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ATTEMPTS TO SYNTHESISE KAINIC ACID

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by  
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To  
Emma

"lateral knowledge is like the archer  
discovering that although he has hit  
the bull's-eye and won the prize,  
his head is on a pillow  
and the sun is coming in the window"

R. M. Pirsig  
(Zen and the Art of Motorcycle  
Maintenance)

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## ABSTRACT

Attempts were made to synthesise a conformationally restricted analogue of kainic acid wherein the double bond was confined in a ring-system. The stratagem involved an intramolecular Diels-Alder reaction but could not be tested as the precursors to the cycloaddition reaction could not be prepared.

Attempts were made to develop a general route to kainic acid and analogues by employing a 1,3-dipolar cycloaddition reaction between aziridines and olefins. Triazolines were used as a precursor to aziridines because of the ease of formation from alkyl azides and olefins. The required dipolar cycloaddition was found to occur but produced various side-products from the triazoline thermolysis. The subsequent Grignard reaction on the cycloaddition product gave problems as the compound epimerised under basic conditions and did not undergo reaction with methyl Grignard or methyl lithium.

An attempt to prepare kainic acid and analogues by an intramolecular 1,3-dipolar cycloaddition or a 1,3-sigmatropic shift reaction failed when the basic precursors for the reaction could not be prepared.

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THE HISTORY AND USES OF KAINIC ACID



## 1. INTRODUCTION

As the title suggests, the review outlines the history of kainic acid, and describes the various uses to which the compound has been put. The review is not comprehensive but covers the important aspects in each section.

The first section deals with the anthelmintic properties of kainic acid. A description of the characteristics of roundworm, and a summary of the drugs used in treatment, are included in this section to qualify the importance of kainic acid in this field. The second section describes the successful syntheses of kainic acid and the naturally occurring isomer,  $\alpha$ -allokainic acid. Section three deals with the neurochemical uses of kainic acid. A vast amount of work has been done in the field of excitatory amino acids, but in this review only aspects of that work dealing with the evaluation and uses of kainic acid are covered.

## 2. DRUGS TO COMBAT ASCARIS

### 2.1 Introduction

"This Wormy World" was the title of a report published in 1947.<sup>1</sup> It was based upon survey data obtained around the world, and gave an estimate of the amount of parasitism of man by helminths. The report suggested that 2,250 million helminthic infections were harboured by a world population of 2,166 million people. Of the many species of worm, infection by Ascaris lumbricoides was found to be the most prevalent.

A vast amount of research has since been undertaken to

find ways of eradicating this and other helminthic diseases, but despite this work, helminthic infections are as widespread as ever. Updated values for prevalence rates<sup>2,3</sup> suggested at least one billion people were infected with the roundworm Ascaris lumbricoides, and that some 58,000 tons of eggs were excreted per annum.

Infection with Ascaris lumbricoides can cause severe abdominal pain, but more serious are the complications which can ensue, such as partial or complete blockage of the intestinal tract, perforation of intestine, and migration of adult worms to extra-intestinal sites.

The normal habitat of adult worms is in the lumen of the small intestine. They are not attached to the mucosa, but can exert pressure on the mucosa, or on other worms, by means of loops or coils, in order to maintain their position or migrate against the peristaltic current.

Female ascarids have an egg-laying capacity of around 200,000 eggs per day. The highly resistant eggs are excreted by the host and within two weeks they have embryonated and are potentially infective. Infection occurs from ingestion of these eggs either in food or water. Larvae hatch from the eggs in the small intestine, enter the portal circulation, and are carried to the liver, then the heart and lungs, where they grow and moult. The parasites then ascend the respiratory tract, pass down the oesophagus and through the stomach for the second time. They mature into adults in the small bowel. Female ascarids reach a length of 20-35 cm and are 3-6 mm in diameter. The male

worms measure about 15-30 cm in length and 2-4 mm in diameter. The period from infection to excretion of new eggs is 2 - 2½ months. Spontaneous loss of infection occurs within 8 - 12 months.

## 2.2 Western drugs

The search for drugs to combat helminthic infections has involved the screening of numerous compounds. Despite the volume of research in this area, there are still remarkably few anthelmintic agents in use. Most compounds showing anthelmintic activity leave much to be desired in their therapeutic effectiveness, lack of side-effects, ease of administration and suitability for mass treatment, and cost. The prime considerations when looking for an anthelmintic drug are, high efficacy, safety, and low cost.

One of the first anthelmintics<sup>4</sup> to be commonly used was piperazine (1). It has since been prepared and used in numerous forms as salts, or derivatives, but none of these have been superior to piperazine itself. The drug has very low toxicity, it is economical, non-staining, palatable, and highly efficient for the treatment of ascariasis and enteriobiasis. Despite the advent of modern anthelmintics, piperazine still remains the drug of choice for these infections.

Piperazine works by paralysing the muscle of the parasite causing it to be swept out by the peristaltic flow. It is thought to work by selectively blocking the response of the ascaris muscle to acetylcholine, while producing only very weak blocking action in mammalian skeletal muscle. It is also thought to alter the permeability of the cell membrane to fatty acids which play an important role in maintaining the membrane

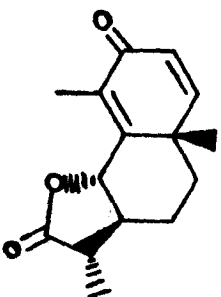
potential of ascaris muscle cells.<sup>5</sup> This effect might also contribute to the paralysing action of the drug.

None of the other early anthelmintics<sup>4,6</sup> compared favourably with piperazine. Santonin (2) which had long been used for its anthelmintic properties was not as effective as piperazine in safe doses, and proved to be toxic in some cases. Hexylresorcinol (3) was a powerful ascaricide, producing necrosis and blisters on contact with the worms, but it was only effective if the intestinal tract was empty before application, and if chewed, the drug produced burning in the mouth. Dithiazanine (4), although more expensive, was the drug of choice if ascaris was accompanied by trichuris or strongyloides, as the drug was active against all three infections. It has now been withdrawn from general use because of severe gastrointestinal side-effects, produced on occasion. Bephenium (5), usually prepared as the hydroxynaphthoate, was introduced in the early sixties and is still used as an effective ascaricide. Pyrantel (6), normally used as the insoluble pamoate, is an extremely powerful ascaricide. It has been shown to be effective against Ascaris suum (pig roundworm) at concentrations of 1:1,000,000 w/v.<sup>7</sup> It is also effective against a variety of other intestinal infections and is thought to act by interfering with the acetylcholinergic system.<sup>8</sup>

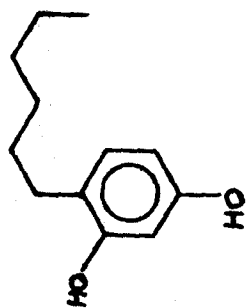
The most important, and truly broad-spectrum anthelmintics, to have been developed in recent years,<sup>8</sup> are the benzimidazoles (7) and levamisole (8). Numerous analogues and derivatives of benzimidazole have been prepared by altering



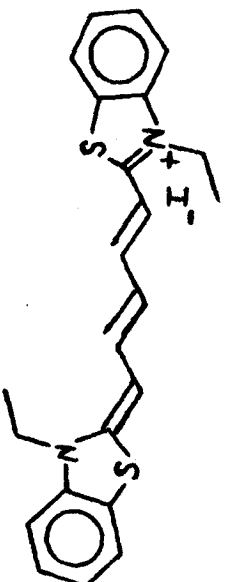
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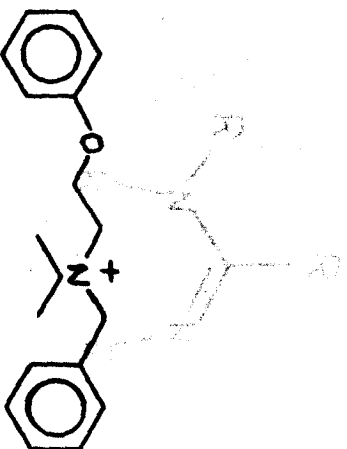
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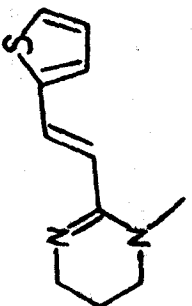
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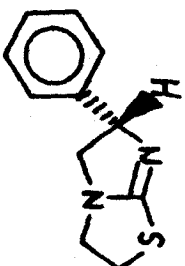
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side-chains on the basic nucleus. Levamisole is the active S(-)-isomer of tetramisole. None of the analogues of tetramisole, so far prepared, have proved to be as potent as the parent compound. These drugs are highly toxic to a wide range of nematodes including ascaris. They are thought to work by inhibiting fumarate reductase.

It should be noted that each of these broad-spectrum anthelmintics possess a common structural feature (Fig.2.1) but it is not yet known how significant this is to the action of the drugs.

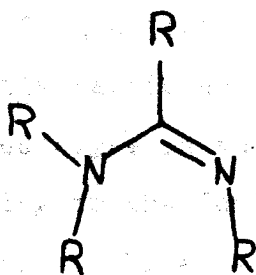


Fig.2.1.

### 2.3 Kainic acid as an ascaricide

In 1935<sup>9</sup> a list of drugs was reported which had been used in China for over 2,000 years, many having been introduced to Japan during the last 1300 years. Among these drugs was an ascaricidal preparation from the seaweed, Digenea simplex.

The raw material, Digenea simplex, is one of the red algae, Rhodomelaceae. In 1950 D.simplex was known to be distributed in the Mediterranean Sea, the Indian Ocean, the waters between mainland China and Taiwan, and around the islands of Japan. At this time Japan was using, not only all the seaweed collected from its own shores, but also most of the production

from other areas. The supply was still short and artificial culture was tried. The decoction of Digenea used medicinally had no side-effects, and was used to eradicate ascariasis in postwar Japan.<sup>10</sup>

The active principle of Digenea simplex was isolated in 1953<sup>11</sup> and called digenic acid after the seaweed. The name was later changed to kainic acid to avoid confusion with other Digenea preparations. The name in Japanese means ghost from the sea, and was derived from the Japanese name for the seaweed, Kainso. Tests showed this substance to be a very strong ascaricidal agent; 20 mg effectively expelled 70% of ascaris.<sup>12</sup>

More recently kainic acid was found to be present in another species of red algae in the Mediterranean, Centroceras clavulatum,<sup>13</sup> belonging to the family of Ceramiales.

The decoction of Digenea used medicinally was found to contain only 0.75-0.83% of the active principle, kainic acid.<sup>14</sup> The procedure developed for extracting kainic acid from the seaweed produced 1g of kainic acid from 1 kg of seaweed.<sup>15</sup>

In the purification of large amounts of kainic acid, small amounts of another substance were obtained. The compound had weak anthelmintic properties. It was first called, T-acid, and then  $\alpha$ -allokainic acid when it was found to be an isomer of kainic acid. Kainic acid kills ascaris by the combined effects of spastic paralysis of the muscle due to an initial neuromuscular excitation,<sup>16</sup> and inhibition of electron transport between NADH and FAD in the muscle.<sup>17</sup> Magnesium salts of kainic acid were shown to produce the strongest paralyzing action,<sup>18</sup>

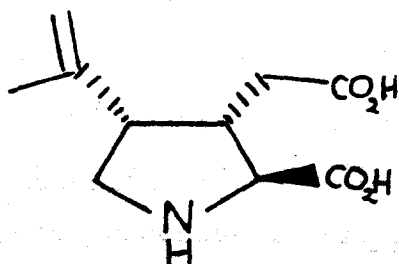
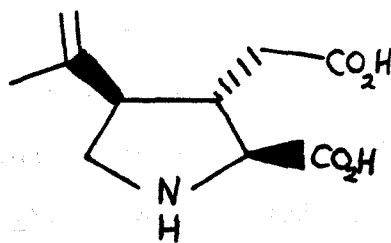
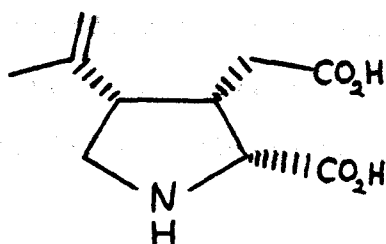
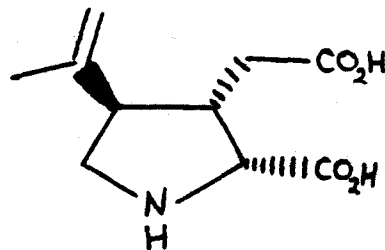
but this is most likely due to an additional separate effect of the magnesium ions.<sup>15</sup> Initial tests showed kainic acid to be three times more powerful than santonin.<sup>19</sup> It has been shown to be effective against Ascaris suum at concentrations of 1:4,000 w/v in vitro.<sup>7</sup>

To increase its spectrum of activity, kainic acid has been used in combination with other anthelmintics. Piperazine kainate<sup>20</sup> is one such preparation which has shown activity against helminths not normally affected by either drug individually.<sup>21</sup> Combinations of kainic acid and santonin have also been shown to be effective against 78% of ascaris infections.<sup>22</sup> Various amide and ester derivatives of kainic acid have been prepared. These were much weaker anthelmintics than the parent amino acid, but they found a use as tasteless anthelmintics.<sup>23</sup>

The correct structure of kainic acid was elucidated in 1955, from chemical degradation studies.<sup>24-26</sup> The stereochemistry was determined<sup>27</sup> from two observations. The first was that neither  $\alpha$ -kainic acid nor  $\alpha$ -allokainic acid formed an anhydride. The  $\beta$ -isomers formed anhydrides easily. This suggested that in the  $\beta$ -isomers the two acid groups are cis, and in the  $\alpha$ -isomers they are trans. The relative stereochemistry between the C-3 and C-4 substituents was then determined by first hydrating the double bond in the isopropenyl side-chain. When  $\alpha$ -kainic acid was hydrated, the resulting alcohol formed a lactone with the C-3 carboxymethyl substituent. Hydration of



the allo-isomer gave an alcohol with no tendency to lactonise. This suggested that the C-3 and C-4 substituents of  $\alpha$ -kainic acid are cis, whereas the allo-isomer substituents are trans. These findings were confirmed when the crystal structures of  $\alpha$ -kainic acid monohydrate and  $\alpha$ -allokainic acid were determined.<sup>28</sup>

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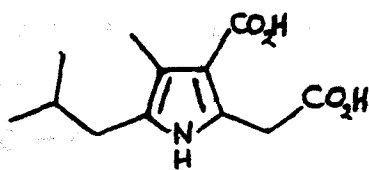
Kainic acid has three asymmetric carbon atoms and therefore eight possible optical isomers. The naturally occurring isomers are L- $\alpha$ -kainic acid (9), the active anthelmintic constituent of Digenea simplex, and L- $\alpha$ -alkokainic acid (10). The stereoisomers, D- $\beta$ -kainic acid (11) and

D- $\beta$ -allokainic acid (12), can be obtained from isomers (9) and (10) respectively.<sup>29</sup>

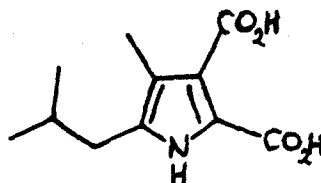
Although  $\alpha$ -allokainic acid was found to have a weak ascaricidal effect against Ascaris lubricoides, it proved to be as effective as  $\alpha$ -kainic acid, when tested for its ability to paralyse Ascaris suum *in vitro*.<sup>7</sup>

Dihydrokainic acid and N-acetylkainic acid exhibited very weak effects when tested for their ability to excite the motility of Ascaris suilla, and neuromuscular preparations of Allobophora foetida.<sup>16</sup> They also failed to inhibit electron transport processes in Ascaris suilla.<sup>17</sup> Kainic acid showed powerful effects in each case.

These tests were carried out on a total of 32 carboxylic acid derivatives of pyrrole and pyrrolidine. Only two compounds other than kainic acid showed a marked activity. These were 5-isobutyl-4-methyl-3-carboxy-2-pyrrole acetic acid (13) and 5-isobutyl-4-methyl-2,3-pyrrole dicarboxylic acid (14).



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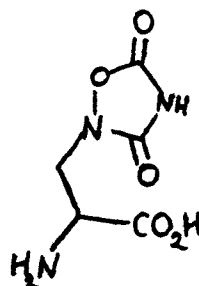
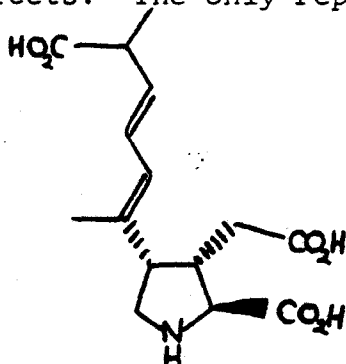
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It would seem that a prerequisite for anthelmintic activity in these compounds is carboxy or carboxymethyl substituents at positions 2 and 3, and a free amino function.

The unsaturation in the C-4 side-chain of kainic acid (9) also appears to be important, although the bulky isobutyl groups in compounds (13) and (14) appear to compensate for the lack of unsaturation. The stereochemistry of the C-4 side-chain of kainic acid appears to be important only against certain species of ascaris.

A seaweed belonging to the same family as Digenea simplex was found to be used as an ascaricide in remote islands of the Kagoshima province in Japan. When the active constituent of this seaweed, Chondria armata, was isolated, it was found to be structurally and stereochemically related to kainic acid. The new compound was called domoic acid<sup>30</sup> and showed high anthelmintic activity,<sup>31</sup> comparable to kainic acid, with no observable side-effects.

Another natural ascaricide which had long been used in China and Japan was the seeds of a green creeping vine found in North Vietnam, Quisqualis chenensis.<sup>32</sup> The active constituent, quisqualic acid, was also found in the leaves of Quisqualis indica.<sup>33</sup> Clinical studies of potassium quisqualate showed the compound to be an effective anthelmintic similar to santonin in its effects. The only reported side-effect was hiccups!



The similarity between domoic acid (15) and kainic acid (9) is obvious. Apart from the acidic and amino functions, it also possesses the unsaturation on the C-4 side-chain, in the desired stereochemistry. The additional chain attached to the olefinic moiety does not seem to impair the action of domoic acid.

The structure of quisqualic acid (16) initially looks far removed from that of kainic acid (9). However, the proton on the ring nitrogen, being flanked by two carbonyls, is actually fairly acidic. When the structure of quisqualic acid is drawn as shown (16), it can be seen that there is a correlation between the possible relative positions in space of the amino and two-acidic functions.

Quisqualic acid obviously lacks the C-4 unsaturation of kainic and domoic acids and was shown in tests against Ascaris suum to be four times weaker than kainic acid.<sup>7</sup>

### 3. SYNTHESIS OF KAINIC ACID

L- $\alpha$ -kainic acid crystallises with one molecule of water to give colourless needles, mp.  $251^{\circ}$ ,  $[\alpha]_D^{24} -14.8^{\circ}$ .<sup>11</sup> The other naturally occurring isomer, L- $\alpha$ -allokainic acid, gives colourless prisms, mp.  $237^{\circ}$ ,  $[\alpha]_D^{26} +8^{\circ}$ .<sup>34</sup> Once the structure of kainic acid had been determined, it became possible to design syntheses of the molecule.

The first synthesis of kainic acid was developed in 1957 and gave the  $\alpha$ -alloisomer.<sup>34</sup> In this synthesis the pyrrolidine ring was constructed from ethyl-2-(1-methyl-2-ethoxyethyl)-cyanoacetate (17) (Fig.3.1). Hydrogenation of the starting material produced a primary amine (18) which was then reacted with ethyl-2-iodoacetate giving the secondary amine (19). The amine was protected with ethylchloroformate and cyclised under Dieckmann conditions to give 1,2-dicarbethoxy-4-(1-methyl-2-ethoxyethyl)-pyrrolid-3-one (21).

The pyrrolidone (21) was set up to be taken on to kainic acid. It possessed the required substituents at positions 1, 2, and 4 in latent form, and had useful functionality at position 3. The stereochemistry was not yet fixed however, as chiral centres C-2 and C-4 were both epimerisable.

The pyrrolidone (21), failing to give a successful condensation with malonate anion, was hydrogenated and acetylated, to give 1,2-dicarbethoxy-3-acetoxy-4-(1-methyl-2-ethoxyethyl)-pyrrolidine (23) (Fig.3.2). This was condensed with diethyl malonate/sodium ethoxide to give a product (24) with  $\alpha$ -allo stereochemistry. The reaction may have proceeded by initial elimination of acetic acid followed by a Michael addition of

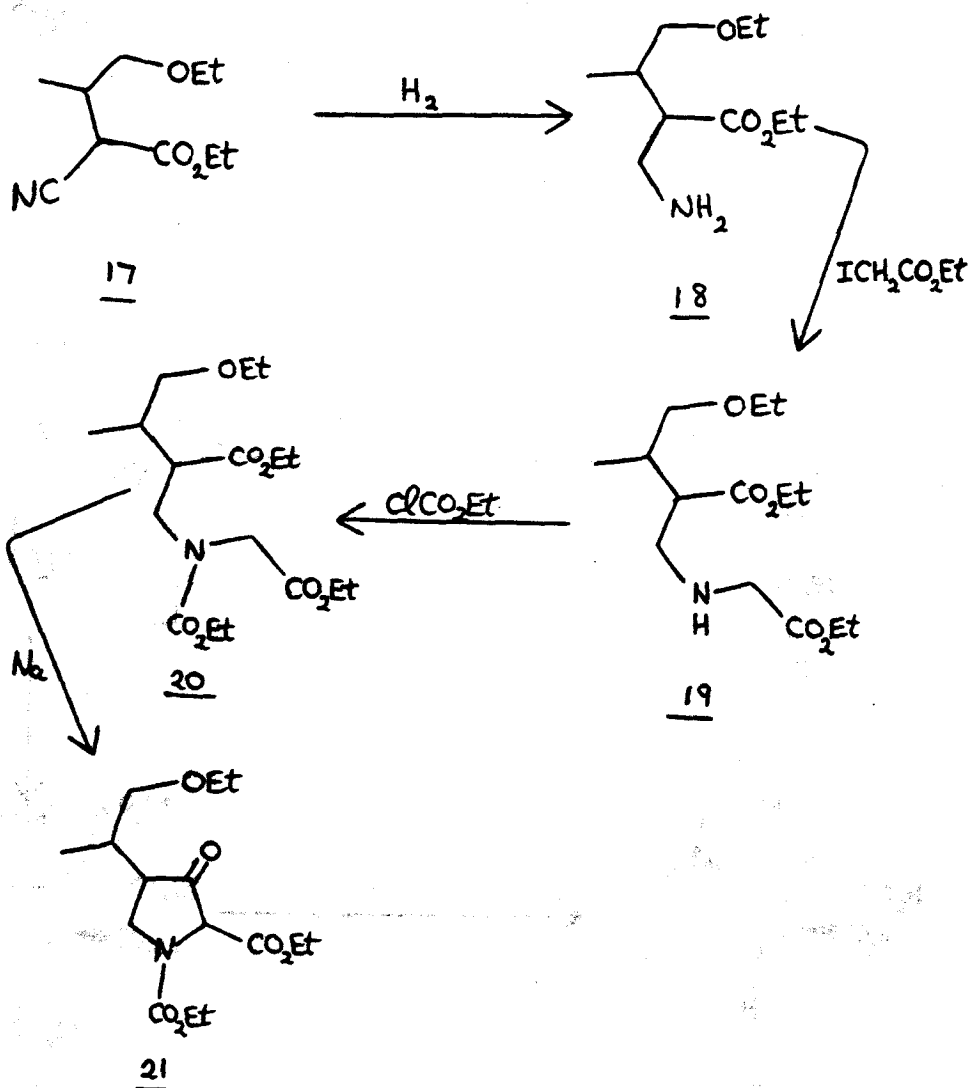


Fig. 3.1

the malonate onto the intermediate  $\alpha,\beta$ -unsaturated ester.

This would have produced the trans arrangement between positions 3 and 4.

Compound (24) required only the generation of the correct side chain functionalities to give  $\alpha$ -allokainic acid. The ether was cleaved by hydrogen bromide and the resulting alcohol, after re-protection of the acid and amino groups, was

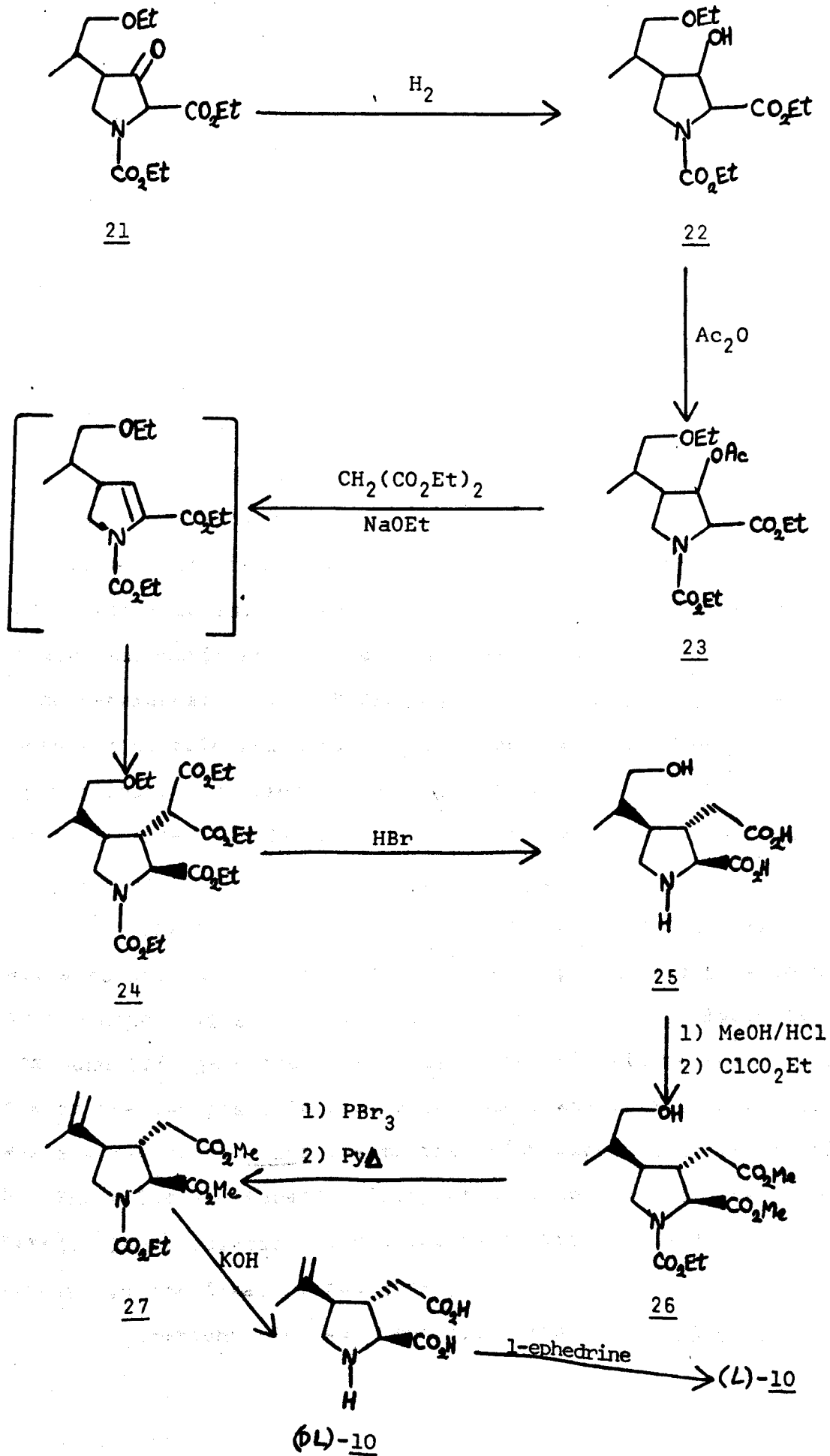


Fig. 3.2

brominated with phosphorus tribromide. Dehydrobromination by heating in pyridine gave the required isopropenyl side-chain. Ester and amide hydrolysis then gave racemic  $\alpha$ -allokainic acid, which was resolved using 1-ephedrine. The same research group then prepared  $\alpha$ -kainic acid<sup>35</sup> from the same starting material. Ethyl-2-(1-methyl-2-ethoxyethyl)-cyanoacetate (17) was used to prepare an intermediate piperidone (32).

The starting material (17) was first hydrolysed to the acid (28) (Fig.3.3). The acid chloride was prepared and condensed with diethyl malonate. The resulting ketone (30) was decarbethoxylated, by heating in acetic acid containing concentrated sulphuric acid, to give ethyl-3-oxo-4-cyano-5-methyl-6-ethoxy-hexanoate (31). Hydrogenation with Raney nickel reduced both the nitrile and carbonyl functions, and the free amine spontaneously cyclised, producing 4-hydroxy-5-(1-methyl-2-ethoxyethyl)-piperid-2-one (32). This compound has two chiral centres and both diastereoisomers were isolated.

The stereoisomeric mixture of compound (32) was acylated and, upon distillation, gave compound (33) by eliminating acetic acid (Fig.3.4). Michael addition of diethyl malonate to compound (33) gave the 4,5-disubstituted piperidone (34). Only one of the two possible diastereoisomers was formed, and this was assumed to be trans due to the bulky substituent at position 5. The relative stereochemistry at positions 4 and 5 was now fixed. These positions would become positions 3 and 4 respectively, in the final pyrrolidine.

Compound (34) was brominated and the product (35)



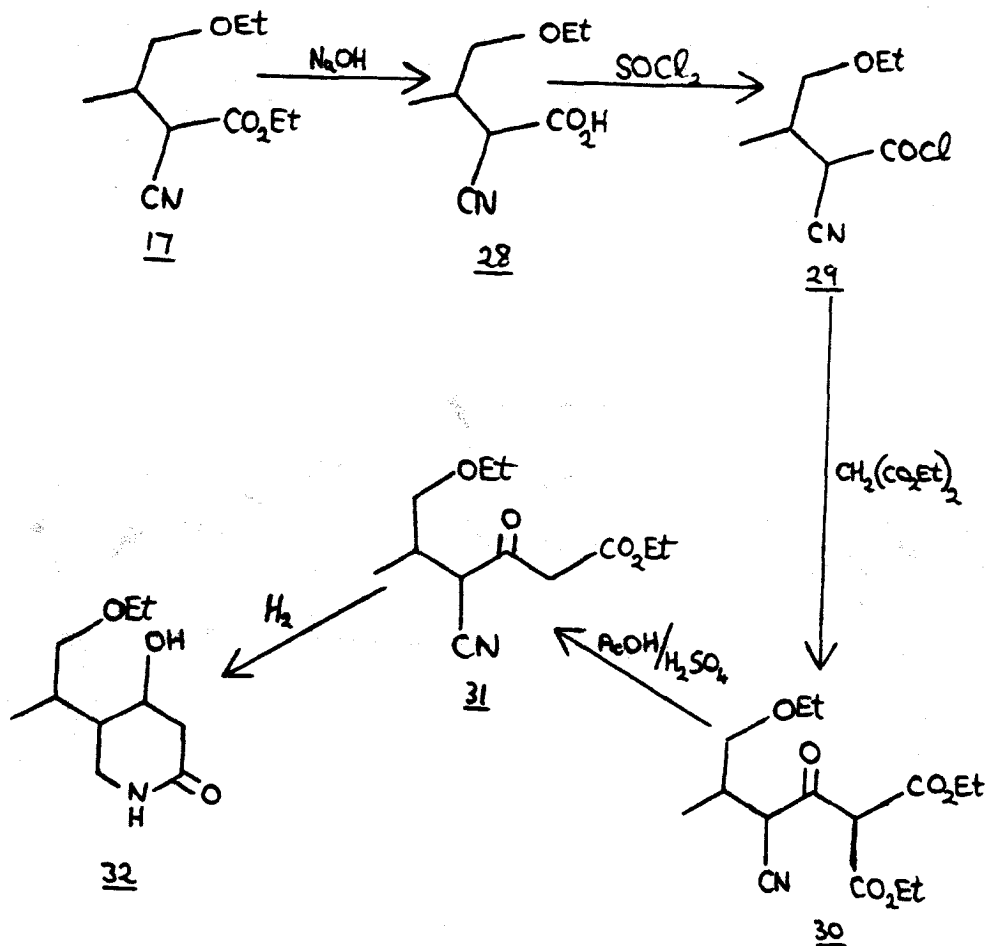


Fig.3.3

hydrolysed with an excess of potassium hydroxide solution. The piperidone ring was opened, giving initially an open-chain amine which ring-closed by displacement of bromide to give DL- $\alpha$ -2-carboxy-3-carboxymethyl-4-(1-methyl-2-ethoxyethyl)-pyrrolidine (36).

The unsaturation was introduced to the C-4 side-chain, and the product was resolved by the same procedures employed for  $\alpha$ -allokainic acid (Fig.3.2).

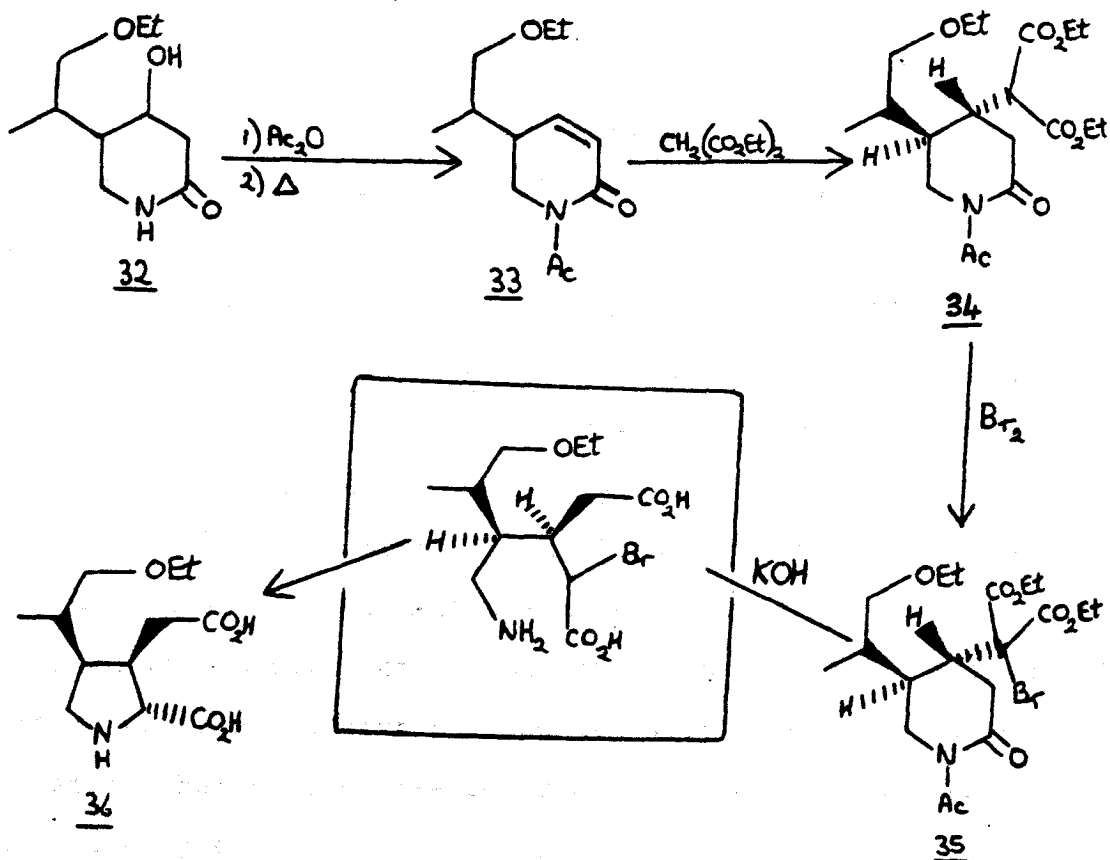
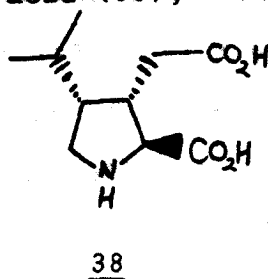
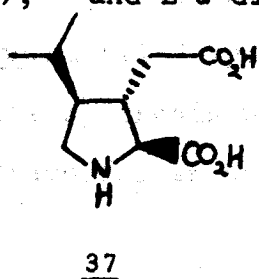


Fig.3.4

In the synthesis of  $\alpha$ -allokainic acid another method was also employed to generate the unsaturation at C-4. A dialkyl amino group was used in place of the ethyl ether. The group was removed by first generating the quaternary ammonium salt and then heating with silver oxide.

The syntheses were also carried out with no ethyl ether or dialkylamino group. This gave L- $\alpha$ -alldihydrokainic acid (37),<sup>33</sup> and L- $\alpha$ -dihydrokainic acid (38),<sup>34</sup> respectively.



In 1965 Murayama and co-workers<sup>36</sup> employed the Wittig reaction as a method of preparing C-4 analogues of kainic acid. They used as a starting material the C-4 methyl ketone of  $\alpha$ -kainic acid (39), or  $\alpha$ -allokainic (52). From this ketone, kainic acid or analogues could be prepared, by first protecting the acid groups, and then reacting with the appropriate triphenylphosphorane (Fig.3.5).

A greater scope for analogue formation was possible for  $\alpha$ -allokainic acid methyl ketone, as this isomer could also be used with phosphonate carbanions. When  $\alpha$ -kainic acid methyl ketone was reacted with phosphonate Wittig reagents, inversion of stereochemistry at C-4 occurred, giving the  $\alpha$ -allokainic acid analogue.

Preparation of the vinyl homologue of  $\alpha$ -kainic acid (44) required the protection of the amino group of dimethyl  $\alpha$ -kainate methyl ketone (40). Initial attempts using the free amine produced the interesting bicyclic kainate analogue (42).

Having generated kainic acid from the methyl ketone by the Wittig reaction, the group then investigated methods for synthesising the methyl ketone compound in order to have a total synthesis of kainic acid. They were successful in synthesising  $\alpha$ -allokainic acid methyl ketone (52),<sup>37</sup> but did not synthesise the methyl ketone of  $\alpha$ -kainic acid.

The methyl ketone of  $\alpha$ -allokainic acid was synthesised from erythro methyl-3-hydroxyglutamate (47) (Fig.3.6). The key step in the synthesis was the formation of the pyrrolidine ring by an intramolecular Michael reaction, (50)  $\rightarrow$  (51). Several

bases were tried and Triton B was found to be the best for catalysing the cyclisation. This reaction effectively dictated the stereochemistry of the final product by producing a trans arrangement at positions 3 and 4, and therefore after hydrolysis, DL- $\alpha$ -allokainic acid methyl ketone (52) was obtained.

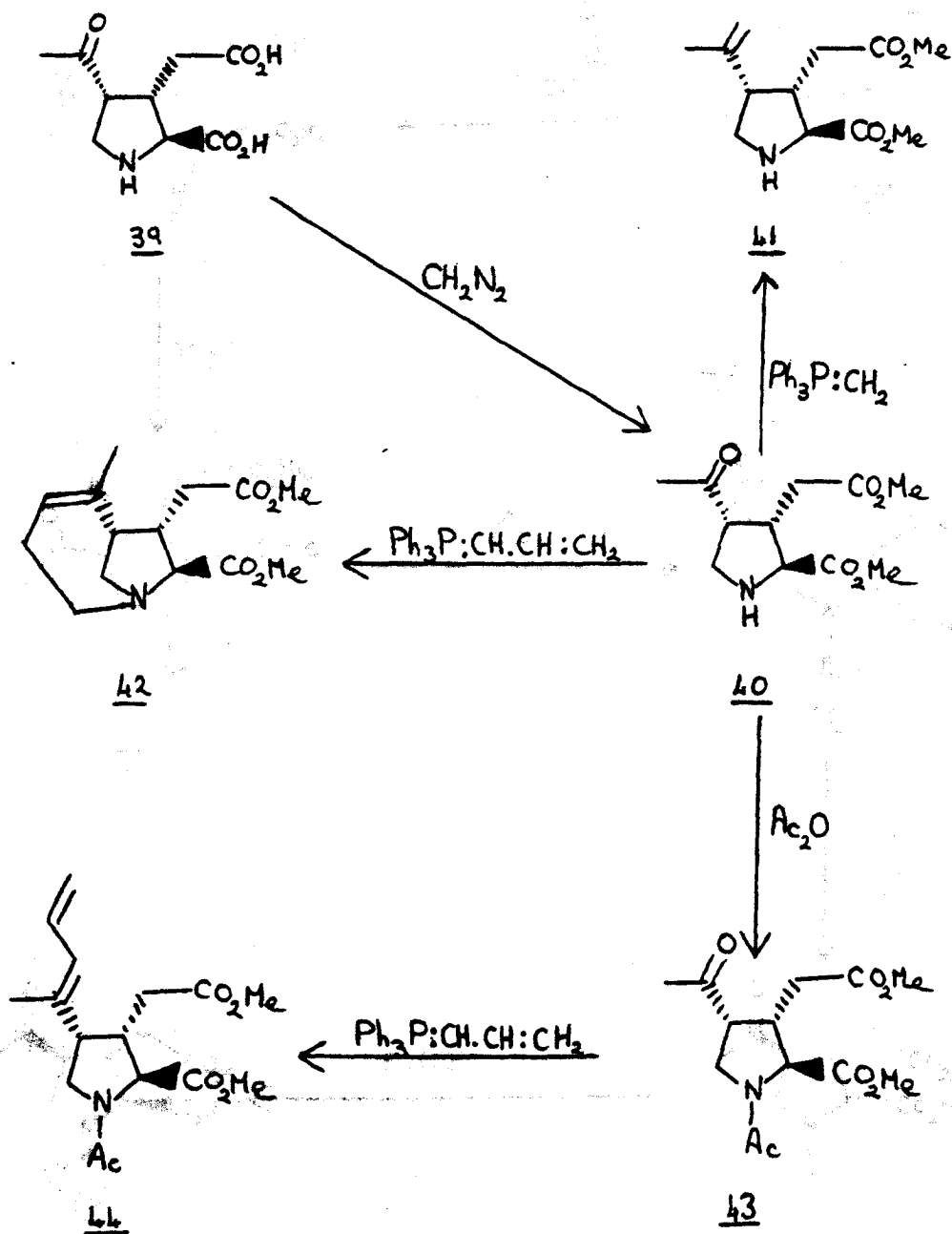


Fig. 3.5

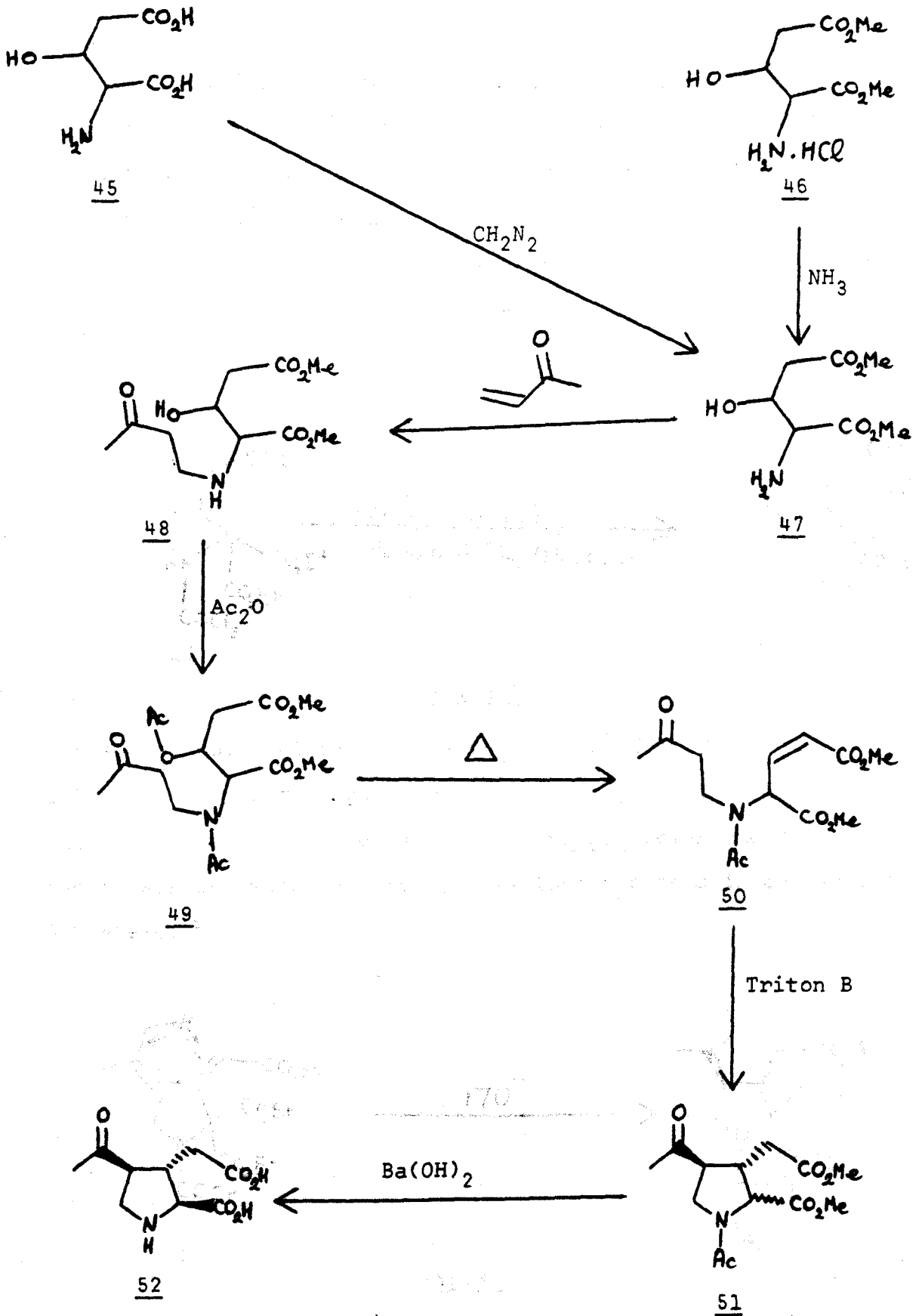


Fig. 3.6

During a study of intramolecular ene-reactions, Oppolzer applied this type of reaction to the synthesis of kainic acid in order to obtain the stereochemical control required for this molecule.

