Synthesis of Enzyme Inhibitors of L-Lysine Biosynthesis

A thesis presented in part fulfilment of the requirement for the Degree of Doctor of Philosophy.

by

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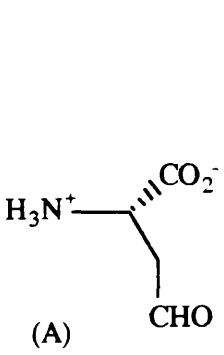
Summary

There are two possible biosynthetic pathways to the essential amino acid L-lysine. The diaminopimelate pathway occurs in higher plants and bacteria and the α-aminoadipate pathway operates in fungi and yeast. This thesis is concerned with the first two steps in the diaminopimelate pathway to L-lysine, catalysed by dihydrodipicolinate synthase (DHDPS) and dihydrodipicolinate reductase (DHDPR).

Synthesis of substrates involved in the DHDPS and DHDPR steps, namely aspartate semialdehyde (A), 2,3-dihydro-dipicolinic acid (B) and 2,3,4,5-tetrahydrodipicolinic acid (C) was attempted. DL-Aspartate semialdehyde was prepared by the ozonolysis of DL-allylglycine for use in the enzyme assay of DHDPS. Attempts were made to synthesise 2,3-dihydro-dipicolinic acid (B) from aspartate semialdehyde and oxaloacetic acid. However the final product was dipicolinic acid, probably formed by oxidation of the unstable 2,3-dihydrodipicolinic acid (B).

The synthesis of 2,3,4,5-tetrahydrodipicolinic acid (C) was achieved. This involved the preparation of dimethyl *N*-tosyl-*cis*-2,6-piperidinedicarboxylate (E). Treatment of the *N*-tosyl



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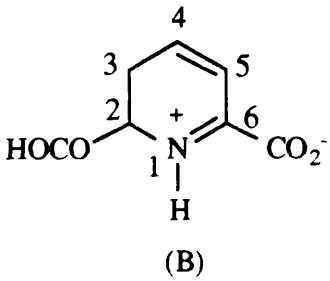

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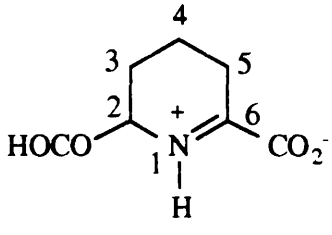

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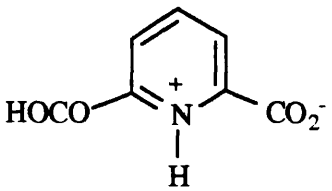
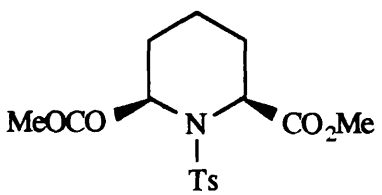


(D)

derivative with potassium t-butoxide resulted in the elimination of toluenesulphinic acid, forming the required imine (C). The imine (C) was found to exist in solution in equilibrium with the enamine and the open chain compound. Imines derived from L-proline and DL-pipecolinic acid were also prepared using the same route.

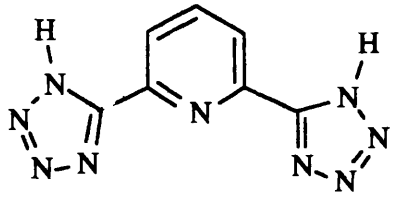


(E)

Inhibition of the biosynthesis of the essential amino acid, L-lysine, is of great interest as inhibitors of the pathway may have herbicidal or antibacterial activity without mammalian toxicity. A number of compounds were synthesised and tested as inhibitors of dihydrodipicolinic acid synthase. Analogues of dipicolinic acid (D) were made. Dipicolinic acid was converted into 2,6-pyridinedicarboxamide. Dehydration of 2,6-pyridine-dicarboxamide gave 2,6-pyridinedinitrile. Using the dinitrile the diimidate and the ditetrazole (F) were prepared for testing as inhibitors of DHDPS.

Using chelidonic acid (G) and chelidamic acid (H), *N*-alkyl chelidamic acids were prepared and tested as enzyme inhibitors of DHDPS. Esterification of these compounds was

carried out using methanol and conc. sulphuric acid to provide more compounds for testing in the enzyme assay.



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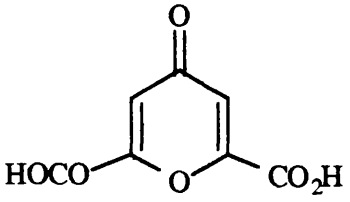

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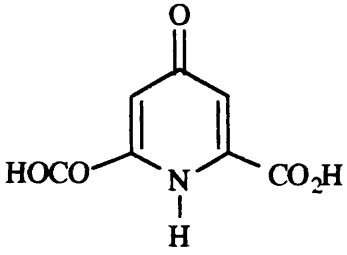
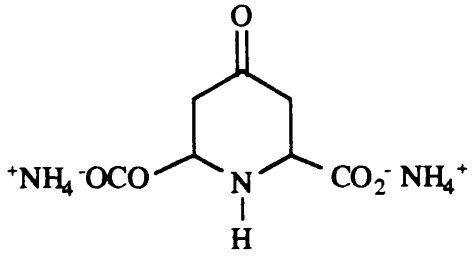


(H)

A number of analogues of 2,6-piperidinedicarboxylic acid were prepared. *N*-Methyl-2,6-piperidinedicarboxylic acid and its *N*-oxide (I) were synthesised from *cis*-2,6-piperidine-dicarboxylic acid. 1,4-Pentadien-3-one-1,5-dicarboxylic acid was prepared from 2-furanacrylic acid. Cyclisation of 2-furan-acrylic acid afforded piperidin-4-one-2,6-dicarboxylic acid (J). Cyclisation with methylamine yielded the *N*-methyl derivative.

All our synthesised compounds were tested as inhibitors of dihydrodipicolinic acid synthase enzyme.



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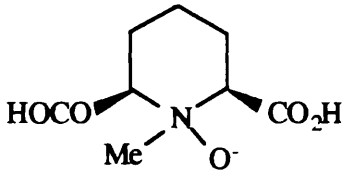


(I)

Abbreviations

ASA	aspartate semialdehyde
d	doublet
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIBAL	diisobutylaluminium hydride
DMF	*N,N*-dimethylformamide
DNP	2,4-dinitrophenylhydrazine
DHDPA	2,3-dihydrodipicolinic acid
DHDPR	2,3-dihydrodipicolinate reductase
DHDPS	2,3-dihydrodipicolinate synthase
IR	infra red
m	multiplet
m / z	mass spectrometry
Ms	methanesulphonate
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
s	singlet
t	triplet
Tf	trifluromethanesulphonate
THDPA	2,3,4,5-tetrahydrodipicolinic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
UV	ultra violet

CHAPTER 1

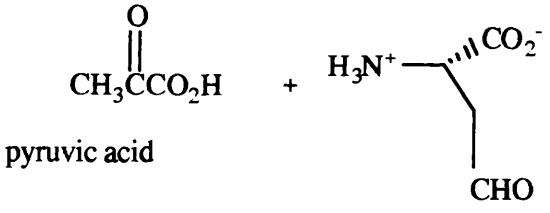
Two distinct pathways to L-lysine (8) have been established in bacteria and fungi. These are called the diaminopimelate pathway (Scheme 1) and the α-aminoadipate pathway (Scheme 2). The latter originates with 2-oxoglutaric acid (9) and involves the formation of α-aminoadipic acid (15) and saccharopine (17) as intermediates. This pathway is characteristic of yeast and fungi and more details will be given in the next Chapter. The diaminopimelate pathway occurs in bacteria and higher plants and is the subject of this thesis.

In the diaminopimelate pathway seven enzyme catalysed reactions are involved in the biosynthesis of L-lysine starting from L-aspartate semialdehyde (1) and pyruvic acid (Scheme 1). These enzymes have been characterised in *Escherichia coli*. However, only enzymes catalysing the first two and last two steps have been isolated from plants and characterised.

The diaminopimelate pathway is of great interest as diaminopimelic acid is an essential building block for peptidoglycan of bacterial cell walls. Compounds which inhibit this pathway could have herbicidal or antibacterial activity. Although plants and micro-organisms are capable of the synthesis of the amino acid L-lysine, mammals lack the ability to synthesise L-lysine. Mammals have to obtain L-lysine from dietary sources. Therefore enzyme inhibitors of lysine biosynthesis should not be toxic to mammals.

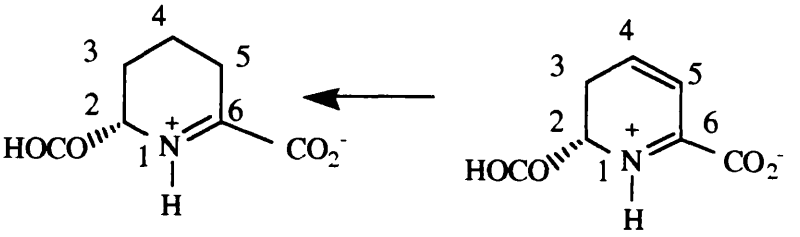
The first step in the diaminopimelate pathway is condensation of pyruvic acid and L-aspartate semialdehyde (1) with dihydrodipicolinate synthase (DHDPS) as the catalyst to

The Diaminopimelate Biosynthetic Pathway To L-Lysine



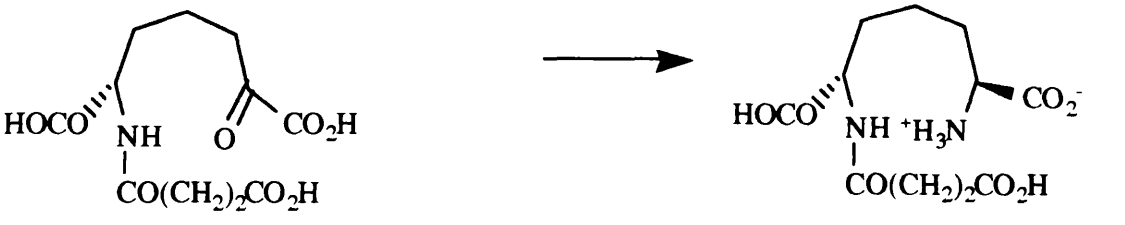
pyruvic acid

(1) L-aspartate semialdehyde



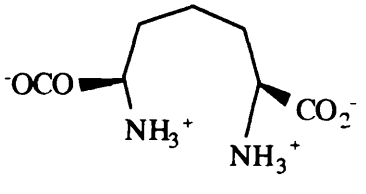
(3) L-2,3,4,5-tetrahydrodipicolinic acid

(2) L-2,3-dihydrodipicolinic acid



(4) L-2-(succinylamino)-6-oxopimelic acid

(5) L-2-(succinylamino)-6-aminopimelic acid



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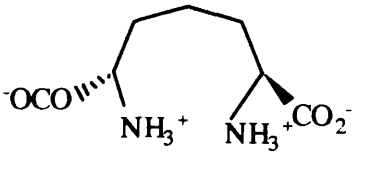
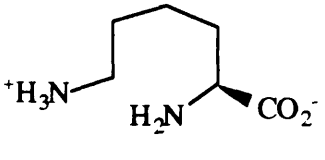
DL-diaminopimelic acid



(6)

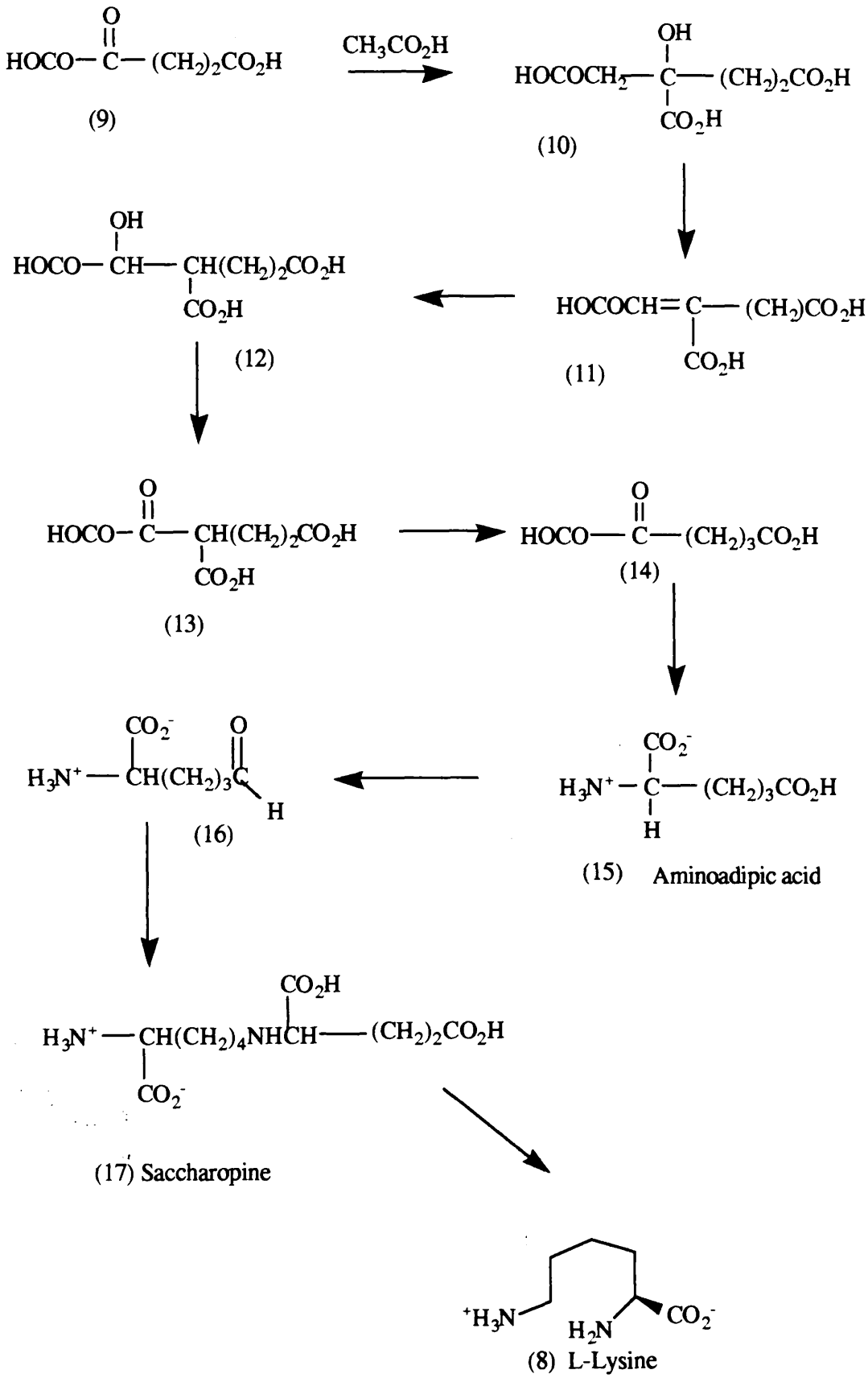
LL-diaminopimelic acid



(8) L-lysine

Scheme 1

The Aminoadipate Pathway To L-Lysine



Scheme 2

give L-2,3-dihydrodipicolinic acid (2). DHDPS has been isolated from bacteria such as *E. coli.*[1] This step has also been demonstrated in plant extracts by Cheshire and Miflin[2] using maize seedlings as the enzyme source. The synthase has also been isolated from spinach leaves[3] and wheat.[4] Aspartate semialdehyde was first prepared by Black and Wright.[5] The condensation of aspartate semialdehyde with pyruvic acid was studied by Kimura in Bacillus subtilis.[6] The second step is the reduction of L-2,3-dihydrodipicolinic acid (2) to L-2,3,4,5-tetrahydrodipicolinic acid (3) with dihydrodipicolinate reductase (DHDPR) as the catalyst and requiring NADPH. This step has been found to occur in maize.[7]

Enzymes which catalyse the next three enzymic reactions in the pathway have not been isolated from plants but have been extracted from micro-organisms. The nicotinamide adenine dinucleotide phosphate (NADP) dependent reduction of L-2,3-dihydrodipicolinic acid (DHDPA) (2) to L-2,3,4,5-tetrahydrodipicolinic acid (THDPA) (3) is followed by acylation of the amino acid. Acylation serves to protect the amino group during the synthesis of diaminopimelate and either acetyl Coenzyme A (CoA) or succinyl CoA is utilised in micro-organisms. The acylated intermediate is aminated at the ε-position in an aminotransferase reaction with glutamate as the amino group donor. Deacylation of the *N*-acyldiaminopimelic acid (5) results in the formation of LL-diaminopimelic acid (6) which is epimerised to DL-diaminopimelic acid (7). Finally decarboxylation at the D-centre gives L-lysine (8). Diaminopimelate decarboxylase has been demonstrated in wheat and maize in the conversion of diaminopimelate into L-

lysine. The diaminopimelate pathway is discussed in more detail in the next Chapter.

The enzyme reactions that we have investigated are the dihydrodipicolinate synthase and dihydrodipicolinate reductase steps of the diaminopimelate pathway found in bacteria. It is assumed that these enzymic reactions in plants are analogous to those in micro-organisms. Therefore inhibitors of the bacterial enzymes may also be inhibitors of the plant enzyme and thus have herbicidal activity.

Very little chemical evidence has been reported for the postulated products of the DHDPA synthase and DHDPA reductase reactions. The synthesis of the intermediates believed to be involved in these two enzymic reactions namely, L-2,3-dihydrodipicolinic acid (2) and L-2,3,4,5-tetrahydrodipicolinic acid (3), was therefore attempted to test them as substrates for the enzymic reactions, and to enable assays to be carried out. This work is described in Chapters 3 and 4.

Aspartate semialdehyde was made for use in the assay of the first enzyme on the L-lysine biosynthetic pathway using the method of Black and Wright.[5] A good assay was required so that selected compounds could be tested as inhibitors. Work on the isolation and purification of this enzyme from *E. coli* was carried out in parallel with this work by Emma Borthwick and Professor J.R. Coggins in the Biochemistry Department, with the support of ICI Agrochemicals Division.

The assay of DHDPS was followed by UV absorbance at 270 nm. This is characteristic of dipicolinic acid which is formed rapidly from L-2,3-dihydrodipicolinic acid (2), the

presumed initial condensation product of L-aspartate semialdehyde and pyruvic acid (Scheme 1).

Much of the effort in this research programme was directed towards the synthesis of potential inhibitors of L-lysine biosynthesis. These targets were modelled on the two early intermediates in the pathway, namely L-2,3-dihydrodipicolinic acid (2) and L-2,3,4,5-tetrahydrodipicolinic acid (3). In Chapters 5 and 6 the synthetic routes carried out to make potential inhibitors are described. These compounds are mainly derivatives of 2,6-piperidinedicarboxylic acid, dipicolinic acid, or chelidamic acid. As well as chemically synthesised compounds, commercially available compounds were tested. Dihydrodipicolinate synthase was isolated from *E. coli* and purified by Emma Borthwick in the Biochemistry Department. Selected compounds were tested for inhibition of the synthase enzyme. This work is described in Chapter 7. Good inhibitors were required for further testing for their herbicidal activity by ICI Agrochemicals. Further work has to be carried out on dihydrodipicolinate reductase enzyme before inhibition studies on this enzyme can be carried out. Enzyme inhibitors have increasing importance in the treatment of many diseases and as agrochemicals such as herbicides.

CHAPTER 2

Introduction

In this Chapter reported studies on each of the enzymes of the diaminopimelate pathway to L-lysine will be reviewed. This includes a discussion on compounds synthesised and tested for enzyme inhibition and for substrate activity with each enzyme on the pathway. A brief review of the α-aminoadipate pathway to L-lysine (Scheme 2) will also be given.

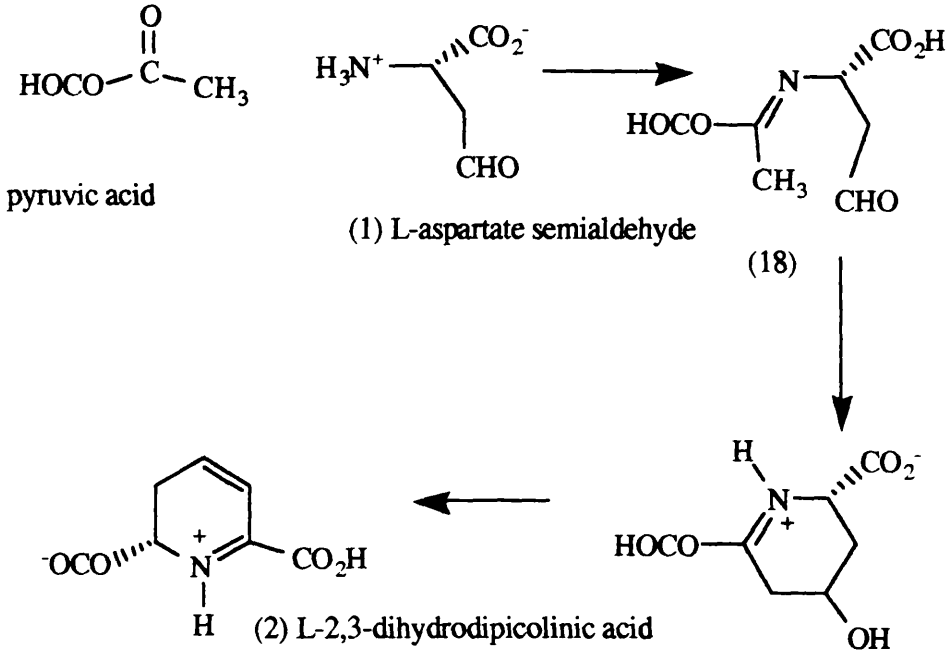
2.1 Dihydrodipicolinate Synthase

Dihydrodipicolinate synthase (DHDPS), a branch point enzyme which leads to L-lysine, catalyses the condensation of pyruvic acid and L-aspartate semialdehyde. This enzyme activity has been detected in bacteria such as *E. coli*[1] and several plants.[2,3,4]

DHDPS is a tetramer, each sub-unit having a molecular weight of 32,000 daltons. The enzyme is subject to feedback inhibition by low concentrations of L-lysine in an allosteric manner.

It is not known if the imine (18) forms first in this condensation reaction (Scheme 3) or if the enolate derived from pyruvic acid adds to the aldehyde (Scheme 4). Studies with various aldolases have shown that imine formation between the keto substrate and the terminal amino group of the L-lysine residue on the enzyme is the obligatory first step in enzyme catalysed aldol reactions. The mechanism (Scheme

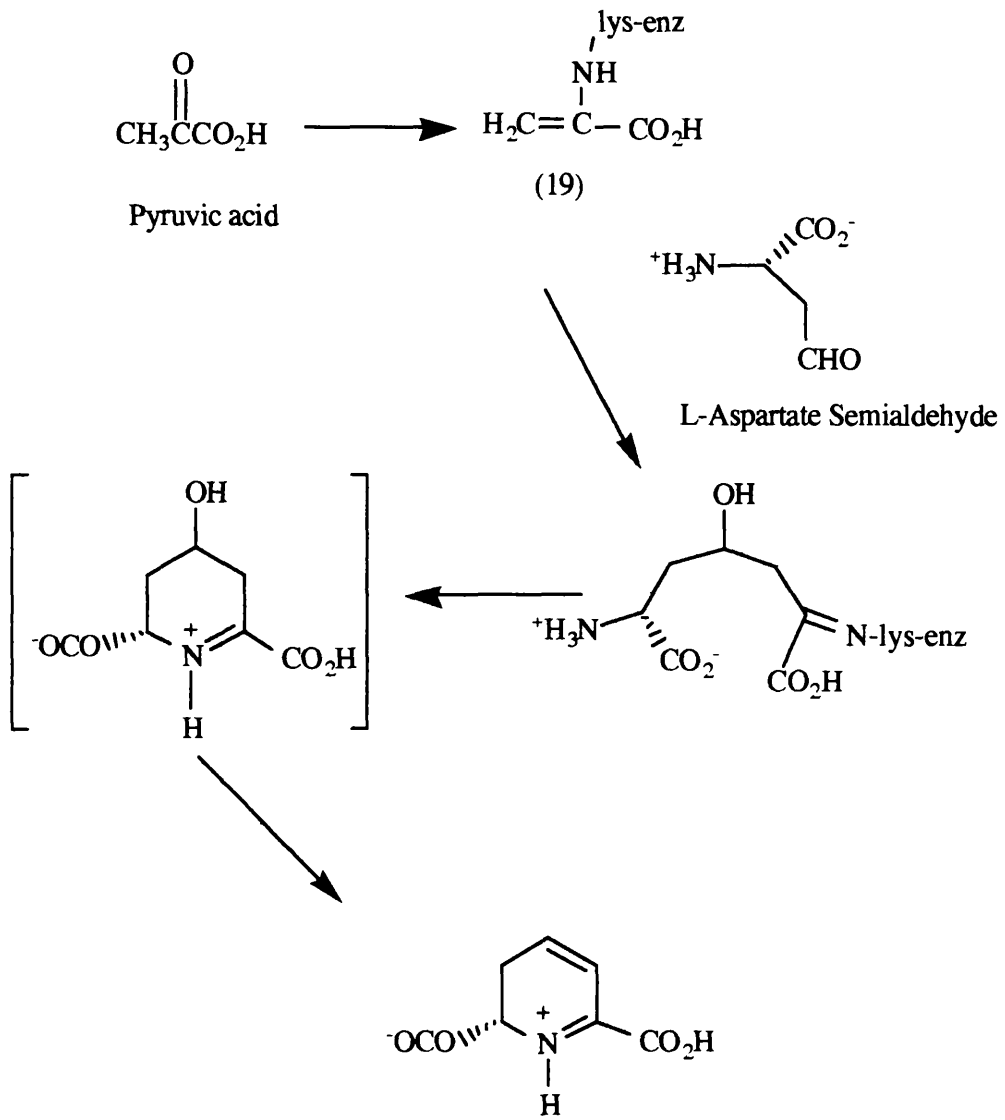
4) is proposed to proceed through a Ping-Pong mechanism in which pyruvic acid binds first to the enzyme to form a Schiff base (19) (K_m 11.76 mM). Water is then released, followed by the binding of aspartate semialdehyde (K_m 0.88 mM). Nucleophilic attack of the pyruvyl enamine on the aspartate semialdehyde aldehyde group then takes place. The formation of L-2,3-dihydrodipicolinic acid (2) involves a number of steps, but the sequence in which they occur is not known. The product of the enzymic condensation is assumed to be L-2,3-dihydrodipicolinic acid (2), although it is possible that L-2,5-dihydrodipicolinic acid is formed.



Scheme 3

Dihydrodipicolinic acid is believed to be unstable and little chemical evidence has been provided for its intermediacy. It was reported by Yugari and Gilvarg[1] that hydrogenation of the condensation reaction product gave 2,6-piperidinedicarboxylic acid which was identified by comparison

of its R_F value with that of authentic material. Dipicolinic acid is probably the isolated product of this enzymic reaction from UV evidence, and could arise from non-enzymic oxidation of L-2,3-dihydrodipicolinic acid.



(2) L-2,3-Dihydrodipicolinic acid (DHDPA)

Scheme 4

The acidic groups of both substrates will be ionised at the assay pH and will probably play a key role in the binding. Suitable functionalities for binders would be arginine residues

in the enzyme active site. The aldehyde carbonyl could be hydrogen bonded to facilitate nucleophilic attack from the enamine (19). There is also likely to be a basic residue in close proximity to the pyruvyl unit to abstract a proton.

2.2 Inactivation of Dihydrodipicolinate Synthase[4]

Kumpaisal, Hashimoto and Yamada[4] studied the properties and reaction kinetics of dihydrodipicolinate synthase using highly purified enzyme from wheat suspension cultures. Aspartate semialdehyde was prepared for use in the DHDPA synthase assay using the method of Black and Wright.[5] Wheat DHDPA synthase, a tetramer, is subject to strong feedback inhibition by low concentrations of L-lysine in an allosteric manner. Bromopyruvic acid, a pyruvic acid analogue, was studied for its effects on wheat DHDPA synthase activity. The assay was developed by Yugari and Gilvarg[1] and utilised *o*-aminobenzaldehyde. The chromophore of the product formed from L-2,3-dihydrodipicolinic acid and *o*-aminobenzaldehyde was observed by its spectrophotomeric absorbance at 540 nm.

Bromopyruvic acid was found to inhibit DHDPA synthase considerably at 1 mM. Results showed that bromopyruvic acid inhibits in a competitive manner with respect to pyruvic acid and in an uncompetitive manner with respect to aspartate semialdehyde. The calculated K_i for bromopyruvic acid was 1.8 mM. From the results of Kumpaisal *et al.*[4] it seemed that bromopyruvic acid irreversibly inactivates DHDPA synthase by alkylating nucleophilic amino acid residues at or near the active site. The competitive type of inhibition with respect to

pyruvic acid, and the protection by pyruvic acid against inactivation indicates that the groups that participate in the enzymic reaction at or near the pyruvate binding site are modified by bromopyruvic acid.

The observation of pseudo first order kinetics for the initial inactivation reaction with bromopyruvic acid is consistent with a two step binding process. The first step may be rapid reversible Schiff base formation between enzyme and inhibitor, followed by a slow irreversible alkylation step. The electron withdrawing bromine in bromopyruvic acid should promote Schiff base formation between the amine group of an amino acid residue of the enzyme and the carbonyl carbon of bromopyruvic acid, resulting in a higher affinity for bromopyruvic acid than pyruvic acid.

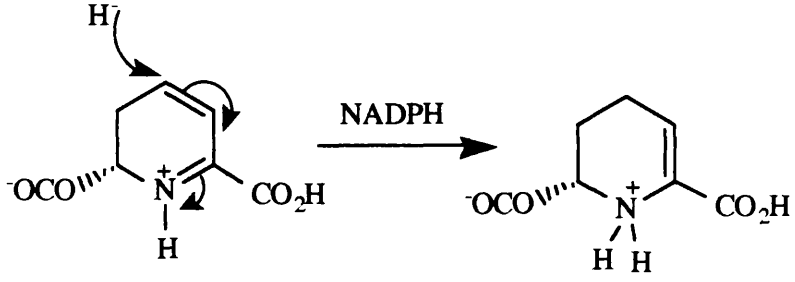
2.3 Dihydrodipicolinic Acid Reductase

Dihydrodipicolinic acid reductase is the enzyme which catalyses the pyridine nucleotide-linked reduction of L-2,3-dihydrodipicolinic acid (2) to L-2,3,4,5-tetrahydrodipicolinic acid (3). Kimura[6] suggested that L-2,3-dihydrodipicolinic acid might exist in equilibrium with L-2,5-dihydrodipicolinic acid and 4-hydroxy-L-2,3,4,5-tetrahydrodipicolinic acid. Theoretically it is also possible for DHDPA to exist as an open chain compound. It has not been established which is the physiological substrate of the reductase enzyme although it has been assumed that it is 2,3-dihydrodipicolinic acid (2).

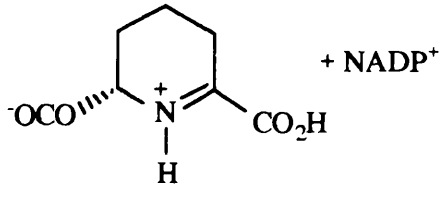
The enzymes that catalyse hydride transfer reactions have been shown to fold into two distinct domains with co-

factor in one and substrate in the other. When a substrate is reduced the hydride anion is transfered from the nicotinamide ring of NADPH to produce $NADP^+$. The reduced co-enzyme has a prochiral centre and the process is stereospecific (Scheme 5).

Tyagi, Henke and Farkas[7] reported the purification of DHDPA reductase from developing maize kernels and compared its properties to those of the bacterial enzyme. The demonstration of this step in maize is additional evidence that the diaminopimelate pathway to L-lysine and not the α-aminoadipate pathway operates in higher plants. Maize DHDPA reductase can use NADPH or NADH equally well.



(2) L-2,3-Dihydrodipicolinic acid



(3) L-2,3,4,5-Tetrtahydrodipicolinic acid

Scheme 5

2.4 Inhibition of Dihydrodipicolinic Acid Reductase

Tyagi, Henke and Farkas[7] reported the synthesis of dihydrodipicolinic acid from aspartate semialdehyde and oxaloacetic acid in alkali. DHDPA was precipitated as its barium salt and stored at -80 °C. The barium salt of dihydrodipicolinic acid was used in the assay with *o*-aminobenzaldehyde developed by Yugari and Gilvarg.[1] The chromophore of the product formed from DHDPA and *o*-aminobenzaldehyde was observed by its spectrophotomeric absorbance of 540 nm. From this the concentration of DHDPA could be determined.

The assay for DHDPA reductase by oxidation of NADPH, in the presence of synthesised DHDPA, could be followed by looking at the disappearance of NADPH which absorbs at 340 nm. Several compounds, with structures similar to dihydrodipicolinic acid, were tested for their effect on the enzymic reduction reaction rate. Of the compounds tested, namely α-picolinic acid, L-pipecolinic acid, isophthalic acid and dipicolinic acid, dipicolinic acid was found to be the most potent of these inhibitors. Dipicolinic acid showed competitive inhibition of DHDPA reductase, and 100% inhibition occurred using 1.5 mM dipicolinic acid. A K_i value of 9.0×10^{-4} M was determined for dipicolinic acid. As only cyclic compounds were found to be good inhibitors of the reductase enzyme this suggests that the substrate, dihydrodipicolinic acid, is cyclic rather than an open chain form. Dipicolinic acid was a better inhibitor than α-picolinic acid showing the importance of both carboxyls in binding to the active site of the enzyme. Unlike DHDPA synthase, which is regulated by feedback inhibition of

L-lysine and diaminopimelic acid, the reductase is not sensitive to those compounds.

DHDPA is unstable at physiological pH which means that the reductase must compete with the non-enzymic reaction that removes DHDPA. This probably occurs by oxidation of DHDPA to the aromatic dipicolinic acid.

Tetrahydrodipicolinic Acid

Shapshak[8] reported the synthesis of D-2,3,4,5-tetrahydrodipicolinic acid by treatment of DL-diaminopimelic acid with L-amino acid oxidase from *Neurospora crassa*. There was no chemical evidence given for the formation of tetrahydrodipicolinic acid.

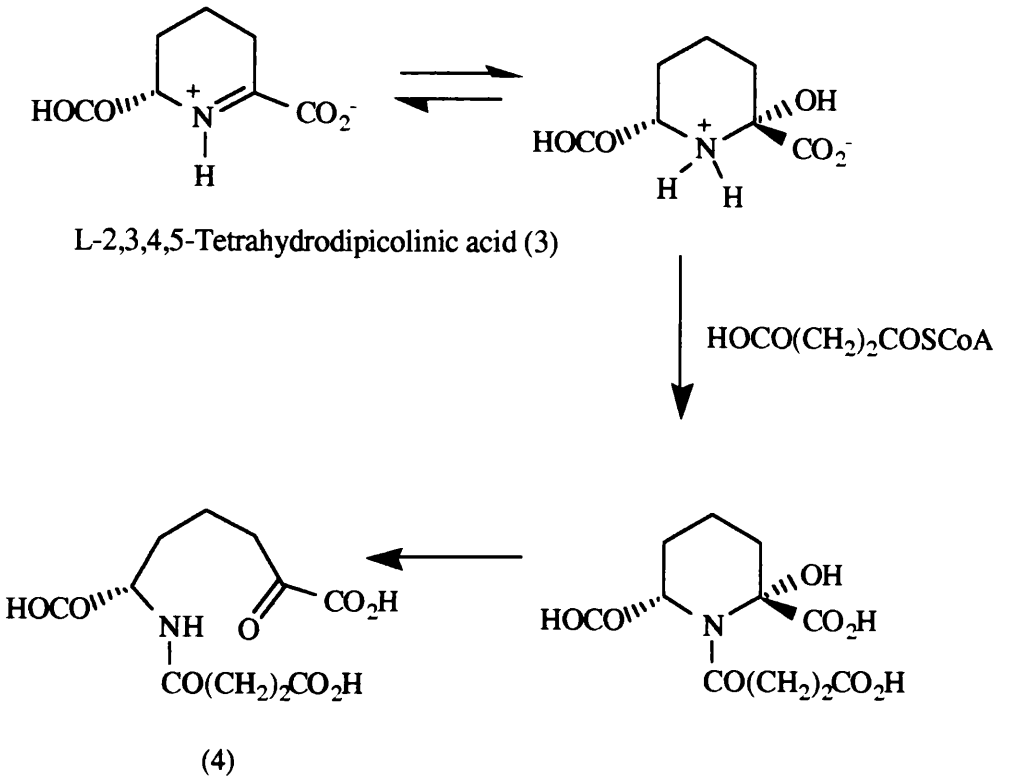
Farkas and Gilvarg[9] investigated the enzymic reduction of L-2,3-dihydrodipicolinic acid (2) to L-2,3,4,5-tetrahydrodipicolinic acid (3). DHDPA reductase was isolated from *E. coli* and partially purified. Reduction of the enzymic reaction product with sodium borohydride gave 2,6-piperidinedicarboxylic acid. This was characterised by comparison of its R_F value with authentic 2,6-piperidinedicarboxylic acid. The solvent system used was methanol-water-pyridine (8 : 1 : 1). The R_F values were identical.

2.5 Succinyl Coenzyme A : Tetrahydrodipicolinate N-Succinyltransferase

Succinyl coenzyme A : Tetrahydrodipicolinate *N*-succinyltransferase catalyses the *N*-succinylation of L-2,3,4,5-tetrahydrodipicolinic acid (THDPA) by succinyl coenzyme A to form L-2-(succinylamino)-6-oxopimelic acid (4). This enzyme has been isolated from *E. coli* by Gilvarg[10] but has not been studied in detail.

Gilvarg and co-workers[11] studied compounds related to THDPA and looked at their interaction with the succinylase. From these results they proposed a stereochemical model for the succinylation of THDPA (Scheme 6). This involves the succinylase enzyme first binding L-THDPA. This is followed by hydration of the imine group where hydroxide adds *cis*- to the C-2 carboxyl group to give 2-hydroxypiperidine-2,6-dicarboxylic acid in which the two carboxyl groups are *trans*. Succinylation then occurs and the ring opens to give the acyclic product L-2-(succinylamino)-6-oxopimelic acid (4).

The carboxyl groups will be the primary ligands involved in the binding of THDPA to the succinylase enzyme. The hydration of the imine will occur enzymically but the enzyme need not necessarily catalyse ring opening. Opening of the ring may occur in a concerted manner during or after succinylation. It is also possible that ring opening occurs after dissociation of the succinylated THDPA from the enzyme.



(4)

Scheme 6

2.6 Succinylase Inhibitors[11]

Gilvarg and co-workers[11] tested the ability of cyclic and acyclic analogues of THDPA to inhibit the succinylase enzyme.

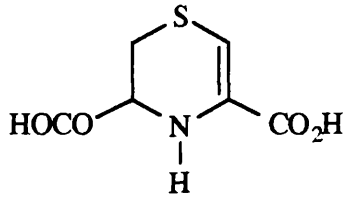
DL- Aminopimelic acid was found previously to be both a substrate and an inhibitor of the succinylase enzyme by Simms.[12] This was investigated further by resolving the racemic mixture and testing the enantiomers separately. L-2-Aminopimelic acid was found to be a good substrate and exhibited a $V_{max}(app)$ similar to the natural substrate, L-THDPA, but had a K_m approximately 50 fold higher. D-2-Aminopimelic acid was not a substrate for the succinylase enzyme, showing the importance of stereochemistry at the α-

carbon. However D-2-aminopimelic acid was a competitive inhibitor of the succinylase enzyme with respect to THDPA (K_i 0.31 mM).

Related acyclic compounds were also tested for inhibitory activity in the succinylase assay. In the succinylase enzyme assay, transfer of the succinyl group from succinyl CoA was followed by measuring the appearance of the free sulphhydryl group of CoASH with 5,5'dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent). The enzyme assay contained 100 mM potassium phosphate, 0.5 mM DTNB, 2,3,4,5-tetrahydrodipicolinic acid and the succinylase enzyme. Compounds were tested initially at 5 mM. The reaction was started by addition of succinyl CoA. The reaction was followed by looking at the spectrophotomeric absorbance at 412 nm. Increasing or decreasing the chain length of 2-aminopimelic acid as in DL-2-aminosuberic acid and DL-2-aminoadipic acid resulted in neither inhibition nor substrate activity. Substituting a sulphur for a methylene as in L-2-amino-4-thiaadipic acid gave a poor K_i value (125 mM) and little substrate activity. 2-Oxopimelic acid was not well accepted by the succinylase enzyme. 2-Hydroxypimelic acid was a good binder, better than 2-aminopimelic acid, although it did not have any substrate activity. DL-2-Amino-6-hydroxypimelic acid was found to be a better substrate than L-2-aminopimelic acid.

A number of cyclic compounds were also tested on the succinylase enzyme. Only one was found to be a substrate, namely 3,4-dihydro-2*H*-thiazine-3,5-dicarboxylic acid (22).

Some cyclic analogues were found to be inhibitors. These compounds are listed in Table 1. Compounds with fully saturated rings, such as 2,6-piperidinedicarboxylic acid were weak inhibitors; however the *trans*-isomer was better than the *cis*-isomer. Compounds with a hydroxyl α to the carboxyl showed good inhibition. The best inhibition was from 2-hydroxytetrahydropyran-2,6-dicarboxylic acid which had a K_i of 0.06 μM.



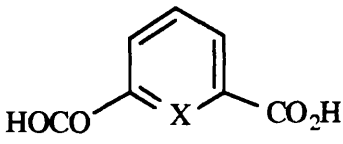
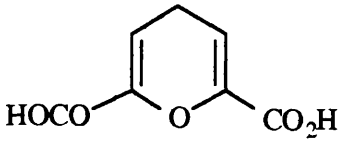
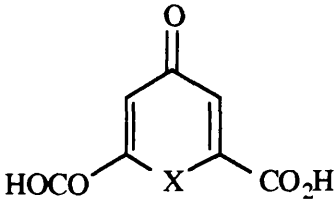
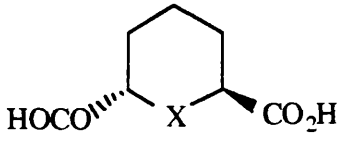
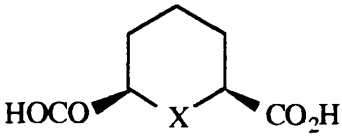
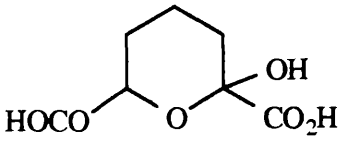
(22)

2.7 N-Succinyl Diaminopimelate Aminotransferase[13]

Succinyl diaminopimelate aminotransferase, catalyses the conversion of L-2-(succinylamino)-6-oxopimelic acid (4) into L-2-(succinylamino)-6-aminopimelic acid (5). The acylated intermediate is aminated at the ε-position with glutamate as the amino group donor.

The enzyme has been isolated from *E. coli* and has been partially purified and characterised by Peterkofsky and Gilvarg.[13] The enzyme is pyridoxal phosphate dependent and like most pyridoxal phosphate dependent enzymes, the aminotransferase is inhibited by hydroxylamine. Treatment at O °C with 0.05M hydroxylamine causes an inhibition of 95%.

TABLE 1

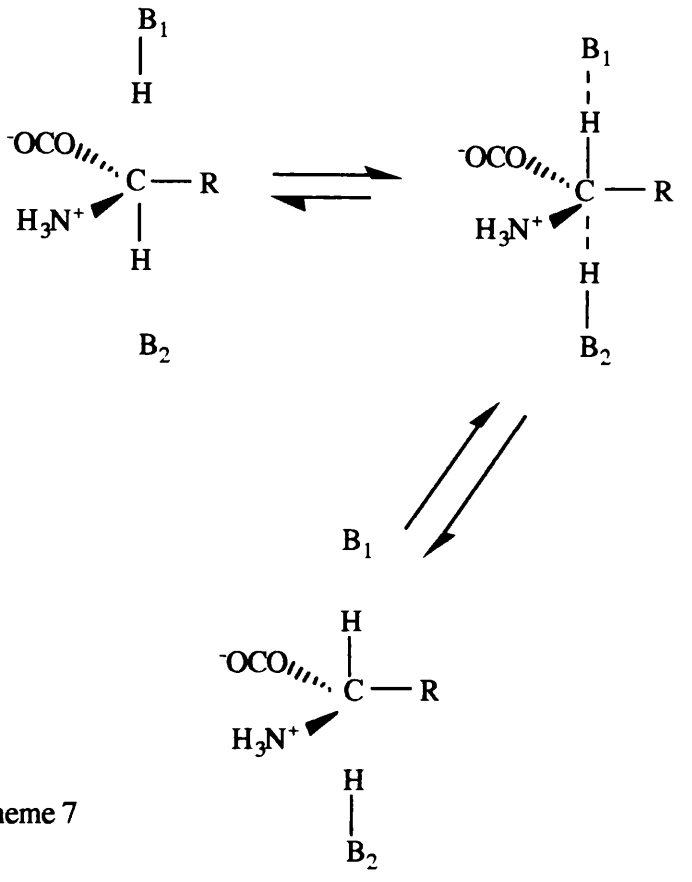
Structure	X	Name	K_i (μM)
	N	Dipicolinic acid	12,800
	O		
		Pyran-2,6-dicarboxylic acid	1,800
	NH	Chelidamic acid	2,600
	O	Chelidonic acid	3,100
	NH	2,6-Piperidinedicarboxylic acid	2000
	O	Tetrahydropyran-2,6-dicarboxylic acid	680
	NH		63,000
	O		3,900
		2-Hydroxy-tetrahydropyran-2,6-dicarboxylic acid	0.06

2.8 Diaminopimelate Dehydrogenase and Diaminopimelate Epimerase

Several variations of *meso*-diaminopimelic acid biosynthesis exist in different bacterial strains. Most bacteria and higher plants convert LL-diaminopimelic acid (6) into the DL-diaminopimelic acid (7) with LL-diaminopimelate epimerase. Some bacteria such as *Bacillus sphaericus* bypass the LL-form by converting THDPA directly into DL-diaminopimelic acid using diaminopimelate dehydrogenase enzyme in a process involving NADPH and ammonia.

2.9 LL-Diaminopimelate Epimerase

Most bacteria employ LL-diaminopimelate epimerase to make DL-diaminopimelic acid. The epimerase was detected over 30 years ago in *E. coli* and has been purified and characterised. It exists as an active monomer of molecular weight 34,000 daltons and requires no co-factor and no metal. It uses an active site thiol group as the source of protons involved in the deprotonation-protonation process, and the mechanism resembles that of proline racemase. Therefore, some anionic character may develop at the α-carbon giving a planar transition state (Scheme 7).



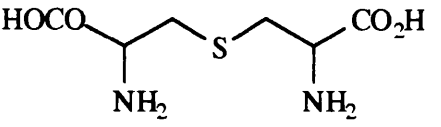
Scheme 7

2.10 LL-Diaminopimelate Epimerase Inhibitors[14]

As most bacteria employ LL-diaminopimelate epimerase it appeared to be an attractive target for producing analogues with antibiotic activity.

Lam and co-workers[14] synthesised analogues of diaminopimelic acid and tested them for inhibition of diaminopimelic acid epimerase from *E. coli*. The epimerase was assayed by coupling the conversion of LL-diaminopimelic acid into DL-diaminopimelic acid with dehydrogenase catalysed oxidation of DL-diaminopimelic acid to THDPA by NADP. NADPH formation was followed by looking at the absorbance at 340 nm.

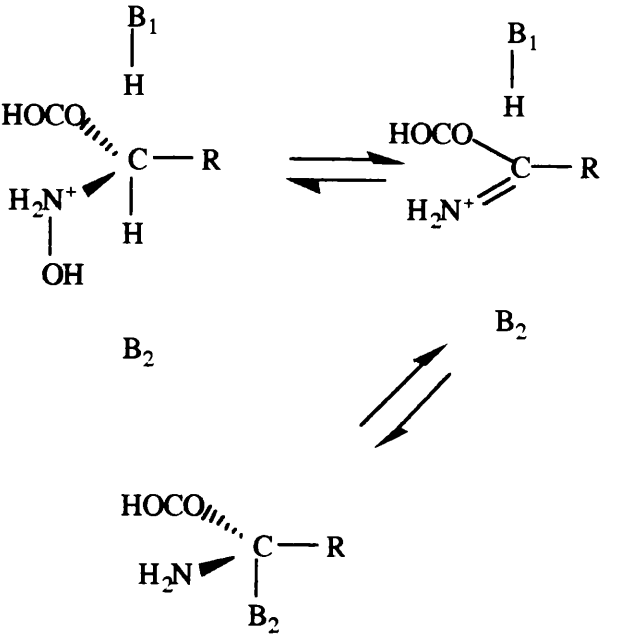
None of the analogues tested was able to inactivate the epimerase irreversibly. *meso*-Lanthionine (23) and its LL-isomer were good competitive inhibitors but the DD-isomer was not, which is in accord with the stereochemical requirement of the enzyme.



(23) lanthionine

Oxidation of the sulphur to a sulphoxide lowered the affinity for the epimerase active site, which could be due to electronic effects or to a change in the overall geometry. *N*-Hydroxydiaminopimelic acid was a very good inhibitor (K_i 0.0056 mM for the mixture of isomers). This could be due to the elimination of water, forming the imine, which could be attacked at the α-carbon by the active site thiol in a reversible fashion (Scheme 8).

N-Aminodiaminopimelic acid was a less effective competitive inhibitor (K_i 2.9 mM) which may be because the terminal amino group of the hydrazone is a poorer leaving group. 4-Methylenediaminopimelic acid was found to be a non-competitive inhibitor (K_i 0.95 mM).



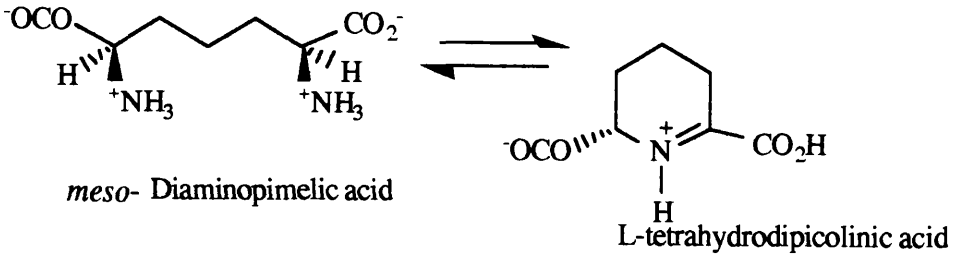
Scheme 8

2.11 meso-Diaminopimelic Acid Dehydrogenase

Studies by Misono and Soda[15] on the dehydrogenase enzyme from *Bacillus sphaericus* showed that it consisted of two identical subunits each of molecular weight 40,000 daltons. The co-factor NADPH donates the 4-*pro-S* hydrogen to the substrate in the reductive reaction and is highly specific for *meso*-diaminopimelic acid (Scheme 9).

In the active site of the dehydrogenase there is a non-essential thiol group and a tryptophan residue. In the oxidative deamination the sequence of addition is $NADP^+$ followed by *meso*-diaminopimelic acid and the release of products is ammonia followed by L-THDPA then NADPH. The dehydrogenase enzyme was investigated by Misono and Soda[15]

as the enzyme provides a convenient assay for LL-diaminopimelic acid epimerase.



Scheme 9

2.12 Inhibitors of meso-Diaminopimelic Acid Dehydrogenase[14]

Analogues of diaminopimelic acid were tested for their effect on the dehydrogenase enzyme by Lam and co-workers.[14] The dehydrogenase was assayed by monitoring NADPH formation spectrophotometrically at 340 nm. They found that the enzyme is sterospecific for *meso*-isomers and barely accommodates substituents on the carbon chain.

The *meso*-isomers of *N*-hydroxy, *N*-amino- and 4-methylene-diaminopimelic acid were found to be good substrates of the dehydrogenase enzyme. Diaminopimelate analogues which were missing a functional group, like α-aminopimelic acid or lysine were not substrates for the enzyme. Lanthionine (23) was not a substrate and neither were the sulphoxide or sulphone.

