals was obtained. Water was added under cooled conditions to decompose acetic anhydride and also to dissolve sodium acetate. This was then filtered, washed with plenty of water and then five times with 95%

ethanol to remove unreacted cinnamaldehyde. The azlactone thus obtained was crystallised from chloroform-ethanol mixture to give orange coloured needles melting at 151-52° (lit.[78] mp 152°) and weighing 33g (60%).

12. 2-Phenyl-4-(2'-furfurylidene)-5-oxazolone:

A mixture of furfuraldehyde, 19.4g (0.2 mol), hippuric acid, 35.8g (0.2 mol), anhydrous sodium acetate, 16.4g (0.2 mol), and acetic anhydride, 56.6 ml (0.6 mol) was heated on a boiling water bath under dry conditions. After 10 min the solution became clear. On cooling it solidified to form an orange mass of crystals. Water was then added to decompose acetic anhydride and to dissolve sodium acetate. The crystalline material was filtered, washed with plenty of water and then five times with 95% ethanol to remove traces of unreacted aldehyde. After crystallisation from benzene, the azlactone was obtained as golden yellow needles, mp 170-71° (lit.[79] mp 171°). It weighed 23g (48.3%).

HYDROLYSIS OF AZLACTONES

II. PREPARATION OF N-BENZOYLAMINO ACID AMIDES

1.DL-N-Benzoylphenylalanine amide:

Powdered 2-phenyl-4-bensal-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this 3g freshly prepared Raney nickel catalyst was added alongwith 10 ml of concentrated ammonia (sp gr 0.888). This was reduced under a hydrogen pressure of 50 lbs/sq inch in a Parr catalytic hydrogenation apparatus. After 9 hr when there was no more absorption of hydrogen, the flask was disconnected and the contents heated on a boiling water bath to dissolve the precipitated benzoylamino acid amide and filtered hot. The catalyst was washed with two 50-ml portions of boiling ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue thus obtained was crystallised from ethanol (95%). The crystalline material was filtered and dried, mp 197-98° (lit.[80,81] mp 196° & 198°), yield 6.1g (95%).

Anal. Calcd for $C_{16}H_{16}O_2N_2$: C,71.64; H,5.97; N,10.44 .

Found C,71.32; H,6.07; N,10.70 .

2. DL-N-Benzoyl-O-methyltyrosine amide:

Powdered 2-phenyl-4-(p-methoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this freshly prepared Raney nickel catalyst (3g) was added alongwith 9 ml of concentrated ammonia. This was reduced under a hydrogen pressure of 37.5 lbs/sq inch. The reduction was complete in 15 hr. The amide was worked up in the usual manner when 2.7g of the crystalline product was obtained, mp 215-16° . As the amide was sparingly soluble in hot alcohol therefore some of the product remained adhering with the catalyst. The catalyst was therefore washed on the Buchner thrice with 10-ml portions of warm glacial acetic acid in which the product was soluble. The acetic acid washings were then diluted with 50 ml of ethanol (95%) and left for crystallisation overnight. The crystalline material which separated was filtered, washed three times with 15-ml portions of ethanol and dried. It weighed 1.95g and melted at 215-16°. Total yield of the amide was 4.65g (77.5%).

Anal. Calcd for $C_{17}H_{18}O_3N_2$: C,68.44; H,6.08; N,9.40 .

Found C,68.05; H,6.08; N,9.54 .

3. DL-N-Benzoylvaline amide:

Powdered 2-phenyl-4-isopropylidene-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this freshly prepared Raney nickel catalyst (3g) was added alongwith 10 ml of concentrated ammonia. This was reduced under a hydrogen pressure of 38 lbs/sq inch. The amide was worked up in the usual manner. The white crystalline material thus obtained melted at 213-14° and weighed 4.4g. A second crop of crystals (1.14g) was also obtained by concentrating the mother liquor. Recrystallisation from ethanol raised the melting point to 220-21° (lit.[72] mp 218-19°). The total yield of the amide was 5.54g (84%).

Anal. Calcd for $C_{12}H_{16}O_2N_2$: C,65.43; H,7.32; N,12.72.

Found C,65.36; H,7.66; N,12.52.

4. DL-N-Benzoylisoleucine amide:

2-Phenyl-4-sec.butylidene-5-oxazolone (6.5g) was suspended in 100 ml of ethanol containing liquor ammonia (10 ml) and freshly prepared Raney nickel catalyst (4g). This was reduced

under a hydrogen pressure of 53 lbs/sq inch. The reduction was complete in 4.5 hr. The contents were boiled on a boiling water bath and filtered hot. The catalyst on the Buchner was washed twice with 50-ml portions of boiling ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue was crystallised from 27 ml of ethanol (35%). The crystalline product was filtered, washed with two 25-ml portions of ethanol (35%) and dried in air. The amide thus obtained melted at 206-7° and weighed 4g. Mother liquor on concentration yielded another 0.65g of the product. The total yield was 4.65g (72.6%). Recrystallisation from ethanol (35%) raised the melting point to 215-16° (lit.[72] mp 215-16°).