Starting from the known compound (53), N-alkylation gave the 1,6-diene (54) in 80% yield.

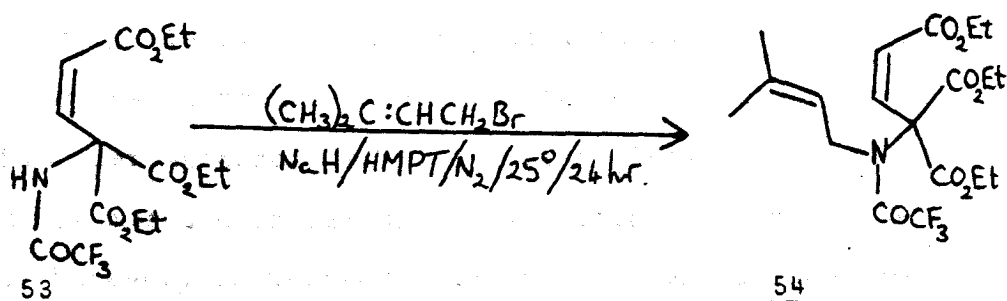


Fig. 3.7

Diene (54) was cyclised by heating to  $170^\circ$  for 10 min. at 1 Torr. Distillation gave the pyrrolidine (55) in 97% yield. This pyrrolidine was exclusively the trans isomer and on hydrolysis and work-up, gave DL- $\alpha$ -allokainic acid in an overall 53% yield.<sup>38</sup>

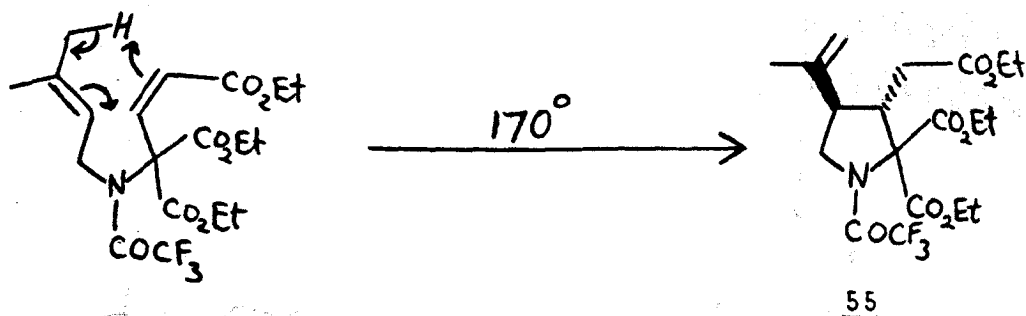


Fig. 3.8

In an attempt to alter the stereochemical course of the cycloaddition so as to obtain the stereochemistry required for  $\alpha$ -kainic acid, Oppolzer decided to use the same type of 1,6-diene system, but with only one ester group adjacent to the nitrogen.<sup>39</sup>

To make this intermediate, methyl N-trifluoroacetyl-glycinate (56) was alkylated with 1-bromo-3-methyl-2-butene (Fig.3.9). The product (57) was then condensed with methyl-2-methylthio-acrylate by Michael addition, to give the precursor to the 1,6-diene, compound (58). The thioether was oxidised with *m*-chloroperoxybenzoic acid, and distillation of the sulphoxide promoted elimination. The initially formed 1,6-diene (59) however, isomerised under the thermal desulphenylation conditions and the isolated product was 1,5-diene (60).

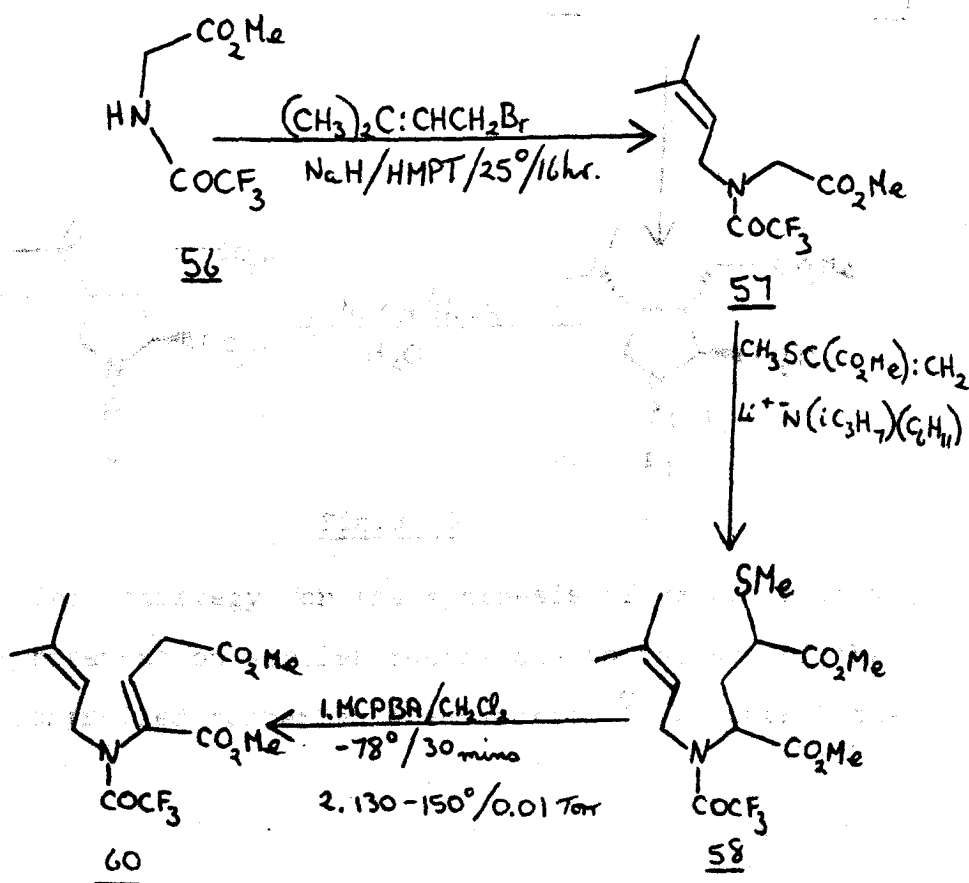


Fig. 3.9

Assuming the isomerisation of 1,6-diene (59) to 1,5-diene (60) to be reversible at high temperatures, the 1,5-diene was heated to  $180^{\circ}$ . This produced the desired ene-reaction and gave a pyrrolidine with the correct stereochemistry to be taken on to  $\alpha$ -kainic acid (Fig.3.10). Hydrolysis of the pyrrolidine (61) produced by the ene-reaction, gave DL- $\alpha$ -kainic acid in overall 41% yield from methyl N-trifluoroacetyl glycinate.

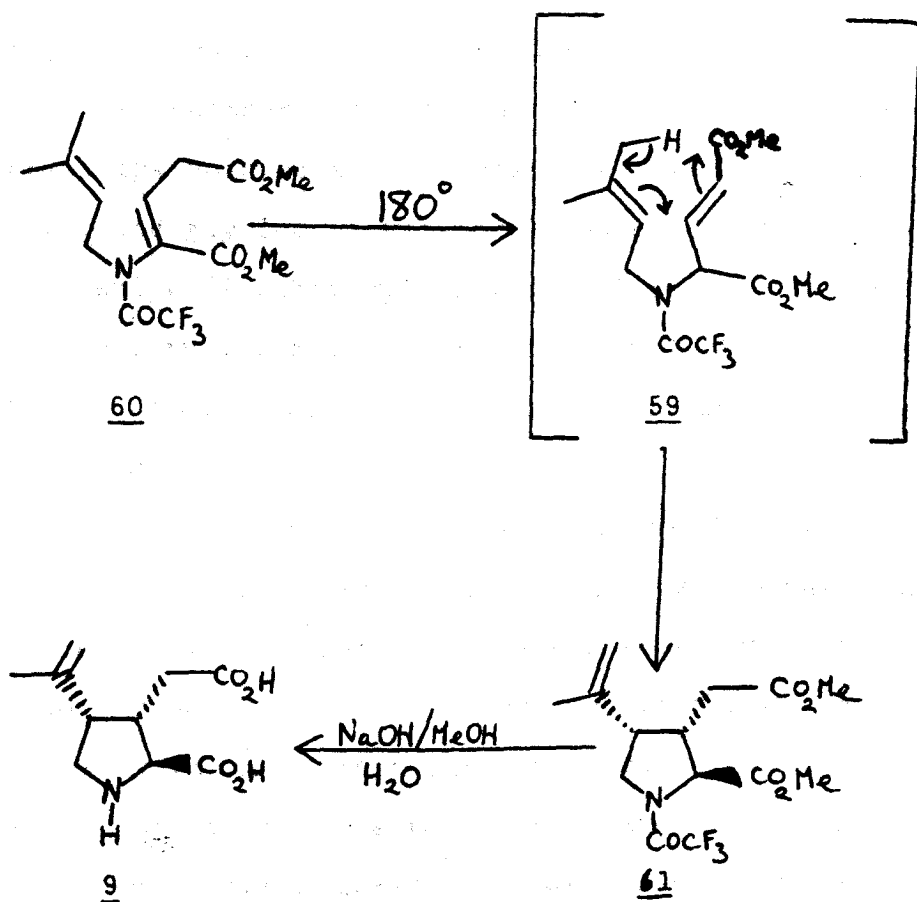


Fig.3.10

This strategy for the synthesis of kainic acid was a vast improvement on earlier routes due to the use of the symmetry-controlled concerted ene-reaction<sup>40</sup> to control the



stereochemistry of the final product and also to produce the unsaturation required in the C-4 side-chain. This method made a much shorter synthetic scheme possible, and therefore gave a much higher overall yield than the previous multistep syntheses.

#### 4. KAINIC ACID IN NEUROCHEMISTRY

##### 4.1 Introduction

Over the last two decades, research with centrally acting compounds has led to an explosive increase in our understanding of the chemistry of the brain. The mode of action of neurotransmitters such as acetylcholine (excitatory), GABA (inhibitory), and glycine (inhibitory), have been fairly well defined. Dopamine (inhibitory), noradrenaline (inhibitory), serotonin (inhibitory), substance P (excitatory), and enkephalin (inhibitory), are also fairly well established as being natural neurotransmitters.

A neurotransmitter is a chemical used by the nervous system to carry a signal across the synaptic gap between nerve cells. There are many variations in their modes of action, but basically when an electrical nerve impulse arrives at the pre-synaptic nerve terminal it stimulates the release of a neurotransmitter. This substance enters the synapse and binds to a specific receptor on the post-synaptic membrane of the adjacent nerve cell. Binding of the neurotransmitter causes a permeability change in the cell, and the ensuing flow of ions results in an electrical imbalance in the cell. This produces an electrical impulse which passes down the cell, and hence the signal has been transmitted from one cell to another. The flow of signals

is then stopped, either by enzymic degradation, as in the case of acetylcholine and enkephalin, or by uptake of the neurotransmitter into the presynaptic nervous tissue by an active transport system.

Glutamic and aspartic acids, because of their potent excitatory actions when applied micro-iontophoretically in the vicinity of neurones, have been proposed as excitatory neurotransmitters at a variety of sites in the brain and nervous system. However, definitive neuropharmacological evidence at the synaptic level has not been forthcoming, and whether or not these amino acids are in fact the natural excitatory neurotransmitters remains speculative and controversial.<sup>41</sup>

Unlike the already established neurotransmitters, conclusive evidence for glutamic acid as a natural transmitter will be very difficult to obtain. The main problem is that no selective antagonists have yet been found for the glutamate receptor. Also glutamic acid is involved in various other functions; protein synthesis, fatty acid synthesis, tricarboxylic acid cycle, GABA synthesis, regulation of ammonia levels, and control of osmotic or ionic balance.

Whether or not glutamic acid is a natural neurotransmitter, it is unquestionable that certain neurones do possess receptors which give rise to excitatory depolarisations in the presence of the amino acid. This receptor is known to be chiral, as L-glutamate is a much more potent excitant than D-glutamate.

The flexibility of the glutamate molecule, together with its many other physiochemical roles, means that very little information can be obtained about this receptor from experiments with glutamic acid. It is therefore necessary to use analogues of glutamic acid, preferably with restricted conformational flexibility, in order to quantify the requirements of this receptor, and elucidate its role in the nervous system.

#### 4.2 Conformationally restricted analogues of L-glutamic acid

Ibotenic acid (64) was the first analogue shown to have excitant action.<sup>42</sup> This isoxazole was first isolated<sup>43</sup> from the mushroom Amanita strobiliformis a fungus used in Northern Japan as a fly-killer. Another mushroom used as a fly-killer, Tricholoma muscarium, contained an active constituent which was a saturated analogue of ibotenic acid. This acid, called tricholomic acid<sup>44</sup> (65), was also found to be a potent neuronal excitant.<sup>45</sup>

Apart from their powerful fly-killing properties, these mushrooms were very tasty and commonly used in food. Tricholomic acid and ibotenic acid were found to have flavour accentuating properties much stronger than monosodium glutamate normally used to accent the flavour of food. As the taste enhancing properties of monosodium glutamate are thought to stem from a depolarisation of taste receptors, a similar effect by ibotenic and tricholomic acid could account for the taste of these mushrooms.<sup>42</sup>

Kainic acid (9) was also seen to be structurally related to glutamic acid, and preliminary tests<sup>45</sup> showed it to

be a powerful excitatory amino acid much more potent than glutamate itself. It was found<sup>46</sup> to be more potent than N-methyl-D-aspartic acid, previously the most potent amino acid excitant reported.<sup>47</sup> Kainic acid has since become the most commonly used and useful neurochemical agent in studies of the glutamate receptor.

The structurally related anthelmintic, domoic acid (15),<sup>48</sup> was also found to be a potent excitant, with effects comparable to kainic acid. It is interesting to note that domoic acid has also been shown to be a powerful fly-killing agent.<sup>49</sup> Quisqualic acid (16), the ascaricidal compound isolated from the genus Quisqualis, is also a potent glutamate agonist.<sup>50</sup>

The structures of these anthelmintic and fly-killing agents are shown in Fig.4.1 along with the synthetic conformationally restricted analogues cis-amino-1,3-dicarboxycyclohexane<sup>46</sup> (66) and cis-amino-1,3-dicarboxycyclopentane<sup>51</sup> (67). The structures of glutamic (63) and aspartic (62) acids are included for comparison.

#### 4.3 Kainic acid and the invertebrate nervous system

Kainic acid, despite its potent anthelmintic properties, only weakly excites invertebrate muscle fibres, and in most cases is less effective than glutamic acid.

Doubt was cast upon the role of glutamate as a transmitter at the excitatory synapses on crustacean muscle fibres when the action of kainic acid on a crayfish nerve-muscle preparation was studied.<sup>52</sup> Kainic acid was found to potentiate

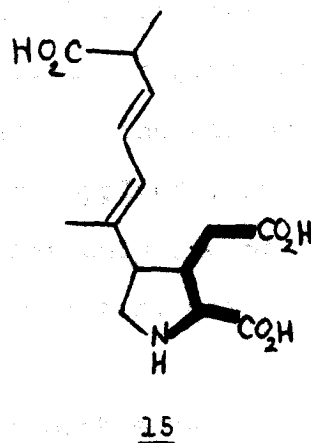
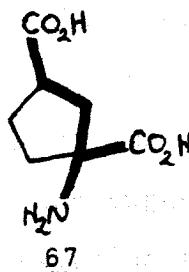
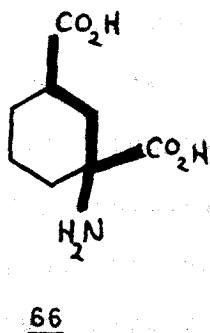
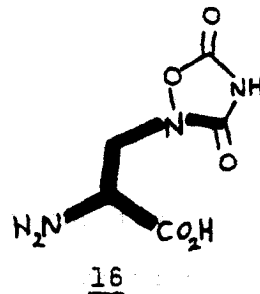
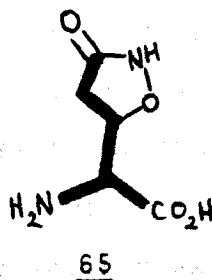
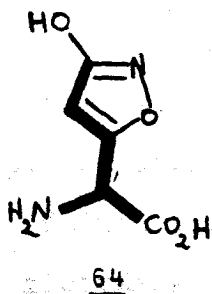
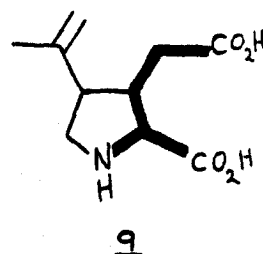
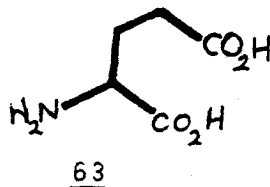
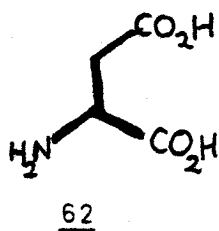


Fig.4.1

the depolarising action of glutamate, but did not affect the amplitude of the excitatory postsynaptic potential, therefore having little direct effect of its own. In snail nerve cells,<sup>53</sup> locust excitatory neuromuscular junction,<sup>54</sup> and the neuromuscular junction of lobster,<sup>55</sup> kainic acid was again found to have only weak agonist activity at glutamate receptors, but strongly potentiated glutamate action. On the walking leg of the crab<sup>56</sup> it was found to be twice as potent as glutamate, but still the most striking effect of kainic acid on these preparations was its potentiation of glutamate activity.

Kainic acid did not inhibit the uptake of tritium-labelled glutamate, therefore another explanation was required to explain this potentiation effect. It was suggested<sup>57</sup> that two glutamate receptors were present; one on the postsynaptic membrane of the neuromuscular junction, and the other at some extrajunctional site. Because kainic acid restored the glutamate potential, depressed by desensitisation of receptors induced by bath-applied glutamate, but did not for glutamate applied iontophoretically at the synapse, it was suggested that only one of these glutamate receptors, the extrajunctional receptor, was kainic acid sensitive.

Quisqualic acid (16) was found to be a much more potent glutamate agonist than kainic acid. It was several hundred times more potent than glutamate on crayfish neuromuscular junction,<sup>50</sup> but unlike glutamate its depolarising action was not potentiated by addition of kainic acid.<sup>57</sup>

It was also found that quisqualic acid could not

potentiate the actions of glutamate.<sup>58</sup> A study was carried out on several analogues of kainic acid, including quisqualic and domoic acids. These compounds were tested for their ability to depolarise glutamate receptors on crayfish opener muscle, and also their abilities to potentiate the action of glutamate. The results, shown in Table 4.1, indicated that kainic and domoic acids have similar actions, with domoic acid being somewhat more potent. Quisqualic acid, because of its different activity characteristics, was thought to be interacting with a different receptor from kainic acid.

TABLE 4.1

Glutamate agonist and potentiating action of kainic acid analogues

<u>Compound tested</u>	<u>Min. conc. (M) for effective:-</u>	
	<u>depolarisation</u>	<u>potentiation</u>
L- $\alpha$ -kainic acid	$3 \times 10^{-4}$	$5 \times 10^{-5}$
$\alpha$ -kainic acid methyl ketone	$1 \times 10^{-5}$	$3 \times 10^{-6}$
$\alpha$ -dihydrokainic acid	-	-
$\alpha$ -kainic acid lactone	-	-
$\alpha$ -N-acetyl kainic acid	-	-
$\beta$ -N-acetyl kainic acid	-	-
$\beta$ -N-acetyl kainic acid anhydride	-	-
Domoic acid	$6 \times 10^{-6}$	$3 \times 10^{-6}$
Quisqualic acid	$1 \times 10^{-7}$	-
L-glutamic acid	$3 \times 10^{-5}$	-

One theory to explain these findings suggested that kainic acid did not affect the sensitivity of the junctional receptor, but the extrajunctional which was not normally sensitive to glutamate, became activated when kainic acid was present in solution. This theory specified that kainic acid did not interact with either receptor directly, but gave no explanation as to how kainic acid might bring about this glutamate sensitising effect.

It was later found that kainic and domoic acids caused slight inhibition of the depolarisations of crayfish opener muscle, induced by quisqualic acid. This led to an updating of the two receptor theory whereby it was suggested<sup>60</sup> that the junctional receptor was sensitive to glutamate and quisqualic acid but not to kainic acid or domoic acid. The extrajunctional receptor was thought to interact with kainic or domoic acid but not glutamate or quisqualic acid. By interaction with the extrajunctional receptor, kainic acid was thought to alter the conformation of the junctional receptor. This change in the junctional receptor was thought to favour glutamate interaction, thereby potentiating the action of glutamate, but it made interaction with quisqualate more difficult and hence caused an inhibition of quisqualate excitation. Tests on lobster muscle fibre<sup>61</sup> suggested that aspartic acid could also act at the extrajunctional receptor.

Kainic acid was also used in structure-activity studies on the excitatory receptor of the hermit crab,<sup>56</sup> the only invertebrate receptor where kainic acid was found to be



more potent than glutamate. In an attempt to obtain some insight into the relative positions of the electrostatic binding sites within this receptor, a selection of conformationally restricted analogues of glutamate were tested for their ability to depolarise the postsynaptic membrane of the nerve-muscle preparation. The results obtained are shown in Table 4.2.

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TABLE 4.2

Effect of glutamate analogues on excitatory receptor of crab

<u>Compound</u>	<u>Relative activity</u>
kainic acid	2
L-glutamic acid	1
<u>cis-cyclo-DL-glutamic acid</u>	0.66
DL-glutamic acid	0.25
L-aspartic acid	0.16
<u>trans-cyclo-N-glutamic acid</u>	0.05
D-glutamic acid	0.01
Ibotenic acid	<0.005

---

Using Dreiding molecular models, the structures of these compounds were studied. Ibotenic acid, which was completely inactive, had a minimum  $\gamma$ -carboxy-amino separation of  $4.6\text{\AA}$ , and a maximum and minimum  $\alpha$ -carboxy- $\gamma$ -carboxy separation of about  $4.2\text{\AA}$ .

Glutamate, kainic acid, and cis-cycloglutamate were able to adopt conformations such that their  $\alpha$ -carboxy- $\gamma$ -carboxy separations were greater than or less than  $4.2\text{\AA}$ . But as a

separation of greater than  $4.2\text{\AA}$  was impossible for aspartic acid, which showed some activity at the receptor, it seemed that these analogues must interact at the receptor with an  $\alpha$ -carboxy- $\gamma$ -carboxy separation of less than  $4.2\text{\AA}$ , and a  $\gamma$ -carboxy-amino separation of less than  $4.6\text{\AA}$ .

L-glutamate, kainic acid, and cis-cyclo $\gamma$ -glutamate were able to adopt superimposable conformations which satisfied these requirements. It was concluded that these analogues and hence glutamate itself interacted with the same excitatory receptor and in a folded conformation.

Invertebrate nerve-muscle preparations have always been popular as models for testing the neurochemical properties of compounds because of their simple structures and ease of use. They have not been as useful as models of the glutamate system as they have been for other neurotransmitters, because of the large variation in effects between different preparations.

In invertebrate systems, glutamate sensitive receptors have been found which have been excitatory and kainic acid sensitive<sup>56</sup>, excitatory and kainic acid insensitive<sup>60</sup>, and inhibitory and kainic acid insensitive.<sup>62</sup> Extrajunctional receptors have been found with structural requirements varying from kainic acid sensitive<sup>60</sup> to ibotenic acid sensitive<sup>63</sup>, and in one preparation, calculations suggested that more than one molecule of glutamate or kainic acid could act at the receptor.<sup>55</sup> Receptors have also been found which were specific for aspartic acid.<sup>64</sup>

#### 4.4 Kainic acid and the mammalian glutamate receptor

##### 4.4.1 Introduction

Early investigations<sup>65</sup> of excitatory amino acids using open-chain analogues of glutamate and aspartate gave rise to the theory that the glutamate sensitive receptor required the three point contact of two acidic and one basic group. These groups had to be the free acids and base and gave optimum results when one acid group was alpha to the base and the other was two or three carbon atoms away from the basic function. It was also suggested that  $\alpha$ -decarboxylation of the excitatory compound would produce a corresponding analogue with inhibitory properties, just as the excitant amino acid, L-glutamate, decarboxylated to give the inhibitory neurotransmitter GABA ( $\gamma$ -aminobutyric acid).

This early theory was mainly supposition and questions dealing with the precise conformation of the receptor site, whether or not there exists separate receptors for glutamate and aspartate, and whether there may be non-specific amino-acid receptors, possibly extrajunctional, in addition to specific synaptic receptors, remain to be established.

Foremost in these investigations has been the use of conformationally restricted analogues of glutamic acid. Examination of their excitatory actions, and correlations with the degree of conformational restraint have provided valuable information about possible receptors.

Although no compounds have yet been found to have absolute antagonistic specificity towards any of these neuronal excitants, some compounds have been found to demonstrate a degree of preferential antagonism. This partial antagonistic

specificity has been used together with conformationally restricted agonist studies, to further the knowledge of the mammalian excitatory nervous system.

Unlike the invertebrate nervous system, mammalian neurons from different species tend to show more consistent effects towards various excitant amino acids. The nervous system even of simple mammals is infinitely more complex than that of crustaceans and insects, and excitant amino acid analogues and antagonists are also used to map the areas where these amino acids are suspected of being neurotransmitters.

#### 4.4.2 The kainic acid receptor

When administered microelectrophoretically into the synapse of mammalian neurons, kainic acid produces a very powerful excitatory effect. The most common explanation for this effect is that kainic acid interacts directly with a post-synaptic receptor on the neuron. Because of the structural similarity between kainic acid and glutamic acid, this receptor is thought to be a glutamate receptor. Glutamate analogues have been tested on numerous nervous tissues, but to date only the structurally related compound, domoic acid,<sup>48</sup> has shown a greater excitatory action than kainic acid on mammalian nerve cells.

Dihydrokainic acid (38) was found<sup>46</sup> to be almost inactive on rat cortical neurons which were strongly excited by kainic acid (9), and  $\alpha$ -allokainic acid (10) was found<sup>45</sup> to be a considerably weaker excitant than  $\alpha$ -kainic acid on cat spinal interneurons. These analogues still possessed the same glutamate-like structural features as  $\alpha$ -kainic acid.

It was suggested<sup>66</sup> that the potency of kainic acid was associated with the unsaturation in the C-4 side-chain, and also the cis-relationship between the C-3 and C-4 substituents. This cis-relationship was thought to result in a mutual steric hindrance which greatly restricted the possible conformations of both substituents. In this relatively fixed conformation, the C-3 side-chain carboxylate group was said to be placed in a particularly well-oriented position with respect to a corresponding electropositive region on the receptor. It was also proposed that the unsaturated group might interact with a lipophilic region on the receptor and so further stabilise the complex.

The cis-orientation requirement of C-3 and C-4 substituents of kainic acid was disputed<sup>67</sup> recently when both the  $\alpha$  and  $\alpha$ -allo isomers of kainic acid methyl ketone (39) and (52) were tested for their ability to increase the permeability of neuronal membranes to sodium ions. Both isomers were found to be almost as potent as  $\alpha$ -kainic acid. Also tested were kainic acid analogues having a carboxylate group at position four. Both isomers of this analogue (68) and (69) proved to be equipotent, and had about one third of the potency of  $\alpha$ -kainic acid.

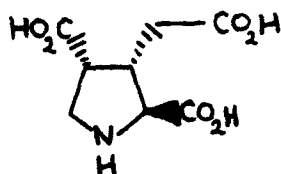
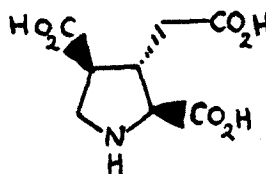
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TABLE 4.3

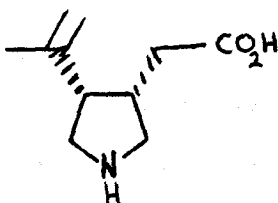
Potencies of some amino acids as neuronal excitants

Amino Acid	Rat <sup>48</sup> Spinal Interneurones	Frog <sup>48</sup> Spinal Motoneurones	Cat <sup>46</sup> Spinal Interneurones	Rat <sup>51</sup> Thalamic Neurones
Domoate	50- > 200	247-373		
Kainate	40- > 200	85-185	8-80	10.62 $\pm$ 1.70
(+)- <u>cis</u> -ADCP				9.74 $\pm$ 1.63
N-methyl-D- aspartate			7-18	
Ibotenate			2-7	7.45 $\pm$ 1.31
Quisqualate	25- > 200	151-614		
(+)- <u>trans</u> -ADCP				1.96 $\pm$ 0.14
L-Glutamate	1	1	1	1
D-Aspartate				0.95 $\pm$ 0.08
L-Aspartate				0.74 $\pm$ 0.03
D-Glutamate				0.41 $\pm$ 0.04
$\alpha$ -allo-kainate	1-3	0.3-0.5		
(+)- <u>cis</u> -ADCH			0.7-0.8	0.13 $\pm$ 0.02
Dihydro-Kainate			0.06-0.6	
(+)- <u>trans</u> -ADCH				0.02 $\pm$ 0.02

ADCP = amino-1,3-dicarboxycyclopentane

ADCH = amino-1,3-dicarboxycyclohexane

Kainic acid has also been  $\alpha$ -decarboxylated<sup>68</sup> in the hope of obtaining a conformationally restricted analogue of GABA. The product (70), when tested microelectrophoretically on feline spinal neurons, was a weak excitant rather than a depressant, suggesting that decarboxylated glutamate agonists do not necessarily interact with inhibitory GABA receptors.



70

Throughout the last decade the potencies of glutamate analogues have been determined on various nervous tissues. Table 4.3 shows the results of three such studies. Although potencies of analogues tend to vary between different species, tissue types, and experimental conditions employed, it can be seen that the potency of each analogue with respect to another, maintains a relatively constant arrangement with kainic and domoic acids in each case being the most powerful neuronal excitants.

Tritium-labelled kainic acid has been prepared<sup>69</sup>, by dissolving kainic acid in glacial acetic acid and stirring at 80° in tritiated water. The reaction was catalysed by 5% rhodium on alumina.

When tritium-labelled kainic acid was bound to synaptic

membranes,<sup>69</sup> the ease with which this labelled compound could be displaced by other glutamate analogues was determined. The ability of analogues to displace the radioactive label varied in roughly the same order as had been determined from neuronal excitation experiments. Of the compounds tested, kainic acid was found to be the best displacer of synaptically bound <sup>3</sup>H-labelled kainic acid. Quisqualic acid had one-third of the potency of kainic acid, and glutamate was weaker than kainate and quisqualate.

The interatomic distances and degree of conformational flexibility have been measured<sup>51</sup> for some excitant amino acids, from computer models of the compounds, constructed from crystallographic data. Assuming the important functional groups for receptor activation to be the amino and two acidic functions, the range of possible interatomic distances between these groups were measured. It can be seen from the results, shown in Table 4.4, that, of the conformationally restricted amino acids, kainic acid retains the greatest degree of conformational freedom due to bond rotation of the C-3 substituent.

Paradoxically, the only compound of comparable potency to kainate is that with the most rigidly fixed conformation, cis-amino-1,3-dicarboxycyclopentane. If the strength of this analogue can be attributed to its high degree of inflexibility in holding the electrostatic centres in the correct spacial array, then the potency of kainate, as was stated previously, must be partly attributable to some effect of the C-4 isopropenyl substituent.

An alternative theory for the high excitatory potency



TABLE 4.4

Interatomic distances in some excitant amino acids. (Å)

<u>Amino acid</u>	$\alpha\text{CO}_2\text{H} - \delta\text{CO}_2\text{H}$		$\delta\text{CO}_2\text{H} - \text{N}$	
	<u>Max.</u>	<u>Min.</u>	<u>Max.</u>	<u>Min.</u>
L-glutamate	5.17	2.31	5.13	2.23
L-aspartate	3.88	2.64	3.87	2.71
Kainate	5.08	4.29	4.62	2.98
Ibotenate	4.61	4.44	4.44	4.26
<u>cis</u> -ADCP	← 4.78 →		← 4.64 →	
<u>cis</u> -ADCH	5.03	2.43	4.32	4.30

of kainic acid and related compounds is that, unlike glutamate and aspartate, there is no powerful uptake system to remove these compounds from the synapse.<sup>70</sup> They are therefore able to act on the receptor for longer periods of time than glutamate and thus exert a stronger effect.

It is thought that a different conformation is required by the uptake system, to that for receptor activation, and the conformationally restricted amino acids are unable to achieve this conformational change. Kainic acid, however, has been shown in one case to be a weak substrate for the uptake system,<sup>71</sup> possibly due to its more flexible structure.

Very little is known about the active transport system<sup>72</sup> for excitatory amino acids, and although it may have a contributory effect on the potency of glutamate analogues such as kainic acid, it is unlikely to be the sole reason for their potency.

#### 4.4.3 The Glutamic acid receptor

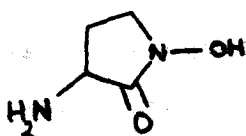
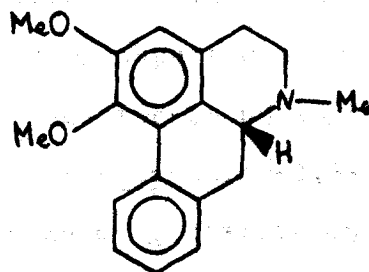
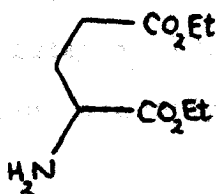
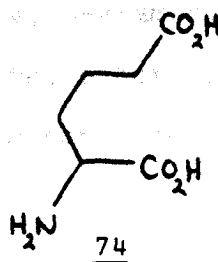
Both glutamic acid and aspartic acid elicit excitatory responses when applied to neurones. It is not known however, whether this effect is the result of interaction at one non-specific amino acid receptor, or whether specific glutamic acid and aspartic acid receptors exist.

Results obtained from experiments on Renshaw cells and spinal interneurons suggested that two receptors did exist.<sup>73</sup> Renshaw cells were known to be more sensitive to aspartate than glutamate, whereas spinal interneurons were more sensitive to glutamate. By using kainic acid as a glutamate analogue unable to interact with aspartate receptors, and N-methyl-D-aspartate as an aspartate analogue unable to act on glutamate receptors, it was found that the results were consistent with the presence of both glutamate and aspartate receptors on spinal interneurons and Renshaw cells, with fewer aspartate receptors on interneurons than on Renshaw cells. These receptors were called glutamate-preferring and aspartate-preferring because, although the amino acids were thought to elicit the strongest response from their own receptor, there was still some degree of overlapping reactivity.

It was stated earlier that no antagonists have yet been found which can give complete and effective blocking of the amino acid receptors. However, some compounds have been found to elicit a partial, reasonably selective, antagonism of these receptors, and when used carefully have given useful additional information on glutamate neurotransmission.

One such antagonist is 1-hydroxy-3-aminopyrrolid-2-one (HA-966) (71).<sup>74</sup> It most likely binds to the receptor via the two oxygen and nitrogen atoms in much the same way as the agonists, but due to electronic or conformational differences, it is unable to evoke the required permeability change in the neuronal membrane. It is not a powerful antagonist, but by depressing the responses of central neurones excited by kainic acid or N-methyl-D-aspartate, it has been possible to amass further evidence for the action of these amino acids on various neurones.

Other compounds have also been found which, although having no absolute antagonistic specificity towards a particular excitant, their appropriate administration has demonstrated that one excitant can sometimes be preferentially antagonised before another.

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Magnesium ions, which were known to inhibit glutamate excitations and were thought to act by a post-synaptic mechanism<sup>75</sup>, were found to depress depolarisations stimulated by N-methyl-D-aspartate, but not those stimulated by kainic acid.<sup>76</sup> On the other hand, (-)-nuciferine (72), an aporphine alkaloid, when administered intravenously, was found to depress the excitations produced by kainic acid, but not those produced by N-methyl-D-aspartate, in cat spinal cord.<sup>77</sup>

These results also point to the existence of both glutamate and aspartate receptors on central neurons, but the use of kainic acid in these studies has recently been questioned.

Some time ago when kainic acid was first found to be a weak glutamate uptake inhibitor<sup>71</sup>, it was suggested that it did not interact with the glutamate receptor. The strong excitations produced by kainic acid were said to be due to the inhibition of uptake of the natural neurotransmitter and that the excitations were therefore elicited by glutamic or aspartic acid. Since then experiments with tritium-labelled receptor-bound kainic acid, and the use of rigid analogues such as (+)-cis-amino-1,3-dicarboxycyclopentane which show no inhibition of glutamate uptake, have largely discounted this theory.

More recently the use of partially selective antagonists has indicated that kainic acid and glutamic acid may not act on the same receptor.<sup>78</sup> By using solutions of  $\alpha$ -kainic acid, L-glutamic acid, N-methyl-D-aspartate, D- $\alpha$ -aminoadipic acid (74), and diethyl glutamate (73) administered electrophoretically from a seven-barrelled micropipette, the neuronal activity changes were

recorded from thirty-four different neurones in the ventrobasal thalamus of anaesthetised rats.

Diethyl glutamate (73) was found to antagonise glutamic acid excitations preferentially to N-methyl-D-aspartate induced excitations, indicating that it was a partially selective glutamate antagonist. D- $\alpha$ -aminoadipate (74) was found to be a partially selective aspartate antagonist by preferentially reducing the actions of N-methyl-D-aspartate relative to that of glutamic acid. Both antagonists were found to reduce the excitatory action of glutamic acid relative to kainic acid induced excitation, which remained unaffected or only minimally reduced. D- $\alpha$ -aminoadipate was able to reduce the excitations elicited by N-methyl-D-aspartate preferentially to those of kainate.

These results were said to demonstrate that the kainic acid receptor and the glutamic acid receptor, which had long been assumed to be one and the same, were in fact different. In the light of these results, it was proposed that the central nervous system of mammals contains, not only specific glutamate and aspartate junctional receptors, but also an unspecific excitatory amino acid receptor which is distinct from the glutamate receptor and may be junctional or, like the invertebrate system, extra-junctional.<sup>51</sup>

The results obtained in these experiments, however, can only be valid if all the compounds tested actually act upon their assumed receptors. The mechanism of action of the antagonists used have not yet been determined<sup>79</sup>, but if their rôle is

found to be not a receptor-mediated event, but a presynaptic mechanism, then the theories constructed from their use could be invalid.

#### 4.5 Cyclic nucleotide accumulation

Kainic acid has been found to stimulate the accumulation of cyclic adenosine monophosphate (cAMP) in the cerebellum. High concentrations of glutamic acid also caused the level of cAMP in the cerebellum to increase, but kainic acid was ten times more potent.<sup>80</sup>

The mode of action of kainic acid was thought to be different from that of glutamic acid, as a three-fold increase in cyclic guanosine monophosphate (cGMP) was also produced, whereas glutamic acid had little or no effect on cGMP levels.

Cyclic-AMP is known to have a regulatory rôle in many cellular processes and has also been connected with synaptic transmission. Noradrenaline and dopamine sensitive adenylate cyclases have been found in various nervous tissues in different mammalian species. It is thought that the neurotransmitters act on a receptor which stimulates the cyclase enzyme to produce a cAMP from adenosine triphosphate, and the increase in cAMP then causes hyperpolarisation of the neurone.<sup>81</sup>

Other glutamate analogues were tested, but kainic acid gave the strongest cAMP level increase. Dihydrokainic acid, proline and hydroxyproline were totally inactive.<sup>82</sup>

N-Methyl-D-aspartate was also inactive, suggesting that only glutamate-like but not aspartate-like transmitters enhanced cAMP levels.

## 4.6 Neurotoxicity of kainic acid

### 4.6.1 Neurotoxic effect

High concentrations of glutamic acid have a toxic effect on brain nerve cells. Kainic acid, being an analogue of glutamate, was tested for its toxic properties and found to be a neurotoxic agent, considerably more powerful than glutamate.<sup>83</sup>

The pattern of neuronal sensitivity to kainic acid in the cerebellum, was consistent with areas suspected of being mediated by glutamic acid.<sup>84</sup> Neuronal sensitivity to kainic acid in rat striatum was found to develop 7-21 days after birth. This development correlated with the appearance and maturational increase in binding sites for tritium-labelled kainic acid. It also correlated with a dramatic increase in glutamatergic innervation in rat striatum found to occur 10-21 days after birth.<sup>85</sup>

It was thought that the toxic effects of kainic acid were produced by the same mechanism that produced neuronal excitatory effects at lower concentrations. The neurotoxic properties of several glutamate analogues were examined<sup>86</sup> and a similarity was found between the neurotoxic and neuroexcitatory properties of the analogues. Kainic acid was by far the most toxic amino acid tested and dihydrokainic acid was 100 times weaker.