2.13 meso-Diaminopimelate Decarboxylase

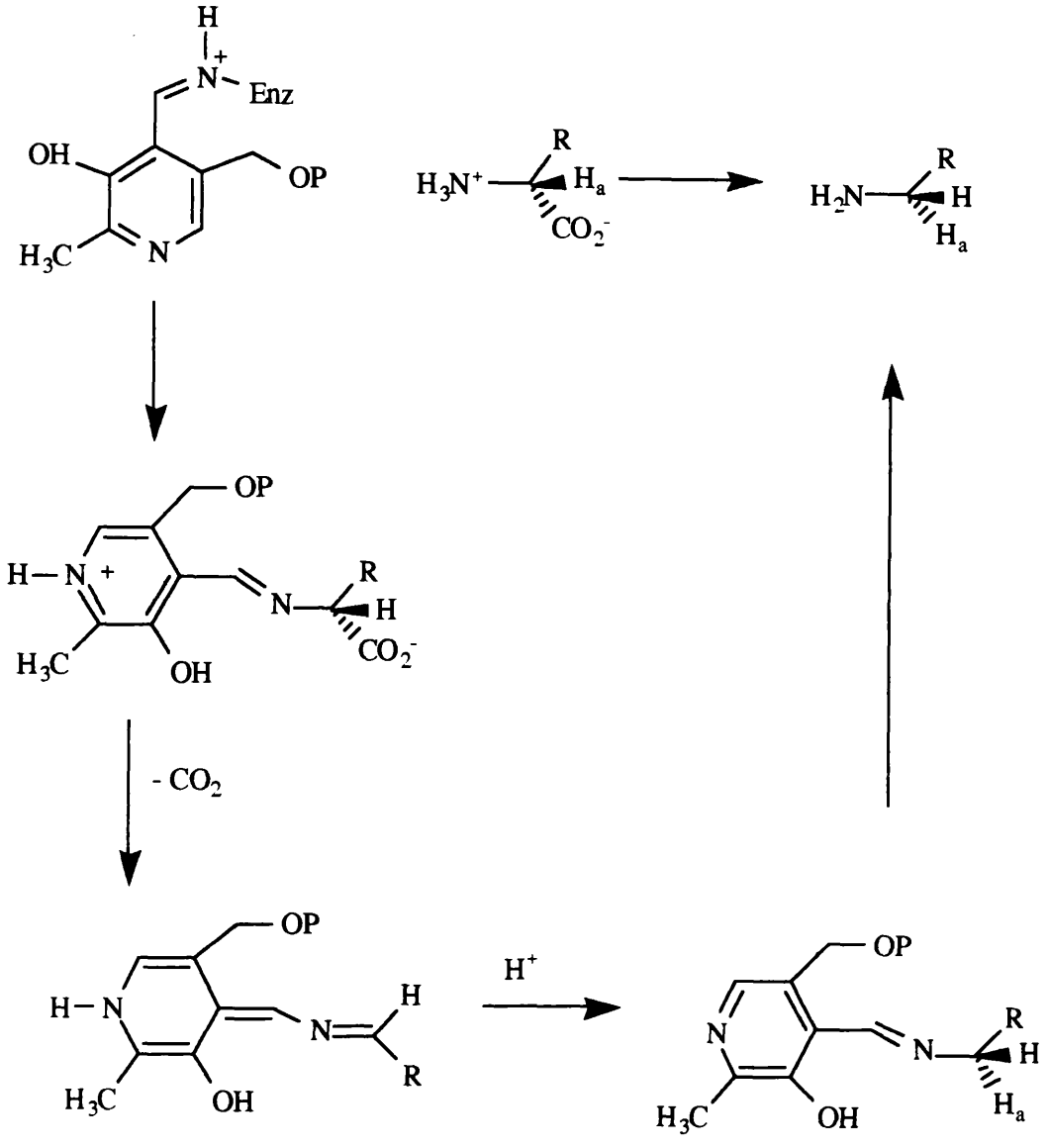
meso-Diaminopimelate decarboxylase is the enzyme that catalyses the decarboxylation of *meso*-diaminopimelic acid (7) to L-lysine (8).

During decarboxylation of α-amino acids by pyridoxal phosphate dependent decarboxylases the bond between the α-carbon and the carboxyl carbonyl of the substrate is expected to be nearly perpendicular to the plane of the conjugated π system of the co-factor.

Formation of the Schiff base between the co-factor and diaminopimelic acid is the first step. The carboxyl group is *trans*-antiparallel with the imine. Carbon dioxide is lost and the co-factor stores the electrons of the cleaved bond until protonation from the solvent can occur and the imine is hydrolysed to give L-lysine. All pyridoxal phosphate dependent α-decarboxylases proceed with retention of configuration except for *meso*-diaminopimelic acid decarboxylase which shows inversion (Scheme 10).

2.14 Inhibition of *meso*-Diaminopimelate Decarboxylase [16]

Vederas and co-workers[16] investigated *meso*-diaminopimelate decarboxylase from *B. sphaericus* and wheat germ and looked at the release of $^{14}CO_2$ from [1,7-^{14}C]diaminopimelic acid. Analogues of diaminopimelic acid were synthesised and tested for their inhibitory effects on L-lysine biosynthesis.



Scheme 10

α-Difluoromethyldiaminopimelic acid was expected to be a potent inhibitor due to extensive precedent with other pyridoxal phosphate dependent α-decarboxylases. This is due to the presence of a fluorine β- to the α-amino acid which can act as a good leaving group. Elimination of the fluorine anion from the stabilised anion of the inhibitor-co-factor complex instead of reprotanation of the imine may occur. This would

result in the formation of an electon deficient conjugated system which could undergo Michael addition reactions with nucleophiles. This might lead to the inhibitor-co-factor complex forming a covalent bond to amino acid residues in the enzyme active site, resulting in irreversible inhibition. However the total lack of irreversible or even strong competitive inhibition shows that the analogue cannot bind to the active site. Both plant and bacterial diaminopimelate decarboxylases enforce the stringent stereochemical requirement for the DL-isomer of the substrate by a tight fit in the region surrounding the α-carbon and do not permit replacement of the α-hydrogen by a larger group. This contrasts to the behaviour of most pyridoxal phosphate dependent α-decarboxylases which easily accommodate an α-methyl or α-difluoromethyl group.

Sulphoxides and sulphones were investigated as inhibitors by Vederas and co-workers.[16] *meso*-Lanthionine (23) is decarboxylated by diaminopimelate decarboxylase at 5% of the rate of the natural substrate. The LL- and *meso*-isomers of lanthionine (23) sulphoxide and sulphone are competitive inhibitors. Sulphoxides were found to be stronger inhibitors than sulphones, maybe due to secondary binding of the sulphoxide functionality.

Following the work of Cooper and Griffiths[17] where *N*-hydroxyglutamate irreversibly inhibited pyridoxal phosphate dependent glutamate decarboxylase, *N*-hydroxydiaminopimelate was tested for inhibition of diaminopimelate decarboxylase and was found to be a good competitive inhibitor of diaminopimelate decarboxylase from

both *B. sphaericus* and wheat germ, which may be due to the formation of a stable nitrone in the enzyme active site. *N*-Aminodiaminopimelic acid was also a good competitive inhibitor (K_i 100 μM for *B. sphaericus* enzyme; K_i 84 μM for wheat germ enzyme).

Conclusions

The diaminopimelate pathway to L-lysine has been studied but not in great detail. Although the main intermediates have been established the mechanisms and stereochemistry of some of the steps have still to be established.

The synthesis of potential enzyme inhibitors of some of the steps has also been carried out. However very little has been done to synthesise analogues of L-2,3-dihydrodipicolinic acid (2) and L-2,3,4,5-tetrahydrodipicolinic acid (3) to test as enzyme inhibitors of the first two enzymic steps.

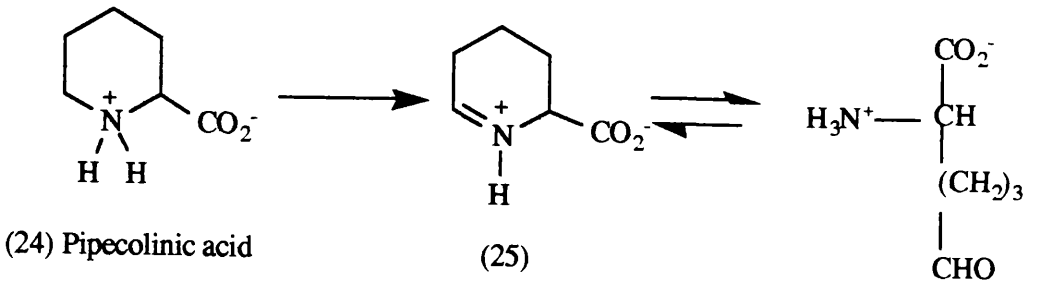
2.15 The α-Aminoadipate Biosynthetic Pathway to L-Lysine[18,19,20]

The α-aminoadipate pathway (Scheme 2) to L-lysine (8) is found in yeast and higher fungi. This pathway has been investigated in detail in *Saccharomyces cerevisiae* where seven enzymes have been partially or fully characterised. Several L-lysine biosynthetic enzymes have also been characterised from *N. crassa.*

The first step in the pathway is the condensation of acetic acid with α-ketoglutaric acid (9) to yield the 7-carbon citrate homologue, homocitric acid (10). Dehydration gives homoaconitic acid (11), followed by hydration to homoisocitric acid (12). Homoisocitrate dehydrogenase converts homoisocitrate into α-ketoadipic acid (14). The next step is an aminotransferase reaction where the α-position is aminated with glutamate as the amino group donor. α-Aminoadipic acid (15) is converted into α-aminoadipate semialdehyde (16) in a multi-step reaction catalysed by aminoadipate semialdehyde dehydrogenase, which is an NADPH dependent enzyme and requires Mg^{2+}. Saccharopine dehydrogenase (glutamate forming) and saccharopine dehydrogenase (lysine forming) catalyse the formation of saccharopine (17) and L-lysine (8) respectively. Kinetic studies on the latter enzyme indicate an ordered mechanism where NAD^+ and saccharopine bind and lysine, α-ketoglutarate, and NADH are released in this order.

Biosynthetic Role of Pipecolinate[21]

Pipecolinic acid (24) can be converted into L-lysine (8) in organisms where pipecolinate oxidase is present to convert pipecolinic acid into 2,3,4,5-tetrahydropyridine-2-carboxylic acid (25). This is in equilibrium with α-aminoadipate semialdehyde (16) (Scheme 11) which can then be converted into L-lysine by the α-aminoadipate pathway.



(24) Pipecolinic acid

(25)

(16)

Scheme 11

CHAPTER 3

3.1 Introduction

The synthesis of L-aspartate semialdehyde (1) and L-2,3-dihydrodipicolinic acid (2), which are substrates in the dihydrodipicolinic synthase and reductase steps of the diaminopimelate pathway to L-lysine (8) (Scheme 1), was previously claimed. However very little chemical evidence for these two compounds was provided.

Synthesis of these substrates was necessary at the start of this work to provide compounds for use in the enzyme assays by our co-workers in the Biochemistry Department.

In this Chapter the methods carried out to synthesise aspartate semialdehyde (1) will be described. The attempted synthesis of 2,3-dihydrodipicolinic acid (2) will also be discussed. In the following Chapter, the attempts to synthesise 2,3,4,5-tetrahydrodipicolinic acid (3) will be described.

3.2 Synthesis of Aspartate Semialdehyde

L-Aspartate semialdehyde (1) is the first substrate on the diaminopimelate pathway to L-lysine (8). Condensation of aspartate semialdehyde with pyruvic acid using dihydrodipicolinate synthase gives dihydrodipicolinic acid (2). Aspartate semialdehyde was required for use in the enzyme assay by our co-workers in the Biochemistry Department.

Black and Wright[5] reported the synthesis of the separate enantiomers of aspartate semialdehyde from DL-allylglycine. Resolution was achieved by enantioselective enzymic

hydrolysis of its *N*-acetyl derivatives. *N*-Acetyl-DL-allylglycine was prepared from DL-allylglycine by Sorrensen's method using 2M NaOH and acetic anhydride. Hydrolysis of *N*-acetyl-DL-allylglycine using hog kidney acylase gave L-allylglycine and unchanged *N*-acetyl-D-allylglycine. L-Allylglycine crystallised on cooling. *N*-Acetyl-D-allylglycine was hydrolysed by heating at reflux in 2M HCl, to give D-allylglycine. On ozonolysis of D- and L-allylglycine D- and L-aspartate semialdehyde were formed. Only the L-isomer was found to be a substrate of dihydrodipicolinate synthase. Although aspartate semialdehyde is reasonably stable in acid solution, Black and Wright[5] found that it deteriorates in neutral solution or in the dry state.

We found that ozonolysis of DL-allylglycine in 1M HCl at 0 ^{o}C gave a mixture of DL-aspartate semialdehyde and formaldehyde (Scheme 12). The ozonide decomposes as it is formed in the acidic solution. The reaction scheme involves hydrolytic cleavage of the ether linkage of the ozonide followed by decomposition to the aldehyde.

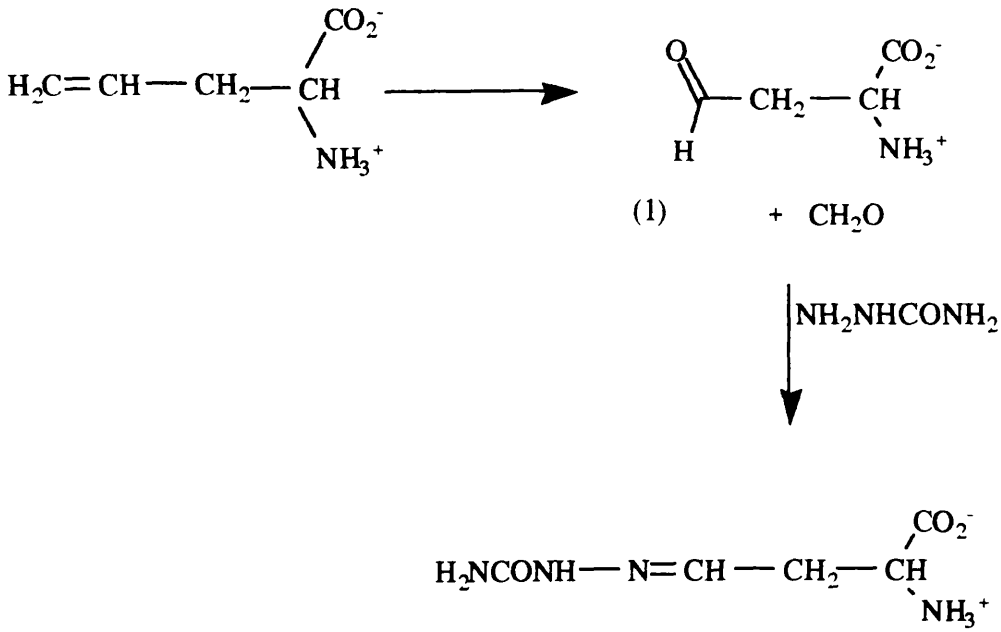
The ozonolysis reaction was repeated in D_2O and DCl and followed by ^{1}H NMR spectroscopy. This showed that aspartic acid was also formed, presumably by oxidation of aspartic semialdehyde. To try and remove the formaldehyde a cation ion exchange column was used. However the concentration of aspartate semialdehyde was found to be less after ion exchange chromatography. This was probably due to the instability of aspartate semialdehyde at room temperature. The concentration of aspartate semialdehyde in acid solution after the ozonolysis was calculated by Emma Borthwick in the

Biochemistry Department by converting aspartate semialdehyde into homoserine using homoserine dehydrogenase. Homoserine is an intermediate on the biosynthetic pathways to threonine and methionine. Homoserine dehydrogenase has been extensively studied and purified. The enzyme activity was monitored by the decrease in absorbance at 340 nm due to the oxidation of NADPH to $NADP^+$. The change in concentration of NADPH during the reaction could be calculated. Since one mole of NADPH reacts with one mole of aspartate semialdehyde, the concentration of aspartate semialdehyde is equivalent to the change in concentration of NADPH. From this, the concentration of aspartate semialdehyde was calculated. The concentration of the solution prepared under similar conditions with different batches of material was between 0.3M and 0.4M.

The synthesis of aspartate semialdehyde derivatives has not been previously reported. Therefore attempts were made to make aldehyde derivatives of aspartate semialdehyde. Treatment of the acidic aspartate semialdehyde solution with 2,4-dinitrophenylhydrazine (DNP) solution gave an orange solid. Characterisation of this solid showed it to be the DNP derivative of formaldehyde. No reaction took place with the ozonolysis products and hydroxylamine hydrochloride and sodium acetate.

Basifying the aspartate semialdehyde solution and adding semicarbazide and sodium acetate gave white crystals of aspartate semialdehyde semicarbazone (Scheme 12). Recrystallisation attempts failed due to the poor solubility of the semicarbazone derivative in any solvent. In the IR

spectrum of the semicarbazone a stretching band was present at 1580 cm^{-1} due to the carboxylate of the amino acid. Bands were also present at 1660 and 1630 cm^{-1} due to the semicarbazone carbonyl. The ^{1}H NMR spectrum contained a multiplet at δ 2.28 due to the methylene protons and a multiplet at δ 4.28 due to the acidic proton next to the carboxylate anion of the amino acid. A multiplet was present at δ 7.15 due to imine proton. The ^{13}C NMR spectrum showed almost entirely one geometric isomer. In the spectrum there was a methylene signal at δ 32.16. The methine signal due to the carbon next to the carboxylate and the ammonium groups appeared at δ 51.60. There were two quaternary signals in the



Scheme 12

spectrum. The signal at δ 172.50 was attributed to the carboxylate carbon. The other quaternary signal present at δ 159.80 was assigned to the carbonyl component of the semicarbazone. The methine signal at δ 142.30 was attributed to the imine carbon.

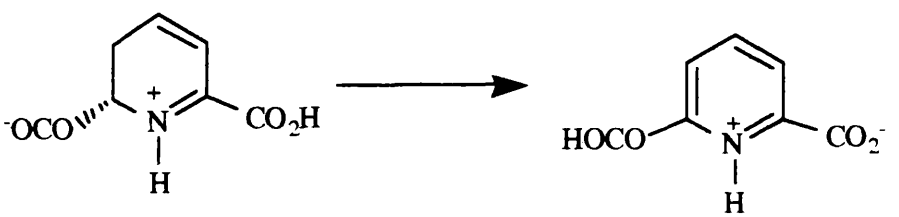
3.3 Synthesis of 2,3-Dihydrodipicolinic Acid (2)

Tyagi *et al.*[7] discussed the synthesis of dihydrodipicolinic acid from oxaloacetic acid (an activated form of pyruvic acid) and L-aspartic semialdehyde. The product was isolated by precipitating it as its barium salt. The solid was unstable and had to be stored at -80 °C. The only evidence given for its formation was the positive rate observed in the dihydrodipicolinate reductase assay.

This procedure was repeated using the crude DL-aspartate semialdehyde solution and oxaloacetic acid in D_2O and NaOD. The reaction was followed by ^1H NMR spectroscopy. A singlet initially appeared at δ 5.40. It is not known for certain what causes this singlet although it could be one of the olefinic protons of the presumed condensation product, 2,3-dihydrodipicolinic acid (2). The signal for the other olefinic proton likely to be present may be under the HOD signal. However it would not be expected that these signals for olefinic protons would be singlets. Another explanation is that both olefinic protons in (2) have the same chemical shift. As the peak at δ 5.40 decreased in size a singlet at δ 8.40 appeared and increased in size until it reached a constant value. This could be the result of air oxidation of 2,3-dihydrodipicolinic

acid (2) to dipicolinic acid (26) (Scheme 13). The singlet at δ 8.40 is characteristic of dipicolinic acid. Further work to be carried out on the isolation of 2,3-dihydrodipicolinic acid should involve the preparation and trapping of (2) as a metal salt at low temperatures as reported in the literature.[7]

Emma Borthwick from the Biochemistry Department isolated the product of enzymic condensation of pyruvic acid and aspartate semialdehyde. The product was examined by ^1H NMR spectroscopy and showed a small singlet at δ 8.40. This agrees with the non-enzymic oxidation of the 2,3-dihydrodipicolinic acid (2) where dipicolinic acid (26) is the final product. The UV absorbance at 270 nm which is used to follow the enzymic reaction is also consistent with the formation of dipicolinic acid.



(2) 2,3-L-Dihydrodipicolinic acid (26) dipicolinic acid

Scheme 13

Conclusions

Aspartate semialdehyde (1), the first substrate on the diaminopimelate pathway to L-lysine (8), was prepared by the ozonolysis of DL-allylglycine. The aldehyde is very unstable and could not be purified. It was stored in the acidic solution at 0 °C. The solution however was satisfactory for use in the

enzyme assay of dihydrodipicolinate synthase. Although the aspartate semialdehyde was a mixture of D- and L-isomers only the L-isomer is a substrate for the synthase enzyme.

Although the synthesis of 2,3-dihydrodipicolinic acid (2) has been reported we were unable to isolate it using the synthetic procedure reported. The compound is too unstable and readily oxidises in air to give dipicolinic acid (26). The stable condensation product arising from the dihydrodipicolinate synthase enzyme reaction was isolated and shown to be dipicolinic acid from its ^{1}H NMR spectrum.

CHAPTER 4. Synthesis of Imines

4.1 Introduction

The synthesis of L-2,3,4,5-tetrahydrodipicolinic acid (3) is important as it is one of the key postulated intermediates in the diaminopimelate pathway to L-lysine. It is the enzymic reduction product of L-2,3-dihydrodipicolinic acid (2). The synthesis of 2,3,4,5-tetrahydrodipicolinic acid could help provide a route for the synthesis of imine analogues to be tested as enzyme inhibitors of the first two enzymes on the diaminopimelate pathway to L-lysine.

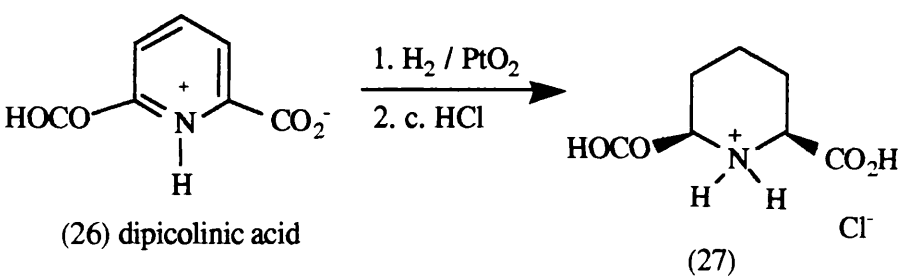
In this Chapter the work carried out to help establish a route for the synthesis of imines derived from L-proline (34), DL-pipecolinic acid (24), and *cis*-2,6-piperidinedicarboxylic acid (27) is described.

4.2. Synthesis of 2,6-Piperidinedicarboxylic Acid

A number of methods have been described in the literature for the synthesis of 2,6-piperidinedicarboxylic acid. Anderson and Soine[22] reported the synthesis of *cis*-2,6-piperidinedicarboxylic acid hydrochloride (27), by hydrogenation of dipicolinic acid (26) in acetic acid using PtO_2 as catalyst, followed by acidification. This method was repeated by us to give a 96% yield of the required *cis*-product (Scheme 14). In the IR spectrum bands were present at 3400 and 1750 cm^{-1} due to the hydroxyl and the carbonyl components of the carboxylic acid groups. The multiplets in the ^1H NMR spectrum at δ 1.60 - 1.85 and 2.31 were due to the

Figure 1. ^{13}C NMR Spectrum of *cis*-2,6-Piperidinedicarboxylic acid (27).

protons on the ring at C-3, C-4 and C-5. The equivalent acidic protons at C-2 and C-6 appeared as a multiplet at δ 3.80. In the ^{13}C NMR spectrum (Figure 1) two methylene signals were present in a ratio of 1 : 2. The signal at δ 23.05 was due to C-4 and the signal present at δ 26.23 was due to C-3 and C-5. The methine signal present at δ 58.05 was due to the equivalent C-2 and C-6. There was one quaternary signal present in the spectrum at δ 172.36 which was attributed to the two carboxylic acid groups. In the mass spectrum the expected molecular ion was observed at *m/z* 173. The largest ion was at *m/z* 84 due to the loss of both carboxylic acid groups.

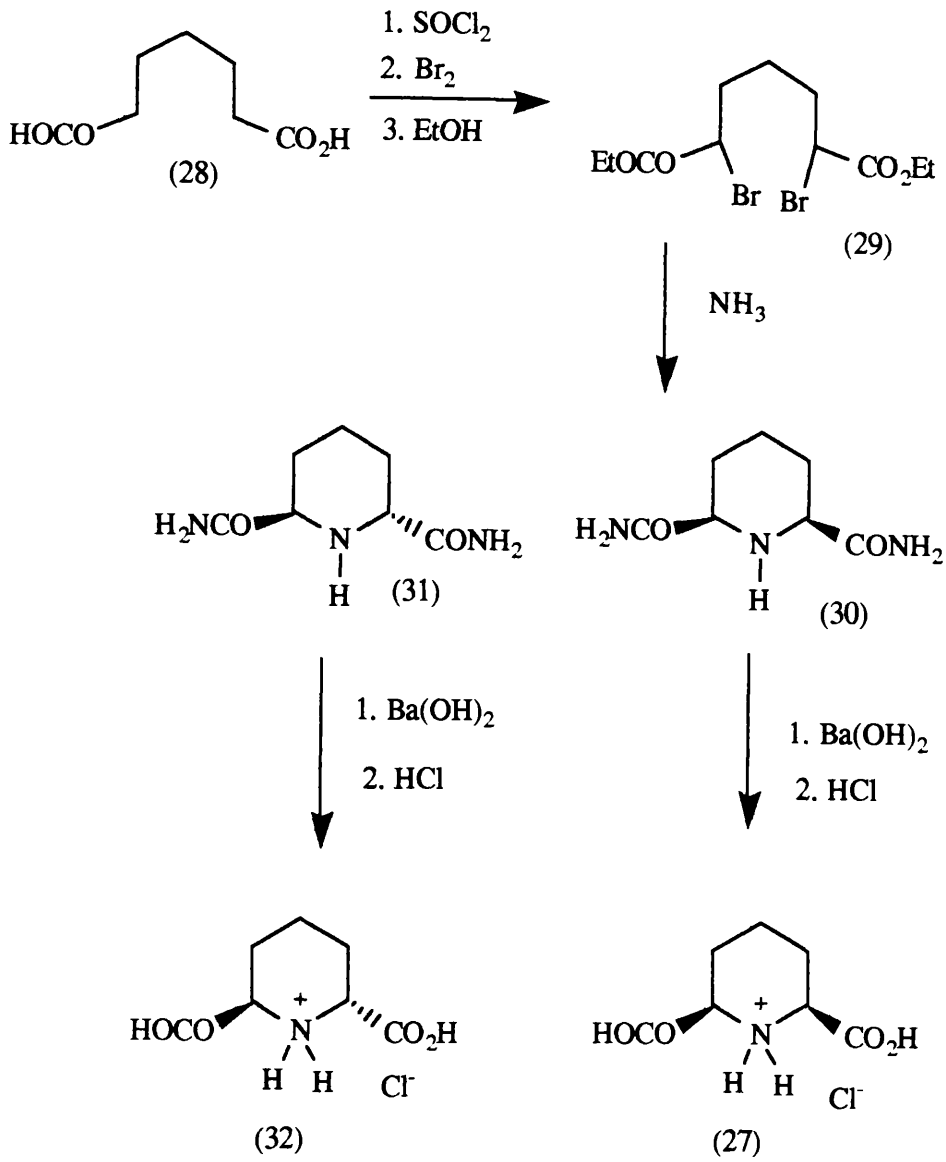


(26) dipicolinic acid

(27)

Scheme 14

A mixture of *cis*- and *trans*-2,6-piperidinedicarboxylic acid has been made by α-bromination of pimelic acid[23] followed by cyclisation with ammonia. This is the method of Fischer.[24] Pimelic acid (28) was converted into the diacid chloride by warming with thionyl chloride. The α-positions were then brominated using bromine, with iodine as a catalyst. This is the Hell-Volhard-Zelinsky reaction where only the carbon α to an acid chloride is halogenated. Addition of ethanol at the end of the reaction converted the diacid chloride into the diethyl ester (29) (Scheme 15).



Scheme 15

Cyclisation of diethyl αα′-dibromopimelate (29) was carried out in a sealed tube with liquid ammonia. After three days the tube was opened and the ammonia was allowed to evaporate off leaving 2,6-piperidinedicarboxamide as a 3 : 1 mixture of isomers, as judged by ^{13}C NMR spectroscopy. Separation of the isomers was achieved by careful crystallisation from water. The *cis*-isomer (30) did not dissolve

in ice water and could be filtered off and dried. The *cis*-isomer was identified by its melting point which was reported in the literature[24] and by the subsequent hydrolysis of the diamide to the diacid which showed the hydrolysis product was the *cis*-isomer of 2,6-piperidinedicarboxylic acid. The *cis*-diacid was identified from its melting point and comparison of its ^{13}C NMR spectrum with that of the product of the hydrogenation of dipicolinic acid which gave *cis*-2,6-piperidinedicarboxylic acid. Concentration and cooling of the filtrate obtained previously gave the *trans*-diamide (31) as white crystals. This isomer was identified by its melting point, as reported in the literature[24] and by its ^{13}C NMR spectrum which was not the same as the spectrum for the *cis*-isomer. The IR spectra of the diamides (30) and (31) showed carbonyl bands at 1680 and 1630 cm^{-1}, which are typical of primary amides and N-H stretching band at 3300 cm^{-1}. The methylene region in the ^{1}H NMR spectrum of both *cis*- and *trans*-diamides was very complicated, between δ 1.70 and 2.05. The α-protons appeared further downfield. The symmetrical *cis*- (*meso*) isomer showed only one multiplet for both α-protons at δ 4.14. The *trans*-isomer has one axial and one equatorial proton α to the carboxyl groups, which appeared as one multiplet at δ 3.95. In the ^{13}C NMR spectrum of the *cis*-diamide (30) two methylenes were observed, one for C-3 and C-5 at δ 25.63, and one for C-4 at δ 17.96. One methine signal was present at δ 54.35 due to the protons at C-2 and C-6 and one carboxyl was observed at δ 171.64. For the *trans*-diamide (31) two methylene signals were observed; one at δ 21.85 for C-4 and one at 25.93 due to C-3 and C-5. One methine signal was observed at δ 57.25 and one carboxyl signal

at δ 171.50. The mass spectra for both isomers contained small molecular ions at *m/z* 171. The largest ion, *m/z* 84, is due to the loss of both carboxyl groups.

The separated *cis*- and *trans*-2,6-piperidinedicarboxamides (30) and (31) were hydrolysed to the coresponding *cis*- (27) and *trans*-2,6-piperidinedicarboxylic acid hydrochlorides (32) by heating at reflux in 10% barium hydroxide solution followed by acidification. The isomers were identified by their different melting points.[22,24] In the IR spectra of both isomers a carbonyl stretching band at 1750 cm^{-1} and a hydroxyl band at 3400 cm^{-1} from the carboxylic acid groups were evident. The ^{1}H and ^{13}C NMR spectra of the diacids (27) and (32) were very similar to the diamide spectra described above. The mass spectra of both diacids showed very small molecular ions at *m/z* 173. The largest ion, *m/z* 84 was due to the loss of both carboxyl groups.

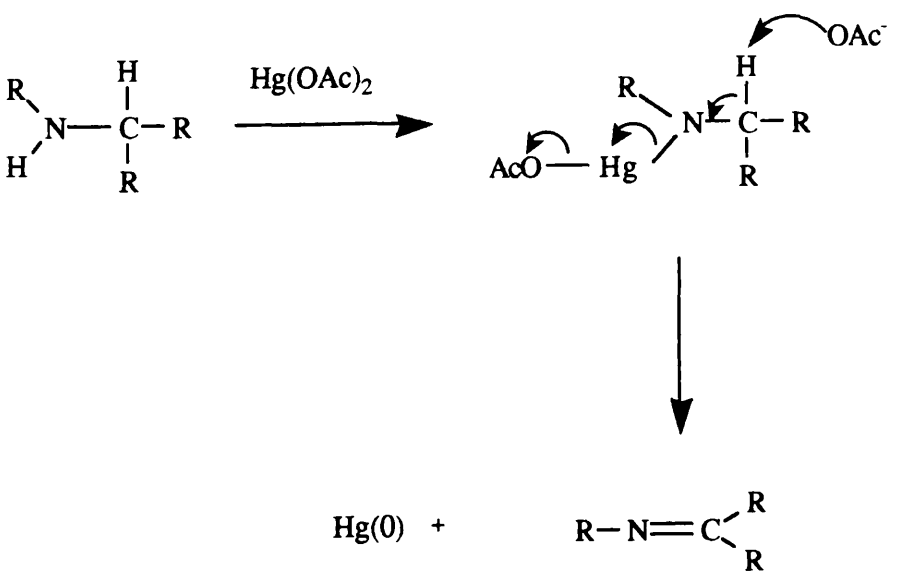
4.3 Synthesis of Imines From Amines

Several methods have been reported in the literature for the oxidation of amines to imines. These methods have not yet been tried on amino acids like L-proline (34), DL-pipecolinic acid (24), and *cis*-2,6-piperidinedicarboxylic acid (27). Therefore the various methods for oxidising amines to imines were carried out using the above amino acids, or their corresponding esters, with the aim of finding a route for the synthesis of 2,3,4,5-tetrahydrodipicolinic acid (3) and its analogues. 2,3,4,5-Tetrahydrodipicolinic acid analogues could be tested as enzyme inhibitors of the dihydrodipicolinate

synthase and reductase steps of the diaminopimelate pathway to L-lysine.

Mercuric Acetate Oxidations

There have been a number of reports of oxidations of secondary amines and tertiary amines to the coresponding imine in dilute acetic acid using mercuric acetate.[25,26] Heating the amine at reflux in dilute acetic acid with four equivalents of mercuric acetate, followed by removal of mercury as mercuric sulphide using hydrogen sulphide gas gave the required imine. The proposed mechanism involves the formation of a mercury complex. The acetate ion removes the α-proton in a concerted β elimination reaction (Scheme 16). This mechanism results in the formation of elemental mercury, which has been reported in some cases.[25] However another



Scheme 16

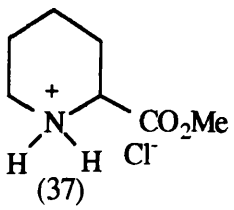
mechanism has also been proposed which involves a series of one electron shifts, in which case Hg(I) and not Hg(0) would be formed initially. *cis*-2,6-Piperidinedicarboxylic acid was heated at reflux with mercuric acetate in 5% acetic acid. The mercury was removed by flushing the system with hydrogen sulphide gas to form mercuric sulphide as a black solid which was filtered off. This step was repeated several times to remove completely the mercury as mercuric sulphide. The filtrate was concentrated to give a white solid which from ^{1}H and ^{13}C NMR spectroscopy was starting material. Starting material was also recovered using *trans*-2,6-piperidine-dicarboxylic acid, L-proline or DL-pipecolinic acid and analogous treatment with mercuric acetate. This is believed to be due to the formation of a mercury salt with the amino acid carboxylic acid group. These amino acids are known to form salts with many metal ions. Flushing the system with hydrogen sulphide gas removed the mercury as mercuric sulphide and left starting material.

Oxidation by Electrophilic Agents

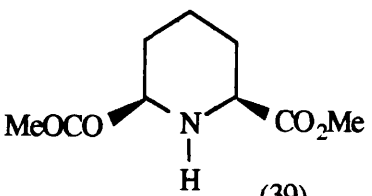
Tertiary amines or amine oxides containing at least one acidic α-hydrogen react with electrophilic agents like *N*-bromosuccinimide (NBS) to form the *N*-halogenated intermediate.[27] These can form imines by elimination of HBr on treatment with base.

Treatment of methyl DL-pipecolinate (37) with *N*-bromosuccinimide (NBS) followed by heating at reflux in potassium hydroxide gave starting material only. The same

reaction was carried out using dimethyl *cis*-2,6-piperidinedicarboxylate (39), but from ^{1}H and ^{13}C NMR spectra a mixture of products was obtained.



(37)



(39)

4.4 Attempted Transaminations of Diaminopimelic Acid

One possible method for preparing imines is the condensation of primary amines with carbonyl compounds. This method was first reported by Schiff, hence the condensation products are often known as Schiff bases.[28] If 2-oxo-6-aminopimelic acid could be synthesised, an intramolecular condensation reaction might take place to give the imine 2,3,4,5-tetrahydrodipicolinic acid. Therefore attempts were made to synthesise 2-oxo-6-aminopimelic acid by the transamination of one of the amino groups of diaminopimelic acid.

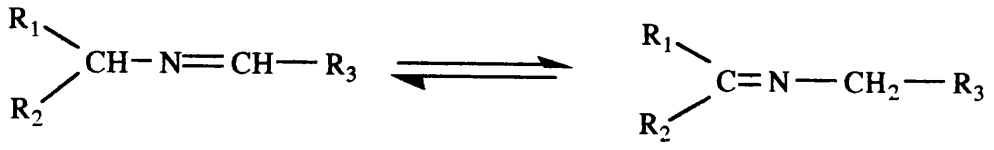
Enzymic Transaminations

Shapshak[8] reported the synthesis of D-2,3,4,5-tetrahydrodipicolinic acid by treatment of *meso*-diaminopimelic acid with the L-amino acid oxidase from *N. crassa*. In this reaction the L-amino group is transaminated to give 2-oxo-6-aminopimelic acid. This could cyclise to form D-2,3,4,5-tetrahydrodipicolinic acid.

DL-Diaminopimelic acid was separated by us from the mixture of DD-, LL- and DL-isomers bought from Sigma, by crystallisation from water and ethanol.[29] DL-Diaminopimelic acid was dissolved in D_2O and NaOD. The pD was adjusted to 6 using DCl. D-Amino acid oxidase (EC. 1.4.3.3), bought from Sigma, was added to the solution. The reaction was followed by ^1H NMR spectroscopy, but after two weeks no changes in the ^1H NMR spectra had occurred, even when the enzyme mixture was heated to 50 ^oC. The reaction was repeated with L-amino acid oxidase (EC. 1.4.3.2) but again no changes in the ^1H NMR occurred.

Chemical Transaminations

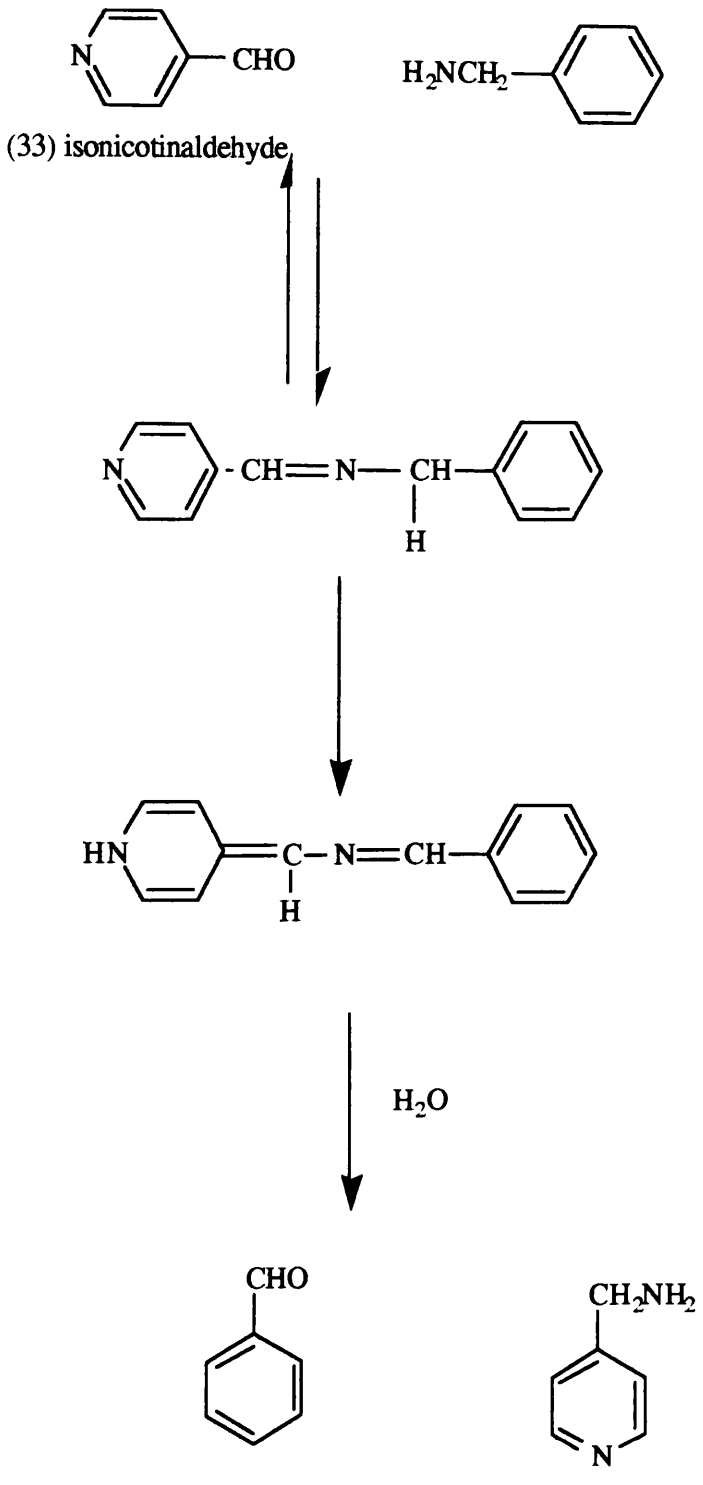
Non-enzymic transaminations have been reported but are not easy to carry out. Reagents such as 3,5-dinitromesitylglyoxal[30] and 6-nitrobenzothiazole-2-carboxaldehyde,[31] which increase the equilibrium constant of the equilibrium (Scheme 17) are not easily available.



Scheme 17

Ohto and Okamoto[32] reported the most convenient method to date using isonicotinaldehyde (33) (Scheme 18). They found that the Schiff base formed between benzylamine and isonicotinaldehyde was converted into benzaldehyde in a quantitative yield by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) followed by acidic hydrolysis.

We tried this reaction by treating a suspension of *meso*-diaminopimelic acid (7) in DMF at 90 °C with isonicotinaldehyde (33) and DBU and stirring for one week. From ^{1}H NMR spectroscopy it could be seen that no reaction had taken place, probably due to the very low solubility of *meso*-diaminopimelic acid (7) in DMF. As diaminopimelic acid is only soluble in basic aqueous solution this method does not appear to be feasible.


N
CHO
H2NCH2
(33) isonicotinaldehyde
N
CH
N
CH
H
HN
C
N
CH
H
H2O
CHO
CH2NH2
N

Scheme 18

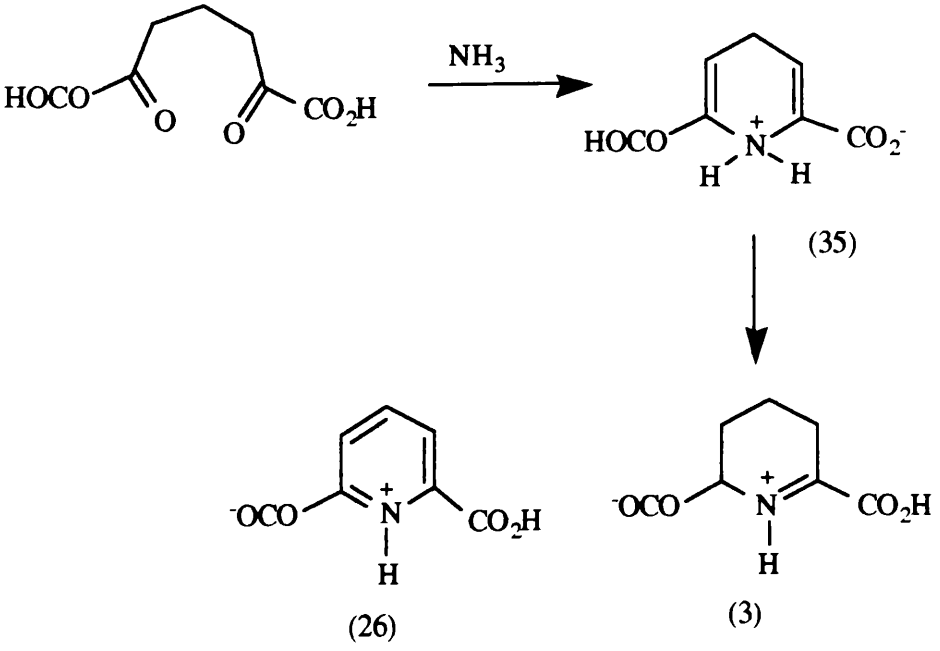
4.5 Cyclisation of α,α′-Dioxopimelic Acid

α,α′-Dioxopimelic acid cyclises on treatment with conc. sulphuric acid and water to give pyran-2,6-dicarboxylic acid as a white solid. By treating α,α′-dioxopimelic acid with ammonia we hoped to get 1,4-dihydrodipicolinic acid (35), which is an isomer of the presumed product (2) of the enzymic condensation step in the diaminopimelate pathway to L-lysine.

Kimura and Sasakawa[33] reported the formation of dipicolinic acid (26) and 2,3,4,5-tetrahydrodipicolinic acid (3) from α,α′-dioxopimelic acid and ammonia. The reaction was carried out under aerobic and anaerobic conditions and they found that the reaction rate did not change. From this result they assumed that dipicolinic acid was not formed by air oxidation of 1,4-dihydrodipicolinic acid (35), the expected initial product of the reaction of α,α′-dioxopimelic acid and ammonia. They proposed that the disproportionation of 1,4-dihydrodipicolinic acid (35) gives dipicolinic acid (26) and 2,3,4,5-tetrahydrodipicolinic acid (3) (Scheme 19). The amount of dipicolinic acid produced in the reaction was estimated by the increase in UV absorption at 270 nm. Reaction products other than dipicolinic acid formed from α,α′-dioxopimelic acid and ammonia were analysed by the ninhydrin reaction and by the formation of complexes with *o*-aminobenzaldehyde. The acid ninhydrin reaction was carried out by adding glacial acetic acid and ninhydrin solution to the reaction products and measuring the absorbance at 420 nm. The reaction with *o*-aminobenzaldehyde was carried out by acidifying the reaction mixture, and adding *o*-aminobenzaldehyde reagent and

measuring the absorbance at 460 nm. The results showed that another product beside dipicolinic acid was formed, which reacted in both colour reactions. This product was assumed to be 2,3,4,5-terahydrodipicolinic acid in equilibrium with 2-oxo-6-aminopimelic acid. The colour reaction with *o*-aminobenzaldehyde is specific for 2,3,4,5-tetrahydropyridine compounds.

Disproportionations of dihydropyridines are known to occur in a number of cases, under certain conditions. Heat, acid, and ammonia often produce pyridine and the reduced pyridine compound from dihydropyridines.[34] In every case pyridine formation was easily seen, from UV and ^{1}H NMR spectroscopic evidence. Although the tetrahydropyridine compound is assumed to be the other product there has been no real evidence provided.



Scheme 19

α,α´-Dioxopimelic acid was synthesised from diethyl oxaloacetate (discussed in Section 6.7) and was treated with liquid ammonia in a sealed tube at -78 °C. The tube was allowed to warm to room temperature overnight. The sealed tube was opened at -78 °C and the ammonia was allowed to evaporate off as the tube warmed to room temperature. The IR spectrum contained very broad bands and showed a broad stretching band at 1600 cm^{-1}, from the carboxylate anions, indicating that the product was formed as an ammonium salt. The yellow solid left was examined by UV and ^{1}H and ^{13}C NMR spectroscopy. The UV spectrum showed an absorbance at 270 nm, which is typical of dipicolinic acid. In the ^{1}H NMR spectrum a singlet at δ 7.79 was probably due to dipicolinic acid. This signal is at higher field than the signal for authentic dipicolinic acid, which normally appears at δ 8.34 as a singlet. This could be due to the product of the reaction of α,α´-dioxopimelic acid and ammonia being formed as an ammonium salt, or due to a difference in the pH at which the NMR spectrum was run. A complicated region appeared in the ^{1}H NMR spectrum between δ 1.50 and 2.00, which could be from the methylenes of a reduced form of dipicolinic acid. There was also a small multiplet at δ 3.40 which was attributed to an acidic α-proton of the reduced form of dipicolinic acid. From integration of the ^{1}H NMR spectrum dipicolinic acid and the reduced dipicolinic acid are in a ratio of 1 : 4. In the ^{13}C NMR spectrum the signals for dipicolinic acid were observed. There were two methines signals present; the signal present at δ 126.18 is due to C-3 and C-5 and the signal at 139.64 was assigned to C-4 of dipicolinic acid. The quaternary carbon

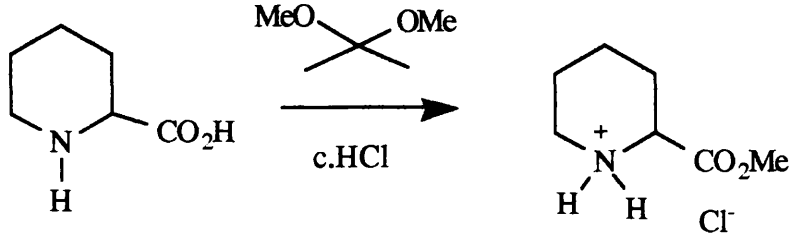
present at δ 153.44 was due to C-2 and C-6 and the signal at δ 178.91 was assigned to the carboxyl of dipicolinic acid. There were three other quaternary signals in the ^{13}C NMR spectrum that could be due to 2,3,4,5-tetrahydrodipicolinic acid; the signal at δ 171.65 might be due to the imine carbon and the signal present at δ 174.97 was attributed to the carboxyl carbon. The quaternary signal present at δ 180.03 might be caused by the carboxylate of the open chain analogue of 2,3,4,5-tetrahydrodipicolinic acid, namely 2-oxo-6-aminopimelic acid. The methylene region of the ^{13}C NMR spectrum was complicated, between δ 18.79 and 25.91. There were two methine signals at δ 40.38 and 41.73, which might be from the C-2 proton of the diammonium salt of 2,3,4,5-tetrahydrodipicolinic acid and to C-6 of its open chain analogue. Separation of products was impossible. A suitable solvent system for TLC could not be found; in all solvent systems tried the R_F values were zero, so chromatography could not be used to separate diammonium dipicolinate and the reduced form of diammonium dipicolinate. Attempts to crystallise the material from methanol or water gave no solid material. Although it was clear dipicolinic acid was formed in the cyclisation reaction, no conclusive evidence could be obtained for 2,3,4,5-tetrahydrodipicolinic acid as the other product.

4.6 Synthesis of *N*-Substituted Amines

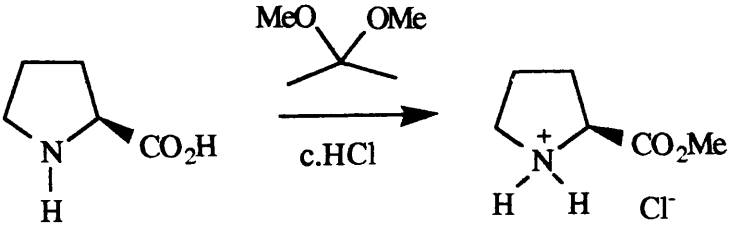
Amines substituted on the nitrogen by a good leaving group, X, eliminate HX easily to form an imine. *N*-

Nitrosoamines eliminate hyponitrous acid on treatment with base to form an imine.[35] *N*-Arylsulphonamides, especially *N*-tosyl derivatives, undergo elimination under the influence of a strong base.[36,37] This reaction requires an easily removable α-hydrogen. Compounds of this type are known to eliminate toluenesulphinic acid on treatment with alkoxide at room temperature to form the imine. Eliminations of HX from *N*-substituted amines are also known to occur where X = OMe or CN to give imines.

Various *N*-substituted analogues of methyl DL-pipecolinate, methyl L-prolinate, and dimethyl *cis*-2,6-piperidinedicarboxylate were prepared and treated with different bases in order to eliminate HX and form imines.