Anal. Calcd for $C_{13}H_{18}O_2N_2$: C,66.64; H,7.74; N,11.96.

Found C,66.37; H,7.51; N,12.08.

5. DL-N-Benzoyl-3,4-dimethoxyphenylalanine amide:

Powdered 2-phenyl-4-(3',4'-dimethoxybensal)-5-oxazolone (3.6g) was suspended in 100 ml of ethanol, Raney nickel catalyst (2g)

was added alongwith 10 ml of concentrated ammonia and it was reduced under a hydrogen pressure of 42 lbs/sq inch. After 3 hr the reduction was complete. The amide was worked up in the usual manner. The residue was crystallised from 50 ml of ethanol (95%). The amide thus obtained melted at 195-96° and weighed 2.0g. Mother liquor on concentration yielded another 0.8g of the product. The total yield was 2.8g (78%).

Anal. Calcd for $C_{18}H_{20}O_4N_2$: C,65.85; H,6.10; N,8.53.

Found C,66.08; H,6.31; N,8.81.

6. DL-N-Benzoyltyrosine amide:

Powdered 2-phenyl-4-(p-acetoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing liquor ammonia (10 ml) and Raney nickel catalyst (3g). This was reduced under a hydrogen pressure of 40 lbs/sq. inch. The reduction was complete in 16 hr. The product was worked up in the usual manner. The residue was crystallised from ethanol (95%). The amide thus obtained on drying melted at 238-39° (lit.[81] mp 238°) and weighed 6.4g. The yield was

nearly quantitative.

Anal. Calcd for $C_{16}H_{16}O_3N_2$: C,67.6; H,5.7; N,9.9 .

Found C,67.41; H,5.62; N,9.69.

7. DL-N-Benzoyl-3-methoxy-4-hydroxyphenylalanine amide:

Powdered 2-phenyl-4-(3'-methoxy-4'-acetoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing liquor ammonia (10 ml), Raney nickel catalyst (3g) and reduced under a hydrogen pressure of 55 lbs/sq. inch. The reduction was complete in 8 hr. The amide was worked up in the usual manner. The residue was crystallised from ethanol (95%). The amide thus obtained melted at 209-10° and weighed 5g (83.5%).

Anal. Calcd for $C_{17}H_{18}O_4N_2$: C,64.96; H,5.73; N,8.91 .

Found C,65.13; H,5.61; N,9.11 .

8. DL-N-Benzoylnorleucine amide:

Powdered 2-phenyl-4-butylidene-5-oxazolone (5g) was suspended in 100 ml of ethanol containing liquor ammonia (10 ml) and freshly prepared Raney nickel catalyst (3g). It was reduced under a hydrogen pressure of 37 lbs/sq. inch. The reduction was complete in 2 hr. The amide was worked up in the previously described manner. The residue was crystallised from ethanol (30%). The crystalline amide thus obtained on drying weighed 4.1g (76%) and melted at 143-44° (lit.[82] mp 140-41°).

Anal. Calcd for $C_{13}H_{18}O_2N_2$: C,66.66; H,7.7; N,11.96.

Found C,66.75; H,7.83; N,11.99.

9. DL-N-Benzoylleucine amide:

Powdered 2-phenyl-4-isobutylidene-5-oxazolone (6g) was suspended in 100 ml of ethanol containing liquor ammonia (10 ml) and freshly prepared Raney nickel catalyst (3g). This was reduced under a hydrogen pressure of 32 lbs/sq. inch. After 1 hr the

reduction was complete. The amide was worked up in the usual manner. The residue was crystallised from ethanol (95%). The amide thus obtained on drying weighed 4.8g (74%) and melted at 171-72° (lit.[83] mp 170°).

Anal. Calcd for $C_{13}H_{18}O_2N_2$: C,66.66; H,7.69; N,11.96.

Found C,66.77; H,7.44; N,11.82.

10. DL-N-Benzoylnorvaline amide:

Powdered 2-phenyl-4-propylidene-5-oxazolone (5g) was suspended in 100 ml of ethanol followed by the addition of liquor ammonia (10 ml) and freshly prepared Raney nickel catalyst (4g). This was reduced under a hydrogen pressure of 41.5 lbs/sq. inch. The reduction was complete in 3 hr. The amide was worked up as before. The residue was crystallised from ethanol (80%). The amide thus obtained on drying weighed 4.1g (75%) and melted at 180-81°.

Anal. Calcd for $C_{12}H_{16}O_2N_2$: C,65.43; H,7.32; N,12.72.

Found C,65.52; H,7.51; N,12.93.

11. DL-N-Benzoyl-δ-phenylnorvaline amide:

Powdered 2-phenyl-4-cinnamylidene-5-oxazolone (5g) was suspended in 100 ml of ethanol. To this freshly prepared Raney nickel catalyst (3g) was added alongwith 10 ml of liquor ammonia. This was then reduced under a hydrogen pressure of 52 lbs/sq inch. The reduction was complete in 5 hr. The flask was disconnected, the contents boiled on a water bath for several minutes and filtered hot. The catalyst was thoroughly washed with boiling ethanol and the combined filtrate and washings were taken to dryness under reduced pressure. The residue thus obtained was crystallised from ethanol (95%). The dried amide weighed 4.0g (75.5%) and melted at 160-61°.

