DEVELOPMENT OF A SYNTHETIC ROUTE TO THE ACTIVE INGREDIENT OF AN AGRICULTURAL HERBICIDE

by

Rory O'Donovan

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Under the supervision of Professor A.C. Pratt

at

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School of Chemical Sciences

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DEDICATION

To my parents and family for their love and support throughout my life.

DECLARATION

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Master of Science, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed:  Date: 04 MAR '99

Rory O'Donovan

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ABSTRACT

A multistep synthesis of 4-amino-3-methyl-6-phenyl-1,2,4-triazin-5-one (Metamitron) from inexpensive phenylglyoxylonitrile and chromatographic systems to accurately monitor each step have been developed. Details of the synthetic steps are as follows:

1. Hydrolysis of phenylglyoxylonitrile to phenylglyoxylic acid amide.
2. The in situ methanolysis of this amide to phenylglyoxylic acid methyl ester.
3. The conversion of this ester to its 2-acetylhydrazone by reaction with acetylhydrazine.
4. The reaction of this 2-acetylhydrazone with hydrazine monohydrate to form phenylglyoxylic acid hydrazide 2-acetylhydrazone.
5. The cyclisation of this hydrazide derivative to 4-amino-3-methyl-6-phenyl-1,2,4-triazin-5-one (Metamitron).

During the synthetic process it was shown that phenylglyoxylic acid methyl ester 2-acetylhydrazone and phenylglyoxylic acid hydrazide 2-acetylhydrazone were present as isomers (E- and Z-) and that isomerisation of the E-isomer of the hydrazide derivative about the carbon-nitrogen double bond must take place prior to cyclisation to Metamitron.

A series of HPLC methods was developed to accurately monitor each stage of the synthesis. This involved the isolation and identification of the isomers of the hydrazone and hydrazide in a high state of purity. These systems enabled the identification and quantitation of each of the components and isomers present in the reaction mixtures and the identification and quantitation of impurities present in the final product (Metamitron).

INTRODUCTION

1.1 Background:

A *Herbicide* in the broadest sense of the word, is any compound which is capable of either killing or severely injuring plants and may thus be used for elimination of plant growth or the killing off of plant parts. The designation *weed* covers a variety of meanings according to the particular situation. According to the general definition a weed is any plant, either a wild or cultivated variety, that is undesired in a particular place. In agriculture and horticulture weeds are thus any plants other than the specific crop being grown. Weeds are conveniently divided into dicotyledonous plants, termed *broadleaf weeds*, and monocotyledonous plants, termed *grass weeds.*

Weed control by chemical means has undergone rapid expansion since the introduction of the selective organic herbicides in the period after the last World War. Strong competitive pressures in the growing of agricultural produce have made rationalisation necessary in the planting, care, and harvesting of cash crops. The trend to large monocultures has led to an increased danger of weed infestation as the earlier farming techniques of proper rotation and intensive cultural practices are being reduced. Simultaneously the shortage and cost of agricultural labour prevent economic control of weeds either by hand or machine and as a result the control of weeds by organic herbicides has become increasingly important throughout the world. The reduction in yields due to weed infestation is of no mean significance. Weeds compete with the crops for food, light and lebensraum above and below the soil surface.

A rough classification of herbicides can be made on the basis of the manner of absorption and the mode of action. A herbicidal agent taken up by the plant via the roots is termed a *soil herbicide,* in contrast to a *foliage*

herbicide, which enters via the green, aerial plant parts. Foliage herbicides that exert their effect directly on the plant parts that have been contacted with the agent are designated *contact herbicides*. All agents that are translocated within the plant after absorption, that is, for which site of absorption and site of action are nonidentical, are called *systemic* or *translocated* herbicides.

Apart from the quantity of herbicide applied, the timing of the application is also important for an optimal control of weeds. Three timings are differentiated according to the state of development of the crop plants when the herbicide is applied, pre-sowing, pre-emergence and post-emergence. The type of herbicide used is selected according to the nature and state of development of the target weed. Soil-applied herbicides are used for weeds not yet emerged; foliar- or soil-applied herbicides are used post-emergence.

Herbicides are split into two groups according to their range of application. The so-called total herbicides can be used for unspecific extermination of vegetation, for suppression of entire plant growth. Naturally, a long residual action of the herbicide is desirable for this indication to avoid the necessity of multiple treatments at short intervals. In the use of total herbicides on agricultural land before or after the growing of the crop, however, a correspondingly short duration of action is required to avoid damage to the subsequent crop.

The so-called semitotal herbicides form a subgroup of the total herbicides. These have a high, unspecific weed action but are well tolerated by woody plants.