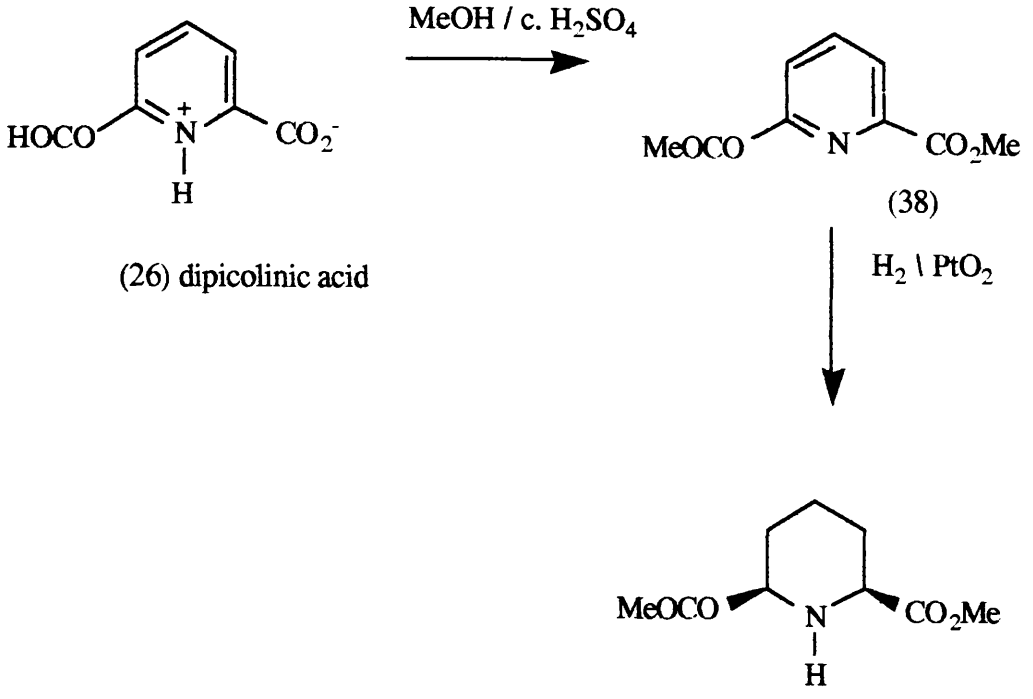
(24) pipecolinic acid (37)



(34) L-proline (36)

(Scheme 20)

L-Proline (34), DL-pipecolinic acid (24), and *cis*-2,6-piperidinedicarboxylic acid (27) were converted into their



(26) dipicolinic acid

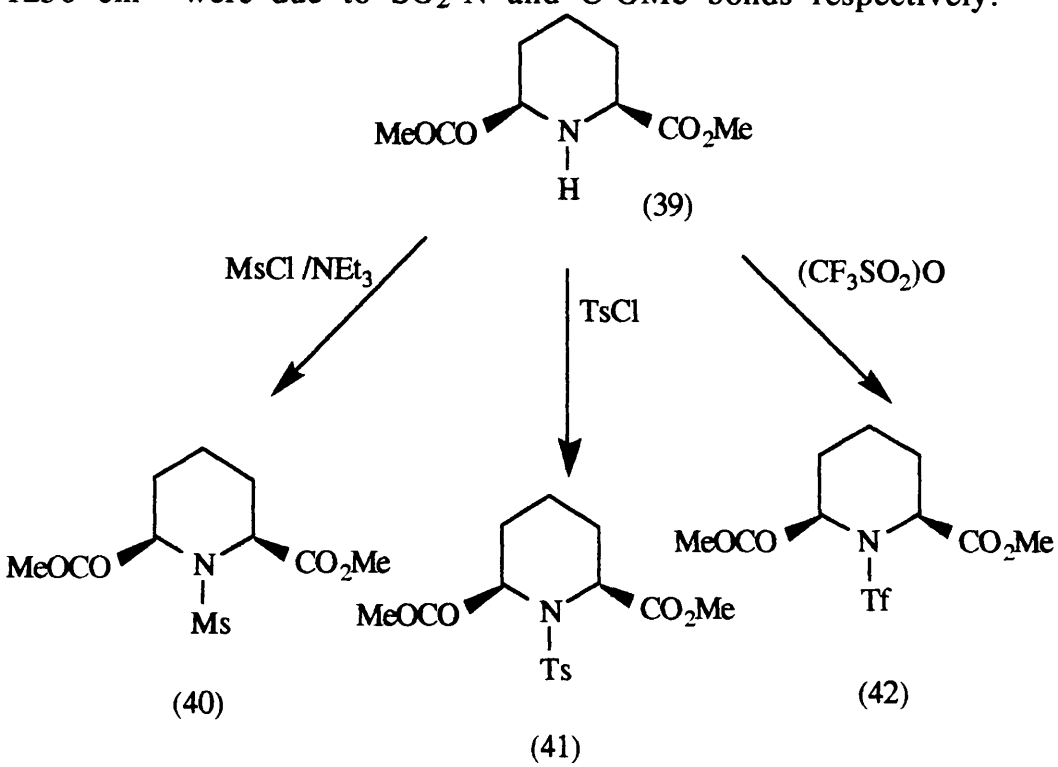
(39)

Scheme 21

methyl esters (36), (37) and (39) respectively to increase their solubility in organic solvents. L-Proline and DL-pipecolinic acid were converted into their methyl ester hydrochloride salts by heating at reflux in 2,2-dimethoxypropane and conc. hydrochloric acid (Scheme 20).

Dimethyl dipicolinate (38) was made from dipicolinic acid (26) by heating at reflux in methanol and conc. sulphuric acid. Hydrogenation of (38) gave dimethyl *cis*-2,6-piperidinedicarboxylate (39) in 94% yield (Scheme 21). In the IR spectrum of (39) stretching bands were present at 1740 and 1150 cm^{-1} due to the carbonyl and ether linkage of the ester groups. The ^1H NMR spectrum was similar to that of *cis*-2,6-piperidinedicarboxylic acid described in Section 4.2, with an extra singlet at δ 3.80 for the methyl groups of the diester. The mass spectrum contained the expected molecular ion at *m/z* 201, and the largest ion observed at *m/z* 84 was due to the loss of both ester groups.

Dimethyl *N*-mesyl-*cis*-2,6-piperidinedicarboxylate (40) was made at -78 °C from dimethyl *cis*-2,6-piperidinedicarboxylate using methanesulphonyl (mesyl) chloride and triethylamine (Scheme 22). The required product (40) was obtained as white needles in 54% yield. In the IR spectrum a band was observed at 1730 cm^{-1} due to the carbonyl of the methyl ester group and bands at 1310 and 1250 cm^{-1} were due to SO_2-N and C-OMe bonds respectively.

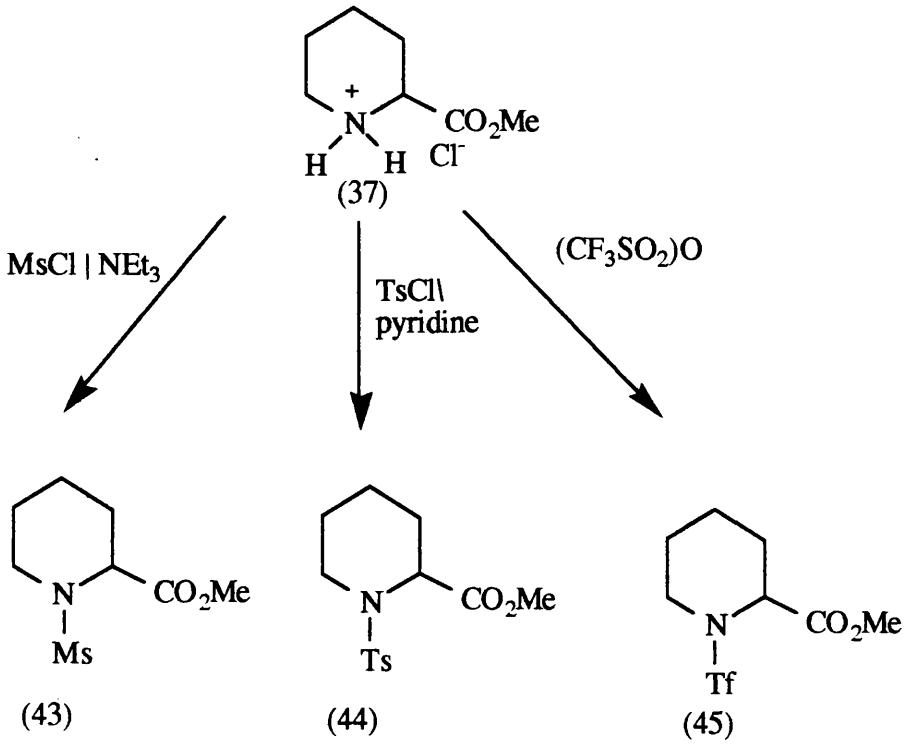


Scheme 22

In the ^1H NMR spectrum a complicated region occurred between δ 1.60 and 2.05 due to the methylenes at C-3, C-4, and C-5. The protons α to the ester groups at C-2 and C-6 appeared further downfield, at δ 4.75 as a multiplet. The methyl group of N-SO_2Me appeared as a singlet at δ 3.08, and the methyl of the ester group gave a singlet at δ 3.62. The mass spectrum

showed only a small molecular ion at *m/z* 279. The largest ion at *m/z* 84 is caused by the loss of both ester groups and the mesyl group.

Methyl *N*-mesyl-DL-pipecolinate (43) was prepared (Scheme 23) using the same procedure as for dimethyl *N*-mesyl-*cis*-2,6-piperidinedicarboxylate. The spectroscopic data for (43) were similar to that for (40), described above.



Scheme 23

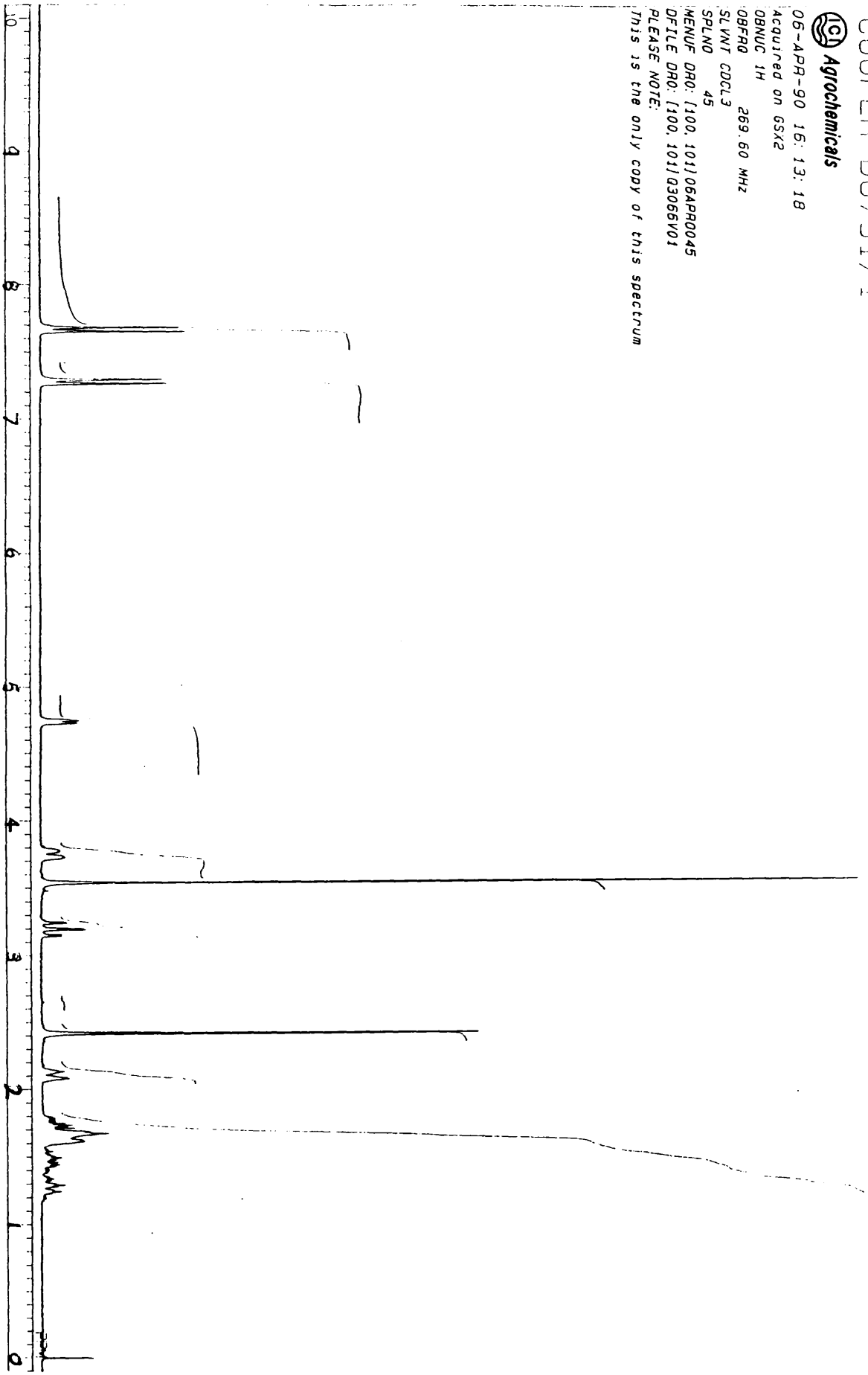
Treatment of dimethyl *cis*-2,6-piperidinedicarboxylate (39) with trifluoromethanesulphonic (triflic) anhydride in pyridine at 0 °C gave dimethyl *N*-triflyl-*cis*-2,6-piperidinedicarboxylate (42) as a yellow oil, which could not be completely purified (Scheme 22). In the IR spectrum a stretching band was present at 1740 cm^{-1} due to the carbonyl group of the methyl ester. The bands at 1360 and 1100 cm^{-1} are due to SO_2-N and C-OMe linkages respectively. The ^1H NMR

spectrum showed a broad complicated region between δ 1.20 and δ 1.80 for the methylenes at C-3, C-4, and C-5. The resonance further downfield at δ 4.00 was due to the protons α to the ester and sulphonamide groups. The methyl groups of the diester appeared as a singlet at δ 3.70. In the mass spectrum no molecular ion was observed; the largest ion at *m/z* 84 was due to the cleavage of both ester groups and the triflyl group.

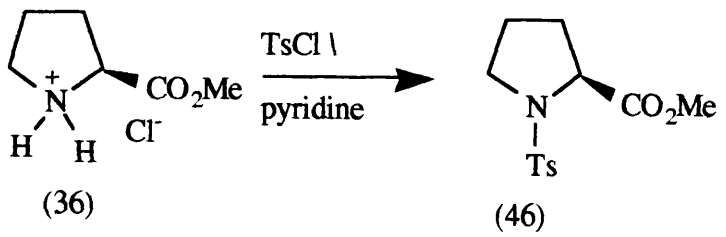
Methyl *N*-triflyl-DL-pipecolinate (45) was prepared (Scheme 23) using the same method as described above. The spectroscopic data for (42) were similar to that of dimethyl *N*-triflyl-*cis*-2,6-piperidinedicarboxylate discussed previously. The mass spectrum, however, showed a molecular ion at *m/z* 275.

Dimethyl *N*-tosyl-*cis*-2,6-piperidinedicarboxylate (41) was synthesised from dimethyl *cis*-2,6-piperidinedicarboxylate (39) by stirring it overnight in pyridine with toluenesulphonyl (tosyl) chloride (Scheme 22). Crystallisation from methanol and water gave the required *N*-tosyl derivative (41) in good yield as white crystals. In the IR spectrum stetching bands were present at 1730 and 1160 cm^{-1} due to the carbonyl and C-O linkage of the ester group. The sulphonamide group gave a stretching band at 1360 cm^{-1}. The ^1H NMR spectrum gave a broad complicated region at δ 1.50 - 1.82 due to the three methylenes at C-3, C-4, and C-5. The protons at C-2 and C-6 appeared further downfield, at δ 4.60 as a multiplet, due to the deshielding effect of the sulphonamide and ester groups. The tosyl group was easily identified by the AA´XX´ system at δ 7.30 and 7.70 and by the singlet at δ 2.30 due to the methyl on

Figure 2. ^{1}H NMR Spectrum of Methyl *N*-Tosyl-DL-pipecolinate (44).



the benzene ring. The mass spectrum showed no molecular ion but an accurate microanalysis was obtained.



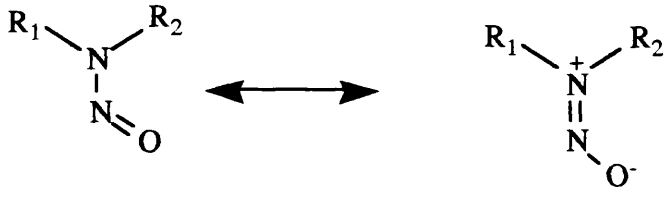
(36) (46)

Scheme 24

Methyl *N*-tosyl-L-prolinate (46) (Scheme 24) and methyl *N*-tosyl-DL-pipecolinate (44) (Scheme 23) were synthesised using the same method as described above, in good yield. The spectra of all *N*-tosyl derivatives, (41), (44), and (46), were similar. The ^1H NMR spectrum of (44) is shown in Figure 2.

Lijinsky, Keefer, and Loo[38] discussed the synthesis of *N*-nitrosopipecolinic acid and *N*-nitrosoproline, by treatment of the amino acid with sodium nitrite in 1M HCl. Addition of methanol to the *N*-nitroso amino acids and concentration with heating gave the corresponding methyl esters. These reactions were repeated by us to give methyl *N*-nitroso-L-prolinate, (49) and (50), and methyl *N*-nitroso-DL-pipecolinate, (47) and (48), as yellow oils. The reaction was also carried out using 2,6-*cis*-piperidinedicarboxylic acid to afford dimethyl *N*-nitroso-*cis*-piperidinedicarboxylate (51) as an oil.

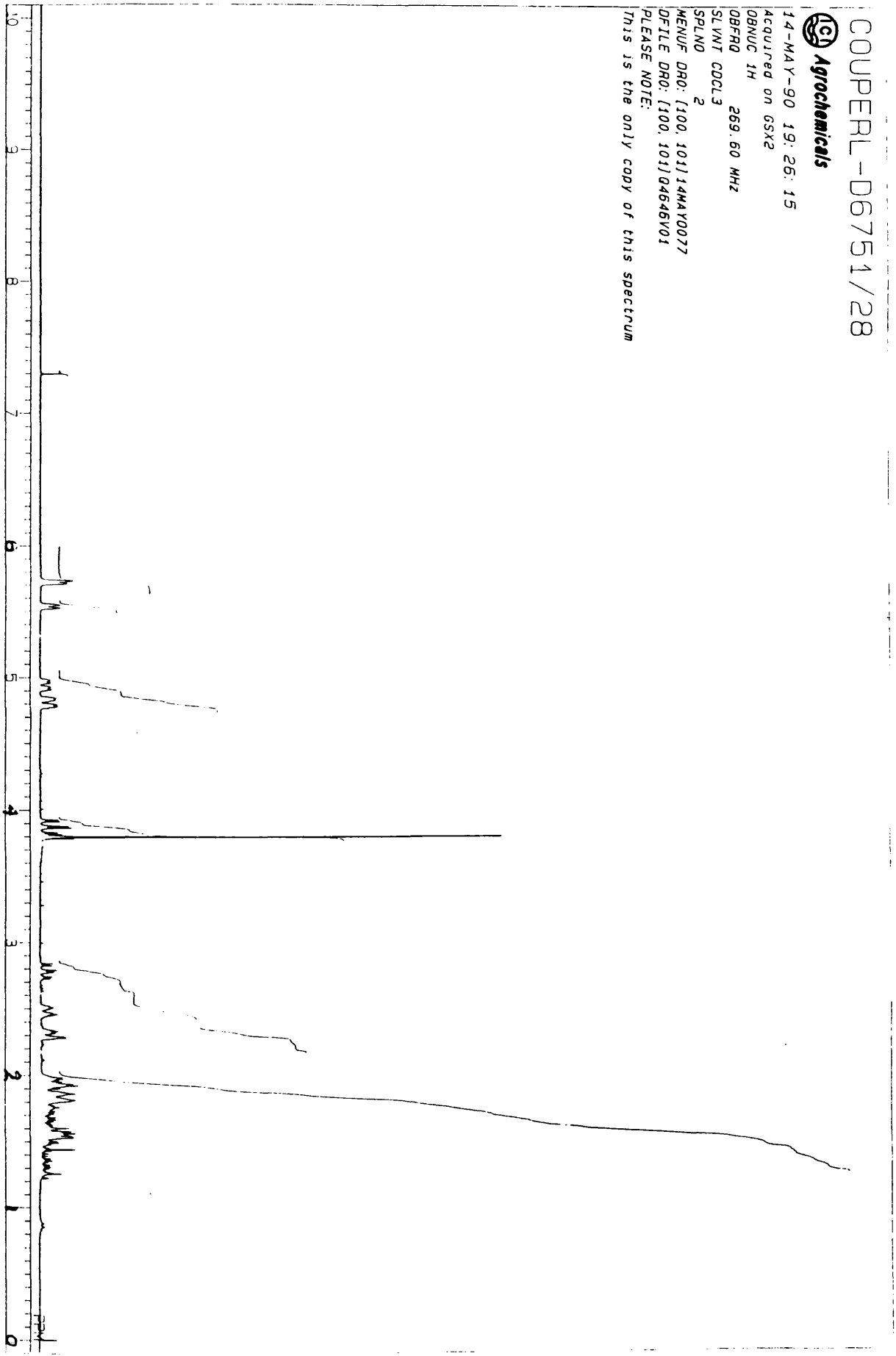
Due to delocalisation and the partial double bond character of the *N*-nitroso linkage, nitrosoamines are planar (Scheme 25).

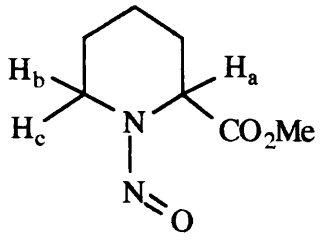


Scheme 25

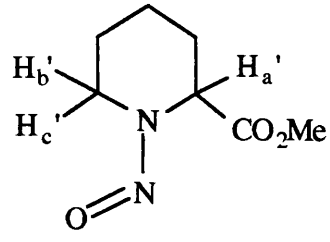
When substituents R_1 and R_2 are non-equivalent, as in methyl *N*-nitroso-L-prolinate and methyl *N*-nitroso-DL-pipecolinate, there are two isomeric forms named *syn-* and *anti-*. These isomers were observed in the ^1H NMR spectra, as in the case of methyl *N*-nitroso-DL-pipecolinate (Figure 3), where a ratio of 1 : 1 of *syn-* (47) and *anti-* (48) isomers was seen. There are six different resonances of almost equal intensity between δ 2.80 and 5.80 due to H_a, H_b, H_c and H_a', H_b', H_c'. The ^1H NMR spectral signal that is furthest downfield is probably due to H_a of the *syn*-isomer. The deshielding effect of the ester and *N*-nitroso group shifted the multiplet downfield. The multiplet next to H_a was assigned to H_a'. As the *N*-nitroso group is *anti-* to H_a' it will have less of a deshielding effect than on H_a.

Figure 3. ^{1}H NMR Spectrum of Methyl *N*-Nitroso-DL-pipecolinate (47) and (48).



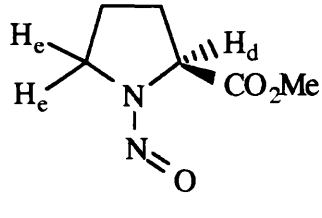


(47) syn

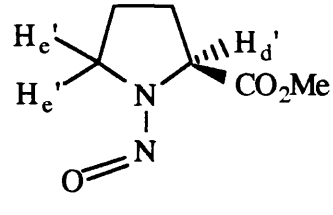


(48) anti

Methyl *N*-nitroso-L-prolinate exists as *syn*- (49) and *anti*-isomer (50). A ratio of almost 1 : 1 of *syn*- and *anti*-isomers can be observed from the ^{1}H NMR spectrum (Figure 4). The resonance furthest downfield at δ 5.28 is due to H_d, which is deshielded by the *N*-nitroso and ester groups. The resonance at δ 4.55 is from H_d', the *N*-nitroso group has less of a deshielding effect than on H_d, as it is *anti* to H_d'. The integrals of these signals were almost equal, therefore there are equal amounts of *syn*- and *anti*-isomers. The multiplet at δ 4.30 is from the H_e' protons, which are deshielded by the N-nitroso group. The multiplet at δ 3.70 is due to the H_e protons of the *syn*-isomer.



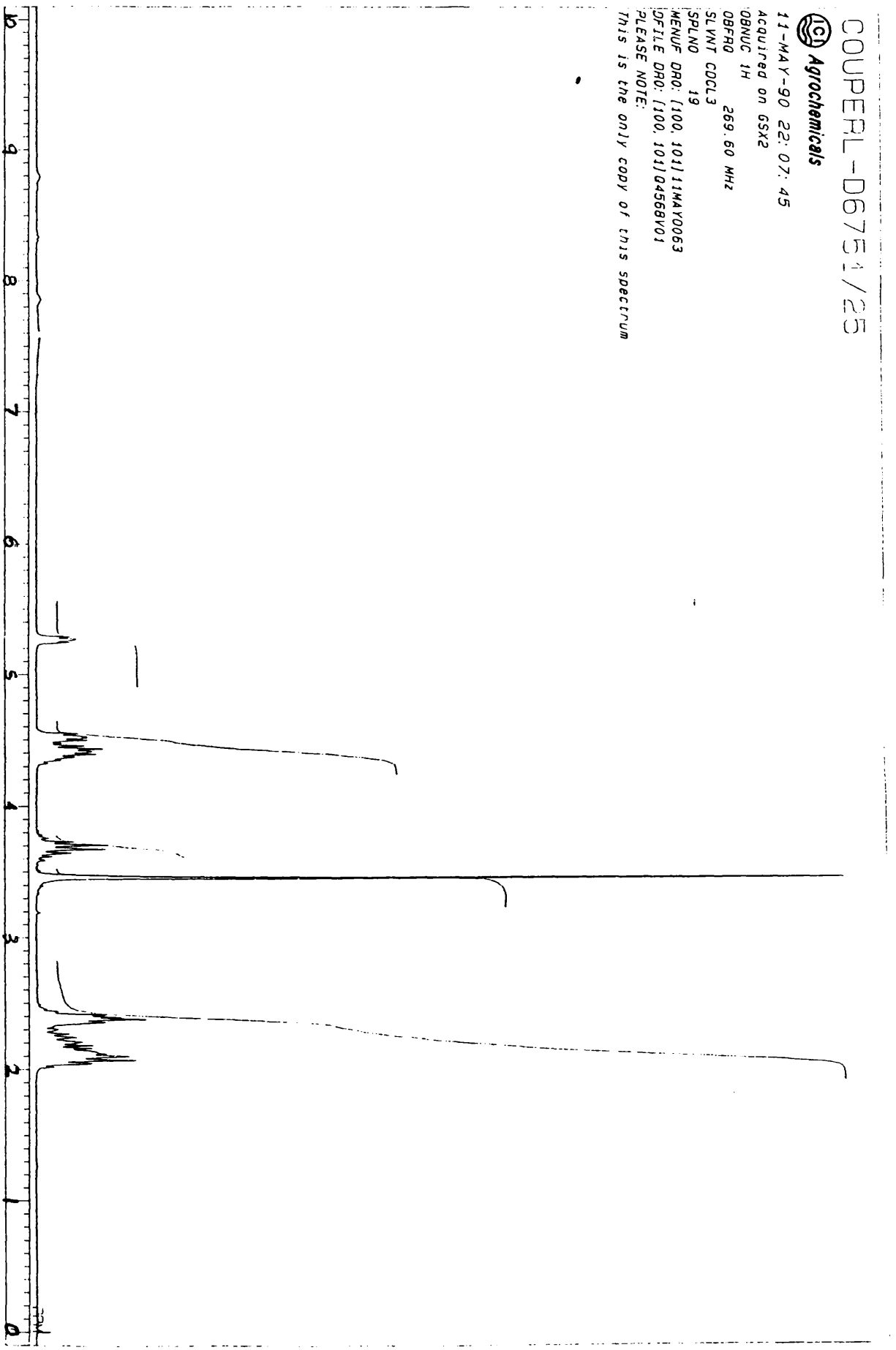
(49) syn



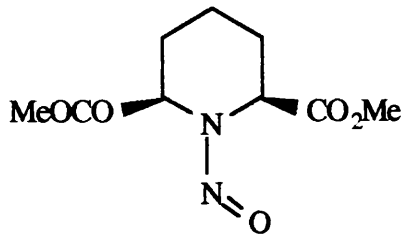
(50) anti

Dimethyl *N*-nitroso-*cis*-2,6-piperidinedicarboxylate (51) exists as only one isomer as $R_1 = R_2$. The ^{1}H NMR spectrum

Figure 4. ^{1}H NMR Spectrum of Methyl *N*-Nitroso-L-prolinate (49) and (50).

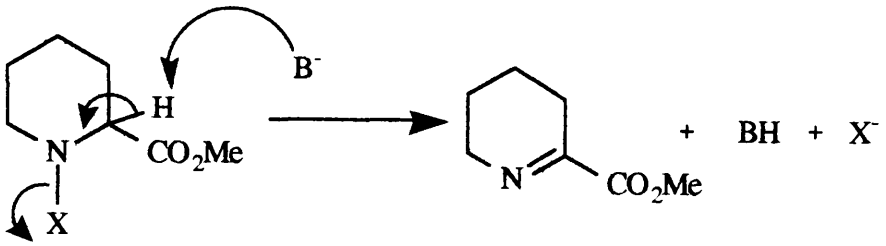


showed a broad complicated region at δ 1.40 - 2.05, due to the three methylene groups at C-3, C-4, and C-5. There was only one multiplet for the two protons α to the ester group at C-2 and C-6. However the ^{13}C NMR spectrum showed that three different methylenes were present at δ 17.21, 24.09, and 26.03. Two methines were observed at δ 48.46 and 54.27 and two quaternary signals were present at δ 168.72 and 169.33 due to the ester carbonyl carbons. The methyl signal for the ester appeared at δ 52.09. This arises because dimethyl *N*-nitroso-*cis*-2,6-piperidinedicarboxylate is not symmetrical.



(51)

The IR spectra of the *N*-nitrosoamino esters were very similar with absorbances at 1450 and 1740 cm^{-1} due to the nitroso and the carbonyl groups respectively. The mass spectra showed the parent ion and ions corresponding to the loss of the ester and nitroso groups.



Scheme 26

4.7 Imine Formation By Elimination

Each of the *N*-substituted heterocyclic methyl esters synthesised was treated with a variety of non-nucleophilic bases in order to eliminate HX (X = Ts, Tf, Ms, or NO) and form the corresponding imine (Scheme 26). The large number of reactions carried out is listed in Table 2 in the Experimental Section.

The *N*-nitroso compounds did not react with any of the bases tried (DBU, DBN, KH, NaH, $KN(TMS)_2$). The strong electron-withdrawing nitroso group and the carboxyl group may stabilise the anion formed to such an extent that it will not react any further. Attempted quenching of the postulated anions formed using methyl iodide was unsuccessful because starting material was recovered. This could be due to steric hindrance at the α-position.

Heating any of the *N*-substituted heterocyclic esters at reflux in the presence of DBU, DBN, or NaH gave starting material.

Methyl *N*-tosyl-DL-pipecolinate (44) was heated at reflux in THF with potassium hydride for four hours. The reaction mixture was cooled and isopropanol was added to destroy excess potassium hydride. The solution was acidified and extracted with dichloromethane. The organic extracts were dried and concentrated to afford toluenesulphinic acid. This was identified from its ^{1}H NMR spectrum which showed an AA´XX´ system at δ 6.90 and 7.20 and a methyl singlet at δ 2.30. The acidic aqueous extracts were freeze dried to leave a white solid. Examination of the solid by ^{1}H and ^{13}C NMR spectroscopy showed that the solid was a mixture of at least three compounds. From this it seemed likely that the elimination reaction had taken place and the imine had formed, but in the acid solution it had decomposed.

When any of the *N*-tosyl compounds were treated with potassium hydride or potassium bis(trimethylsilyl)amide and worked up as described above the organic extracts yielded toluenesulphinic acid and the aqueous acidic solution was freeze dried to give a mixture of products. This suggests that the imine is formed in these reactions but under the acidic conditions it decomposes.

Dimethyl *N*-tosyl-*cis*-2,6-piperidinedicarboxylate (41) in dichloromethane was treated with potassium *t*-butoxide under nitrogen at room temperature. An exothermic reaction took place and a yellow solid precipitated after several hours. The solid was filtered, dried and examined by ^{1}H and ^{13}C NMR spectroscopy. Although the tosyl signals were still there the solid was not starting material. The ester group had been cleaved, as there was no singlet in the ^{1}H NMR spectrum that

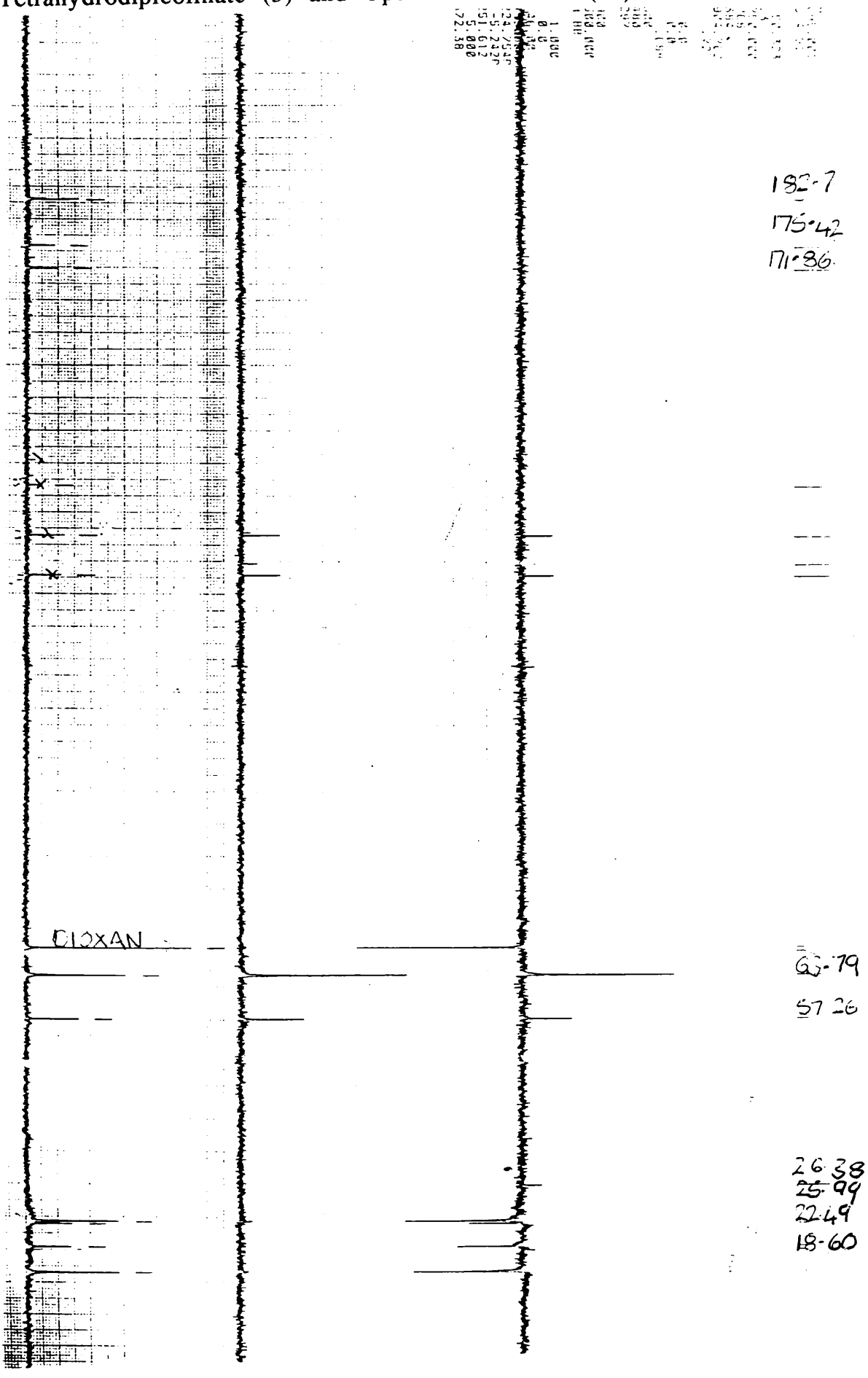
could correspond to the methyl groups of the diester. Ester cleavage probably occurred after ester exchange with the butoxide to form the t-butyl ester.

Attempts were made to remove the toluenesulphinic acid from the solid reaction product. The solid was dissolved in water and the solution was acidified. Extraction of the aqueous acid solution with dichloromethane, followed by drying and concentration of the organic extracts afforded toluenesulphinic acid. This was easily identified by ^{1}H NMR spectroscopy which showed an AA´XX´ system at δ 6.90 and 7.20 and the methyl singlet at δ 2.30. Freeze drying of the acidic aqueous solution and examination by ^{1}H and ^{13}C NMR spectroscopy showed that the reaction product had decomposed to give a mixture of products.

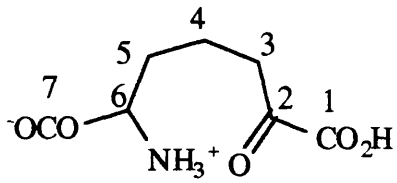
An alternative method for the separation of the toluenesulphinic acid had to be found that did not involve the use of acid. The solid filtered off in the reaction of dimethyl *N*-tosyl-*cis*-2,6-piperidinedicarboxylate (41) and potassium *t*-butoxide, was dissolved in distilled water and stirred for twenty-four hours at room temperature with the weak anion exchange resin Amberlite IR-45 (hydroxide form). The resin was filtered off and the aqueous solution was concentrated under reduced pressure to leave a yellow solid. Methanol was added to the solid and the insoluble material, mainly KCl, was filtered off. Addition of diethyl ether to the methanol solution precipitated a yellow hydroscopic solid. The solid was filtered off and dried over P_2O_5 under vacuum. The IR spectrum was very broad and gave little information. A broad band at 1600 cm^{-1} was assigned to the carboxylate anion group showing that

the product was formed as a potassium salt and the ester group was cleaved during the reaction. Initial studies at Glasgow University on the ^{1}H and ^{13}C NMR spectra showed that the reaction product was a mixture of the potassium salts of the required imine, 2,3,4,5-tetrahydrodipicolinic acid (3) and its open chain analogue (52). The ^{1}H NMR spectrum was very complicated showing a broad multiplet at δ 1.40 - 2.30 due to the methylenes of both compounds (3) and (52). A multiplet appeared at δ 4.02, which was assigned to the C-2 proton of potassium 2,3,4,5-tetrahydrodipicolinate and the C-6 proton of 2-oxo-6-aminopimelic acid. There was no singlet that could correspond to the methyl groups of the diester. In the ^{13}C NMR spectrum (Figure 5) four methylene signals were present at δ 18.60, 22.49, 25.99 and 26.38 in a ratio of 2 : 1 : 1 : 2 which were assigned to the protons at C-3, C-4, and C-5 of the potassium salt of (3) and its open chain analogue (52). The signal at 18.60 could be attributed to the C-4 of both compounds (3) and (52) and the signal present at δ 26.38 was due to C-5 of (3) and C-3 of (52). The other two signals were probably due to C-3 of (3) and C-5 of (52). There were two methine signals at δ 57.26 and 63.79 which would be due to the acidic C-2 proton of (3) and the acidic C-6 proton of its open chain analogue, 2-oxo-6-aminopimelic acid (52). There were four quaternary carbons in the spectrum. The signal at δ 171.86 was assigned to the imine carbon at C-6 of 2,3,4,5-tetrahydrodipicolinic acid and the signal at δ 175.42 and 182.7 to the carboxylate group of the potassium salts of (3) and (52). The signal at δ 216.72 indicates a ketone is present which is

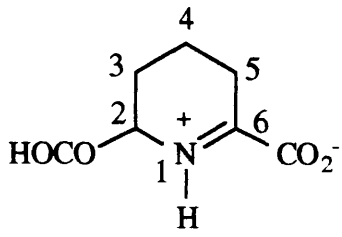
Figure 5. ^{13}C NMR Spectrum of Potassium 2,3,4,5-Tetrahydrodipicolinate (3) and Open Chain Form (52).



additional evidence for the imine being in equilibrium with the open chain compound (52).

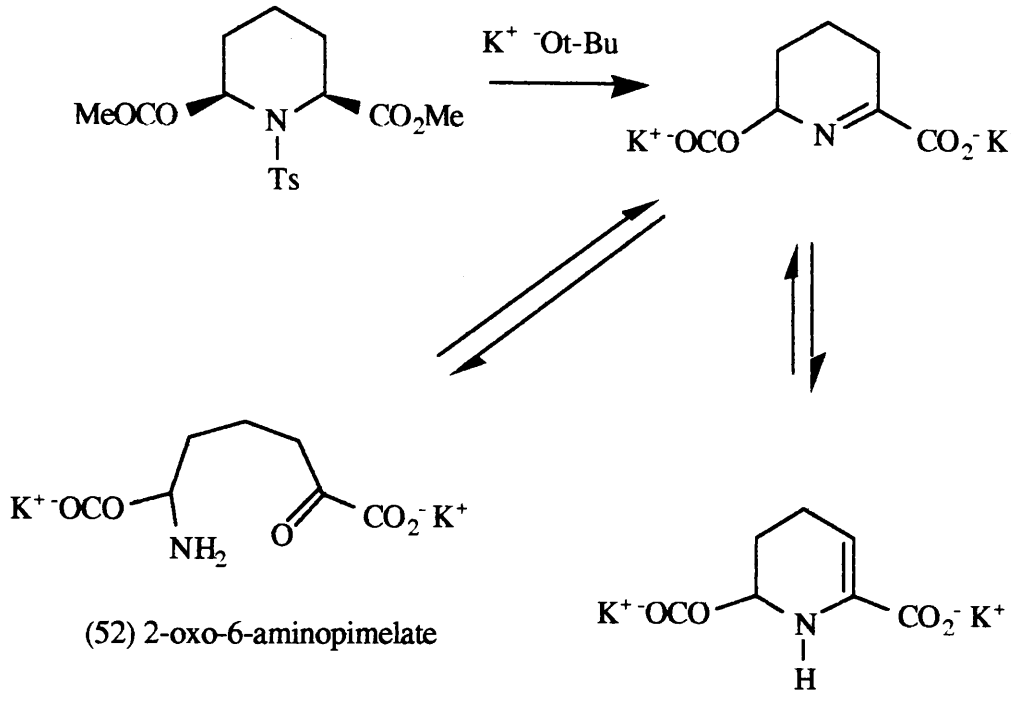


(52)



(3)

Further work was carried out on the ^1H NMR spectrum of our synthesised 2,3,4,5-tetrahydrodipicolinic acid at ICI Agrochemicals on the 250 MHz NMR spectrometer. The sample used for this work had been prepared a month beforehand. The 250 MHz ^1H NMR spectrum showed additional signals compared to our spectrum, which was run earlier. A doublet of doublets appeared at δ 5.20 in the 250 MHz NMR spectrum run at ICI. These signals were assigned to



(52) 2-oxo-6-aminopimelate

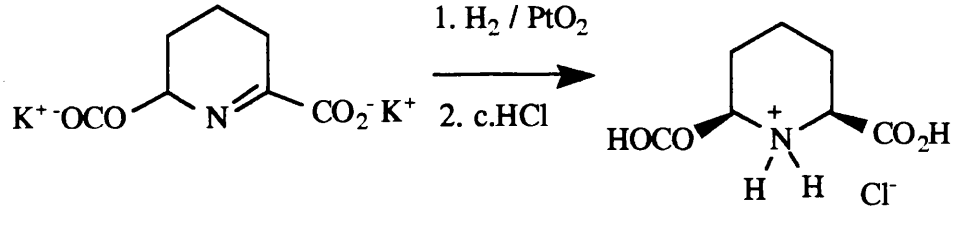
(53)

Scheme 27

the olefinic protons of the enamine (53) of tetrahydrodipicolinic acid. Also a small singlet at δ 8.34 indicated that some oxidation of tetrahydrodipicolinic acid had occurred to give dipicolinic acid. The FAB mass spectrum showed a molecular ion at *m/z* 171 which could correspond to the imine (3), or the enamine (53). The product from the reaction of potassium *t*-butoxide is therefore a mixture of three products. The initially formed imine (3) establishes an equilibrium with the enamine (53) and the open chain compound (52) (Scheme 27). The ratio of these compounds was found to be 2.8 : 3.7 : 1. This was calculated from the 250 MHz ^{1}H NMR spectrum taken at ICI on a month old sample of our synthesised potassium 2,3,4,5-tetrahydrodipicolinate.

The ^{1}H and ^{13}C NMR spectra were repeated using D_2O and DCl to examine the effect of pH on potassium 2,3,4,5-tetrahydrodipicolate. However in acid or at neutral pH the spectra changed completely and showed that tetrahydrodipicolinic acid decomposed readily at neutral or acidic pH. This explains why methods which used these conditions to generate (3) have failed.

Hydrogenation of the potassium salt of 2,3,4,5-tetrahydrodipicolinic acid was carried out in water using PtO_2 as catalyst, followed by acidification, to give *cis*-2,6-piperidinedicarboxylic acid hydrochloride (27) in 95% yield. This was identified by its ^{1}H and ^{13}C NMR spectra and by its melting point which were identical to those of authentic material (Scheme 28). This is taken as good evidence for the prescence of the imine (3) in equilibrium with enamine (53) and open chain forms (52).



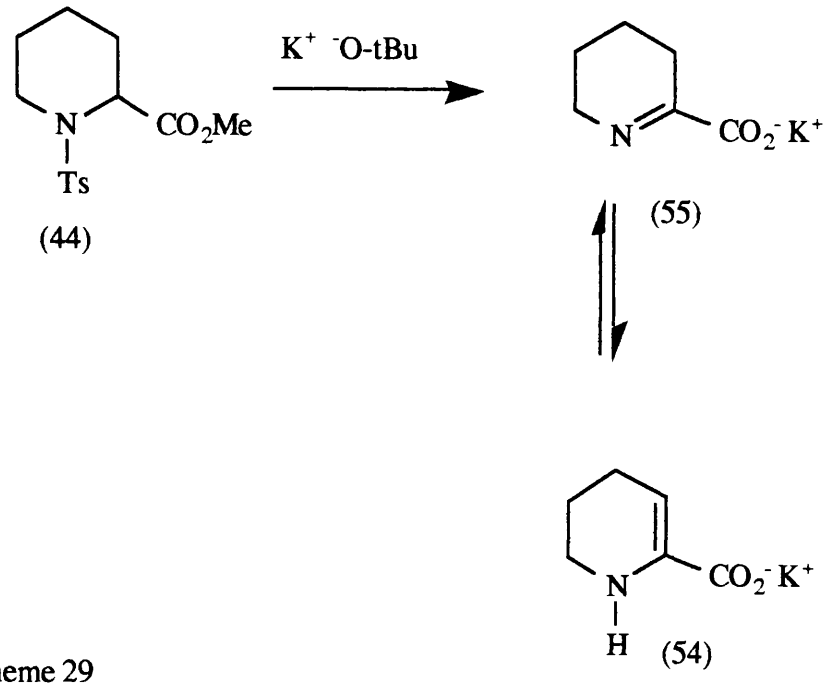
Scheme 28 (27)

Further evidence for the formation of the imine, 2,3,4,5-tetrahydrodipicolinic acid was provided by work carried out by Emma Borthwick in the Biochemistry Department. When the potassium salt of our synthesised THDPA (3) was used as a substrate in the *meso*-diaminopimelic acid dehydrogenase enzyme assay a positive rate was observed. The dehydrogenase enzyme is used by some bacteria in the

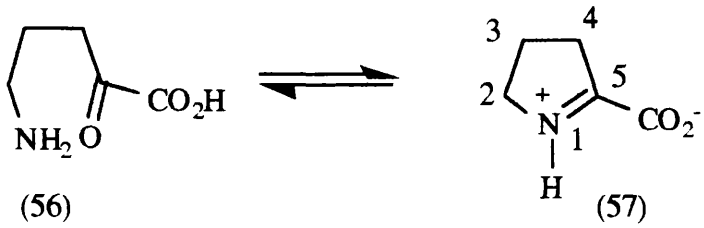
biosynthesis of L-lysine.[14,15] 2,3,4,5-Tetrahydrodipicolinic acid (3) is converted directly into DL-diaminopimelic acid (7) using NADPH and ammonia bypassing LL-diaminopimelic acid (6). The enzyme reaction can be followed by looking at the disappearance of NADPH which absorbs at 340 nm. More information on this enzyme was given in Section 2.12.

Methyl *N*-tosylpipecolinate (44) was treated with potassium *t*-butoxide in dichloromethane, using the same procedure as described for dimethyl *N*-tosyl-*cis*-2,6-piperidinedicarboxylate. The solid that precipitated during the reaction was filtered off, dried and examined by ^{1}H and ^{13}C NMR spectroscopy. The spectra showed that the solid was not starting material, as the multiplet for the acidic proton at C-2 and the methyl ester singlet were no longer there. However the toluenesulphinic acid signals were still present. This could be observed by the AA'XX' system at δ 6.90 and 7.20 and by the methyl singlet at δ 2.30 in the ^{1}H NMR spectrum. A small doublet of doublets at δ 5.48 could be caused by the olefinic proton of the enamine of tetrahydropicolinic acid (55). The multiplet at δ 3.32 was assigned to the C-2 methylene group of (55). The ^{13}C NMR spectrum showed four methylenes at δ 16.82, 21.60, 42.14, and 48.80. Two quaternary carbons at δ 170.92 and 175.16 were assigned to the imine at C-6 and the carboxylate groups respectively. There were also signals that correspond to toluenesulphinic acid. These results suggested that the elimination reaction had taken place and the imine (55) in equilibrium with the enamine (54) had been formed (Scheme 29). Most of the toluenesulphinic acid was removed by stirring the solid, formed in the reaction with methyl *N*-

tosyl-pipecolinate and potassium *t*-butoxide, with Amberlite IR-45 anion exchange resin (OH form) in distilled water. The resin was filtered off and the filtrate was concentrated under reduced pressure. The solid left was crystallised from methanol and diethyl ether to give a yellow hydroscopic solid. The IR spectrum contained very broad bands and a band at 1600 cm^{-1} was assigned to the carboxylate anion. The product was therefore a potassium salt and gave a positive flame test. The ^{1}H NMR spectrum gave a broad multiplet at δ 1.40 - 2.00. This could be caused by the three ring methylenes at C-3, C-4, and C-5. The multiplet at δ 3.34 was asigned to the C-2 methylene of (55). The ^{13}C NMR spectrum was similar to the spectrum, described above, before the toluenesulphinic acid was removed but was more complicated due to some decomposition of the product, tetrahydropicolinic acid. Although the imine, tetrahydropicolinic acid (55), in equilibrium with the enamine, (54) was initially formed by elimination of toluenesulphinic acid (Scheme 29) it was unstable and deteriorated during the removal of toluenesulphinic acid using the anion exchange resin.



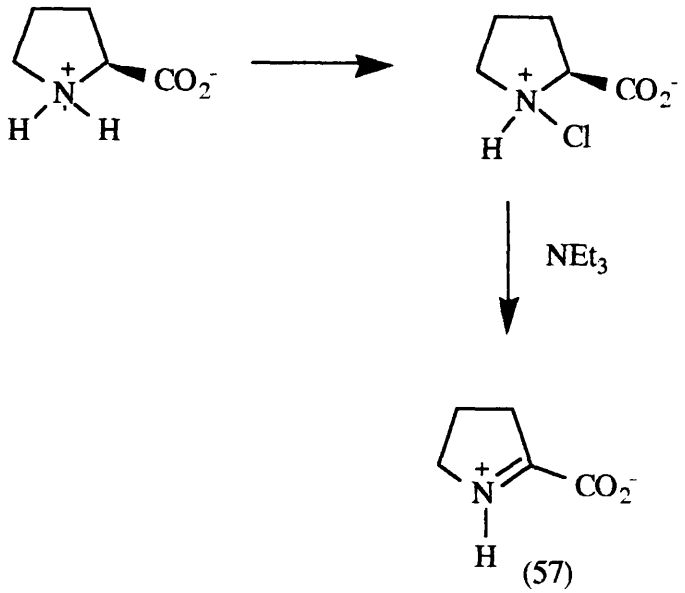
Scheme 29



Scheme 30

The synthesis of 3,4-dihydro-2*H*-pyrrole-5-carboxylate (57) has been previously discussed. Stewart *et al.*[39] reported the synthesis of (57) from α-keto-δ-aminovaleric acid (56). This compound spontaneously cyclised to the imine (57) between pH 4.6 and 9.4 (Scheme 30). They prepared 3,4-dihydro-2*H*-pyrrole-5-carboxylic acid while investigating the reduction of Schiff bases using NADPH.

Hausler and Schmidt[40] reported the synthesis of (57) by the oxidation of proline using *t*-butyl hypochlorite and triethylamine (Scheme 31).