Anal.Calcd for $C_{18}H_{20}O_2N_2$: C,73.0; H,6.75; N,9.46 .

Found C,72.9; H,6.55; N,9.66 .

12. DL-N-Benzoyl-β-(2-furyl)-alanine amide:

Powdered 2-phenyl-4-(2-furfurylidene)-5-oxazolone (5g)

was suspended in 100 ml of ethanol. To this freshly prepared Raney nickel catalyst (3g) and 10 ml of liquor ammonia were added. This was hydrogenated under a hydrogen pressure of 45 lbs/sq inch. It took 5 hr to complete the reduction. The flask was then disconnected, contents boiled on a water bath and filtered hot. The catalyst was thoroughly washed with boiling ethanol and the combined filtrate and washings were evaporated to dryness under reduced pressure. The residue thus obtained was crystallised from ethanol (95%). The crystalline amide melted at 198-99° with decomposition and weighed 3.5g. Mother liquor on concentration yielded 0.5g more of the product. The total yield was 4.0g (74%).

Anal. Calcd for $C_{14}H_{14}O_3N_2$: C,65.11; H,5.42; N,10.85 .

Found C,65.42; H,5.44; N,10.6 .

III. PREPARATION OF N-BENZOYLAMINO ACIDS

1. DL-N-Benzoylphenylalanine:

DL-N-Benzoylphenylalanine amide (2g) was taken in 40 ml of concentrated hydrochloric acid in a round bottomed flask and warmed on a water bath until a clear solution was obtained. The flask was left at room temperature for 18 hr. Afterwards the crystalline product was filtered, washed with water and dried, when it weighed 1.8g. The filtrate was diluted with 60 ml water when a second crop of crystals (0.18g) was obtained. The total yield was 1.98g (98.8%). On crystallisation from ethanol-water mixture (20:1) the benzoylamino acid melted at 184-85° (lit.[84,16] mp 187-88° & 182°).

Anal. Calcd for $C_{16}H_{15}O_3N$: C,71.36; H,5.61; N,5.20.

Found C,71.23; H,5.80; N,5.31.

2. DL-N-Benzoyl-O-methyltyrosine:

DL-N-Benzoyl-O-methyltyrosine amide (2g) was taken

in 40 ml of concentrated hydrochloric acid, heated on a sand bath for 5 min and then left at room temperature for 4 hr. After this period it was diluted with 30 ml of water and again left at room temperature overnight. Crystalline benzoylamino acid thus obtained was filtered, washed thoroughly with water and dried, mp 170-71°, yield 1.4g. A second crop of crystals (0.1g, mp 165-66°) was obtained by concentrating the mother liquor under reduced pressure to about 20 ml. The total yield of the benzoylamino acid was 1.5g (75%). Recrystallisation from 70% ethanol raised the melting point to 175-76°(lit.[85,73] mp 178-79° & 136-37°).

Anal. Calcd for $C_{17}H_{17}O_4N$: C,68.23; H,5.68; N,4.68.

Found C,68.51; H,5.83; N,4.89.

3. <u>DL-N-Benzoylvaline</u>:

DL-N-Benzoylvaline amide (2.25g) was suspended in 45 ml of concentrated hydrochloric acid, warmed on a boiling water bath till a clear solution was obtained and left at room temperature

overnight (12 hr). The contents were diluted with 40 ml of water and again left at room temperature for 6 hr. White transparent needles were filtered, washed with three 20-ml portions of water and dried. The benzoylamino acid thus obtained melted at 147-48° (lit.[86] mp 132.5°) and weighed 1.25g. Mother liquor on concentration yielded another 0.76g of the product. The total yield was 2.01g (89%).

Anal. Calcd for $C_{12}H_{15}O_3N$: C,65.14; H,6.83; N,6.33.

Found C,65.32; H,6.81; N,6.24.

4. DL-N-Benzoylisoleucine:

DL-N-Benzoylisoleucine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid. This was warmed on a water bath till a clear solution was obtained and then left at room temperature for 4 hr. The contents were diluted with 30 ml of water and again left at room temperature overnight. This was evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (80%). The benzoylamino acid

obtained on filtration weighed 1.5g and melted at 135-36° (lit.[87,88] mp 138-39° & 118°). A second crop of crystals (0.4g) was obtained on concentrating the mother liquor. The total yield was 1.9g (95%).

Anal. Calcd for $C_{13}H_{17}O_3N$: C,66.38; H,7.23; N,5.95.

Found C,66.43; H,7.51; N,6.1.

5. DL-N-Benzoyl-3,4-dimethoxyphenylalanine:

Powdered dl-N-benzoyl-3,4-dimethoxyphenylalanine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid, warmed on a water bath until a clear solution was obtained and then left at room temperature for 4 hr. The contents were then diluted with 25 ml of water and again left at room temperature overnight. The crystalline benzoylamino acid was filtered, washed with three 10-ml portions of water and dried in an oven at 80°. The dry crystalline benzoylamino acid weighed 1.9g (95%) and melted at 175-76°. On recrystallisation from 20% ethanol, the benzoylamino

acid melted at 180-81°.