It is generally thought that kainic acid toxicity results from interaction with postsynaptic glutamate receptors.<sup>87</sup> A presynaptic mechanism was discounted when specific <sup>3</sup>H-labelled kainic acid binding sites were found on membrane fragments of rat striatum.<sup>88</sup> Neurons having these receptors are depolarised

for prolonged periods, due to poor kainate uptake. The chronic excitation soon becomes irreversible and the ensuing ionic imbalance results in cell death. It is also possible that the increased levels of cAMP and cGMP could be responsible for the toxic effects of kainic acid, as high concentrations of cyclic nucleotides can result in cell death.

It has also been suggested that kainic acid neurotoxicity might occur by an indirect mechanism involving glutamic acid release and inhibition of reuptake.<sup>89</sup> The greater potency of kainic acid relative to glutamic acid was explained by the powerful glutamate uptake system, preventing high concentrations of glutamate from forming in the synapse when kainic acid was not present.

Injection of kainic acid into the striatum, after surgical lesioning of the cortex, has provided strong support for this mechanism of neurotoxicity.<sup>85</sup> The striatum receives a major excitatory input from the cerebral cortex and is thought to use glutamate as a transmitter. Decortication was found to abolish the effects of injected kainic acid, suggesting that glutamic acid release is the important factor in the neurotoxicity of kainic acid.

Injection of kainic acid into rat striatal tissue caused a reduction in the release of acetylcholine and GABA.<sup>90</sup> This was due to the destruction of neurons containing choline acetyltransferase (CAT), and neurons containing glutamic acid decarboxylase (GAD). It was therefore inferred that GABAergic and cholinergic neurons in the striatum possessed glutamate receptors.<sup>91</sup>



Injection into various parts of the brain has given valuable information about glutamate receptor locations and glutamatergic pathways. Kainic acid has proved the most useful analogue in the neurotoxic field. Analogues, such as quisqualic acid and ibotenic acid, having similar excitatory potences, have proved to be much weaker neurotoxic agents. Quisqualate and ibotenate, when injected into rat striatum in similar concentrations to kainic acid, produced no significant drop in CAT or GAD levels.

#### 4.6.2 Neurochemical lesions

When injected into rat brain, kainic acid destroyed local cells around the area of injection, but did not damage axons passing through or terminating in the injected area.<sup>92</sup>

Injection of kainic acid into the substantia nigra of rat brain, resulted in a decrease in dopamine activity in the striatum, but GABAergic nerve terminals in the substantia nigra were unaffected. When kainic acid was injected into the striatum, GABA activity in the substantia nigra was decreased, but dopamine activity in the striatum remained unchanged.<sup>93</sup>

This phenomenon is what would be expected for a post-synaptic receptor-mediated effect, as axons passing through, and presynaptic termini in the injected area, do not possess glutamate receptors and are unaffected by kainic acid. The effect has provided a general technique of chemiolesioning of the brain where previously surgical lesions would have been required.<sup>94</sup>

This technique has found uses in neurochemical studies totally unrelated to glutamate. For example, kainic acid lesions

were used in an investigation of the antagonism, by opiates, of prostaglandin E<sub>2</sub>-stimulated synthesis of cAMP.<sup>95</sup> The experiment showed that morphine completely suppressed cAMP induced synthesis by prostaglandin E<sub>2</sub>. But after kainic acid was injected into the corpus striatum, causing degeneration of cell bodies in that region, morphine gave very poor inhibition of prostaglandin E<sub>2</sub> stimulation. This suggested that opiate receptors involved in PGE<sub>2</sub> stimulation of cAMP synthesis were located within the corpus striatum.

#### 4.6.3 Animal model for Huntingtons chorea

The symptoms of Huntingtons chorea, an hereditary movement disorder, result from degeneration of neurones primarily in the basal ganglia. Several neurochemical abnormalities have been identified in the brains of people dying with this disorder, but no animal system with similar neuropathological changes has yet been described.

Kainic acid, when injected into rat striatum, was found to cause neuronal degeneration, neurochemical changes, and behavioural responses resembling those found in Huntingtons chorea. It was therefore thought that kainic acid could provide an animal model for the study of the disease.<sup>96</sup>

The striatal lesions produced by kainic acid have particular relevance to Huntingtons chorea because the major site of neuropathological change in this disorder occurs in the striatum. This, together with the fact that <sup>3</sup>H-labelled kainic acid binds to striatal tissue more strongly than any other area of rat brain, lends support to the theory that the massive cortico-striatal pathway in the brain is glutamatergic and that Huntingtons chorea could result from an overstimulation of

these neurons.<sup>97</sup>

Injection of kainic acid into rat striatum produced a large decrease in the number of dopamine and muscarinic acetylcholine receptors which was very similar to the loss of these receptors found in Huntingtons chorea - diseased brains.<sup>98</sup> The density of tritiated kainic acid binding of diseased brains, when compared with control human brains, was found to be significantly reduced and similar to a reduction in binding sites found in kainic acid lesioned rat striatum.<sup>99</sup>

The reasons for the excitotoxic effects which occur in Huntingtons chorea are not known, but the use of a kainic acid generated animal model, is of considerable use for the study and treatment of this disease.

#### 4.6.4 Toxicity to host in anthelmintic use

It is paradoxical that a substance, which only weakly excites invertebrate muscle preparations, but is extremely toxic to mammalian neurones, should be found to effectively kill worms by spastic paralysis, while causing no observable side effects in the host.

Mass therapy trials conducted in Japan<sup>22</sup>, on up to 28,000 school children, noted no central nervous system side effects due to kainic acid. In such tests no child received more than 0.3 mg of kainic acid per kg of body weight. This was one-tenth the oral dose found to cause toxic effects in infant mice.<sup>83</sup> As high concentrations of kainic acid are not required for anthelmintic use, this may account for its reputation as a safe drug.

In the light of the knowledge of the neuroexcitatory effects of kainate and glutamate, the use of this drug in countries where monosodium glutamate is a popular food additive, is thought to be potentially hazardous.<sup>83</sup> An excess of monosodium glutamate in the diet has been found to be responsible for a phenomenon known as Chinese Restaurant Syndrome.<sup>100</sup> This condition produces headache and chest pain and it is thought that kainic acid could have an additive or potentiating effect on the dietary glutamate, and so produce more serious results.

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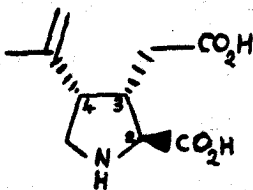
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STUDIES TOWARDS THE DEVELOPMENT OF A  
GENERAL SYNTHETIC ROUTE TO KAINIC ACID  
AND STRUCTURALLY RELATED ANALOGUES

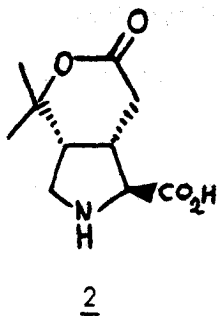
1. ATTEMPTS TO SYNTHESISE A CONFORMATIONALLY RESTRICTED ANALOGUE OF KAINIC ACID BY AN INTRAMOLECULAR DIELS-ALDER REACTION

Kainic acid, the active anthelmintic constituent of the red algae Digenea simplex<sup>1</sup>, has in recent years been found to be a very useful chemical tool for investigating certain aspects of the nervous system.<sup>2</sup> A key consideration in these studies is the active conformation in which kainic acid elicits its neuropharmacological effects. In order to ascertain the active conformation of kainic acid, analogues with restricted conformational flexibility are required for testing.

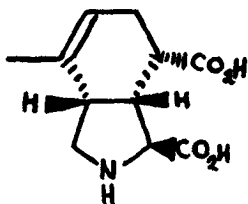
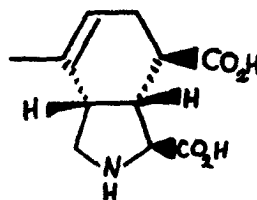
The pharmacological activity of kainic acid (1) depends upon the molecule possessing a free N-H group, two free carboxylic acids, and a side-chain containing a double bond at position 4. The stereochemistry of the molecule is critical as only a trans relationship between C2 and C3 substituents, and a cis relationship between the C3 and C4 substituents produces strong pharmacological responses. The important feature to be considered, when designing a synthesis of kainic acid, is the cis relationship between the C3 and C4 substituents. The chiral centre at position 2 is readily epimerisable and therefore the thermodynamically more favourable trans relationship, between the C2 and C3 substituents, can be easily obtained.



The conformational freedom of the active groups in kainic acid are severely restricted by the pyrrolidine ring, but there is still a fair degree of conformational flexibility associated with the acid group at position 3 and the double bond at position 4 due to rotation of the acetic acid and isopropenyl substituents respectively. A simple way of fixing the positions of these groups would be to incorporate them in a ring. A ring has previously been constructed across positions 3 and 4 by hydrating the double bond and forming the bicyclic kainic acid lactone<sup>3</sup> (2). This compound was totally inactive as it had lost both the C4 double bond and the C3 free acid functions.



In this project an attempt was made to synthesise a similar bicyclic system, which would also incorporate the required groups, with the correct stereochemistry about the pyrrolidine ring. The only conformational changes possible in this system would be the flexing of the rings. A further degree of "fine tuning" of the conformation could be obtained by preparing two stereoisomers of the bicyclic system, compounds (3) and (4). Testing of these compounds could provide valuable information about the conformation required at the active site of various kainic acid sensitive neurochemical systems.

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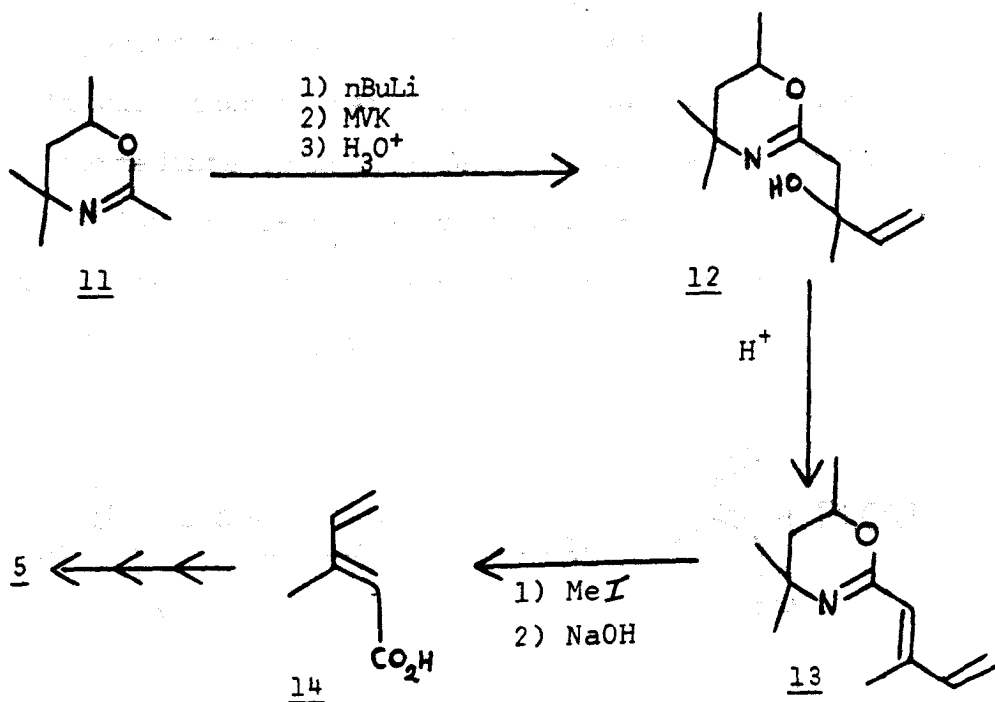
It was decided to construct the bicyclic system using an intramolecular Diels-Alder reaction. This reaction has been found to be a very efficient method for constructing 5,6-ring systems.<sup>4</sup> The reaction is stereospecific due to the concerted, symmetry-controlled cycloaddition, and usually proceeds in high yield, due to the favourable entropy factor of an intramolecular reaction. In syntheses, the intramolecular Diels-Alder is also favoured over the intermolecular reaction because the regiochemistry of the reaction is controlled by the geometric confines of the system.

Scheme 1.1 shows the intramolecular Diels-Alder reaction proposed for the synthesis of analogues (3) and (4). Compound (7) should cyclise very easily to give a mixture of isomers (8) and (9), corresponding to endo and exo cycloaddition products. The endo cycloaddition product (8), has the correct stereochemistry to be taken on to compound (3), but the exo addition product (9), has a trans-fused ring system and must first be epimerised to the thermodynamically more stable cis-fused product (10) before it can be taken on to compound (4).





Analogous reactions<sup>5,6</sup> have shown that this type of cycloaddition, where the dienophile is activated by an ester group, usually produces almost exclusively the endo-mode of addition, which would give the cis-fused ring system. This suggests that Scheme 1.1 may only give rise to compound (3).

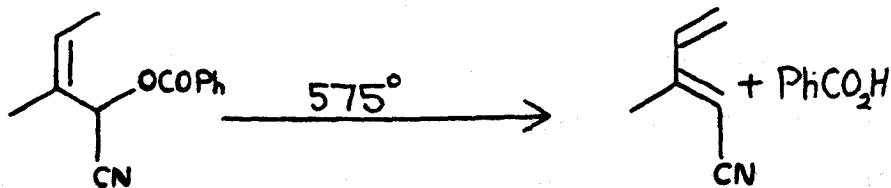


Scheme 1.2

Before Scheme 1.1 could be attempted it was first necessary to develop a synthesis for compound (5). Work towards the synthesis of amino-diene (5) was started using the route shown in Scheme 1.2. The oxazine reagent developed by Meyers,<sup>7,8</sup>

2,4,4,6-tetramethyl-5,6-dihydro-oxazine (11), was lithiated and allowed to react with methyl vinyl ketone. The resulting alcohol (12) was obtained in low yield and when the reaction was scaled up (X5), the product was mainly polymeric and no alcohol was obtained. The conversion of compound (12) to compound (13) was attempted by acid catalysis of the alcohol in refluxing benzene, but no dehydration occurred. Because of these unpromising first steps, and also the dubious nature of the transformation of acid (14) to amino-diene (5), it was decided to abandon the route shown in Scheme 1.2.

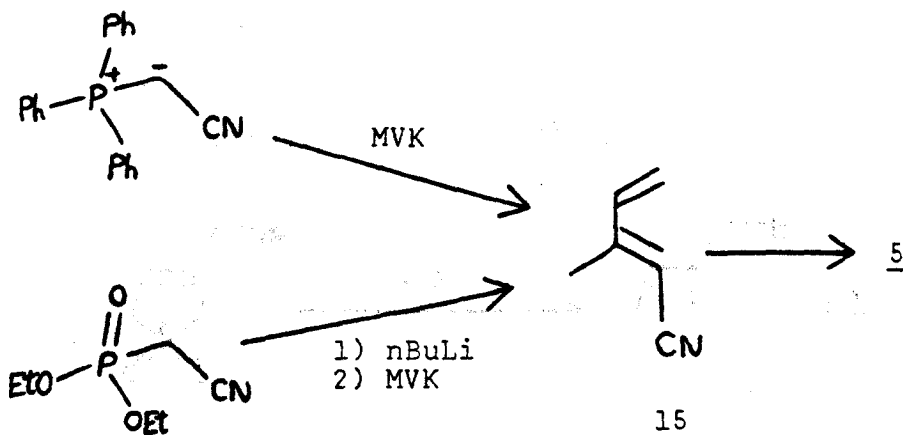
It was thought that a nitrile would make a more suitable intermediate to amino-diene (5) than a carboxylic acid. The required nitrile, 1-cyano-2-methyl-1,3-butadiene (15), has been prepared previously by the gas-phase pyrolysis of the benzoate of tiglic aldehyde cyanohydrin.<sup>9</sup>



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Facilities for a gas-phase pyrolysis were not available. It was decided, instead, to synthesise the nitrile by a Wittig reaction as shown in Scheme 1.3. The phosphorane used, triphenylcyanomethylene phosphorane, was prepared by a reported method.<sup>10</sup> The Wittig reaction between the phosphorous

ylid and methyl vinyl ketone produced triphenyl phosphine oxide, suggesting that the reaction must have occurred. However, no nitrile could be isolated from the reaction mixture. The reaction was repeated several times under various reaction conditions and work-up procedures, but no nitrile could be obtained.

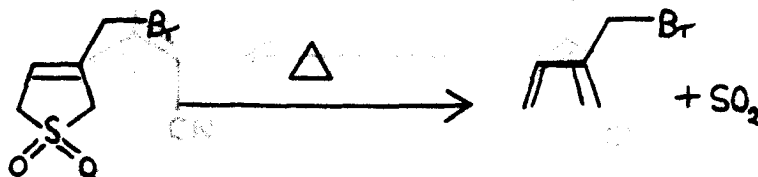


Scheme 1.3

The reaction had been carried out in refluxing benzene as the Wittig reagent requires elevated temperatures in order to react. It is possible that at such temperatures the cyano-diene (15) might be unstable and therefore the Wittig reaction may have produced the desired product which then polymerised leaving no isolable product. In order to carry out the reaction at low temperature the much more reactive phosphonate anion, obtained from diethyl phosphonoacetonitrile,<sup>11,12</sup> was used, but again the desired cyanodiene (15) was not obtained. The phosphonate reaction was repeated using a solid/liquid two phase system<sup>13</sup> whereby the phosphonate and ketone were dissolved

in THF, and stirred with finely powdered potassium hydroxide. This reaction gave a brown gum which once again did not contain the desired product.

Another attempt to prepare compound (15) was made using 3-bromomethyl-2,5-dihydrothiophen-1,1-dioxide (16). This sulphone was prepared by heating isoprene and sulphur dioxide in a sealed autoclave,<sup>14</sup> followed by allylic bromination of the resulting unsaturated sulphone.<sup>15,16</sup>

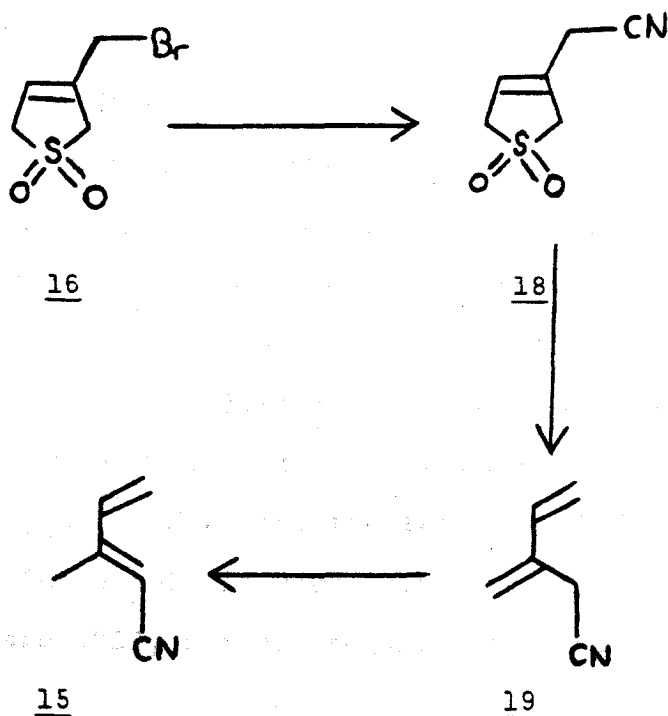


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The reaction between sulphur dioxide and dienes is reversible, and heating compound (16) under reduced pressure produced 2-bromomethyl-1,3-butadiene (17) by extrusion of sulphur dioxide. Cyclic sulphones can therefore act as masked dienes.

This strategy was employed in Scheme 1.4 whereby compound (16) was first converted to the cyano derivative, which could then act as a precursor for cyanodienes (19) and (15). The displacement of bromide from compound (16), by cyanide, to give the cyano-cyclic sulphone (18), proved to be a difficult reaction. The standard conditions for this type of displacement (heating the alkyl halide with a cyanide salt in DMSO<sup>17</sup>) gave only a polymerised product. This was thought to be due to overheating, but when the reaction was repeated at



Scheme 1.4

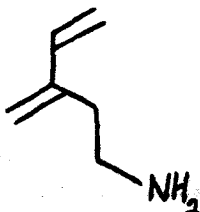
room temperature, a similar black polymeric mixture was obtained. The reactions were repeated using the bromo-diene (17) instead of sulphone (16), but again only polymer was obtained in each case. The reactions were repeated using HMPA<sup>18</sup> as solvent, but were unsuccessful. A solid/liquid two-phase system was then tried. The sulphone (16) or diene (17) was dissolved in dichloromethane and stirred with solid potassium cyanide. The reactions were catalysed using a crown ether<sup>19</sup> which coordinated the potassium ions, so partially solubilizing the salt, and leaving highly reactive free cyanide ions in solution. In both cases the starting material was recovered unchanged even after refluxing for several hours. Only the diene (17) showed

signs of having reacted slightly. The crown ether reaction was also tried using HMPA as solvent, but as in the previous reactions with this solvent the starting material was degraded. The problem was eventually overcome by using a liquid/liquid two-phase system. The displacement of bromide by cyanide ion was effected by vigorously stirring a solution of the sulphone (16) or diene (17) in dichloromethane, with an aqueous solution of sodium cyanide and using tetrabutylammonium bromide as phase transfer catalyst.<sup>20</sup> However, the sulphone (18) obtained, was found to be soluble only in very polar aprotic solvents such as acetonitrile and DMSO, but dilute solutions could be obtained in THF.

The mass spectrum of sulphone (18), like that for sulphone (16), showed the major fragmentation to be the elimination of sulphur dioxide to give a diene. However, when the nitrile was heated under the same conditions as for compound (16), no cyanodiene (19) was obtained. The contents of the flask appeared to polymerise and char during the pyrolysis and no distillate was produced. This seemed to suggest that the temperature required for the retro-Diels-Alder was greater than that at which the sulphone (16), or perhaps the diene (19), was stable.

A method has been reported whereby, sulphones that would normally require high temperatures to undergo cycloelimination, can be made to eliminate sulphur dioxide under mild conditions, by reaction with lithium aluminium hydride.<sup>21</sup> However, if this reaction was applied to compound (18) it would also cause the nitrile to be reduced.<sup>22</sup> It was decided to try

this reaction of the cyano-sulphone (18) with lithium aluminium hydride in the hope that the hydride would, not only reduce the nitrile and eliminate sulphur dioxide, but also isomerise the exo double bond to give the required amino-diene (5). For this isomerisation to occur, the elimination reaction would have to precede the reduction of the nitrile. In this way cyano-diene (19) would be produced initially and would be expected to isomerise very easily to the system of greater conjugation, cyanodiene (15), which would then be reduced to amino-diene (5). If the nitrile reduction were to occur before the elimination of sulphur dioxide, it is unlikely that the isomerisation would take place, and amino-diene (20) would be the expected product.



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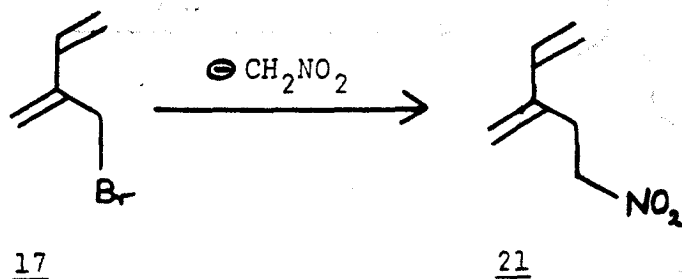
The actual direction the reaction took was not established, as when the reaction was attempted, the product obtained was a polymeric residue. It is therefore not known whether the reaction produced an unspecified diene which then polymerised under the reaction conditions, or whether the starting material was degraded by the hydride instead of reacting cleanly.

When the cyanide substitution reaction was attempted on the bromo-diene (17), using the liquid/liquid phase transfer method catalysed by tetrabutylammonium bromide, the reaction



would not proceed to completion. In each attempt the reaction product was a mixture of the bromo-diene (17) and cyanodiene (19). Increasing the reaction time and the speed of agitation did not improve the product ratio and the best yield of cyanodiene obtained was 35%, as calculated from the n.m.r. spectrum of the mixture. The product was not isolated, and an isolated yield would probably have been much lower.

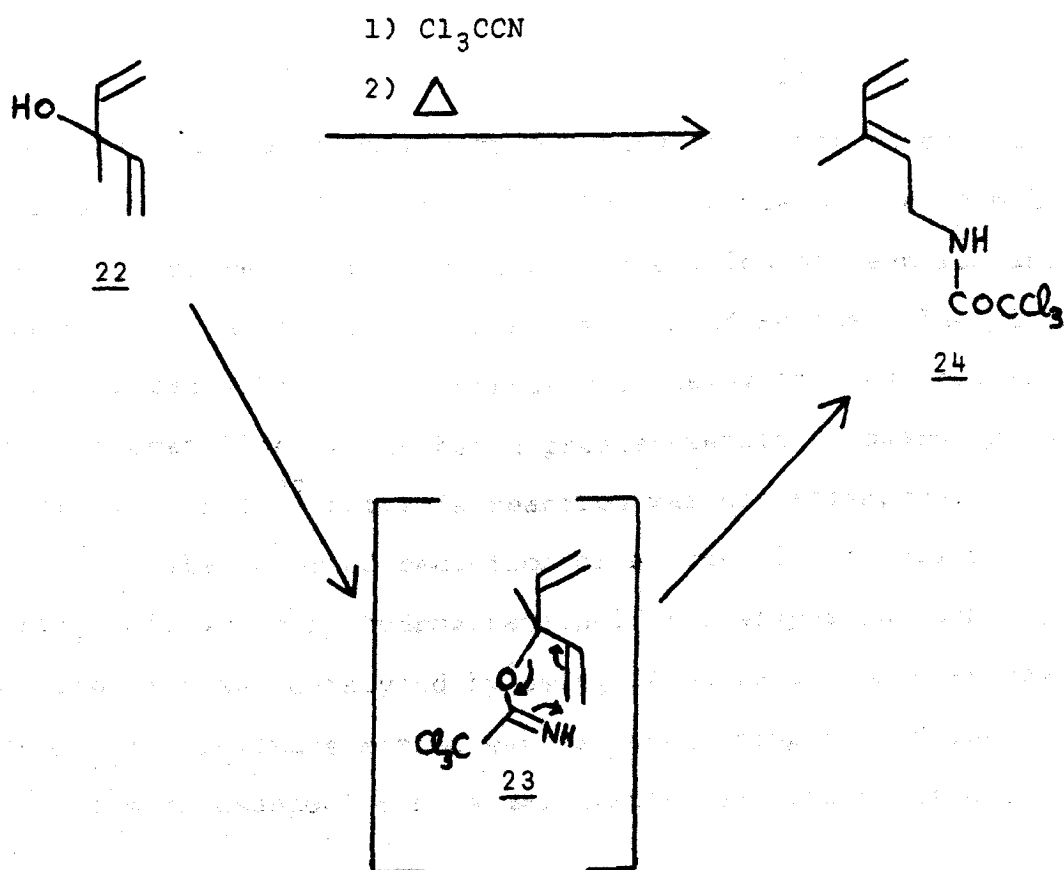
The bromo-diene (17) was also reacted with the anion of nitromethane in an attempt to displace the bromide, but once again only a polymeric residue was obtained. The i.r. spectrum of this residue suggested the presence of a nitro group, but this may have been due to some residual nitromethane as no product could be isolated from the mixture.



Formation of a Grignard reagent from the bromocyclic sulphone (16) and bromodiene (17) was attempted, using standard methods for Grignard formation, but the allylic halides appeared to be unreactive towards Grignard formation. In the one case where a reaction did occur, when a Grignard exchange reaction was attempted on the sulphone, the product obtained from a test reaction with carbon dioxide did not give a carboxylic acid. An unstable solid was produced which was possibly the result of

a base-catalysed rearrangement or reaction of the sulphone. At this point work on the sulphones was abandoned as the reactions showed no promise.

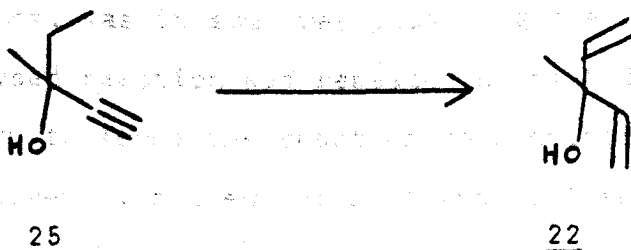
It was decided to apply a general method for the synthesis of amines, developed by Overman,<sup>23</sup> to the synthesis of amino-diene (5). The method involves the conversion of an allylic alcohol into an allylic trichloroacetimidate, which is then thermally rearranged to give an allylic trichloroacetamide. This can then be converted under mild conditions (dilute NaOH/ room temperature) to the free amine. The reaction that would be required to obtain compound (5) is shown in Scheme 1.5.



Scheme 1.5

A divinyl carbinol was used so as to produce the required diene (24). Reaction of the carbinol with trichloroacetonitrile should have given the trichloroacetimidate (23), which, being symmetrical could then rearrange via either double bond.

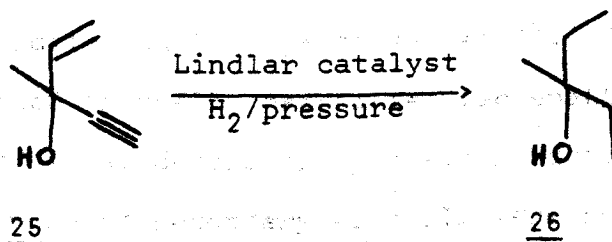
A method had been reported in the literature for the preparation of the divinyl carbinol (22). In this method sodium acetylide<sup>24</sup> was reacted with methyl vinyl ketone to give 3-methylpent-4-en-1-yn-3-ol (25).<sup>25</sup> This alkyne was then partially hydrogenated<sup>26</sup> to give compound (22).



The acetylide reaction was tried using the reported method, and gave carbinol (25) in 28% yield. Low yields are normal in this type of reaction because of competition between 1,2 and 1,4 modes of attack on the  $\alpha,\beta$ -unsaturated ketone. The yield could probably have been increased by using the less reactive calcium acetylide, which has a greater tendency towards attack at the carbonyl,<sup>27</sup> but this reaction was not attempted.

The reported reduction of alkyne (25) to divinyl carbinol (22) was by hydrogenation of the alkyne in methanol. The reaction was catalysed by using 2% palladium on charcoal. When this literature method was repeated using 5% and 10% palladium on charcoal (no 2% was available), the reaction, at

best, was very sluggish, and after extended reaction times the mixture still contained mainly the unreacted alkyne with only a small amount of the divinyl carbinol (22). Catalytic amounts of Lindlar catalyst gave no reaction. The freshness of the catalyst, was tested using 1-hexyne, and proved to be active by quantitatively converting the alkyne to 1-hexene. Some measure of reaction was obtained when the catalyst was used in a 1:2 w/w ratio with the alkyne (25), but after several days the reaction mixture still contained mainly starting material. These results could not be accounted for, unless compound (25), or perhaps the reaction product, was in some way poisoning the catalyst. The Lindlar catalysed reaction was repeated using hydrogen under pressure to try to force the reaction to completion. The use of pressure, however, caused the catalyst to lose its reactive selectivity, and alkyne (25) was hydrogenated to the saturated alcohol, 3-methyl-pentan-3-ol (26).



3-Methylpenta-1,4-dien-3-ol (22) can also be prepared from the reaction of ethyl acetate with two moles of vinyl magnesium chloride.<sup>28</sup> This reaction involves an initial displacement of ethoxide by the Grignard reagent to give methyl vinyl ketone, which is then attacked by the second mole of Grignard to form the divinyl compound (22). In this reaction there is again the possibility of 1,2 or 1,4-attack on the  $\alpha,\beta$ -unsaturated ketone, and therefore a high yield was not

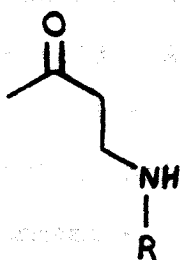
expected. A solution of vinyl magnesium chloride in THF was prepared<sup>29</sup> and reacted with ethyl acetate according to the described literature method. The reaction produced mainly polymeric material, and the best yield of divinyl carbinol (22) obtained was 27% (crude).

The carbinol was subjected to the Overman procedure, as shown in Scheme 1.5. This entailed an initial conversion of the carbinol to an alkoxide by addition of a metal hydride. The alkoxide was then reacted with trichloroacetonitrile at 0°. This step caused the solution to turn black, and after work-up, only polymer and some starting material was obtained. A further solution of alkoxide was prepared and added dropwise to a solution of trichloroacetonitrile at -20°. Each drop of alkoxide turned black immediately upon hitting the solution of trichloroacetonitrile, and again only a polymeric residue and some starting material was obtained.

In all the examples cited by Overman, the formation of trichloroacetimidate had been performed at 0°. The resulting imidates were then thermally rearranged (Scheme 1.5) under a variety of conditions, depending on their structure. Imidates formed from primary or secondary alcohols were rearranged by refluxing in xylene, but tertiary imidates were found to rearrange at a convenient rate in refluxing benzene. In all the examples given, except one, the imidates were stable compounds and could be isolated and purified. The one unstable imidate was obtained from a bisallylic secondary alcohol. This imidate was found to undergo facile rearrangement at room temperature and only the final trichloroacetamide was isolated from the reaction mixture.

Analogously it seemed highly likely that carbinol (22), being a tertiary alcohol and bis-allylic, would rearrange spontaneously at  $0^\circ$  and possibly even at  $-20^\circ$ , when converted to the imidate. This would have caused the final product, diene (24), to be formed immediately upon reaction of the alkoxide of carbinol (22) with trichloroacetonitrile. If this was the case, then the reaction must have failed due to the final product being unstable to the reaction conditions.

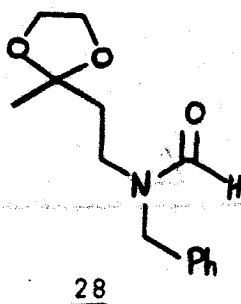
The lack of success in synthesising amino-diene (5), meant that the strategy for constructing the intramolecular Diels-Alder precursor, as outlined in Scheme 1.1, had to be abandoned. Instead it was decided to construct the Diels-Alder precursor in such a way that the formation of the diene was the last reaction, prior to cycloaddition. In this way any instability due to the diene would be minimised. The synthetic strategy adopted is shown in Scheme 1.6.

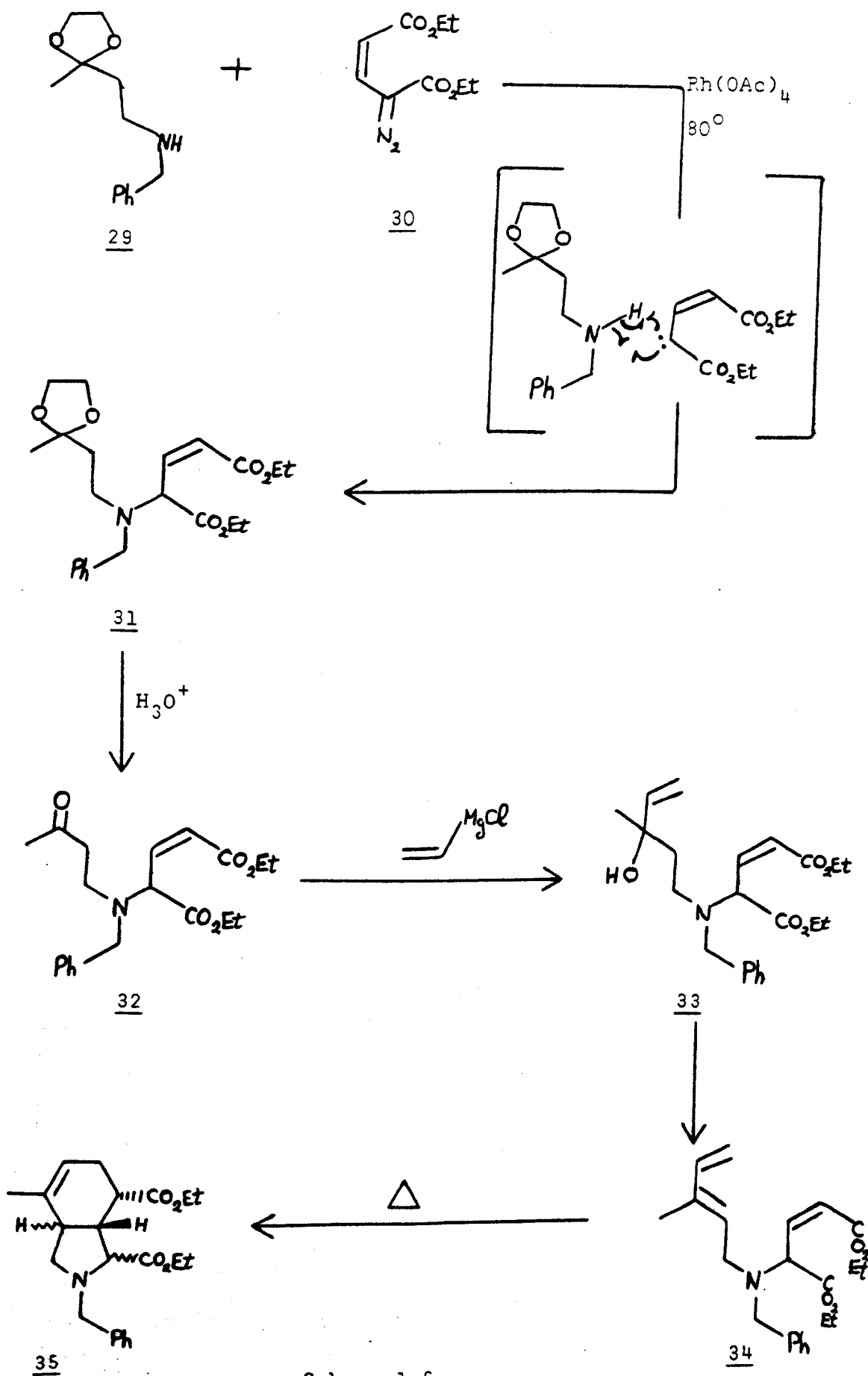


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The keto-amine (27) was used to prepare ketal (29). Two attempts were made to prepare this ketone by Michael-type condensation to methyl vinyl ketone. The first used methyl carbamate and the second benzylamine. Both reactions were unsuccessful, but it was later found that the benzylamine addition had probably given the correct product. The residue

from this reaction was distilled but gave only benzylamine as a distillate, and some viscous high boiling material. The ketone (27) (R=benzyl) was eventually prepared by a very low yielding Mannich reaction<sup>30</sup> involving benzylamine hydrochloride, formaldehyde, and acetone. This reaction gave compound (27) as the crystalline hydrochloride, from which the free amine could be liberated by neutralisation with sodium hydroxide. This amine could not be distilled, but instead decomposed, giving a distillate of benzylamine and leaving a polymeric residue in the distillation pot. This observation suggested that the Michael reaction between methyl vinyl ketone and benzylamine might have worked and that the product was degraded by attempted distillation. The compound was also degraded when an attempt was made to prepare the ketal (29) by the standard method of refluxing in benzene with ethylene glycol and 4-toluene sulphonic acid. The ketal was instead prepared using the hydrochloride of compound (27) and refluxing in benzene with ethylene glycol and some concentrated hydrochloric acid. An alternative method using the hydrochloride of (27) in ethylene glycol with 4-toluene sulphonic acid and triethyl orthoformate did not give ketal (29) but the ethylene ketal formamide (28).





Scheme 1.6



The diazo compound (30) was prepared easily and in good yield from the reaction of diethylglutaconate with tosyl azide<sup>32</sup> in the presence of triethylamine. The use of rhodium acetate to catalyse the formation of carbenes from diazo compounds has been used successfully in  $\beta$ -lactam chemistry<sup>33</sup> and is a well proven method for generating carbenes. The standard procedure used, in  $\beta$ -lactam chemistry, to insert a diazo compound, via the carbene, into an N-H bond, is to react the diazo and amino functions, in the presence of rhodium acetate, in refluxing benzene. The reactions are usually intramolecular and therefore give a good yield with very little carbene dimerisation.

To avoid the formation of dimers in an intermolecular carbene insertion reaction, such as the one proposed in Scheme 1.6, it is essential to have the amine present in a very large excess. This was achieved by using a five-fold excess of the amine (29), and adding the diazo compound (30) to the amine very slowly, over a long period of time. The effective excess of amine was further enhanced by heating the neat amine to 80° in an oil bath, instead of refluxing in benzene, and the diazo compound was added as a dilute solution in benzene. The addition was carried out over 5 hours, after which the reaction was stopped when no diazo band could be detected in the i.r. spectrum.

The reaction mixture obtained was very dark and gave a highly streaked t.l.c. with numerous spots. After work-up the major product was a distillable yellow oil. This oil was not the desired product as n.m.r. spectroscopy indicated that no coupling to the amine had occurred. The spectrum contained ethyl

ester and olefin resonances, but there were no peaks which could have been attributed to the amine. The oil also gave a resonance signal at  $\tau$ -1.06, suggesting a carboxylic acid or enolic proton was also present. The i.r. spectrum showed the presence of the ester carbonyl and the double bond but gave no indication of an acid or enol being present. It is thought that this product must have been formed by a dimerisation, or intramolecular insertion reaction, of the glutaconate carbene, followed by a rearrangement. The structure of this product was not determined however, as no structure could be devised to fit the n.m.r., i.r., and mass spectral data. A crystalline minor product was also isolated from this reaction. The n.m.r. spectrum of this solid showed no ester resonances but contained signals which correlated with the structure of the amine. The main difference in the spectrum of the solid, from that of the starting amine, was that the aromatic protons no longer gave a sharp singlet, but were split into a series of multiplets. The aromatic protons also integrated for 10 rather than 5, and there were an additional two singlet resonances at  $\tau$  7.73 and 8.65. There were no low field acidic protons in the spectrum and yet the i.r. spectrum showed a strong broad acidic-like absorption. The mass spectrum did not help in assigning a structure to this product, and like the oil, it remains unidentified.

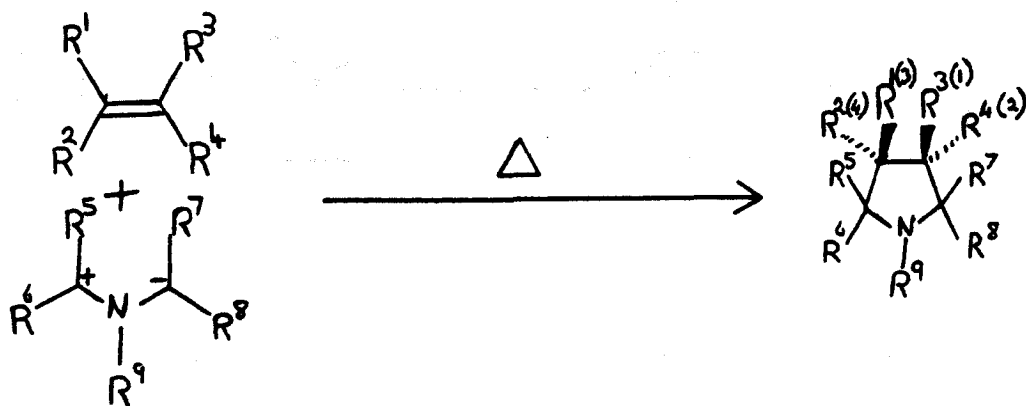
The reaction, therefore, appeared to have transformed both the amine (29) and the diazoglutaconate (30), to some extent. The products from the reaction could not be identified and no N-H carbene insertion product was isolated. At this stage, work on the synthesis of the Diels-Alder precursor was stopped.