Scheme 31

(57)

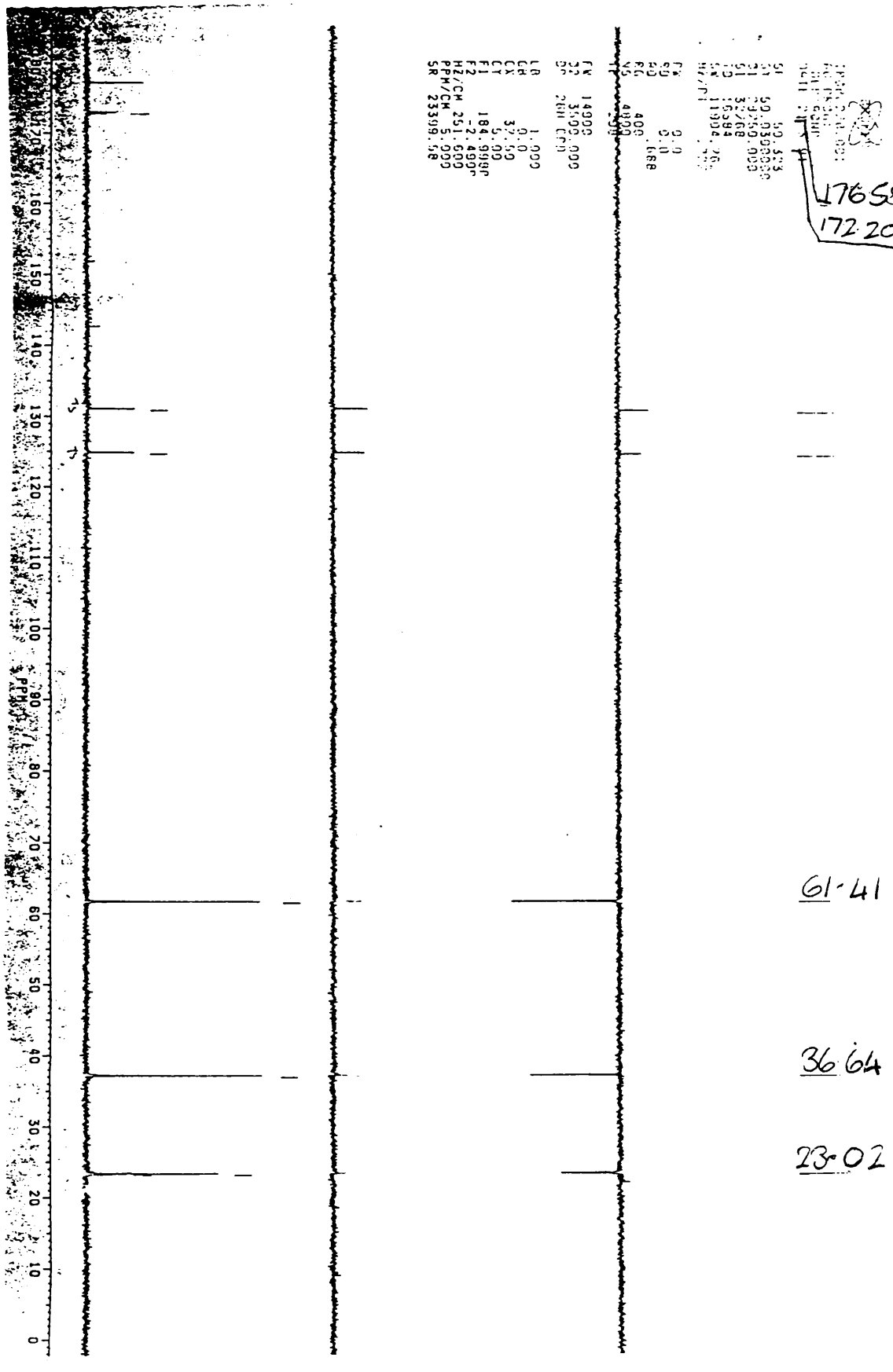
Methyl *N*-tosyl-L-prolinate (46) in dichloromethane was treated with potassium *t*-butoxide and a white solid precipitated. The solid was filtered off and examined by ^{1}H and ^{13}C NMR spectroscopy. In the ^{1}H NMR spectrum the AA´XX´ system at δ 6.90 and 7.20 and the methyl singlet at δ 2.30 due to toluenesulphinic acid were present. The ^{1}H NMR spectrum did not contain a singlet that could correspond to a methyl ester indicating that the ester had been cleaved during the reaction. In the ^{1}H NMR spectrum three multiplets were present for the methylenes at C-2, C-3, and C-4. The ^{13}C NMR spectrum showed no methine signals, indicating that the acidic proton at C-5 had been lost.

Most of the toluenesulphinic acid was removed by the anion exchange resin, Amberlite IR-45, by stirring the solid in distilled water with the resin. The resin was filtered off and the filtrate was concentrated under reduced pressure. The

solid residue was crystallised from methanol and diethyl ether to give a white solid. From ^{1}H and ^{13}C NMR spectroscopy about 1% of the solid was toluenesulphinic acid which could not be removed even by crystallisation. The remainder of the solid was the required potassium 3,4-dihydro-2*H*-pyrrole-5-carboxylate (57). The ^{1}H NMR spectrum contained three multiplets in a ratio of 1 : 1 : 1 at δ 1.75, 2.55, and 3.70. The multiplet at δ 1.75 was due to the methylene protons at C-3 on the ring coupled to the methylene protons at C-2 and C-4. The methylene protons at C-2 and C-4 appeared as multiplets due to coupling from the C-3 protons. The ^{13}C NMR spectrum (Figure 6) showed three different methylene groups at δ 23.02, 36.64, and 61.41, and two quaternary carbons. The signal at δ 172.20 was assigned to the imine carbon at C-5 and the signal at δ 176.58 to the carboxylate carbon. In the mass spectrum the expected molecular ion was observed at *m/z* 113 and a larger ion present at *m/z* 69 was due to the loss of a carboxylate group. Microanalysis did not give accurate results probably due to the small impurities of potassium chloride and toluenesulphinic acid.

The imine of L-proline is the only product in the reaction of methyl *N*-tosyl-L-prolinate and potassium *t*-butoxide. There was no evidence for the formation of the enamine or the open chain compound (56) as found with the six membered rings, potassium tetrahydrodipicolinate and potassium tetrahydropicolinate. This could be due to the five membered ring being flat and the bond angle being close to the stable tetrahedral angle of 109.5°. The six membered rings have a flattened chair conformation and so the bonds can have the

Figure 6. ^{13}C NMR Spectrum of Potassium 3,4-Dihydro-2*H*-pyrrole-5-carboxylate (57).



correct allignment to tautomerise. As the five-membered ring is flat the imine is less likely to tautomerise to the enamine as the bonds will not have the correct antiperiplanar allignment for a proton to be removed and the carbon carbon double bond to shift as for the six-membered rings.

Conclusions

cis-2,6-Piperidinedicarboxylic acid could be easily prepared in good yield by hydrogenation of dipicolinic acid. A mixture of *cis*- and *trans*-2,6-piperidinedicarboxylic acid was made and separated at the diamide stage after cyclisation of diethyl α,α′-dibromopimelate with ammonia, followed by hydrolysis of the diamides to the diacids.

The oxidation of the amino acids, L-proline (34), DL-pipecolinic acid (24), and *cis*-2,6-piperidinedicarboxylic acid (27) to their cyclic imine was eventually achieved by elimination of toluenesulphinic acid from the *N*-tosyl derivatives. In the case of the reaction of *N*-tosyl-L-prolinate and potassium *t*-butoxide the cyclic imine potassium 3,4-dihydro-2*H*-pyrrole-5-carboxylate was the only product. However in the reaction of the compounds with six-membered rings, (41) and (44), a mixture of compounds was obtained. An equilibrium was established between the imine and enamine tautomers and in the case of dipotassium 2,3,4,5-tetrahydrodipicolinate the open chain form was produced as well. The differences observed between the behaviour of the five and the six-membered rings is probably due to the different conformations of the rings.

CHAPTER 5

Analogues of Dipicolinic Acid and Chelidamic Acid

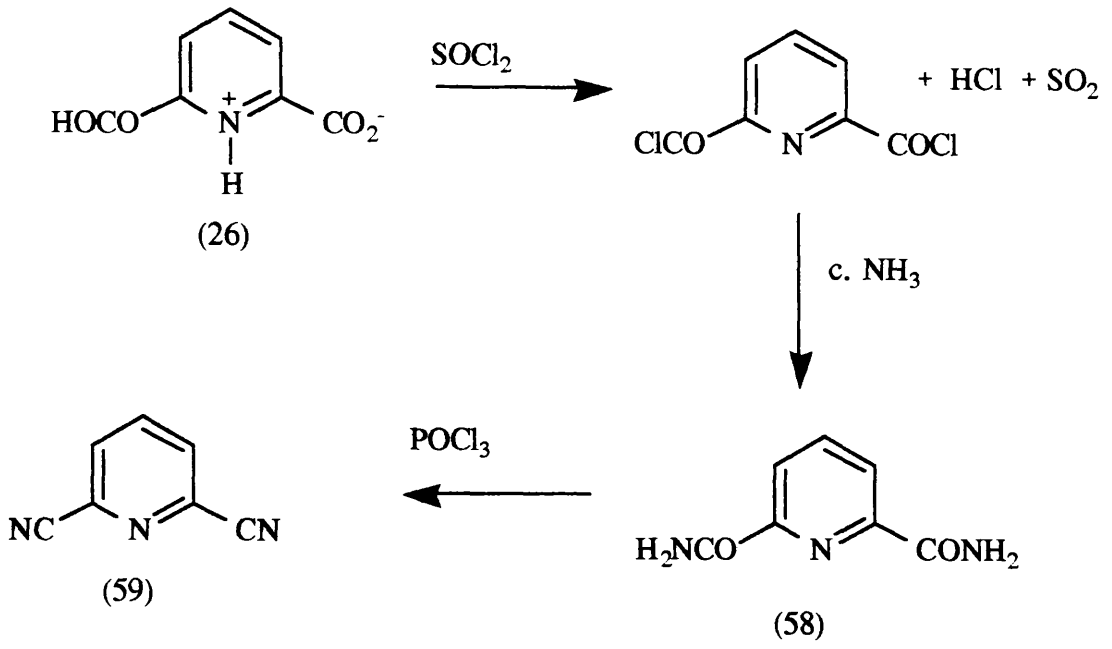
5.1 Introduction

Dipicolinic acid (26) has some structural similarities to the enzymic condensation product, dihydrodipicolinic acid (2), which lies in the diaminopimelate pathway to L-lysine (8). Dipicolinic acid was tested as an enzyme inhibitor of dihydrodipicolinate reductase by Tyagi *et al.*[7] and was found to be a competitive inhibitor of the enzyme with K_i 9 x 10^{-4} M. Therefore we proposed to synthesise analogues of dipicolinic acid to be tested as enzyme inhibitors of dihydrodipicolinate synthase, along with commercially available dipicolinic acid.

5.2 Synthesis of 2,6-Pyridinedinitrile

Dipicolinic acid can be converted into the diacid chloride by heating at reflux in thionyl chloride. The excess thionyl chloride was removed under reduced pressure. The diacid chloride was added to conc. ammonia solution at 0 °C to precipitate 2,6-pyridinedicarboxamide (58) as a white solid in 51% yield (Scheme 32). In the IR spectrum a stretching band was present at 1670 cm^{-1}, which is typical of amides and a band at 1590 cm^{-1} was due to the pyridine ring. The ^1H NMR spectrum showed a singlet at δ 7.81 due to the three aromatic protons of the pyridine ring and a singlet at δ 9.10 was assigned to the amide protons. In the mass spectrum a molecular ion was observed at the expected *m/z* 181 (100%),

and a smaller ion at *m/z* 122 was due to the loss of an amide group.

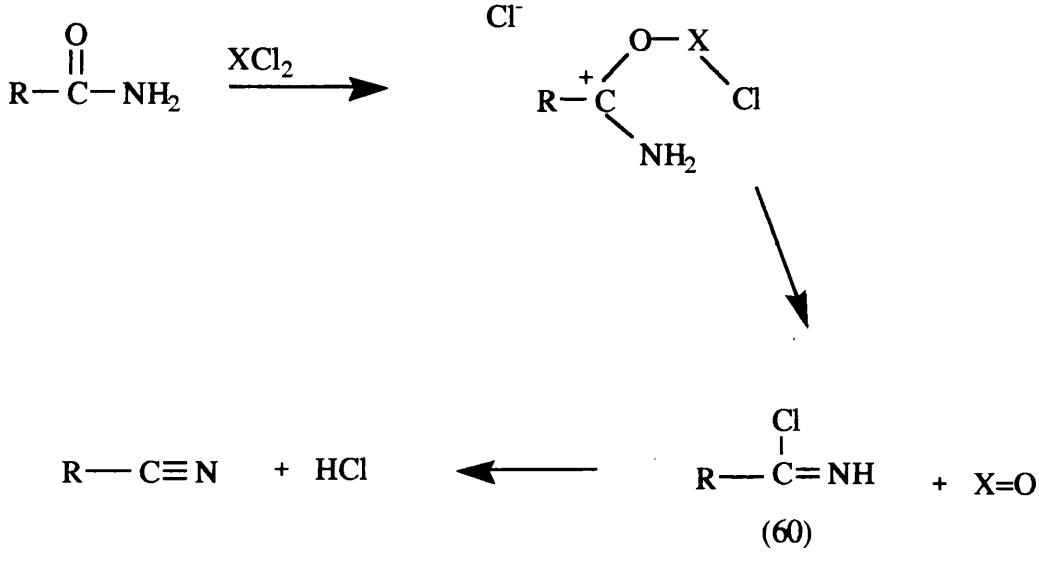


Scheme 32

There a number of ways of preparing nitriles, including addition of cyanide to multiple bonds (eg. carbonyls), reaction of halogen compounds with metal cyanides, and displacement of diazonium salts (Sandmeyer reaction). Another method is the conversion of the carboxylic acid into the carboxamide, followed by dehydration to the nitrile. Dehydrating agents commonly used include phosphorus pentoxide, phosphorus pentachloride and phosphorus oxychloride.

In cases where the nitrile is sufficiently volatile to allow purification by distillation, P_2O_5 may be used as the dehydrating agent. Another effective reagent is PCl_5. The mechanism is proposed to involve the formation of an imidoyl chloride (60), which is cleaved to give the nitrile and phosphoryl chloride.

The general mechanism for these dehydration reactions involves the inorganic acid halide (phosphorus pentachloride, thionyl chloride, phosgene, or phosphorus oxychloride) combining with the amide. The O-complex between the amide and the acid halide polarises the C-O bond so that nucleophilic substitution followed by elimination of XO (where X= PCl_3, SO, CO, or POCl) can take place to form an imidoyl chloride (60). Loss of HCl from the imidoyl chloride (60) gives the nitrile (Scheme 33). Basic solvents are often employed in these reactions to remove the acidic by-products.



Scheme 33

The reagent of choice for the dehydration of 2,6-pyridinedicarboxamide to give the corresponding nitrile is phosphorus oxychloride (Scheme 32). As 2,6-pyridinedinitrile (59) is not volatile P_2O_5 cannot be used. Phosphorus oxychloride was readily available and less toxic than some of the other dehydrating agents. A number of nitriles have been prepared using phosphorus oxychloride. Solvents such as

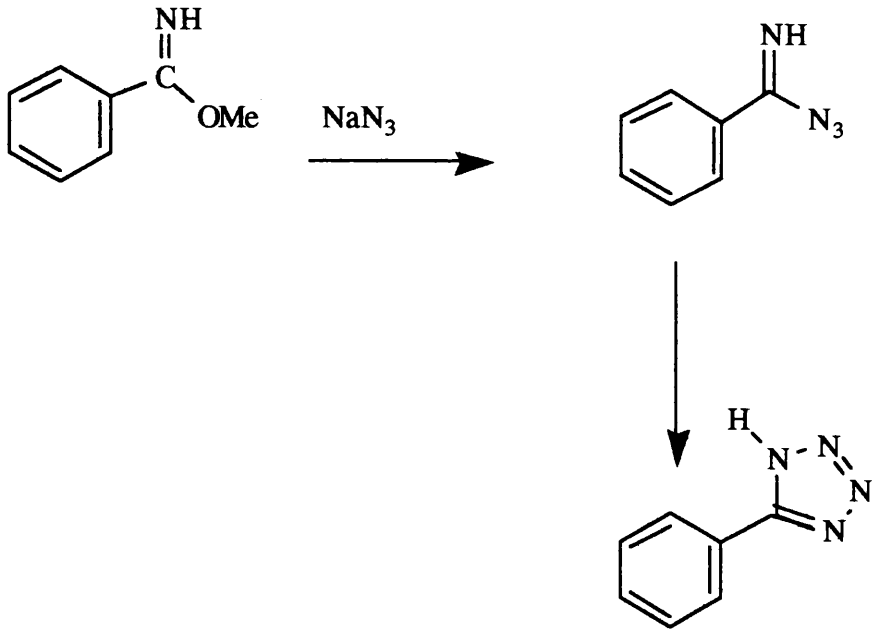
acetonitrile or 1,2-dichloroethane are often employed. Sometimes a base such as pyridine is used to remove the acidic by-products. Other advantages in using $POCl_3$ are that it is cheap, and does not attack carbonyl groups. However hydroxy substituents may be converted into the corresponding chloro groups.

Pearse and Wisowaty[41] originally reported the synthesis of 2,6-pyridinedinitrile (59) by the reaction of 2,6-dibromopyridine with copper cyanide in DMF. The product was obtained in a 10% yield. Banks and Brookes[42] reported an improved synthesis involving the dehydration of 2,6-pyridinedicarboxamide (58) using phosphorus oxychloride in tetrachloroethane (Scheme 32). We repeated the method of Banks and Brookes[42] by heating at reflux 2,6-pyridinedicarboxamide and phosphorus oxychloride in tetrachloroethane. 2,6-Pyridinedinitrile (59) was obtained as white crystals in 26% yield. A band at 2120 cm^{-1} observed in the IR spectrum is characteristic of a nitrile group. The band at 1590 cm^{-1} was assigned to the pyridine ring. The ^1H NMR spectrum showed two multiplets; the signal at δ 7.91 was due to the protons at C-3 and C-5 and the signal at δ 8.09 is caused by the proton at C-4. The integrals were in a ratio of 2 : 1. The ^{13}C NMR spectrum showed quaternary carbons at δ 135.12, for the nitrile and at δ 115.31 for C-2 and C-6 of the ring. There were methine signals for the aromatic ring carbons. The signal at δ 131.15 was due to C-4 and the signal present at δ 138.94 was due to C-3 and C-5. The mass spectrum gave the expected molecular ion at *m/z* 129 (100%) and a fragment ion at *m/z* 103 from the loss of a nitrile group.

5.3 Tetrazoles

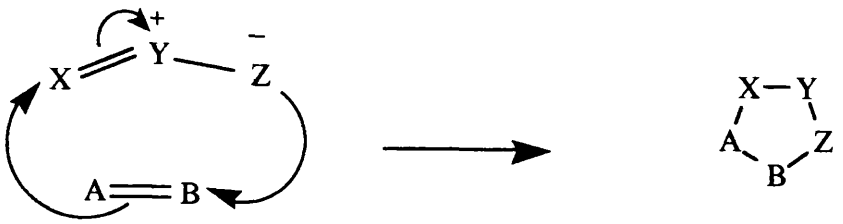
An area where there has been a recent rapid growth in interest, due to biological importance, is in the synthesis of tetrazoles. Tetrazoles are of interest as they have some similarities to carboxylic acids. A tetrazole occupies a similar position in space to the carboxylic acid group, but is metabolically more stable. Many tetrazoles have been synthesised and shown to possess biological activity. Tetrazoles have been prepared that act as anti-inflammatory agents,[45] antibacterial compounds,[46] and anti-fertility agents.[47] As Tyagi *et al.*[7] found dipicolinic acid to be a good competitive inhibitor of the dihydrodipicolinate reductase enzyme, we decided to convert the carboxylic acid groups of dipicolinic acid into tetrazoles to produce a new compound for testing as a potential inhibitor of dihydrodipicolinic acid synthase enzyme.

A number of methods for the synthesis of tetrazoles have been reported. These include the reaction of an imidate with an azide to give an imino azide followed by cyclisation to afford the tetrazole (Scheme 34).[43]



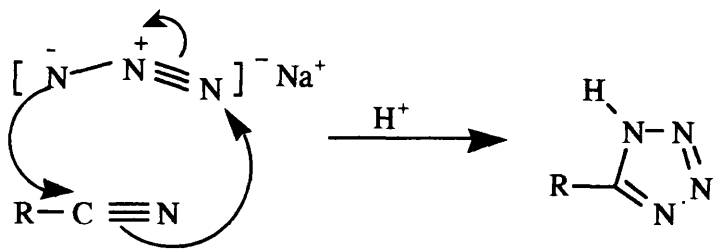
Scheme 34

Another method involves the reaction of a nitrile with an azide. This method has been successful in a number of cases.[44] The general route involves the condensation of the nitrile with the azide ion in a 1,3-dipolar cycloaddition reaction. The azide ion is the 1,3-dipole. This is a system with 4 π electrons spread over three atoms. The azide group can be represented as zwitterionic and can display both electrophilic and nucleophilic character. 1,3-Dipoles undergo cycloaddition reactions (Scheme 35). Compounds which react with them are unsaturated compounds containing groups such as C=N, C=C, or C=O. The first step in an ozonolysis reaction of an olefin to form an ozonide is another example of this type of reaction. The general reaction is a concerted process and is stereoselective (Scheme 35).



Scheme 35

In the formation of the tetrazole (61) the azide ion acts as the 1,3-dipole and adds in a concerted manner to the nitrile (Scheme 36). Only electron deficient nitriles have been found to react in this way.

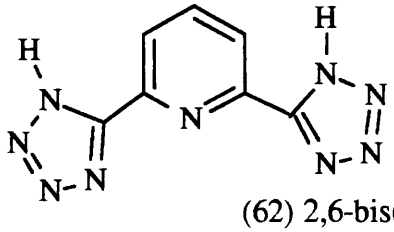


(61)

Scheme36

2,6-Pyridinedinitrile in DMF was treated with ammonium chloride and sodium azide. Addition of diethyl ether at the end of the reaction precipitated a white solid, 2,6-bis(1′*H*-tetrazol-5′-yl)pyridine (62) in 90% yield. In the UV spectrum absorbances at λ 292 nm (ε 9750) and 221 nm (ε 20,450) were ascribed to the pyridine ring and the tetrazole ring. The IR spectrum was broad and gave little helpful information. The band at 1600 cm^{-1} could be due to the pyridine ring. The ^{1}H NMR spectrum showed a singlet at δ 8.30 for the aromatic ring protons. In the ^{13}C NMR spectrum two quaternary carbons were observed; one for C-2 and C-6 of the pyridine ring at δ

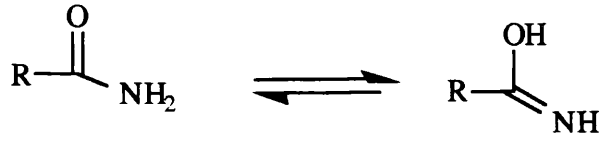
148.42 and one for the carbons of the tetrazole ring at δ 162.47. The aromatic methine signal at δ 122.59 was assigned to C-4 and the methine signal at δ 139.60 to C-3 and C-5. These two signals were in a ratio of 2 : 1. The mass spectrum contained the expected molecular ion at *m/z* 215.



(62) 2,6-bis(1'*H*-tetrzol-5'-yl)pyridine

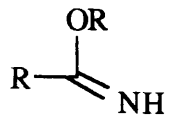
5.4 Imidates

Imidic acids (63) are the tautomeric forms of carboxamides (Scheme 37). Their esters (64) are called alkyl imidates. Imidates have found recent application in agricultural products,[48] bactericides,[49] and pesticides.[50] The synthesis of imidates was first carried out by Pinner using a nitrile and an alcohol, phenol or thiol under acid conditions and is known as the Pinner synthesis.[51] The reaction normally requires an excess of the alcohol.



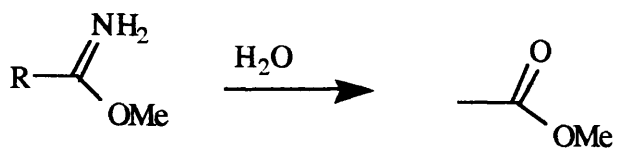
(63)

Scheme 37

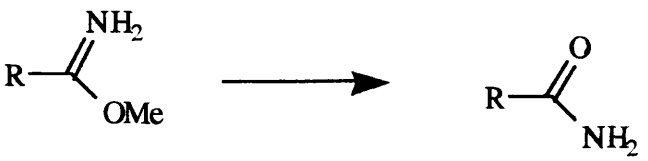


(64)

Two side reactions sometimes cause problems. Water can hydrolyse the imidate to give the ester, hence the necessity for anhydrous conditions (Scheme 38). Decomposition to the amide can also occur, hence the temperature should be kept at 0 °C (Scheme 39).

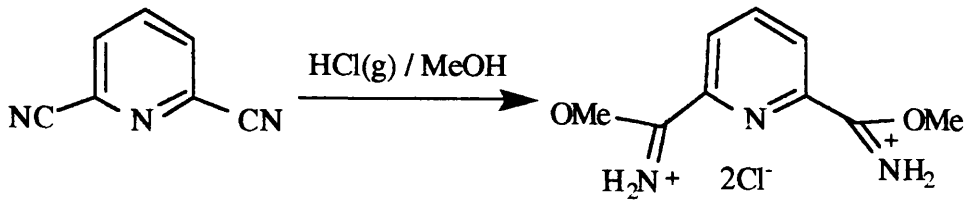


Scheme 38



Scheme 39

Using 2,6-pyridinedinitrile the Pinner synthesis was carried out. Dry HCl gas was bubbled through a solution of 2,6-pyridinedinitrile in methanol. The white solid that precipitated was found to be dimethyl 2,6-pyridinediimidate dihydrochloride (65) (Scheme 40).



Scheme 40

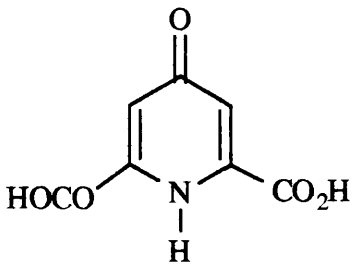
(65)

The IR spectrum showed a band at 3350 cm^{-1} due to the NH group. The C=N stretching band appeared at 1660 cm^{-1}. This is characteristic of imidates. The band at 1590 cm^{-1} was due to the pyridine ring. In the ^1H NMR spectrum a singlet at δ 4.12 and a multiplet at δ 8.38 were present in a ratio of 2 : 1. These are for the two methyl groups and the three aromatic protons respectively. The ^{13}C NMR spectrum showed a methyl signal at δ 54.18, and aromatic methines at δ 129.58 for C-3 and C-5 and at δ 140.77 for C-4. The quaternary signal at δ 147.81 was assigned to C-2 and C-6 which are equivalent and the imidate signal was present at δ 166.65.

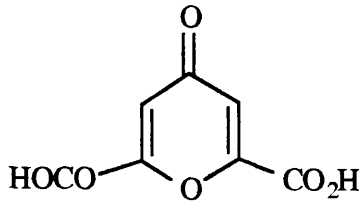
5.5 Chelidamic Acid, Chelidonic acid and Analogues

Chelidamic acid (66) and chelidonic acid (67) have some structural similarity to the intermediates of the first two enzymic steps of the diaminopimelate pathway to L-lysine, namely L-2,3-dihydrodipicolinic acid (2) and L-2,3,4,5-tetrahydrodipicolinic acid (3). For this reason, and as they are commercially available, (66) and (67) were tested as enzyme inhibitors, and they were used as starting materials for the

synthesis of analogues, which were also tested as enzyme inhibitors of DHDPA synthase.



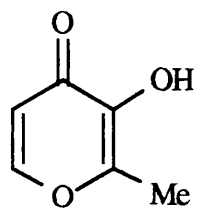
(66)



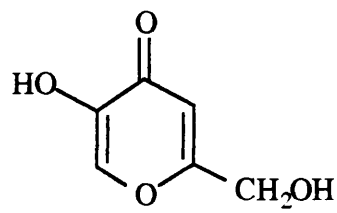
(67)

Chelidonic Acid

Chelidonic acid is an example of a γ-pyrone. Several natural accurring γ-pyrones are known, like maltol (68) from pine needles, and kojic acid (69) which is produced by moulds of the genus *Aspergillus*.



(68) maltol



(69) kojic acid

These compounds can be regarded as vinylogous lactones. Although the γ-pyrone system is not aromatic, it can be aromatised by reaction of an electrophile with the carbonyl group to give the pyrylium salt (Scheme 41).

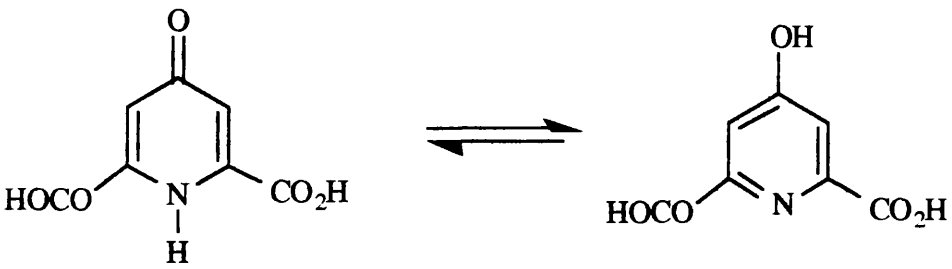
The carbonyl groups of γ-pyrones do not show typical ketonic properties, in that neither oximes nor hydrazones can be formed. However, nucleophiles attack the γ-pyrone at C-2

or C-6, because the conjugation and the electron-withdrawing carbonyl group make the carbon-carbon double bond susceptible to nucleophilic attack. On the other hand the electron-withdrawing carbonyl group deactivates the double bond towards electrophilic attack.

From X-ray analysis of a crystal of the copper salt of chelidonic acid it exists as the γ-pyrone structure (67) and is almost flat (D. Tudor, unpublished results).

Chelidamic Acid

Chelidamic acid could exist as two tautomeric species, the γ-pyridone structure (66) and the hydroxypyridine structure (70) (Scheme 42).



(66) (70)

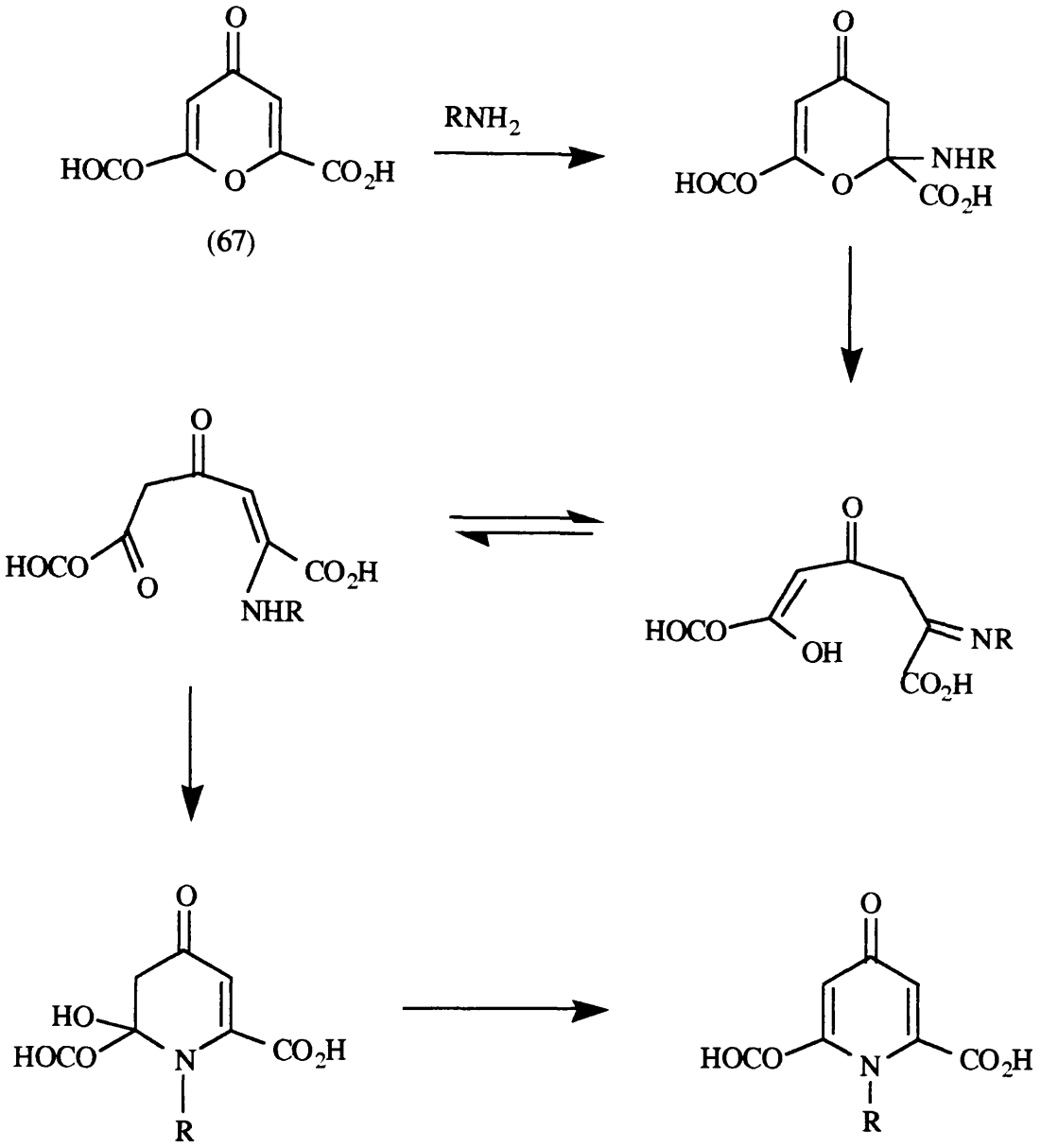
Scheme 42

Chelidamic acid is commercially available and it can be prepared from chelidonic acid and ammonia.[54] This reaction was carried out by us, by heating chelidonic acid at reflux in conc. ammonia solution for five hours. Addition of conc. hydrochloric acid gave chelidamic acid in 35% yield. The IR spectrum of chelidamic acid showed stretching bands at 3400 and 1740 cm^{-1} due to the hydroxyl and carbonyl components

of the carboxylic acid group. The band at 1580 cm^{-1} was assigned to the carbon-carbon double band and that at 1630 cm^{-1} was due to the carbonyl group at C-4. The ^{1}H NMR spectrum showed a singlet at δ 6.68 which was assigned to the two olefinic protons. In the ^{13}C NMR spectrum a methine signal was observed at δ 117.50. A quaternary carbon at δ 183.46 was due to the carbonyl group at C-4 and another at δ 167.59 was due to the two carboxylic acid groups. Another quaternary carbon at δ 144.52 was assigned to C-2 and C-6 of the pyridone ring. Chelidamic acid was found to have a chromophore at λ 274 nm. From the spectroscopic data it seems likely that chelidamic acid exists mainly as one tautomer, 4-(*1H*)-pyridone-2,6-dicarboxylic acid.

X-ray analysis of a crystal of the strontium salt of chelidamic acid, showed it to have the pyridone structure (66) (D. Tudor, unpublished results).

The reaction mechanism for the formation of chelidamic acid from chelidonic acid and ammonia is shown in Scheme 43. Michael addition of ammonia to the αβ-unsaturated carbonyl compound occurs at C-2, followed by the ring opening. Closure of the ring by attack of the nitrogen lone pair at C-6 followed by elimination of water results in the formation of chelidamic acid (66).



Scheme 43

Amines can also react in the same way to give *N*-substituted chelidamic acid analogues. The more basic and less hindered the amine the better is the yield. The reaction requires high temperatures, but above 150 °C this may cause decarboxylation.

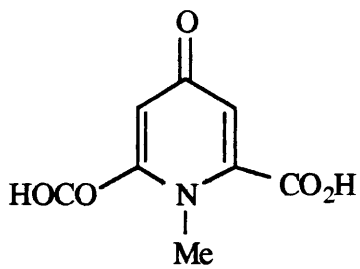
Chelidamic acid Analogues

A number of *N*-substituted chelidamic acids have been previously synthesised. Katritzky and co-workers[53] found that in the reaction of chelidonic acid and an aryl amine the product was either *N*-arylchelidamic acid, *N*-aryl-4-pyridone or *N*-aryl-4-pyridone-2-carboxylic acid, depending on the conditions. Heating the reaction mixture at reflux for long periods of time resulted in decarboxylation. Decarboxylation could be avoided by heating at reflux in aqueous hydrochloric acid instead of water. Using this method Katritzky and co-workers synthesised *N*-*p*-nitrophenylchelidamic acid.

Another *N*-substituted chelidamic acid that was previously prepared is *N*-methylchelidamic acid (71). Reinhard and Riegel[55] first made *N*-methylchelidamic acid in 1926 by treating chelidonic acid with methylamine. We carried out this reaction by heating chelidonic acid at reflux in 30% methylamine solution for five hours. The solution was cooled and conc. hydrochloric acid was added to precipitate *N*-methylchelidamic acid in 80% yield. The IR spectrum was similar to that of chelidamic acid. The ^{1}H NMR spectrum showed two singlets in a ratio of 2 : 3. The singlet at δ 6.59 was assigned to the olefinic protons at C-3 and C-5. The singlet at δ 2.65 was due to the N-methyl group. The ^{13}C NMR spectrum showed a methyl signal at δ 41.40 and a methine at δ 112.72. A quaternary signal at δ 175.02 was attributed to the carbonyl at C-4 and the signal at δ 168.06 was assigned to the carboxylic acid groups. The signal at δ 154.25 was assigned to the

quaternary carbons at C-2 and C-6. *N*-Methylchelidamic acid has a UV absorbance at λ 265 nm (ε 24,200) which is due to the dieneone-diacid system.

As chelidamic acid and *N*-methylchelidamic acid have similar spectra, it can be assumed that their structures are also similar. Therefore, *N*-methylchelidamic acid probably also has the γ-pyridone structure (71).

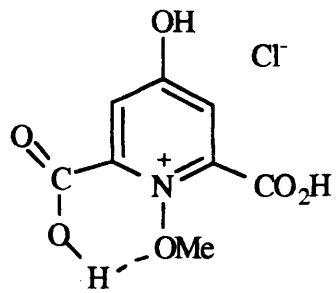


(71)

N-methoxychelidamic acid (72) has not been previously synthesised. We heated chelidonic acid at relux in 25% methoxylamine hydrochloride solution for four hours. On cooling the solution a yellow solid precipitated, without the addition of acid. *N*-Methoxychelidamic acid was obtained in 69% yield. The IR spectrum gave stretching bands at 3500 and 1725 cm^{-1} due to the hydroxyl and the carbonyl of the carboxylic acid groups. Bands were also present at 1600 and 1575 cm^{-1}, which were assigned to the aromatic ring. The ^1H NMR spectrum showed three signals at δ 3.95, 6.50 and 6.95 in a ratio of 3 : 1 : 1. The signal at δ 3.95 was a singlet and is due to the N-O-methyl group. The signals at δ 6.50 and 6.95 were multiplets with the same splitting and are from the protons at C-3 and C-5. These could be olefinic or aromatic depending on

whether the structure is the γ-pyridone (72) or the hydroxypyridinium structure (73). In the ^{13}C NMR spectrum (Figure 7) five quaternary carbons were observed at δ 167.12, 167.09, 153.42, 151.21 and 149.58. The signals at δ 167.12 and 167.09 must be from the carboxylic acid groups, however there is no signal that could correspond to the carbonyl at C-4 of an αβ-unsaturated system. One of the other quaternary carbons could be from a C-OH on the aromatic ring at C-4. There are two methine signals at δ 103.10 and 109.14 for C-3 and C-5, suggesting that the compound is not symmetrical. The ^{13}C NMR spectrum indicates that *N*-methoxychelidamic acid exists as the hydroxypyridinium structure (73) and is not a γ-pyridone (72).

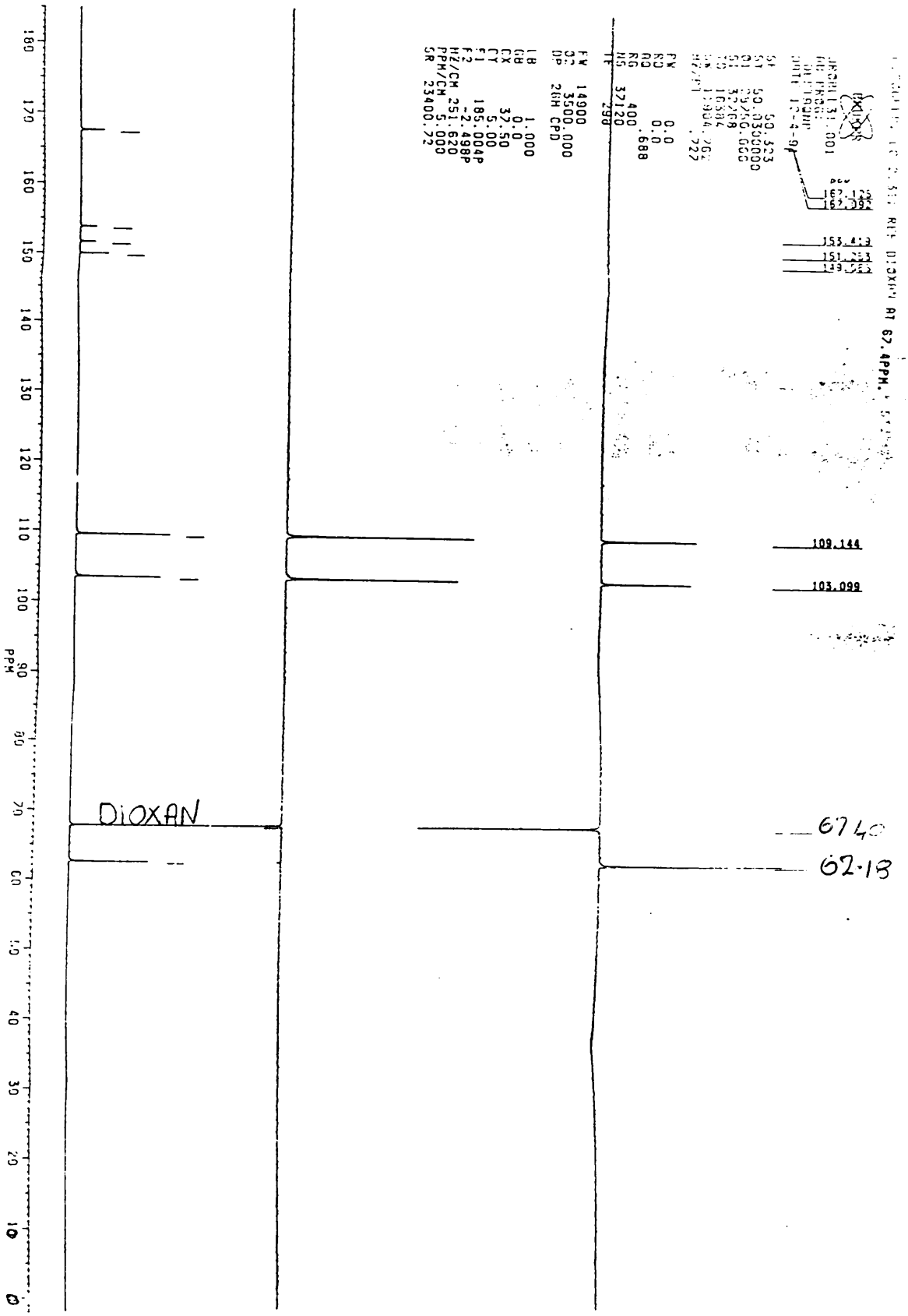
The lack of symmetry of *N*-methoxychelidamic acid could be due to hydrogen bonding between the O-Me and the acidic proton of the carboxylic acid, forming a 6-membered ring and holding the structure in a fixed conformation (74).



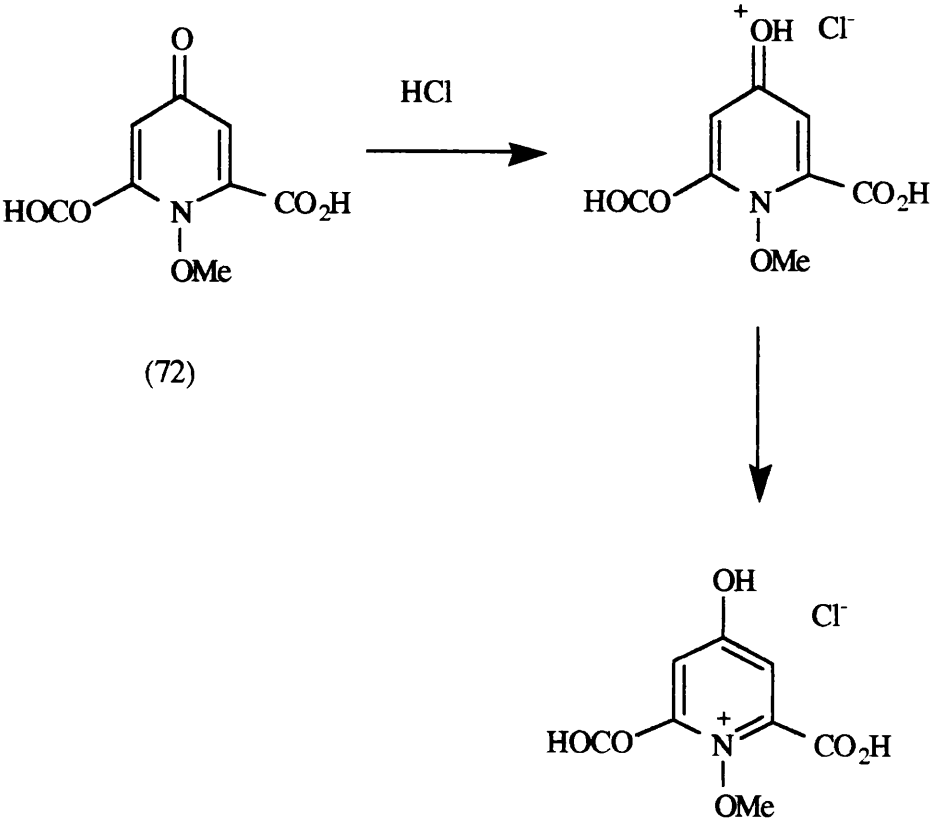
(74)

In the reaction of chelidonic acid and methoxylamine hydrochloride, the carbonyl oxygen could be protonated resulting in the formation of *N*-methoxy-4-hydroxy-2,6-pyridinedicarboxylic acid hydrochloride (73) (Scheme 44). In the UV spectrum of this compound (73) an absorbance at λ 299 (ε 16,300) was observed. This λ_{max} value is considerably higher than those of chelidamic acid and *N*-methylchelidamic acid,

Figure 7. ^{13}C NMR Spectrum of *N*-Methoxychelidamic acid (73).



suggesting that *N*-methoxychelidamic acid has a different structure from (66) and (71). This evidence also supports the formation of the hydroxypyridinium structure (73) and not the γ-pyridone (72).



(72)

(73)

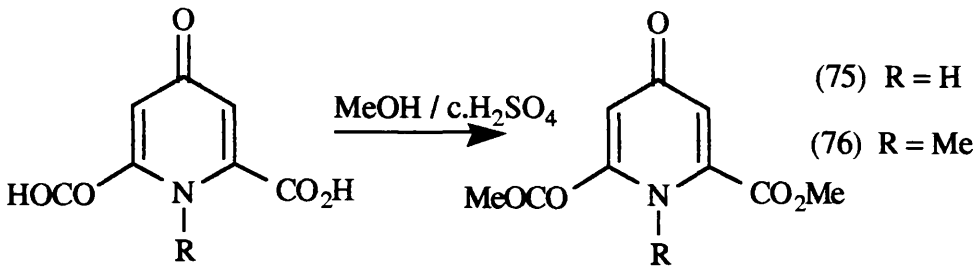
Scheme 44

Esterifications

Chelidamic acid (66) can be converted into its dimethyl ester, as previously reported, by acid catalysed esterification (Scheme 45).[54] Chelidamic acid was heated at reflux in methanol and conc. sulphuric acid to give dimethyl chelidamate (75) in 60% yield. Stretching bands at 1740 and 1200 cm^{-1} were observed in the IR spectrum due to the carbonyl and ether linkage of the ester groups. The ^{1}H and ^{13}C NMR spectra

were similar to those of chelidamic acid. The ^{1}H NMR spectrum had an additional singlet at δ 3.98 from the methyl groups of the ester, and the ^{13}C NMR spectrum had a methyl signal at δ 41.40.

Dimethyl *N*-methylchelidamate (76) was prepared from *N*-methylchelidamic acid using the same procedure as for dimethyl chelidamate, in 60% yield and gave spectroscopic data similar to those of *N*-methylchelidamic acid (Scheme 45).

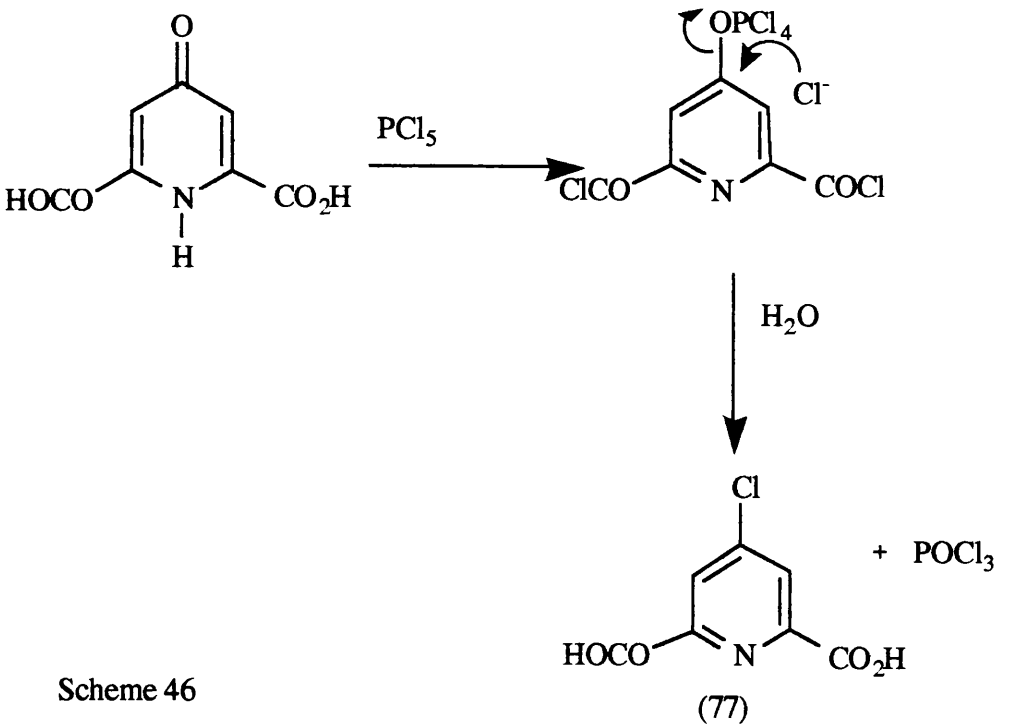


Scheme 45

5.6 Synthesis of 4-Chlorodipicolinic Acid

Although the carbonyl group in γ-pyridone derivatives does not behave like a normal keto group it is possible for nucleophiles to attack at C-4. This type of reaction probably involves the displacement of a leaving group from a pyrylium ion.

An example of this reaction is the formation of 4-chlorodipicolinic acid from chelidamic acid and phosphorus pentachloride by heating them at reflux in chloroform for three days (Scheme 46).

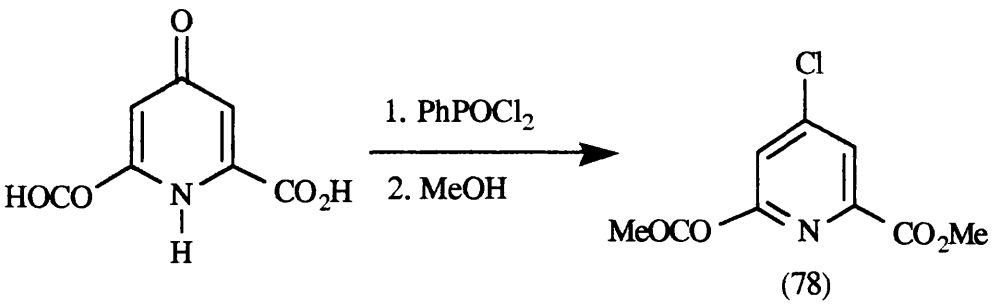


Scheme 46

(77)

The carboxylic acid groups were converted into the acid chlorides during the reaction with PCl_5. Addition of water at the end of the reaction reformed the dicarboxylic acid. 4-Chlorodipicolinic acid (77) was obtained as white needles in 59% yield. 4-Chlorodipicolinic acid was also prepared by heating chelidamic acid at reflux in phenylphosphonic dichloride for two hours. The product was obtained as white needles in 70% yield. The reaction mechanism is the same as in the reaction with PCl_5 (Scheme 46). The preferred method is therefore the latter reaction of chelidamic acid and phenylphosphonic dichloride due to the better yield and shorter time of reflux. The IR spectrum for 4-chlorodipicolinic acid showed a carbonyl stretching band at 1730 cm^{-1}. In the ^1H NMR spectrum a multiplet at δ 7.50 was observed for the aromatic ring protons. In the mass spectrum a small molecular ion appeared at *m/z* 201 together with ions at *m/z* 166 from loss of a chlorine and *m/z* 121 from the loss of the carboxylate and the chlorine.