Anal. Calcd for $C_{18}H_{19}O_5N$: C,65.64; H,5.82; N,5.20.

Found C,65.84; H,5.60; N,5.42.

6. DL-N-Benzoyltyrosine:

Finely powered dl-N-benzoyltyrosine amide (4 g) was suspended in 60 ml of concentrated hydrochloric acid. The amide immediately went into solution. This was left at room temperature for 4 hr and then diluted with 40 ml of water and again left at room temperature overnight. The contents were evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (80%). Benzoylamino acid obtained on drying in an oven at 80°, melted at 194-95° (lit.[20,16] mp 195-97° & 182°) and weighed 3.6g. The mother liquor on concentration yielded another 0.05g of the product. The total yield was 3.65g (89%).

Anal. Calcd for $C_{16}H_{15}O_4N$: C,67.36; H,5.26; N,4.91.

Found C,67.57; H,5.39; N,4.73.

7. DL-N-Benzoyl-3-methoxy-4-hydroxyphenylalanine:

DL-N-Benzoyl-3-methoxy-4-hydroxyphenylalanine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid. The mixture was warmed on a sand bath till a clear solution was obtained and then left at room temperature for 14 hr. The crystalline benzoylamino acid was filtered, washed with three 10-ml portions of water and dried. It melted at 160-61° and weighed 1.4g (70%). Recrystallisation from ethanol raised the melting point to 162-63° (lit.[89] mp 164°).

Anal. Calcd for $C_{17}H_{17}O_5N$: C,64.76; H,5.4; N,4.44.

Found C,64.58; H,5.21; N,4.29.

8. DL-N-Benzoylnorleucine:

DL-N-Benzoylnorleucine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid, warmed on a boiling water bath till a clear solution was obtained and then left at room temperature overnight. The crystalline material thus separated

was filtered and washed with water free of hydrochloric acid. On drying it weighed 1.6g (80%) and melted at 135-36° (lit.[90] mp 134°).

Anal. Calcd for $C_{13}H_{17}O_3N$: C,66.38; H,7.23; N,5.95.

Found C,66.19; H,7.24; N,5.88.

9. DL-N-Benzoylleucine:

DL-N-Benzoylleucine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid. This was warmed on a boiling water bath till a clear solution was obtained and then left at room temperature for 4 hr. The solution was diluted with 30 ml of water and again left at room temperature overnight. The crystalline material thus separated was filtered and dried. It weighed 1.66g (83%) and melted at 138-39° (lit.[90] mp 137-41°).

Anal. Calcd for $C_{13}H_{17}O_3N$: C,66.38; H,7.23; N,5.95.

Found C,66.57; H,7.34; N,5.78.

10. DL-N-Benzoylnorvaline:

DL-N-Benzoylnorvaline amide (2g) was suspended in 40 ml of concentrated hydrochloric acid, warmed on a luke warm water bath till a clear solution was obtained and then left at room temperature overnight. The crystalline benzoylamino acid separated was filtered, washed with two 10-ml portions of water and dried. The dried material weighed 1.7g (85%) and melted at 151-52° (lit.[86] mp 152.5°).

Anal. Calcd for $C_{12}H_{15}O_3N$: C,65.14; H,6.83; N,6.3 .

Found C,65.21; H,6.97; N,6.35 .

11. DL-N-Benzoyl-δ-phenylnorvaline:

DL-N-Benzoyl-δ-phenylnorvaline amide (2g) was suspended in 40 ml of concentrated hydrochloric acid. This was warmed on a boiling water bath till a clear solution was obtained and then left at room temperature overnight. The crystalline material thus separated was filtered, washed with three 10-ml portions of water

and dried. It melted at 189-90° and weighed 1.8g (90%). Recrystallisation from ethanol raised the melting point to 191-92° (lit.[98] mp 187.5°).

Anal. Calcd for $C_{18}H_{19}O_3N$: C,72.72; H,6.4; N,4.72.

Found C,72.57; H,6.42; N,4.9.

12. DL-N-Benzoyl-β-(2-furyl)-alanine:

DL-N-Benzoyl-β-(2-furyl)-alanine amide (2g) was warmed on a free flame with 15 ml of 30% sodium hydroxide solution. The heating was stopped when the evolution of ammonia ceased. The contents were acidified by dropwise addition of concentrated hydrochloric acid with vigorous shaking and thorough cooling. Benzoylamino acid crystallised out on cooling in a refrigerator. The crystalline material was filtered, washed several times with water and dried. The crude benzoylamino acid weighed 1.62g (80%) and melted at 155-56°. Recrystallisation from water raised the melting point to 162-63° (lit.[79] mp 163°).

Anal. Calcd for $C_{14}H_{13}O_4N$: C,64.86; H,5.02; N,5.4.

Found C,64.66; H,5.11; N,5.43.