2. ATTEMPTS TO DEVELOP A GENERAL METHOD FOR THE SYNTHESIS OF KAINIC ACID AND STRUCTURAL ANALOGUES BY 1,3-DIPOLAR CYCLOADDITION

When contemplating the feasibility of producing a general method for the synthesis of kainic acid analogues, the cis stereochemistry of the C3 and C4 substituents on the pyrrolidine ring, had to be given prime consideration. This had been the main problem in the previous synthesis of kainic acid, and an elaborate synthetic sequence was required to overcome it.<sup>34</sup> The resulting synthesis contained several low yielding steps and was a long and inefficient method for producing kainic acid. The scheme was also highly specific for the parent compound and could not easily have been adapted to the production of kainic acid analogues.

Of the many methods available for synthesising pyrrolidines, that of 1,3-dipolar cycloaddition seemed to be the only method which might satisfy the criteria set by this project. The 1,3-dipolar cycloaddition, developed in the early 1960's by Huisgen,<sup>35</sup> is an extremely versatile general method for the synthesis of five-membered heterocycles. Pyrrolidines can be obtained by this method when an azomethine ylid undergoes cycloaddition with an alkene. The precise reaction mechanism has not been conclusively determined,<sup>36,37</sup> but it is generally believed to be a concerted mechanism<sup>38,39</sup> which obeys the Woodward-Hoffman rules<sup>40</sup> for orbital-symmetry-allowed cycloaddition reactions. The reactions are therefore stereospecific. When an azomethine ylid and a cis-substituted alkene are allowed to react, the pyrrolidine formed is exclusively the isomer in which the C3 and C4 substituents are cis. The 1,3-dipolar

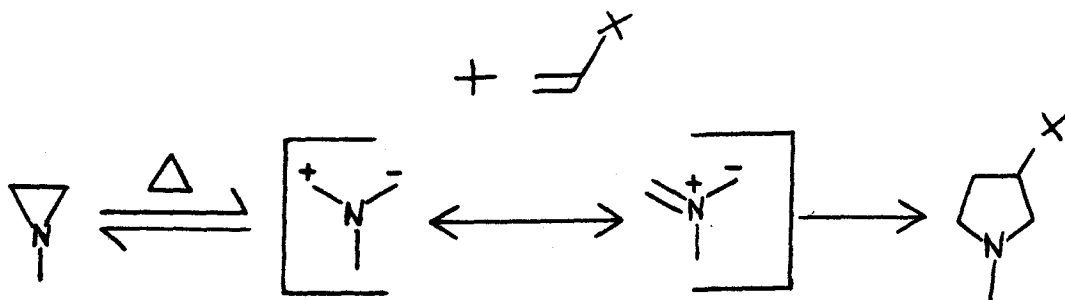
cycloaddition reaction is therefore a general method for preparing the kainic acid ring system with the correct stereochemistry at positions 3 and 4. By altering the substituents on the azomethine ylid and the alkenes it should then be possible to have a highly flexible synthesis for numerous kainic acid analogues. The only problem with this generalised method, as illustrated below, is the regiochemistry of the reaction, but this can be easily controlled by selecting appropriate R groups.



Shortly after work on this project commenced, a new synthesis of kainic acid was announced by Oppolzer.<sup>41</sup> This was an efficient high yielding synthesis, based on an intramolecular ene reaction, to obtain the required stereochemical control. However, the reaction was very sensitive to the side chains present on the ene reaction precursor, and to the reaction conditions. The reaction was therefore specific for kainic acid alone and a need still existed for a flexible synthetic route to kainic acid analogues.

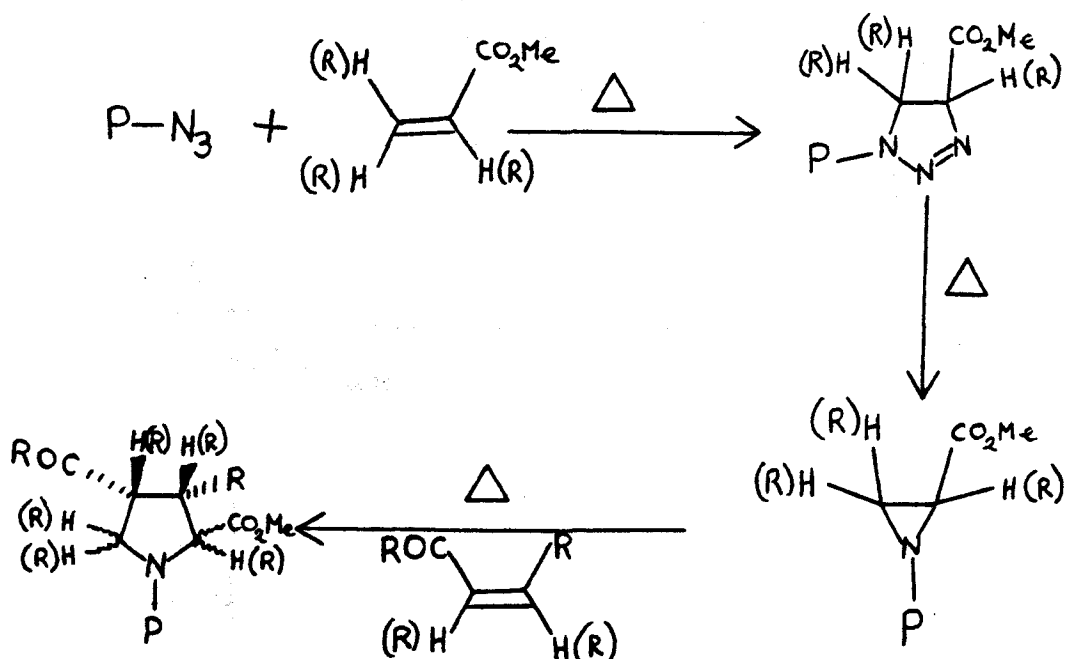
Azomethine ylids have been prepared by the action of base on immonium salts,<sup>35</sup> and more recently by heating the imine derivatives of  $\alpha$ -amino acids.<sup>42</sup> However, the most common method of generating azomethine ylids is to heat aziridines. This causes

the carbon-carbon bond to weaken, and above a certain temperature the aziridine exists in equilibrium with the open-chain ylid. If the aziridine is heated in the presence of a 1,3-dipolarophile, such as an activated alkene, the transient azomethine ylid can be trapped by cycloaddition to give a pyrrolidine.<sup>43-48</sup>



Of the many methods available for synthesising aziridines, it was decided to utilise the thermolysis of  $\Delta^2$ -1,2,3-triazolines.<sup>49-53</sup> These can be prepared by the 1,3-dipolar cycloaddition of an azide with an alkene,<sup>50</sup> or a diazoalkane with an imine.<sup>54</sup> When heated, these compounds eliminate nitrogen to give aziridines. Using triazolines as intermediates to aziridines, and therefore pyrrolidines, means that, in theory at least, complicated pyrrolidine structures can be prepared from very simple azide and alkene starting materials.

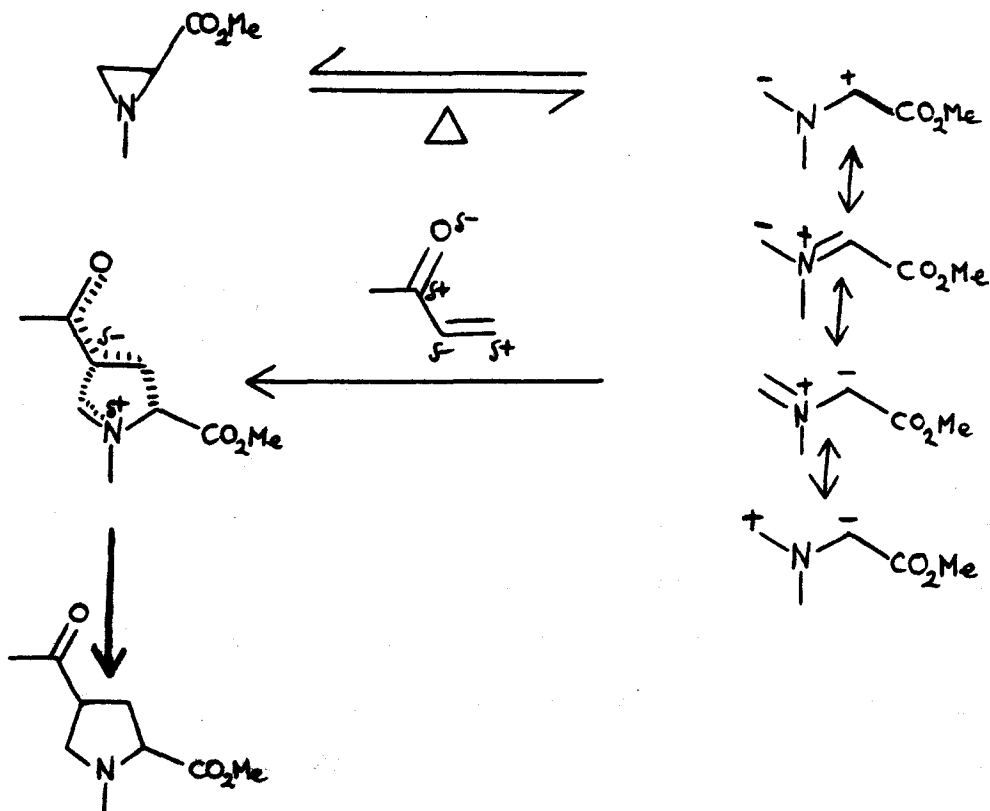
Scheme 2.1 shows the generalised route which was proposed for the synthesis of kainic acid analogues. The symbol P represents any protecting group which can easily be removed, at a subsequent stage, to give the free amine. The ester substituent on the aziridine ring, apart from being required to produce the acid group at position two in the final pyrrolidine,



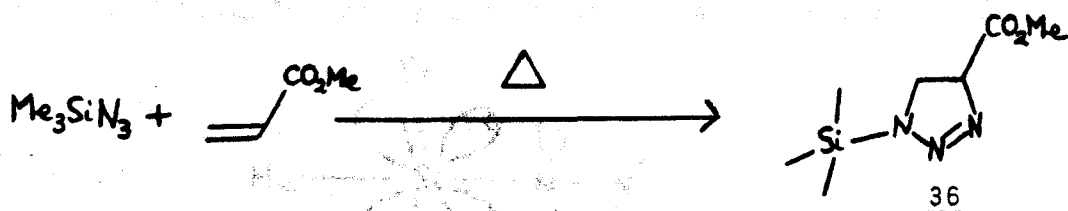
Scheme 2.1

is also important in controlling the regiochemistry of the 1,3-dipolar cycloaddition. The ester does this by stabilizing the negative charge on the azomethine ylid, and therefore makes that end of the 1,3-dipole more nucleophilic. The carbonyl on the  $\alpha,\beta$ -unsaturated ketone is also important in controlling the regiochemistry of the cycloaddition as it polarises the double bond of the 1,3-dipolarophile and stabilises the partial negative charge formed in the transition state due to unequal rates of bond formation.

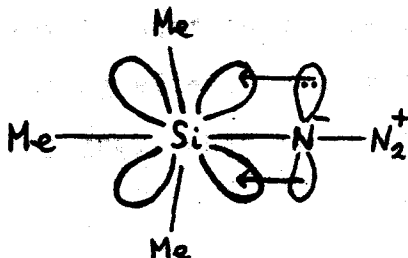
The electronic effects of other substituents on the 1,3-dipole and dipolarophile can also affect the direction of cycloaddition, and steric effects also play an important part in determining regiochemistry. The mechanism shown below is therefore an example of the ideal case, and in certain circumstances when other substituents are present, the regioselectivity of the reaction may be lost.



To test the viability of Scheme 2.1, it was decided to attempt the preparation of the parent compound,  $\alpha$ -kainic acid, and then to investigate the flexibility of the method for analogue production. The substituents on positions 4 and 5 of the triazolone intermediate in the synthesis, eventually become the substituents on positions 2 and 5 of the final pyrrolidine. To obtain the required substituents for kainic acid, the triazolone should be prepared by the cycloaddition of an azide with methylacrylate. It was decided to use trimethylsilyl azide, as the trimethylsilyl protecting group was easily removable by hydrolysis.

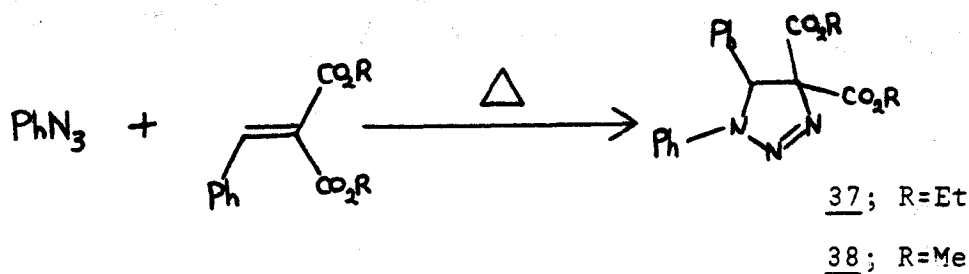


In a previous study,<sup>55</sup> when trimethylsilylazide was reacted with powerful dipolarophiles, the reactions proved to be vigorous and exothermic, but the products were thought to have been obtained by some mechanism other than 1,3-dipolar cycloaddition. There was therefore some doubt as to whether triazoline (36) would be formed from the reaction of trimethylsilyl azide with methylacrylate. When the reaction was performed the outcome was surprising in that no reaction whatsoever was observed. The reactants were left at 50° for one week, after which, n.m.r. spectroscopy showed no change from the initial mixture. This result was unexpected as silicon, which is less electronegative than nitrogen, should have activated the azide towards cycloaddition. Butylazide<sup>51</sup> is known to be much more reactive than phenyl azide<sup>49</sup> in cycloaddition reactions with ethylacrylate. This is thought to be due to the +I effect of the butyl group activating the  $\alpha$ -nitrogen of the azide by increasing the electron density around it, whereas the -I effect of the phenyl ring tends to stabilise the azido group and so reduces the reactivity towards cycloaddition. The +I effect of the trimethylsilyl group should therefore have made the azide very reactive. It is thought that the lack of reactivity in this case must have been due to backbonding of p electrons on the  $\alpha$ -nitrogen of the azide into empty d orbitals on the silicon. In this way electron density would be removed from the azide making it less reactive towards cycloaddition with electron-poor olefins.





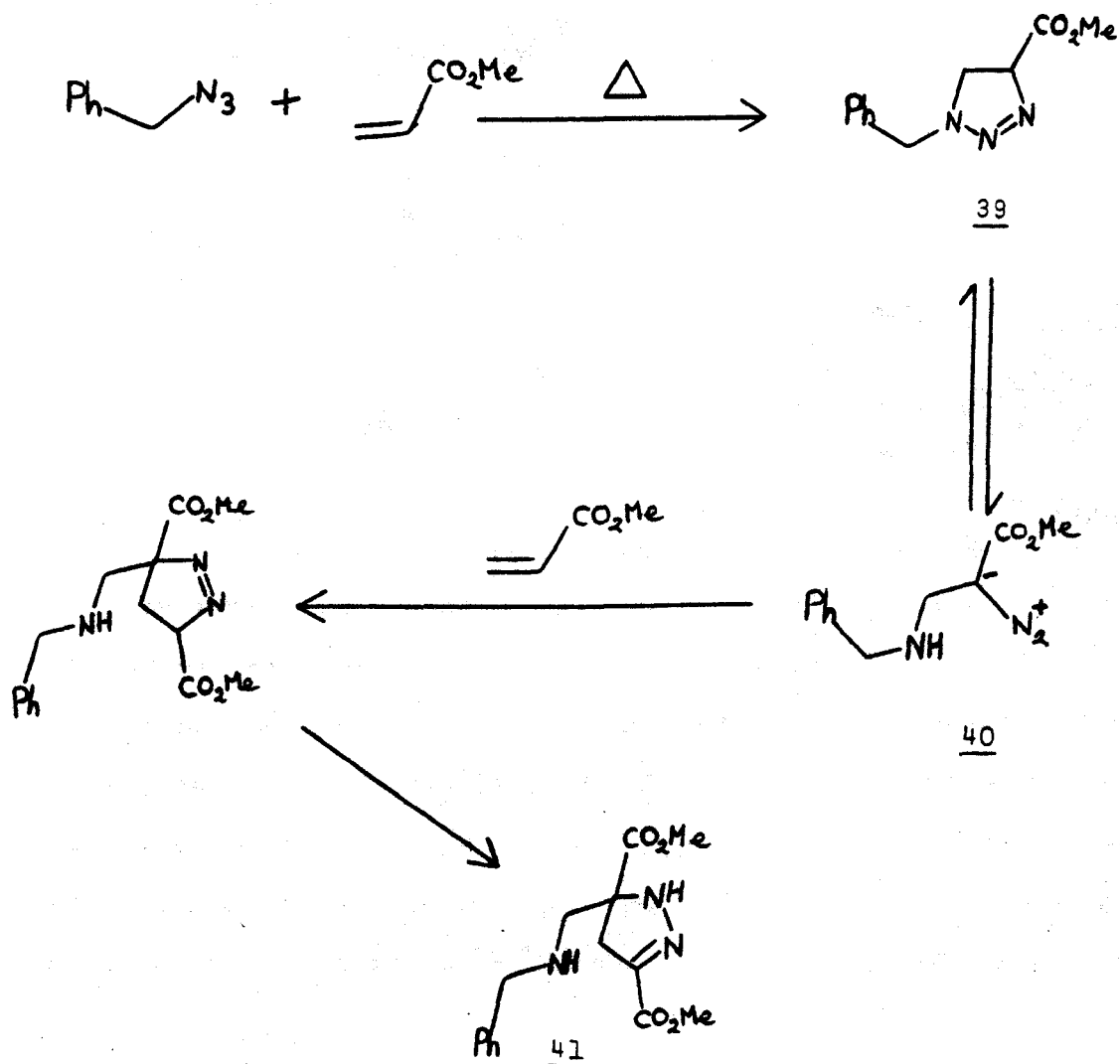
A further check on the lack of reactivity of trimethylsilylazide towards cycloaddition was made by attempting to react it with diethylbenzalmalonate.<sup>56</sup> This olefin was known to react<sup>52</sup> with phenylazide<sup>57</sup> and therefore provided another comparison of the effects imposed upon the azido group by trimethylsilyl and phenyl substituents. The azides were reacted with the benzal malonate by stirring equimolar amounts of dipole and dipolarophile in an oil bath at 60°. For comparison; ethyl azidoformate,<sup>57</sup> which would be expected to be less reactive than phenyl azide due to the electron withdrawing effect of the ester group, was also reacted with diethyl benzalmalonate.



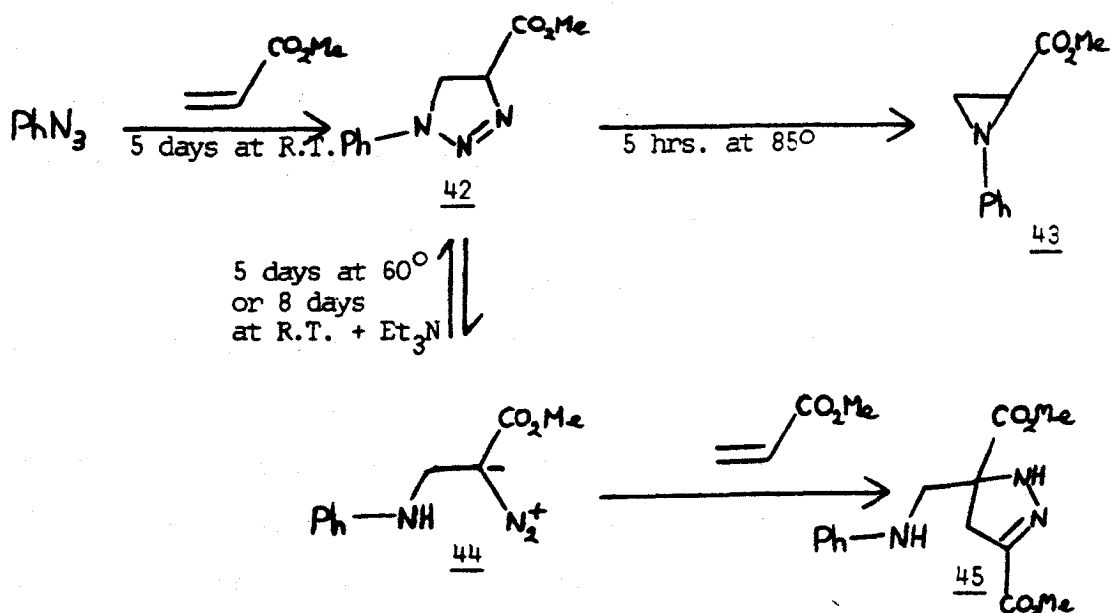
The mixtures were left for one month, after which the phenyl azide reaction was found to have produced triazoline (37). The other two reactions were found to contain only unreacted diethyl benzalmalonate and azide decomposition products. It would therefore seem that trimethylsilyl azide, like ethyl azidoformate, is an electron-poor 1,3-dipole and is not suitable for cycloaddition with electron-poor olefins.

It was thought that a benzyl group would exert the required activating effect on an azide, and would also make a suitable leaving group when the synthesis was complete.<sup>61,62</sup>

The 1,3-dipolar cycloaddition to methylacrylate was tried again, this time using benzylazide as the 1,3-dipole. As with the trimethylsilyl azide reaction, this reaction also gave surprising results. Instead of producing the desired triazoline, 1-benzyl-4-carbomethoxy- $\Delta^2$ -1,2,3-triazoline (39), the reaction gave a  $\Delta^2$ -pyrazoline (41). These compounds are known to occur when triazolines have an electron withdrawing group at position four. The triazoline can exist in equilibrium with an open-chain diazoester (40), which can then undergo a second 1,3-dipolar cycloaddition with excess dipolarophile.



When phenyl azide and methylacrylate were reacted at  $60^{\circ}$ , 5-anilinomethyl-3,5-dicarbomethoxy- $\Delta^2$ -pyrazoline (45) was formed by the same mechanism. Huisgen and workers reacted phenyl azide and methylacrylate at room temperature and obtained 1-phenyl-4-carbomethoxy- $\Delta^2$ -1,2,3-triazoline (42).<sup>49</sup> At room temperature this triazoline was stable in the presence of methylacrylate and only slowly formed a pyrazoline by base catalysis. When heated to  $85^{\circ}$  the triazoline eliminated nitrogen to give an aziridine (43).<sup>58</sup>



It has also been reported<sup>51</sup> that butyl azide reacts with an excess of ethylacrylate at room temperature to give pure 1-butyl-4-carbomethoxy- $\Delta^2$ -1,2,3-triazoline in 24 hours, but when left a further 10 days at room temperature, 5-butylaminomethyl-3,5-carbomethoxy- $\Delta^2$ -pyrazoline was formed. These observations suggested that it should be possible to isolate the triazoline (39) formed in the benzyl azide reaction, if the equilibrating reaction to the open-chain diazoester (40) could be slowed down.

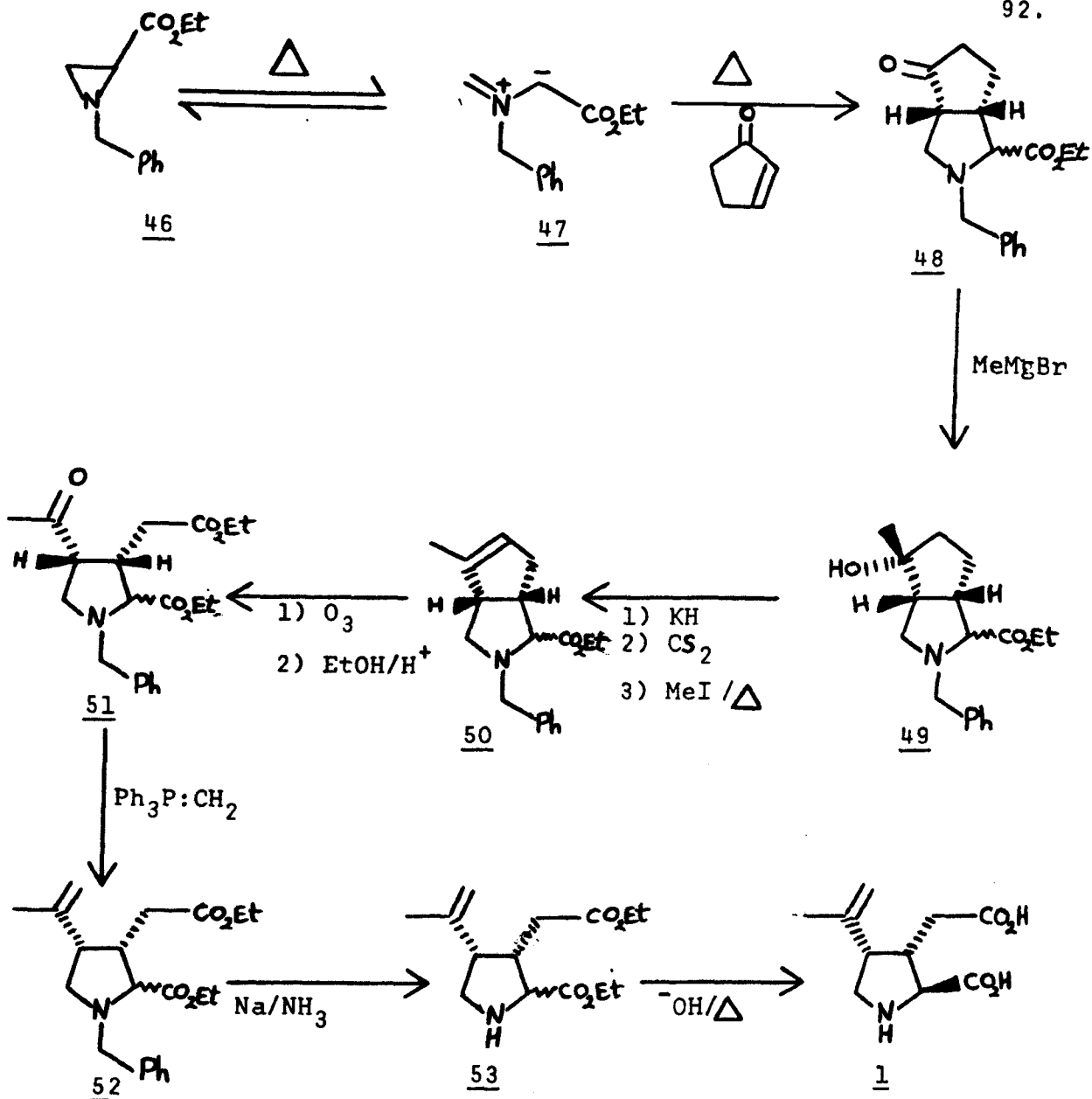
As the rate of isomerisation from triazoline to diazoester depends upon the nucleophilicity of the nitrogen at position one, the acidity of the proton at position four, and the temperature, it should be possible to isolate triazoline (39) by reducing the temperature to a value where the initial cycloaddition can still occur but isomerisation to compound (40) is minimised.

Several test reactions were performed at various temperatures, with or without solvent; using equimolar amounts of azide and olefin to ensure that if the triazoline was formed initially then there would be no excess acrylate available to form the pyrazoline (41). It was found that when the reactants were mixed and left for fourteen days at room temperature, the only product was pyrazoline, whether the reactants were neat, or a dilute solution in chloroform. When the same reactions were tried at  $4^{\circ}$ , the chloroform solution, after fourteen days, was found by n.m.r. spectroscopy to contain approximately 50% starting materials and 50% pyrazoline. The neat reactants, at  $4^{\circ}$ , gave a final mixture containing 40% starting materials and 60% pyrazoline. Even at  $-20^{\circ}$  where very little reaction had occurred after 14 days, the only detectable product from n.m.r. spectroscopy was the pyrazoline (41). It would seem from these results that the triazoline (39) must be very labile and opens to the diazoester as soon as it has formed. The instability of this triazoline cannot be explained in terms of the nucleophilicity of the nitrogen at position one, as the adduct from the butyl azide and ethylacrylate reaction, mentioned above, was easily isolated even although it contained a nitrogen of

comparable base strength. The only explanation for the instability of 1-benzyl-4-carbomethoxy- $\Delta^2$ -1,2,3-triazoline (39), relative to 1-butyl-4-carbomethoxy- $\Delta^2$ -1,2,3-triazoline, might be the acidity of the proton at position four. If the proton was more acidic when alpha to a carbomethoxy group than when alpha to a carbomethoxy group, then this might explain the difference in stabilities. The reaction between benzyl azide and ethyl acrylate was not carried out however, so this theory was not confirmed.

It was decided instead, to prepare 1-benzyl-2-carbomethoxyaziridine (46) by a different method,<sup>59</sup> so that the crucial 1,3-dipolar cycloaddition reaction between an azomethine ylid and an olefin, could be tested. In order to prepare kainic acid from aziridine (46), the corresponding azomethine ylid (47) should ideally have been reacted with ethyl 5-ketohex-3-enoate,<sup>60</sup> to give pyrrolidine (51) directly. This cycloaddition was not tried however, as it was decided to use cyclopentenone as 1,3-dipolarophile. It was proposed that cyclopentenone could be used to prepare kainic acid, according to Scheme 2.2. This olefin was chosen because it could not only be used to produce kainic acid, but also a series of analogues. By reacting adduct (48) with various Grignard reagents, or increasing the size of the olefin ring to cyclohexenone etc., or placing other substituents on the ring, many analogues are possible. Bicyclic olefin (50) would also be an interesting kainic acid analogue.

Aziridine (46) and cyclopentenone were refluxed in *o*-xylene but no cycloaddition product (48) was obtained. The reaction produced a polymeric gum. The aziridine was also heated

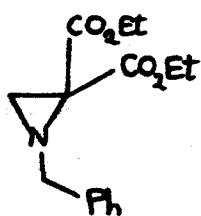
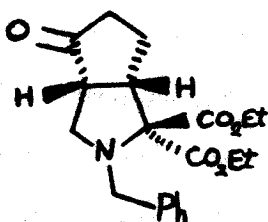
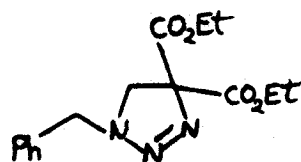


Scheme 2.2

to 100° in an excess of diethylmaleate, but again no cycloaddition product was obtained, and only polymer could be reclaimed from the reaction mix. It seems unlikely that any azomethine ylid (47) was formed during these reactions, as this species would almost certainly have been trapped by the dipolarophile. To test the thermal stability of 1-benzyl-2-carbethoxyaziridine (46), the compound was refluxed in *o*-xylene for 30 minutes, after which the material was found to contain some aziridine but was mainly polymeric. This suggested that

the aziridine was breaking down by some mechanism which was more facile than a thermal conrotatory ring cleavage to give the azomethine ylid. A disrotatory ring cleavage was also attempted by irradiating the aziridine in excess diethylmaleate at  $0^\circ$ . In this reaction very little polymer was produced, but no cycloaddition product was obtained. After irradiation for 24 hours the mixture was still predominantly starting materials.

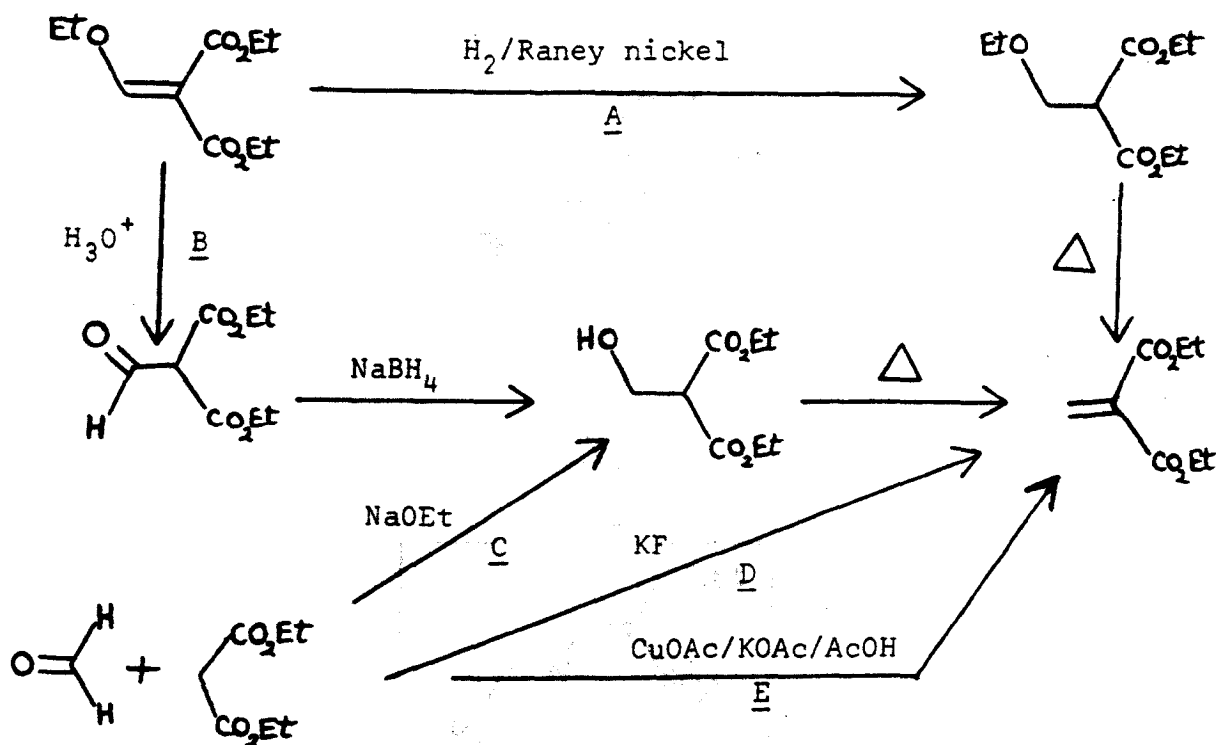
In order to reduce the amount of energy required for aziridine ring fission, it was thought that an additional ester group on the aziridine might stabilise the azomethine ylid species sufficiently to bring about equilibration of the two forms at a lower temperature. With this aim in mind, it was decided to substitute 1-benzyl-2,2-dicarbethoxyaziridine (54) for aziridine (46) in Scheme 2.2, while still using cyclopentenone as dipolarophile, so as to produce the bicyclic structure (55). The gem-diester would spontaneously decarboxylate during the final saponification step, therefore no additional reactions would be required to accommodate aziridine (54) into Scheme 2.2.

545556 R = Me57 R = Et

The triazoline precursor of aziridine (54), 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57), was made easily, from the reaction of benzyl azide with diethyl methylene malonate. No problems were encountered as with the methyl acrylate cycloaddition because triazoline (57), unlike triazoline (39), has no proton at position four and therefore cannot isomerise to a diazoester structure. However, there were problems in trying to prepare the starting olefin, diethyl methylene malonate. A standard preparation<sup>63</sup> (A) was known for this compound, but could not be made to work. The reaction was repeated numerous times, precisely according to the reported procedure; it was also tried with freshly prepared Raney nickel catalyst,<sup>64</sup> and in several attempts the pressure or temperature of the hydrogenation reaction were varied, but in every case the procedure was found to be useless. Another literature method<sup>65</sup> (D) was found to be of little preparative use, as the best yield obtained using the procedure, was 6%. Two further attempts (B) and (C) were made to prepare the olefin, but they were unsuccessful. The methylene malonate was eventually prepared by method (E).<sup>66</sup> The olefin was a clear liquid which polymerised to a clear solid after several weeks at  $-20^\circ$ . The solid depolymerised when heated strongly and the olefin could be reclaimed by distillation.<sup>67</sup> Dimethyl methylene malonate was also prepared by method (E) but was less stable than the diethyl ester and polymerised when left overnight at  $-20^\circ$ .

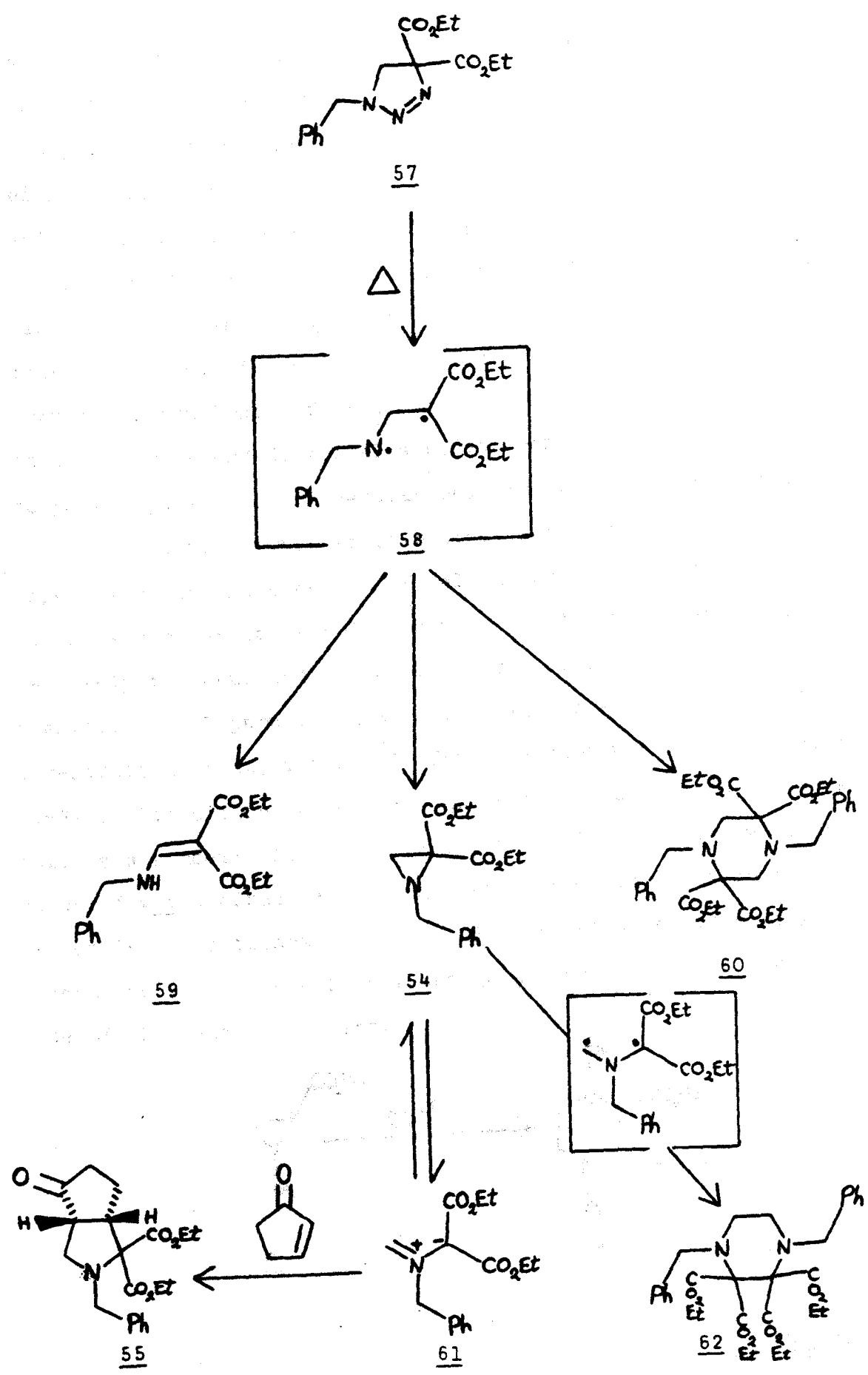
Once triazoline (57) had been prepared, it was decided to use it as the starting material for the in situ generation of the corresponding azomethine ylid (61), instead of first converting it into aziridine (54). With this in mind,





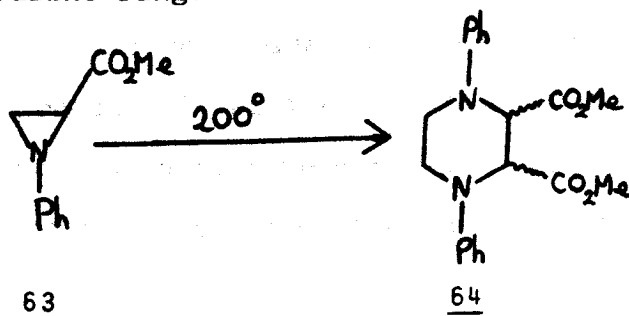
triazoline (57) was reacted with cyclopentenone in refluxing toluene. The mass spectrum of the resulting gum suggested that the desired cycloaddition product, compound (55), was present. T.l.c. of the gum showed three main spots: these components were separated by p.l.c. The material corresponding to the upper spot was obtained as a crystalline solid and was thought to be piperazine (60). This piperazine was obtained in 3.6%. The material corresponding to the lower spot on t.l.c. was also isolated as a solid and found to be diethyl benzylaminomethylene malonate (59). This enamine was obtained in 28.8% yield and its structure was confirmed by comparison of its physical properties with those of the product from a reported synthesis using benzylamine and diethyl ethoxymethylenemalonate.<sup>69</sup>

The formation of enamine (59) was predictable as it is well documented that when triazolines are thermolysed, they produce enamines or anils in addition to aziridines, the ratio of enamine to aziridine produced being dependent upon the



substituents on the triazoline.<sup>51,53</sup> The thermal extrusion of molecular nitrogen from triazolines is thought to produce a highly reactive diradical intermediate (58), which can then either ring-close to give an aziridine, or rearrange to an anil or enamine. By this mechanism, the formation of piperazine (60) could be accounted for in terms of a dimerisation of two diradical species. However no reports have been found, to date, of any piperazine structures being obtained from the thermolysis of triazolines. This would seem to suggest that the transient diradical species does not exist long enough to participate in a bimolecular reaction with another diradical.

Certain types of aziridines are known to form piperazines in the presence of halide ions,<sup>70,71</sup> but the reaction requires a polar solvent in order to be effective, and would not be likely to occur under the conditions used for the triazoline thermolysis. Piperazines have also been obtained by thermal dimerisation of aziridines.<sup>72</sup> This reaction was described as a (3+3)-cycloaddition between two azomethine ylid species, but as this is a symmetry-forbidden reaction, it is more likely to have occurred via a diradical intermediate. The dimerisation of 1-phenyl-2-carbomethoxyaziridine (63) was reported as giving piperazine (64), where both ester groups were found on the same side of the piperazine ring.



The formation of compound (64) also suggested a diradical mechanism might be involved and indicated that the piperazine obtained from the thermolysis of triazoline (57) might not have been structure (60), but rather structure (62). The conditions used did not appear to be extreme enough for this diradical reaction to take place, but from spectral analysis it was not possible to determine whether the piperazine obtained was structure (60) or (62).