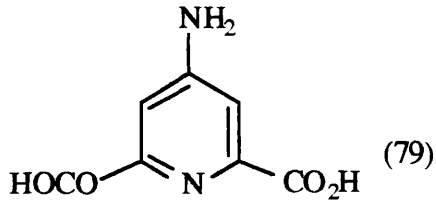
During the formation of 4-chlorodipicolinic acid the acid groups were converted into the acid chloride. Therefore, it is possible to make the dimethyl ester by additon of methanol, instead of water at the end of the reaction (Scheme 47). Chelidamic acid was heated at reflux in phenylphosphonic dichloride for two hours. Addition of methanol to the cooled solution resulted in the precipitation of a white solid. The solid was filtered off and dried to give dimethyl 4-chlorodipicolinate (78) in 88% yield. The spectra were similar to that of 4-chlorodipicolinic acid, but they contained the expected additional signals for the methyls of the ester groups.



(78)

Scheme 47

4-Aminodipicolinic acid (79) has previously been synthesised from 4-chlorodipicolinic acid and ammonia.[57] The reaction was carried out at high pressure in a bomb at 150 °C. We tried to repeat this reaction using a sealed tube instead of a bomb. 4-Chlorodipicolinic acid was treated, in a sealed tube, with liquid ammonia and stirred for seven days at room temperature. However, after the ammonia had evaporated only starting material was left.



(79)

Conclusions

Analogues of dipicolinic acid were prepared for testing as enzyme inhibitors of the dihydrodipicolinic acid synthase enzyme. Dipicolinic acid was converted into the diamide by adding the diacid chloride to ammonia. Dehydration of 2,6-pyridinedicarboxamide gave 2,6-pyridinedinitrile. The dinitrile was used to prepare the dimethyl imidate and the ditetrazole compounds for testing as inhibitors.

Using chelidonic acid, chelidamic acid and its *N*-substituted analogues were prepared. Good inhibition was observed with chelidonic acid and chelidamic acid in preliminary screening therefore their analogues were attractive targets for testing in the enzyme assay. These test results are listed in Chapter 7.

CHAPTER 6

Analogues of 2,6-Piperidinedicarboxylic Acid

6.1 Introduction

The synthesis of 2,6-piperidinedicarboxylic acid can be easily carried out by a number of methods, which were discussed in Chapter 3. These methods should allow the preparation of analogues of 2,6-piperidinedicarboxylic acid. The synthesis and testing of 2,6-piperidinedicarboxylic acid analogues as enzyme inhibitors was also carried out to investigate if enzyme inhibitors could only be unsaturated compounds like the natural enzyme substrate, L-2,3,4,5-tetrahydrodipicolinic acid (THDPA) (3) and L-2,3-dihydrodipicolinic acid (DHDPA) (2).

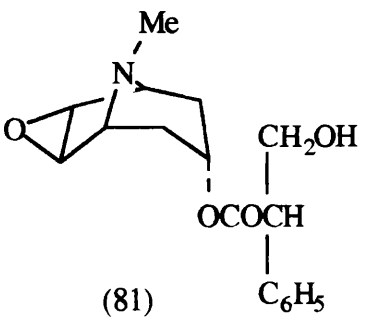
L-2,3,4,5-Tetrahydrodipicolinic acid (3), an intermediate on the diaminopimelate pathway to L-lysine, was synthesised as a dipotassium salt by the elimination of toluenesulphinic acid from dimethyl *N*-tosyl-2,6-piperidinedicarboxylate. The synthesis of analogues of (3) is feasible by elimination of toluenesulphinic acid from the *N*-tosyl derivatives of 2,6-piperidinedicarboxylic acid analogues with an acidic α-proton. Analogues of 2,3,4,5-tetrahydrodipicolinic acid might be substrates for the DHDPA synthase or reductase enzymes or they might inhibit the enzyme. Enzyme inhibitors may have herbicidal or antibacterial activity without mammalian toxicity.

In this Chapter the synthesis and attempted synthesis of 2,6-piperidinedicarboxylic acid analogues will be discussed.

This includes the attempted synthesis of 4-substituted 2,6-piperidinedicarboxylic acids.

6.2 *N*-Substituted Analogues

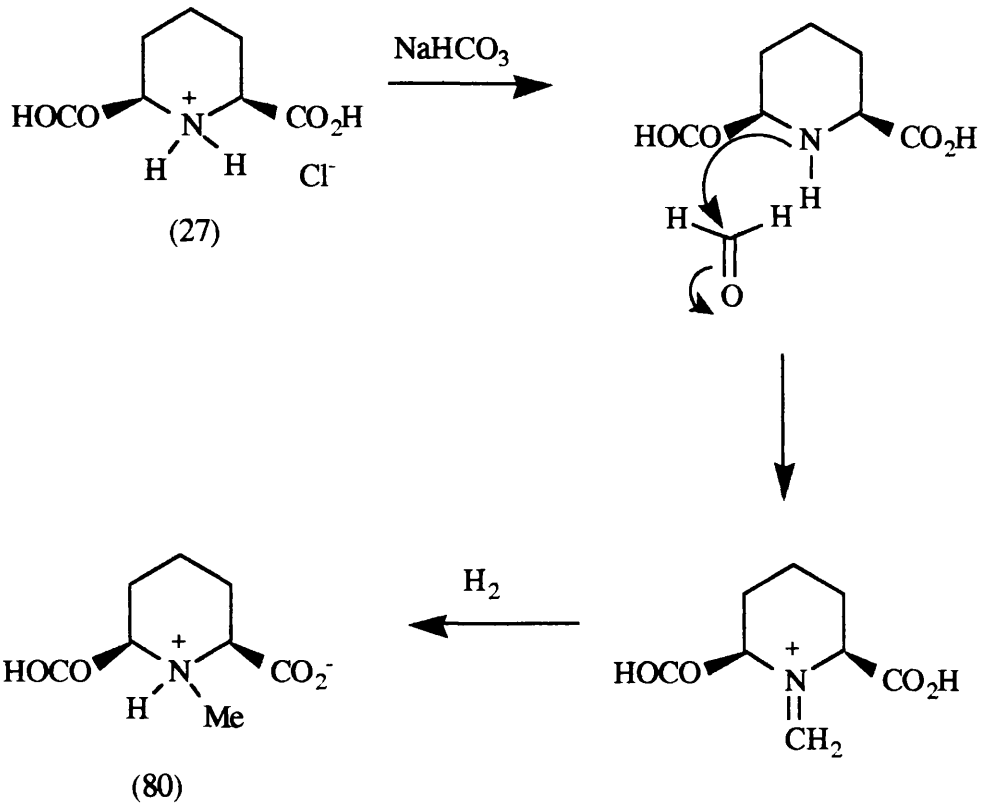
N-Methyl-2,6-piperidinedicarboxylic acid (80) has been prepared by a number of methods. One method used was to heat the tetraethyl ester of dibromopentane tetracarboxylic acid with methylamine in a sealed tube, followed by treatment of the product with barium hydroxide.[58] Hess and Suchier[59] prepared *N*-methyl-*cis*-2,6-piperidinedicarboxylic acid by the careful oxidation of scopolamine (81). They believed the product was the *cis*-isomer as its spectra were very similar to the naturally occurring *N*-methyl-*cis*-2,6-piperidinedicarboxylic acid (scopolinic acid).



(81)

A more convient method is the one used by Anderson and Soine.[22] *cis*-2,6-Piperidinedicarboxylic acid was prepared by the catalytic hydrogenation of dipicolinic acid (26). The product was obtained as the hydrochloride salt (27) in good yield. *N*-Methylation was carried out by heating at reflux a mixture of *cis*-2,6-piperidinedicarboxylic acid, formic acid, formaldehyde and sodium bicarbonate. The first step in the

reaction is the conversion of the hydrochloride salt into the free base using sodium bicarbonate. The nitrogen lone pair can then attack the formaldehyde and this is followed by dehydration to give the immonium ion. Formic acid acts as a hydrogen donor and reduces the immonium group to give the required *N*-methyl derivative (Scheme 48).



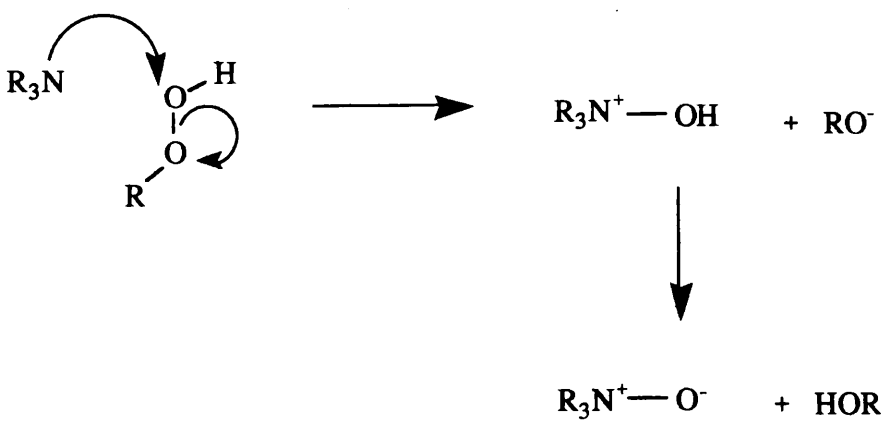
Scheme 48

cis-2,6-Piperidinedicarboxylic acid was prepared by the hydrogenation of dipicolinic acid (26) as described in Section 3.2. *N*-Methylation was carried out using the method of Anderson and Soine.[22] The sodium chloride formed during the reaction was filtered off and the filtrate was concentrated and the residue was crystallised from methanol to give *N*-methyl-

cis-2,6-piperidinedicarboxylic acid (80). In the IR spectrum bands were present at 3450 and 1740 cm^{-1} due to the hydroxyl and carbonyl components of the carboxylic acid groups. The ^{1}H NMR spectrum was similar to that of *cis*-2,6-piperidinedicarboxylic acid (27) but with the expected *N*-methyl signal at δ 3.10. The ^{13}C NMR spectrum was similar to that of the diacid (27) with an additional methyl signal at δ 42.95. In the mass spectrum the molecular ion at *m/z* 187 was observed and an ion at *m/z* 84 due to the loss of the methyl and acid groups was present.

N-Oxidation

Amines and other basic nitrogen compounds react readily with with hydrogen peroxide or peracids to give the *N*-oxide. The mechanism is shown in Scheme 49. The reaction depends on the nucleophilicity of the amine and the electrophilicity of the peroxide. Pyridine and other less nucleophilic nitrogen heterocycles require a peracid such as trifluroperacetic acid.



Scheme 49

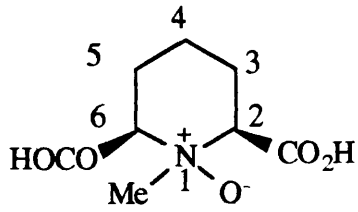
Organic peracids can be made from the carboxylic acid, like acetic acid or trifluroacetic acid, and 30% hydrogen peroxide solution (Scheme 50). Strong acids like trifluroacetic acid and formic acid rapidly develop the equilibrium concentrations of the peracid. Weaker acids may require a catalytic quantity of mineral acid. In the case of trifluroacetic acid, the peracid can be prepared *in situ*, so the peracid does not need to be previously prepared. As the peracid is used the equilibrium is displaced to the right (Scheme 50) and more peracid is formed.

Scheme 50

N-Methyl-*cis*-2,6-piperidinedicarboxylic acid (80) was oxidised by heating it at reflux in trifluroacetic acid with 30% hydrogen peroxide. Trifluroacetic acid had to be used due to the poor solubility of *N*-methyl-*cis*-2,6-piperidinedicarboxylic acid in most solvents. *N*-Methyl-*cis*-2,6-piperidinedicarboxylic acid *N*-oxide (82) was precipitated by addition of diethyl ether to the cooled reaction mixture to afford the product in 57% yield. Trifluroperoxyacetic acid probably acted as the oxidising agent during the reaction.

In the IR spectrum of (82) there were bands present at 3400 and 1750 cm^{-1} from the hydroxyl and the carbonyl components of the carboxylic acid groups. The band at 970 cm^{-1} was assigned to the N-oxide bond. The ^1H NMR spectrum showed a broad multiplet at δ 1.20 - 1.60 due to the

methylenes at C-3, C-4, and C-5. The *N*-methyl group appeared as a singlet at δ 2.00. The acidic C-2 and C-6 protons gave a multiplet at δ 2.50. The ^{13}C NMR spectrum showed that there were signals due to methylenes in two different enviroments in a ratio of 2 : 1. The C-3 and C-5 methylenes are equivalent and gave a signal at δ 28.41. The C-4 methylene appeared at δ 22.01. The methine signal for C-2 and C-6 appeared at δ 77.33 and the carboxylate carbon gave a quaternary signal at δ 183.66. In the mass spectrum there was a molecular ion *m/z* 203. The largest ion at *m/z* 142 was due to the loss of an oxygen and a carboxylic acid group. As there was only one set of carbon signals in the ^{13}C NMR spectrum this indicated that although two diastereoisomers could be formed, the reaction yielded only one isomer.



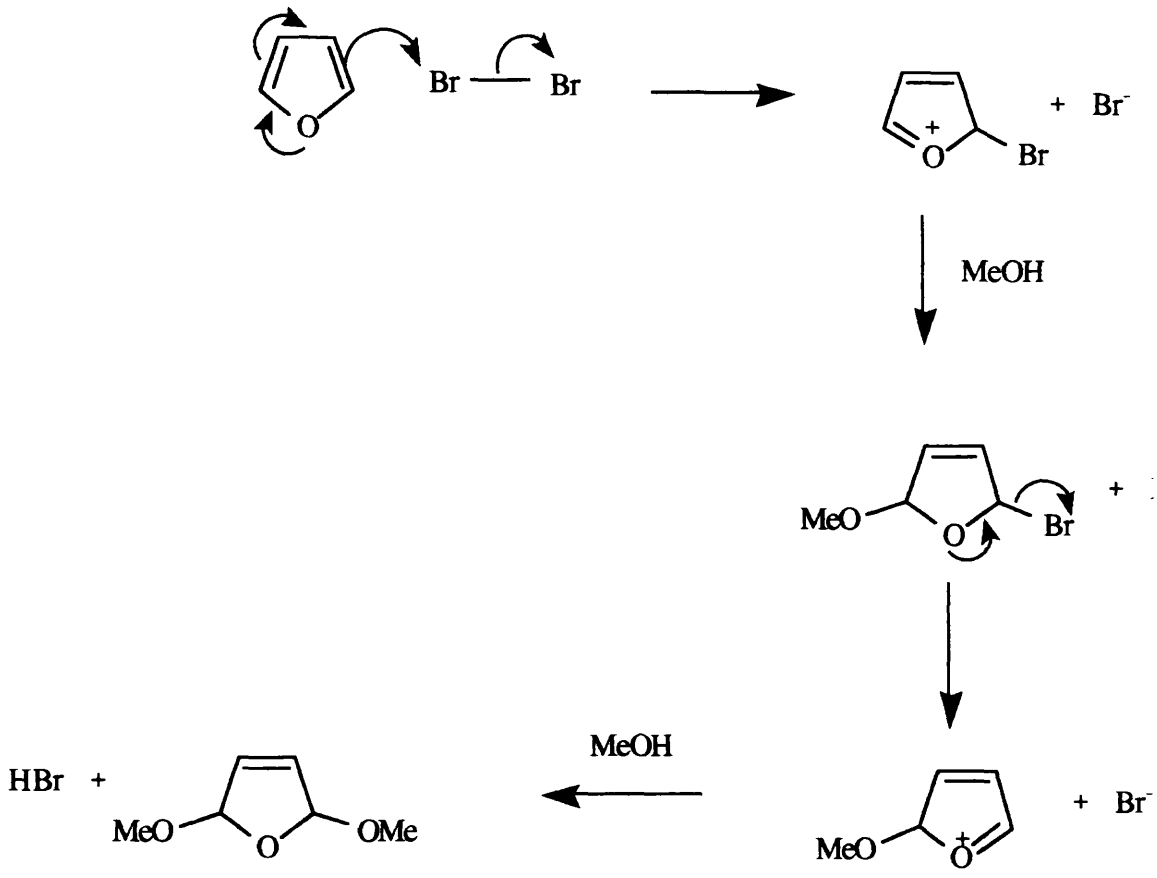
(82)

6.3 Synthesis of Piperidin-4-one-2,6-dicarboxylic Acid

Midorikawa[60] reported the synthesis of 1,4-pentadien-3-one-1,5-dicarboxylic acid (86) from 2-furanacrylic acid using hydrogen peroxide and conc. hydrochloric acid.

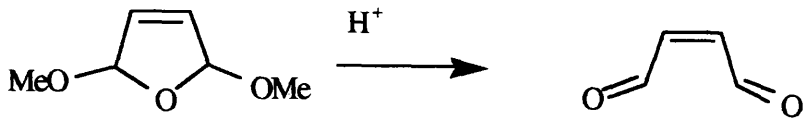
Although furan is aromatic it is less aromatic than benzene and can react with electrophiles to give addition products rather than substitution. If bromination is carried out

in methanol an acetal is formed. This is the result of an initial addition process (Scheme 51).

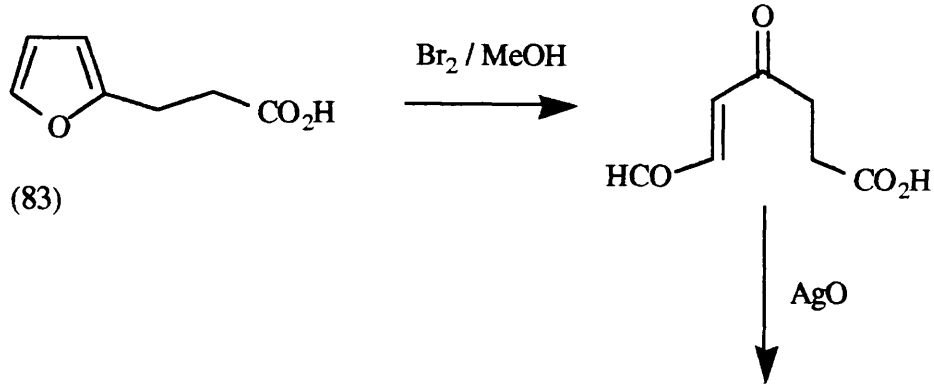


Scheme 51

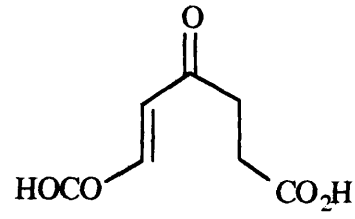
The acetal can ring open on addition of acid to give the enedione (Scheme 52). An example of this type of reaction is the conversion of 2-furanpropionic acid (83) into furanoic acid (84).[61] Addition of bromine results in ring opening to give the aldehyde. Subsequent oxidation with silver oxide yields furanoic acid (Scheme 53).



Scheme 52



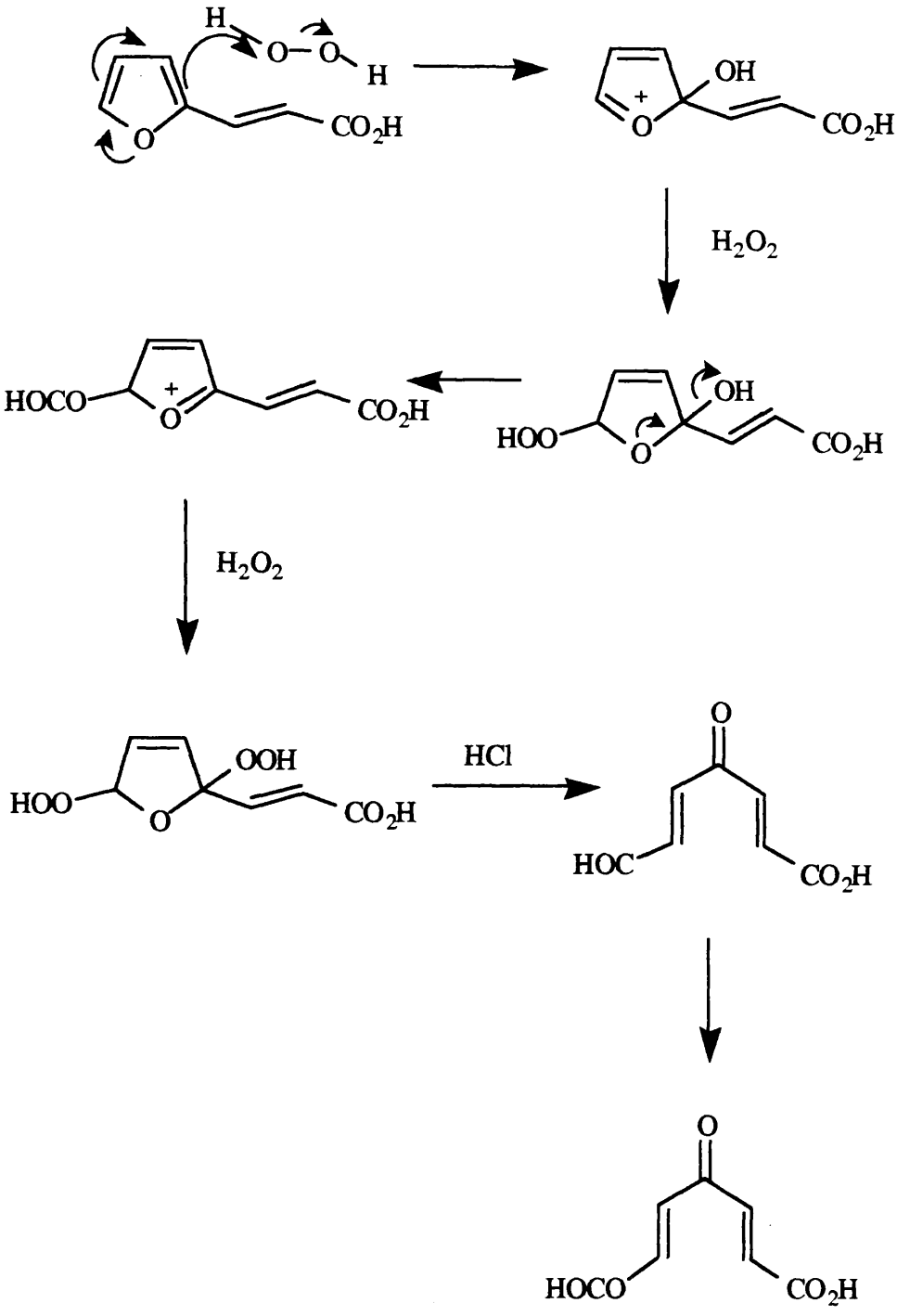
(83)



(84)

Scheme 53

The reaction of 2-furanacrylic acid (85) with hydrogen peroxide and conc. hydrochloric acid to yield 1,4-pentadien-3-one-1,5-dicarboxylic acid (86) is analogous to the reaction of 2-furanpropionic acid and bromine. Furans are unstable to oxidation so that the furan ring is oxidised before the side chain. Oxidation by hydrogen peroxide occurs at C-2 and C-5 of the furan ring. The mechanism is similar to the reaction of furan with bromine and methanol and is shown in Scheme 54.



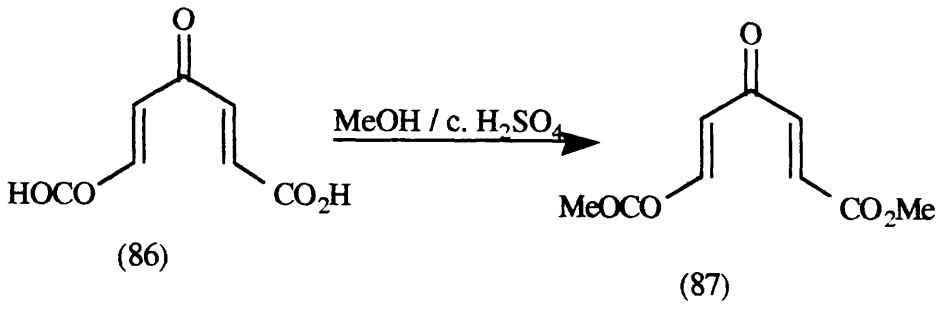
(86)

Scheme 54

We carried out the procedure of Midorikawa[60] by stirring a suspension of 2-furanacrylic acid in hydrogen peroxide and conc. hydrochloric acid for five days at room temperature. The orange crystals that formed were filtered off and washed with methanol, to remove unchanged 2-furanacrylic acid. 1,4-

Pentadien-3-one-1,5-dicarboxylic acid (86) was obtained in 40% yield. The IR spectrum gave carbonyl bands at 1700 and 1670 cm^{-1} for the carboxylic acid and ketone groups respectively. The band at 1640 cm^{-1} was assigned to the carbon-carbon double bond. 1,4-Pentadien-3-one-1,5-dicarboxylic acid was insoluble in every NMR solvent tried therefore no ^{1}H and ^{13}C NMR spectra could be obtained. In the mass spectrum a parent ion was present at *m/z* 170 and ions due to the loss of the carboxylic acid groups were observed.

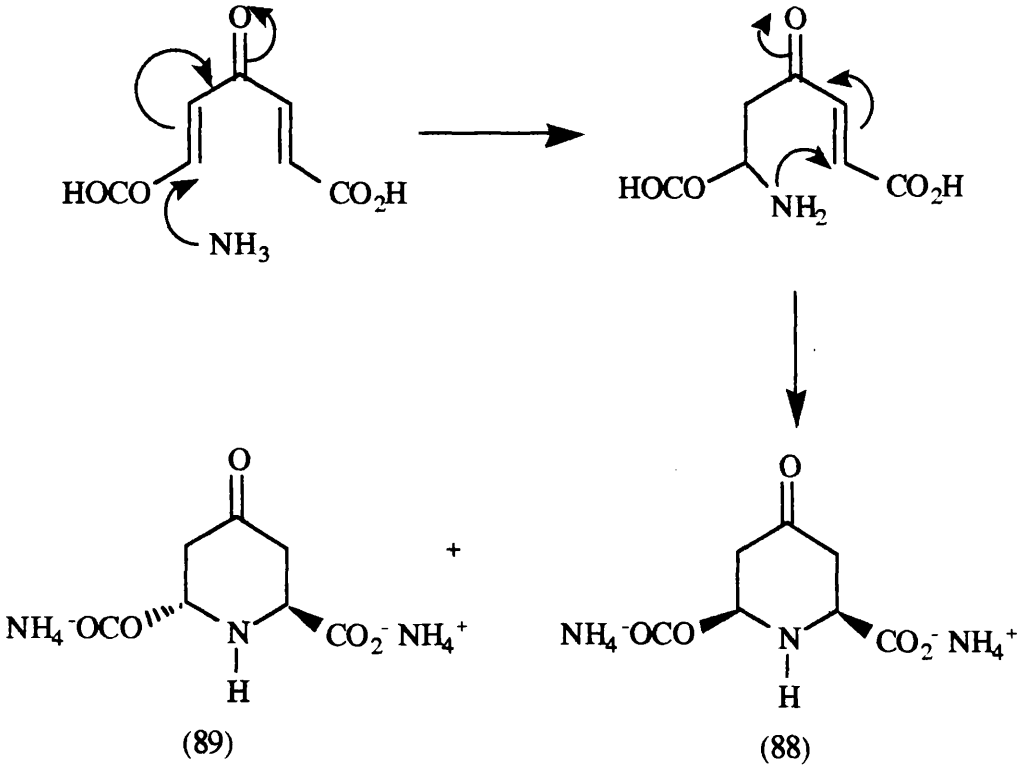
1,4-Pentadien-3-one-1,5-dicarboxylic acid (86) was converted into the dimethyl ester by heating it at reflux in methanol and conc. sulphuric acid for thirty minutes (Scheme 55). Dimethyl 1,4-pentadien-3-one-1,5-dicarboxylate (87) was obtained in 96% yield. In the IR spectrum carbonyl stretching bands were present at 1720 and 1670 cm^{-1} from the ester and ketone groups. The band at 1640 cm^{-1} was due to the carbon-carbon double bond. The ^{1}H NMR spectrum gave a singlet at δ 3.69 from the methyls of the ester groups. The AB system at δ 6.60 and 7.15 was due to the olefinic protons. The coupling constant of *J* 15 Hz indicates that the double bonds are *trans*-. The ^{13}C NMR spectrum showed a methyl group at δ 51.88 from the ester groups, and methine signals at δ 132.02 and 137.03 due to the carbon-carbon double bonds. The quaternary signals at δ 170.54 and 195.51 were assigned to the ester carbonyls and the C-4 carbonyl respectively. The mass spectrum contained the expected parent ion at *m/z* 198 (100%).



(86) (87)

Scheme 55

1,4-Pentadien-3-one-1,5-dicarboxylic acid (86) contains an α,β-unsaturated carbonyl system which can undergo Michael additions with nucleophiles such as ammonia.



(89) (88)

Scheme 56

Ammonia adds to the double bond at C-2. This is then followed by intramolecular cyclisation by addition of the amine to C-6 to give piperidin-4-one-2,6-dicarboxylic acid (Scheme 56).

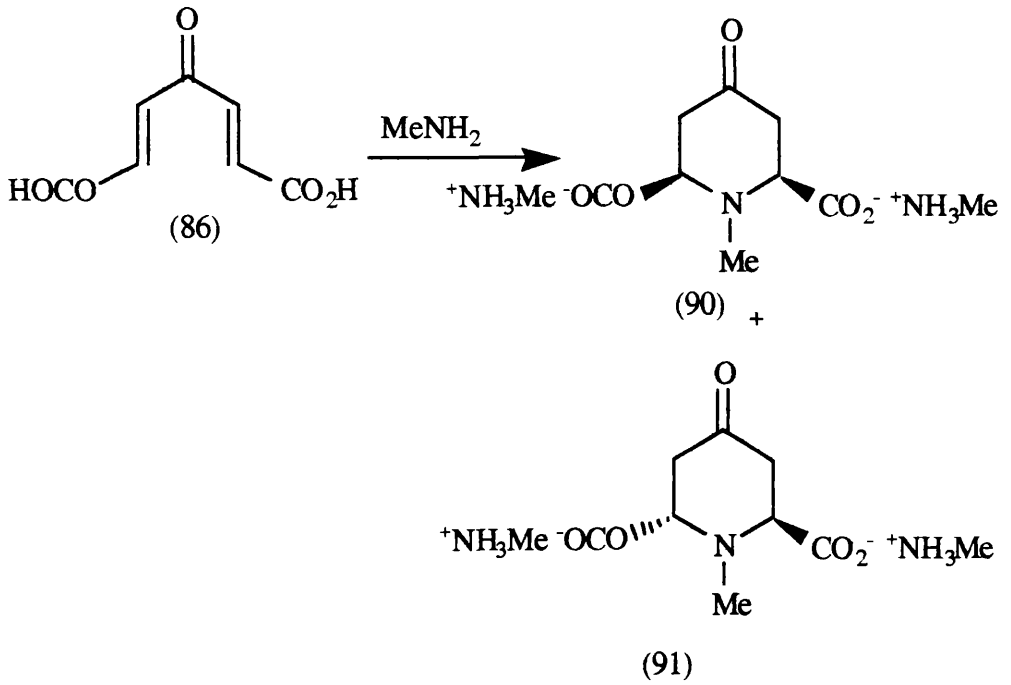
This reaction was carried out by Hermann and Dreiding[62] to give a 3 : 2 mixture of the diammonium salts of *cis*- (88) and *trans*-piperidin-4-one-2,6-dicarboxylic acid (89). We repeated this reaction by heating at reflux 1,4-pentadien-3-one-1,5-dicarboxylic acid (86) in conc. ammonia solution for two hours. Concentration of the solution under reduced pressure gave diammonium *cis*- (88) and *trans*-piperidin-4-one-2,6-dicarboxylic acid (89) as a yellow solid in 91% yield. A suitable solvent could not be found for TLC. In all solvent systems tried the R_F value was zero. Attempted crystallisation from water or aqueous methanol gave no solid material. Therefore it was not possible to purify the mixture or separate the *cis*- and *trans*-isomers (88) and (89).

In the IR spectrum of the mixture bands were present at 1690 cm^{-1} and 1590 cm^{-1}, for the carbonyls at C-4 and the carboxylate anions respectively. The ^1H NMR spectrum showed a broad multiplet at δ 2.28 - 2.32 due to the C-3 and C-5 protons of both *cis*- and *trans*-isomers. The multiplet at δ 4.20 was assigned to the C-2 and C-6 protons of the major isomer, and the multiplet at δ 4.00 was due to the C-2 and C-6 protons of the minor isomer. These signals were in a ratio of 2 : 1, indicating that this is the ratio of the *cis*- and *trans*-isomers. The ^{13}C NMR spectrum of the major isomer gave a methylene signal at δ 37.43 and a methine signal at δ 55.71. The quaternary carbons at δ 171.34 and 176.85 were assigned to the carboxylate and C-4 carbonyls. The minor isomer showed a methylene signal at δ 31.70 due to the C-3 and C-5 methylene groups. The methine group appeared at δ 52.94. The carboxylate and C-4 carbonyl were present as a quaternary

signal at δ 170.63 and 176.85 respectively. The mass spectrum of the mixture of isomers (88) and (89) gave a molecular ion at *m/z* 187 and additional ions at *m/z* 143 and *m/z* 99 from the loss of the carboxyl groups. Although it is not known for certain which isomer is the major isomer it will probably be the *cis*-isomer as both carboxyls are likely to be equatorial. The *trans*-isomer has one axial and one equatorial carboxyl group. This arrangement is less favorable due to the destabilising 1,3-diaxial interactions suffered by the axial carboxyl group.

Cyclisation of 1,4-pentadien-3-one-1,5-dicarboxylic acid (86) was next carried out using 30% methylamine solution (Scheme 57). 1,4-Pentadien-3-one-1,5-dicarboxylic acid was heated at reflux in 30% methylamine solution for four hours. Concentration of the reaction mixture gave the diammonium salt of *N*-methylpiperidine-4-one-2,6-dicarboxylic acid as a mixture of *cis*- (90) and trans-isomers (91) in a 94% yield. In the IR spectrum bands were present at 1620 and 1590 cm^{-1} due to the C-4 carbonyl and the carboxylate anions. The ^{1}H NMR spectra of the two isomers were identical. The methylene groups at C-3 and C-5 appeared as a multiplet at δ 2.25 - 2.86. The singlet at δ 2.90 was due to the *N*-methyl group. The acidic protons at C-2 and C-6 appeared as a multiplet at δ 3.85. However the ^{13}C NMR spectrum of the *cis*- and *trans*-isomers were not the same. There was only one methylene signal for C-3 and C-5 of both isomers at δ 31.97 and one methyl signal at δ 39.86. The methine group of the major isomer was present at δ 65.29. The signal at δ 67.50 was due to the methine of the minor isomer. The two methine signals were in a ratio of 2 : 1,

indicating that this is the ratio of the *cis*- and *trans*-isomers. There were three quaternary signals present in the ^{13}C NMR spectrum. The signal at δ 172.23 was assigned to the carboxylate of the major isomer and the signal at δ 172.90 to the minor isomer. The quaternary signal for C-4 was the same for both isomers and appeared at δ 178.34. In the mass spectrum the expected molecular ion was observed at *m/z* 202. Ions due to the loss of the methyl and the carboxyl groups were also present. Attempts to separate the two isomers by crystallisation in water or methanol and water gave no solid material. Chromatography could not be employed to separate the isomers as a suitable solvent system could not be found. The R_F value in all solvent systems tried was zero.

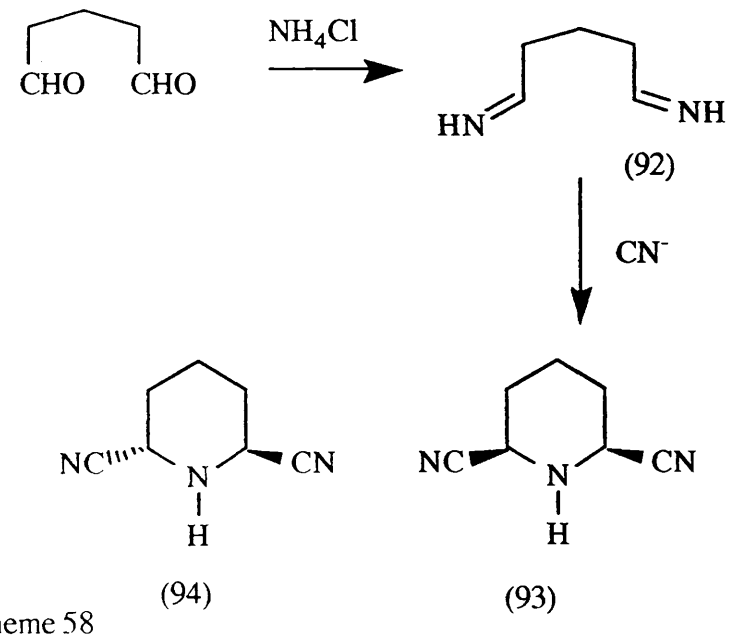


Scheme 57

6.4 Synthesis of 2,6-Piperidinedinitrile

The synthesis of 2,6-piperidinedinitrile was discussed by R.A. Henry.[63] The reaction was carried out using glutaraldehyde, sodium cyanide and ammonium chloride to give a 30% yield of the required product. The product was obtained as a mixture of isomers but these were not separated.

The reaction mechanism (Scheme 58) may proceed via the di-imine (92) followed by cyclisation and addition of the cyanide ion to the resulting cyclic imine (two times) to afford a mixture of *cis*- (93) and *trans*-2,6-piperidinedinitrile (94).



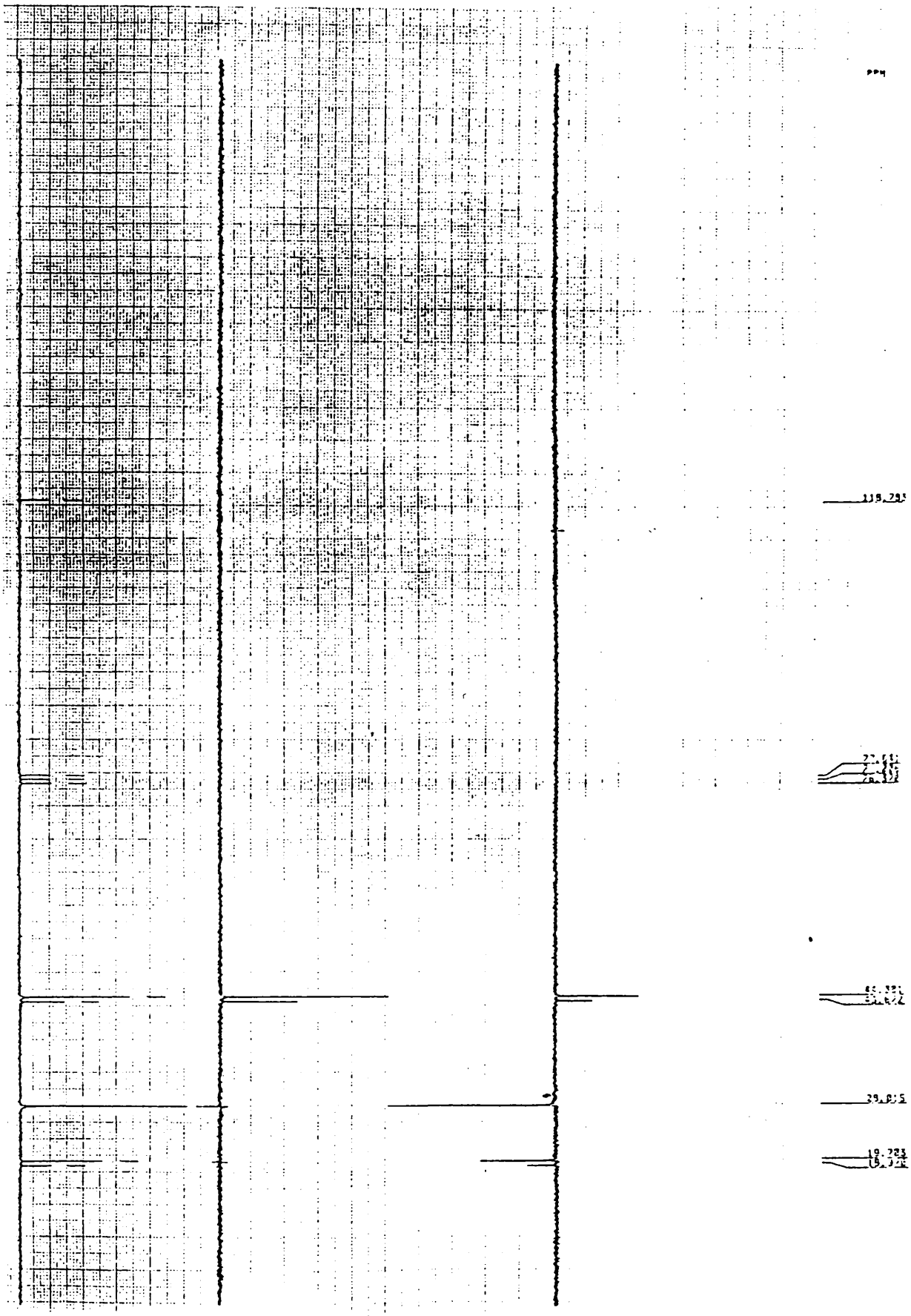
Scheme 58

We carried out this synthesis by stirring a solution of glutaraldehyde sodium bisulphite addition compound in water with sodium cyanide and ammonium chloride at room

temperature for ten days. The organic extracts afforded 2,6-piperidinedinitrile as a mixture of *cis*- and *trans*-isomers. The ^{13}C NMR spectrum of the mixture is shown in Figure 8. Trituration of the solid product with diethyl ether removed the minor isomer. The major isomer did not dissolve in diethyl ether. The different isomers gave different melting points. The total reaction yield was 53% with 30% of one isomer and 23% of the other.

The IR spectra of the separated isomers were identical and contained a stretching band due to the nitrile group at 2230 cm^{-1}. The N-H bond gave a stretching band at 3310 cm^{-1}. The ^{1}H NMR spectra of the separated isomers were the same. The multiplets present at δ 2.79 - 1.84 and at 2.45 were due to the three methylene group protons at C-3, C-4 and C-5. The multiplet at δ 4.02 was assigned to the protons α to the nitrile groups at C-2 and C-6. However, as seen in Figure 8, the ^{13}C NMR spectra of the separated isomers were not the same. The major isomer showed two methylene signals in a ratio of 2 : 1. The methylene signal at δ 27.94 was attributed to the equivalent carbons at C-3 and C-5. The signal present at δ 19.34 was due to C-4. The methine signal at δ 45.53 was due to C-2 and C-6. The quaternary signal at δ 118.87 was assigned to the nitrile carbons. It would be expected that the major isomer was *cis*-2,6-piperidinedinitrile as both nitrile groups will probably be equatorial, which is a more favorable arrangement than the *trans*-isomer which would have one of its nitrile groups axial. In the mass spectrum the expected molecular ion was observed at *m/z* 135.

Figure 8. ^{13}C NMR Spectrum of 2,6-Piperidinedinitrile as a Mixture of *cis*- and *trans*-Isomers.



The ^{13}C NMR spectrum of the minor isomer showed two different methylenes at δ 19.89 due to C-4 and at 27.94 due to C-3 and C-5 in a 1 : 2 ratio. One methine signal for C-2 and C-6 was present at δ 43.32. The quaternary signal present at 118.95 was due to the nitrile carbon. In the mass spectrum a molecular ion at *m/z* 135 was present.

The separated isomers were hydrolysed to *cis*- and *trans*-2,6-piperidinedicarboxylic acid, by heating at reflux in 10% barium hydroxide solution. The major isomer afforded *cis*-2,6-piperidinedicarboxylic acid when hydrolysed and the minor isomer gave the *trans*-diacid on treatment with barium hydroxide. The synthesis of amino acids from the aldehyde, sodium cyanide and ammonium chloride followed by hydrolysis of the nitrile group is known as the Strecker synthesis. The *cis*- and *trans*-2,6-piperidinedicarboxylic acid isomers were identified by mixed melting points with authentic material and from their ^{13}C NMR spectra. The major isomer therefore must be *cis*-2,6-piperidinedinitrile. This would be expected as both nitrile groups can be equatorial in the *cis*-isomer. The *trans*-isomer will have one equatorial and one axial nitrile group. The axial nitrile will be destabilised by 1,3-diaxial interactions.

6.5 Attempted Synthesis of Substituted Glutaraldehydes

Attempts were made to synthesise substituted glutaraldehydes with the aim of cyclising them using the Strecker synthesis, described in the last Section, to give 4-

substituted-2,6-piperidinedicarboxylic acids. Cyclisation of the dialdehydes with sodium cyanide and ammonium chloride should give the 4-substituted-2,6-piperidinedinitriles as a mixture of *cis*- and *trans*-isomers which might be separated and the nitriles could be hydrolysed to the corresponding diacids.

The methods used in attempts to synthesise substituted glutaraldehydes will be described in this Section. These methods include the oxidation of a primary diol, reduction of a diester and the oxidative ring opening of a 1,2-diol.

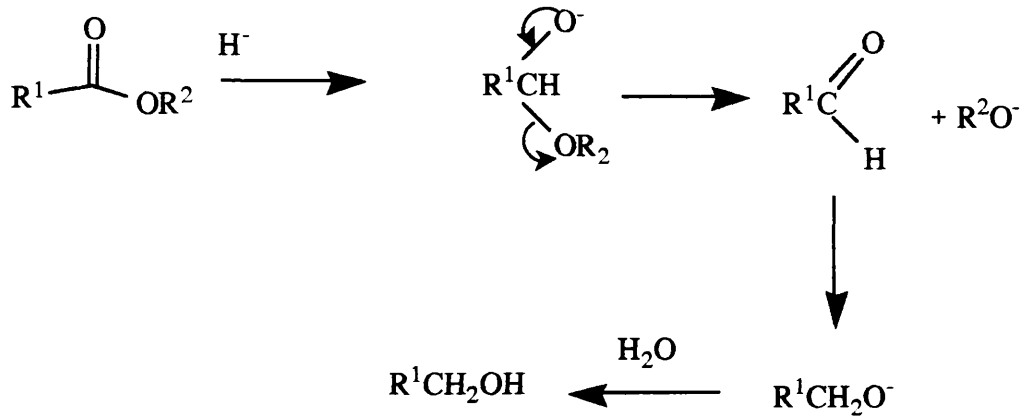
Reduction of Diesters

There are a number of commercially available substituted glutaric acids. These include 3,3-dimethylglutaric acid. This was converted into dimethyl 3,3-dimethylglutarate by heating at reflux in thionyl chloride, followed by the addition of the diacid chloride to methanol to give the ester in a 75% yield. In the IR spectrum bands were present at 1705 and 1150 cm^{-1} due to the carbonyl and the C-O linkage of the ester group. The band at 1385 cm^{-1} was assigned to $C(CH_3)_2$ stetching. The ^1H NMR spectrum contained three singlets. The singlet at δ 0.90 was due to the methyl groups and the one at δ 2.45 was due to the C-2 and C-4 protons. The methyls of the ester appeared as a singlet at δ 3.70.

By the use of aluminium hydrides and complex hydrides esters can be reduced to aldehydes. These reactions are ussually carried out at sub-zero temperatures. Lithium aluminium hydride is often too powerful and can reduce esters

to alcohols. Diisobutylaluminium hydride (DIBAL) is less reactive and is often used to make aldehydes from methyl or ethyl esters.[64]

The first stage in the reaction involves the addition of hydride to the carbonyl bond to generate the hemiacetal. The hemiacetal can eliminate one molecule of alcohol to afford the alkoxide and the aldehyde. It is possible for the aldehyde to be further reduced to give the alcohol (Scheme 59).



Scheme 59

We attempted to carry out the DIBAL reduction of dimethyl 3,3-dimethylglutarate to give 3,3-dimethylglutaraldehyde. Dimethyl 3,3-dimethylglutarate was treated with DIBAL in dichloromethane at -78 ^{o}C. The solids were filtered off at the end of the reaction and the filtrate was concentrated to an oil. Examination of the oil by ^{1}H and ^{13}C NMR spectroscopy showed that mainly starting ester was present. However some 3,3-dimethylpentane-1,5-diol was also formed. Although the ester was reduced to the aldehyde, it

was immediately reduced further by DIBAL to give the diol. The aldehyde is probably more reactive than the ester towards DIBAL, and is therefore reduced further by the DIBAL. Use of limited amounts of DIBAL might lead to the formation of the dialdehyde.

Oxidation of Diols

Another method for preparing aldehydes is by oxidation of an alcohol. This method has the problem of overoxidation.

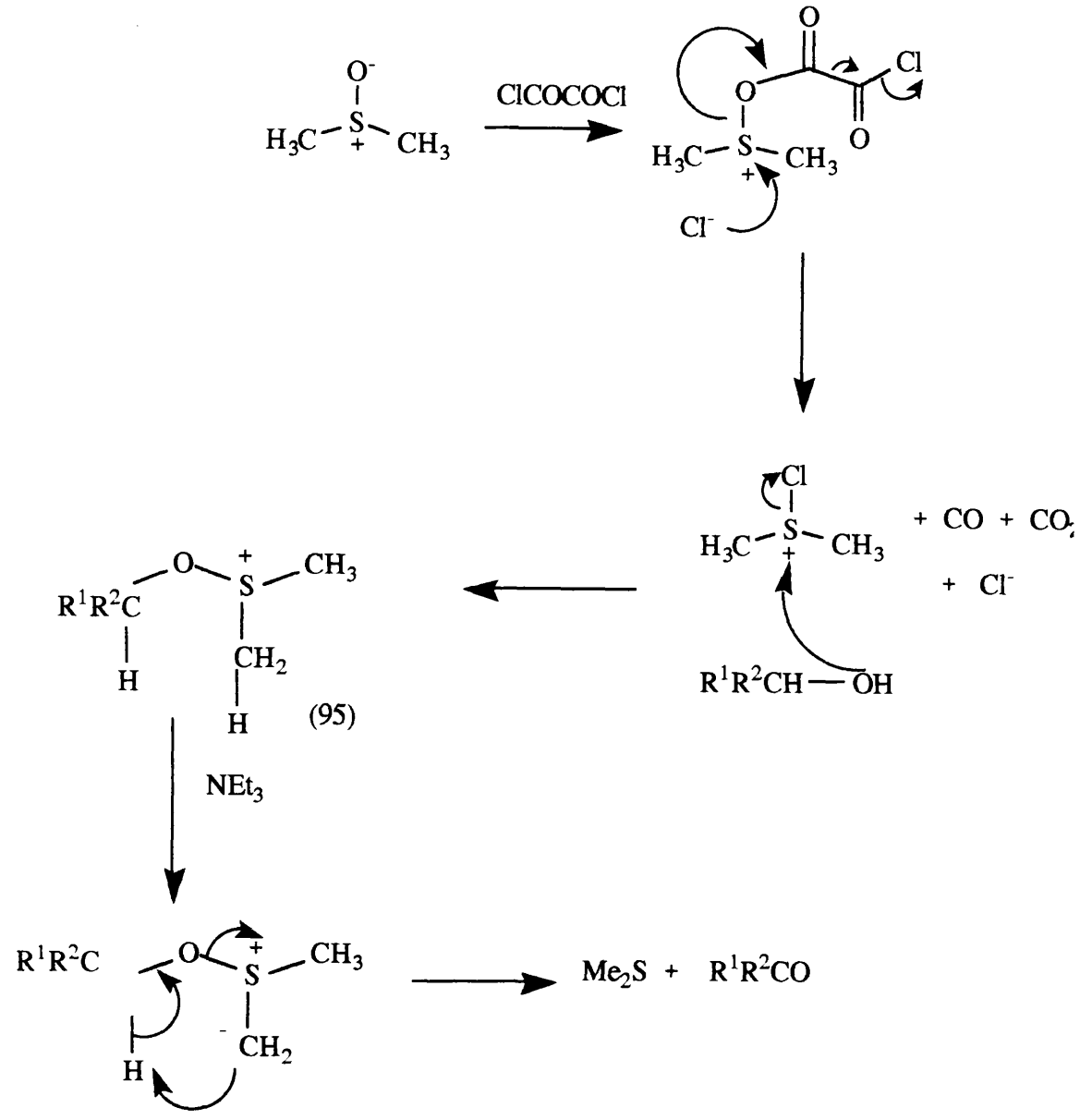
Ley and co-workers[65] recently developed a reagent for the oxidation of alcohols to aldehydes or ketones. This reagent, tetrapropylammonium perruthenate (TPAP), is a mild catalytic oxidant and can be used along with 4-methylmorpholine *N*-oxide (NMO) to oxidise primary alcohols to aldehydes and secondary alcohols to ketones. The advantages in using TPAP are it can be used with acid sensitive groups, and it does not cause racemisation of optically active compounds.