IV. PREPARATION OF AMINO ACIDS

1. DL-Phenylalanine:

DL-N-Benzoylphenylalanine amide (2g) was refluxed with 60 ml of concentrated hydrochloric acid for 5 hr and then left at room temperature overnight. Next morning benzoic acid which separated was filtered and washed three times with 10-ml portions of water. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in 30 ml water and treated with silver oxide (2g). The contents were warmed on a water bath for 1 hr with frequent shaking and filtered. The filtrate was treated with water-washed hydrogen sulphide gas to remove traces of silver, boiled on a free flame for few minutes and filtered. The clear filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 15 ml water, ethanol (40 ml) was then added and the solution left for crystallisation. The amino acid which crystallised out was filtered, washed three times with 10-ml portions of ethanol (95%), dried and weighed, 1.11g (90%), mp 274-75° dec (Lit.[16] mp 263-65°).

Anal. Calcd for $C_9H_{11}O_2N$: C,65.45; H,6.66; N,8.48.

Found C,65.55; H,6.87; N,8.68.

2. DL-O-Methyltyrosine:

DL-N-Benzoyl-O-methyltyrosine amide (2g) was refluxed with 60 ml of concentrated hydrochloric acid for 4 hr and then left at room temperature overnight. Benzoic acid which separated was filtered, washed with three 10-ml portions of water and the filtrate evaporated to dryness under reduced pressure. This process of evaporation under reduced pressure was repeated twice by adding 20-ml portions of water each time. The residue thus obtained was worked up in the manner as described in the case of phenylalanine. The residue thus obtained was dissolved in excess of water till a clear solution was formed. The solution was concentrated till crystallisation started and then kept in a refrigerator overnight to complete the crystallisation. The crystalline amino acid obtained in this manner was filtered, washed with three 10-ml portions of ethanol (95%) and dried when it weighed 0.75g and melted at 264-65° dec (lit.[91] mp 277-79°). A second crop

of crystals (0.25g) was obtained on concentrating the mother liquor to a small volume and then diluting it with ethanol (95%). The total yield was 1.0g (76%).

Anal. Calcd for $C_{10}H_{13}O_3N$: C,61.53; H.6.66; N,7.18.

Found C,61.31; H,6.57; N,6.99.

3. DL-Valine:

DL-N-Benzoylvaline amide (3g) was refluxed with 80 ml of concentrated hydrochloric acid for 1.5 hr and then left at room temperature overnight. Benzoic acid thus separated was filtered and washed with two 15-ml portions of water. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was worked up in the manner as described before. The filtrate thus obtained after removal of silver ions was evaporated to dryness under reduced pressure. The residue was dissolved in minimum amount of water, ethanol (40 ml) was added and this was left for crystallisation. The amino acid thus crystallised was filtered, washed with three 10-ml portions of ethanol (95%) and

dried in an oven at 80°; mp 291-92° dec, (lit.[92] mp 293°), yield 1.5g (nearly quantitative).

Anal. Calcd for $C_5H_{11}O_2N$: C,51.26; H,9.46; N,11.96.

Found C,51.13; H,9.49; N,12.03.

4. DL-Isoleucine:

DL-N-Benzoylisoleucine amide (2g) was refluxed with 60 ml of concentrated hydrochloric acid for 2 hr and then left at room temperature overnight. Benzoic acid thus separated was filtered and washed with three 10-ml portions of water. The filtrate was evaporated to dryness under reduced pressure. The residue was worked up in the usual manner. The residue thus obtained after the removal of silver ions was dissolved in minimum amount of water followed by the addition of 50 ml of ethanol (95%) and kept for crystallisation. The amino acid thus crystallised was filtered, washed with three 10-ml portions of ethanol (95%) and dried in an oven at 80°. This weighed 0.75g and melted at 270-71°(lit.[93] mp 278-80°dec).

Another 0.25g of the product melting at 260-61° was obtained on concentration of the mother liquor. The total yield of the amino acid was 1.0g (90%).

Anal. Calcd for $C_6H_{13}O_2N$: C,54.96; H,9.92; N,10.68.

Found C,55.03; H,9.8%; N,10.5.

5. DL-3,4-Dimethoxyphenylalanine:

DL-N-Benzoyl-3,4-dimethoxyphenylalanine amide (2g) was refluxed with 60 ml of concentrated hydrochloric acid for 6 hr and then left at room temperature overnight. The reaction mixture was worked up by the general procedure adopted in the case of phenylalanine. The crystalline amino acid thus obtained was filtered, washed four times with 10 ml of ethanol (95%) and dried when it weighed 0.9g and melted at 241-42° with decomposition (lit.[94] mp 249-50° dec). Mother liquor on concentration yielded another 0.25g of the product. The total yield was 1.15g (84%).

Anal. Calcd for $C_{11}H_{15}O_4N$: C,58.7; H,6.66; N,6.22.

Found C,59.03; H,6.96; N,6.15.

6. DL-Tyrosine:

DL-N-Benzoyltyrosine amide (2g) was refluxed with 60 ml of concentrated hydrochloric acid for 5 hr and then left at room temperature overnight. Benzoic acid which separated was filtered and washed with three 10-ml portions of water. The reaction mixture was worked up as in the case of phenylalanine. The filtrate thus obtained after removal of silver ions was evaporated to dryness under reduced pressure. The residue thus obtained was dissolved in minimum amount of water followed by the addition of 50 ml of ethanol (95%) and left for crystallisation in a refrigerator overnight. The amino acid thus crystallised was filtered, washed with three 10-ml portions of ethanol and dried when it melted at 306-7° with decomposition (lit.[16] mp 295°) and weighed 0.9g. Mother liquor on concentration gave another 0.22g of the amino acid. The total yield was 1.12g (88%).