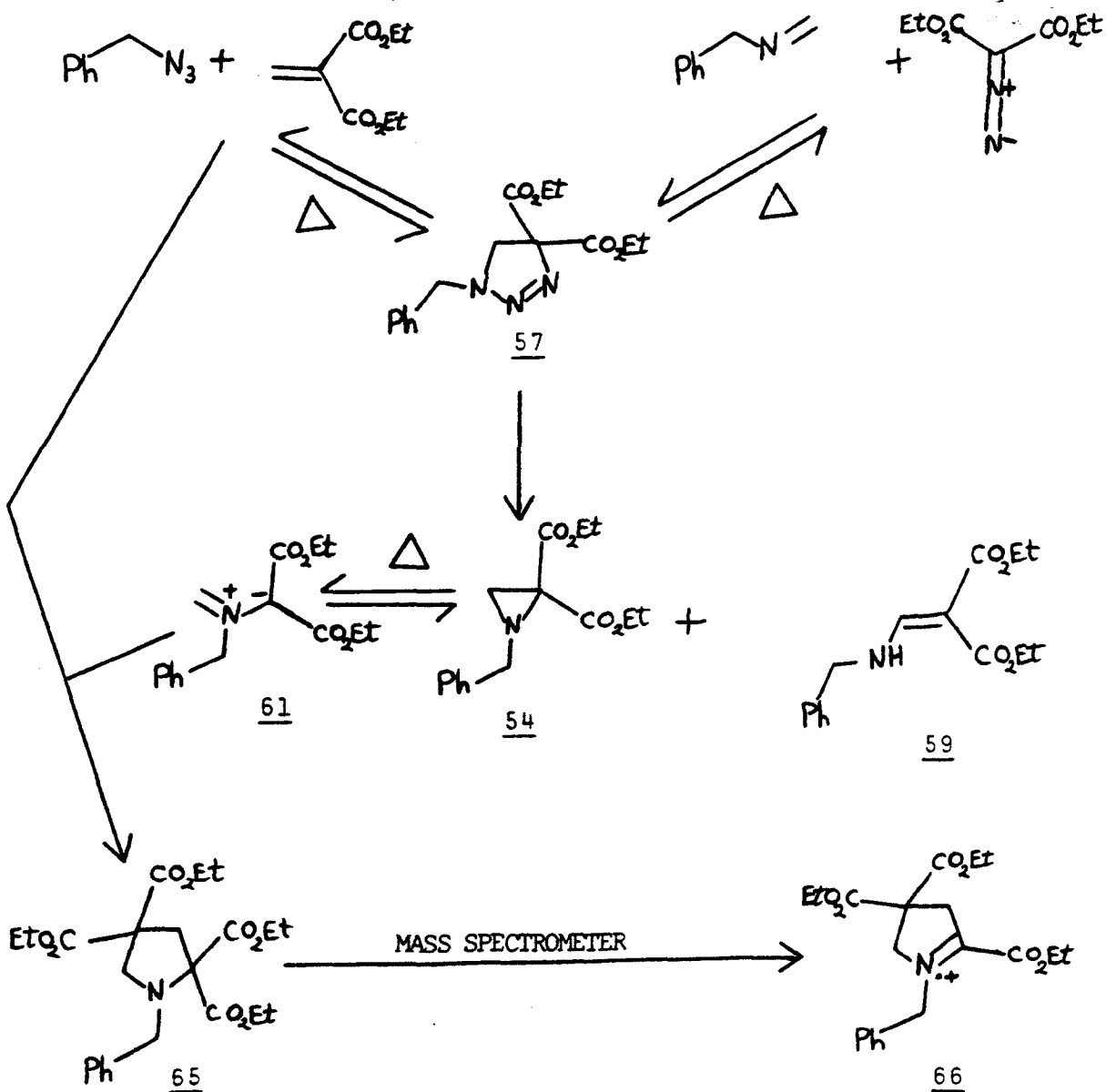
After the piperazine and enamine had been removed from the crude reaction mixture a light brown viscous gum was left. This gum ran as one spot on t.l.c. and was the main product from the reaction (67.6%). The mass spectrum of this gum showed an  $m/e$  value corresponding to the molecular ion of compound (55), and also a strong signal for  $M^+-(CO_2Et)$ . However, the n.m.r. spectrum was poorly resolved and suggested a mixture was present. The spectrum exhibited benzyl, ethyl ester, and methylene resonances, and there were also multiplets at around  $\tau = 2$  which might have been due to the cyclopentanone protons in compound (55), but the overall spectrum was complex and contained more protons than were required for compound (55). The crude gum was distilled but the pale yellow gum obtained gave the same complex spectrum.

A direct thermolysis of triazoline (57) was carried out and the product mixture separated. This reaction appeared to give the same product mix as the attempted cycloaddition. When the piperazine and enamine were removed from the mixture, a gum remained which was similar to the material obtained from

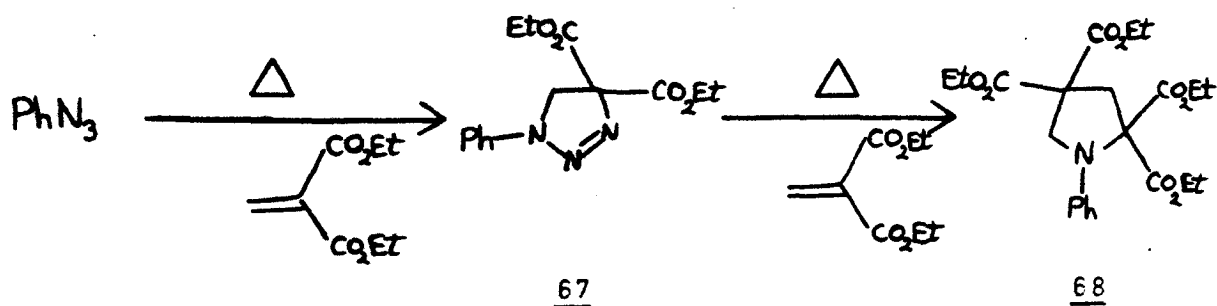
the cyclopentenone triazoline cycloaddition except that the n.m.r. spectrum was not as complex and the mass spectrum did not have an m/e value corresponding to compound (55). The highest m/e value found in the spectrum of this gum was 376. This m/e value was also found in the spectrum of the cycloaddition reaction gum. A high resolution mass scan on m/e 376 correlated this mass with the formula,  $C_{20}H_{26}NO_6$ . As this empirical formula did not represent a possible compound, it was thought that it must be a fragment from an unknown product of higher mass. The gum from the cycloaddition reaction was subjected to further mass spectral analysis, by running a metastable scan on the ion with m/e 286. This m/e corresponded to  $M^+-(CO_2Et)$  for compound (55) and the scan detected mass 359, the molecular ion of compound (55), thus indicating that the two mass values were connected. The scan did not pick up any trace of mass 376, thereby suggesting that mass 359 was not connected with this unknown mass fragment at 376.

From the n.m.r. spectrum of the gum from the thermolysed triazoline, it was thought that the main constituent might be pyrrolidine (65). This compound could have arisen via a 1,3-dipolar cycloaddition of the azomethine ylid (61) with diethyl methylene malonate. For this to occur the triazoline would have had to undergo a retro-1,3-dipolar cycloaddition. Dipolar cyclo-reversal reactions are well known,<sup>73</sup> and triazoline, apart from eliminating molecular nitrogen when heated, can also exist in equilibrium with the retro-cycloaddition fragments, azides and alkenes, or diazoalkanes and azomethines. If triazoline (57) can exist in equilibrium with benzyl azide and diethyl methylene

malonate when heated, then any azomethine ylid (61), produced by the thermolysis of the triazoline, would immediately be trapped by the methylene malonate which is a much stronger dipolarophile than cyclopentenone. The mass spectrum of the gum did not indicate the presence of a molecular ion for compound (65), but this structure would be expected to decarboxylate very easily in a mass spectrometer, therefore the observed mass fragment at 376 might have corresponded to structure (66). A metastable scan on the molecular ion at  $m/e$  376 detected a weak intensity peak at  $m/e$  449.

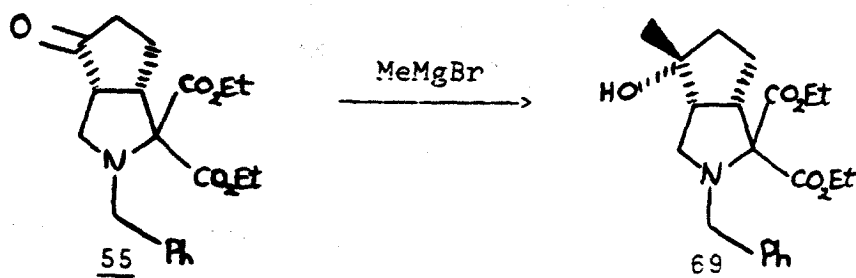


Further proof that structure (65) was the material produced from thermolysis of triazoline (57) was obtained by preparing 1-phenyl-2,2,4,4-tetracarboethoxypyrrolidine (68) from the cycloaddition of phenyl azide with diethylmethylene malonate followed by thermolysis of the resulting triazoline (67) in an excess of methylene malonate. This pyrrolidine gave an n.m.r. standard for the positions of the ring methylene protons, and the spectrum of the unknown thermolysis gum was found to be similar, suggesting that the main constituent of the gum probably was compound (65).



From these investigations it appeared that the gum obtained from the reaction of triazoline (57) with cyclopentenone was a mixture of the desired cycloaddition product (55), pyrrolidine (65), and possibly some other impurities arising from cycloreversal side-reactions. As this mixture gave only one spot on t.l.c., and was not separated by distillation, it was decided to use the crude mixture in the subsequent Grignard reaction which compound (55) was required to undergo, according to Scheme 2.2. It was thought that as compound (55) should have been the only component of the mixture to contain a ketone function, this would react first with the Grignard reagent, and the resulting carbinol (69) would then be separable from the

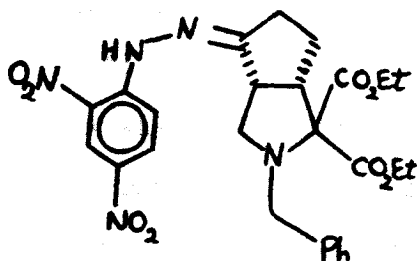
bulk of the mixture.



An excess of Grignard reagent was used, by assuming the gum to be pure (55), and adding a mole equivalent of reagent. The reaction was attempted several times, but in each case, the product obtained was identical to the initial gum, and the mass spectrum of the product still contained the molecular ion from compound (55). It seemed highly unlikely that a ketone would not have reacted with a Grignard reagent under the conditions used, therefore it seemed possible that the cycloaddition gum might not have contained compound (55) after all. To determine whether the bicyclic ketone (55) was present, the mixture was reacted with 2,4-dinitrophenyl hydrazine, and the hydrazone produced was isolated. The n.m.r. spectrum of the solid obtained was consistent with that expected for the DNP-derivative of compound (55), and the mass spectrum contained a molecular ion which also suggested that the derivative (70) had been produced.

Compound (70) was obtained from the cycloaddition gum in 16% yield, thus indicating that compound (55) had been produced in 10% yield from the reaction of triazoline (57) with cyclopentenone. Apart from this being a low yielding reaction,



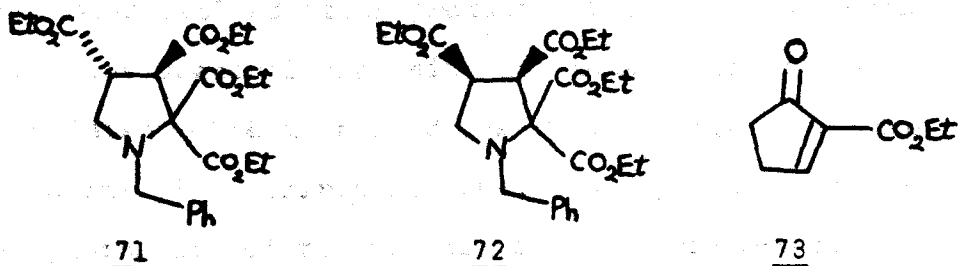


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there was also the question of why the Grignard reaction did not work. It was thought that, as the low yield had been due to the side-reactions incurred by thermolysing the triazoline, and as the lack of Grignard reactivity may also have been due to these side-products, it would be better to abandon the triazoline thermolysis method, and obtain (55) by other means.

The thermolysis of triazoline (57) was also carried out using diethyl maleate, diethyl fumarate, and maleic anhydride as dipolarophiles, to determine whether the low yield of compound (55) was due to the low dipolarophilic strength of cyclopentenone. Apart from the anhydride, which produced mainly polymer and some thermolysis products, these reactions gave the same results as the reaction with cyclopentenone. Once again the cycloaddition products were obtained as a mixture with the gum from the straight triazoline thermolysis. T.l.c. of the anhydride reaction product was highly streaked and contained several spots, but the t.l.c.'s of the fumarate and maleate reactions showed the characteristic three spot system. As with the cyclopentenone cycloaddition, the lower spot was enamine (59), the faint upper spot was piperazine (60) or (62), and the strong central spot was the mixture of cycloaddition product and tri-

azoline thermolysis side products. The maleate reaction was tried using the olefin itself as solvent but the same product mix was obtained as when the olefin and triazoline were equimolar in *o*-xylene. Cupric fluoroborate<sup>74</sup> was added to a reaction of triazoline (57) and maleate in *o*-xylene, but instead of increasing the yield of cycloaddition product by electrophilic catalysis, the reaction gave a very dark crude product mixture. The reaction produced the same products as the straight cycloaddition without catalyst, but it also produced some tarry polymeric material which contaminated the main products thus making purification difficult.

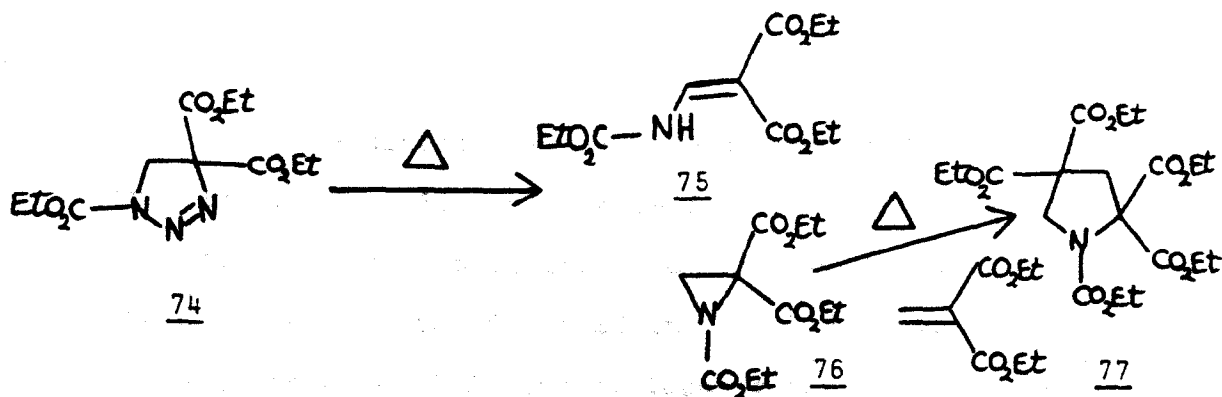


None of these olefins are as strong dipolarophiles as diethyl methylene malonate and therefore they could not compete effectively with the triazoline cycloreversal product for the azomethine ylid (61). It was hoped that the copper ions might have activated the maleate sufficiently to increase the ratio of product (72) to side product (65), but this was not realised. It might have been possible to achieve this effect by increasing the activation of cyclopentenone with an ester group on position two, but a cycloaddition between triazoline (57) and 2-carbethoxy-cyclopent-2-en-1-one (73) was not attempted.

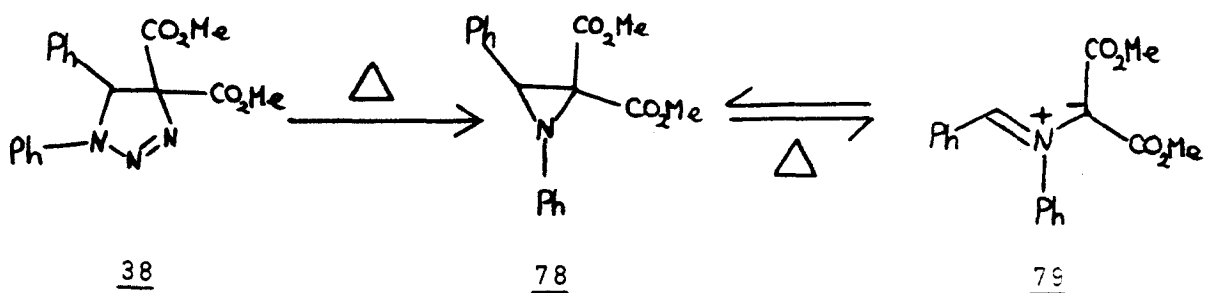
It has been reported that when triazolines are photolysed, they produce aziridines and do not form any of the side products obtained by thermolysis.<sup>75-77</sup> The photolysis of triazoline (57) should therefore provide a ready supply of 1-benzyl-2,2-dicarbethoxyaziridine (54). With this in mind, an ether solution of the triazoline was irradiated with a medium pressure mercury lamp, but the resulting mixture was found to be mainly polymeric and contained no major products. The photolysis was repeated using benzophenone as a sensitizer. Cyclopentenone was also added so that if the aziridine was formed, and underwent a light-induced ring-opening to the azomethine ylid species (61), it could be trapped by the olefin to give the bicyclic compound (55). The reaction did appear to produce some 1,3-dipolar cycloaddition product, as compound (55) was detected by mass spectral analysis, but none of the material could be isolated. The main products from the reaction were polymer, enamine (59), aziridine (54), and piperazine (60) or (62). The photolysis of the triazoline was therefore no better than the thermolysis.

It was thought that the problem must lie with the type of triazoline system being used. Most of the reported clean transformations of triazoline to aziridine involve systems which have an aryl substituent at position one. To see whether it might be an electron-withdrawing effect which favours aziridine formation over enamine, 1,4,4-tricarbethoxy- $\Delta^2$ -1,2,3-triazoline (74) was prepared from ethyl azidoformate and diethyl methylene malonate. When this triazoline was thermolysed,

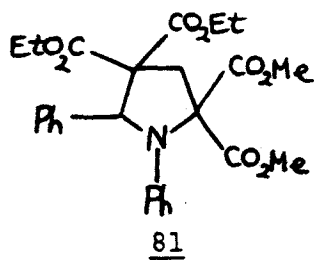
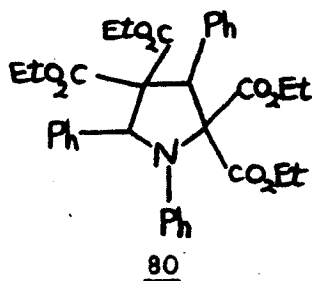
only enamine (75) was produced. Even when heated with diethyl methylene malonate, only enamine (75) was obtained. No cycloaddition product (77) or aziridine (76) could be detected in the product mixture.



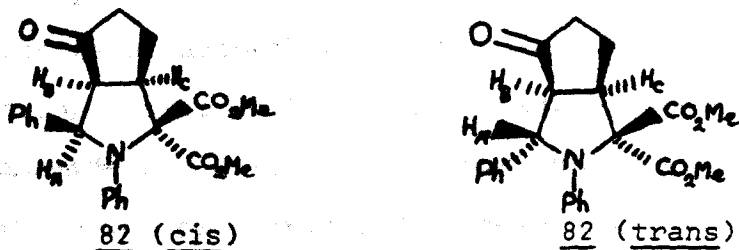
The lack of success with the triazolines which have been tried suggested that the triazoline to aziridine transformation was certainly not a general reaction but must be very sensitive to the substituents on the triazoline. In order to investigate the optimum conditions for aziridine formation and reaction, it was decided to utilize the well tried triazoline, 1,5-diphenyl-4,4-dicarbomethoxy- $\Delta^2$ -1,2,3-triazoline (38). This material had already been prepared when testing the dipolar cycloaddition of phenyl azide with dimethyl benzal malonate. The triazoline (38) was thermolysed by heating to  $170^\circ$  under vacuum in a Kugelrohr apparatus until nitrogen evolution had ceased. The temperature was then increased to  $250^\circ$  and a deep purple liquid distilled leaving only a trace of residue. The aziridine (78) cooled to a purple gum which formed a light red solution when dissolved in dichloromethane. The colour was due to the aziridine (78) being in equilibrium with the ring-opened azomethine ylid (79).



The almost quantitative formation of aziridine (78) from triazoline (38) and the facile ring-opening of aziridine (78) to form the azomethine ylid (79) meant that triazoline (38) would be very useful for studying the 1,3-dipolar cycloaddition of an azomethine ylid with cyclopentenone. This triazoline has already been extensively studied<sup>46,47,48</sup> and reacted with many activated olefins to produce pyrrolidines but no reports have been found of 1,3-dipolar cycloaddition with cyclopentenone. Triazoline (38) and the diethyl ester analogue (37) have been used to generate the azomethine ylid species (79) in situ by refluxing in toluene. Two reactions were carried out to test these conditions, using diethyl benzal malonate and diethyl methylene malonate respectively, as the 1,3-dipolarophiles. The reaction of diethyl benzal malonate with triazoline (37) was a literature reaction, and as reported, gave pyrrolidine (80) as the main product. Diethyl methylene malonate and triazoline (38) also reacted in refluxing toluene to give pyrrolidine (81).

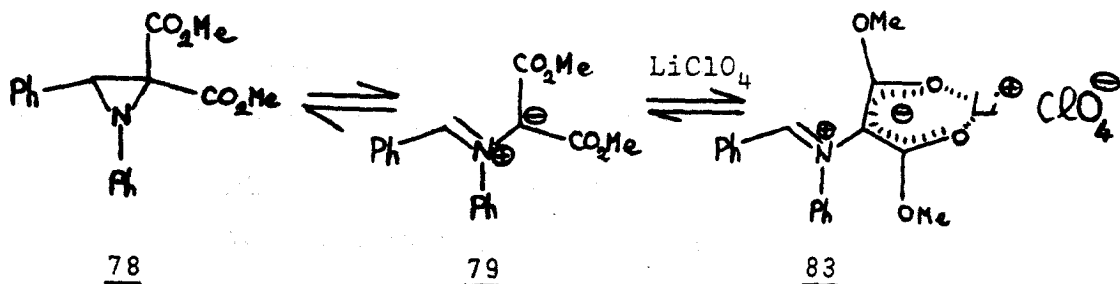


The reaction of triazoline (38) with cyclopentenone in refluxing toluene gave the desired cycloaddition product, compound (82). The reaction was low yielding and produced a mixture of cis and trans isomers. The main product was obtained in 13.3% and was assigned the cis stereochemistry. The minor product, the trans isomer was obtained in only 1.7%. The stereochemical assignments were made on the basis of the proton n.m.r. spectra. The major product was found to have a  $J_{AB}$  splitting value which was double that for the minor product. Molecular models indicated that the cis isomer should have the largest splitting value, therefore the major product was deemed to be the cis isomer. The  $J_{BC}$  splitting values were large for both isomers. This was consistent in each case with the cis ring-junction stereochemistry expected from a 1,3-dipolar thermal cycloaddition.



The same reaction was tried in benzene using a low pressure mercury vapour lamp as the energy source. In this case the triazoline appeared to have been photolysed to aziridine but this aziridine did not under these conditions equilibrate with the open-chain azomethine-ylid species. When the reaction was worked-up, the cyclopentenone was recovered unchanged but only

the hydrolysis products of the aziridine, benzaldehyde and dimethyl anilinomalonate, were obtained. As the reaction conditions were thoroughly anhydrous, it is not known how the aziridine could have hydrolysed.



The thermal reaction in refluxing toluene was repeated in the presence of lithium perchlorate. This salt is reported<sup>78</sup> to form a relatively stable complex (83) with the azomethine ylid of aziridine (78) and effectively shifts the equilibrium of the aziridine ring-opening reaction in favour of the ylid species. The reaction produced the characteristic deep red colour indicative of the presence of azomethine ylid in the mixture, and after overnight reaction a pale yellow solution was produced. On work-up only benzaldehyde and diethyl anilinomalonate were isolated. No cyclopentenone was recovered and no molecular ion corresponding to the cycloaddition product was detected in the mass spectrum of the residue. Once again it is not known how the aziridine could have hydrolysed but it is likely that the cyclopentenone may have been lost by polymerisation induced by the lithium perchlorate.

Another attempt to increase the yield of the reaction involved using a Lewis acid catalyst as a means of further activating the double bond of the dipolarophile. Cyclopentenone was

added to boron trifluoride etherate and the mixture was added to an equilibrating mixture of aziridine (78) and azomethine ylid (79) at 80°. The red solution instantly decolourised and the colour did not return. This instantaneous reaction appeared to indicate the increased reactivity of the dipolarophile but the effect did not increase the yield which was still low at 13.4%. However the use of boron trifluoride did have a marked effect upon the stereoselectivity of the reaction. The product obtained was entirely the trans isomer of compound (82), no cis isomer was isolated or even detected by t.l.c. This was a total reversal of the stereoselectivity of the standard thermal cycloaddition in refluxing toluene which gave predominantly the cis isomer. It is unlikely that this effect would have been due to the electronic changes at the double bond of cyclopentenone caused by coordination of the carbonyl oxygen to boron. It is most likely a steric effect whereby the boron trifluoride attached to the oxygen of cyclopentenone caused the bulky phenyl substituent on the azomethine ylid to be repelled.

A final attempt to improve the yield of the cycloaddition reaction with cyclopentenone proved more successful and gave compound (82) in 44.4%. The triazoline and enone were heated strongly with no solvent present. The triazoline melted and the solution effervesced as nitrogen was evolved. The reaction by this method gave a 1:1 ratio of cis and trans isomers.

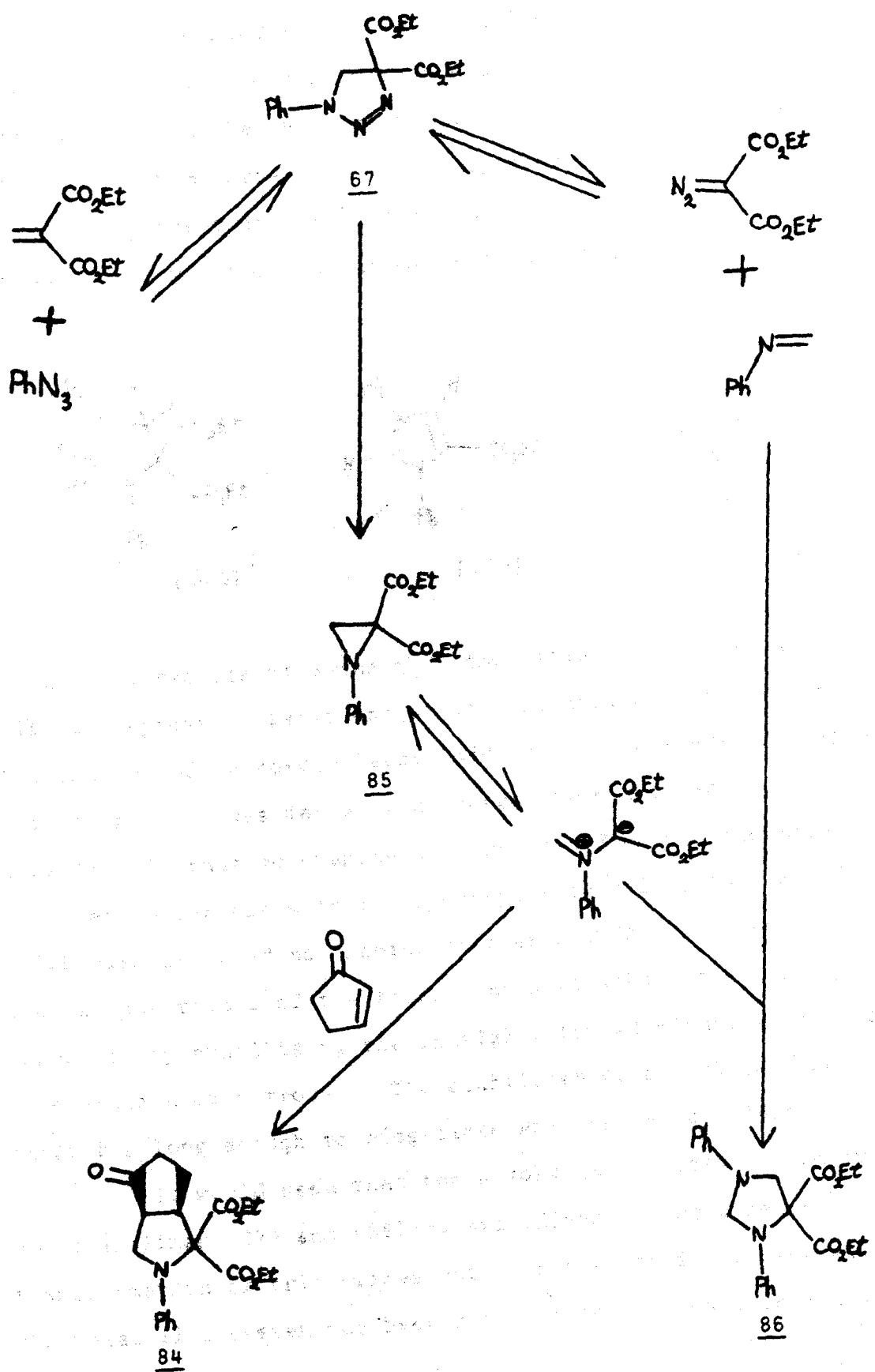
Now that a relatively efficient method had been developed for reacting cyclopentenone with triazoline (38), the reaction conditions were employed in the reaction of triazoline (57) with cyclopentenone. The heterogeneous mixture was heated



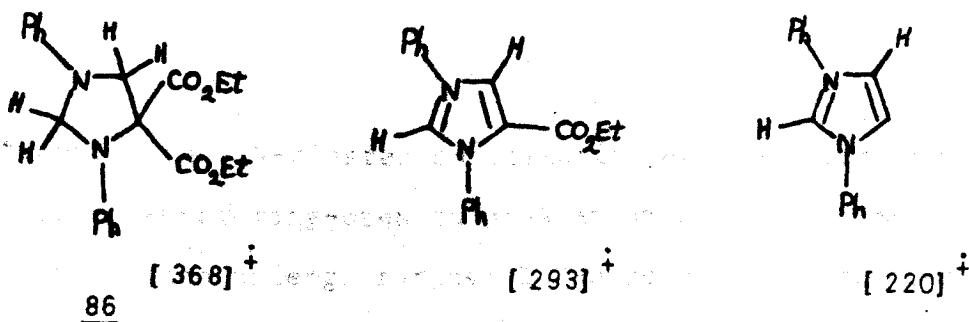
without solvent until all the triazoline had thermolysed. The mixture was then refluxed for several hours in xylene. The reaction again produced enamine (59) and an oil containing a mixture of the cycloaddition products (55) and (65). The amount of 1,3-dipolar cycloreversion taking place in the triazoline did not appear to have been reduced under these conditions and therefore the reaction was still of no synthetic use.

To check whether the extreme difference in reactivity between triazoline (38) and triazoline (57) might be due to the effect of a phenyl substituent at position 1 instead of a benzyl substituent, cyclopentenone was reacted with 1-phenyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (67) in refluxing toluene. This reaction gave a crude oil which appeared from mass spectral data to contain the desired cycloaddition product, compound (84). Another oil which was isolated from the same mixture gave a mass spectrum which indicated that some 1,3-dipolar cycloreversion may also have occurred. In this case however the triazoline did not revert to an azide and alkene as was the case with triazoline (57). It is thought that triazoline (67) was actually in equilibrium with diethyl diazomalonate and N-phenyl-azomethine which competed with cyclopentenone as a 1,3-dipolarophile.

Triazoline (67) was also refluxed in methyl crotonate but the mass spectrum of the product did not indicate the presence of a cycloaddition product with the alkene. The spectrum was remarkably similar to that for the cyclopentenone and triazoline (67) reaction, without the mass fragments relating to the cyclopentenone cycloaddition product (84).

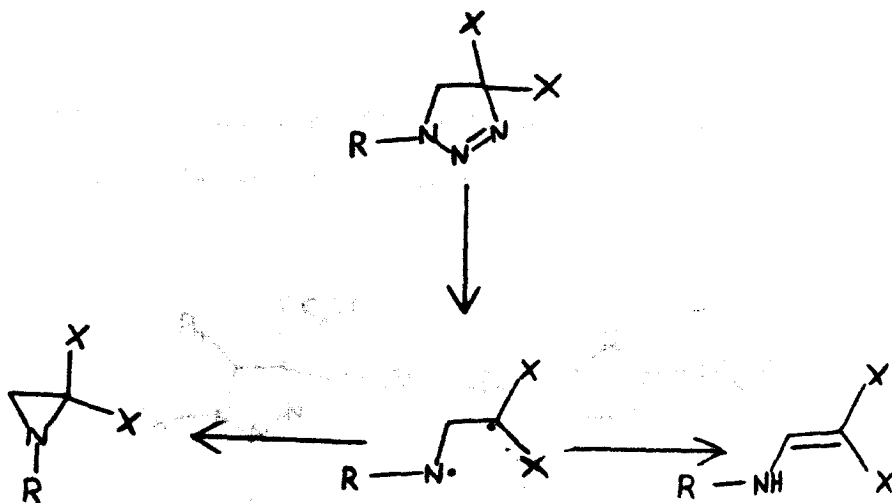


The evidence for the formation of compound (86) is scant and is based entirely on the presence of  $m/e$  values 368, 293, and 220 in the mass spectrum. The n.m.r. spectra of the crude oils from these reactions suggested that compound (86) might have been present in the mixtures but the samples were not pure enough for any conclusive evidence to be obtained.



From these results it seems that the replacement of an N-benzyl with an N-phenyl substituent on the triazoline does not remove the problem of retro-dipolarcycloaddition. However, the N-phenyl triazoline (67) was markedly different from the N-benzyl triazoline (57) in that no enamine was produced from the thermolysis. This was comparable with 1,5-diphenyl triazolines (37) and (38) which also produced no enamine on thermolysis. A phenyl substituent at position 1 of the triazoline most likely inhibits enamine formation by stabilising the initially formed diradical produced on extrusion of nitrogen. The stabilised diradical can then exist for long enough to ring-close and form an aziridine.

It would seem that the almost quantitative thermolysis of triazolines (37) and (38) to aziridines is not a general transformation of triazolines but is rather an ideal case whereby the triazoline system has been set-up to avoid possible side-

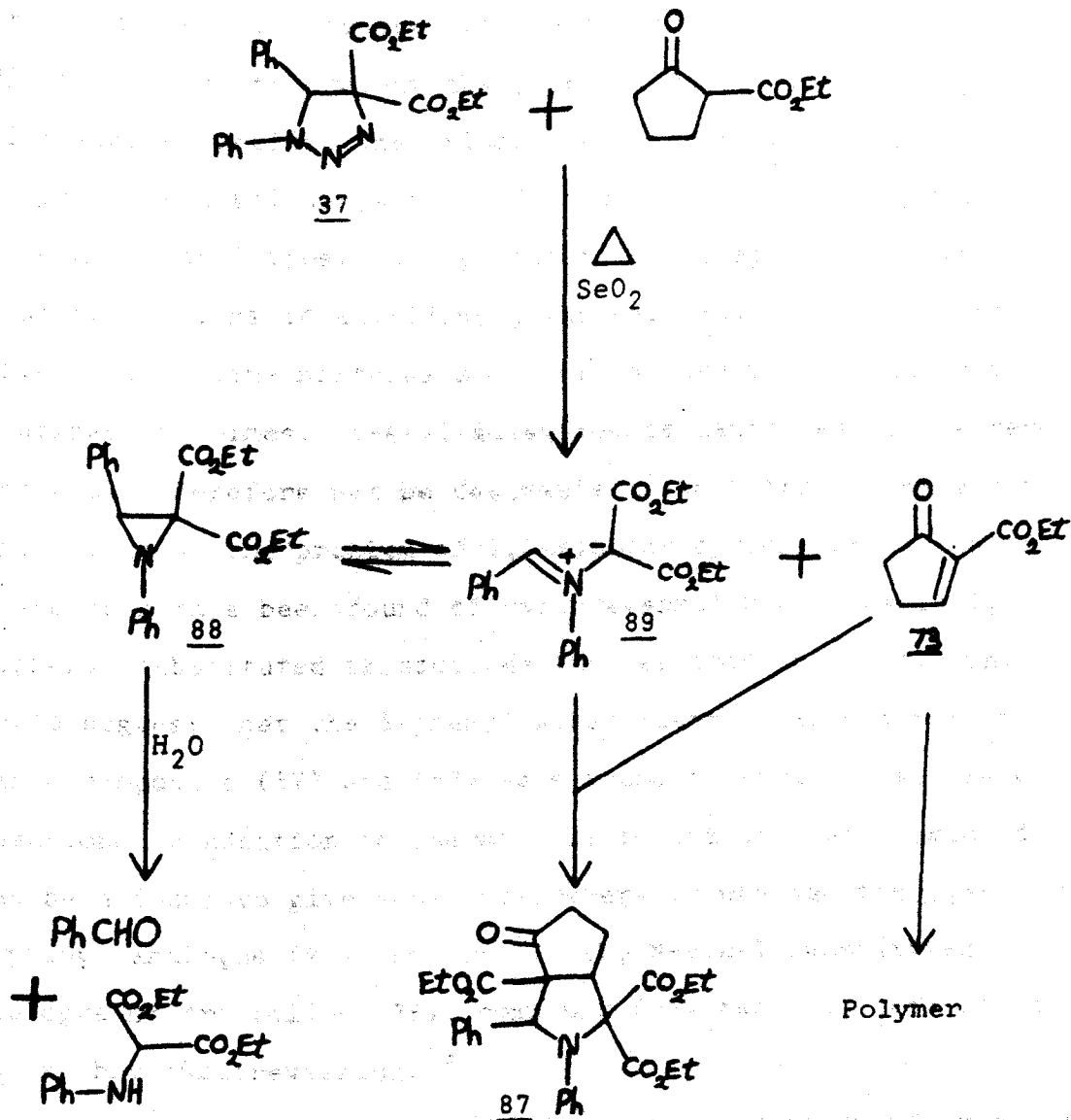


reactions. The 4,4-diester substitution ensures that the triazoline cannot ring-open to give an amino-diazo compound which could then undergo further 1,3-dipolar cycloaddition. The phenyl substituent at position 1 appears to increase the ratio of aziridine to enamine dramatically and the phenyl substituent at position 5 may also be important in reducing the tendency towards retro-1,3-dipolar cycloaddition.

A final attempt to overcome the problem of retro-1,3-dipolar cycloaddition was made by using 2-carbethoxycyclopentenone (73) as dipolarophile. This olefin being more strongly activated than cyclopentenone should have been able to compete much more successfully with diethylmethylene malonate, the retro-cycloaddition product from triazoline (57), for the azomethine ylid (61).

2-Carbethoxycyclopentanone, produced by a Dieckmann reaction of diethyl adipate, was refluxed in dioxan with selenium dioxide to give 2-carbethoxycyclopent-2-en-1-one (73). This enone was found to polymerise rapidly and so was prepared in situ.

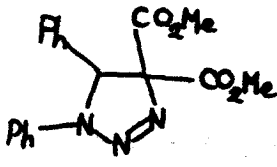
Triazoline (37) was used for a trial reaction and was refluxed in dioxan with 2-carbethoxycyclopentanone and selenium dioxide.



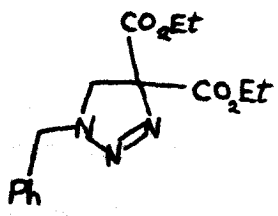
The reaction mixture produced polymer, some aziridine and the hydrolysis products of aziridine. No cycloaddition product (87) was detected. The aziridine appears to have been stable to the reaction conditions, therefore the enone (73) must have polymerised completely before any cycloaddition could occur.

At this point it was decided to abandon the strategy as outlined in Scheme 2.2. It was obvious that  $\Delta^2$ -1,2,3-triazolines were not the ideal starting materials as had been envisaged at the outset of this project. Apart from requiring 4,4-disubstitution to prevent the possibility of ring-opening to diazo-amine species, the triazolines also require electron donating N-substituents to produce a good ratio of aziridine to enamine or imines. N-Aryl substituents appear to give the best conversions to aziridines, whereas N-alkyl substituents give aziridine/enamine mixtures and N-carbalkoxy triazolines give 100% enamines or imines. N-Aryl substituents cannot easily be removed and would therefore not be desirable as an N-protecting group. There is also the problem of 1,3-dipolar cycloreversal reactions. No reports have been found of cycloreversal reactions in 1,5-diphenyl substituted triazolines such as compound (38). This would suggest that the 5-phenyl substituent is also important since compounds (57) and (67) were found to exhibit cycloreversal reactions in addition to thermolysis to aziridines. Compound (91) has been found to give some cycloreversion whereas the 1,5-diphenyl analogue (90) has not.<sup>53</sup> The N-alkyl substituted analogue of triazoline (38), compound (92) has also been found to exhibit cycloreversion.<sup>73</sup>

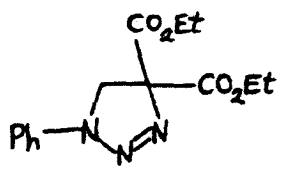
It would appear that for a good conversion of triazoline to aziridine, the triazoline should be: N-aryl substituted to avoid enamine or imine formation, 4,4-disubstituted to avoid ring-opening to diazo-amines, and 1,5-diaryl substituted to avoid retro-1,3-dipolar cycloaddition. These precise requirements mean that triazolines cannot be used as general starting materials for the production of kainic acid analogues.



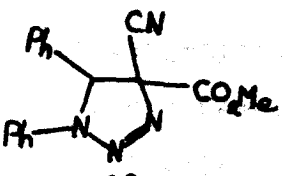
38



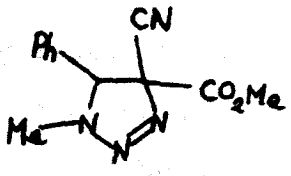
57



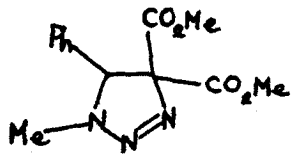
67



90

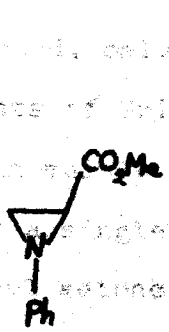


91

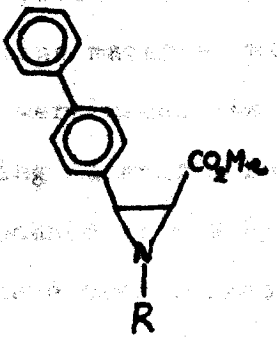


92

The majority of reported 1,3-dipolar cycloaddition reactions between aziridines and olefins involve 1,3-diaryl substituted aziridines. These aromatic substituents probably help to stabilise the intermediate azomethine ylid species and so make ring-opening of the aziridine more facile. There are reported 1,3-dipolar cycloadditions with aziridines having an N-aryl substituent but no substituent in position 3 such as compound (43).<sup>85</sup> There are also examples where the aziridines have N-alkyl substituents and aryl substituents in position 3 such as compounds (93) and (94),<sup>45</sup> but no reports have been found of 1,3-dipolar cycloadditions with aziridines of the N-alkyl, 2-carbalkoxy-type such as compound (46).