We carried out this reaction with 3,3-dimethylpentane-1,5-diol, which was prepared by the diborane reduction of 3,3-dimethylglutaric acid (discussed later). A mixture of 3,3-pentane-1,5-diol, NMO and 5 mol % of TPAP in dichloromethane with 4 Å molecular sieves was stirred at room temperature for twenty-four hours. The inorganic material was removed by several aqueous washings. However evaporation of the organic solvent yielded starting material only. Further work was not carried out on this reaction due to the expense of TPAP.

Swern Oxidation

There a number of mild oxidants based on dimethyl sulphoxide which have been used for the conversion of alcohols into carbonyl compounds. One of the most effective methods is the Swern oxidation, which uses dimethyl sulphoxide and oxalyl chloride.[66] The Swern oxidation often gives quantitative yields of carbonyl compounds, irrespective of steric factors. The first stage in the reaction is the activation of dimethyl sulphoxide with oxalyl chloride. This step is exothermic and is carried out at low temperatures. Chlorodimethylsulphonium chloride is formed in this step. This can react with the alcohol to give the sulphonium ion (95). The last step involves the removal of a proton by a base like triethylamine to give the carbonyl compound (Scheme 60). The Swern oxidation has been used for both primary and secondary alcohols. The oxidation of diols to dicarbonyl compounds has also been achieved using this method. The oxidation of a primary-primary diol to the corresponding dialdehyde formed the final step in the synthesis of the potent antifeedant polygodial.[67]

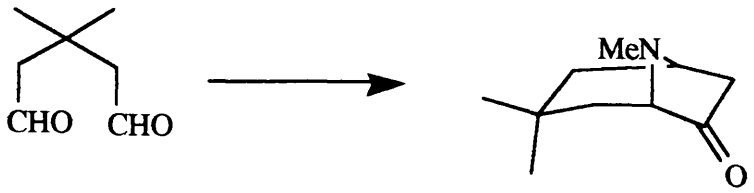
We attempted to carry out the Swern oxidation of 3,3-dimethylpentane-1,5-diol. The activation of dimethyl sulphoxide was carried out in dry dichloromethane at -78 ^{o}C using oxalyl chloride. 3,3-Dimethylpentane-1,5-diol was then added followed by the base triethylamine. However extraction of the aqueous layer yielded unreacted diol.



Scheme 60

Oxidative Cleavage of 1,2-Diols

The synthesis of 3,3-dimethylglutaraldehyde (96) was discussed by Meinwald and Lee.[68] The dialdehyde was an intermediate in the synthetic route to 7,7-dimethylpseudopelletierine (97) (Scheme 61).

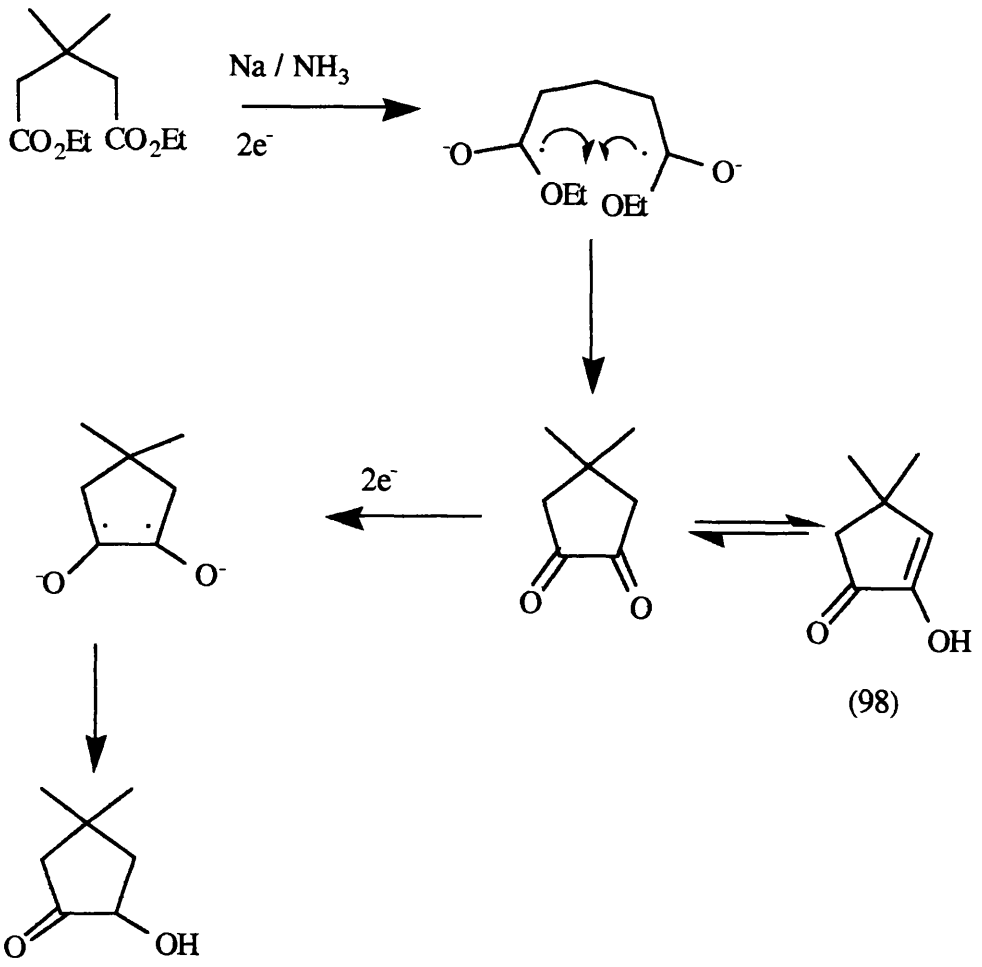


(96) (97)

Scheme 61

Two methods were used for the synthesis of the dialdehyde (96). The first synthesis depended on the selective reduction of the bis-*N*-methylanilide of 3,3-dimethylglutaric acid with lithium aluminium hydride. No satisfactory conditions for this reaction could be found; at best a mixture of starting material, dialdehyde and diacid was recovered. Therefore another route to the dialdehyde had to be found. Diethyl 3,3-dimethylglutarate was treated with sodium in liquid ammonia-ether solution. This step is an acyloin condensation and sodium and ammonia provide electrons. The acyloin condensation is a useful route for formation of five and six membered rings, from a suitable diester. The electrons convert the ester into a radical anion. This cyclises to give the diketo compound, which exists with one of the cabonyls in the enol form (98). This can be reduced further by two more electrons to give the α-hydroxyketone (99) (Scheme 62).

A mixture of 4,4-dimethyl-2-hydroxycyclopentanone (99) and 4,4-dimethylcyclopentane-1,2-dione (98) was obtained by Meinwald and Lee[68] in the acyloin condensation of diethyl 3,3-dimethylglutarate in a 1 : 1 ratio. Two reduction steps gave *cis*-4,4-dimethylcyclopentane-1,2-diol (100). Subsequent cleavage of the diol by sodium bismuthate afforded

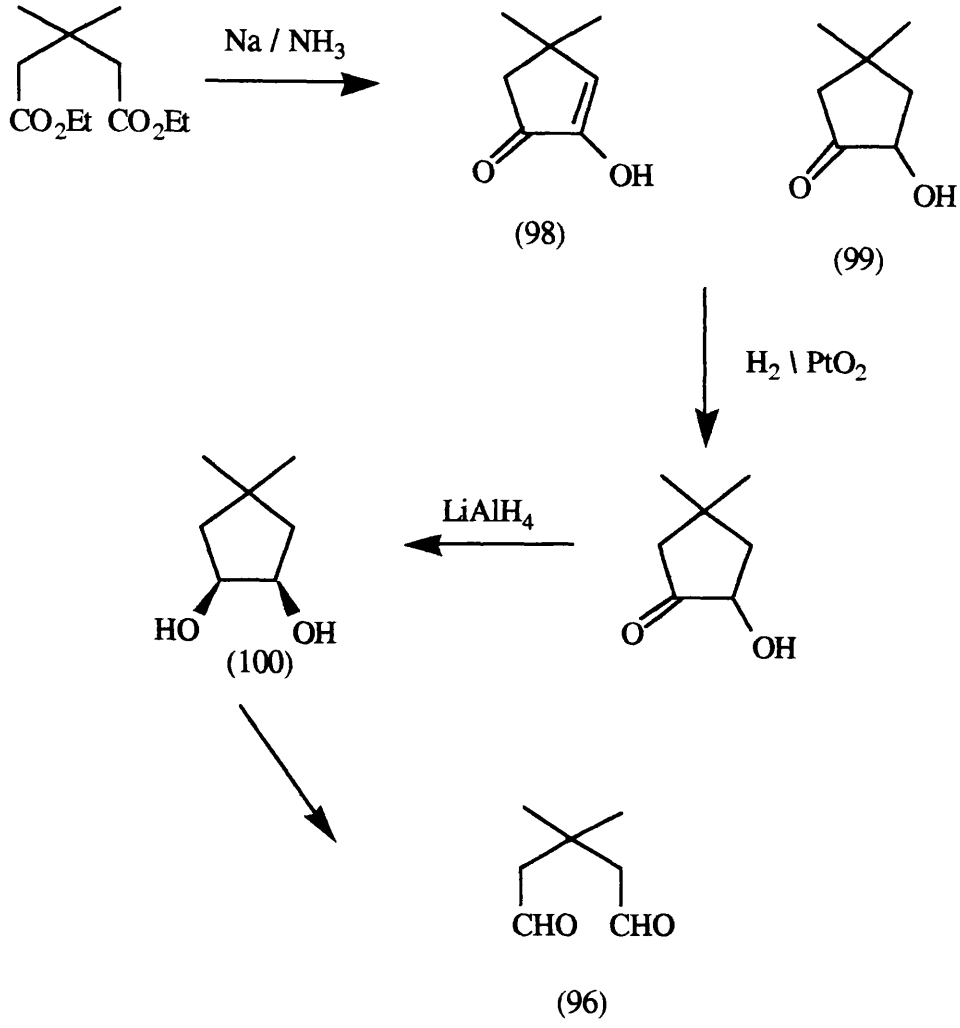


(98)

(99)

Scheme 62

3,3-dimethylglutaraldehyde. This was identified by making its DNP derivative. This aldehyde (96) was found by Meinwald and Lee to be very unstable and had to be used immediately in the next stage of their synthesis of (97), without isolation or purification. This series of reactions used to prepare (96) is shown in Scheme 63.



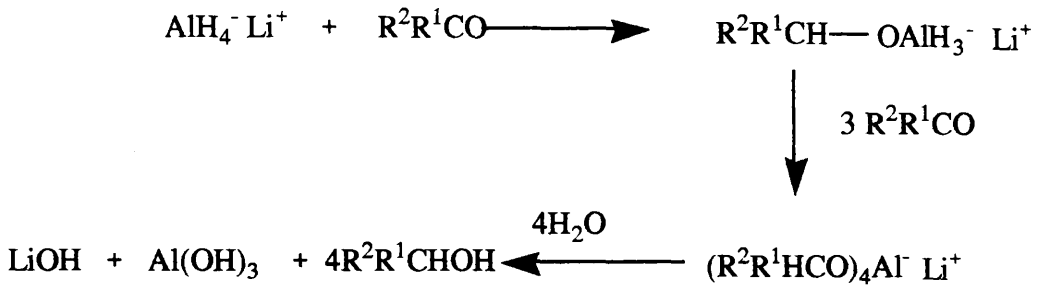
Scheme 63

We attempted to carry out this synthesis of 3,3-dimethylglutaraldehyde. Readily available 3,3-dimethylglutaric acid was converted into its diethyl ester by heating at reflux in thionyl chloride, followed by addition of the diacid chloride to ethanol. Diethyl 3,3-dimethylglutarate was prepared in 75% yield.

Sodium was added to diethyl 3,3-dimethylglutarate in diethyl ether and liquid ammonia. The solution became a deep blue colour showing that solvated electrons had been produced. The ammonia was allowed to evaporate off, and methanol was

added to destroy the sodium. The solution was acidified and extracted to give a mixture of 4,4-dimethyl-2-hydroxycyclopentanone (99) and 4,4-dimethylcyclopentane-1,2-dione (98). This appeared as two spots on TLC using chloroform and ethanol as the solvent system. In the IR spectrum of the mixture there were stretching bands present at 3400 cm^{-1} for the hydroxyl, 1730 cm^{-1} for the carbonyls and 1630 cm^{-1} for the carbon-carbon double bond. In the ^1H NMR spectrum the methyls appeared as a singlet at δ 0.90. The singlet at δ 6.65 was assigned to the olefinic proton of 4,4-dimethylcyclopentane-1,2-dione. This compound exists with one carbonyl mainly in the enol form (98). The mass spectrum showed ions that could correspond to the molecular ion of (98) at *m/z* 126 and (99) at *m/z* 128. The two compounds were not separated or purified. The mixture was hydrogenated at room temperature and atmospheric pressure using PtO_2 as catalyst. The double bond of the enol system of 4,4-dimethylcyclopentan-1,2-dione was hydrogenated to give 4,4-dimethyl-2-hydroxycyclopentanone (99). TLC using chloroform and ethanol as the solvent system showed one spot. In the IR spectrum bands were present at 3400 cm^{-1} due to the hydroxyl and 1730 cm^{-1} due to the carbonyl. The ^1H NMR spectrum showed a singlet at δ 0.90 due to the methyl groups. The multiplet at δ 1.20 was assigned to the methylene at C-3. The methylene protons at C-5 appeared as a singlet. The multiplet present at δ 3.82 was due to the proton at C-2 which is deshielded by the hydroxyl group. The single at δ 4.90 was exchangeable with deuterium and therefore was due to the acidic hydroxyl protons.

The carbonyl of 4,4-dimethylcyclopentanone can be reduced using lithium aluminum hydride, as described in the literature by Meinwald and Lee.[68] The reaction involved the nucleophilic attack of the hydride ion which is transferred from the metal to the carbonyl compound. Four molecules of ketone react with one of AlH_4 (Scheme 64).



Scheme 64

The aluminiumhydride approaches from the less hindered side of the carbonyl compound. Therefore the *cis*-isomer is the main product. The reaction was carried out under anhydrous conditions using THF as the solvent. The inorganic solids were filtered off and the filtrate was concentrated to give *cis*-4,4-dimethylcyclopentane-1,2-diol (100). The IR spectrum showed a stretching band at 3350 cm^{-1} due to the hydroxyl group. The methyl groups gave a symmetrical doublet at 1380 cm^{-1}. In the ^1H NMR spectrum a singlet was present at δ 1.10 due to the methyl groups. The methylene groups at C-1 and C-5 appeared as a multiplet at δ 1.42. The broad singlet at δ 3.20 disappeared on addition of D_2O, showing that this signal was due to the acidic alcohol protons. The multiplet at δ 3.40 was assigned to the C-1 and C-2 protons, which would be deshielded by the hydroxyl groups. The ^{13}C

NMR spectrum showed a methyl signal at δ 16.95. The quaternary signal present at δ 27.43 was due to C-4. The methylene groups at C-3 and C-5 appeared at δ 28.50. The methine signal present at δ 68.46 was assigned to C-1 and C-2. In the mass spectrum the expected molecular ion was observed at *m/z* 130. Ions due to the loss of the methyl groups were also present.

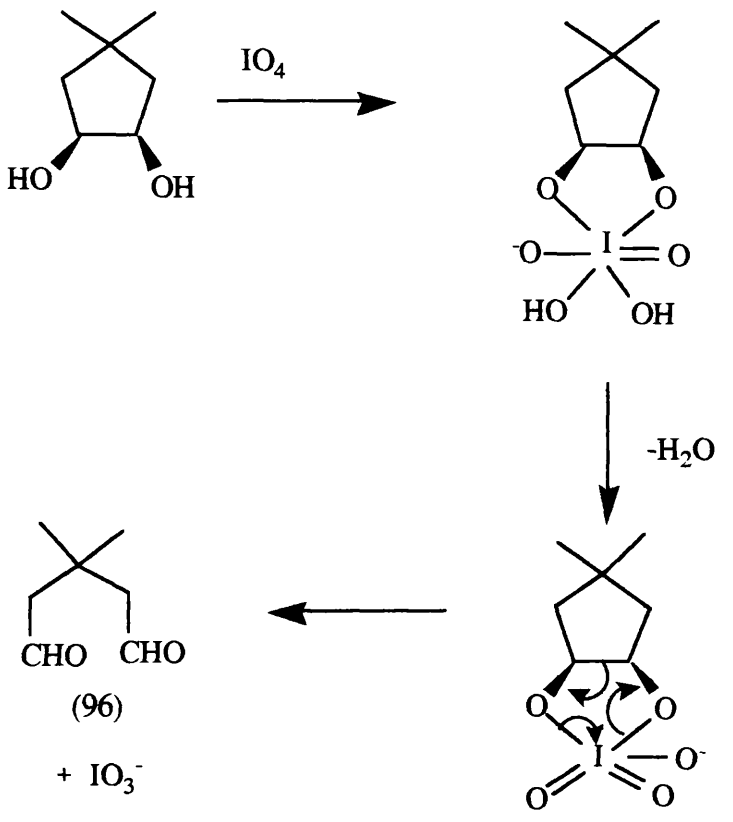
Cleavage of 1,2-diols

1,2-Diols (glycols) can be cleaved by a number of reagents. The preferred reagents for this oxidative cleavage reaction are periodic acid, potassium or sodium periodate and lead tetraacetate. This type of reaction is important in carbohydrate chemistry and has been extensively reviewed.[70,71,72] Other reagents that have been employed in this type of reaction are bismuthate derivatives and calcium hypochlorite.

Meinwald and Lee[68] used sodium bismuthate to cleave the diol (100) to synthesise 3,3-dimethylglutaraldehyde. We tried this method but could not isolate the dialdehyde. The reaction was carried out in phosphoric acid. Extraction of the aqueous layer gave 3,3-dimethylglutaric acid. Therefore, we decided to investigate other methods for the cleavage of the diol to the dialdehyde.

Two of the most important reagents for the cleavage of 1,2-diols are lead tetraacetate and periodate. The advantage in using periodate is the ease of handling as it is available as a crystalline compound. Periodate is better than lead

tetraacetate as the latter can cause other reactions and overoxidation is more likely to occur. Also periodate is less toxic. Therefore periodate is the preferred reagent.



Scheme 65

Periodic acid, (H_5IO_6), sodium periodate, ($NaIO_4$) and potassium periodate (KIO_4) may be used in aqueous media with water soluble glycols. Normally a slight excess of oxidant is employed and the reaction is carried out at room temperature as the iodate decomposes at high temperatures. The mechanism of the oxidation has been extensively studied and evidence suggests a cyclic periodate is formed (Scheme 65). The requirement for a cyclic ester to be formed explains the non-reactivity of certain 1,2-diols toward periodate oxidation.

Thus, in the case of five membered systems *cis*-diols are more reactive than *trans*-diols.

Treatment of *cis*-4,4-dimethylcyclopentane-1,2-diol with periodic acid in THF was carried out at room temperature. Examination of the product by ^{1}H NMR and IR spectroscopy showed that the product was 3,3-dimethylglutaric acid. The IR spectrum showed a band at 3450 cm^{-1} due to the hydroxyl group of the acid. The melting point and ^{1}H and ^{13}C NMR spectra corresponded to that of authentic 3,3-dimethylglutaric acid. Although oxidative cleavage of the diol had taken place, the aldehyde which was initially formed oxidised readily in air to give the diacid.

6.6 Synthesis of Substituted Pimelic acids

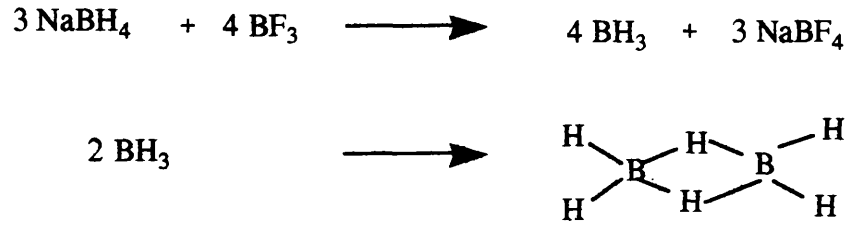
The bromination of pimelic acid followed by cyclisation and hydrolysis to give *cis*- and *trans*-2,6-piperidinedicarboxylic acid was discussed in Section 3.2. Elimination of toluenesulphinic acid from dimethyl *N*-tosyl-*cis*-2,6-piperidinedicarboxylate (41) gave 2,3,4,5-tetrahydrodipicolinic acid (3). We intended to synthesise substituted pimelic acid which would be brominated and the products cyclised to give substituted *cis*- and *trans*-2,6-piperidinedicarboxylic acids. Therefore, using this route it might be possible to synthesise analogues of THDPA.

There are a number of substituted glutaric acids commercially available. By carrying out a sequence of reactions on the acid groups of glutaric acid it should be possible to extend the chain length from five to seven to give

substituted pimelic acids. The reaction scheme includes reduction, substitution and hydrolysis and is discussed in this Section. The glutaric acids used were 3,3-dimethylglutaric acid (101), 3-methylglutaric acid (102), 3-phenylglutaric acid (104) and 3,3-tetramethyleneglutaric acid (103). The sequence of reactions is shown in Scheme 66.

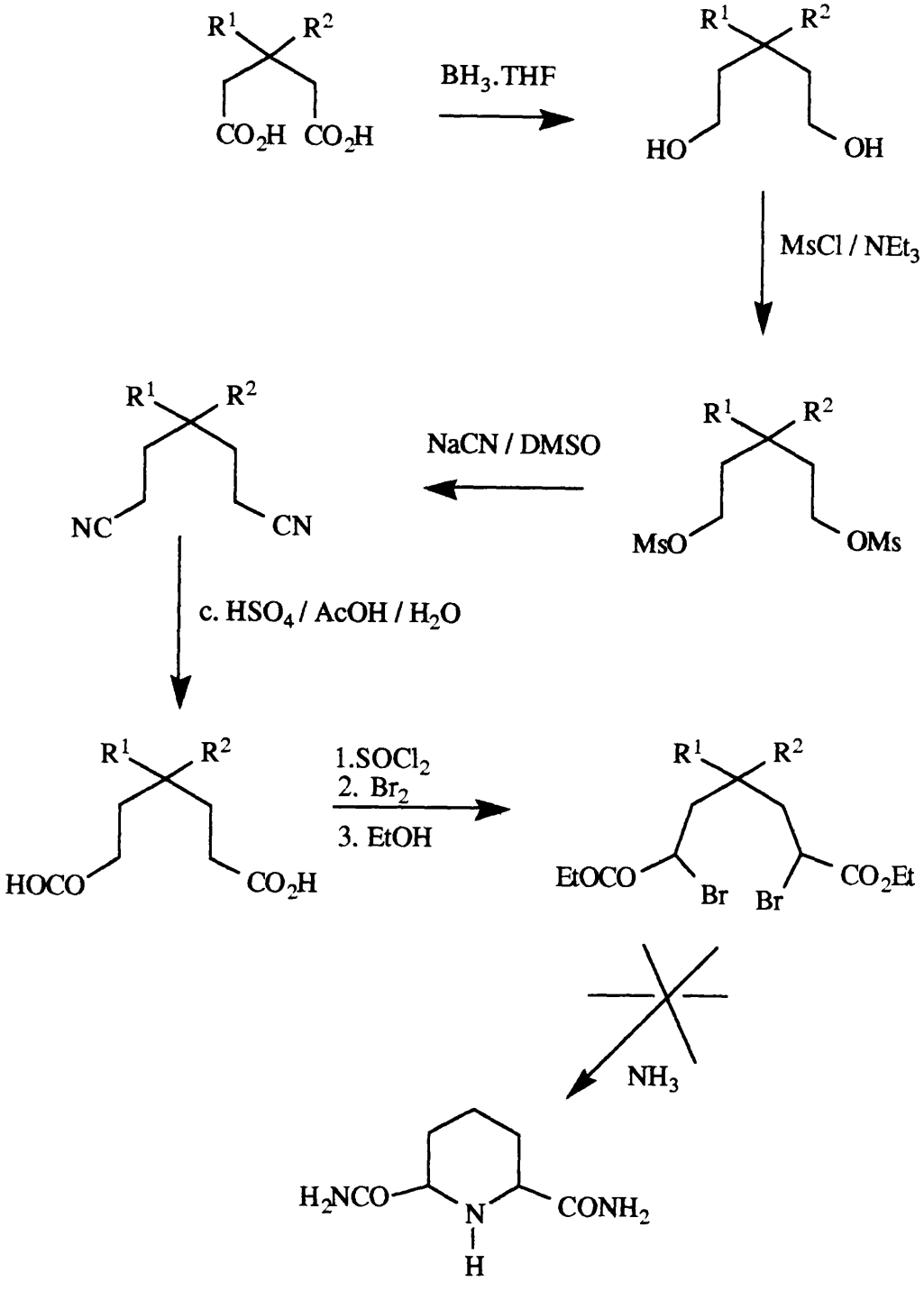
Diborane Reduction

Sodium borohydride does not reduce the free carboxylic acid group but diborane prepared from borontrifluoride etherate in THF converts aliphatic acids into alcohols in 89 - 100% yields (Scheme 67). Diborane is a dimer although it acts as if it were the monomer BH_3. In THF, however the reagent exists as the monomer, in the form of an acid-base complex with the solvent.



Scheme 67

Unlike complex hydride anions, which are nucleophilic, boranes are electrophilic and combine with the lone pair of the oxygen. The mechanism for the reduction of carboxylic acids using diborane was first established by Brown *et al.* (Scheme 68).[74] Each acid group requires three active hydrides equivalents. The reagent is insensitive to steric effects.



(101) $R^1 = R^2 = Me$

(102) $R^1 = Me\ R^2 = H$

(103) $R^1, R^2 = (CH_2)_4$

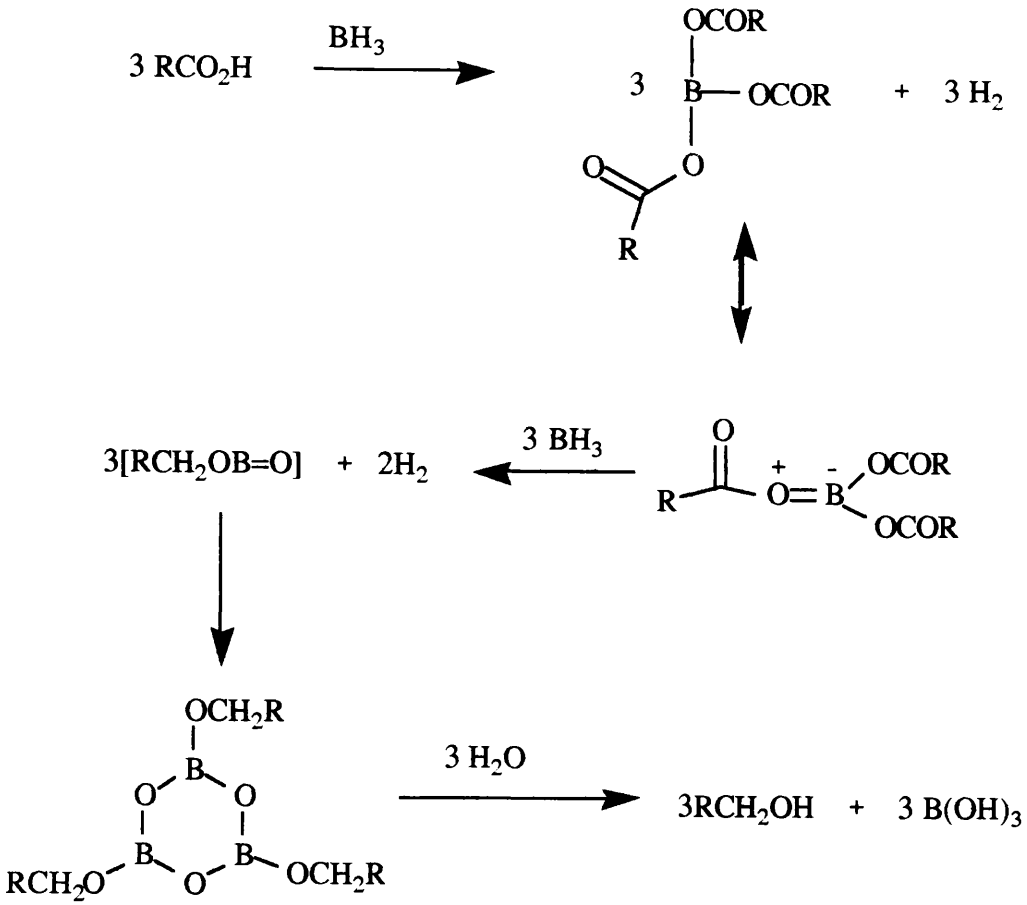
(104) $R^1 = H\ R^2 = Ph$

Scheme 66

3,3-Dimethylglutaric acid was heated at reflux in THF. IM Borane in THF was added carefully, and the solution was heated at reflux for 18 hours. Potassium carbonate was added to the aqueous solution to neutralise the boric acid formed during the reaction. Extraction and distillation afforded 3,3-dimethylpentane-1,5-diol in good yield. The IR spectrum contained a hydroxyl stretching band at 3460 cm^{-1}. The band at 1385 cm^{-1} was assigned to $C(CH_3)_2$ stretching. The ^1H NMR spectrum showed a singlet at δ 0.90 due to the methyl groups. The triplet at δ 1.52 was assigned to its C-2 and C-4 protons and the triplet present at δ 3.62 was due to the C-1 and C-5 protons, which are deshielded by the hydroxyl groups. The broad singlet at δ 3.00 disappeared when D_2O was added, showing that this signal was due to acidic hydroxyl protons. In the ^{13}C NMR spectrum methyl groups were present at δ 18.08. The quaternary C-3 gave a signal at δ 21.49. There were two methylene groups in the spectrum; one at δ 43.75 due to C-2 and C-4 and the other at δ 59.13 due to C-1 and C-5. The mass spectrum did not contain the expected molecular ion, but microanalysis gave correct results.

In order to extend the carbon chain the hydroxyl groups have to be replaced by nitriles. To achieve this the hydroxyls were converted into better leaving groups. An alkyl sulphonate is a good leaving group and is prepared easily from the alcohol.

3,3-Dimethylpentane-1,5-diol in THF was treated with methanesulphonyl chloride and triethylamine. Triethylamine acts as a base and takes up the hydroxyl protons.



Scheme 68

Addition of water and extraction of the aqueous layer afforded 3,3-dimethylpentane-1,5-diol dimethylsulphonate. In the IR spectrum bands were present at 1475 cm^{-1} for 0-SO_2 stretching and at 1400 cm^{-1} due to $C(CH_3)_2$ stretching. The band present at 1170 cm^{-1} was assigned to S=0 stretching. The mass spectrum did not contain the expected molecular ion, but the microanalysis gave the required results.

Treating mesylates with metal cyanides in aprotic solvents results in an SN_2 reaction with CN^- as the nucleophile and MsO^- as the leaving group. Dipolar aprotic solvents are often employed in these reactions, to solvate the cation and to

make the free anion a better nucleophile. This type of reaction proceeds with inversion of configuration.

3,3-Dimethylpentane-1,5-diol dimethylsulphonate in DMSO was heated with sodium cyanide for 18 hours. The solution was diluted with dichloromethane and the organic layer was washed with brine to remove the DMSO and inorganic material. Concentration of the organic layer yielded 3,3-dimethylpentane-1,5-dinitrile as an oil. Chromatography using a silica column was employed to purify the dinitrile. The IR spectrum showed the nitrile stretching band at 2235 cm^{-1} and a $C(CH_3)_2$ stretching band was present at 1440 cm^{-1}.

In the ^1H NMR spectrum of the dinitrile a singlet was present at δ 0.90 due to the methyl groups. Two triplets appeared in the spectrum. The methylenes α to the nitrile appeared as a triplet at δ 2.28 and the methylene protons at C-2 and C-4 gave a triplet at δ 1.60. The mass spectrum contained the expected molecular ion at *m/z* 150.

One method of preparing carboxylic acids is the hydrolysis of nitriles in acid solution. 3,3-Dimethylpentane-1,5-dinitrile was heated at reflux in equal volumes of acetic acid, sulphuric acid and water for 18 hours. Concentration of the reaction mixture followed by crystallisation from methanol gave 4,4-dimethylpimelic acid. The IR spectrum contained stretching bands at 3400 cm^{-1} and 1730 cm^{-1} due to the hydroxyl and carbonyl components of the carboxylic acid group. The band at 1450 cm^{-1} was assigned to $C(CH_3)_2$ stretching. In the ^1H NMR spectrum there was a singlet present at δ 0.90 due to the methyl groups. The triplet at δ 1.62 was assigned to the methylene protons of C-3 and C-5, and

the triplet at δ 2.31 was due to the C-2 and C-6 protons. The ^{13}C NMR spectrum contained a methyl signal at δ 16.48. The quaternary signal at δ 20.43 was assigned to C-4. There were two methylene signals present; the signal at δ 29.84 was due to C-3 and C-5, and the signal at δ 48.38 was caused by C-2 and C-6. In the mass spectrum a molecular ion was observed at *m/z* 188. Ions were also present due to the loss of the methyl and carboxyl groups.

In Section 3.2 the synthesis of diethyl α,α′-dibromopimelate from pimelic acid was discussed. The same procedure discussed in that Section was applied to 3,3-dimethylpimelic acid. Attempted distillation of the product, diethyl 2,6-dibromo-4,4-dimethylpimelate was unsuccessful due to the high boiling point of the oil. A poor yield of the distilled compound was obtained as most of the oil decomposed at these high temperatures (b.p. 220 °C at 4 mm Hg). Therefore a silica column was employed to purify the dibromoester. The IR spectrum showed stretching bands at 1740 and 1220 cm^{-1} for the carbonyl and C-O bond of the ester. The band at 1385 cm^{-1} was assigned to $C(CH_3)_2$ stretching. In the ^1H NMR spectrum a singlet was present at δ 0.92 due to the methyl groups. The triplet at δ 1.30 was attributed to the methyl groups of the ethyl ester. The OCH_2 components of the ethyl esters appeared as overlapping quartets at δ 4.30. The methylene protons at C-3 and C-5 gave a multiplet at δ 2.30. The multiplet at δ 3.98 was attributed to the C-2 and C-6 protons. In the mass spectrum a molecular ion was observed at *m/z* 404, 403, 402. This is due to the two

different isotopes of bromine, 79 and 81, which are present in a 1 : 1 ratio.

The sequence of reactions used to extend the chain of 3,3-dimethylglutaric acid to give 4,4-dimethylpimelic acid, followed by the bromination step, were repeated using 3-methylglutaric acid (102), 3-phenylglutaric acid (104) and 3,3-tetramethyleneglutaric acid (103). The spectroscopic data for the intermediates in the synthesis of substituted diethyl α,α′-dibromopimelates were as expected when compared to those described at each stage when starting from 3,3-dimethylglutaric acid.

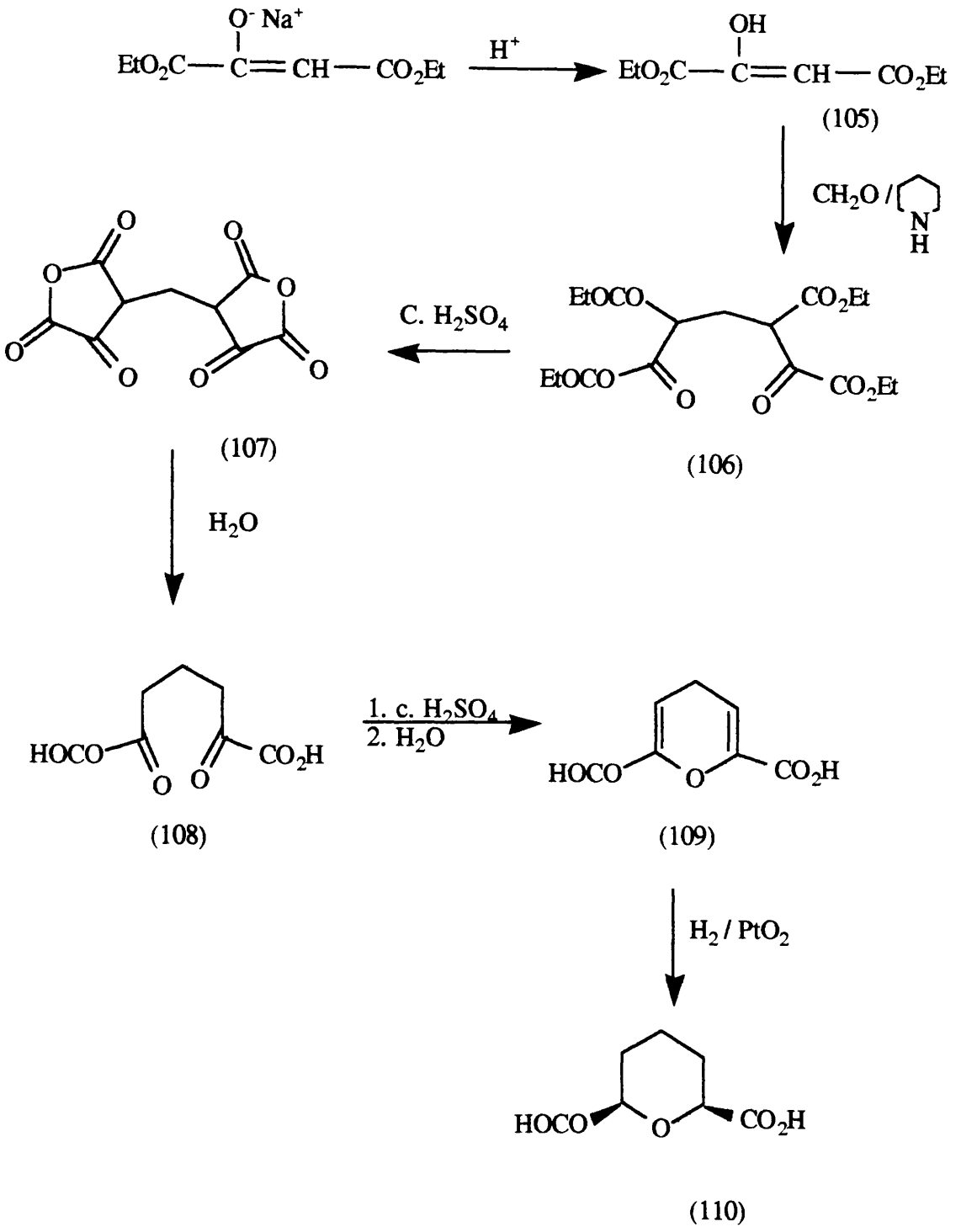
The cyclisation of diethyl α,α′-dibromopimelate was discussed in Section 3.2. Hydrolysis of the separated *cis*- and *trans*-2,6-piperidinedicarboxamides afforded *cis*- and *trans*-2,6-piperidinedicarboxylic acid. We hoped to cyclise and hydrolyse the products the from substituted diethyl α,α′-dibromopimelates to give substituted 2,6-piperidinedicarboxylic acids. The 4- and 4,4- disubstituted α,α′-dibromopimelates were treated with liquid ammonia in a sealed tube, using the conditions described for diethyl α,α′-dibromopimelate. However examinations of the residues after the ammonia had evaporated showed that the required products were not formed. TLC showed a mixture of products was present. The conditions if the reaction was varied. Longer and shorter reaction times were used. Ammonium acetate instead of ammonia was also employed but in every case a black tar was formed. This could be the result of polymerisation of the dibromoesters.

6.7 Synthesis of Tetrahydropyran-2,6-dicarboxylic Acid

Cope and Fourier[75] discussed the synthesis of *cis*- and *trans*-1,5-cyclooctanediols which involved the preparation of pyran-2,6-dicarboxylic acid and *cis*-tetrahydropyran-2,6-dicarboxylic acid. α,α′-Dioxopimelic acid (108) was prepared from diethyl oxaloacetate (105) and cyclised by conc. sulphuric acid to give pyran-2,6-dicarboxylic acid (109). Hydrogenation of (109) afforded *cis*-tetrahydropyran-2,6-dicarboxylic acid (110). This series of reactions is shown in Scheme 69.

By repeating this procedure we planned to synthesise pyran-2,6-dicarboxylic acid to test as an enzyme inhibitor of DHDPA synthase. Pyran-2,6-dicarboxylic acid is an analogue of chelidonic acid which showed good inhibition of the enzyme in preliminary screening of compounds by E. Borthwick. The synthesis of *cis*-tetrahydropyran-2,6-dicarboxylic acid is also of interest to investigate if the presence of an oxygen instead of a nitrogen in the ring has an effect on the inhibition of the enzyme.

Diethyl oxaloacetate was bought from Aldrich as the sodium salt. Diethyl oxaloacetate was regenerated by stirring the salt in 5M sulphuric acid and distillation to give the product as a clear oil, which was used immediately in the next stage. Two moles of diethyl oxaloacetate reacted with one mole of formaldehyde using piperidine as a base to afford methylene bis(diethyl oxaloacetate) (106). In the IR spectrum bands were



Scheme 69

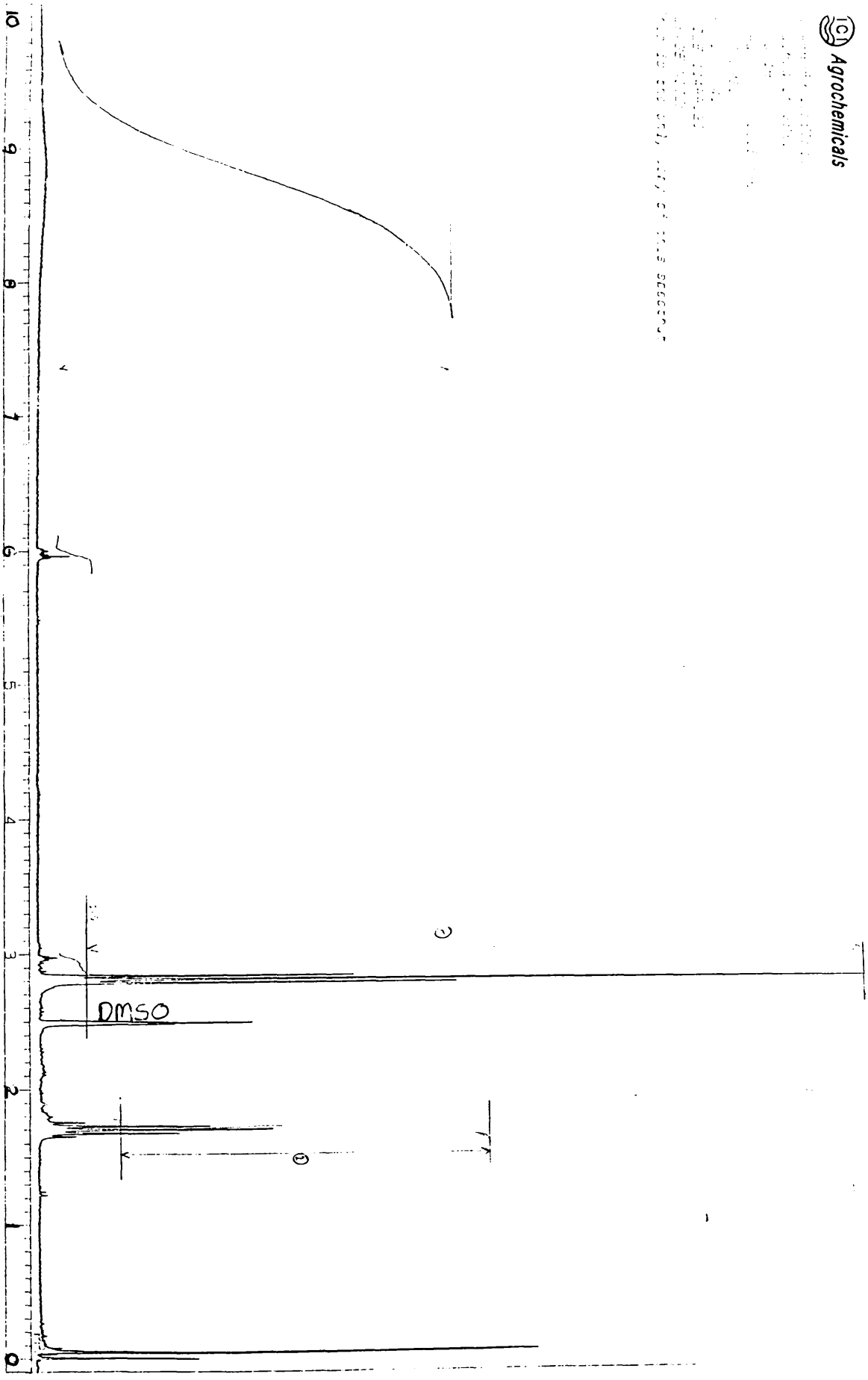
present at 1730 and 1150 cm^{-1} due to the carbonyl and C-O bond of the ester group. The band present at 1640 cm^{-1} was due to the carbonyls at C-2 and C-6. The ^1H NMR spectrum

showed a triplet at δ 1.42 due to the methyls of the ethyl ester groups and a quartet at δ 4.30 was due to the methylenes of the ethyl esters. The multiplet at δ 3.40 was assigned to the protons α- to the ester groups and the multiplet at δ 2.80 was due to the methylene protons. The ^{13}C NMR spectrum contained two quaternary signals at δ 168.84 and 169.16 which were assigned to the ester carbonyls. The keto carbonyl was not observed possibly because it exists mainly as the enol form which is stabilised by conjugation. Two ester methylene groups were observed at δ 61.09 and 62.78 and the methyl group of the ester gave a signal at δ 13.74. A methine signal was present at δ 45.19 and a methylene was observed at δ 17.89. The mass spectrum did not show the expected molecular ion but the required microanalysis results were obtained.

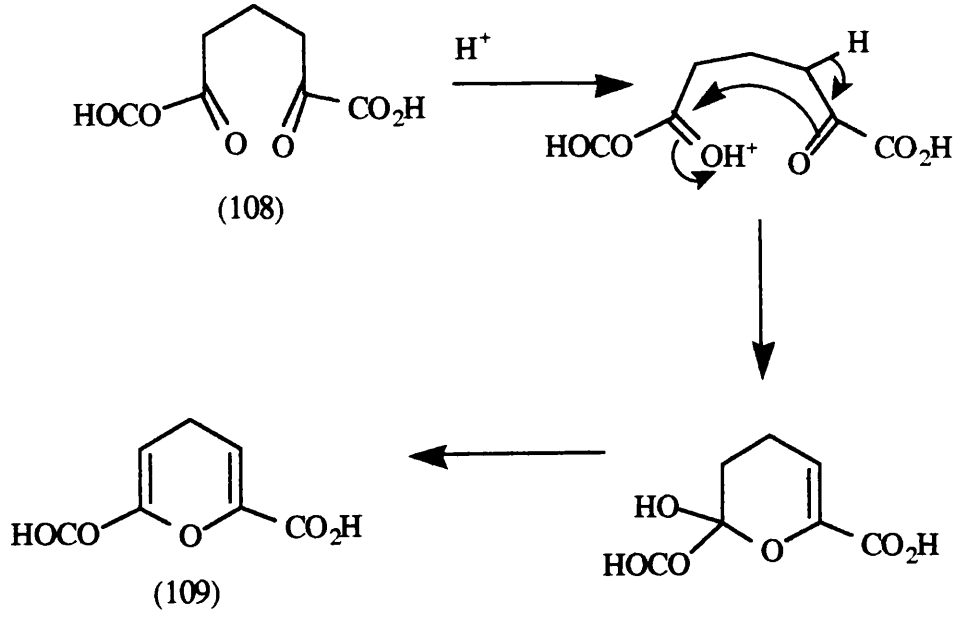
Hydrolysis and decarboxylation of methylene bis(diethyl oxaloacetate) (106) yields α,α′-dioxopimelic acid. This was achieved by treating the ester (106) with conc. sulphuric acid to give the anhydride (107). This crystallised out during the reaction and was filtered off and used immediately in the next step. Formation of anhydrides from dicarboxylic acids occurs easily especially in cases where five or six membered rings are formed.

The solid anhydride (106) was heated at reflux in water for thirty minutes. Effervescence due to loss of CO_2 was observed. Concentration of the solution gave α,α′-dioxopimelic acid (108). In the IR spectrum bands were present at 3400 and 1720 cm^{-1} due to the hydroxyl and carbonyl components of the carboxylic acid group. The ^{1}H NMR spectrum of (108)

Figure 9. ^{1}H NMR spectrum of α,α´-Dioxopimelic Acid (108)



(Figure 9) showed a multiplet at δ 1.72 due to the C-4 protons coupled to C-3 and C-5 protons. The protons at C-3 and C-5 coupled to C-4 protons appeared as only one triplet as they are equivalent. A small multiplet at δ 6.00 was assigned to the olefinic protons of the enol system, showing that there is keto-enol tautomerisation present, but amounts of the enol are small. In the ^{13}C NMR spectrum a quaternary signal was present at δ 196.34 due to the C-2 and C-6 carbons. Another quaternary signal was present at δ 162.77 and was attributed to the carboxylic acid groups. The methylene signal present at δ 16.38 was due to C-4 and the signal at δ 17.72 was assigned to C-3 and C-5. These signals were in a ratio of 1 : 2. In the mass spectrum the expected molecular ion was observed at *m/z* 188. The fragment ion at *m/z* 144 was due to the loss of a carboxylic acid group.



Scheme 70

α,α′-Dioxopimelic acid (108) was cyclised on treatment with conc. sulphuric acid followed by addition to water to yield pyran-2,6-dicarboxylic acid (109). This mechanism is shown in Scheme 70.

In the IR spectrum of (109) bands were observed at 3400 cm^{-1} and 1710 cm^{-1} due to the hydroxyl and carbonyl components of the dicarboxylic acid. The bands at 1670 and 1630 cm^{-1} were attributed to the keto carbonyl and the carbon-carbon double bond respectively. The ^{1}H NMR spectrum showed a multiplet at δ 2.90 due to the C-4 methylene protons. The multiplet at δ 5.90 was attributed to the olefinic protons. In the ^{13}C NMR spectrum a quaternary signal was present at δ 162.14 due to the carboxylic acid groups. The quaternary signal at δ 141.98 was assigned to C-2 and C-6, and the methine signal present at δ 110.07 was due to C-3 and C-5. The C-4 methylenes showed a signal at δ 21.42. A chromophore was observed in the UV spectrum at 279 nm due to the conjugated π system.

Hydrogenation of pyran-2,6-dicarboxylic acid as described by Cope and Fourier,[75] gave *cis*-tetrahydro-2,6-pyrandicarboxylic acid (110) in 96% yield (Scheme 69). In the IR spectrum of (110) bands were present at 3400 and 1700 cm^{-1} due to the hydroxyl and carbonyl components of the carboxylic acid groups. The band at 1100 cm^{-1} was attributed to the ether linkage. The ^{1}H NMR spectrum showed multiplets at δ 1.70 and 2.05 due to the protons at C-3, C-4 and C-5. A multiplet was present at δ 4.10 due to the acidic protons α to the carboxylic acid groups. Two methylene signals were present in the ^{13}C NMR spectrum; one at δ 24.70 was due to C-4

and one at δ 34.48 was due to C-3 and C-5. The acidic protons α to the carboxylic acid groups appeared at δ 76.83. The quaternary signal at δ 175.78 was attributed to the carboxyl carbons. In the mass spectrum the expected molecular ion was observed at *m/z* 175 and ions at *m/z* 129 and *m/z* 85 were due to the loss of the carboxyl groups.

Conclusions

The synthesis of analogues of 2,6-piperidinedicarboxylic acid was carried out. *N*-Substituted analogues of the *cis*-isomer were easily prepared. The cyclisation of 1,4-pentane-3-one-1,5-dicarboxylic acid with ammonia or methylamine gave the diammonium salts of *cis*- and *trans*-piperidin-4-one-2,6-dicarboxylic acid and their *N*-methyl analogues.