Anal. Calcd for $C_9H_{11}O_3N$: C,59.66; H,6.07; N,7.73.

Found C,59.98; H,6.43; N,7.96.

7. DL-3-Methoxy-4-hydroxyphenylalanine:

DL-N-Benzoyl-3-methoxy-4-hydroxyphenylalanine amide (2g) was refluxed with 60 ml of concentrated hydrochloric acid for 6 hr and then left at room temperature overnight. The isolation of the amino acid was done in the same manner as in the case of phenylalanine. The amino acid thus obtained was filtered, washed with three 10-ml portions of ethanol (95%) and dried in an oven at 80°. The amino acid on drying weighed 0.8g and melted at 245-46° with decomposition (lit.[94] mp 255-56° dec). Mother liquor on concentration yielded another 0.27g of the amino acid. The total yield was 1.07g(80%).

Anal. Calcd for $C_{10}H_{13}O_4N$: C,56.87; H,6.16; N,6.63.

Found C,56.99; H,6.3; N,6.94.

8. DL-Norleucine:

DL-N-Benzoylnorleucine amide (2.5g) was heated under reflux with 60 ml of concentrated hydrochloric acid for 4 hr and then left at room temperature overnight. Benzoic acid which separated was

filtered and washed with three 10-ml portions of water. The combined filtrate and washings were worked up as described in the previous cases. The amino acid obtained in this manner melted at 284-85° with decomposition (lit.[95] mp 297-300°) and weighed 1.2g (85%).

Anal. Calcd for $C_6H_{13}O_2N$: C,54.96; H,9.92; N,10.68.

Found C,55.15; H,9.73; N,10.64.

9. DL-Leucine:

DL-N-Benzoylleucine amide (2.5g) was refluxed with 60 ml of concentrated hydrochloric acid for 3 hr and then left at room temperature overnight. The reaction mixture was then worked up in the usual manner. The amino acid obtained in this way weighed 1.2g (88%) and melted at 286-87° with decomposition (lit.[90] mp 293-95° dec).

Anal. Calcd for $C_6H_{13}O_2N$: C,54.96; H,9.92; N,10.68.

Found C,54.87; H,9.95; N,10.59.

10. DL-Norvaline:

DL-N-Benzoylnorvaline amide (2.4g) was heated under reflux with 60 ml of concentrated hydrochloric acid for 2 hr and then left at room temperature overnight. Benzoic acid thus separated was filtered and washed with three 10-ml portions of water. The combined filtrate and washings were taken to dryness under reduced pressure. The residue was dissolved in minimum amount of water and neutralised with ammonia. Ethanol (40 ml) was added to this solution and it was kept for crystallisation in a refrigerator overnight. The white shining plates of the amino acid were filtered, washed with three 10-ml portions of ethanol (95%) and dried in an oven at 80°. The amino acid thus obtained weighed 1.05g (82%) and melted at 284-85° (lit.[86] mp 291-5° dec).

Anal. Calcd for $C_5H_{11}O_2N$: C,51.26; H,9.46; N,11.96.

Found C,51.47; H,9.53; N,12.14.

11. DL-δ-Phenylnorvaline:

DL-N-Benzoyl-δ-phenylnorvaline amide (2g) was refluxed with 100 ml of sodium hydroxide solution (30%) for 24 hr. The mixture was then neutralised by dropwise addition of dilute hydrochloric acid accompanied by thorough cooling and vigorous shaking. The solution was concentrated under reduced pressure until the volume was about 30 ml. Ethanol (10 ml) was added and this was then left for crystallisation in a refrigerator overnight. The crystalline amino acid thus obtained was filtered, washed with two 10-ml portions of ethanol (95%) and dried when it melted at 239-40° with decomposition (lit.[96] mp 203-6° dec) and weighed 0.9g. Mother liquor on concentration yielded 0.35g more of the amino acid. The total yield was 1.25g (96%).

Anal. Calcd for $C_{11}H_{15}O_2N$: C,68.39; H,7.77; N,7.25.

Found C,68.28; H,7.51; N,7.28.

12. DL-β-(2-Furyl)-alanine:

DL-N-Benzoyl-β-(2-furyl)-alanine amide (2g) was refluxed with barium hydroxide solution (16g in 100 ml water) for 24 hr. At the end of this period it was diluted with 100 ml of water and carbon dioxide was passed to remove barium as barium carbonate. This was filtered, washed with water and the combined filtrate and washing were evaporated to dryness under reduced pressure. The residue thus obtained was crystallised from ethanol (80%). The crystalline amino acid so obtained melted at 256-57° with decomposition (lit.[97] mp 260° dec) and weighed 0.8g (73%).

Anal. Calcd for $C_7H_9O_3N$: C,54.19; H,5.85; N,9.03.