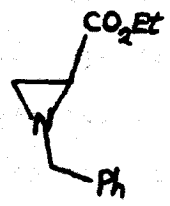


43



93 R = iso-propyl

94 R = cyclohexyl



46

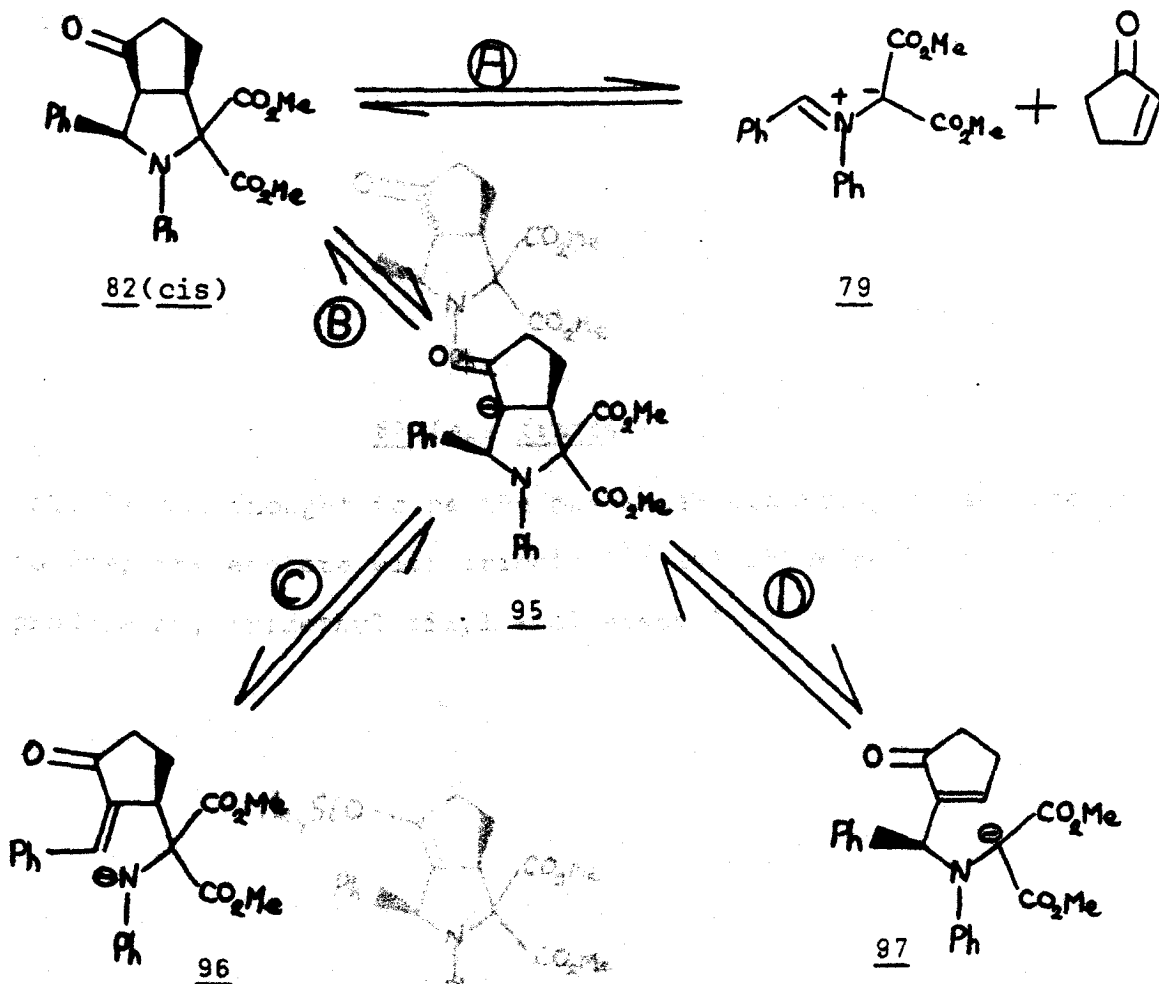
It was found that aziridine (46) polymerised instead of ring-opening to an azomethine ylid. It may be necessary therefore to block the 3-position to avoid this polymerisation unless an aryl substituent is present, as in compound (43), to assist the ring-opening reaction under milder conditions.

There remains the anomaly of the Grignard reaction on compound (55) which could not be made to work. If the gum obtained from the reaction of triazoline (57) with cyclopentenone did contain compound (55) then it is strange that the addition of Grignard reagent did not produce alcohol (69) or the dehydrated analogue. To test this reaction the bicyclic compound (82) obtained from cycloaddition of triazoline (38) and cyclopentenone was used. This compound is more sterically hindered than structure (55) and so if a Grignard reagent was able to react with it, then it should also react with compound (55).

The reaction of the bicyclic compound (82) with methyl magnesium iodide or methyl magnesium bromide appeared to have no effect unless a large excess of reagent was employed, and then as expected the reaction gave numerous products, none of which were isolated. When only one mole equivalent was used the reaction mixture turned milky white suggesting that some transformation had occurred. T.l.c. of this solution showed only starting material to be present and when the reaction mixture was quenched, only starting material was obtained. When two mole equivalents of Grignard were used, the reaction product was found to contain mainly starting material, but the n.m.r. spectrum contained a singlet resonance at  $\tau$  8.43 which suggested that some methyl ketone may have been formed by reaction of the Grignard on the ester groups.

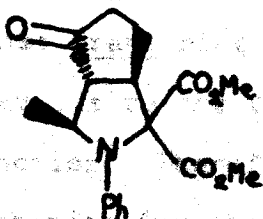


When the cis isomer of compound (82) was reacted with one equivalent methyl magnesium bromide or methyl lithium, the product was a 50:50 mixture of cis and trans isomers of the starting material. Possible reaction pathways by which epimerisation could occur are shown below.



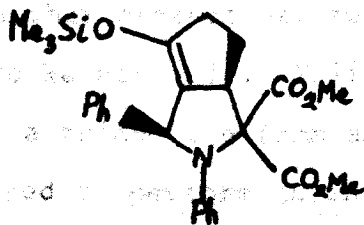
Pathway A would be a retro-1,3-dipolarcycloaddition to the azomethine ylid (79) and cyclopentenone which could then recombine to give a mixture of cis and trans isomers. This reaction is thought not to occur, partly because the isomers were found to be thermally stable and also because the epimerisations can occur at low temperature. The requirement of a strong

base (methyl Grignard or methyl lithium) suggests that the abstraction of a proton as in route B must be the initial reaction. If this enolisation were the only process occurring then a mixture of isomers would only be produced if the enolate reprotonated to give a trans ring junction. The product would therefore be a mixture of the all cis isomer of compound 82 and the all trans isomer.



82 (all trans)

This is not thought to be the mechanism occurring as an attempt to trap the enolate with trimethyl silyl chloride<sup>86</sup> did not produce any trimethyl silyl enol ether (98).



98

Of the postulated ring-opening mechanisms C and D, route D is thought to be the most likely. The carbanion in structure (97) is stabilised by the geminal esters whereas structure (96), with a negatively charged nitrogen, would be

highly reactive. It is also unlikely that pathway C would produce any trans compound (82) from cis starting isomer as the stereo effects of structure (96) would dictate the direction of attack on the double bond. The only isomeric mix possible would again be the original cis (82) isomer and the all trans (82) isomer.

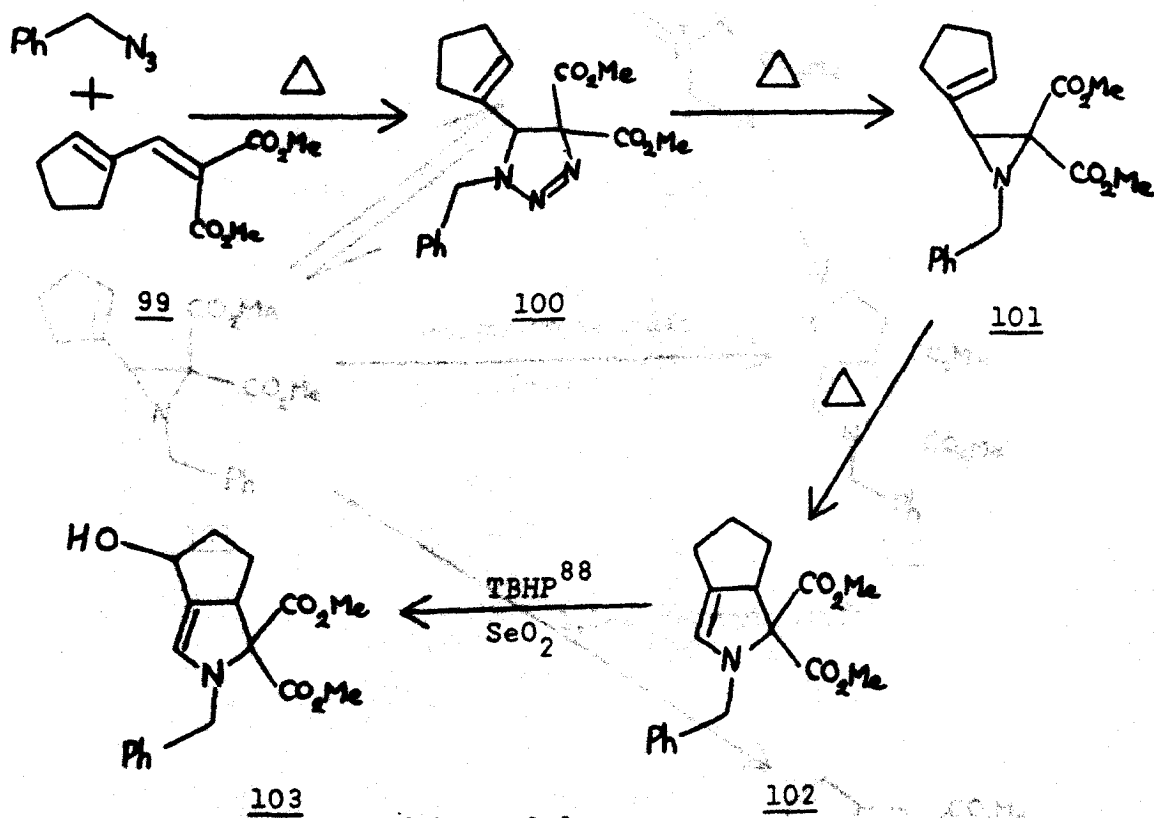
Structure (97) could ring close from either side of the double bond and so generate a mixture of cis and trans isomers of compound (82) with cis ring junction stereochemistry. It is thought unlikely that trans ring junctions would be produced in this bicyclic system due to the thermodynamic instability relative to the cis ring junction. Therefore pathway D is thought to be the most likely mechanism for the epimerisation of compound (82). This epimerisation could also be applied to compound (55) as the two phenyl substituents of (82) are not implicated in the mechanism. The driving force for the reaction appears to be the stability of the malonate-type carbanion produced.

3. An attempt to prepare kainic acid and its structural analogues by intramolecular 1,3-dipolar cycloaddition

A further attempt was made at constructing the bicyclic intermediate to kainic acid. This route was also based on the preparation of a triazoline from an azide and olefin but in this case it was hoped to perform an intramolecular 1,3-dipolar cycloaddition reaction when the triazoline was thermolysed. The advantages of intramolecular reactions have already been discussed in Section 1 and have been found to be useful when applied to 1,3-dipolar cycloadditions. <sup>87</sup>

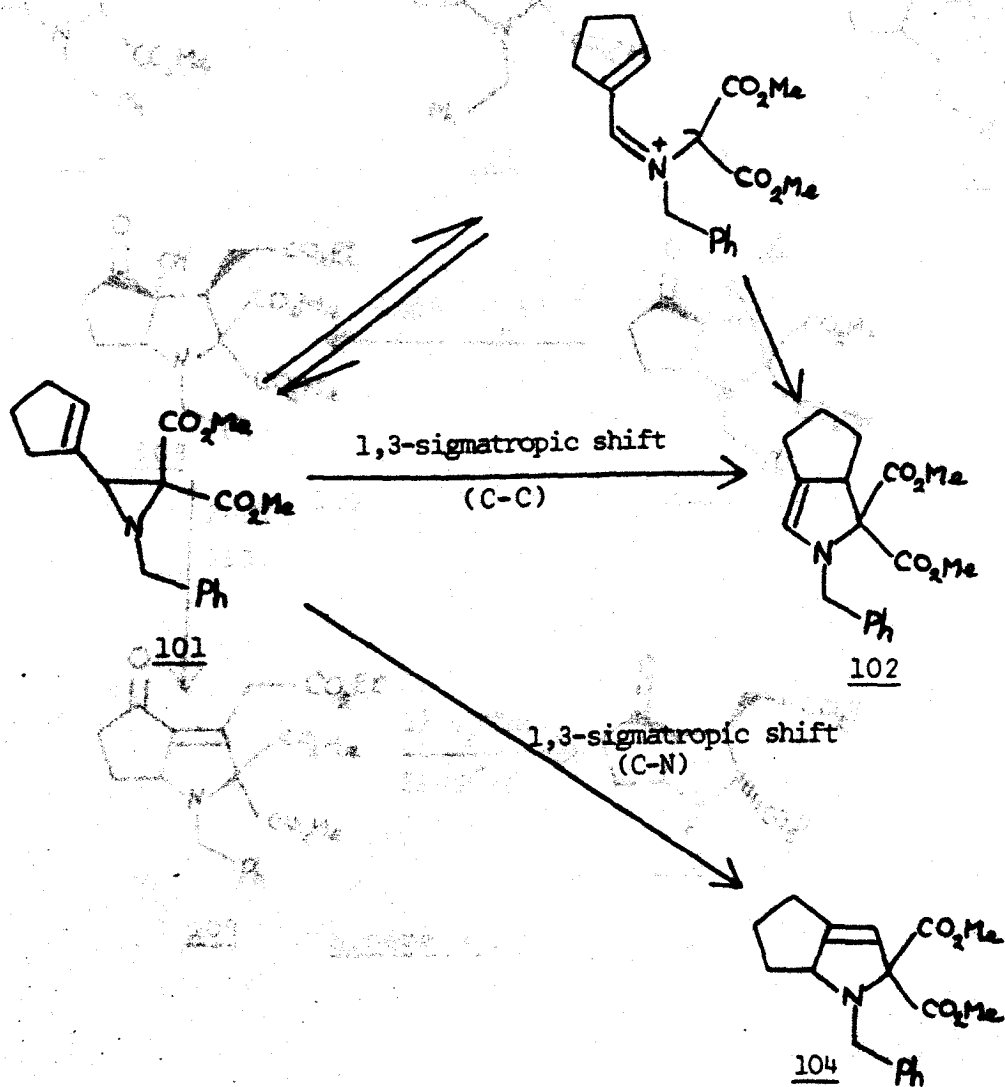
The strategy adopted is shown in Scheme 3.1. It was thought that this route might have more chance of success than the intermolecular triazoline reactions attempted previously.

The reasoning for this is that compound (100) has disubstitution at position four therefore no diazo-amine formation would be likely to occur. Some enamine formation would be expected during thermolysis because of the N-alkyl substituent and for the same reason, some cycloreversion might occur although this might be reduced by the effect of the substituent at position 5. The percentage of aziridine (101) which did form would be likely to react almost exclusively with the double bond of the cyclopentenyl substituent because of its close proximity and therefore the production of side products due to cycloreversion would be minimised. The unsaturated substituent on the 3-position of aziridine (101) might also help to induce a more facile ring-opening reaction to the azomethine ylid.

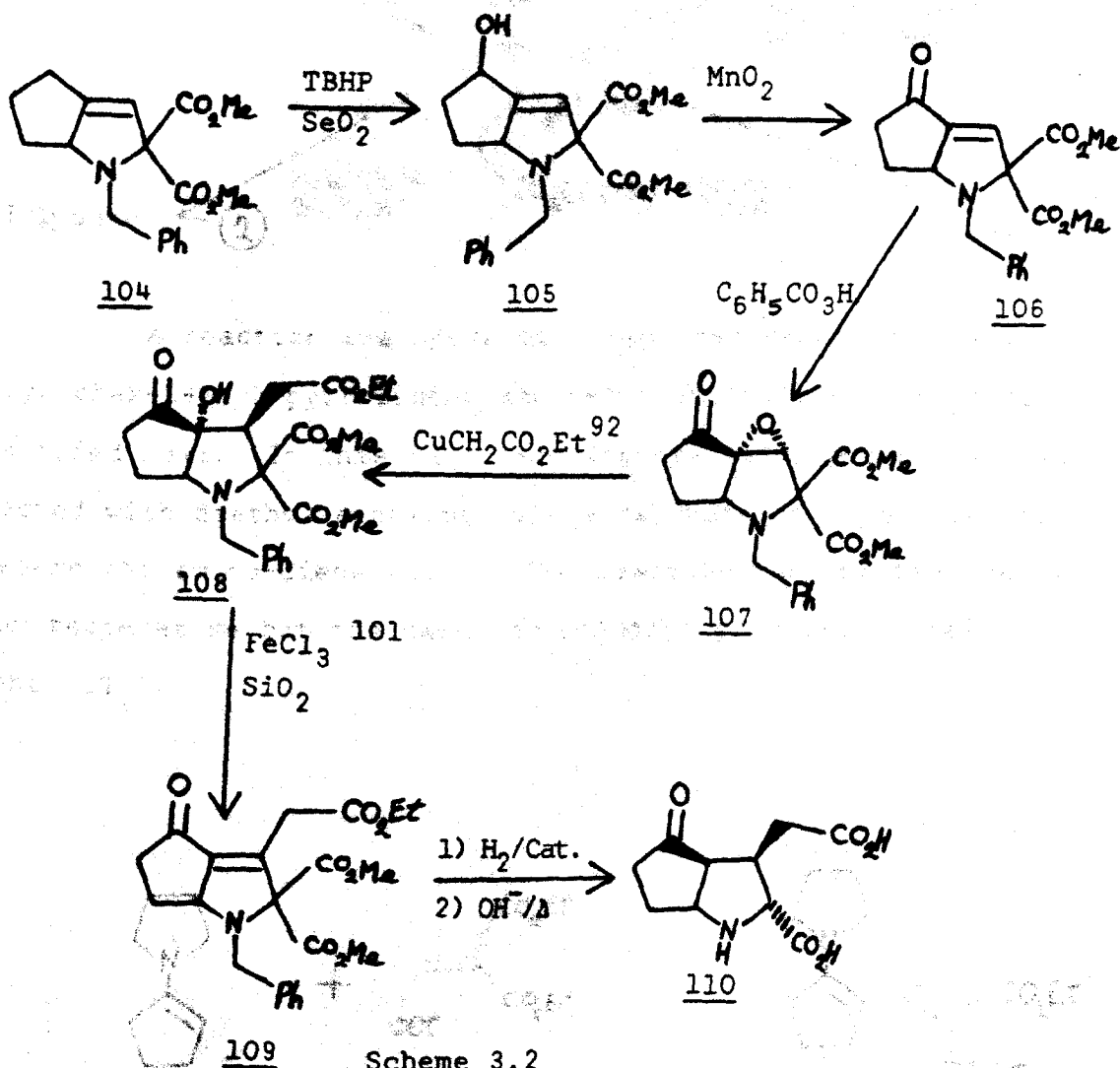


Scheme 3.1

Intramolecular 1,3-dipolar cycloadditions of this type between an aziridine and an olefin have not been reported in the literature. However compound (101) is also set up for a vinylcyclopropane-cyclopentene type 1,3-sigmatropic rearrangement and this reaction could also result in the formation of compound (102). The possibility of an intramolecular 1,3-dipolar cycloaddition occurring cannot be completely ruled out as 1,3-sigmatropic shifts have high activation energies<sup>40</sup> and therefore require elevated temperatures in order to proceed. It is feasible that an aziridine which is activated towards ring-opening could react via the open-chain azomethine ylid species preferentially to undergoing a thermal 1,3-sigmatropic shift.

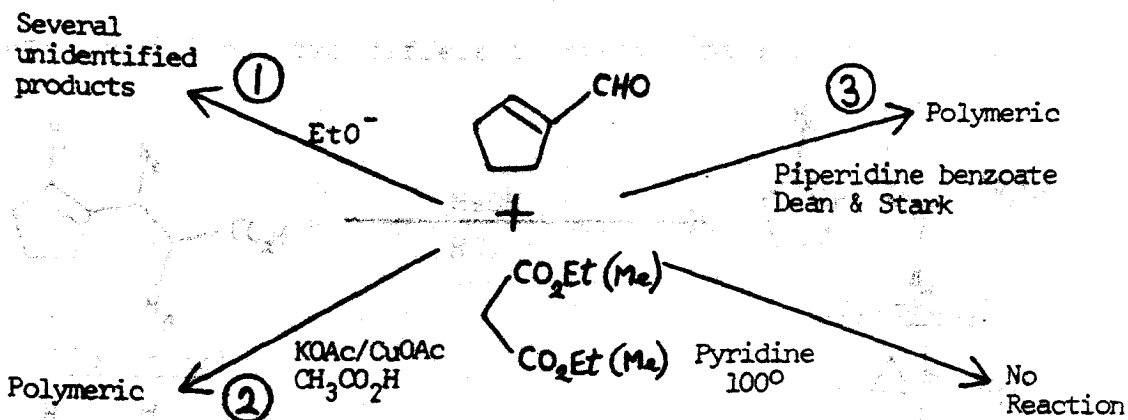


Two 1,3-sigmatropic rearrangements are possible for compound (101) by either the migration of a C-C bond to form compound (102) or a C-N bond to give compound (104). Two reported rearrangements of vinyl aziridines<sup>89,90</sup> were found to occur by C-N bond migration, but these aziridines were not activated for ring-opening and it was hoped that the gem-diester substituents on compound (101) would either encourage azomethine ylid formation or lower the activation energy for C-C bond migration to take place. In either case compound (102) would be formed.

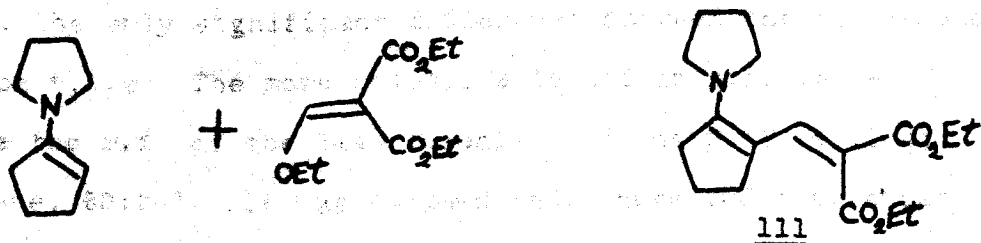


Compound (104) would be of no use for synthesising kainic acid but as Scheme 3.2 shows, it might be possible to make an interesting kainic acid analogue using compound (104) as starting material.

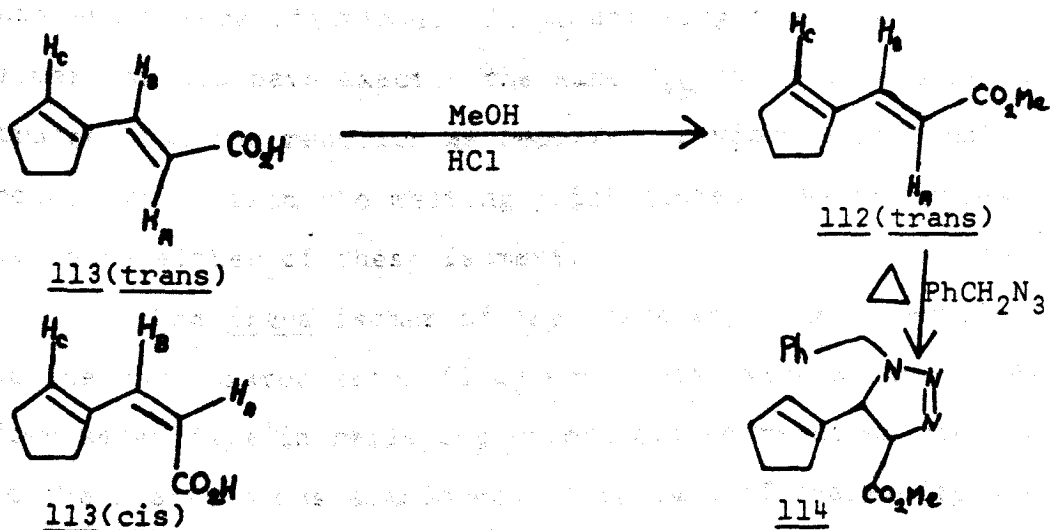
Several attempts were made to prepare compound (99) using cyclopent-1-ene aldehyde<sup>81</sup> as starting material. These methods were all unsuccessful as the diagram below shows.



A reaction analogous to a reported reaction<sup>91</sup> between 1-cyclohex-1-enyl-pyrrolidine and methoxymethylene malononitrile was tried next. In this case 1-cyclopent-1-enyl-pyrrolidine was reacted with diethyl ethoxymethylene malonate in an attempt to prepare the amino-diene (111). The reaction was spontaneous at room temperature but the dark red solution produced contained no diene (111).



As the diene diester (99) could not be prepared, it was decided to make the monoester diene (112) instead. The corresponding free acid (113) has been reported<sup>82</sup> and was prepared by heating cyclopent-1-ene aldehyde in pyridine with malonic acid at 100° for 3 hours. The report did not mention the stereochemistry of the product, but only one compound was obtained which melted at 162°. When this reaction was repeated under the same conditions, two different crystalline solids were obtained.

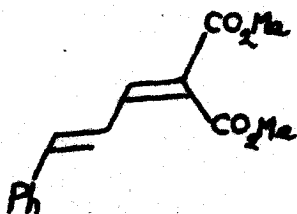
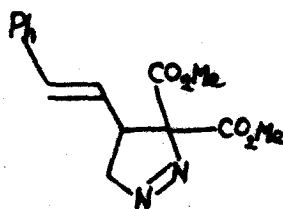


One of these solids was soluble in ethyl acetate but the other was insoluble. The infra-red and mass spectra of the two materials were virtually identical and the n.m.r. spectra were identical apart from slight chemical shift differences in protons H<sub>B</sub> and H<sub>C</sub>. The  $J_{AB}$  coupling constants for the two compounds were identical (16 Hz). Apart from the difference in the melting points of the two substances which may or may not have been due to differences in purity, the only significant difference between the two compounds was seen on t.l.c. The more soluble acid had an r.f. value of 0.83 while the r.f. of the less soluble acid was 0.62 (silica; ether/hexane, 80:20). It was assumed that these two substances



must be the cis and trans isomers of acid (113). The less soluble acid was assumed to be the trans isomer because of its higher melting point and an infra-red absorption at  $982\text{ cm}^{-1}$  which may have been due to the carbon-hydrogen deformation in a trans disubstituted double bond. The more soluble isomer had an infra-red absorption at  $720\text{ cm}^{-1}$  which was indicative of carbon-hydrogen deformation in a cis substituted double bond. Apart from these two absorptions, the infra-red spectra of the two substances were identical. It is striking that these two stereoisomers should have exactly the same  $J_{AB}$  coupling constants and strange that the reaction as reported previously gave only one product which from the melting point quoted does not appear to have been either of these isomers.

The trans isomer of the diene acid was esterified and the unsaturated ester (112) was heated with benzyl azide. After seven days in refluxing chloroform no reaction had occurred and the reaction was abandoned. This lack of reactivity was not expected as the corresponding  $\alpha,\beta$ -unsaturated ester where the cyclopentenyl ring is replaced by a phenyl ring, i.e. methyl cinnamate, reacts with organic azides to give mixtures of triazolines and ring-opened diazocompounds.<sup>53</sup> Also a similar conjugated diene, compound (115), has been shown<sup>93</sup> to specifically add diazomethane to the activated double bond of the diene to give the pyrazoline (116).

115116

The cycloaddition would have been more likely to have proceeded had the doubly activated diene, compound (99), been used. There would also have been no likelihood of forming open-chain diazo-products with this starting material. However, the lack of success in preparing compound (99), and also lack of time meant that the work had to be stopped at this stage.



<sup>1</sup>H nmr spectra were recorded as dilute solutions in the given solvent, on a Perkin-Elmer R24 at 60 MHz or a Perkin-Elmer R32 at 90 MHz. <sup>13</sup>C nmr spectra were recorded as solutions in deuteriochloroform on a Bruker WP80.

Infra-red spectra were recorded on a Perkin-Elmer 577 Grating Infra-red Spectrophotometer. Melting points were recorded on a Kofler block and are uncorrected.

Analytical tlc was carried out using glass plates coated with Merck Kieselgel GF<sub>254</sub> (Type 60) and column chromatography was carried out using Merck Kieselgel HF<sub>254</sub> in an adaptation of the pressure method of Still<sup>102</sup>.

ANALYTICAL DATA AND PHYSICAL PROPERTIES OF THE POLYMER

The polymer was obtained as a white solid. The yield was 10.5 g (90%). The polymer was soluble in chloroform, dichloromethane, and nitrobenzene. The glass transition temperature was 105°C. The melting point was 145°C. The polymer was collected as only starting materials and the product.

PREPARATION OF MONOMER AND POLYMERIZATION

Monomer was prepared by the reaction of 10.0 g (0.05 mol) of 1,2-dichloroethane with 10.0 g (0.10 mol) of sodium hydroxide in a 100 ml round-bottom flask equipped with a magnetic stirrer and a reflux condenser. The mixture was refluxed for 24 hours. The product was collected as only starting materials and the product. The yield was 7.5 g (75%).

Preparation of 2-(2-methyl-2-hydroxybut-3-enyl)-4,4,6-trimethyl-5,6-dihydro-oxazine (12)

2,4,4,6-Tetramethyl-5,6-dihydro-oxazine (5.64g, 0.04 mol.) was dissolved in dry THF (60 ml) and cooled to  $-78^{\circ}$ . Butyl lithium (31.1 ml of 1.35M; 0.042 mol.) was added under nitrogen and the mixture was stirred for one hour. Methyl vinyl ketone was added dropwise and the mixture was allowed to warm to room temperature. The reaction was left overnight, then poured into water (400 ml) and acidified with dilute hydrochloric acid. The solution was extracted with ether/pentane (1:1) and the organic layer discarded. The acid solution was neutralised with sodium bicarbonate (sat.), extracted with ether, dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated. The crude product was distilled (bp.  $78-82^{\circ}/2$  mm) yielding 2.75g (32.6%) of a viscous colourless liquid;  $\tau(\text{CDCl}_3)$  3.8 (1H,s), 4.05-5.15 (3H,m), 5.7-6.1 (1H,m), 7.75 (2H,s), 8.0-9.2 (14H,m);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3400-3200, 1650, 1150, 1000, and  $900\text{ cm}^{-1}$ ;  $m/e$  211 ( $\text{M}^+$ ); Found  $\text{M}^+ = 211.1573$ ,  $\text{C}_{12}\text{H}_{21}\text{NO}_2$  requires  $\text{M}^+ = 211.1573$ .

Attempted preparation of 2-(2-methyl-1,3-butadienyl)-4,4,6-trimethyl-5,6-dihydro-oxazine (13)

The alcohol (12) (2.94g, 13.9 mmol.) was dissolved in benzene (50 ml) and refluxed with a catalytic amount of toluene-4-sulphonic acid (0.5g). The flask was equipped with a Dean and Stark trap and the mixture refluxed for four days. No water was collected and only starting material was recovered.

Preparation of maleic acid monoethyl ester<sup>94</sup>

Sublimed maleic anhydride (19.04g, 0.194 mol.) was refluxed in ethanol (150 ml) for 6.5 hours. Concentration and distillation (bp.  $90-98^{\circ}/0.5$  mm.) gave 28g (90%) of maleic acid monoethyl ester;  $\tau(\text{CDCl}_3)$  2.25 (1H,s), 3.6 (2H,s), 5.75 (2H,q), and 8.65 (3H,t).

Preparation of triphenylcyanomethylenephosphorane<sup>10</sup>

Chloroacetonitrile (9.55g, 126.5 mmol.) was made up to 60 ml. with anhydrous benzene and added to a boiling solution of triphenylphosphine (35.43g, 135 mmol.) in 120 ml. of anhydrous benzene. The mixture was refluxed for 30 min. during which time a precipitate formed. The flask was left to cool and the precipitate collected by filtration, washed with benzene and dried under vacuum. Yield 2.4g. By repeating the process of refluxing and filtering of the successive filtrates, the total yield was increased to 17.64g (41.4%) of triphenylcyanomethyl phosphine chloride, m.p. 192-3°,  $\tau(\text{D}_2\text{O})$  2.2-2.7 (15H,m), 5.5 (2H,s).

Sodium hydroxide ( $1.55 \times 10^{-3}\text{M}$ ) was added dropwise to a stirring solution of triphenylcyanomethyl phosphine chloride (8g, 23.7 mmol.) in water (228 ml.). A milky-white precipitate formed with each drop. Addition was complete at the neutralisation point and the mixture was filtered. The colourless precipitate was washed with water and dried at 60° in vacuo (6.34g, 80%), m.p. 134° (lit.<sup>10</sup> 140°);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3500-3100, 2150, 1125, and 1000  $\text{cm}^{-1}$ ;  $\tau(\text{CDCl}_3)$  2.1-2.7 (15H,m), and 8.0 (1H,s).

Attempted Wittig reaction of triphenylcyanomethylenephosphorane and methyl vinyl ketone

The phosphorus ylid (5.42g, 18 mmol.) was added to a solution of methyl vinyl ketone (1.53g, 21.8 mmol.) in benzene (75 ml.). The mixture was refluxed under nitrogen for 4 hours. The solution was washed with aqueous sodium bisulphite (100 ml.), sodium carbonate (100 ml.), water (100 ml.) and dried ( $\text{Na}_2\text{SO}_4$ ). The mixture was passed through a column (silica) but only triphenylphosphine oxide was obtained, and the nitrile could not be isolated, if present.

The reaction was repeated and the product vacuum distilled after removal of the solvent. The distillate obtained was shown, from t.l.c., to be a mixture of several components. The n.m.r. spectrum suggested that the desired product was not present.

The reaction was repeated using ethylene glycol dimethyl ether as solvent. The mixture was refluxed for 30 hours, adding additional ketone at various intervals. A thick gum was obtained. T.l.c. indicated several components. Vacuum distillation gave a viscous pale yellow oil. This was a mixture of three main components (t.l.c.). The i.r. spectrum indicated the presence of olefinic and nitrile groups, but the n.m.r. spectrum was not consistent with the desired product.

#### Preparation of diethyl phosphonoacetonitrile<sup>79</sup>

Triethyl phosphite (20g, 120.5 mmol.) and chloroacetonitrile (9.1g, 120.5 mmol.) were refluxed under N<sub>2</sub> for 2 hours. The apparatus was adjusted for distillation and unreacted triethyl phosphite and chloroacetonitrile were removed at atmospheric pressure. The product was distilled under vacuum, b.p. 102°/0.4 mm (lit.<sup>79</sup> 127-131°), 16.38g (77%);  $\tau(\text{CDCl}_3)$  5.84 (4H,q), 7.05 (2H,d), and 8.62 (6H,t);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3000, 2260, 1270, 1163, and 1020 cm<sup>-1</sup>; m/e 177 (M<sup>+</sup>).

#### Attempted Wittig reaction of diethyl phosphonoacetonitrile and methyl vinyl ketone

(1) Diethyl phosphonoacetonitrile (8.76g, 60 mmol.) was added to a solution of n-butyl lithium (30 mmol.) in THF (240 ml.) at -78° under dry nitrogen. Methyl vinyl ketone (2.1g, 30 mmol.) was added dropwise over a period of 1 hour. The addition caused an initial blue colouration which disappeared quickly. After addition the mixture was stirred for 30 mins. and then allowed to warm to

room temperature. Water (1L) was added and the mixture extracted with dichloromethane and dried ( $\text{MgSO}_4$ ). Concentration gave a viscous yellow oil (3.52g). The oil contained two main components (t.l.c.), one of which was unreacted phosphonate. N.m.r. spectrum showed no olefinic resonances, therefore the desired product could not have been present. The product obtained was not isolated.

(2) The phosphonate (0.885g, 5 mmol.) and methyl vinyl ketone (0.35g, 5 mmol.) were mixed in dry THF (5 mls.) and added to a suspension of powdered potassium hydroxide (0.5g, 10 mmol.) in dry THF (15 mls.) at  $-78^\circ$ . The mixture was stirred overnight with the temperature slowly rising to  $-50^\circ$ . T.l.c. indicated that all the phosphonate had been consumed and the mixture was filtered to give a pale yellow-green solution which was then concentrated. The residue was distilled (Kugelrohr  $100^\circ/25$  mm.) giving a small amount of clear liquid. The residual light brown gum would not distil under high vacuum. The i.r. spectrum of the distillate showed no carbonyl or nitrile absorptions and the n.m.r. showed no olefinic resonances but did contain ethoxy resonances. The liquid was thought to be a phosphate moiety.

#### Preparation of 2,5-dihydro-3-methylthiophene-1,1-dioxide<sup>14</sup>

To a 1.2L steel autoclave (precooled before use with methanol and dry ice) was added freshly distilled isoprene (352 ml., 3.52 mol.), liquid sulphur dioxide (160 ml., 3.52 mol.), methanol (176 ml.), and hydroquinone (8g). The vessel was sealed and heated slowly to  $85^\circ$ . After 4 hours the vessel was allowed to cool overnight. The liquid obtained was concentrated to give the crude cyclic sulphone. Recrystallisation from methanol gave 355.5g (76.5%) of colourless crystals, m.p.  $60-63^\circ$  (lit.<sup>14</sup>  $63.5-64^\circ$ );



$\tau(\text{CDCl}_3)$  4.4 (1H,m), 6.4 (4H,m), and 8.15 (3H,m);  
 $\nu_{\text{max}}^{\text{CHCl}_3}$  1655, 1305, and 900  $\text{cm}^{-1}$ .

Preparation of 3-bromomethyl-2,5-dihydrothiophene-1,1-dioxide (16)<sup>16</sup>

2,5-Dihydro-3-methylthiophene-1,1-dioxide (132g, 1 mol.), N-bromosuccinimide (178g, 1 mol.), and benzoyl peroxide (12g), were refluxed overnight in dry dichloromethane (1L). The mixture was allowed to cool and then concentrated to half its original volume. The solution was cooled to 5° and the succinimide precipitate removed by filtration. The filtrate was dissolved in an equal volume of ethanol and the product crystallised out at 5°. Recrystallisation from ethanol gave 91.1g (43%), m.p. 85-88° (lit.<sup>15</sup> 87-88°);  $\tau(\text{CDCl}_3)$  4.05 (1H,m), 6.05 (2H,s), and 6.2 (4H,s);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1725, 1325, 1130, and 900  $\text{cm}^{-1}$ ; m/e 210 and 212.

Preparation of 3-cyanomethyl-2,5-dihydrothiophene-1,1-dioxide (18)

(1) The bromocyclic sulphone (16) (1g, 4.74 mmol.) was dissolved in dichloromethane (3 ml.) and potassium cyanide (0.31g, 4.77 mmol.) was added. The mixture was stirred with a catalytic amount of 18-crown-6 ether. No reaction occurred after 24 hours. The mixture was refluxed for 24 hours but still only starting material was present.

(2) The above reaction was repeated using a quantitative amount of crown ether. Concentration of the mixture after 24 hours refluxing gave a viscous gum which contained only crown ether and starting material.

(3) The bromocyclic sulphone (16) (2.11g, 10 mmol.) was dissolved in HMPA (20 ml.) and the solution stirred with a catalytic amount of 18-crown-6 ether. Dry potassium cyanide

(1.08g, 16.6 mmol.) was added and the mixture stirred at room temperature for 5 days and then at 60° overnight. The mixture was poured into water (100 ml.) and extracted with dichloromethane. The organic layer was washed several times with water and dried (MgSO<sub>4</sub>). Concentration gave a residue containing mainly HMPA, crown ether, and polymer (n.m.r.). No nitrile absorptions were detected by i.r. spectroscopy.

(4) The sulphone (16) (0.9g) was stirred in DMSO (3 ml.) under nitrogen. Sodium cyanide (0.23g, 4.7 mmol.) was added and the mixture stirred at 80° for 24 hours. Water was added and the product extracted into ethyl acetate. Drying (MgSO<sub>4</sub>), and concentration, gave a gummy residue which showed no nitrile absorptions in the i.r. spectrum.

(5) The above reaction was repeated at room temperature for 24 hours. Again only a polymeric product, containing no nitrile groups, was obtained.

(6) The sulphone (16) (2g, 9.5 mmol.) was dissolved in dichloromethane (15 ml.), and tetrabutylammonium bromide (4 mg.) was added. The mixture was stirred under nitrogen, cooled to 0° and a solution of sodium cyanide (0.5g, 10.2 mmol.) in water (1 ml.) was added. The solution was stirred vigorously for two days. Water was added and the mixture extracted with dichloromethane, washed twice with water, dried (MgSO<sub>4</sub>) and concentrated, giving 1.1g (74%) of pale cream crystals. The product was recrystallised by adding just enough acetonitrile to a mixture of the solid in refluxing ethanol, to give a solution, m.p. 135-139°;

$\tau$ (DMSO-d<sup>6</sup>) 3.15 (1H,m), 6.0 (4H,m), and 6.28 (2H,s);

$\nu_{\text{max}}^{\text{DMSO}}$  2255 cm<sup>-1</sup>; m/e 157 (M<sup>+</sup>). The crystals were found to dissolve only in acetonitrile and dimethyl sulphoxide.

Preparation of 2-bromomethyl-1,3-butadiene (19)<sup>15</sup>

3-Bromomethyl-2,5-dihydrothiophene-1,1-dioxide (24.75g, 0.157 mol.) was heated under reduced pressure (100 mm.) in a flask equipped with a still head. The solid melted, evolving sulphur dioxide, and the remaining diene was distilled into an ice-cooled receiver, b.p. 80-100°/100 mm. (lit.<sup>15</sup> 80-90°/100 mm.), 15.64g (91%),  $\tau(\text{CDCl}_3)$  3.54-4.0 (1H,m), 4.58-4.98 (4H,m), and 6.0 (2H,s);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1590  $\text{cm}^{-1}$ ; m/e 146 and 148.

Attempted preparation of 2-cyanomethyl-1,3-butadiene (18)

- (1) The cyanocyclic sulphone (17) (1g), was heated under reduced pressure (100 mm) in a flask equipped with a still head. The solid melted and charred, but no liquid distilled into the ice-cooled receiver.
- (2) The bromodiene (19) (1g, 6.8 mmol.) was dissolved in dry dichloromethane (1.5 ml.) and dry potassium cyanide (0.44g, 6.8 mmol.) was added along with dry 18-crown-6 ether (7 mg.). The apparatus was flushed with dry nitrogen and the mixture refluxed overnight. The mixture was passed through silica gel (10 cm of Kieselgel HF<sub>254</sub>) with dichloromethane and the solution concentrated. The residue (0.6g) contained mainly starting material with a very small amount of cyanodiene (indicated by i.r.).
- (3) Dry sodium cyanide (0.37g, 7.5 mmol.) was stirred in dry DMSO. The system was flushed with nitrogen and heated to 90°. The heat was removed and the bromodiene (19) (1g, 6.8 mmol.) in DMSO (2 ml.) was added. The mixture was allowed to cool and water was added. The aqueous solution was extracted with dichloromethane, and the organic extracts washed with saturated sodium chloride and dried (MgSO<sub>4</sub>). Concentration gave a solid brown

polymeric residue containing no cyanodiene or starting material (n.m.r., i.r., t.l.c.).

(4) The bromodiene (19) (1g, 6.8 mmol.) and sodium cyanide (0.5g, 10 mmol.) were stirred in HMPA (15 ml.) at room temperature for 24 hours. The mixture was extracted with dichloromethane, washed with saturated sodium chloride, and dried ( $\text{MgSO}_4$ ).