Another route for the synthesis of 2,6-piperidinedicarboxylic acid is the Strecker synthesis, which involved the cyclisation of glutaraldehyde to give 2,6-piperidinedinitrile which was hydrolysed to afford the *cis*-diacid (27) and the *trans*-diacid (32). Attempts were made to synthesise substituted glutaraldehydes which when cyclised and hydrolysed would yield 4-alkyl substituted analogues of the diacid (27). However all the methods tried to synthesise substituted glutaraldehydes failed mainly due to the instability of the dialdehydes.

Another route for the synthesis of 4-alkyl substituted analogues of (27) is from the 4-substituted pimelic acids, which were prepared by extending the chain of the corresponding substituted glutaric acid. These analogues of pimelic acid were

brominated using the same method used to brominate pimelic acid. Attempts were made to cyclise the 4-substituted diethyl α,α′-dibromopimelates using ammonia but this final step in the synthesis failed.

It was hoped that the analogues of 2,6-piperidinedicarboxylic acid could be oxidised to the corresponding imine, which would be analogues of THDPA (3). However due to the problems in finding a suitable route for the synthesis of THDPA (3) this compound was synthesised only recently. Future work could involve the synthesis of imines of the analogues of 2,6-piperidinedicarboxylic acid that we prepared, which would be tested as enzyme inhibitors of DHDPA synthase and reductase enzymes.

The analogues of 2,6-piperidinedicarboxylic acid that we prepared were tested as enzyme inhibitors of DHDPA synthase by Emma Borthwick in the Biochemistry Department. This work will be discussed in the next Chapter.

CHAPTER 7

Preliminary Compound Screening With Dihydrodipicolinic Acid Synthase

Work on the isolation and purification of the dihydrodipicolinate synthase from *E. coli* was carried out by Professor J.R. Coggins and E. Borthwick of the Biochemistry Department.[76] Work was also carried out on the DHDPA reductase enzyme, however further work has to be carried out before inhibitory studies can be carried out using this enzyme.

Two different assays have been reported in studies on the synthase enzyme. Yugari and Gilvarg[1] developed the *o*-aminobenzaldehyde assay. The spectrophotomeric absorbance at 540 nm was used to follow the enzymic reaction.

Yugari and Gilvarg also employed the 270 nm assay which was the assay used by E. Borthwick in this work. Aspartate semialdehyde and pyruvic acid condense using DHDPA synthase to give the unstable enzymic product, dihydrodipicolinic acid. Non-enzymic oxidation of dihydrodipicolinic acid affords dipicolinic acid, which has a spectrophotomeric absorbance at 270 nm. This can be used to follow the enzyme reaction.

Synthesised compounds, which include dipicolinic acid analogues, chelidamic acid analogues and 2,6-piperidine-dicarboxylic acid analogues along with some shelf compounds were tested in the 270 nm assay for DHDPA synthase by E. Borthwick.

The assay screen consists of:-

1. The standard assay: 100 mM imidazole buffer; 1 mM aspartate semialdehyde; 1 mM pyruvic acid; and 16 units DHDPA synthase.
2. Three concentrations of the compound being tested, 10 mM, 5 mM, 1 mM, and when required 0.5 mM and 0.1 mM were used.

The level of inhibition was measured as a percentage of the standard rate as shown in the following equation:

$$\frac{(a-x)}{a} \times 100 = \% \text{ Inhibition}$$

where x = rate of assay with compound and a = rate of standard assay.

Chelidamic acid and its *N*-substituted analogues showed good inhibition at low concentrations. *N*-Methylchelidamic acid showed the best inhibition, 63% inhibition was observed at 0.1 mM. The diesters of chelidamic acid and its *N*-substituted analogues also showed good inhibition.

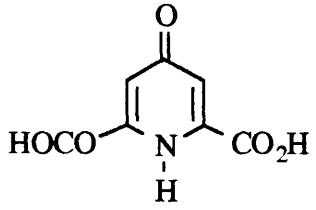
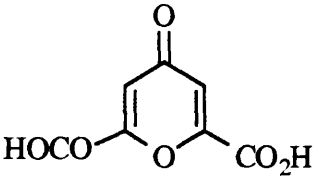
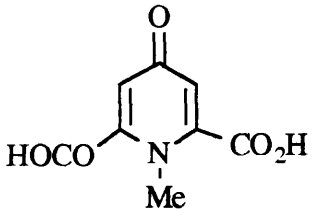
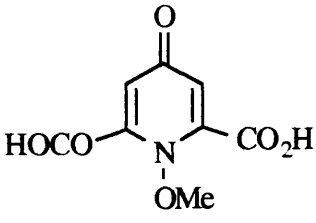
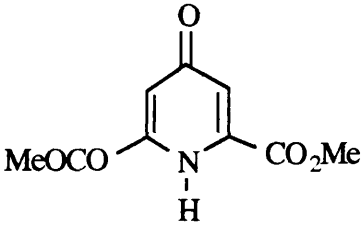
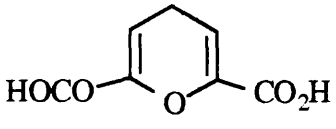
Dipicolinic acid showed significant inhibition at 0.5 mM, as did some of its analogues. Changing the diacid to a diester, a dicyano, a diimidate or a ditetrazole derivative had little effect on the amount of inhibition. Poor inhibition was observed with the fully saturated 2,6-piperidinedicarboxylic acid analogues. However piperidine-4-one-2,6-dicarboxylic acid and its *N*-methyl derivative showed good inhibition.

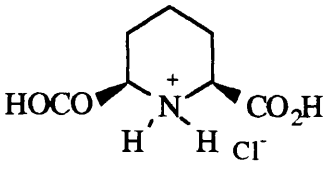
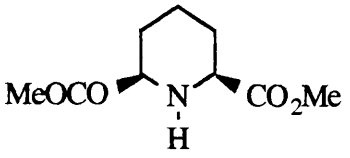
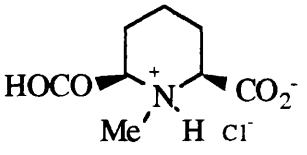
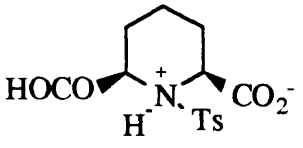
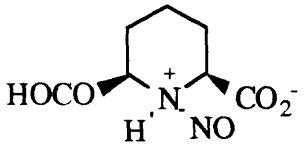
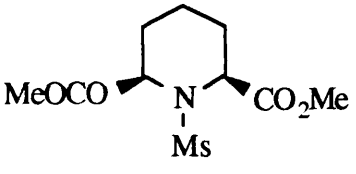
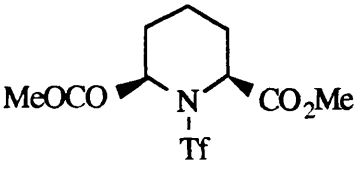
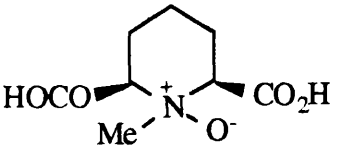
Compounds with only one acid group such as pipecolinic acid and proline and their analogues showed little or no

inhibition, showing the importance of two carboxylic acid groups in binding to the active site of the enzyme.

The compounds tested in the DHDPA assay at different concentrations are listed in the following table.

Preliminary Compound Screening with DHDPA Synthase

	% Inhibition				
	10 mM	5 mM	1 mM	0.5 mM	0.1 mM
	100	100	100	72	0
	100	100	100	96	0
	100	100	100	100	63
	100	100	100	95	58
	100	100	100	0	
	No inhibition				

	% Inhibition				
	10 mM	5 mM	1 mM	0.5 mM	0.1 mM
	No inhibition				
	100	100	100	0	0
	No inhibition				
	100	100	100	-ve	
	100	100	25		
		25	21		
	81	22	11		
	No inhibition				

	% Inhibition				
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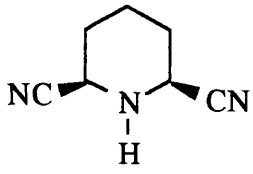
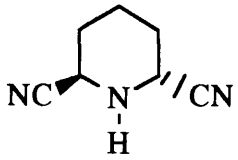
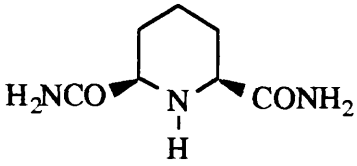
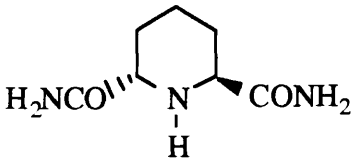
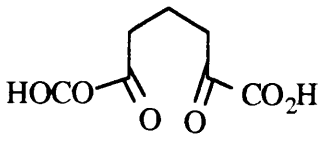
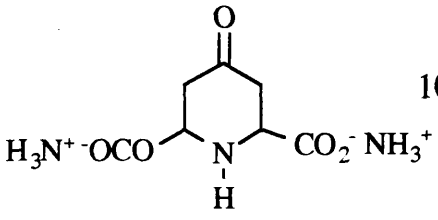
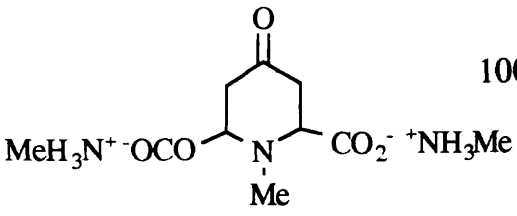
	61	42	8		
	90	37	3		
	16	5	3		
	23	13	0		
	100	100	100	75	
	100	100	72	30	
	100	100	87	54	

	% Inhibition				
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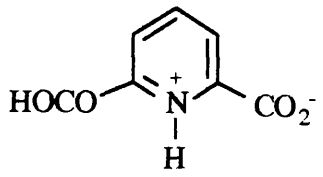
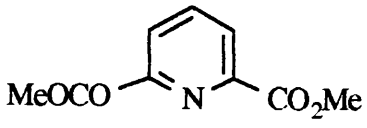
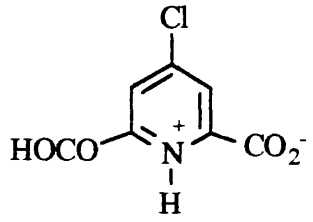
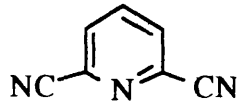
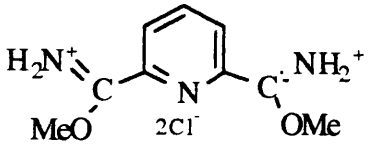
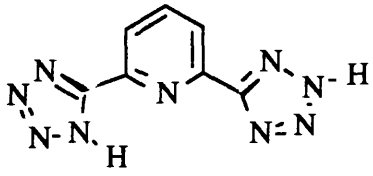
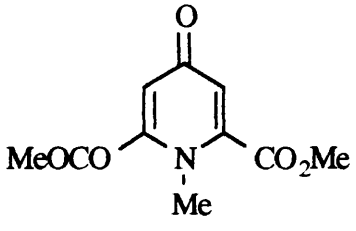
	100	100	100	56	16
	100	100	94	24	0
	100	97	6		
	100	100	100	70	13
	100	100	100	92	3
	100	100	100	75	8
	100	100	100	46	

	% Inhibition				
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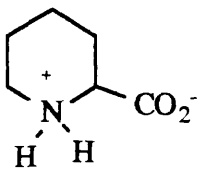
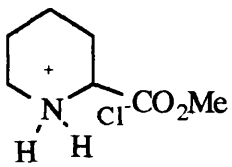
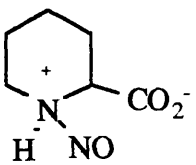
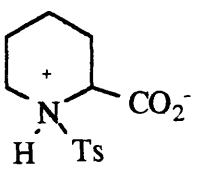
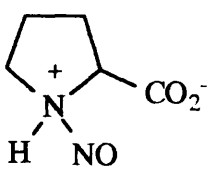
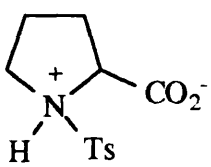
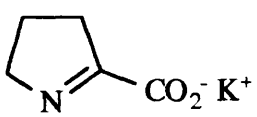
	0	0	0		
	0	0	0		
	100	80	0		
	60	4	10		
	90	10	0		
	60	7	0		
	43		7		

CHAPTER 8

Experimental

8.1 General Notes

All melting points were measured with a Kolfer hot-stage apparatus and are uncorrected. The optical rotations were measured with an optical Activity Ltd AA-10 polarimeter. Infra red spectra were obtained on a Perkin Elmer 580 spectrophotometer and ultra violet with a Pye-Unicam SP-100 spectrophotometer. All IR spectra were run on KBr discs unless otherwise stated. Nuclear magnetic resonance spectra were recorded with a Perkin Elmer R 32 spectrometer operating at 90 MHz (δ_H) or with a Bruker WP-200 SY spectrometer operating at 200 MHz (δ_H) or 50 MHz (δ_C). Mass spectra were determined with A. E. I. MS 12 or 902 spectrometer. TLC were carried out on Kieselgel 60 F_{254} plastic sheets of 2 mm thickness.

THF was distilled from KOH and then from sodium-benzophenone under nitrogen prior to use. DMSO and dichloromethane were distilled from CaH_2. Organic solvents were dried with sodium sulphate.

8.2 Experimental to Chapter 3

DL-Aspartic Semialdehyde[5] *(1)*

Ozone was bubbled through a solution of DL-allylglycine (0.50 g, 4.3 mmol) in 1M HCl (4.3 ml) at 0 °C for 3 h to give mainly

DL-aspartic semialdehyde (1) and formaldehyde. The product was not isolated or purified as it is unstable. The acidic solution was stored at O °C. Aliquots of the solution were used in the assay for the first enzyme, dihydrodipicolinic acid synthase, where a positive rate was observed.

The ozonolysis reaction was repeated in D_20 and DCl and followed by ^1H NMR spectroscopy, δ_H (200 MHz) (D_20 + DCl) 1.38 (2H, m) and 3.37 (1H, m) and impurities at δ 2.24 (m) and δ 3.51 (m).

DL-Aspartic Semialdehyde Semicarbazone

Semicarbazide hydrochloride (0.16 g) and sodium acetate (0.24 g) in water (1 ml) were added to the above aspartate semialdehyde solution (1.5 ml). The solution was basified to pH 8 with 1M NaOH solution. After cooling, aspartic semialdehyde semicarbazone crystallised and was filtered off and dried (0.12 g), m.p. 222 - 225 °C; υ_{max} 3520, 3000, 1660, 1630 and 1580 cm^{-1}; δ_H (200 MHz) (D_20 + DCl) 2.28 (2H, m) 4.28 (1H, m) and 7.15 (1H, m); δ_C (50 MHz) (D_2O + DCl) 32.16 (t), 51.60 (d), 142.32 (d), 159.80 (s), and 172.5 (s).

Attempted Synthesis of Aspartate Semialdehyde Dinitrophenylhydrazone

Dinitrophenylhydrazine (0.57 g) in 1M HCl (2 ml) and ethanol (2 ml) was added to a portion of aspartate semialdehyde solution (1.5 ml). After cooling an orange solid precipitated (0.12 g), m.p. 176 - 178 °C. From its melting point

and ^{1}H and ^{13}C NMR spectroscopy the solid is the dinitrophenylhydrazone of formaldehyde.

Formation of Dipicolinic Acid (26) from Aspartate Semialdehyde (1)

Aspartate semialdehyde (1) (approx. 0.2 g) in 1M HCl was cooled to 0 °C. The solution was basified to pH 9 using 1M NaOH solution, and oxaloacetic acid (0.50 g) was added. The solution was given to our co-workers in the Biochemistry Department to test in the assay for the second enzyme, dihydrodipicolinic acid reductase.

The same mixture was made up using aspartate semialdehyde in 1M DCl and this was basified with NaOD. The reaction was followed by ^{1}H NMR spectroscopy. An intermediate was initially formed, which could be 2,3-dihydrodipicolinic acid (2), δ_H (200 MHz) (D_2O + NaOD) 5.47 (s) but was readily converted into dipicolinic acid (26), δ_H (200 MHz) (D_2O + NaOD) 8.34 (s). After 135 min. the singlet at δ 5.47 had disappeared while the singlet at δ 8.34 had increased in size and reached a constant value.

8.3 Experimental to Chapter 4

cis-*2,6-Piperidinedicarboxylic Acid (27)*

Dipicolinic acid (26) (1.50 g, 8.7 mmol) in glacial acetic acid (50 ml) was hydrogenated at atmospheric pressure and room temperature for 30 h using PtO_2 (0.15 g) as catalyst. The

catalyst was removed by filtration through Celite, and the filtrate was acidified with conc. HCl to precipitate a white solid. Crystallisation from water gave *cis*-2,6-piperidinedicarboxylic acid (27) as a white powder (1.51 g, 96%), m.p. 290 - 295 °C, (lit.[22], 290 - 295 °C); υ_{max} 3400, 2990 and 1750 cm^{-1}; δ_H (200 MHz) (D_20) 1.80 - 1.85 (4H, m), 2.31 (2H, m) and 3.80 (2H, m); δ_C (50 MHz) (D_20) 23.05 (t), 26.27 (t), 58.05 (d) and 172.36 (s); *m/z* 173 (M^+ , 31%), 128 (26%) and 84 (100%) (Found: C, 39.98; H, 5.72; N, 6.65. $C_7H_{11}0_4N$ requires C, 40.10; H, 5.77; N, 6.68%).

Diethyl α,α´-Dibromopimelate (29)

Pimelic acid (5.40 g, 34 mmol) in thionyl chloride (6.09 ml) was heated at 40 °C for 18 h. Iodine (0.10 g) and bromine (4.02 ml, 75 mmol) were added and the mixture was heated at 80 °C for 6 h. After cooling the solution was added to ethanol (30 ml). Water (75 ml) was added and the aqueous solution was extracted with diethyl ether (3 x 25 ml). The combined ether extracts were washed with 10% sodium thiosulphate solution (5 x 20 ml). The ether layer was dried, filtered, and concentrated to an oil. Distillation gave diethyl α,α´-dibromopimelate (29) as a clear oil (60% yield), b.p. 165 - 170 °C (4 mmHg); R_F (hexane) 0.68; υ_{max} (liq. film) 2980, 1740, 1425, 1230 and 1100 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.30 (6H, t), 1.62 (2H, m), 2.10 (4H, m), 4.00 (2H, m), and 4.20 (4H, q); *m/z* 222 (62%); 166 (70%) and 73 (100%) (Found: C, 35.82; H, 5.07. $C_{11}H_{18}O_4Br_2$ requires C, 35.51; H, 5.05%).

cis- *(30) and* trans-*2,6-Piperidinedicarboxamide (31)*

Diethyl α,α´-dibromopimelate (29) (1.0 g, 2.9 mmol) in liquid ammonia in a sealed tube was left standing at room temperature for 3 d. The tube was opened at -78 °C and allowed to warm to room temperature. Ice water (5 ml) was added to the solid residue. The remaining solid was filtered off and dried to give *cis*-2,6-piperidinedicarboxamide (30) (0.15 g, 30%), m.p. 226 - 228 °C (lit.[24], m.p. 226 - 228 °C); υ_{max} 3300, 2980, 1680, 1630 and 1400 cm^{-1}; δ_H (200 MHz) (D_20) 1.48 - 1.53 (4H, m), 1.81 (2H, m), and 4.14 (2H, m); δ_C (50 MHz) (D_20) 17.96 (t), 25.64 (t), 54.35 (d), and 171.64 (s); *m/z* 171 (M^+, 20%), 128 (42%) and 84 (100%) (Found: M^+, 171.0769. $C_7H_{11}0_4N$ requires M^+, 171.1110). Concentration of the filtrate gave crystals of *trans*-2,6-piperidinedicarboxamide (31) (0.10 g, 20%), m.p. 264 - 268 °C (lit.[24], 265 - 269 °C); υ_{max} 3300, 2980, 1680, 1630 and 1400 cm^{-1}; δ_H (D_2O) (200 MHz) 1.26 - 1.60 (4H, m), 1.85 (2H, m), and 3.72 (1H, m); δ_C (50 MHz) (D_2O) 21.85 (t), 25.93, (t), 57.26 (d), and 171.50 (s); *m/z* 171 (M^+, 26%), 128 (39%) and 84 (100%) (Found: M^+, 171.1009 $C_7H_{13}O_2N_3$ requires M^+, 171.1100).

cis-*2,6-Piperidinedicarboxylic Acid (27)*

cis-2,6-Piperidinedicarboxamide (30) (0.40 g, 2.3 mmol) in 10% barium hydroxide solution was heated at reflux for 2 h. Carbon dioxide was added to the solution and the solid barium carbonate was filtered off. The filtrate was acidified with conc. HCl and concentrated to leave *cis*-2,6-piperidinedicarboxylic

acid (27) as a white solid (0.32 g, 79%), m.p. 292 - 295 °C (lit.[22], 290 - 295 °C); υ_{max} 3300, 2990 and 1750 cm^{-1}; δ_H (200 MHz) (D_20) 1.30 - 1.84 (4H, m), 2.31 (2H, m), and 3.80 (2H, m); δ_C (50 MHz) (D_20) 23.09 (t), 26.24 (t), 58.10 (d) and 172.40 (s); *m/z* 173 (M^+, 24%), 128 (62%), and 84 (100%) (Found: M^+, 173.0686. $C_7H_9O_4N$ requires M^+ 173.0951).

trans-*2,6-Piperidinedicarboxylic Acid*

The above hydrolysis procedure was repeated using *trans*-2,6-piperidinedicarboxamide (31) in 10% barium hydroxide solution to give *trans*-2,6-piperidinedicarboxylic acid (32) as a white powder (14% yield), m.p. 276 - 279 °C (lit.[24], 275 - 278 °C); υ_{max} 3300, 2950, 1750 and 1150 cm^{-1}; δ_H (200 MHz) (D_2O) 1.29 - 1.83 (4H, m), 2.28 (2H, m), and 4.30 (1H, m); δ_C (50 MHz) (D_2O) 22.44 (t), 26.06 (t), 58.49 (d), and 172.48 (s); *m/z* 173 (M^+, 12%), 128 (81%), and 84 (100%) (Found: M^+, 173.0791. $C_7H_9O_4N$ requires M^+, 173.0951).

Attempted Imine Formation from Methyl Pipecolinate

N-Bromosuccinimide (NBS) was added with stirring to an ice cool solution of methyl pipecolinate (37) (0.64 g, 4.5 mmol) and triethylamine (0.80 g, 7.9 mmol) in dry dichloromethane. After 30 min more triethylamine was added (2.40 g). After 12 h stirring, with the temperature allowed to rise from 0 °C to room temperature the solution was extracted with 1M HCl (2 x 15 ml). KI (0.5 g) was added to the acid extracts followed by sodium thiosulphate (1.00 g). The solution was basified with

1M NaOH and extracted with dichloromethane (3 x 20 ml). The combined organic extracts were dried, filtered and concentrated to a yellow oil. Crystallisation from methanol and ether gave starting material (0.62 g).

Attempted Imine Formation Using Dimethyl Piperidinedicarboxylate and NBS

Dimethyl piperidinedicarboxylate (39) (1.00 g) was treated with NBS and triethylamine in dichloromethane as described above. Concentration of the organic extracts gave a brown oil. Examination of the oil by TLC using dichloromethane as the solvent gave a streak. ^{1}H and ^{13}C NMR spectroscopy showed a mixture of products had been formed which could not be separated.

Attempted Synthesis of Tetrahydrodipicolinic Acid

A mixture of *cis*-2,6-piperidinedicarboxylic acid (0.60 g, 3.4 mmol) in 5% acetic acid (15 ml) with mercuric acetate (4.02 g, 12 mmol) was heated at reflux for 4 h. The mixture was cooled and the mercury salts were filtered off. The filtrate was saturated with H_2S gas and the black solid that formed was filtered off through Celite. This treatment with H_2S gas was repeated twice. The filtrate was acidified with conc. HCl and concentrated to afford a white solid (0.31 g, 53%). From ^{1}H and ^{13}C NMR spectroscopy this was starting material. *trans*-2,6-Piperidinedicarboxylate was treated with mercuric acetate as in the above procedure and also gave starting material.

Attempted Transamination of Diaminopimelic Acid

Isonicotinaldehyde (33) (0.38 g, 3.5 mmol) and 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) (5.49 g, 9 mmol) were added to a suspension of diaminopimelic acid (0.57 g, 3 mmol) in DMF (20 ml). The suspension was stirred for 18 h at room temperature. The solid was filtered off (0.57 g) and ^{1}H NMR spectroscopy indicated that this was starting material.

Separation of meso-*Diaminopimelic Acid (7) from DD- and DL-Diaminopimelic Acid*[29]

Diaminopimelic acid (5.0 g), bought from Sigma as a mixture of isomers and was dissolved in boiling water (125 ml). The solution was allowed to cool to room temperature and ethanol (100 ml) was added, giving a turbid solution. This was left overnight and the solid that crystallised was filtered off to give *meso*-diaminopimelic acid (7) (2.7 g, 52%) m.p. > 300 °C (lit.[29], > 300 °C); υ_{max} 3040, 2500, 1630, 1600 and 1530 cm^{-1}; δ_H (90 MHz) (D_2O + DCl) 1.89 (2H, m), 2.19 (4H, m) and 4.20 (2H, m); *m/z* 190 (M^+, 1%), 128 (23%) and 56 (100%).

Attempted Enzymic Transamination of meso-*Diaiminopimelic Acid (7)*

meso-Diaminopimelic acid (7) (70 mg) was dissolved in D_2O and DCl. The pD was adjusted to 6 using NaOD. D-Amino acid oxidase enzyme (EC 1.4.3.3.) (6 mg, 1.62 units) from Sigma was

added. The reaction was followed by ^1H NMR spectroscopy, but after 7 d no change had taken place. The reaction was repeated usind L-amino acid oxidase (EC 1.4.3.2.) but no reaction took place.

Cyclisation of α,α′-Dioxopimelic Acid[33]

α,α′-Dioxopimelic acid (108) (1.00 g, 5.32 mmol) in a sealed tube with liquid ammonia was left standing at room temperature for 18 h. The tube was opened at -78 °C and allowed to warm to room temperature. The residue was examined by ^1H and ^{13}C NMR spectroscopy, which showed dipicolinic acid (26) had been formed as its diammonium salt and another compound was present which could be tetrahydrodipicolinic acid (3), λ_{max} (H_2O) 273 (ε 78,600); υ_{max} 3300, 1600, 1580 and 1250 cm^{-1}; δ_H (200 MHz) (D_20) for the reduced form of dipicolinic acid, 1.50 - 2.00 (6H, m) and 3.40 (1H, m); δ_H (200 MHz) (D_2O) for dipicolinic acid, 7.79 (1H, s); δ_C (50 MHz) (D_2O), for the reduced form of dipicolinic acid, 18.79 (t), 22.22 (t), 25.91 (t), 41.77 (d), 45.32 (d), 171.65 (s), 171.65 (s) and 180.03; δ_H (50 MHz) (D_2O) for dipicolinic acid, 126.18 (d), 139.64 (d), 153.44 (s) and 178.91 (s); *m/z* too involatile.to measure.

Dimethyl Dipicolinate (38)

A solution of dipicolinic acid (26) (4.00 g, 24 mmol) in methanol (50 ml) and conc. sulphuric acid (10 ml) was heated for 18 h. Water (30 ml) was added and the aqueous solution

was neutralised with sodium carbonate. The solution was acidified with conc. HCl and extracted with chloroform (4 x 25 ml). The combined extracts were dried, filtered and concentrated to leave a white solid. Crystallisation from chloroform gave dimethyl dipicolinate (38) as a white powder (2.87 g, 96%), m.p. 117 - 119 °C; R_F ($CHCl_3$ - conc. NH_3, 99:1) 0.78; υ_{max} 2990, 1740, 1590, 1250 and 1110 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 4.00 (6H, s), 8.05 (1H, t) and 8.34 (2H, d); *m/z* 195 (M^+, 24%), 87 (62%) and 79 (100%) (Found: C, 55.45; H, 4.60; N, 7.18. $C_9H_9O_4N$ requires C, 55.43; H, 4.65; N, 7.18%).

Dimethyl cis-*2,6-Piperidinedicarboxylate (39)*

A solution of dimethyl dipicolinate (38) (1.0 g, 5.1 mmol) in chloroform (20 ml) was hydrogenated at atmospheric pressure and room temperature for 24 h with PtO_2 (0.10 g) as catalyst. The catalyst was removed by filtration through Celite. The filtrate was concentrated to afford a white solid. Crystallisation from methanol gave dimethyl *cis*-2,6-piperidinedicarboxylate (39) as a white powder (0.94 g, 94%), m.p. 210 - 212 °C; υ_{max} 2990, 1740, 1320 and 1150 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.38 - 1.83 (4H, m), 2.40 (2H, m) 3.80 (6H, s) and 4.20 (2H, m); *m/z* 201 (M^+, 28%), 147 (42%) and 84 (100%) (Found: C, 53.78; H, 7.53; N, 6.96. $C_9H_{15}O_4N$ requires C, 53.77; H, 7.52; N, 6.96%).

Methyl DL-Pipecolinate Hydrochloride (37)

A solution of DL-pipecolinic acid (24) (1.0 g, 7.75 mmol) in distilled 2,2-dimethoxypropane (16 ml) with conc. HCl (8.8 ml)

was heated at reflux for 30 min, then stirred at room temperature overnight. The solution was concentrated to give a yellow solid. Crystallisation from methanol and diethyl ether gave methyl DL-pipecolinate (37) as a white powder (1.10 g, 93%), m.p. 182 - 186 °C; R_F ($CHCl_3$) 0.58; υ_{max} 2990, 1750, 1250 and 1100 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.30 - 1.72 (5H, m), 2.12 (1H, m), 2.82 (1H, m), 3.22 (1H, m), 3.55 (3H, s) and 3.75 (1H, m); δ_C (50 MHz) ($CDCl_3$) 22.17 (t), 22.32 (t), 26.51 (t) 44.75 (d), 53.42 (q), 57.67 (d) and 172.59 (s); *m/z* 143 (M^+, 42%) and 84 (100%) (Found: C, 58.80; H, 9.15; N, 9.79. $C_7H_{13}O_2N$ requires C, 58.79; H 9.16; N, 9.79%).

Methyl L-Prolinate Hydrochloride (36)

L-Proline (2.50 g, 21 mmol) was treated with 2,2-dimethoxypropane and conc. HCl as in the above procedure to give methyl L-prolinate hydrochloride (36) as a white powder (2.2 g, 81%), m.p. 228 - 232 °C; R_F ($CHCl_3$) 0.49; $[\alpha]_D$ -30°; υ_{max} 2990, 1750, 1250 and 1100 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.98 - 2.10 (2H, m), 2.42 (2H, m), 3.58 (2H, m), 3.85 (3H, s), 4.52 (1H, m) and 5.80 (1H, s); *m/z* 130 (M^+, 61%) and 72 (100%) (Found: C, 55.51; H, 9.32; N, 10.78. $C_6H_{12}O_2N$ requires C, 55.54; H, 9.30; N, 10.78%).

Dimethyl N-*Nitroso*-cis-*2,6-piperidinedicarboxylate*

A mixture of *cis*-2,6-piperidinedicarboxylic acid (27) (1.0 g, 5.8 mmol) and sodium nitrite (0.67 g, 10 mmol) in 1M HCl (15 ml) was stirred for 1 h at room temperature. The aqueous solution

was extracted with dichloromethane (3 x 20ml). The combined extracts were dried, filtered and concentrated to give a yellow solid. Addition of methanol and concentration of the solution with heating gave dimethyl *N*-nitroso-*cis*-2,6-piperidinedicarboxylate (51) as a yellow oil (0.8 g, 63%); R_F ($CHCl_3$) 0.38; υ_{max} (liq. film) 2990, 1740, 1480 and 1160 cm^{-1} ; δ_H (250 MHz) ($CDCl_3$) 1.10 (4H, m), 1.52 (2H, m) and 6.05 (2H, m); δ_C (50 MHz) ($CDCl_3$) 17.21 (t), 24.89 (t), 26.03 (t), 48.46 (d), 52.09 (q), 54.27 (d), 168.72 (s), 169.33 (s); *m/z* 230 (M^+, 21%,) 172 (14%) 114 (100%) and 84(32%); (Found: M^+, 230.0980. $C_9H_{14}O_5N_2$ requires M^+, 230.1520).

Methyl N-Nitroso-DL-pipecolinate (47) and (48)[38]

DL-Pipecolinic acid (24) was treated with sodium nitrite in 1M HCl as described above to give methyl *N*-nitroso-DL-pipecolinate as a mixture of *syn*- (47) and *anti*- (48) isomers as a yellow oil (70% yield), R_F ($CHCl_3$) 0.42; υ_{max} (liq. film) 2980, 1740, 1430 and 1180 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.62 (12H, m), 2.31 (1H, m), 3.75 (6H, m), 3.85 (1H,m), 4.80 (1H, m), 4.95 (1H, m), 5.55 (1H, m) and 5.72 (1H, m); δ_C (250 MHz) ($CDCl_3$) 20.42 (t) 23.37 (t), 25.90 (t), 39.36 (t), 48.69 (t), 50.15 (q), 61.14 (d) and 170.85 (s); *m/z* 172 (M^+, 42%), 142 (54%) and 84 (100%); (Found: M^+, 172.1342. $C_7H_{12}O_3N_2$ requires M^+, 172.173).

Methyl N-*Nitroso*-L-*prolinate (49) and (50)*[38]

L-Proline (34) was treated with sodium nitrite in 1M HCl as described previously to give methyl *N*-nitroso-L-prolinate as a

mixture of *syn-* (49) and *anti-* (50) isomers as a yellow oil (75% yield); R_F ($CHCl_3$) 0.39; $[\alpha]_D$ -114 °; υ_{max} (liq. film) 2950, 1740, 1440 and 1150 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 2.15 (4H, m), 2.40 (4H, m), 4.35(2H, m), 3.50 (3H, s), 3.72 (1H, m), 4.58 (2H, m) and 5.30 (1H, m); *m/z* 158 (M^+, 29%) and 69 (100%) (Found: M^+, 158.095. $C_6H_{10}O_3N_2$ requires M^+, 158.1400).

*Dimethyl N-Tosyl-*cis*-2,6-piperidinedicarboxylate (41)*

Dimethyl *cis*-2,6-piperidinedicarboxylate (39) (2.50 g, 12.5 mmol) in pyridine (25 ml) with *p*-toluene sulphonyl chloride (tosyl chloride) (4.80 g, 24 mmol) was stirred at room temperature for 24 h. The reaction mixture was poured into water (25 ml) and the aqueous solution was extracted with dichloromethane (3 x 30 ml). The combined extracts were dried, filtered and concentrated to an oil. Crystallisation from methanol and water gave dimethyl *N*-tosyl-*cis*-2,6-piperidinedicarboxylate (41) (2.90 g, 65%), m.p. 58 - 61 °C; R_F ($CHCl_3$) 0.61; υ_{max} 2930, 1730, 1360 and 1160 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.52 (3H, m), 1.80 (3H, m), 2.30 (3H, s), 3.46 (6H, s), 4.60 (2H, m), 7.30 and 7.70 (4H, AA´XX´ system, *J* 8Hz); δ_C (50 MHz) ($CDCl_3$) 15.94 (t), 21.39 (t), 21.55 (t), 51.85 (q), 52.89 (d), 124.87 (d), 126.67 (d), 138.67 (s), 142.54 (s) and 170.73 (s); *m/z* 296 (M^+,21%), 236 (18%) and 84 (100%) (Found: C, 53.99; H, 5.96; N, 3.94. $C_{16}H_{19}O_6SN$ requires C, 54.00; H, 5.96; N, 3.94%).

Methyl N-*Tosyl*-DL-*Pipecolinate (44)*

Methyl DL-pipecolinate was treated with tosyl chloride in pyridine as described above to give methyl *N*-tosyl-DL-pipecolinate (44) as white crystals (61% yield), m.p. 69 - 71 °C, R_F ($CHCl_3$) 0.41; υ_{max} 2980, 1740, 1360 and 1150 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.47 - 1.51 (5H, m), 2.15 (1H, m), 2.40 (3H, s), 3.20 (1H, m), 3.61 (3H, s), 3.78 (1H, m), 4.80 (1H, m), 7.25 and 7.68 (4H, AA´XX´ system *J* 8Hz); δ_C (50 MHz) ($CDCl_3$) 19.77 (q), 21.31 (t), 24.36 (t), 27.50 (t), 42.35 (t), 51.55 (q), 54.76 (d), 126.90 (d) 129.22 (d), 136.68 (s), 142.95 (s) and 171.00 (s); *m/z* 298 (M^+, 51%) and 69 (100%) (Found: C, 56.61, H, 6.42; N, 4.71. $C_{14}H_{19}O_4NS$ requires C, 56.60; H, 6.44; N, 4.71%).

Methyl N-*Tosyl*-L-*Prolinate (46)*

Methyl L-prolinate was treated with tosyl chloride in pyridine as described above to give methyl *N*-tosyl-L-prolinate (46) as white crystals (75% yield), m.p. 74 - 76 °C, R_F ($CHCl_3$) 0.28; υ_{max} 2980, 1750, 1350 and 1150 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.70 (1H, m), 2.00 (3H, m), 2.42 (3H, s), 3.21 (1H, m), 3.48 (1H, m), 3.60 (3H, s), 4.20 (1H, m), 7.21 and 7.80 (4H, AA´XX´, *J* 8Hz); δ_C (50 MHz) ($CDCl_3$) 21.45 (q), 24.53 (t), 30.79 (t), 48.34 (t), 52.32 (q), 60.27 (d), 127.38 (d), 129.85 (d), 134.97 (s), 143.56 (s) and 172.52 (s); *m/z* 284 (MH^+, 61%) and 130 (100%) (Found: C, 54.97; H, 6.05; N, 4.95. $C_{13}H_{17}O_4NS$ requires C, 54.98; H, 6.06; N, 4.94%).

Dimethyl N-*Mesyl*-cis-*2,6-piperidinedicarboxylate (40)*

A mixture of dimethyl *cis*-2,6-piperidinedicarboxylate (0.75 g, 3.75 mmol), methanesulphonyl chloride (mesyl chloride) (0.38 ml, 5.6 mmol) and triethylamine (0.50 ml, 3.75 mmol) was stirred for 18 h at room temperature under nitrogen. Water (20 ml) was added and the aqueous solution was extracted with dichloromethane (3 x 25 ml). The combined extracts were dried, filtered, and concentrated to an oil. Crystallisation from diethyl ether gave dimethyl *N*-mesyl*cis*-2,6-piperidinedicarboxylate (40) as white needles (0.54 g, 43%), m.p. 102 - 105 °C; υ_{max} 2970, 1730, 1310, and 1245 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.60 (4H, m), 2.05 (2H, m), 3.08 (3H, s), 3.62 (6H, s) and 4.75 (2H, m); *m/z* 279 (M^+, 6%), 221 (20%) 142 (60%) and 84 (100%) (Found: C, 43.07; H, 6.15; N, 5.02. $C_{10}H_{17}O_6SN$ requires C, 43.05; H, 6.14; N, 5.02%).

Methyl N-*Mesyl*-DL-*pipecolinate (43)*

Methyl DL-pipecolinate was treated with mesyl chloride and triethylamine in dichloromethane as described above to give methyl *N*-mesyl-DL-pipecolinate (43) as white needles (68% yield), m.p. 91 - 93 °C; R_F ($CHCl_3$) 0.89; υ_{max} 2980, 1720, 1310, and 1250 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.74 (5H, m), 2.20 (1H, m), 3.00 (3H, s), 3.18 (1H, m), 3.68 (1H, m), 3.70 (3H, s) and 4.75 (1H, m); *m/z* 222 (M^+, 25%), 164 (59%) and 84 (100%) (Found: C, 43.30; H, 5.92; N, 6.30. $C_8H_{15}O_4SN$ requires C, 43.28; H, 5.90; N, 6.31%).

Dimethyl N-*Triflyl*-cis-*2,6-piperidinedicarboxylate (42)*

A mixture of dimethyl *cis*-2,6-piperidinedicarboxylate (1.00 g, 5 mmol) and trifluromethanesulphonic (triflic) anhydride (1.41 g, 5 mmol) in pyridine (30 ml) was stirred at 0 °C for 4 h. The reaction mixture was poured into ice water (30 ml) and the aqueous solution was extracted with dichloromethane (3 x 20 ml). The combined extracts were dried, filtered and concentrated to an oil. Purification was achieved by a silica column, eluting with hexane and adding increasing proportions of dichloromethane to give dimethyl *N*-triflyl-*cis*-2,6-piperidinedicarboxylate (42) as a yellow oil (1.20 g, 72%); R_F ($CHCl_3$) 0.48; υ_{max} (liq film) 2980, 1740, 1600, 1360, 1250 and 1100 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.24 (2H, m), 1.50 (2H, m), 1.83 (2H, m), 3.40 (6H, s) and 4.00 (2H, m); *m/z* 201 (6%), 142 (84%) and 84 (100%).

Methyl N-*Triflyl*-DL-*pipecolinate (45)*

Methyl DL-pipecolinate was treated with triflic anhydride in pyridine as described above to give methyl *N*-triflyl-DL-pipecolinate (45) as a yellow oil (69% yield), R_F ($CHCl_3$) 0.42; υ_{max} (liq. film) 2980, 1740, 1600, 1360 and 1100 cm^{-1}; δ_H (250 MHz) ($CDCl_3$) 1.74 - 1.80 (5H, m), 2.25 (1H, m) 3.12 (1H, m) 3.60 (1H, m), 3.80 (3H, s) and 4.00 (1H, m); *m/z* 275 (M^+, 2%), 142 (24%) and 84 (100%).

General Procedure for Attempted Imine Formation using KH

To the *N*-substituted, heterocyclic esters (2 mmol) in dry THF (25 ml) was added KH (0.30 g). The suspension was heated at reflux for 4 h, then cooled and poured into isopropanol (30 ml). Water (20 ml) was added and the solution was extracted with dichloromethane (3 x 15 ml). The combined extracts were dried, filtered, and concentrated. The residue was studied by ^{1}H NMR spectroscopy. The aqueous layer was freeze dried and the residue was examined by ^{1}H and ^{13}C NMR spectroscopy. The range of compounds used is shown in Table 2.

General Procedure for Attempted Imine Formation using DBU Or DBN

A solution of DBU or DBN (4 mmol) in toluene (2 ml) was added to a solution of *N*-substituted heterocyclic ester (3 mmol) in toluene (8 ml). The mixture was heated at reflux for 72 h. The solution was cooled to room temperature and poured into ice water (15 ml) and the aqueous solution was extracted with dichloromethane (3 x 10ml). The combined extracts were dried, filtered and concentrated. The DBU or DBN was removed from the residue by flash chromatography on silica, eluting with dichloromethane. Elution with methanol gave starting material. The range of compounds used is shown in Table 2.

General Procedure for Attempted Imine Formation using NaH

A mixture of *N*-substituted heterocyclic ester (2 mmol) and NaH (3 mmol) in DMSO (10 ml) was heated at reflux for 72 h. The reaction mixture was cooled and poured into isopropanol (30 ml). Water (20 ml) was added and the aqueous solution was acidified with 1M HCl and extracted with dichloromethane (3 x 15ml). The combined extracts were dried, filtered, and concentrated to give starting material. The range of compounds used is shown in Table 2.

Attempted Imine Formation using Potassium bis(trimethylsilyl)amide

A mixture of the *N*-substituted heterocyclic ester and potassium bis(trimethylsilyl)amide (0.5M solution in toluene, 6.5 ml) in DMSO (20 ml) was heated at 60 °C for 4 h under nitrogen. The solution was poured into water (15 ml) and acidified with 1M HCl. The aqueous solution was extracted with dichloromethane (3 x 15 ml). The combined extracts were dried, filtered, and concentrated. The residue was examined by ^{1}H and ^{13}C NMR spectroscopy. The aqueous layer was freeze dried and the residue was examined by ^{1}H and ^{13}C NMR spectroscopy. The range of compunds used is shown in Table 2.

Attempted Imine Formation using Potassium t-butoxide

The *N*-substituted heterocyclic ester (4 mmol) in dry dichloromethane (20 ml) under nitrogen was treated with

potassium t-butoxide (12 mmol). The solution was stirred for 24 h at room temperature, then the solution was washed with 1M HCl (2 x 15 ml). The organic layer was dried, filtered, and concentrated and the residue examined by ^{1}H and ^{13}C NMR spectroscopy. Compounds used are shown in Table 2.

Potassium Tetrahydrodipicolinate

A mixture of *N*-tosyl-*cis*-2,6-piperidinedicarboxylate (41) (4.0 g, 13 mmol) and potassium t-butoxide (4.50 g, 40 mmol) in dry dichloromethane (25 ml) under nitrogen was stirred for 4 h. The solid was filtered off, dissolved in water (25 ml) and stirred with Amberlite 1R-45 anion exchange resin (OH form) (30 g) for 24 h. The resin was filtered off and the filtrate was concentrated to a solid. Crystallisation from methanol and ether afforded a a yellow hydroscopic solid (1.30 g, 41%). From ^{1}H and ^{13}C NMR spectroscopy this was mainly a mixture of 2,3,4,5-tetrahydrodipicolinic acid (3) and 2-oxo-6-aminopimelic acid (52) as their potassium salts. m.p. >300 ^{o}C; υ_{max} 3300, 1600 and 1400 cm^{-1}; δ_H (200 MHz) (D_20) 1.55 - 1.72 (8H, m), 2.25 (4H, m) and 4.30 (2H, m); δ_C (50 MHz) (D_20) 18.60 (t), 22,49 (t), 25.99 (t), 26.38 (t), 57.26 (d), 63.79(d), 171.86 (s), 175.42 (s), 182.74 (s) and 216.72 (s); *m/z* (FAB) 171 (M^+).

cis-*2,6-Piperidinedicarboxylic Acid*

2,3,4,5-Tetrahydrodipicolinic acid (3) (0.50 g, mmol) in water (10 ml) was hydrogenated at atmosperic pressure and room

temperature for 12 h using PtO_2 (0.005 g) as catalyst. The catalyst was removed by filtration through Celite, and the filtrate acidified with conc. HCl to precipitate a white solid. The solid was filtered off and dried over P_2O_5 to give *cis*-2,6-piperidinedicarboxylic acid (27) (0.49 g, 97%), m.p. 290 - 295 °C (lit.[22], 290 - 295 °C); ^1H and ^{13}C spectroscopy same as before.

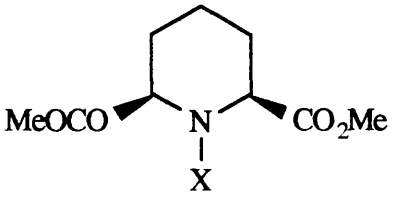
Potassium 3,4-Dihydro-2H-pyrrole-5-carboxylate (57)

Methyl *N*-tosyl-L-prolinate (46) in dichloromethane was treated with potassium t-butoxide as described above to give potassium 3,4-dihydro-2*H*-pyrrole-5-carboxylate (57) as a white powder (65% yield) m.p. > 250 °C; υ_{max} 2980, 1630, 1600 and 1100 cm^{-1}; δ_H (200 MHz) (D_20) 1.75 (2H, m), 2.55 (2H, m) and 3.70 (2H, m); δ_C (50 MHz) (D_2O) 23.02 (t), 36.64 (t), 61.41 (t), 172.70 (s) and 176.58 (s); *m/z* 113 (M^+, 2%) and 69 (46%).

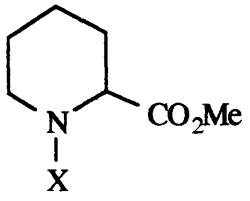
Potassium Tetrahydropicolinate (55)

Methyl *N*-tosyl-DL-pipecolinate in dichloromethane was treated with potassium *t*-butoxide as described above to give a mixture of the potassium salts of 2,3,4,5-tetrahydropicolinic acid (55) and the enamine compound (54) as a yellow hydroscopic powder (35% yield); m.p. > 300 °C; υ_{max} 2990, 1630, 1600 and 1100 cm^{-1}; δ_H (200 MHz) (D_20) 1.40 - 1.62 (10H, m), 3.34 (4H, m) and 6.65 (1H, dd); (50 MHz) (D_20) 19.88 (t), 21.61 (t), 22.96 (t), 48.84 (t), 171.02 (s) and 174.87 (s).

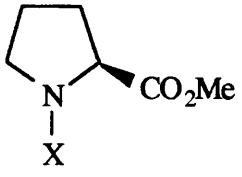
Compounds used for Attempted Imine Formation



(40) X = Ms
(41) X = Ts
(42) X = Tf
(51) X=NO



(43) X = Ms
(44) X = Ts
(45) X = Tf
(47), (48) X=NO



(46) X = Ts
(49, (50) X = NO

Each of the above *N*-substituted heteroyclic ester was treated with a variety of bases. The following Table shows the bases used in their reaction with each compound and the result obtained. The general procedures used in the attempted imine formation are described for each base used.

Table 2

Base	DBU	DBN	NaH	KH	$KNTMS_2$	KO^tBu
	a	a	c	b	d	e, f
Compd.						
40	SM	SM	SM	MP	MP	
41	SM	SM	SM	MP		P[f]
42			SM	MP	MP	
43	SM	SM	SM	MP	MP	
44	SM	SM	SM	MP		P[f]
45	SM	SM	SM		MP	
46	SM	SM	SM	MP	MP	P[f]
47, 48	SM	SM	SM	SM	SM	SM[e]
49, 50	SM	SM	SM	SM	SM	SM[e]
51	SM	SM	SM	SM	SM	SM[e]

SM = Starting material recovered in organic extracts.

MP = Aqueous layer yielded a mixture of products.

P = Desired product.

a = Procedure on page 174, b = Procedure on page 174 c = Procedure on page 175, d = Procedure on page 175, e = Procedure on page 175, f = Procedure on page 176 - 177.

Attempted Synthesis of Dimethyl N-Nitroso-2-methyl-cis-2,6-piperidinedicarboxylate

Dimethyl *N*-nitroso-*cis*-2,6-piperidinedicarboxylate (0.46 g, 2 mmol) in dry THF (25 ml) with KH (0.30 g) was heated at reflux for 4 h. The solution was cooled to room temperature and methyl iodide (0.28 g) was added and the suspension was stirred for 2 h. Isopropanol was added to destroy the excess KH. Water (30 ml) was added and the solution was extracted with dichloromethane (3 x 25 ml). The combined extracts were

dried, filtered, and concentrated to give a yellow oil. From ^1H NMR spectroscopy this was starting material.

8.4 Experimental to Chapter 5
DIPICOLINIC ACID ANALOGUES

2,6-Pyridinedicarboxamide (58)

Dipicolinic acid (26) (1.50 g, 9 mmol) in thionyl chloride was heated at reflux for 18 h. The solution was cooled and excess thionyl chloride was removed under reduced pressure. The yellow oil was added to conc. ammonia solution cooled to O °C. The precipitate was filtered off and dried to give 2,6-pyridinedicarboxamide (58) as a white powder (0.75 g, 51%), m.p > 300 °C (lit.[42], 320 - 323 °C), υ_{max} 3400, 3100, 1670, and 1590 cm^{-1}; δ_H (200 MHz) (d_6-DMSO) 7.82 (3H, m) and 9.10 (4H, s); *m/z* 181(M^+, 100%) and 122 (48%) (Found: M^+, 165.0531; C, 50.92; H, 4.27; N, 25.42. $C_7H_7O_2N_3$ requires M^+, 165.1260; C, 50.94; H, 4.27; N, 25.43%).

2,6-Pyridinedinitrile (59)

2,6-Pyridinedicarboxamide (58) (1.00 g, 6.0 mmol) in tetrachloroethane (10 ml) was heated to 110 °C. Phosphorus oxychloride (11.5 ml, 10 equiv.) was added over 10 min and the mixture was heated at reflux for 30 min. The solution was decanted from the solid and concentrated under reduced pressure. Chloroform (20 ml) was added and the solution was washed with 10% sodium carbonate solution (3 x 15 ml) and

water (2 x 15 ml). The chloroform layer was dried, filtered, and concentrated. 2,6-Pyridinedinitrile (59) was obtained as white crystals from chloroform (0.20 g, 26%); m.p. 128 - 131 °C (lit.[42], 128 - 132 °C); υ_{max} 2120, 1570 and 1450 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 7.91 (2H, m) and 8.09 (1H, m); δ_C (50 MHz) ($CDCl_3$) 115.31 (s), 131.15 (d), 135.12 (s) and 138.94 (d); *m/z* 129 (M^+, 100%) and 103 (48%) (Found: M^+, 129.0325. $C_7H_3N_3$ requires M^+, 129.0311).