Found C,54.38; H,5.73; N,9.14.

REFERENCES

REFERENCES

1. Carter, H.E., Org. Reactions, Vol.III, 202 (1947).

2. Davies, J.S.H., Jones, W.O., and Imperial Chemical Industries., Brit. 583190, 1946.

3. Galat, A., J.Am.Chem.Soc., 72, 4436 (1950).

4. MacDonald, S.F., J.Chem.Soc., 1948, 376.

5. Nowak, K., Siemion, I.Z., and Siemieniewski, H., Roczniki Chem., 36, 557 (1962).

6. Weygand, F., and Glockler, U., Chem.Ber., 89, 653 (1956).

7. Shen, L.M.C., and Hartung, W.H., J.Org. Chem., 23, 96 (1958).

8. Crawford, M., and Little, W.T., J.Chem. Soc., 1959, 729.

9. Behringer, H., Ger. 1,095,833 (Cl.12p), Dec.29.1960, Appl. May 29, 1954; 4pp.

10. Baltazzi, E., and Davis, E.A., Chem. Ind. (London), 1962, 929.

11. Behringer, H., and Taul, H., Chem. Ber., 90, 1398 (1957).

12. Steglich, W., and Hurnaus, R., Tetrahedron Letters, 1966(4), 383.

13. Bodea, C., and Oprean, I., Rev. Roum. Chim., 1968,13(12), 1647.

14. Woodward, R.B., and Woodman, D.J., J.Org.Chem., 1969, 34(9),2742.

15. Harry, L.S., and Norman, L.W., U.S.Patent No.3,275,648(1966).

16. Erlenmeyer, E.Jr., and Halsey, J.T., Ann., 307, 138 (1899).

17. Erlenmeyer, E.Jr., Ann., 275, 13 (1893).

18. Erlenmeyer, E.Jr., and Kunlin, J., Ann., 307, 163 (1899).

19. Erlenmeyer, E.Jr., Ann., 337, 205 (1904).

20. Fischer, E., Ber., 32, 3638 (1899).

21. Deulofeu, V., Anales Soc. espan.fis.quim., 32, 152 (1934).

22. Sonn, A., Muller, E., Bulow, W., and Meyer, W., Ber., 58, 1103 (1925).

23. Schaaf, F., and Labouchere, A., Helv. Chim.Acta, 7, 357 (1924).

24. Dey, B.B., and Rajagopalan, S., Arch. Pharm., 277, 359, 377 (1939).

25. Restelli, E., Anales asoc. quim. argentina, 23, 58 (1935).

26. Ellinger, A., and Flamand, C., Ber., 40, 3029 (1907).

27. Ellinger, A., and Flamand, C., Z.physiol. Chem., 55, 8 (1908).

28. Fischer, H., and Zerweck, W., Ber., 56, 519 (1923).

29. Barger, G., and Ewins, A.J., Biochem. J., 11, 58 (1917).

30. Ellinger, A., and Matsuoka, Z., Z.physiol. Chem., 91, 45 (1914).

31. Erlenmeyer, E.Jr., Ber., 31, 2238 (1898).

32. Harington, C.R., and Barger, G., Biochem. J., 21, 169 (1927).

33. Lamb, J., and Robson, W., Biochem. J., 25, 1231 (1931).

34. Harington, C.R., and McCartney, W., Biochem. J., 21, 852 (1927).

35. Gillespie, H.B., and Snyder, H.R., Org. Syntheses, Coll.Vol.2, 489(1943).

36. Schiemann, G., and Roselius, W., Ber., 65, 1439 (1932).

37. Schuegraf, K., Helv. Chim. Acta, 12, 405 (1929).

38. Schmalfuss, H., and Peschke, W., Ber., 62, 2591 (1929).

39. Baltazzi, E., and Davis, J.W., Compt. rend., 240, 208 (1955).

40. Bergmann, M., Stern, F., and Witte, C., Ann., 449, 277 (1926).

41. Carter, H.E., Handler, P., and Melville, D.B., J.Biol. Chem., 129,359(1939).

42. Harwood, H.J., and Johnson, T.B., J.Am.Chem. Soc., 56, 468 (1934).

43. Herbst, R.N., and Shemin, D., Org. Syntheses, Coll.Vol. 2, 491 (1943).

44. Fischer, H., and Hofmann, H.J., Z.physiol. Chem., 245, 139 (1936-1937).

45. Attenburrow, J., Elliot, D.F., and Penny, G.F., J.Chem. Soc.,310 (1948).

46. Erlenmeyer, E.Jr., Ann., 271, 137 (1892).

47. Erlenmeyer, E.Jr., Ber., 30, 2976 (1897).

48. Erlenmeyer, E.Jr., and Halsey, J.T., Ber, 30, 2981 (1897).

49. Sus, O., and Schafer, W., Ger.patent 831,999 (1952).

50. Weisblat, D.I., and Lyttle, D.A., J.Am. Chem. Soc., 71, 3079(1949).

51. Lyttle, D.A., and Weisblat, D.I., J.Am. Chem. Soc., 69, 2118(1947).