Concentration gave a dark brown residue containing only HMPA and polymer (n.m.r., t.l.c.).

(5) The bromodiene (19) (1g, 6.8 mmol.) and tetrabutylammonium bromide (2 mg.), were dissolved in dichloromethane and stirred at  $0^\circ$  under nitrogen. Sodium cyanide (0.34g, 7 mmol.) was dissolved in water (0.5 ml.) and the solution added to the mixture and stirred. Vigorous stirring at room temperature was maintained overnight and the mixture was extracted with dichloromethane, washed with water, dried ( $\text{MgSO}_4$ ), and concentrated. The residue (0.35g) was a mixture of starting material (37%), and cyanodiene (63%), giving a 35% yield of product;  $\tau(\text{CDCl}_3)$  3.5-4.05 (1H,m), 4.6-5.1 (4H,m), and 6.83 (2H,s);  $\nu_{\text{max}}^{\text{CHCl}_3}$  2260, and  $1600 \text{ cm}^{-1}$ .

(6) Lithium aluminium hydride (1.68g, 44.25mmol.) was stirred in dry ether (70 ml.). Solid cyanocyclic sulphone (17) (1.44g, 9.17 mmol.) was added and the mixture refluxed under nitrogen overnight. Excess hydride was destroyed by addition of wet ether and the mixture was poured into a solution of sodium potassium tartrate. Extraction with ether, and concentration, gave a polymeric residue containing some starting material.

(7) The cyanocyclic sulphone (17) (0.9g, 5.73 mmol.) was dissolved in dry THF (30 ml.) and added dropwise under nitrogen to a suspension of lithium aluminium hydride (0.5g, 13 mmol.) in dry ether (30 ml.). The mixture turned a yellow-green colour as

the addition was complete (10 min.). The mixture was stirred for 30 min. and excess hydride was quenched with ethyl acetate. The mixture was washed with saturated sodium sulphate and extracted with THF and then ether. The combined extracts were dried ( $\text{MgSO}_4$ ), and concentrated, to give a brown polymeric residue.

Attempted preparation of 1-nitro-3-methylene-pent-4-ene (21)

A solution of sodium ethoxide was prepared from sodium (0.17g, 7.5 mmol.) in ethanol (5 ml.). Nitromethane was added (0.5 ml., 9.2 mmol.) under nitrogen and a white precipitate formed. The bromodiene (1g, 6.8 mmol.), in nitromethane, was added at room temperature and the mixture stirred overnight. Water was added and the solution extracted with ether, washed with water, dried ( $\text{MgSO}_4$ ), and concentrated. The residue (0.43g) contained a complex mixture of components (t.l.c.). N.m.r. suggested some starting material was still present. I.r. showed some absorption bands which may have been due to a nitro group, but these could have been due to some residual nitromethane. No separation of the mixture was attempted.

Attempts to form the Grignard reagent of 3-bromomethyl-2,5-dihydro-thiophene-1,1-dioxide (16)

(1) The bromocyclic sulphone (16) (5g, 23.7 mmol.) and magnesium turnings (0.57g, 23.7 mmol.) were refluxed in dry THF (50 ml.) under nitrogen. No reaction occurred and a crystal of iodine was added. Still no reaction occurred and a few drops of methyl iodide were added, but no Grignard reagent was formed.

(2) Magnesium turnings (0.3g) were placed in dry ether and a few drops of methyl iodide were added. When the reaction between these had commenced, the ether was decanted and the activated magnesium covered with dry THF (15 ml.). The bromocyclic sulphone

(16) (2.5g) was dissolved in dry THF (15 ml.) and added under nitrogen. The mixture was refluxed but after 1 hour no reaction had occurred. A crystal of iodine was added but no reaction occurred and experiment was abandoned.

(3) Methyl magnesium bromide was prepared using magnesium turnings (0.576g) in ether containing an excess of methyl bromide. A crystal of iodine initiated the reaction and Grignard formation was complete after 30 mins. The bromocyclic sulphone (16) (5g) was dissolved in dry THF (70 ml.) and added to the Grignard reagent at room temperature. The mixture was refluxed for two hours and carbon dioxide was bubbled through the resulting solution for several hours at room temperature. The solution was poured into dilute hydrochloric acid and the mixture extracted with dichloromethane, washed with water, and dried ( $MgSO_4$ ). Concentration gave a viscous gum which solidified to a white solid. I.r. spectrum suggested this was not a carboxylic acid. When left overnight at room temperature in a sealed flask the product turned a dark brown colour and a strong smell of acetic acid was released when the flask was opened. The remaining solid was insoluble in all solvents and could not be identified.

Attempts to form the Grignard reagent of 2-bromomethyl-1,3-butadiene (19)

Procedures (1) and (2) as applied above to the bromocyclic sulphone were also carried out using the bromodiene (19). In each case no Grignard reagent was formed.

Preparation of 3-methylpent-4-en-1-yn-3-ol (25)<sup>25</sup>

Liquid ammonia (1L) was run into a two litre flask equipped with a cold finger condenser. Sodium (1g) and finely powdered ferric nitrate (0.5g) was added and the mixture stirred

until the blue colour had turned to black. Dry nitrogen was bubbled through the liquid and the remainder of the sodium (35g) was added with continual stirring. When the reaction mixture had changed colour from blue to light grey, dry acetylene was added to the nitrogen being passed through the liquid. After 2½ hours the solution turned black due to formation of the acetylide. Methyl vinyl ketone (24g, 0.34 mol.) in ether (250 ml.) was added over a period of 1 hour and the mixture was left to stir for a further 2 hours. Ammonium chloride (100g.) was added to quench the reaction and the reaction vessel was then left open overnight to allow the ammonia to evaporate. The residue was added to water and extracted with ether. The organic extract was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated. The residue was distilled through a vigreux column (30 x 1.5 cm.) (b.p.  $60^\circ/85$  mm. lit.<sup>25</sup> 63.5-64.5/100 mm) to give 9.24g (28%) of a colourless liquid;  $\tau(\text{CDCl}_3)$  3.9-5.1 (3H,m), 6.75 (1H,s(broad)), 7.5 (1H,s), and 8.5 (3H,s);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3580, and 3300  $\text{cm}^{-1}$ .

Attempts to partially hydrogenate 3-methylpent-4-en-1-yn-3-ol to 3-methylpenta-1,4-dien-3-ol (22)

(1) The carbinol (25) (1.92g, 0.02 mol.) was stirred in methanol (75 ml.) containing 10% palladium on charcoal catalyst (0.1g) under hydrogen for 1 hour. The mixture was filtered and water (100 ml.) was added. The aqueous solution was extracted with ether, and the ethereal solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated, giving a viscous, pale yellow liquid (1.26g). T.l.c. of this liquid showed several components to be present. N.m.r. showed the acetylenic resonance intensity was considerably reduced relative to the olefinic resonances, but the spectrum was not favourable and suggested unselective hydrogenation had occurred.

- (2) The reaction was repeated as above using 5% palladium on charcoal as catalyst (0.5g). In one hour 16 units of hydrogen were taken up. The reaction was left a further 24 hours and the product isolated and distilled (b.p.  $60^{\circ}/100$  mm.). The distillate contained mainly starting material with about 20% reduction product (n.m.r.).
- (3) The reduction was repeated as above using Lindlar catalyst (0.1g). After 24 hours no hydrogen had been taken up and only starting material was obtained after work-up.
- (4) The reduction was attempted as above using ethanol as solvent. Again no hydrogen was taken up.
- (5) The reduction was repeated as above using Lindlar catalyst (1g.) in methanol (8 ml.). The reaction was left for three days. Water was added and the aqueous solution was extracted with ether. The ethereal solution was dried and concentrated and the residue distilled ( $70^{\circ}/100$  mm.). The distillate (1.37g) was found, by n.m.r., to contain about 55% starting material and 45% product. A separation of these two components was attempted by chromatography, but was unsuccessful.
- (6) The residues from previous reduction attempts were combined to give 4.25g containing 70% 3-methylpent-4-en-1-yn-3-ol and 30% 3-methylpenta-1,4-dien-3-ol. The mixture was dissolved in dry ethanol (50 ml.) and placed in a steel reaction vessel. Lindlar catalyst (1g) was added and the vessel was sealed and pressurised to 37 atms with hydrogen. The mixture was stirred overnight at room temperature. The pressure was released and the mixture filtered and concentrated. The residue was distilled through a short vigreux column (b.p.  $40-50^{\circ}/90-100$  mm, lit.<sup>96</sup>  $122.1-122.9^{\circ}/760$  mm) to give 0.6g of clear liquid which appeared to be 3-methylpentan-3-ol;  $\tau(\text{CDCl}_3)$  8.2 (1H,s,broad),



8.55 (4H,q), 8.9 (3H,s) and 9.15 (6H, $\tau$ );  $\nu_{\text{max}}^{\text{CHCl}_3}$  3430  $\text{cm}^{-1}$ ;  
 m/e 84, 73, and 55.

Preparation of vinyl magnesium chloride<sup>29</sup>

Vinyl chloride (47.7g, 0.76 mol.) was dissolved in dry THF (200 ml.) in a dropping funnel. Dry magnesium turnings (14.58g, 0.6 mol.) were placed in a flange flask (1L) under nitrogen, and 10 ml. of the vinyl chloride solution were added. Ethyl iodide (1 ml.) was introduced and the mixture heated with an air gun until the reaction initiated. The remaining vinyl chloride solution was added over a period of 15 mins., the temperature during the addition being maintained between 30-35°. The mixture was then heated to 50° for one hour and then allowed to cool.

Preparation of 3-methyl penta-1,4-dien-3-ol<sup>28</sup>

The solution of vinyl magnesium chloride, prepared above, was cooled (ice/salt), and dry ethyl acetate (26 ml., 0.27 mol.) in dry THF (50 ml.) was added at such a rate that the temperature did not exceed 5° (1.25 hr.). The mixture was stirred for a further 1.5 hours and then allowed to reach room temperature. Saturated ammonium chloride (250 ml.) was added and the THF layer was separated. The aqueous layer was extracted twice with THF and the combined extracts were concentrated. Distillation of the residue gave 7.2g of crude product (b.p. 40-60°/100 mm. lit.<sup>26</sup> 68-72°/120 mm.). The material was purified by passing through a column of Kieselgel H, using pentane/ether (80:20) as eluent;  $\tau(\text{CDCl}_3)$  3.9-5.15 (6H,m), 7.29 (1H,s), and 8.65 (3H,s);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3595 and 1640  $\text{cm}^{-1}$ .

Reaction of 3-methyl penta-1,4-dien-3-ol with trichloroacetonitrile

(1) The carbinol (0.49g, 5 mmol.) was stirred in dry THF under nitrogen at room temperature. Potassium hydride (0.03g, 0.75 mmol.) (washed twice with dry pentane) was added dropwise as a slurry in pentane, to the stirring carbinol solution. The mixture was stirred for 5 mins. and then drawn into a syringe and added dropwise to an ice-cooled stirring solution of trichloroacetonitrile (0.72g, 5 mmol.) in dry THF (5 ml.). The mixture was stirred at 0° for 1.5 hours and then concentrated. The residue was shaken with a mixture of pentane (25 ml.) and methanol (0.05 ml.), and filtered. The filtration removed a quantity of black polymeric material. The filtrate was concentrated to a yellow oil (0.35g) which appeared to be mainly starting material (tlc, nmr, and ir).

(2) The above reaction was repeated at -20° but gave the same result.

Attempted preparation of N-carbomethoxy-4-aminobutan-2-one (27)

Sodium hydride (2g of 80% dispersion in oil, 66.6 mmol.) was washed with hexane under nitrogen. The hexane was decanted and replaced by benzene. A solution of methyl carbamate (5g, 66.6 mmol.) in benzene (30 ml.) was added and the mixture evolved hydrogen and gave a thick white precipitate. The mixture was refluxed and methyl vinyl ketone (5.4 ml., 66.6 mmol.) in benzene (10 ml.) was added slowly. The reaction mixture turned brown and a thick yellow solid formed. After refluxing for 1 hour and allowing to cool, water was added to the mixture. The solid would not dissolve in the organic or the aqueous phase. The mixture was filtered, giving an orange solid. This material

would not dissolve in any cold solvents, and only reluctantly in a few hot solvents. The material was thought to be polymeric.

Attempted preparation of N-benzyl-4-aminobutan-2-one (27)

Benzylamine (10.7g, 0.1 mol.), methyl vinyl ketone (7g, 0.1 mol.), and triethylamine (10.1g, 0.1 mol.) were stirred at room temperature in dry benzene (50 ml.). The reaction was exothermic. After stirring for 24 hours the solvent and triethylamine were removed by distillation. The residue was distilled under high vacuum, giving 8.15g of a yellow liquid. N.m.r. of this liquid showed it to be a crude mixture containing benzylamine. The mixture was redistilled, giving benzylamine (2.1g) and a thick yellow oil. This material was not identified.

Preparation of N-benzyl-4-aminobutan-2-one (27)<sup>30</sup>

Benzylamine hydrochloride (17.95g, 0.125 mol.), paraformaldehyde (7.5g, 0.25 mol.), and acetone (100 ml.) were refluxed overnight, to give a clear, pale yellow solution. The solution was concentrated to a viscous gum which was dissolved in acetone and cooled to  $-20^{\circ}$ . After 30 mins. a white solid (1.1g) was filtered off, and the filtrate was again chilled. A further crop (0.7g) was obtained, giving a total yield of 1.8g (6.75%) of the hydrochloride;  $\tau(D_2O)$  2.46 (5H,s), 5.75 (2H,s), 6.65-7.05 (4H,m), and 7.76 (3H,s);  $\nu_{\max}^{KBr}$  2850-2320 and  $1710\text{ cm}^{-1}$ .

The free amine was liberated by dissolving the hydrochloride in water and adding a solution of sodium hydroxide until the solution was slightly alkaline. The amine was extracted into dichloromethane, dried ( $MgSO_4$ ), and concentrated;  $\tau(CDCl_3)$  2.98 (5H,s), 6.49 (2H,s), 7.35-7.75 (4H,m), 8.12 (3H,s), and 8.61 (1H,s(broad));  $\nu_{\max}^{film}$   $1710\text{ cm}^{-1}$ . When this liquid was

distilled, it decomposed, giving a distillate containing mainly benzylamine. Distillation was possible, however, using a short-path Kugelrohr apparatus.

Attempts to prepare N-benzyl-4-aminobutan-2-one ethylene ketal (29)

(1) The amino-ketone (27) (1.37g, 7.74 mmol.) was added to a mixture of ethylene glycol (20 ml.) and benzene (200 ml.). A catalytic amount of toluene-4-sulphonic acid was added and the mixture was refluxed under a Dean & Stark water separating apparatus. After 8 hours the mixture was cooled and concentrated. The residue was extracted with ether, washed with potassium hydroxide solution and dried (KOH pellets). Concentration gave 1.1g of brown oil which showed no distinct spots on t.l.c. (the plate showed a continuous streak).

(2) The hydrochloride of amino-ketone (27) (1.65g, 7.74 mmol.) was dissolved in ethylene glycol (50 ml.) and triethyl orthoformate was added (10 ml.). A catalytic amount of toluene-4-sulphonic acid was added and the mixture heated to 100° in an oil bath. The reaction flask was fitted with a distillation apparatus and left at 100° overnight. A distillate (1 ml.) of ethanol and ethylformate was obtained. The residue was concentrated to remove excess orthoformate, and the glycol solution was poured into aqueous potassium hydroxide and extracted with ether. The ethereal extract was washed with potassium hydroxide solution and dried over KOH pellets. Concentration and short-path distillation of the residue, gave N-benzyl-N-formyl-4-aminobutan-2-one ethylene ketal (28);  $\tau(\text{CDCl}_3)$  1.82 (1H,d), 2.77 (5H,s), 5.58 (2H,d), 6.2 (4H,m), 6.7 (2H,m), 8.1 (2H,m), and 8.75 (3H,s);  
 $\nu_{\text{max}}^{\text{film}}$  1675  $\text{cm}^{-1}$ ;  $m/e$  249 ( $\text{M}^+$ ); Found  $\text{M}^+ = 249.1351$ ,  $\text{C}_{14}\text{H}_{19}\text{NO}_3$  requires  $\text{M}^+ = 249.1365$ .

Preparation of N-benzyl-4-aminobutan-2-one ethylene ketal (29)

The hydrochloride of amino-ketone (27) (9.6g, 45 mmol.) was dissolved in ethylene glycol (20 ml.) and benzene (250 ml.). A few drops of concentrated hydrochloric acid were added and the mixture was refluxed under a Dean & Stark for 24 hours. Benzene was removed and the glycol solution was made slightly alkaline by addition of a sodium hydroxide solution, and the product was extracted into ether and concentrated. The residue was distilled from NaOH pellets in a short-path distillation apparatus to give a clear liquid, 8.93g (90%);  $\tau(\text{CDCl}_3)$  2.80 (5H,s), 6.25 (4H,s), 6.32 (2H,s), 7.33 (2H,t), 8.19 (2H,t), 8.28 (1H,s(broad)), and 8.77 (3H,s);  $\nu_{\text{max}}^{\text{film}}$  3320  $\text{cm}^{-1}$ .

Diazotisation of diethyl glutaconate

Diethyl glutaconate (1.86g, 0.01 mol.), tosyl azide (1.97g, 0.01 mol.), and triethylamine (1.2g, 0.01 mol.) were stirred in ethanol (25 ml.) at room temperature. The solution turned red and the reaction was complete in under 18 hours. The mixture was concentrated and addition of ether caused the side-product, toluene-4-sulphonamide, to precipitate. The mixture was filtered and the filtrate concentrated to give a red oil. The oil was purified by column chromatography (ether/hexane; 1:1), and gave a yellow oil, 1.8g (85%);  $\tau(\text{CDCl}_3)$  2.71 (1H,d), 4.28 (1H,d), 5.75 (4H,dq), and 8.72 (6H,dt);  $\nu_{\text{max}}^{\text{CHCl}_3}$  2101, 1703, and 1615  $\text{cm}^{-1}$ ;  $m/e$  212 ( $\text{M}^+$ ); Found  $\text{M}^+ = 212.0804$ ,  $\text{C}_9\text{H}_{12}\text{N}_2\text{O}_4$  requires 212.0798.

Attempted insertion reaction between N-benzyl-4-aminobutan-2-one ethylene ketal (29) and diethyl diazo-glutaconate (30)

The amine (2.6g, 11.8 mmol.) was stirred in a dry flask under nitrogen at 80°. Rhodium acetate (~ 5 mg) was added

and the solution turned violet. The diazo compound (0.5g, 2.36 mmol.) was dissolved in dry benzene (10 ml.) and added dropwise to the amine over a period of 5 hours. The mixture was left to stir at 80° for a further hour and then cooled and concentrated. An i.r. spectrum of the crude reaction product showed no diazo band. The dark brown solution was heated to 130° at 0.1 mm. pressure in a Kugelrohr apparatus to remove the excess starting amine. The residue, a dark brown gum, was taken up in ether. T.l.c. of this solution suggested the reaction had been very messy as numerous spots and much streaking was shown on the plate. Left overnight the solution deposited some colourless crystals (22 mg.), m.p. 120-1°. The remaining gum was heated to 150°/0.1 mm. in a Kugelrohr, and a pale yellow oil was obtained (155 mg.). The oil was redistilled but still contained a small amount of the starting amine as a major impurity (n.m.r., t.l.c.). This oil was not the desired product and could not be identified;  $\tau(\text{CDCl}_3)$  -1.06 (1H,s(broad)), 2.73 (2H,m), 5.64 (4H,q), 6.16 (1H,m) and 8.66 (6H,t);  $\nu_{\text{max}}^{\text{film}}$  1722 and 1562  $\text{cm}^{-1}$ ; m/e 212, 178, 167, and 140.

The crystalline solid obtained as a minor product was also not identified;  $\tau(\text{CDCl}_3)$  1.9-3.0 (10H,m), 5.91 (2H,s), 6.3 (4H,s), 7.1 (2H,t), 7.73 (3H,s), 7.98 (2H,t), 8.65 (1H,s), and 8.92 (3H,s);  $\nu_{\text{max}}^{\text{CH}_2\text{Cl}_2}$  3300-2200, 1728, 1678, and 1605  $\text{cm}^{-1}$ ; m/e 178, 172, 120, and 106.

Attempted preparation of 1-trimethylsilyl-4-carbomethoxy- $\Delta^2$ -1,2,3-triazoline (36)

Methyl acrylate (2.24g, 26 mmol.) and trimethylsilylazide (3g, 26 mmol.) were stirred at room temperature under dry nitrogen, and the reaction monitored by n.m.r. After 5 days no change had occurred and the mixture was heated to 50° in an oil

bath. No reaction had occurred after 7 days at 50° and the reaction was abandoned.

#### Preparation of diethyl benzal malonate<sup>56</sup>

Diethyl malonate (100g, 0.63 mol.), benzaldehyde (71g, 0.66 mol.), piperidine (3.25 ml.), and benzoic acid (2g.) were refluxed in benzene (200 ml.) through a Dean & Stark apparatus. The reaction was continued until no more water was collected (18 hours); the mixture was cooled, and 100 ml. of benzene was added. The solution was washed with sodium bicarbonate (sat.), water, and dried (Na<sub>2</sub>SO<sub>4</sub>). The solution was concentrated and the residue distilled, b.p. 124°/0.1 mm. (lit.<sup>56</sup> 140-2°/4 mm.), to give a colourless liquid;  $\tau$ (CDCl<sub>3</sub>) 2.42 (s,1H), 2.78 (s,5H), 5.85 (q,4H), and 8.85 (t,6H).

#### Attempted cycloaddition of trimethylsilyl azide and diethyl benzal malonate

Trimethylsilyl azide (0.026 mol.) and diethyl benzal malonate (0.026 mol.) were stirred under nitrogen at 60°. After 15 hours a sample was removed and n.m.r. showed only resonances attributable to the starting materials. After one month n.m.r. showed the olefin to be unchanged, but several new resonances in the trimethylsilyl region of the spectrum suggested that the azide had decomposed. As no reaction had occurred, the reaction was abandoned.

#### Preparation of ethyl azidoformate<sup>57</sup>

Ethyl chloroformate (35g, 0.32 mol.) was added to a solution of sodium azide (50g, 0.77 mol.) in water (250 ml.). The mixture was stirred vigorously for 2½ hours and extracted with ether: all the colour remained in the aqueous layer. The clear ether solution was dried (MgSO<sub>4</sub>), concentrated, and

distilled, b.p.  $24^{\circ}/15$  mm. (lit.<sup>57</sup>  $25^{\circ}/2$  mm.), giving a clear oil (29.4g, 80%);  $\tau(\text{CDCl}_3)$  5.7 (2H,q) and 2.67 (3H,t);  $\nu_{\text{max}}^{\text{film}}$  2170, 2125 and  $1720 \text{ cm}^{-1}$ .

Attempted cycloaddition of ethyl azidoformate and diethyl benzal malonate

Ethyl azidoformate (16.8 mmol.) and diethyl benzal malonate were stirred in dry toluene (10 ml.) containing a crystal of hydroquinone. The mixture was heated, under nitrogen, to  $70^{\circ}$  for one month. A dark brown solution was obtained, from which t.l.c. and n.m.r. indicated that only diethyl benzal malonate and polymeric material was present.

Preparation of phenyl azide<sup>57</sup>

Phenyl hydrazine hydrochloride (47.5g) was added to water (300 ml.) together with concentrated hydrochloric acid (21 ml.). The mixture was cooled to  $0^{\circ}$  and ether (100 ml.) added. A solution of sodium nitrite (25g.) in water (30 ml.) was added at such a rate that the temperature did not rise above  $5^{\circ}$ . The mixture was steam distilled until 250 ml. of distillate had been collected. The ether layer was separated and the aqueous layer extracted with ether. The combined ether solutions were dried ( $\text{CaCl}_2$ ) and concentrated. Distillation gave a pale yellow liquid, (21.6g, 55%), b.p.  $23^{\circ}/0.75$  mm. (lit.<sup>57</sup>  $41-3^{\circ}/5$  mm.);  $\tau(\text{CDCl}_3)$  3-3.9 (multiplet).

Preparation of 1,5-diphenyl-4,4-diethoxycarbonyl- $\Delta^2$ -1,2,3-triazoline (37)<sup>52</sup>

Phenyl azide (9.6g, 80.6 mmol.) and diethyl benzal malonate (20g, 80.6 mmol.) were maintained at  $60^{\circ}$  under nitrogen for one month. The mixture was cooled and petroleum ether (40-60) was added. The white precipitate was filtered off and



recrystallised from ethanol, (8g, 29.4%), m.p. 95-6° (lit.<sup>52</sup> 98°);  $\tau(\text{CDCl}_3)$  2.6-3.2 (5H,m), 2.84 (5H,s), 4.18 (1H,s), 5.75 (2H,m), 6.42 (2H,dq), 8.8 (3H,t), and 9.22 (3H,t); m/e 367 ( $\text{M}^+$ ) and 339 (base peak).

Preparation of 1,5-diphenyl-4,4-dimethoxycarbonyl- $\Delta^2$ -1,2,3-triazoline (38)<sup>52</sup>

Phenyl azide (93.4g, 0.78 mol.) and dimethyl benzal malonate (175g, 0.79 mol.) were stirred under nitrogen at 70°. After one month the mixture had solidified. The solid was slurried in ether/hexane, filtered and washed with ether. The white crystals were dried in vacuo, (100g, 37%), m.p. 158° decomp. (lit.<sup>52</sup> 171° decomp.)  $\tau(\text{CDCl}_3)$  2.79 (5H,s), 2.84 (5H,s), 4.12 (1H,s), 6.11 (3H,s), and 6.83 (3H,s);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1742 and 1598  $\text{cm}^{-1}$ .

Preparation of benzyl azide<sup>96</sup>

Benzyl chloride (20.24g, 0.16 mol.) was added to a stirring solution of sodium azide (20.8g, 0.32 mol.) in water (30 ml.) Aliquat 336 (methyl trioctylammonium chloride) (3.24, 0.008 mol.) was added and the mixture stirred vigorously at 100° for 24 hours. The mixture was cooled, extracted with ether, dried ( $\text{MgSO}_4$ ), and concentrated. The residue was distilled, b.p. 40°/2 mm. (lit.<sup>95</sup> 74°/11 mm.) to give a clear oil (16.04g, 75%);  $\tau(\text{CDCl}_3)$  2.93 (5H,s), and 5.95 (2H,s);  $\nu_{\text{max}}^{\text{CHCl}_3}$  2100 and 905  $\text{cm}^{-1}$ .

Cycloaddition of benzyl azide and methyl acrylate

Benzyl azide (9.7g, 73 mmol.) and methyl acrylate (6.3g, 73 mmol.) were stirred at room temperature and the reaction followed by n.m.r. After four days, all the methyl acrylate had been consumed and the viscous liquid was placed

under vacuum to remove any last traces of olefin. The gum was triturated with methanol and a white crystalline solid was obtained, (5.35g, 48%). Recrystallisation from ethanol gave 5-benzylaminomethyl-3,5-dicarbomethoxy- $\Delta^2$ -pyrazoline (41), m.p. 101.5-102° (lit.<sup>84</sup> 100-101°),  $\tau(\text{CDCl}_3)$  2.75 (5H,s), 3.02 (1H,s,broad), 6.22 (3H,s), 6.225 (2H,s), 6.29 (3H,s), 6.81 (2H,d), 7.08 (2H,d), 7.08 (2H,d), and 8.4 (1H,s,broad);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3360, 1740, and 1580  $\text{cm}^{-1}$ ; m/e 305 ( $\text{M}^+$ ).

#### Cycloaddition of phenyl azide and methyl acrylate

Phenyl azide (3.09g, 0.026 mol.) and methyl acrylate (2.24g, 0.026 mol.) were mixed and placed in an oil bath at 60°. After 5 days, n.m.r. indicated that no methyl acrylate was present, but i.r. still showed a strong azide absorption. An excess of methyl acrylate was added (5 ml.) and the mixture left a further 8 days. The resulting mixture was concentrated to remove excess methyl acrylate and passed down a column (alumina: pet.ether/chloroform, 8:2) to give a white solid, which was recrystallised from ethanol, giving 5-(anilino-methyl)-3,5-dicarbomethoxy- $\Delta^2$ -pyrazoline (44), m.p. 86-88° (lit.<sup>49</sup> 85.5-86.5°);  $\tau(\text{CDCl}_3)$  2.7-3.6 (5H,m), 5.95 (1H,broad), 6.21 (3H,s), 6.3 (3H,s), 6.51 (2H,s) and 6.81 (2H,d);  $\nu_{\text{max}}^{\text{CHCl}_3}$  3350, 1720, 1600 and 1580  $\text{cm}^{-1}$ ; m/e 291 ( $\text{M}^+$ ).

#### Cycloaddition of benzyl azide and methyl acrylate under various conditions

Benzyl azide (1g, 7.5 mmol.) and methyl acrylate (0.65g, 7.5 mmol.) were left in a sealed flask for 14 days;

1. At room temperature: the product was evacuated to remove residual olefin. N.m.r. of residue showed only benzyl azide and 5-benzylaminomethyl-3,5-dicarbomethoxy- $\Delta^2$ -pyrazoline to be present.

2. At room temperature in chloroform (10 ml.): as for 1.
3. At 4°: as above, but reaction had not gone to completion. N.m.r. indicated approximately 60% pyrazoline formed.
4. At 4° in chloroform (10 ml.): as above, but only 50% pyrazoline formed.
5. At -20°: very little reaction occurred. Only slight pyrazoline resonances detected by n.m.r. No triazoline was detected.
6. At -20° in chloroform (10 ml.): as for 5.

In none of these reactions was the presence of triazoline (39) indicated by n.m.r.

#### Preparation of ethyl 2,3-dibromopropionate

Ethyl acrylate (100g, 1 mol.) was stirred in carbon tetrachloride (200 ml.) and the mixture heated to 70°. Bromine was added at such a rate as to maintain the temperature between 70° and 80°. When addition was complete the solvent was removed and the residue distilled, (247.9g, 95%), b.p. 110°/25 mm. (lit.<sup>97</sup> 112°/23 mm.);  $\tau(\text{CDCl}_3)$  5.5-6.5 (5H,m) and 8.7 (3H,t);  $\nu_{\text{max}}^{\text{film}}$  1740  $\text{cm}^{-1}$ .

#### Preparation of N-benzyl-2-carbethoxyaziridine (46)<sup>59</sup>

Ethyl 2,3-dibromopropionate (39g, 0.15 mol.) was cooled to 5° in dry benzene (100 ml.). A solution of benzylamine (16.1g, 0.15 mol.) and triethylamine (30.1g, 0.3 mol.) in dry benzene, was added. A thick precipitate formed during the addition. The mixture was refluxed for 3 hours, cooled, and filtered. The filtrate was washed with water and dried ( $\text{MgSO}_4$ ). The solution was concentrated and the residue distilled, b.p. 96°/0.05 mm.

giving 23.13g (75%) of aziridine,  $\tau(\text{CDCl}_3)$  2.88 (5H,s), 5.95 (2H,q), 6.6 (2H,s), 7.82 (2H,m), 8.39 (1H,dd), and 8.8 (3H,t);  $\nu_{\text{max}}^{\text{film}}$  1715  $\text{cm}^{-1}$ ;  $m/e$  205 ( $M^+$ ).

Attempted cycloaddition of N-benzyl-2-carbethoxyaziridine with cyclopentenone

The aziridine (2g, 9.75 mmol.) and cyclopentenone (1g, 12.2 mmol.) were refluxed in dry *o*-xylene (5 ml.) under nitrogen for 14 hours. The xylene and unreacted cyclopentenone were removed by short-path distillation to leave a polymeric residue. T.l.c. of this material was highly streaked and no individual products could be isolated from the mixture.

Attempted cycloaddition of N-benzyl-2-carbethoxyaziridine with diethylmaleate

1. Thermal.

The aziridine (0.5g) was stirred in diethyl maleate (3 ml.) at 100° under nitrogen for 24 hours. The excess maleate was removed by short-path distillation to leave a dark brown viscous gum. T.l.c. of this material was streaked, showing no distinct spots. No cycloaddition product could be detected from a mass spec. analysis of the mixture.

2. Photolytic

The aziridine (3g, 14.6 mmol.) and diethyl maleate (2.52g, 14.6 mmol.) were dissolved in dry benzene (300 ml.) and irradiated, under nitrogen at 0°, with a medium pressure mercury lamp. After 24 hours, t.l.c. indicated only starting materials. The solution was removed from the photolysis reactor vessel and the vessel was found to be coated with a thin clear layer of polymer. The solution was concentrated and addition of ether caused a small amount of polymer to precipitate. The remaining

solution contained only aziridine and diethyl maleate (t.l.c. and n.m.r.). No cycloaddition product was detected by mass spec. analysis.

Preparation of diethyl ethoxymethylene malonate<sup>68</sup>

Triethyl orthoformate (200g, 1.35 mol.), acetic acid (252g, 2.46 mol.), diethyl malonate (192g, 1.2 mol.), and anhydrous zinc chloride (0.1g), were placed in a 1L flask equipped with a thermometer, a gas inlet tube, and a 15 inch Vigreux column. The mixture was agitated for 5 minutes with a stream of dry air, and then heated to reflux. As the reaction proceeded, the volatile products were collected from the top of the column by distillation. The mixture was refluxed until the temperature reached 155° and the reaction mix was allowed to cool. A suspension of zinc salts was removed by filtration and the residue was distilled under reduced pressure (15-20 mm. Hg) until the still-head temperature reached 100°. Distillation was continued under high vacuum and the product obtained, b.p. 120°/1 mm. (lit.<sup>68</sup> 108-110°/0.25 mm.), (105g, 40%).

Attempts to prepare diethyl methylene malonate

A.<sup>64</sup> Diethyl ethoxymethylene malonate (101g, 0.435 mol.) was dissolved in ethanol (94 ml.) and placed in a high pressure hydrogenation vessel along with Raney nickel (15g. of wet catalyst). The pressure was raised to 100 Atms. with hydrogen and the mixture stirred at 45° for 3 days. The vessel was allowed to cool and the liquid concentrated to leave a product which should have been diethylethoxymethylmalonate (85.6g). This oil appeared to be the correct product from n.m.r. but when pyrolysed in the recommended

apparatus,<sup>66</sup> only a very small amount of ethanol was obtained and eventually a liquid distilled which was thought to be diethyl methyl malonate. The hydrogenation was repeated several times but gave the same results each time. The reaction was also tried at lower temperature, with lower pressure, and with higher pressure but in each case the outcome was the same. The pyrolysis was tried using toluene-4-sulphonic acid, and also using sodium ethoxide, to catalyse the elimination of ethanol, but no additional ethanol was obtained.

- B. Diethyl ethoxymethylene malonate (5g, 23.51 mmol.) was stirred overnight at room temperature with dilute hydrochloric acid (2M). Extraction with ether and drying ( $\text{MgSO}_4$ ), gave a clear oil (2.6g, 60%) which was thought to be diethyl formyl malonate,  $\tau(\text{CDCl}_3)$  1.73 (1H,s,broad), 5.75 (5H,m), and 8.7 (6H,m);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1730, 1648, and 1598  $\text{cm}^{-1}$ . This liquid was stirred in methanol (40 ml.) with sodium borohydride (0.6g, 15.95 mmol.) at room temperature for 20 hours. The mixture was poured into water and extracted with ether and dried ( $\text{MgSO}_4$ ). Concentration gave a clear oil which was found to be diethyl malonate.
- C. Diethyl malonate (57g, 0.356 mol.) was stirred in dry THF and a solution of sodium ethoxide (0.356 mol.) in ethanol (150 ml.) was added. The resulting mixture was slowly added to a refluxing mixture of paraformaldehyde (10.69g) in dry THF. The solution turned orange as the addition proceeded. The mixture was refluxed for 1½ hours, cooled and concentrated. The residue was poured into water and extracted with dichloromethane. Concentration gave a yellow

oil (22g) which was distilled (Kugelrohr:250°/760 mm Hg.) to give a clear liquid which was thought to be 1,1,3,3-tetracarbethoxypropane;  $m/e$  332 ( $M^+$ ).

D.<sup>65</sup> Diethyl malonate (80g, 0.5 mol.), paraformaldehyde (15g, 0.5 mol.) and potassium fluoride (29g, 0.5 mol.) were stirred in ether (150 ml.) at room temperature for 5 hours. The solution was filtered and concentrated and the excess malonate was removed by distillation to leave a polymeric residue. The polymer was cracked to give 34.8g of distillate. This product was found to be about 15% diethylmethylene malonate and therefore meant a non-isolated yield of 6% product.

E.<sup>66</sup> Diethyl malonate (80g, 0.5 mol.), paraformaldehyde (30g, 1 mol.), copper acetate (5g.) and potassium acetate (5g), were mixed into acetic acid (200g.) and heated to 100° for 2 hours. The mixture was distilled under reduced pressure, from the reaction vessel, until the temperature reached 120°/35 mm. Distillation was continued and the fraction collected from 120°/35 mm. to 140°/35 mm. at which point the contents of the distillation pot charred. The high boiling fraction was redistilled, giving diethylmethylene malonate (24.9g, 29%), b.p. 85-93/10 mm. (lit.<sup>65</sup> 90-93°/7 mm.)  
 $\tau(\text{CDCl}_3)$  3.65 (2H,s), 5.8 (4H,q), and 8.73 (6H,t);  
 $\nu_{\text{max}}^{\text{film}}$  1730 and 1625  $\text{cm}^{-1}$ .

#### Preparation of dimethyl methylene malonate

Dimethyl malonate (66g, 0.5 mol.), paraformaldehyde (30g, 1 mol.), cupric acetate (5g), and potassium acetate (5g), were mixed into acetic acid (200g) and heated to 100° for 2 hours. The mixture was distilled under reduced pressure until the

temperature reached 125°/30 mm. This fraction was discarded and distillation continued until no more liquid was obtained. The high boiling fraction was redistilled, b.p. 97°/8 mm. (lit.<sup>98</sup> 195-205°), (10.5g, 12%), giving crude dimethyl methylene malonate,  $\tau(\text{CDCl}_3)$  3.48 (2H,s), and 6.15 (6H,s). This liquid polymerised to a clear solid when left overnight at -20°.

Preparation of 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57)

Benzyl azide (44g, 0.331 mol.) and diethyl methylene malonate (57g, 0.331 mol.) were stirred at room temperature in dry benzene (250 ml.). The reaction had gone to completion after 5 hours (t.l.c.) but was left a further 3 days at room temperature. Addition of hexane caused a white solid to precipitate. The crystals were filtered and dried, giving 86g (85%) of triazoline (57), m.p. 59.5-60°;  $\tau(\text{CDCl}_3)$  2.72 (5H,s), 5.15 (2H,s), 5.75 (4H,q), 6.45 (2H,s) and 8.72 (6H,t);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1735  $\text{cm}^{-1}$ ; m/e 277 ( $\text{M}^+-\text{N}_2$ ) and 204 ( $\text{M}^+-\text{N}_2-\text{CO}_2\text{Et}$ );  $\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_4$  requires: 59.02%C, 6.23%H and 13.77%N; found: 59.11%C, 6.34%H, and 13.82%N.

Preparation of 1-benzyl-4,4-dicarbomethoxy- $\Delta^2$ -1,2,3-triazoline (56)

Benzyl azide (3.86g, 29 mmol.) and dimethyl methylene malonate (5g, 29 mmol.) were stirred at room temperature in dry benzene (4 ml.). The mixture was stirred overnight and then concentrated. The clear oil crystallised after standing for several days at -20°. The solid was recrystallised from isopropanol, m.p. 45-47°,  $\tau(\text{CDCl}_3)$  2.9 (5H,s), 5.3 (2H,s), 6.3 (6H,s) and 6.5 (2H,s);  $\nu_{\text{max}}^{\text{CH}_2\text{Cl}_2}$  1730  $\text{cm}^{-1}$ ; m/e 249 ( $\text{M}^+-\text{N}_2$ ) and 190 ( $\text{M}^+-\text{N}_2-\text{CO}_2\text{CH}_3$ ) (found: C, 56.17; H, 5.51; N, 15.15).  $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_4$  requires C, 51.15; H, 4.92; N, 13.77%.



Reaction between 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57) and cyclopentenone, in refluxing toluene

The triazoline (57) (18.68g, 60.98 mmol.) was dissolved in dry toluene (150 ml.) containing hydroquinone (a few crystals). The apparatus was flushed with dry nitrogen and cyclopentenone (5g, 60.97 mmol.) was added. The mixture was refluxed overnight and allowed to cool. The pale yellow solution was concentrated to a light brown oil (22.29g). T.l.c. showed three components to be present. The material was passed through a pressure column (500g. of silica) but no separation was obtained. The oil (15.5g.) was separated by p.l.c. (30 40 x 20 cm. plates, each containing 60g. of silica, eluted with ether/hexane; 4:1). Two bands were obtained from the plates: the lower spot on t.l.c. was obtained clearly as the lower band on p.l.c., but the middle and top spots on t.l.c. were very close together, and were collected as one band on p.l.c. The material from the lower band was washed off the silica with dichloromethane and concentrated to give an oil (4.44g, 28.8%). Trituration of this oil in methyl cyclohexane gave a waxy solid, and four recrystallisations from hexane gave white crystals of diethyl benzylaminomethylene malonate (59), m.p. 69-70° (lit.<sup>99</sup> 74°),  $\tau(\text{CDCl}_3)$  2.0 (1H,d,J=14 Hz), 2.78 (5H,s), 5.56 (2H,d,J=6 Hz), 5.81 (2H,q), 5.85 (2H,q), 8.7 (3H,t) and 8.74 (3H,t);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1685, 1655, and 1609  $\text{cm}^{-1}$ ; m/e 277 ( $\text{M}^+$ ) (found: C,65.18; H,6.88; N,5.23.  $\text{C}_{15}\text{H}_{19}\text{NO}_4$  requires C,64.98; H, 6.86; N,5.05%). The upper band from p.l.c. was washed with dichloromethane and concentrated to give 11.0g of pale brown gum. The gum was dissolved in ether and placed in the freezer compartment of a refrigerator for one hour. A white precipitate was obtained and this was removed by filtration. The solid (0.56g, 3.6%) was

recrystallised three times from methylcyclohexane to give white crystals, m.p. 136-137°. This material was thought to be either piperazine (60) or (62);  $\tau(\text{CDCl}_3)$  2.83 (10H,s), 6.0 (8H,m), 6.14 (4H,s), 6.74 (4H,s) and 8.93 (12H,t);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1730  $\text{cm}^{-1}$ ; m/e 554 ( $\text{M}^+$ ) (found: C,65.11; H,6.93; N,5.20,  $\text{C}_{30}\text{H}_{38}\text{N}_2\text{O}_8$  requires C,64.98; H,6.86; N,5.05%).

The remaining ether solution was concentrated to a gum (10.44g, 67.6%). This material showed only one spot on t.l.c. Mass spec. analysis showed that the molecular ion for the desired bicyclic product (55) was present, m/e 359, but there was also a mass fragment at 376 which could not be accounted for. The oil was distilled (Kugelrohr, 230°/2 mm.) giving a pale yellow gum. This gum gave only one spot on t.l.c. but was still thought to be a mixture as both 359 and 376 mass fragments were still present in the mass spectrum, and the n.m.r. was too complex to interpret. An accurate mass calculation on 359 gave  $\text{C}_{20}\text{H}_{25}\text{NO}_5$ . An accurate mass on 376 gave  $\text{C}_{20}\text{H}_{26}\text{NO}_6$ . A metastable ion scan from the base peak (286) showed 359 to be the parent mass of the 286 fragment. Mass 376 was not detected in the metastable scan.