2,6-Bis(1´H-tetrazol-5´-yl)pyridine (62)

A mixture of 2,6-pyridinedinitrile (59) (0.50 g, 3.87 mmol), NaN_3 (0.58 g, 8.9 mmol), and ammonium chloride (0.41 g, 8.9 mmol) in DMF (10 ml) under nitrogen was heated at 70 °C for 24 h. The solution was cooled and added to diethyl ether (50 ml). The precipitate was filtered off, washed with ether, and dried to give 2,6-bis(1´H-tetrazol-5´-yl)pyridine (62) as a white powder (0.75 g, 90%), m.p. > 300 °C; λ_{max} (MeOH) 292 nm (ε 9570) and λ_{max} 221 nm (ε 20450); υ_{max} 3000, 2500 and 1600 cm^{-1}; δ_H (200 MHz) (D_2O) 8.30 (s); δ_C (50 MHz) (D_2O) 122.59 (d), 139.60 (d), 148.42 (s) and 162.47 (s); *m/z* 215 (M^+, 26%) and 187 (100%) (Found: M^+, 215.0672. $C_7H_3N_9$ requires M^+, 215.0472).

Dimethyl 2,6-Pyridinediimidate (65)

Dry HCl gas was bubbled through a solution of 2,6-pyridinedinitrile (0.50 g, 3.9 mmol) in methanol (10 ml). The white crystalline solid that was filtered off and dried.

Recrystallisation from water gave methyl 2,6-pyridinediimidate (65) (0.56 g, 46%), m.p. > 300 °C; υ_{max} 2960, 1660, 1590, 1310 and 1100 cm^{-1}; δ_H (200 MHz) (D_2O) 7.34 (m); δ_C (50 MHz) (D_2O) 54.18 (q), 129.58 (d), 140.77 (d), 147.81 (s) and 166.65 (s); *m/z* 196 (M^+, 4%), 165 (20%), 136 (41%) and 80 (100%) (Found: C, 40.59; H, 4.92; N, 15.76. $C_9H_{13}O_2N_3Cl_2$ requires C, 40.62; H, 4.92; N, 15.79%).

4-Chlorodipicolinic Acid (77)

Method 1

A mixture of chelidamic acid (1.0 g, 5.46 mmol) and phenylphosphonic dichloride (4.32 g, 21 mmol) was heated at reflux for 2 h. The solution was cooled to 0 °C and water (20 ml) was added. The solution was basified with 1M NaOH solution. Additional water was added to dissolve the pink wax and addition of conc. HCl caused a white solid to precipitate. This was filtered off and recrystallisation from acetic acid gave white needles of 4-chlorodipicolinic acid (77) (0.75 g, 70%), m.p. 209 - 211°C (lit.[56], 208.5 °C); υ_{max} 3500, 3100, 1730, 1200 and 700 cm^{-1}; δ_H (90 MHz) (D_2O) 7.50 (m); *m/z* 201 (M^+, 16%); 166 (42%) and 121 (100%) (Found: C, 41.57; H, 2.49; N, 6.88. $C_7H_4O_4NCl$ requires C, 41.55; H, 2.51; N, 6.88%).

Method 2

Chelidamic acid (66) (1.50 g, 8.3 mmol) and phosphorous pentachloride (5.70 g) in of chloroform (50 ml) were heated at

reflux for 72 h. The resulting acid chloride was hydrolysed by adding the solution to cold water (20 ml). The solid that precipitated was filtered off and washed with cold water to remove the phosphoric acid. Recrystallisation from acetic acid gave 4-chlorodipicolinic acid (77) as white needles (0.75 g, 59%); m.p. 209 - 211 °C; spectroscopy as above.

Dimethyl 4-Chlorodipicolinate (78)

A mixture of chelidamic acid (1.0 g, 5.46 mmol) and phenylphosphonic dichloride (4.32 g, 21 mmol) was heated at reflux for 2 h. The solution was cooled to 0 °C and methanol (20 ml) was added to dissolve the wax. On cooling a white solid precipitated. The solid was filtered off and recrystallised from chloroform to give dimethyl 4-chlorodipicolinate (78) as white needles (1.12 g, 88%), m.p. 144 - 146 °C; R_F ($CHCl_3$) 0.36; υ_{max} 3100, 2980, 1720, 1590, 1440 and 1340 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 4.00 (6H, s) and 8.25 (2H, s); *m/z* 231 (M^+, 18%), 229 (M^+, 40%), 173 (100%) (Found: C, 47.06; H, 3.51; N, 6.11. $C_9H_8O_4Cl$ requires C, 47.07; H, 3.51; N, 6.10%).

Attempted Synthesis of 4-Aminodipicolinic Acid (79)

4-Chlorodipicolinic acid (0.30 g, 1.48 mmol) was sealed in a tube with liquid ammonia (10 ml) and left standing at room temperature for 3 d. The tube was cooled to -78 °C before opening and then allowed to warm to room temperature. The white solid left was dissolved in water (5 ml) and conc. HCl (1

ml) was added. The white solid that precipitated was filtered off and dried to give starting material (0.30 g).

8.4 SYNTHESIS OF CHELIDAMIC ACID AND ITS DERIVATIVES

Chelidamic Acid (66)

Chelidonic acid (67) (1.50 g, 7.5 mmol) in conc. ammonia solution (15 ml) was heated at reflux for 5 h. The solution was acidified with 1M HCl. A white solid precipitated and was recrystallised from boiling water to give chelidamic acid (66) (0.48 g, 35%), m.p. 242 - 246 °C (lit.[52], 248 °C); R_F($CHCl_3$-MeOH-AcOH: 74 : 24 : 1) 0.58; λ_{max} (H_2O) 274 nm (ε 12,400); υ_{max} 3400, 2850, 1730, 1640 and 1580 cm^{-1}; δ_H (200 MHz) (D_2O) 6.89 (s); δ_C (50 MHz) (D_2O) 117.50 (d), 144.52 (s), 166.59 (s), and 183.46 (s); *m/z* 183 (M^+, 17%), 139 (48%) and 95 (100%) (Found : C, 45.92; H, 2.74; N, 7.65. $C_7H_4O_5N$ requires C, 45.90; H, 2.72; N, 7.65%).

Dimethyl Chelidamate (75)

Chelidamic acid (2.00 g, 11 mmol) in methanol (25 ml) with conc. H_2SO_4 (5 ml) was heated at reflux for 18 h. Water (40 ml) was added and the solution was neutralised with sodium carbonate. The aqueous solution was acidified with conc. HCl and extracted with chloroform (3 x 20 ml). The combined organic extracts were dried, filtered and concentrated to leave a yellow oil. Crystallisation from methanol and diethyl ether

gave dimethyl chelidamate (75) as a white powder (1.69 g, 73%) m.p. 130 - 132 °C (lit.[54], 131 - 133 °C); υ_{max} 3440, 1740, 1600, 1580, 1450 and 1260 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 3.98 (6H, s) and 6.86 (2H, s); *m/z* 211 (M^+, 22%) and 152 (100%) (Found: M^+, 211.0477. $C_9H_9O_5N$ requires M^+, 211.171).

N-Methylchelidamic Acid (71)

Chelidonic acid (1.50 g, 7.5 mmol) in 30% methylamine solution (20 ml) was heated at reflux for 5 h. The solution was cooled and acidified with 1M HCl. A white solid precipitated and was recrystallised to give *N*-methylchelidamic acid (71) as a yellow powder (1.20 g, 80%), m.p. 198 - 200 °C (lit.[55], 197 - 200 °C), λ_{max} (H_2O) 265 nm (ε 24,230); υ_{max} 3400, 2850, 1730, 1630, and 1580 cm^{-1}; δ_H (200 MHz) (D_2O + NaOD) 2.65 (3H, s), and 6.78 (2H, s); δ_C (50 MHz) (D_2O + NaOD) 41.40 (q), 112.72 (s), 154.25 (d), 168.00 (s) and 175.00 (s); *m/z* 197 (M^+, 6%), 153 (34%) and 81 (100%) (Found: C, 48.70; H, 3.57; N, 7.10. $C_8H_7O_5N$ requires C, 48.67; H, 3.55; N, 7.11%).

Dimethyl N-*Methylchelidamate (76)*

N-Methylchelidamic acid (1.0 g, 5 mmol) in methanol (25 ml) with conc. H_2SO_4 (0.80 g) was heated at reflux for 18 h. The solution was cooled and water (20 ml) was added. The aqueous solution was basified with sodium carbonate. The solution was acidified with conc. HCl and extracted with chloroform (3 x 15 ml). The combined organic extracts were dried, filtered and concentrated to give dimethyl *N*-methylchelidamate (76) as a

yellow powder (0.68 g, 60%), m.p. 170 - 172 °C; υ_{max} 2960, 1750, 1630, 1480, and 1320 cm^{-1}; δ_H (200 MHz) (d_6-DMSO) 2.30 (3H, s), 3.98 (6H, s), and 6.89 (2H. s); δ_C (50 MHz) (d_6-DMSO) 39.91 (q), 53.74 (q), 120.15 (d), 144.08 (s), 162.59 (s) and 176.76 (s); *m/z* 225 (M^+, 25%), 166 (61%) and 108 (100%) (Found: M^+, 225.7635. $C_{10}H_{11}O_5N$ requires M^+, 225.8235).

N-Methoxychelidamic Acid (73)

A mixture of chelidonic acid (1.50 g, 7.5 mmol) in 25 - 30% methoxylamine hydrochloride solution (10 ml) with triethylamine (1.1 ml, 1 equiv) was heated at reflux for 5 h. The yellow solid that formed was filtered off and dried to give *N*-methoxychelidamic acid (73) as a yellow powder (1.10 g, 69%); m.p. > 250°C; λ_{max} (H_20) 299 nm (ε 16332); υ_{max} 3500, 3100, 1725, 1600, 1575, 1225 and 1050 cm^{-1}; δ_H (200 MHz) (D_2O + NaOD) 3.95 (3H, s), 6.50 (1H, m) and 6.95 (1H, m); δ_C (50 MHz) (D_2O + NaOD) 62.18 (q), 103.10 (d), 109.14 (d), 149.58 (s), 151.25 (s), 167.09 (s), and 167.12 (s); *m/z* 213 (M^+, 100%), 198 (20%) and 167 (32%) (Found: M^+, 213,0261. $C_8H_7O_6N$ requires M^+, 213.1361).

8.5 Experimental to Chapter 6

PIPERIDINE ANALOGUES

N-*Methyl*-cis-*2,6-piperidinedicarboxylate (80)*

A mixture of *cis*-2,6-piperidinedicarboxylic acid hydrochloride (0.50 g, 2.9 mmol), 37% formaldehyde solution (0.48 g), 90%

formic acid (0.60 ml) and sodium bicarbonate (0.25 g, 2.9 mmol) was heated at reflux for 17 h. Precipitated sodium chloride was filtered off and the filtrate was concentrated to an oil. Crystallisation from methanol gave *N*-methyl *cis*-2,6-piperidinedicarboxylic acid (80) as white crystals (0.36 g, 72%), m.p. 212 - 216 °C (lit.[22], 212 - 215 °C), υ_{max} 3450, 2990, 1740 and 1050 cm^{-1}; δ_H (200 MHz) (D_2O + NaOD) 1.75 - 1.82 (6H, m), 3.10 (3H, s) and 3.75 (2H, m); δ_C (50 MHz) (D_2O + NaOD) 22.01 (t), 28.41 (t), 42.95 (q), 69.24 (d) and 173.42 (s); *m/z* 187 (M^+, 6%), 142 (42%) and 82 (100%) (Found: C, 42.90; H, 6.27; N, 6.27. $C_8H_{13}O_4N$ requires C, 42.90; H, 6.30; N, 6.26%).

N-*Methyl*-cis-*2,6-piperidinedicarboxylic Acid N-Oxide (82)*

N-Methyl-*cis*-2,6-piperidinedicarboxylic acid (0.90 g, 5.3 mmol) in TFA (11.0ml) with 30% hydrogen peroxide (1.50 ml) was heated at reflux for 3 h. The solution was cooled and added to diethyl ether (250 ml). The precipitate was filtered off and dried to give *N*-methyl-*cis*-2,6-piperidinedicarboxylic acid *N*-oxide (82) as a white powder (0.60 g, 57%), υ_{max} 3400, 2990, 1750, 1200 and 970 cm^{-1}; δ_H (200 MHz) (D_2O + NaOD) 1.20 - 1.60 (6H, m), 2.00 (3H, s) and 2.50 (2H, m); δ_C (50 MHz) (D_2O + NaOD) 23.70 (t), 30.59 (t), 43.54 (q), 71.33 (d) and 183.66 (s); *m/z* 203 (M^+, 6%), 187 (42%), 142 (100%) and 82 (10%) (Found: C,47.79; H, 6.51; N, 6.97. $C_8H_{13}O_5N$ requires C, 47.80; H, 6.52; N, 6.97%).

Attempted Synthesis of 4-Chloro-cis-2,6-piperidinedicarboxylic Acid

4-Chloro-2,6-pyridinedicarboxylic acid (0.30 g, 1.48 mmol) in glacial acetic acid (15 ml) was hydrogenated at room temperature and atmospheric pressure with PtO_2 (0.30 g) as catalyst for 30 h. The catalyst was removed by filtration through Celite. The filtrate was acidified with conc. HCl (4 ml) to precipitate a white solid. The solid was filtered off and dried (0.25 g, 97%), m.p. 290 - 295 °C. From ^1H and ^{13}C NMR spectroscopy the product was found to be *cis*-2,6-piperidinedicarboxylic acid.

1,4-Pentadien-3-one-1,5-dicarboxylic acid (86)

2-Furanacrylic acid (85) (4.80 g, 34 mmol) in 30% aqueous hydrogen peroxide (50 ml) and conc. HCl (5 ml) was stirred at room temperature for 5 d. The orange crystals that formed were filtered off, washed with methanol, and dried to afford 1,4-pentadien-3-one-1,5-dicarboxylic acid (86) (2.3 g, 40%), m.p. 228 - 230 °C (lit.[60], 236 °C); υ_{max} 3425, 3000, 1700, 1670, 1640, 1400, 1310 and 1270 cm^{-1}; no suitable solvent was found for NMR spectroscopy; *m/z* 170 (M^+, 16%), 126 (100%) and 82(32%) (Found : M^+ 170.021. $C_7H_6O_5$ requires M^+ 170.055).

Dimethyl 1,4-Pentadien-3-one-1,5-dicarboxylate (87)

1,4-Pentadien-3-one-1,5-dicarboxylic acid (1.00 g, 5.8 mmol) in methanol (6 ml) and conc. H_2SO_4 (0.4 g) were heated at reflux for 30 min. The solution was concentrated to a yellow solid. Crystallisation from ethyl acetate gave dimethyl 1,4-pentadien-3-one-1,5-dicarboxylate (87) (0.87 g, 96%) m.p. 171 -172 °C (lit.[60], 171 - 172°C); υ_{max} 2990, 1720, 1670, 1640, 1250, and 1100 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 3.69 (6H, s), 6.60 and 7.15 (2H, AB system, *J* 15 Hz); δ_C (50 MHz) ($CDCl_3$) 51.88 (q), 132.02 (d), 137.03 (d), 170.54 (s) and 195.51 (s); *m/z* 198 (M^+ 100%) (Found: C, 54.56; H, 5.03. $C_9H_{10}O_3$ requires C, 54.52; H, 5.08%).

*Diammonium Piperidin-4-one-2,6-dicarboxylate (*cis- (88) *and* trans- *(89) isomers)*

1,4-Pentadien-3-one-1,5-dicarboxylic acid (0.6 g, 3.4 mmol) in conc. ammonia solution (15 ml) was heated at reflux for 3 h. The solution was concentrated to an oil. Drying over P_2O_5 gave diammonium piperidin-4-one-2,6-dicarboxylate as a mixture of *cis-* (88) and *trans-* (89) isomers as a light brown powder (0.58 g, 91%), m.p. > 300 °C (lit.[62], m.p. > 300 °C); υ_{max} 3450, 2970, 1620, 1590, and 1250 cm^{-1}; δ_H (200 MHz) (D_2O) for major isomer: 2.28 - 2.32 (4H, m) and 4.20 (2H, m); and minor isomer: 2.28 - 2.32 (4H, m) and 4.00 (2H, m); δ_C (50 MHz) (D_2O) for major isomer: 37.43 (t), 55.71 (d), 92.23 (d), 171.34 (s) and 176.85 (s); and minor isomer: 31.70 (t), 52.94 (d), 91.60 (d), 170.63 (s) and 176.85 (s); *m/z* 187 ($M^{\cdot+}$, 19%), 143 (42%) and

99 (100%) (Found: M^+, 187.1396. $C_7H_8O_5N$ requires M^+, 187.1421).

Diammonium N-Methylpiperidin-4-one-2,6-dicarboxylate (cis- (90) *and* trans- (91) *isomers)*

1,4-Pentadien-3-one-1,5-dicarboxylic acid (0.60 g, 3.4 mmol) in 30% methylamine solution (10 ml) was heated at reflux for 4 h the solution was concentrated to an oil under reduced pressure. Drying over P_2O_5 gave diammonium *N*-methylpiperidin-4-one-2,6-dicarboxylate as a mixture of *cis*- (90) and *trans*- (91) isomers as a light brown powder (0.61 g, 94%), m.p. > 300 °C; υ_{max} 3450, 2970, 1620, 1590, and 1250 cm^{-1}; δ_H (200 MHz) (D_2O) 2.25 - 2.86 (6H, m), 2.90 (3H, s), and 3.85 (2H, m); δ_C (50 MHz) (D_2O) 31.97 (q), 39.86 (t), 65.29 (d), 67.50 (d), 172.23 (s), 172.90 (s), and 178.34 (s); *m/z* 202 (12%), 187 (20%), 143 (45%), and 99 (100%) (Found: M^+, 202.1532. $C_8H_{11}O_5N$ requires M^+, 202.0560).

cis- *(93) and* trans-*2,6-Piperidinedinitrile (94)*[63]

A solution of glutaraldehyde sodium bisulphite addition compound (14 g, 45 mmol) in water (100 ml) with sodium cyanide (6.0 g, 120 mmol) and ammonium chloride (6.56 g, 120 mmol) was stirred for 10 d at room temperature. The aqueous solution was extracted with diethyl ether (3 x 30 ml) and hot toluene (3 x 30 ml). The combined organic extracts were dried, filtered and concentrated to give an oily solid. The solid was triturated with diethyl ether (3 x 8 ml) and the remaining solid

was a white powder of *cis*-2,6-piperidinedinitrile (93) (1.80 g, 30%), m.p. 66 - 68 °C; υ_{max} 3310, 2980, and 2230 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.79 - 1.84 (4H, m), 2.45 (2H, m) and 4.02 (2H, m); δ_C (50 MHz) ($CDCl_3$) 19.34 (t), 27.94 (t), 43.53 (d) and 118.87 (s); *m/z* 135 (M^+, 38%) and 80 (100%) (Found: M^+ 135.0794; C, 62.19; H, 6.71; N, 31.15. $C_7H_9N_3$ requires M^+ 135.1420; C, 62.22; H, 6.71;N, 31.09%). On cooling the filtrate gave *trans*-2,6-piperidinedinitrile (94) as white crystals (1.40g, 23%), m.p. 58 - 60 °C; υ_{max} 3310, 2980, and 2230 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.79 - 1.84 (4H, m), 2.45 (2H, m) and 4.02 (2H, m); δ_C (50 MHz) ($CDCl_3$) 19.85 (t), 27.94 (t), 44.31 (d), and 118.95 (s); *m/z* 135 (M^+, 5%), 108 (100%) and 80 (85%) (Found: M^+, 135.0793; C, 62.20, H, 6.72; N, 31.12. $C_7H_9N_3$ requires M^+, 135.1420; C, 62.22, H, 6.71; N, 31.09%).

cis-*2,6-Piperidinedicarboxylic Acid (27)*

A mixture of *cis*-2,6-piperidinedinitrile (0.50 g, 3.7 mmol) and barium hydroxide (0.40 g) in water (10 ml) was heated at reflux for 18 h. The reaction mixture was allowed to cool and solid carbon dioxide was added. The solid barium carbonate was filtered off and the filtrate acidified and concentrated under reduced pressure to give *cis*-piperidinedicarboxylic acid (27) (0.62 g, 97%), m.p. 290 - 295 °C (lit.[22], 290 - 295 °C); υ_{max} 3400, 3000, 2990, 1760, 1390, and 1200 cm^{-1}; δ_H (200 MHz) (D_2O) 1.48 - 1.52 (4H, m), 2.15 (2H, m), and 3.80 (2H, m); δ_C (50 MHz) (D_2O) 23.11 (t), 26.27 (t), 58.12 (d), and 172.42 (s); *m/z* 173 (M^+, 4%), 129 (21%), and 84 (100%) (Found: C, 39.98; H, 5.72; N, 6.68. $C_7H_{11}O_4N$ requires C, 40.10; H, 5.77; N, 6.65%).

trans-*2,6-Piperidinedicarboxylic Acid (32)*

A mixture of *trans*-2,6-piperidinedinitrile (0.50 g, 3.7 mmol) and barium hydroxide (0.40 g) was heated at reflux for 18 h. The reaction mixture was allowed to cool and solid carbon dioxide was added. The solid barium carbonate was filtered off and the filtrate was acidified and concentrated under reduced pressure to give *trans*-2,6-piperidinedicarboxylic acid hydrochloride (32) (0.61 g, 96%); m.p. 264 - 266 °C; υ_{max} 3300, 2990, 1760, 1390, 1290, and 1200 cm^{-1}; δ_H (200 MHz) (D_2O) 1.48 - 1.52 (4H, m), 2.15 (2H, m), and 4.15 (1H, m); δ_C (50MHz) (D_2O) 22.48 (t), 26.06 (t), 58.45 (d), and 172.41 (s); *m/z* 173 (M^+, 8%), 129 (31%), and 84 (100%) (Found: C, 39.97; H, 5.74; N, 6.68. $C_7H_{11}O_4N$ requires C, 40.10; H, 5.74; N, 6.65%).

Dimethyl 3,3-Dimethylglutarate

3,3-Dimethylglutaric acid (101) (0.50 g, 3 mmol) in thionyl chloride was heated at reflux for 45 min. The solution was cooled and added to methanol (7 ml) at 0 °C. Water 10 ml was added and the aqueous solution was extracted with diethyl ether (3 x 10 ml). The combined organic extracts were dried, filtered and concentrated to an oil. Distillation gave dimethyl 3,3-dimethylglutarate as a clear oil (0.42 g, 72 %), b.p. 70 - 74 °C (2 mm Hg); υ_{max} (liq. film) 2990, 1700, 1430, 1335, and 1150 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 0.90 (6H, s), 2.43 (4H, s), 3.65 (6H, s); δ_C (50 MHz) ($CDCl_3$) 17.05 (q), 20.15 (s), 52.76 (t), 58.97

(q); *m/z*, 88 (M^+, 100%), 73 (32%), and 58 (26%) (Found: C, 57.52; H, 8.55. $C_9H_{16}O_4$ requires C, 57.49; H, 8.58%).

Attempted Synthesis of 3,3-Dimethylglutaraldehyde
Method 1

Dimethyl 3,3-dimethylglutarate (0.25 g, 1.3 mmol) in dry CH_2Cl_2 (10 mml) was cooled to -78 °C. Diisobutylaluminum hydride (DIBAL) (3.13 ml) was added dropwise to the solution under nitrogen. The solution was stirred for 20 min at this temperature. Analar methanol (2.34 ml) was added and the solution was stirred for 45 min at room temperature The reaction mixture was filtered to remove the white solid and the filtrate was concentrated to an oil. From NMR spectroscopy this was mainly starting material.

Attempted Synthesis of 3,3-Dimethylglutaraldehye
Method 2

A mixture of 3,3-dimethylpentane-1,5-diol (0.50 g, 3.8 mmol), 4-methylmorpholine *N*-oxide (0.74 g, 1.5 equiv), tetrapropylammonium perruthenate (TPAP) (0.15 g, 0.5 mole %) and 4 Å molecular sieves in dry CH_2Cl_2 (15 ml) was stirred for 24 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (20 ml) and washed with 10% aqueous sodium sulphite (2 x 15 ml), brine (2 x 15 ml), and 10% aqueous copper(II) sulphate (2 x 15 ml). The organic layer was dried, filtered, and concentrated to give starting material (0.19 g).

Attempted Synthesis of 3,3-Dimethylglutaraldehyde
Method 3

A mixture of dry CH_2Cl_2 (25 ml) and distilled oxalyl chloride (2.0 ml, 22 mmol) under nitrogen was cooled to -60 °C. Distilled DMSO (3.40 ml, 44 mmol) diluted with CH_2Cl_2 (15 ml) was added and the solution was stirred for 2 min. 3,3-Dimethylpentane-1,5-diol in dry CH_2Cl_2 (5 ml) was added over 5 min and the mixture was stirred for 15 min. Distilled triethylamine (14.0 ml, 100 mmol) was added over 5 min and the solution was allowed to reach room temperature. Water (5 ml) was added and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 ml). The combined organic extracts were washed with brine (3 x 10 ml). The organic layer was dried, filtered and concentrated to give unreacted diol.

Diethyl 3,3-Dimethylglutarate

3,3-Dimethylglutaric acid (0.50 g, 3 mmol) in thionyl chloride (2.2 ml) was heated at reflux for 45 min. The solution was cooled and added to ethanol (7 ml) at O °C. Water (10 ml) was added and the aqueous solution was extracted with diethyl ether (3 x 10 ml). The combined ether extracts were dried, filtered and concentrated to an oil. Distillation gave diethyl 3,3-dimethylglutarate as a clear oil (0.49 g, 75%), b.p. 90 - 94 °C (2 mmHg); υ_{max} (liq. film) 2990, 1705, 1430, 1335, and 1150 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 0.90 (6H, s), 1.31 (6H, t), 2.45 (4H, s) and 4.10 (4H, q); δ_C (50 MHz) ($CDCl_3$) 16.40 (q), 20.09 (s), 26.41

(q), 51.62 (t), 84.60 (t) and 175.20 (s); *m/z* 216 (M^+, 100%), 143 (40%) and 71 (26%) (Found: C, 76.60; H, 11.69, $C_{11}H_{20}O_4$ requires C, 76.62; H, 11.66%).

4,4-Dimethyl-2-hydroxycyclopentanone (99) and 4,4-Dimethylcyclopentan-1,2-dione (98)

Dry diethyl ether (50 ml) and liquid ammonia (75 ml) were added to a dry three necked flask fitted with a nitrogen inlet and a dry ice condenser. Sodium (0.54 g) was added and the system was swept with nitrogen. A solution of diethyl 3,3-dimethylglutarate (1.00 g, 4.6 mmol) in diethyl ether (25 ml) was added with stirring over 1 h. The ammonia was allowed to evaporate off overnight. A mixture of methanol (1 ml) and ether (1 ml) was added to destroy the sodium. The resulting mixture was acidified with 6M HCl (20 ml). Water (5 ml) was added and the solution was extracted with diethyl ether (4 x 30 ml). The combined ether extracts were dried, filtered and concentrated to an oil. The oil was a 1 : 1 mixture of 4,4-dimethyl-2-hydroxycyclopentanone (99) and 4,4-dimethylcyclopentan-1,2-dione (98), R_F ($CHCl_3$-ethanol, 75:25) 0.58 and 0.63; υ_{max} (liq. film) 3400, 2960, 1730, 1630 and 1380 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) for 4,4-dimethyl-2-hydroxycyclopentanone (99): 0.90 (6H, s), 1.20 (2H, m), 2.25 (2H, s), and 3.82 (1H, m); δ_H (90 MHz) ($CDCl_3$) for 4,4-dimethylcyclopentan-1,2-dione (98): 0.90 (6H, s), 2.25 (2H, s), and 6.65 (1H, s); *m/z* 128 (M^+, 24%), 126(M^+, 23%), 111 (42%) and 98 (100%).

4,4-Dimethyl-2-hydroxycyclopentanone (99)

The mixture of 4,4-dimethyl-2-hydroxycyclopentanone and 4,4-dimethylcyclopentan-1,2-dione (1.00 g, 7.8 mmol) in ethanol (25 ml) was hydrogenated at room temperature and atmospheric pressure for 4 h with PtO_2 (0.05 g) as catalyst. The catalyst was removed by filtration through Celite and the filtrate was concentrated to give 4,4-dimethyl-2-hydroxycyclopentanone (99) as a clear oil (0.96 g, 96%), R_F (CH_2Cl_2) 0.59; υ_{max} (liq. film) 3350, 2980, 1730 and 1380 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 0.90 (6H, s), 1.20 (2H, m), 2.25 (2H, s), 3.82 (1H, m) and 4.90 (1H, s); *m/z* 128 (M^+, 29%), 113 (40%) and 99 (100%) (Found: M^+, 128.0549. $C_7H_{12}O_2$ requires M^+, 128.1730).

cis-*4,4-Dimethylcyclopentane-1,2-diol (100)*

4,4-Dimethyl-2-hydroxycyclopentanone (1.00 g, 7.8 mmol) was added to a mixture of lithium aluminium hydride (0.60 g, 17 mmol) in dry THF (12 ml) under nitrogen. The mixture was stirred for 18 h at room temperature. Water (3 ml) was added and the inorganic salts were filtered off and washed with ether. The filtrate was dried, filtered and concentrated to give *cis*-4,4-dimethylcyclopentane-1,2-diol (100) as a clear oil (0.93 g, 91%), R_F (CH_2Cl_2) 0.71; υ_{max} (liq film) 3350, 2990 and 1380 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.10 (6H, s), 1.42 (4H, d), 3.20 (2H, s) and 3.40 (2H, m); δ_C (50 MHz) ($CDCl_3$) 16.95 (q), 27.43 (s), 28.50 (t) and 68.44 (d); *m/z* 130 (M^+, 41%), 115 (20%) and 99 (100%) (Found: C, 64.59; H, 10.78. $C_7H_{14}O_2$ requires C, 64.62; H, 10.77%).

Attempted Synthesis of 3,3-Dimethylglutaraldehye (96)
Method 4

Periodic acid (0.387 g, 1.69 mmol) in THF (5 ml) was added to a solution of *cis*-4,4-dimethylcyclopenta-1,2-diol (0.20 g, 1.54 mmol) in THF (5 ml). A white solid formed immediately and stirring was continued for 20 min. The solid was filtered off and the filtrate was concentrated to an oil that crystallised on standing to give 3,3-dimethylglutaric acid, m.p. 100 - 102 °C; υ_{max} 3450, 2990, 1710 and 1380 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.10 (6H, s), 2.49 (4H, s) and 11.90 (2H, s).

3,3-Dimethylpentane-1,5-diol

3,3-Dimethylglutaric acid (101) (1.0 g, 6.25 mmol) in dry THF was brought to reflux under nitrogen. 1M Borane in THF (12.5 ml) was added dropwise and the mixture was heated at reflux for 18 h. The solution was cooled and water (20 ml) was added. The aqueous solution was saturated with potassium carbonate and extracted with diethyl ether (4 x 20 ml). The combined extracts were dried, filtered and concentrated to an oil. Distillation gave 3,3-dimethylpentane-1,5-diol as a clear oil (0.68 g, 81%), b.p. 140 °C (1 mmHg); υ_{max}(liq film), 3460, 2960, 1385 and 1250 cm^{1}; δ_H (200 MHz) ($CDCl_3$) 0.90 (6H, s), 1.52 (4H, t), 3.00 (2H, s) and 3.62 (4H, t); δ_C (50 MHz) ($CDCl_3$) 18.08 (q), 21.49 (s), 43.75 (t) and 59.13 (t); *m/z* 99(100%) and 87(42%) (Found: C, 64.58; H, 12.19. $C_7H_{16}O_2$ requires C, 63.59; H 12.20%).

3,3-Dimethylpentane-1,5-diol Dimethyl-sulphonate

3,3-Dimethylpentane-1,5-diol (1.50 g, 11 mmol) in dry THF (22 ml) was cooled to -78 °C. Methanesulphonyl chloride (1.76 ml, 56 mmol) followed by triethylamine (2.32 g, 22 mmol) were added to the solution and the mixture was stirred for 18 h. Water (100 ml) was added and the aqueous solution was extracted with dichloromethane (3 x 75 ml). The combined extracts were dried, filtered, and concentrated to an oil. Crystallisation from diethyl ether gave 3,3-dimethylpentane-1,5-diol dimethylsulphonate as white needles (2.1 g, 64%), m.p. 61 - 63 °C; υ_{max} 2900, 1300 and 1170 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 0.95 (6H, s), 1.70 (4H, t), 2.96 (6H, s), and 4.28 (4H, t); *m/z* 97 (100%) and 81 (59%) (Found : C, 37.49; H, 6.97. $C_9H_{20}O_6S_2$ requires C, 37.48; H, 6.99%).

3,3-Dimethylpentane-1,5-dinitrile

3,3-Dimethylpentane-1,5-diol dimethylsulphonate (0.50 g,1.77 mmol) with sodium cyanide (0.17 g, 3.5 mmol) in dry DMSO (6.0 ml) under nitrogen was heated at 85 - 90 °C for 18 h. The reaction mixture was cooled and dichloromethane (15 ml) was added. The solution was washed with brine (6 x 15 ml). The organic layer was dried, filtered, and concentrated to an oil. Purification was achieved using a silica column eluting with hexane and increasing proportions of dichloromethane to give 3,3-dimethylpentane-1,5-dinitrile as a yellow oil (0.15 g, 54%); $R_F(CH_2Cl_2)$ 0.51; υ_{max}(liq.film) 2980, 2235, 1440,and 1100 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 0.90 (6H, s), 1.60 (4H, t),and 2.28 (4H, t);

m/z 150 (*M*, 22%), and 97 (100%) (Found: M^+, 149.8492. $C_7H_{14}N_2$ requires M^+, 150.2271).

4,4-Dimethylpimelic Acid

3,3-Dimethyl-1,5-dinitrile (0.50 g, 3.3 mmol) in water (1 ml), conc. H_2SO_4 (1 ml), and glacial acetic acid (1 ml) was heated at reflux for 18 h. The solution was cooled and basified with 1M NaOH solution. The aqueous layer was acidified with conc. HCl and extracted with ethyl acetate (5 x 20 ml). The combined ethyl acetate extracts were dried, filtered, and concentrated to an oil. Crystallisation from acetone gave 4,4-dimethylpimelic acid as a white powder (0.45 g, 79%), m.p. 85 - 87 °C; R_F(acetone-toluene, 5:1) 0.49; υ_{max} 3400, 3000, 2960, 1730, 1450, and 1250 cm^{-1}; δ_H(200 MHz) ($CDCl_3$) 0.88 (6H, t), 1.62 (4H, t), and 2.31 (4H, t); δ_C (50 MHz) ($CDCl_3$) 16.48 (q), 20.43 (s), 29.84 (t), 48.38 (t), and 172.31 (s); *m/z* 188 (M^+, 21%), 172 (30%), 157 (25%), and 97 (100%) (Found: C, 57.38; H, 9.18. $C_9H_{16}O_4$ requires C, 57.43; H, 9.25%).

Diethyl 2,6-Dibromo-4,4-dimethylpimelate

4,4-Dimethylpimelic acid (2.50 g, 13 mmol) in thionyl chloride (2.42 ml) was heated at 40 °C for 18 h. Iodine (0.50 g) and bromine (1.60 ml, 28 mmol) were added and the mixture was heated at 80 °C for 6 h. After cooling the solution was added to ethanol (15 ml). Water (30 ml) was added and the aqueous solution was extracted with diethyl ether (3 x 15 ml). The combined ether extracts were washed with 10% sodium

thiosulphate solution (5 x 20 ml). The ether layer was dried, filtered, and concentrated to an oil. Purification was achieved using a silica flash column and eluting with hexane and increasing proportions of dichloromethane to give diethyl 2,6-dibromo-4,4-dimethylpimelate as a yellow oil (3.4 g, 64%), R_F (hexane) 0.72; υ_{max} (liq. film) 2995, 1740, 1385, and 1220 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 0.92 (6H, s), 1.30 (6H, t), 2.30 (4H, m), 3.98 (2H, m), and 4.30 (4H, q); *m/z* 404 (M^+, 6%), 403 (M^+, 7%), 40 (M^+, 6%), and 149 (100%) (Found: M^+, 402.0486. $C_{13}H_{22}O_4Br_2$ requires M^+, 402.1246).

3,Methylpentane-1,5-diol

3-Methylglutaric acid was treated with 1M borane in THF as desribed prevously to give 3-methylpentane-1,5-diol in 82% yield, b.p. 120 - 125 °C (2 mmHg); υ_{max} (liq. film) 3400, 2960, 1375, 1150 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 0.96 (3H, d), 1.45 (5H, m), 3.10 (2H, s) and 3.52 (4H, t); *m/z* 103 (21%), 87 (100%) (Found: C, 60.95; H, 11.96. $C_6H_{14}O_2$ requires C, 60.98; H, 11.94%).

3-Methylpentane-1,5-diol Dimethylsulphonate

3-Methylpentane-1,5-diol was treated with methanesulphonyl chloride and triethylamine in THF as described previously to give 3-methylpentane-1,5-diol dimehylsulphonate as white needles (62% yield), m.p. 56 - 58 °C; υ_{max} 2990, 1475, 1400, 1170, and 1040 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 0.90 (3H, d), 1.40 (1H, m), 1.65 (4H, m), 3.00 (6H, s), and 4.21 (4H, t); *m/z* 97

(61%), 81 (100%), and 69 (25%) (Found: C, 35.04; H, 6.59. $C_8H_{12}O_6S_2$ requires C, 35.05; H, 6.60%).

3-Methylpentane-1,5-dinitrile

3-Methylpentane-1,5-diol dimethylsulphonate was treated with sodium cyanide in DMSO as described previously to give 3-methylpentane-1,5-dinitrile as a yellow oil (61% yield), R_F (CH_2Cl_2) 0.65; υ_{max} 2980, 2230, and 1380 cm^{-1}; δ_{max} (90 MHz) ($CDCl_3$) 0.98 (3H, d), 1.58 (5H, m), and 4.30 (4H, t); *m/z* 136 (M^+, 36%), 109 (23%), and 83 (100%) (Found: M^+, 136.2094. $C_8H_{12}N_2$ requires M^+, 136.1760).

4-Methylpimelic Acid

3-Methylpentane-1,5-dinitrile was hydrolysed with sulphuric acid and acetic acid as previously described to give 4-methylpimelic acid as a white solid (72% yield), m.p. 72 - 74 °C; R_F (acetone-toluene, 5:1) 0.51; υ_{max} 3400, 2960, 1720 and 1430 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 0.85 (3H, d), 1.48 (5H, m), and 2.35 (4H, t); δ_C (50 MHz) ($CDCl_3$) 19.75 (q), 28.37 (t), 29.76 (d), 46.26 (t), and 171.43 (s); *m/z* 174 (M^+, 8%), 160 (52%), 116 (52%) and 72 (100%) (Found: M^+, 174.1560. $C_8H_{14}O_4$ requires M^+, 174.1920).

Diethyl 2,6-Dibromo-4-methylpimelate

4-Methylpimelic acid was treated with thionyl chloride, bromine, and ethanol as described previously to give diethyl

2,6-dibromo-4-methylpimelate as a yellow oil (68% yield), R_F (hexane) 0.58; υ_{max} (liq. film) 2990, 1740, 1450, 1200, and 1020 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 0.90 (3H, d), 1.30 (6H, t), 1.80 (5H, m), 3.82 (2H, m), 4.30 (4H, q); *m/z* 390 (M^+, 2%), 389 (M^+, 2%), 388 (M^+, 2%), and 135 (100%) (Found: M^+, 388.0021. $C_{12}H_{20}O_4Br_2$ requires M^+, 388.0740).

3-Phenylpentane-1,5-diol

3-Phenylglutaric acid (104) was treated with 1M borane in THF as previously described to give 3-phenylpentane-1,5-diol as a clear oil (69% yield), b.p. 150 °C (2 mmHg); υ_{max} 2990, 1590, 1450, and 1170 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.90 (4H, m), 3.20 (2H, s), 3.53 (4H, t), 3.75 (1H, m), and 6.70 (5H, m); *m/z* 103 (29%), and 56 (100%) (Found: C, 73.35; H, 9.01. $C_{11}H_{16}O_2$ requires C,73.30; H, 8.95%).

3-Phenylpentane-1,5-diol Dimethylsulphonate

3-Phenylpentane-1,5-diol was treated with methanesulphonyl chloride and triethylamine in THF as described previously to give 3-pentane-1,5-diol dimethylsulphonate as white needles (59% yield), m.p. 78 - 80 °C; υ_{max} 2990, 1590, 1430, and 1170 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.20 (4H, m), 3.00 (6H, s), 3.80 (1H, m), 4.20 (4H, t), and 7.20 (5H, m); *m/z* 337 (MH^+, 31%), 241 (69%), and 97 (100%) (Found: C, 46.58; H, 5.86. $C_{13}H_{20}O_6S_2$ requires C, 46.61; H, 5.86%).

3-Phenylpentane-1,5-dinitrile

3-Phenylpentane-1,5-diol dimethylsulphonate was treated with sodium cyanide in DMSO as previously deacribed to give 3-phenylpentane-1,5-dinitrile as a yellow oil (72% yield), R_F (CH_2Cl_2) 0.84; υ_{max}(liq. film) 2290, 2235, 1590, and 1100 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.80 (4H, m), 2.10 (4H, t), 3.40 (1H, m), and 7.20 (5H, m); *m/z* 198 (M^+, 8%), 121 (39%), and 97 (100%) (Found: M^+, 198.2108. $C_{13}H_{14}N_2$ requires M^+, 198.2420).

4-Phenylpimelic Acid

3-Phenylpentane-1,5-dinitrile was hydrolysed with sulphuric acid and acetic acid, as described previously for 3,3-dimethylpentane-1,5-dinitrile, to give 4-phenylpimelic acid as a white powder (64% yield), m.p. 120 - 124 °C; R_F (acetone-toluene, 5:1) 0.78; υ_{max} 3400, 3010, 1720, and 1590 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.69 (4H, m), 2.15 (4H, t), 3.80 (1H, t), and 7.30 (5H, m); δ_C (50 MHz) ($CDCl_3$) 27.31 (t), 47.54 (t), 74.58 (d), 134.69 (d), 138.08 (d), 139.54 (d), 143.68 (s), and 173.45 (s); *m/z* 236 (M^+, 11%), 159 (45%), and 115 (100%) (Found: M^+, 236.4005. $C_{13}H_{16}O_4$ requires M^+, 236.2559).

Diethyl 2,6-Dibromo-4-phenylpimelate

4-Phenylpimelic acid was treated with thionyl chloride, bromine, and ethanol, as described previously 4,4-dimethylpimelic acid, to give diethyl 2,6-dibromo-4-phenylpimelate as a yellow oil (60% yield), R_F (hexane) 0.84;

υ_{max} (liq. film) 2980, 1740, 1590, 1280, and 1020 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.25 (6H, t), 1.82 (4H, m), 3.80 (1H, m), 4.00 (2H, m), 4.20 (4H, q), and 7.75 (5H, m); *m/z* 452 (M^+, 1%), 451 (M^+, 1%), 450 (M^+, 1%), 213 (20%), and 140 (100%) (Found: M^+, 449.8496. $C_{17}H_{22}O_4Br_2$ requires M^+, 450.1668).

3,3-Tetramethylenepentane-1,5-diol

3,3-Tetramethyleneglutaric acid (103) was treated with 1M borane in THF as described previously to give 3,3-tetramethylenepentane-1,5-diol as a clear oil (72% yield), b.p. 130 °C (2 mmHg); υ_{max} (liq. film) 3390, 2980, 1400, and 1100 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.10 (8H, m), 1.71 (4H, t), 3.70 (4H, t), and 4.32 (2H, s); *m/z* 158 (M^+, 10%) and 56 (100%) (Found: C, 60.99; H, 12.01. $C_9H_{18}O_2$ requires C, 60.98; H, 11.95).

3,3-Tetramethylenepentane-1,5-diol Dimethylsulphonate

3,3-Tetramethylenepentane-1,5-diol was treated with mesyl chloride and triethylamine in THF, as described previously for 4,4-dimethylpentane-1,5-diol, to give 3,3-tetramethylenepentane-1,5-diol dimethylsulphonate as white needles (65% yield), m.p. 78 - 80 °C; υ_{max} 2990, 1530, 1180, and 1050 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.20 (4H, t), 1.50 (4H, m), 1.80 (4H, m), 3.00 (6H, s), and 4.20 (4H, t); *m/z* 315 (MH^+, 24%), 219 (17%), and 97 (100%) (Found: C, 42.05; H, 7.09. $C_{11}H_{22}O_6S_2$ requires C, 42.02; H,7.05%).

3,3-Tetramethylenepentane-1,5-dinitrile

3,3-Tetramethylenepentane-1,5-diol dimethylsulphonate was treated with sodium cyanide in DMSO, as previously described for 3,3-dimethylpentane-1,5-diol, to give 3,3-tetramethylenepentane-1,5-dinitrile as a yellow oil (58% yield), R_F (CH_2Cl_2) 0.70; υ_{max} (liq. film) 2970, 2235, 1495, and 1000 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.29 (8H, m), 1.50 (4H, t), 2.35 (4H, t); *m/z* 177 (M^+, 18%), 149 (40%), and 122 (100%) (Found: M^+, 176.1853. $C_{11}H_{16}N_2$ requires M^+, 178.2380).

4,4-Tetramethylenepimelic Acid

3,3-Tetramethylenepentane-1,5-dinitrile was hydrolysed in sulphuric acid and acetic acid, as previously described for 3,3-dimethylpentane-1,5-dinitrile, to give 4,4-tetramethylenepimelic acid as a white powder (69% yield), m.p. 94 - 96 ^oC; R_F (acetone-toluene, 5 : 1) 0.63; υ_{max} 3400, 2970, and 1740 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.42 (4H, q), 1.58 (8H, m), and 2.25 (4H, t); δ_C (50 MHz) ($CDCl_3$) 25.40 (t), 26.59 (s), 27.05 (t), 28.45 (t), 48.08 (t), and 171.43 (s); *m/z* 214 (M^+, 7%), 170 (20%), and 97 (100%) (Found: M^+, 214.3365. $C_{11}H_{18}O_4$ requires M^+, 214.2543).

Diethyl 2,6-Dibromo-4,4-tetramethylenepimelate

4,4-Tetramethylenepimelic acid was treated with thionyl chloride, bromine, and ethanol, as previously described 4,4-dimethylpimelic acid, to give diethyl 2,6-dibromo-4,4-

tetramethylenepimelate (65% yield), R_F (hexane) 0.68; υ_{max} (liq. film) 2990, 1740, 1375, and 1050 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.30 (6H, t), 1.78 - 1.84 (10H, m), 4.00 (2H, m), and 4.25 (4H, q); δ_C (50 MHz) ($CDCl_3$) 13.70 (q), 14.96 (s), 20.47 (t), 36.73 (t), 37.40 (t), 45.87 (d), 61.60 (t), and 169.82 (s); *m/z* 430 (M^+, 2%), 429 (M^+, 2%), 428 (2%), 383/382/381 (30%), and 166 (100%).

Attempted Synthesis of 4- or 4,4-substituted Piperidine-2,6-dicarboxylic Acid

The 4- or 4,4-substituted diethyl 2,6-dibromopimelate (2.5 mmol) were treated with liquid ammonia (15 ml) in a sealed tube for 3 d. The tube was cooled to -78 °C before opening and allowed to warm to room temperature. The black oils left were examined by TLC and ^1H and ^{13}C NMR spectroscopy which showed them to be a mixture of products.

Diethyl Oxaloacetate (105)

A mixture of the sodium salt of diethyl oxalacetate (100 g, 0.54 mol) in 2.5M H_2SO_4 (200 ml) and diethyl ether (150 ml) was stirred for 1 h at room temperature. The two layers were seperated and the aqueous layer was extracted with diethyl ether (2 x 50 ml). The combined ether extracts were dried, filtered, and concentrated to afford an oil. Distillation gave diethyl oxaloacetate (105) as a clear oil, (50 g, 57%), b.p. 110 °C (2.6 mmHg), R_F ($CHCl_3$) 0.42; υ_{max} (liq. film) 2990, 1740, 1650, and 1250 cm^{-1}; δ_H (90 MHz) ($CDCl_3$) 1.20 (12H, t), 3.70 (1H, t),

4.20 (8H, q) and 5.90 (1H, s); *m/z* 188 (M^+, 100%), and 115 (48%) (Found: C, 44.66; H,6.38. $C_8H_{12}O_5$ requires C, 44.68; H, 6.38%).

Methylene Bis(diethyl oxaloacetate) (106)

A mixture of distilled diethyl oxaloacetate (50 g, 0.26 mmol), 30% formaldehyde solution (10.6 g, 0.13 mmol), piperidine (0.1 ml), and ethanol (5 ml) was stirred for 48 h at room temperature. The white solid was filtered off and dried to give methylene bis(diethyl oxaloacetate) (106) (35 g, 70%), m.p. 80 - 82 °C (lit.[75], 80 - 84 °C); R_F ($CHCl_3$) 0.15; υ_{max} 2990, 1740, 1640, and 1210 cm^{-1}; δ_H (200 MHz) ($CDCl_3$) 1.42 (12H, t), 2.80 (2H, m), 3.40 (2H, m), and 4.30 (8H, q); δ_C (50 MHz) ($CDCl_3$) 13.72 (q), 17.91 (t), 45.20 (d), 61.09 (t), 62.75 (t), 94.78 (s), 168.32 (s), and 169.21 (s); *m/z* 84 (100%) (Found: C, 52.57; H, 6.23. $C_{17}H_{24}O_{10}$ requires C, 52.53; H, 6.22%).

α,α′-Dioxopimelic Acid (108)

Methylene bis(diethyl oxaloacetate) (12.10 g, 31 mmol) in conc. H_2SO_4 (13 ml) was stirred at room temperature for 7 d. The solid was filtered off, washed with toluene (20 ml), and dried to give the anhydride (107) as a white crystalline solid (5.0 g) which was used immediately. The anhydride (107) (5.0 g) in water (15 ml) was heated at reflux for 30 min. The solution was concentrated under reduced pressure and dried over P_2O_5 to give α,α′-dioxopimelic acid (108) as a white solid (2.6 g, 45%), m.p. 125 - 127 °C (lit.[75], 127 °C); R_F ($CHCl_3$ - MeOH - AcOH,

74 : 24 :1) 0.42; υ_{max} 3400, 2990, 1740, 1720, and 1100 cm^{-1}; δ_H (200 MHz) (d_6-DMSO) 1.72 (2H, m), and 2.95 (4H, t); δ_C (50 MHz) (d_6-DMSO) 16.39 (t), 27.74 (t), 162.75 (s), and 196.34 (s); *m/z* 188 (100%), and 142 (42%) (Found: M^+, 188.2096. $C_7H_8O_6$ requires M^+, 188.4112).

2,6-Pyrandicarboxylic Acid (109)

α,α´-Dioxopimelic acid (108) (0.50 g, 2.6 mmol) in conc. H_2SO_4 was stirred at 0 °C for 3 h. The solution was poured into ice water (4 ml) and the precipitate was filtered off and dried to afford 2,6-pyrandicarboxylic acid (109) as a white powder (0.36 g, 81%), m.p. > 300 °C (lit.[75], 320 °C); λ_{max} (MeOH) 279 (ε 80,100); δ_{max} 3400, 1710, 1670, 1630, 1450, 1270, and 1130 cm^{-1}; δ_H (200 MHz) (d_6-DMSO) 2.90 (2H, m), and 5.90 (2H, m); δ_C (50 MHz) (d_6-DMSO) 21.41 (t), 110.00 (d), 141.95 (s), and 162.40 (s); *m/z* 170 (M^+, 22%) and 125 (100%) (Found: C, 49.92; H, 3.56. $C_7H_6O_5$ requires C, 49.94; H, 3.57%).

cis-*Tetrahydropyran-2,6-dicarboxylic Acid (110)*

2,6-Pyrandicarboxylic acid (0.50 g, 2.9 mmol) in ethanol (15 ml) was hydrogenated for 18 h at room temperature and atmospheric pressure using PtO_2 as catalyst. The catalyst was removed by filtration through Celite, and the filtrate was concentrated to give a white solid which was dried over P_2O_5. *cis*-Tetrahydropyran-2,6-dicarboxylic acid (110) was obtained as a white powder from methanol (0.48 g, 96%), m.p. > 300 °C (lit.[75], 306 °C); υ_{max} 3400, 1700, 1450, and 1100 cm^{-1}; δ_H (200

MHz) (D_2O) 1.64 (3H, m), 2.05 (3H, m), and 4.10 (2H, m); δ_C (50 MHz) (D_2O) 24.77 (t), 34.46 (t), 76.83 (d), and 175.86 (s); *m/z* 174 (M^+, 35%), and 129 (100%) (Found: M^+, 174.0942, $C_7H_{10}O_5$ requires M^+, 174.1575).

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