52. Sus, O., and Schafer, W., Ger. patent 830,049 (1952).

53. Mori, Y., Kondo, S., and Kumagae, H., J.Chem.Soc.Japan, 78, 1174(1957).

54. Akabori, S., Izumi, Y., and Okuda, T., J.Chem. Soc. Japan, 77, 490(1956).

55. Gaudry, and McIvor, R.A., Canadian J.Chem., 29, 427 (1951).

56. Izumi, Y., and Konisi, I., J. Chem. Soc. Japan, 74, 957 (1953).

57. Izumi, Y., and Kawasaki, M., J.Chem. Soc. Japan, 78, 660 (1957).

58. Warner, D.T., and Moe, O.A., J.Am. Chem. Soc., 70, 2765 (1948).

59. Knoop, F., and Oesterlin, H., Z.physiol. Chem., 170, 186-211 (1927).

60. Aubel, E., and Bourguel, M., Compt. rend.acad. Sci. U.R.S.S., 186, 1844 (1928).

61. Desmuelle, P., and Fromageot, C., Bull. Soc. Chim. France, 5, 1, 700-2(1934).

62. Schoenheimer, R., and Ratner, S., J.Biol. Chem., 127, 301 (1939).

63. Greenstein, J.P., and Winitz, M., Chem. of Amino Acids, Vol.3, 2761, John Wiley, New York (1961).

64. Izumiya, N., Nagamatsu, A., and Ota, S., Kyushu Mem.Med.Sci., 4, 1 (1953).

65. Haulin, K.E., and Hartung, W.H., J.Biol.Chem., 145, 349 (1942).

66. Hartung, W.H., J.Am.Chem.Soc., 50, 3370 (1928).

67. Waters, K.L., and Hartung, W.H., J.Org. Chem., 10, 524 (1945).

68. Naseem H.Khan, Ph.D.Thesis, A.M.U., Aligarh, India (1963).

69. Birkofer, L., Chem.Ber., 80, 83 (1947).

70. Anker, H.S., J. Biol. Chem., 187, 167 (1950).

71. Vanzyle, G., Van Tamelen, E.E., Zuidema, G.D., J.Am.Chem.Soc., 73, 1765 (1951).

72. Kidwai, A.R., and Devasia, G.M., J.Org. Chem., 27, 4527 (1962).

73. Dakin, H.D., J.Bio. Chem., 8, 11,19 (1910).

74. Ramage, G.R., and Simonsen, J.L., J.Chem. Soc., 1935, 532.

75. Doyle, F.P., Holland, D.O., Marflit, W., Nayler, J.H.C., and O'Connor, C.M., J.Chem.Soc., 1955, 1719.

76. Bick, J.S., and Ide, W.S., Org. Syntheses, Coll.Vol.2,55 (1943).

77. Finar I.L., and Libman, D.D., J.Chem. Soc., 1949, 2728.

78. Erlenmeyer, E.Jr., and Matter, O., Ann., 337, 271 (1904).

79. Flatow, L., Z.physiol. Chem., 64, 367, 387 (1910).

80. Mohr, E., and Stroschein, F., Ber., 42, 2521 (1909).

81. Bergmann, M., and Fruton. J.S., J.Bio. Chem., 124, 321 (1938).

82. Kanewskaja, S.I., Chemisches Zentralblatt, 1, 1026 (1928).

83. Max, J., Ann., 369, 276 (1909).

84. Fischer, E., and Mouneyrat, A., Ber., 33, 2383 (1900).

85. Carter, H.E., and Hirman, J.W., J.Biol.Chem., 178,403 (1948).

86. Slimmer, M.D., Ber, 35, 401 (1902).

87. Itschner, K.F., Drechsler, E.R., Warner, C., and Fox, S.W., Arch. Biochem. Biophys., 53, 294 (1954).

88. Bouveault, L., and Locquin, R., Compt. rend. Acad.Sci., 141,117(1905).

89. Bloch, B., Z.physiol. Chem., 98, 236 (1916).

90. Fischer, E., Ber., 33, 2382, 2370 (1900).

91. Dakin, H.D., J.Biol. Chem., 9, 151 (1911).

92. Feofilaktov, V.Y., and Zaitseva, V.N., J.Gen.Chem.U.S.S.R., 10, 1391 (1940).

93. Marvel, C.S., Org.Syntheses, Coll.Vol.3, 495 (1955).

94. Johnson, T.B., and Bengis, R., J.Am.Chem.Soc., 35, 1613, 1615 (1913).

95. Adams, R., and Marvel, C.S., J.Am.Chem.Soc., 42, 320 (1920).

96. Braun, J.V., and Kruber, O., Ber., 45, 389 (1912).

97. Sasaki, T., Ber., 54, 2056 (1921).

98. Walter, R., and Heins, B., Ber., 88, 34 (1955).

99. Erlenmeyer, E., Ber., 33, 2036 (1900).

100. Erlenmeyer, E., Ann., 337, 265 (1904).

101. Erlenmeyer, E., and Stadlin, W., Ann., 337, 283 (1904).

102. Erlenmeyer, E., and Wittenberg, F., Ann., 337, 294 (1904).