The reaction between triazoline (57) and cyclopentenone was also carried out in refluxing *o*-xylene, methyl cyclohexane and an excess of cyclopentenone at 120°. In each case the reaction gave a mixture of enamine (59), piperazine (60 or 62) and the unknown gum which seemed to contain bicyclic compound (55). The ratio of products was not determined.

#### Thermolysis of 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57)

The triazoline (3g, 9.84 mmol.) was heated in dry *o*-xylene (9 ml.) under nitrogen until effervescence began. When

no more gas was evolved (5 min.) the heating was removed and the mixture concentrated (Kugelrohr, 50°/2 mm.) to give a viscous oil (2.72g, 99.8%). A portion of this oil (1.05g) was separated by p.l.c. (eluted with ether/hexane, 4:1) into two bands. The lower band gave 0.14g (13%) of a waxy solid. This material had spectral characteristics identical to those of enamine (59). The upper band gave a white precipitate (0.14g, 13%) on addition of ether. This solid was identical to the piperazine obtained in the above triazoline reaction. The remaining gum (0.57g, 54%) was distilled (Kugelrohr, 150°/0.5 mm) to give a yellow oil. This oil gave a mass spectrum which contained 376 as the highest indicated mass. No mass fragments of 359 or 286 were detected. From the spectral data, this oil was thought to be crude 1-benzyl-2,2,4,4-tetracarbethoxypyrrolidine (65);  $\tau(\text{CDCl}_3)$  2.82 (5H,s), 5.76 (4H,q), 5.98 (4H,q), 6.01 (2H,s), 6.21 (2H,s), 6.79 (2H,s), 8.71 (6H,t) and 8.92 (6H,t);  $\nu_{\text{max}}^{\text{CDCl}_3}$  1730  $\text{cm}^{-1}$ ; m/e 376, metastable scan from 376 detected 449 as weak intensity peak.

Preparation of 1-phenyl-2,2,4,4-tetracarbethoxypyrrolidine (68)

Phenyl azide (2g, 16.8 mmol.) and diethyl methylene malonate (2.9g, 16.8 mmol.) were stirred in dry toluene (10 ml.), containing hydroquinone (a few crystals), under nitrogen at 70°. After three days the solution was cooled and concentrated. The residual oil (4.78g) showed two main spots on t.l.c. From n.m.r. this crude product appeared to contain mainly 1-phenyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (67).  $\tau(\text{CDCl}_3)$  2.7-3.45 (5H,m), 5.94 (4H,q), 7.20 (2H,s), and 8.92 (6H,t). The crude triazoline (1g, 3.44 mmol.) and diethyl methylene malonate (0.86g, 5 mmol.), were refluxed in dry toluene (10 ml.)

under nitrogen for two days. The mixture was concentrated and trituration of the oil in pentane gave a crude solid. This material was recrystallised from ethanol to give white crystals, m.p. 78-81<sup>o</sup>, (0.3g 20%);  $\tau(\text{CDCl}_3)$  2.65-3.3 (5H,m), 5.8 (4H,q), 5.85 (4H,q), 5.94 (2H,s), 6.64 (2H,s), 8.77 (6H,t) and 8.88 (6H,t);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1730  $\text{cm}^{-1}$ ;  $m/e$  435 ( $M^+$ ) (found  $M^+ = 435.1886$ ,  $\text{C}_{22}\text{H}_{29}\text{NO}_8$  requires  $M^+ = 435,1898$ .)

Attempted reaction of bicyclic compound (55) with methyl magnesium bromide

The gum, obtained from the reaction of triazoline (57) with cyclopentenone, which was thought to contain compound (55), was dissolved in dry ether (2.0g in 30 ml.). The solution was cooled to -78<sup>o</sup> and a solution of methyl magnesium bromide (15 ml. of 0.196M, 5.88 mmol.) was added by syringe. The mixture turned milky yellow but t.l.c. indicated no change had occurred. The mixture was allowed to reach room temperature overnight. Dilute hydrochloric acid was added and the mixture extracted with ether. The ethereal solution was dried ( $\text{MgSO}_4$ ), and concentrated to a viscous gum (1.1g). This gum gave the same t.l.c. as the starting material and the mass spectrum contained the molecular ion for compound (55) ( $m/e$  359) but no mass fragments corresponding to the desired product, compound (69), were observed.

The Grignard reaction was also attempted in tetrahydrofuran, but gave the same results as above.

Formation of dinitrophenyl hydrazone derivative of bicyclic compound (55)

The gum, obtained from the reaction of triazoline (57) and cyclopentenone, (1.85g) was shaken in a solution of methanol

containing 2,4-dinitrophenylhydrazine (2.0g) and conc. sulphuric acid (4 ml.). The solution was left to stand overnight. The methanol was removed under reduced pressure and replaced with dichloromethane. The solution was washed with water and dried ( $\text{MgSO}_4$ ). The solution was concentrated and the solid residue recrystallised from methanol to give orange crystals, m.p.  $170-1^\circ$ ;  $\tau(\text{CDCl}_3)$  0.92 (1H,d), 1.72 (1H,dd), 2.16 (1H,d), 2.68 (5H,m), 5.66 (2H,q), 5.7 (2H,s), 5.8 (2H,q), 6.09 (1H,d), 6.74 (1H,d), 6.94 (1H,m), 7.28 (2H,d), 7.45 (2H,m), 8.08 (2H,m), 8.64 (3H,t) and 8.73 (3H,t);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1750, 1638, and 1616  $\text{cm}^{-1}$ ; m/e 539 ( $\text{M}^+$ ) (found  $\text{M}^+ = 539.2023$ ,  $\text{C}_{26}\text{H}_{29}\text{N}_5\text{O}_8$  requires  $\text{M}^+ = 539.2017$ ).

Yield = 0.45g, therefore the crude gum contained approximately 16% of compound (55), and the original reaction mixture contained about 10% of the desired bicyclic compound (55). The reaction between triazoline (57) and cyclopentenone was repeated and the crude reaction product was shaken with acidic, methanolic hydrazine solution as above. In this case numerous solids of varying colours from red to deep brown were obtained and the desired derivative (70) could not be isolated.

Reaction between 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57) and diethyl fumarate

The triazoline (0.5g) was stirred in diethyl fumarate (2 ml.) under nitrogen at  $80^\circ$  for 24 hours. T.l.c. indicated that triazoline was still present and the temperature was raised to  $115^\circ$ . After a further 24 hours the excess fumarate was removed (Kugelrohr  $80\%$ , 5 mm.), leaving a residue which showed two main spots on t.l.c. Mass spec. analysis showed that the mixture contained the desired cycloaddition product plus triazoline thermolysis products.

Reaction between 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57) and diethyl maleate

1. The triazoline (0.5g) was stirred in diethyl maleate (2 ml.) under nitrogen for 24 hours at 115<sup>o</sup>. The excess maleate was removed (Kugelrohr 80<sup>o</sup>/0.5 mm.) giving a residue showing two main spots on t.l.c. Mass spec. analysis confirmed the presence of a cycloaddition product as well as the products from triazoline thermolysis.
2. The triazoline (0.5g, 1.64 mmol.) and diethyl maleate (0.28g, 1.64 mmol.) were refluxed in toluene (3 ml.) under nitrogen. After 24 hours the toluene and any residual maleate was removed to give a residue which showed two main spots on t.l.c. The fractions were separated by p.l.c. (ether/hexane, 4:1) giving a clear oil for the top fraction (0.5g) and a crystalline solid (0.12g) for the bottom fraction. The solid was found to be enamine (59) from triazoline thermolysis and the top fraction was found, from mass spec. analysis, to contain the cycloaddition product as well as the other products from triazoline thermolysis.
2. The triazoline (0.5g) and diethyl maleate (0.28g) were refluxed in dry *o*-xylene (3 ml.) containing a catalytic amount of cupric fluoroborate. On heating the mixture, the colour changed from pale green to red. After 13 hours the mixture was concentrated and the fractions separated by p.l.c. (ether/hexane, 4:1). The top fraction was found to contain the cycloaddition product and triazoline thermolysis products, but there were also many other impurities present. The bottom fraction was enamine (59).

Reaction between 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57) and maleic anhydride

The triazoline (0.5g) and maleic anhydride (0.16g, 1.64 mmol.) were refluxed in *o*-xylene under dry nitrogen.

During initial heating the reaction mixture changed colour from a clear solution to black. After 13 hours t.l.c. showed the two main spots obtained from triazoline thermolysis and also a strongly fluorescing spot. The three components were isolated by p.l.c. (ether/hexane, 4:1) but mass spec. analysis of each fraction did not detect any cycloaddition product. Apart from some triazoline thermolysis products, the reaction produced mainly polymer.

Photolysis of 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57)

The triazoline (1.29g, 4.23 mmol.) was dissolved in dry ether (500 ml.) containing a few crystals of hydroquinone. The solution was irradiated at room temperature under nitrogen for 21 hours. T.l.c. showed that all the triazoline had been removed and three new spots were present. When the solution was concentrated, the t.l.c. showed marked streaking and numerous other spots were present. A separation was not attempted.

Sensitised photolysis of 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57) in the presence of cyclopentenone

The triazoline (4g, 13.11 mmol.) was dissolved in dry toluene (400 ml.) and some benzophenone (spatula tip-full) was added. The vessel was evacuated and filled with dry nitrogen. Cyclopentenone (1g, 12.2 mmol.) was added and the mixture was irradiated at room temperature using a medium pressure mercury lamp. After 24 hours the solution was concentrated to an oil (4.6g). Separation of 4g of this oil (p.l.c.; ether/hexane, 4:1) gave 1.2g (30%) of enamine (59), and 1.95g of an oil. This oil was a mixture showing 4 spots on t.l.c., three of which were minor. The oil was distilled (Kugelrohr; 155<sup>o</sup>/0.5 mm.), giving

0.95g (21%) of a clear liquid. This liquid was thought to be crude aziridine (54),  $\tau(\text{CDCl}_3)$  2.8 (5H,m), 5.88 (4H,q), 6.23 (2H,s), 7.64 (2H,s) and 8.82 (6H,t);  $\nu_{\text{max}}^{\text{film}}$  1720  $\text{cm}^{-1}$ ; m/e 277 ( $\text{M}^+$ ). Addition of ether to the residue from the distillation, caused the precipitation of a small amount of piperazine (60 or 62) (t.l.c. and m.p.). The mass spectrum of the crude reaction product had shown the presence of some cycloaddition product (m/e 359), but apart from the materials isolated, the remaining residues appeared to be mainly polymeric, and no cycloaddition product was isolated.

Attempted preparation of 1,2,2-tricarbethoxyaziridine (76)

Ethyl azidoformate (16.8 mmol.) and diethyl methylene malonate (16.8 mmol.) were stirred at 70° in dry toluene (10 ml.) containing a few crystals of hydroquinone. After 24 hours the reaction mixture was cooled and concentrated. Addition of ether caused a white solid (100 mg.) to precipitate. This was found to be polymerised diethyl methylene malonate. The remaining ether solution was concentrated to give a yellow oil (3.67g). The oil was a mixture of two components (t.l.c.). These were separated by p.l.c. (ether/hexane, 3:2) and found to be triazoline (74) plus a small amount of enamine (75). The triazoline was obtained as a yellow oil,  $\tau(\text{CDCl}_3)$  5.65 (6H,m), 5.91 (2H,s), and 8.65 (9H,m);  $\nu_{\text{max}}^{\text{film}}$  1725  $\text{cm}^{-1}$ .

The crude triazoline (1g, 3.48 mmol.) was refluxed in toluene for two days, cooled and concentrated. The resulting oil was distilled (Kugelrohr; 200°/1 mm.) to give a clear oil. This oil contained mainly enamine (75),  $\tau(\text{CDCl}_3)$  1.64 (1H,d), 5.71 (4H,dq), 5.8 (2H,q), 8.68 (6H,dt), and 8.72 (3H,t);  $\nu_{\text{max}}^{\text{film}}$  3290, 1720, 1665 and 1605  $\text{cm}^{-1}$ ; m/e 259 ( $\text{M}^+$ ) (found



$M^+ = 259.1049$ ,  $C_{11}H_{17}NO_6$  requires  $M^+ = 259.1056$ .) A small amount of polymer was also present.

When triazoline (74) was refluxed in toluene with diethyl methylene malonate only enamine (75) was obtained. No pyrrolidine was detected.

When triazoline (74) was refluxed in methyl crotonate overnight only enamine (75) was produced. No pyrrolidine was detected.

Thermolysis of 1,5-diphenyl-4,4-dimethoxycarbonyl- $\Delta^2$ -1,2,3-triazoline (38)

The triazoline (1g, 2.95 mmol.) was heated to  $170^\circ$  under vacuum in a Kugelrohr short-path distillation apparatus. The triazoline melted and decomposed, evolving nitrogen. The temperature was increased to  $250^\circ$  and a deep purple liquid distilled and cooled to a purple gum. The gum was dissolved in dichloromethane giving a light red solution. Concentration gave 0.9g (98%) of 1,3-diphenyl-2,2-dicarboxymethyl aziridine (78),  $\tau(\text{CDCl}_3)$  2.5-3.2 (10H,m), 5.74 (1H,s), 6.49 (3H,s) and 6.54 (3H,s);  $\nu_{\text{max}}^{\text{CH}_2\text{Cl}_2}$  3035, 2959, 1740, 1704 and  $1601 \text{ cm}^{-1}$ ; (lit.<sup>52</sup>  $\tau_{\text{Hcyclic}}$  5.73,  $\tau_{\text{CH}_3}$  6.44 and 6.50;  $\nu_{\text{C=O}}^{\text{film}}$   $1748 \text{ cm}^{-1}$ ).

Reaction of 1,5-diphenyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (37) with diethyl benzal malonate

The triazoline (37) (0.5g, 1.36 mmol.) was added to diethyl benzal malonate (0.338g, 1.36 mmol.) in dry toluene (3.4 ml.). The mixture was refluxed for 7 days and the solvent removed by evaporation. The residue was taken up in ethanol from which the product crystallised as 1,3,5-triphenyl-2,2,4,4-tetracarbethoxypyrrolidine (80), m.p.  $163-165^\circ$  (lit.<sup>46</sup>  $165^\circ$ ), yield 0.30g (37.5%).

Reaction of 1,5-diphenyl-4,4-dicarbomethoxy- $\Delta^2$ -1,2,3-triazoline (38) with diethyl methylene malonate

The triazoline (38) (0.5g, 1.47 mmol.) and diethyl methylene malonate (0.275g, 1.6 mmol.) were dissolved in dry toluene (2 ml.) and the mixture refluxed overnight. The mixture was concentrated and the product isolated by column chromatography (hexane, ethyl acetate 80:20, Kieselgel HF silica) to give 1,5-diphenyl-2,2-dicarbomethoxy-4,4-dicarbomethoxypyrrolidine (81), m.p. 102-104°.

$\tau(\text{CDCl}_3)$  2.3-3.4 (10H,m), 4.1 (1H,s), 5.75 (2H,dq), 6.05 (3H,s), 6.42 (2H,q), 6.49 (3H,s), 7.86 (2H,s), 8.22 (3H,t) and 9.14 (3H,t);  $\nu_{\text{max}}^{\text{CHCl}_3}$  1739  $\text{cm}^{-1}$ ; m/e 483 ( $\text{M}^+$ ) (found  $\text{M}^+ = 483.1845$ ,  $\text{C}_{26}\text{H}_{29}\text{NO}_8$  requires  $\text{M}^+ = 483.1894$ .)

Reaction of 1,5-diphenyl-4,4-dicarbomethoxy- $\Delta^2$ -1,2,3-triazoline (38) with cyclopentenone

1. The triazoline (38) (10g, 29.5 mmol.) and cyclopentenone (2.5 ml, 29.9 mmol.) were dissolved in dry toluene (50 ml.) containing a crystal of hydroquinone and the mixture was refluxed overnight under a blanket of nitrogen. The mixture was concentrated and the cycloaddition product isolated by column chromatography (ether/hexane 80:20, Kieselgel HF). The column separated the mixture into three main fractions. The first and fastest running fraction gave 3.27g of dark brown oil. This fraction did not contain any cycloaddition product (mass spec.) and was not identified. The final fraction gave 0.8g of a polymeric sludge and contained no cycloaddition product. The middle fraction gave 5.57g of a dark brown gum. Addition of ether to the gum caused a solid to precipitate, 1.54g (13.3%) and recrystallisation from methanol gave m.p. 167-9°. This was the cis-isomer of the cycloaddition product, compound (82),  $\tau(\text{CDCl}_3)$  2.5-3.5 (10H,m),

5.21 (1H,d; J=8Hz), 6.10 (3H,s), 6.56 (3H,s), 6.95 (1H,dd,J=8 and 12Hz), and 7.3-8.03 (5H,m);  $^1\text{H}$ -coupled  $^{13}\text{C}$   $\delta$  23.54 (t), 36.79 (t), 49.96 (d), 52.30 (q), 53.24 (q), 58.61 (d), 71.16 (d), 79.56 (s), 117.55 (d), 119.59 (d), 126.05 (d), 127.47 (d), 128.17 (d), 129.01 (d), 142.55 (s), 144.55 (s), 168.20 (s), 170.61 (s), and 214.48 (s);  $\nu_{\text{max}}^{\text{CH}_2\text{Cl}_2}$  1750, and 1605  $\text{cm}^{-1}$ ; m/e 393 ( $\text{M}^+$ ), 334 (base peak) (found  $\text{M}^+$  = 393.1544,  $\text{C}_{23}\text{H}_{23}\text{NO}_5$  requires  $\text{M}^+$  = 393.1576.)

After one month the remaining gum crystallised and was twice recrystallised from methanol to give a white solid, m.p. 132-3 $^\circ$ , 193 mg (1.7%). This was the trans-isomer of the cycloaddition product, compound (82),  $\tau(\text{CDCl}_3)$  2.4-3.6 (10H,m), 4.76 (1H,d,J 4Hz), 6.12 (3H,s), 6.49 (3H,s), 7.20 (1H,dd, J 4 and 10Hz), and 7.5-7.95 (5H,m);  $^1\text{H}$ -coupled  $^{13}\text{C}$   $\delta$  21.48 (t), 36.11 (t), 49.49 (d), 52.04 (q), 52.95 (q), 60.16 (d), 66.76 (d), 78.33 (s), 115.74 (d), 118.71 (d), 125.95 (d), 127.22 (d), 128.40 (d), 128.98 (d), 143.06 (s), 144.55 (s), 168.93 (s), 170.41 (s), and 214.34 (s);  $\nu_{\text{max}}^{\text{CH}_2\text{Cl}_2}$  1750, and 1605  $\text{cm}^{-1}$ ; m/e 393 ( $\text{M}^+$ ), 334 (base peak) (found  $\text{M}^+$  = 393.1560,  $\text{C}_{23}\text{H}_{23}\text{NO}_5$  requires  $\text{M}^+$  = 393.1576.)

2. The triazoline (1.5g, 4.42 mmol.) was dissolved in benzene (30 ml.) and placed in a 3-necked flask equipped with a cold finger, nitrogen inlet, and guard tube. Cyclopentenone (370  $\mu\text{L}$ , 4.42 mmol.) was added and the mixture stirred under a continuous flow of nitrogen. The mixture was irradiated using a low pressure mercury lamp as the light source and the walls of the flask acted as a pyrex filter. After 30 mins. the triazoline began to crystallise on the cold finger and dichloromethane was added to keep the material in solution. After overnight irradiation the

mixture was found to contain no triazoline (t.l.c.) but cyclopentenone was still present (i.r. spectrum). After 4 hrs. no further change was observed and the mixture was concentrated and distilled (Kugelrohr) to give a clear liquid ( $50^{\circ}/1$  mm.) and a pale yellow oil ( $150^{\circ}/1$  mm.). The clear liquid was thought, from its n.m.r. spectrum, to be a mixture of benzaldehyde and cyclopentenone. The yellow oil was thought to be dimethyl anilinomalonate,  $\tau(\text{CDCl}_3)$  2.7-3.6 (5H,m), 5.12 (1H,s), 5.27 (1H,s), and 6.34 (6H,s);  $m/e$  223 ( $M^+$ );  $\nu_{\text{max}}^{\text{film}}$  3380, and  $1739 \text{ cm}^{-1}$ .

3. The triazoline (0.5g, 1.36 mmol.) (37) was dissolved in dry toluene (5 ml.) and lithium perchlorate (anhydrous, 0.145g, 136 mmol.) was added. The mixture was refluxed under a blanket of dry nitrogen and a deep red colour was formed. After refluxing overnight the solution was pale yellow and the aziridine was found to have hydrolysed to benzaldehyde and diethyl anilinomalonate. No  $m/e$  421 was found in the mass spectrum and the cyclopentenone appeared to have polymerised as none was found in the mixture. The reaction was carried out three times, taking care to ensure moisture-free conditions but in each case the hydrolysis products of aziridine (78) were obtained.

4. The triazoline (1.93g, 5.7 mmol.) (38) was dissolved in dry benzene (20 ml.) and refluxed until no triazoline could be detected by t.l.c. A benzene solution containing cyclopentenone (0.477 ml, 5.7 mmol.) and boron trifluoride etherate (0.7 ml, 5.7 mmol.) was added to the deep red solution of aziridine and the solution instantly decolourised. The mixture was refluxed overnight and passed through one inch of silica gel to remove

inorganic salts. The resulting pale yellow solution was concentrated and purified by column chromatography (Kieselgel H, ether) giving 0.3g (13.4%) of the trans-isomer of compound (82). No cis-isomer was obtained.

5. The triazoline (14.5g, 42.77 mmol.) (38), cyclopentenone (7g, 85.36 mmol.) and hydroquinone (few crystals) were heated by bunsen flame until the temperature reached 160°. The mixture liquefied giving a homogeneous pale yellow solution which turned deep red on further heating. The temperature was maintained at 150-160° until the effervescing due to nitrogen evolution had subsided. The mixture was cooled to 100° and toluene (10 mls.) was added. The mixture was refluxed overnight and then concentrated to remove toluene and excess cyclopentenone. The residual dark brown gum was purified by column chromatography (Kieselgel H, 400g; ether/pet.ether 40-60°, 50:50) giving 3.84g (22.8%) of the cis-isomer of compound (82), and 3.63g (21.6%) of the trans-isomer.

Reaction of 1-benzyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (57) with cyclopentenone by heating the heterogeneous mixture without solvent

The triazoline (57) (2.0g, 6.55 mmol.) and cyclopentenone (2.85g, 34.75 mmol.) were placed in a pear-shaped flask under dry nitrogen and heated strongly with a bunsen flame until all the triazoline had melted. The heating was continued until no more nitrogen was evolved from the solution. o-Xylene was added and the mixture refluxed for 3 hours. The solvent and excess cyclopentenone were removed by short-path distillation under vacuum leaving a residual gum which contained two main components as indicated by t.l.c. These components were separated by column chromatography (Kieselgel H; ether/hexane, 50:50)

to give 0.78g of material corresponding to the top spot on t.l.c., and 0.55g of material corresponding to the bottom spot on t.l.c. The lower spot material was found to be diethyl benzylaminomethylene malonate (59) (30.3%) (tlc., nmr., ir). The top spot material was distilled (Kugelrohr 230°/0.2 mm) to give a viscous pale yellow oil. This oil appeared, from the n.m.r. and mass spectra, to be a mixture of compounds (55) and (65).

Reaction of 1-phenyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (67) with cyclopentenone

The triazoline (67) (0.5g) and a large excess of cyclopentenone were refluxed in dry toluene (20 ml.) under nitrogen for 24 hours. The resulting mixture showed marked streaking on t.l.c. but contained two main spots. These were separated by preparative layer chromatography (Kieselgel G; ether/hexane, 60:40). The top band from p.l.c. gave a crude brown oil. The mass spectrum of this oil did not contain a molecular ion or mass fragments corresponding to the cycloaddition product (84), m/e 368, 293, and 220. The lower band from p.l.c. was also a crude brown oil. The mass spectrum of this oil indicated that the cycloaddition product (84) was present, m/e 345 ( $M^+$ ), 272 (base peak), 244, and 200. As for the material from the upper band, fragments m/e 293 and 220 were also present but of lower intensity,  $\tau$ ( $CDCl_3$ ) 2.7-2.95 (m), 3.1-3.45 (m), 4.92 (s), 5.40 (s), 5.6-5.95 (m), 7.3-7.45 (m), 7.65-7.77 (m), and 8.6-8.91 (m).

Reaction of 1-phenyl-4,4-dicarbethoxy- $\Delta^2$ -1,2,3-triazoline (67) with methyl crotonate

The triazoline (0.5g) was refluxed in methyl crotonate (5 ml.) overnight. The t.l.c. of the resulting mixture was

highly streaked. The only significant spot which was evident in the streaking was isolated by p.l.c. giving a dark brown oil. The mass spectrum of this oil did not indicate the presence of any cycloaddition product or fragments thereof but gave a spectrum which was very similar to that produced by the oil isolated from the top layer in the reaction between triazoline (67) and cyclopentenone,  $m/e$  368, 293, and 220,  $\tau(\text{CDCl}_3)$  2.7-2.95 (m), 3.1-3.45 (m), 4.92 (s), 5.1-5.3 (m), 5.40 (s), 5.6-5.95 (m), 8.6-8.93 (m).

#### Preparation of 2-carbethoxycyclopentanone<sup>80</sup>

Sodium metal (23g, 1 mol.) was stirred in dry toluene (400 ml.) under a blanket of nitrogen. Diethyl adipate (202g, 1 mol.) was added slowly and the mixture was heated. When half the ester had been added the mixture was refluxed for 15 mins. and the solution began to thicken. Ester addition was continued and the mixture began to solidify in parts. Heating was removed and the mixture continued to reflux by the reaction exotherm. When the reaction had subsided (1 hour), ethanol (30 ml.) was added. Acetic acid (50 mls. glacial in 100 ml. water) was added with vigorous stirring and the organic layer was separated. The aqueous layer was washed with toluene and the combined organic fraction distilled, firstly at atmospheric pressure until the toluene, ethanol and water were removed and then under vacuum to give the product as a colourless oil, b.p. 80-90°/1 mm. (lit.<sup>80</sup> 83-88°/5 mm.), 74.4g (47.6%).

#### Preparation of 2-carbethoxycyclopent-2-en-1-one (73)<sup>83</sup>

2-Carbethoxycyclopentanone (1.33g) was refluxed in dioxan (2.2 ml.) with selenium dioxide (0.95g), for 25 mins. The mixture was cooled and filtered. The presence of the enone

in the mixture was detected by  $\tau$  1.8 ( $\tau$ ). A complete n.m.r. spectrum could not be obtained because of the presence of polymer in the mixture.

#### Polymerisation of 2-carbethoxycyclopent-2-en-1-one (73)

The preparation of enone (73) detailed above was repeated with aliquots being removed from the reaction mix and worked-up at five minute intervals. The samples were checked for enone content by the intensity of the  $\tau$  1.8 triplet in the n.m.r. spectrum. The enone concentration was maximum in the first sample taken 5 minutes after the reaction had started. The enone level dropped rapidly with time and after two hours no 2-carbethoxycyclopentenone could be detected in the reaction mixture.

#### Attempted in situ reaction of 1,3-diphenyl-2,2-dicarbethoxyaziridine with 2-carbethoxycyclopent-2-en-1-one

2-Carbethoxycyclopentanone (1.27g, 8.2 mmol.), 1,5-diphenyl-4,4-dicarbethoxytriazoline (37) (3g, 8.2 mmol.) and selenium dioxide (0.91g, 8.2 mmol.) were refluxed in dioxan (6 ml.) for three days. The dioxan was removed under reduced pressure and the residue passed through a short column of silica gel to remove the selenium residues. The product mixture was partially separated by column chromatography and found to contain some 2-carbethoxycyclopentanone, 1,3-diphenyl-2,2-dicarbethoxyaziridine, benzaldehyde, diethyl anilinomalonate and polymer. No evidence for the presence of a cycloaddition product was detected by mass spectroscopy.

#### Reaction of bicyclic compound (82) with methyl magnesium iodide

A mixture of cis/trans compound (82) (100 mg, 0.25 mmol.) was stirred in dry THF under nitrogen. The solution was



cooled to  $-78^{\circ}$  and a solution of methyl magnesium iodide in diethyl ether was added (0.25 mmol.). The mixture was allowed to warm to room temperature. T.l.c. of the reaction mixture suggested no reaction had occurred. The solution was again cooled and a further addition of Grignard reagent was made. When the mixture warmed to room temperature, t.l.c. again showed starting material to be present. A large excess of reagent was added and the mixture stirred overnight at room temperature. T.l.c. indicated that all the starting material was gone and the mixture was shaken with saturated ammonium chloride. The aqueous layer was extracted with ether and the combined organic layer was dried ( $\text{MgSO}_4$ ) and concentrated to a yellow gum (79 mg.). T.l.c. of this gum was highly streaked and contained numerous spots. No major products could be isolated and no alcohol was detected by infra-red or mass spectroscopy.

Reaction of bicyclic compound (82) with methyl magnesium bromide

A.

Dry magnesium (31g, 1.27 mmol.) was placed in a 3-necked flask equipped with dry nitrogen inlet, cold finger condenser and rubber septum. Methyl bromide (large excess) was added and when the reaction commenced, dry ether (20 ml.) was added. The mixture was stirred at room temperature until reaction was complete (30 mins.). Dry THF was added and the mixture was distilled (via a side-arm under a slight nitrogen flow) until the temperature at the still head reached  $60^{\circ}$ .

In another 3-necked flask equipped with a water condenser, dry nitrogen inlet, and rubber septum, compound (82) (250 mg, 0.64 mmol., mixture of cis and trans isomers) was dissolved in dry THF. The solution was cooled to  $-15^{\circ}$  and half

of the prepared Grignard reagent was added dropwise. The mixture was allowed to warm to room temperature and after 2 hours t.l.c. indicated that no reaction had occurred. The mixture was refluxed but still t.l.c. showed no change. The second half of the Grignard solution was added but still no reaction was indicated by t.l.c. A large excess of Grignard was added and the solution turned dark green. T.l.c. still indicated no change. A saturated solution of ammonium chloride was added and the mixture extracted with THF and concentrated. The residue was taken up in dichloromethane, dried ( $\text{MgSO}_4$ ), and concentrated to a yellow gum. T.l.c. of this material showed three spots, two of which were the isomeric mix of starting material. No alcohol absorption was found in the i.r. spectrum and the unidentified spot on t.l.c. was not isolated.

**B.**

Compound (82) (100 mg, 0.25 mmol., mixture of cis and trans isomers) was dissolved in dry THF and stirred at room temperature with methyl magnesium bromide (0.25 mmol.). Methyl iodide (0.25 mmol.) was added and the mixture was stirred for two days. T.l.c. indicated no reaction had occurred.

**C.**

Compound (82) (100 mg, 0.25 mmol., mixture of cis and trans isomers) was dissolved in dry THF and stirred at room temperature with methyl magnesium bromide (0.25 mmol.). After one hour, trimethylsilyl chloride (47.5  $\mu\text{L}$ , 0.37 mmol.) and triethylamine (52.2  $\mu\text{L}$ , 0.375 mmol.) were added. The mixture was filtered through celite and concentrated. The residue was dissolved in deuteriochloroform and the n.m.r. spectrum indicated

that the material was the trans isomer of compound (82),  $\tau$  4.76 (1H,d; J=4Hz). No cis isomer was detected in the product.

D.

Compound (82) (100 mg, 0.25 mmol.) was stirred in dry ether (50 ml.) until dissolved (1½ hr.). Methyl magnesium bromide (0.25 mmol.) in ether was added from a syringe via a rubber septum. The mixture gave an immediate white precipitate on addition. T.l.c. indicated only starting material. A further mole equivalent of Grignard reagent was added but still t.l.c. indicated that no reaction had occurred. A large excess of reagent was added and t.l.c. showed the starting material to have almost completely gone and one major spot was present. The mixture was worked-up by addition of saturated ammonium chloride and the aqueous layer extracted with dichloromethane. The combined organic layers were dried and concentrated to an oil. T.l.c. of this oil contained numerous minor components and four major spots, two of which had the same  $R_f$  as the isomers of compound (82). The other two major components were isolated by p.l.c. but did not appear from i.r. (no alcohol absorption) or n.m.r. spectra to be the desired product and were not identified.

E.

Compound (82) (100 mg, 0.25 mmol.) (cis isomer) was dissolved in dry ether (50 ml.) and methyl magnesium bromide (0.25 mmol.) in ether was added at room temperature. The solution produced a white precipitate and the mixture was stirred under nitrogen overnight. The reaction was worked up as above and the n.m.r. spectrum showed the product to be a 1:1 mixture of cis and trans isomers of compound (82),  $\tau$ (CDCl<sub>3</sub>) 4.76 (1H,d;

J=4Hz) and 5.21 (1H,d; J=8Hz).

F.

Compound (82) (100 mg, 0.25 mmol.) was dissolved in dry ether (50 ml.) and methyl magnesium bromide (0.50 mmol.) in ether was added at room temperature. The solution produced a white precipitate and then turned pale yellow. The mixture was stirred under nitrogen overnight and then worked-up as above. The n.m.r. spectrum of the product showed that the material consisted mainly of starting material (both isomers) but the cis isomer was the major component and a new resonance at  $\tau(\text{CDCl}_3)$  8.43 (singlet) was observed.

#### Reaction of bicyclic compound (82) with methyl lithium

The cis isomer of compound (82) (0.1g, 0.25 mmol.) was dissolved in dry ether (35 ml.) and methyl lithium (150  $\mu\text{L}$  of 1.66M, 0.25 mmol.) was added dropwise at  $-20^\circ$  with stirring under dry nitrogen. After 30 mins. the mixture was warmed to room temperature. T.l.c. indicated that the solution contained a mixture of cis and trans isomers of compound (82), no other spots were observed.

#### Thermal stability of bicyclic compound (82)

A. The trans isomer of compound (82) (0.5g) was refluxed in dry toluene (20 ml.) under nitrogen overnight. T.l.c. of the resulting solution indicated that no reaction had occurred and no cis isomer was present.

B. The cis isomer of compound (82) (0.5g) was refluxed in dry toluene (20 ml.) under nitrogen overnight. T.l.c. of the resulting solution indicated that no reaction had occurred and no trans isomer was present.

Preparation of cyclopent-1-ene aldehyde<sup>81</sup>

Sodium metaperiodate (7.38g, 34.5 mmol.) was dissolved in water (100 ml.) and stirred. Cyclohexan-1,2-diol (4g, 34.5 mmol.) was added and the temperature rose from 23° to 33°. The mixture was stirred for 10 minutes and then cooled to 25°. The mixture was left stirring at room temperature overnight. Diethyl ether (100 ml.) and sodium hydroxide (50 ml., 3M) were added and the mixture was stirred vigorously for 30 mins. during which time the colour darkened to a deep yellow. The ether layer was separated and the aqueous layer extracted with ether (100 ml. x 2). The combined ethereal layer was dried (MgSO<sub>4</sub>) and concentrated. The residue was distilled, b.p. 35°/9 mm. (lit.<sup>81</sup> 52°/20 mm.) to give 1.7g (51%) of product,  $\tau$ (CDCl<sub>3</sub>) 0.32 (1H,s), 3.18 (1H,m), 7.5 (4H,m), and 7.95 (2H,m);  $\nu_{\text{max}}^{\text{film}}$  1665 and 1610 cm<sup>-1</sup>.

Reaction of cyclopent-1-ene aldehyde with dialkyl malonate

1. Sodium metal (0.5g, 21.74 mmol.) was placed in a dry 100 ml. flask and dry ethanol (3 ml.) was added. Dry THF (10 ml.) was added, followed by diethyl malonate (3.48g, 21.75 mmol.) in THF (10 ml.). The mixture was refluxed for 15 mins. and then cyclopent-1-ene aldehyde (3g, 31.25 mmol.) in THF (10 ml.) was added and the mixture refluxed for 30 mins. The mixture darkened to a deep green/brown solution which displayed a blue fluorescence to direct light. The mixture was concentrated and diethyl ether was added (50 ml.). The solution was quenched with dilute hydrochloric acid. The aqueous layer was extracted with ether (20 ml.) and the combined ether layer was washed with a solution of sodium bicarbonate, and then water, dried (MgSO<sub>4</sub>), and concentrated to give a yellow oil, 4.22g. T.l.c. suggested this oil was a

mixture of several components. The n.m.r. spectrum did not look favourable and the i.r. spectrum showed no olefinic absorptions.

2. Cyclopent-1-ene aldehyde (5g, 52.08 mmol.), diethyl malonate (4.16g, 26.04 mmol.), potassium acetate (0.26g) and copper acetate (0.26g) were added to glacial acetic acid (10.5g) and the mixture stirred at 100° for 2 hr. Distillation of the mixture gave only acetic acid, cyclopent-1-ene aldehyde, and diethyl malonate. When the reaction time was extended to overnight at 100°, distillation gave only acetic acid and a little cyclopent-1-ene aldehyde. The residue was a black charred mass of polymer.

3. Cyclopent-1-ene aldehyde (3g, 31.25 mmol.), diethyl malonate (4.8g, 30 mmol.), piperidine (0.16 ml.), benzoic acid (0.1g) and benzene (10 ml.) were refluxed in a Dean & Stark apparatus. When 0.45 ml. of water had been collected the reaction was stopped and concentrated. The residue was a black polymeric material which would not distil under high vacuum.

4. Cyclopent-1-ene aldehyde (1.0g, 10.4 mmol.) and dimethyl malonate (4.12g, 31.2 mmol.) were stirred in pyridine (0.98g, 14.6 mmol.) at 100°. The mixture turned dark after 30 min. but after 4 hours t.l.c. indicated only starting materials to be present. The mixture was stirred at 100° for 5 days but still no reaction was indicated by t.l.c.

#### Preparation of 1-cyclopent-1-enyl-pyrrolidine

Cyclopentanone (84g, 1 mol.) was refluxed in benzene (250 ml.) with pyrrolidine (71g, 1.01 mol.) and a small amount of p-toluene sulphonic acid in a Dean & Stark apparatus. The

reaction was stopped when no more water was collected (4 hr.) and the mixture concentrated. The residue was distilled, b.p.  $88^{\circ}/11$  mm. (lit.<sup>100</sup>  $81.5-82^{\circ}/5$  mm.), to give the enamine, 84.65g (62%). The crude product was redistilled from a flask packed with glass wool, b.p.  $100^{\circ}/20$  mm,  $\nu_{\text{max}}^{\text{CHCl}_3}$   $1620$   $\text{cm}^{-1}$ .

Reaction of 1-cyclopent-1-enyl-pyrrolidine with diethyl ethoxymethylene malonate

The enamine (1.08g, 10 mmol.) and olefin (2.16g, 10 mmol.) were stirred in dry THF (20 ml.) under nitrogen at room temperature. The solution turned yellow immediately upon addition of the starting materials and then slowly turned red. After 2 hrs. the solution was bright red. The mixture was left overnight at room temperature and then concentrated. T.l.c. showed numerous components were present in the mixture but distillation under high vacuum gave only a small amount of unreacted enamine, b.p.  $48^{\circ}/0.6$  mm. The reaction was repeated, adding the reactants at  $-15^{\circ}$  and then stirring the mixture at  $-10^{\circ}$  for several hours before allowing to warm to room temperature. After 24 hrs. at room temperature the mixture was concentrated and distilled but again only a small amount of enamine was obtained.

Reaction of cyclopent-1-ene aldehyde with malonic acid<sup>82</sup>

Cyclopent-1-ene aldehyde (14.66g, 152.7 mmol.) and malonic acid (47.64g, 458.1 mmol.) were stirred in pyridine (16.94g, 214 mmol.) at  $100^{\circ}$ . The mixture effervesced slowly and continued to do so for the duration of the reaction. After  $2\frac{1}{2}$  hrs. the effervescence subsided and after 3 hrs. the mixture was allowed to cool and immediately solidified. Sulphuric acid (30% V/v) was added and the solid collected by filtration and washed with water. The material was dissolved in THF and

extracted with saturated sodium bicarbonate solution (3 x 100 ml.). The bicarbonate solution was neutralised with aqueous hydrochloric acid (2M) and further acid was added to adjust the pH to 1-2. The solid obtained was collected by filtration, washed with water and dried in vacuo over  $P_2O_5$ , 13.44g (63.8%). The solid was stirred in ethyl acetate and then filtered. The solid was dried in vacuo giving 8.64g (41.0%) of crystals which sublimed above  $100^\circ$  and then recrystallised as highly rhombic crystals which formed a glass at  $139-140^\circ$  and melted at  $140-145^\circ$ ,  $\tau(CDCl_3 + d^6-DMSO)$  1.18 (1H,s,broad), 2.56 (1H,d,J=16Hz), 3.87 (1H,s), 4.32 (1H,d,J=16Hz), 7.55 (4H,m), and 8.05 (2H,m);  $\nu_{max}^{CHCl_3}$  3300-2400, 1685, 1620, and  $982\text{ cm}^{-1}$ ; m/e 138 ( $M^+$ ). The filtrate was concentrated and the solid dried in vacuo giving 4.8g (23%) of crystals, m.p.  $136-140^\circ$ ,  $\tau(CDCl_3)$  -1.13 (1H,s,broad), 2.40 (1H,d,J=16Hz), 3.72 (1H,s), 4.26 (1H,d,J=16Hz), 7.50 (4H,m), and 7.98 (2H,m);  $\nu_{max}^{CHCl_3}$  3300-2400, 1685, 1620, and  $720\text{ cm}^{-1}$ ; m/e 138 ( $M^+$ ), (lit.<sup>82</sup> only one product obtained, m.p.  $162^\circ$ ).

#### Esterification of trans-3-cyclopent-1-enyl prop-2-enoic acid (113)

The acid (5.69g, 41.2 mmol.) was refluxed in methanol (100 ml.) containing concentrated hydrochloric acid (2 ml.). The reaction was maintained at reflux overnight and then concentrated. The solid residue was dissolved in ether and washed with saturated sodium bicarbonate, then twice with water and dried ( $MgSO_4$ ). Concentration gave a crude solid which was recrystallised from hexane at  $-20^\circ$  to give a white solid, 4.38g (70%), m.p.  $38-39^\circ$ ,  $\tau(CDCl_3)$  2.53 (1H,d,J=16Hz), 3.87 (1H,m), 4.3 (1H,d,J=16Hz), 6.29 (3H,s), 7.55 (4H,m) and 8.03 (2H,m);  $\nu_{max}^{CHCl_3}$  1705, 1630 and  $979\text{ cm}^{-1}$ .



Reaction of *trans* methyl-3-cyclopent-1-enyl prop-2-enoate  
with benzyl azide

The diene ester (1.52g, 10 mmol.) was dissolved in chloroform (20 ml.) and stirred under nitrogen. Benzyl azide (1.33g, 10 mmol.) was added and the mixture stirred at room temperature. After 24 hrs. at room temperature, t.l.c. indicated that no reaction had occurred. The temperature was increased to 50° and after 24 hrs. t.l.c. still indicated that no reaction had occurred. The mixture was refluxed for 7 days after which time t.l.c. indicated that the solution still contained only starting materials.

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