By far the largest and economically most important group of agents used today is that of the selective herbicides, which find application in all crops. A selective herbicide has high activity against the target weed but is well tolerated by the crop plant. Because it is seldom possible to control all

the target weed species simultaneously with one agent, various combinations are used in practice to achieve consistent control success.

The characteristics described here for the classification of herbicides, although useful in practice, are not suitable for a clear division since various agents do not adhere strictly to them. Many herbicides are absorbed by the plant both via the roots and via the foliage. They can act as contact agents and can also be translocated, or they can be applied at different states of development. Even the division between total and selective herbicides is not clear-cut. Almost total weed control can also be achieved by raising the application rates of selective agents.[1]

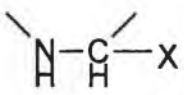
Selectivity of herbicides is very important in weed control in crops. Selectivity is based on *biochemical, morphological* and *physiochemical* differences between weeds and crop plants. Biochemical differences are such that the crops have a unique defence mechanism. The ability of maize to detoxify triazine herbicides by enzymatic hydrolysis is not present in weeds and as a result triazines are extensively used for weed control in this crop. Morphological differences are such that dicotyledonous plants, e.g. broad leafed weeds, have a larger surface area, with the meristemic tissue exposed to the herbicidal spray. On the other hand, monocotyledonous plants, e.g. grasses, possess narrow upright leaves which form a protective sheath around the meristematic tissue: this partly accounts for the selective toxicity of the phenoxyacetic acid herbicides, eg. MCPA and 2,4-D, towards broad-leaved weeds.

Physicochemical factors may be important: with soil-applied herbicides like triazines, the low aqueous solubility means that the chemical only penetrates some five centimetres downwards in the soil and so germinating weed seeds are killed but more deep-rooted crops are unaffected. The bipyridinium herbicides, e.g. paraquat and glyphosate, are strongly

bound to soil colloids by cation exchange. Consequently they enter the soil solution which means that only those plants directly hit by the spray are damaged and there is no uptake of the toxicant from the soil.

The purpose of this project was to synthesise the active ingredient of the selective herbicide, *Metamitron.* This is an unsymmetrical triazine herbicide, which is used as a pre- and post-emergence herbicide for the selective control of annual grasses and broad-leaved weeds in sugar beet. This herbicide was first discovered by Bayer AG and was patented in Ireland in May 1973. The main benefit of this herbicide is its high selectivity towards broad leaf and grass weeds in fodder and sugar beets. Metamitron containing herbicides act by inhibiting electron transport in photosynthesis. Two well known types of triazine herbicides are simazine and atrazine which when used in large concentrations act as total weed killers, but in lower concentrations can be used for the selective control of many germinating weeds in a variety of crops such as maize and sugar cane which are resistant to the herbicidal action of triazines because these plants contain an enzyme that detoxifies the compounds by hydrolysis in the plant tissues. The products of this enzymatic hydrolysis are not herbicidal, so these triazines are very valuable for selective weed control in these crops.[2,3]

Triazines kill plants by interfering with photosynthesis and it seems clear that, like the urea herbicides, the primary site of action is inhibition of the Hill reaction of photosynthetic electron transport.[3-6] Triazines are potent inhibitors of the Hill reaction in isolated chloroplasts; all the herbicides inhibiting the Hill reaction possess the common structural feature:



in which X is an atom possessing a lone pair of electrons (either N or O). It is possible that this grouping represents the essential toxophore in these herbicides and is responsible for binding them to a vital enzyme involved in the Hill reaction, thus preventing the photolysis of water and so depriving the plant of its energy supply.[7]

The first results of the investigation of the herbicidal properties of 1,2,4-triazin-5-ones were published in 1968.[8] It was found that compounds which were substituted by Y-alkyl (Y=O, S, NH) in the 3-position, by an amino group in the 4-position, and by alkyl, cycloalkyl or phenyl in the 6-position were particularly active. One compound, 4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one, was chosen for further development.[9] Under the common name metribuzin it was used as a herbicide against a wide range of weeds in soyabeans, potatoes and tomatoes. With several other crops, however, e.g. sugar beet, it was found to be incompatible.

A quantitative structure-activity study led to the conclusion that the substituent Y-alkyl in the 3-position with Y=O, S, NH might be replaceable by alkyl.[6] In the course of further investigations chemical synthesis of these new 3-alkyl-1,2,4-triazin-5-ones was effected and their herbicidal activity confirmed.[10,11] Some compounds of the new triazinone group were excellently tolerated by sugar beet. The relationships between chemical structure, selectivity and herbicidal activity of these compounds have been described.[12] These investigations showed the existence, within the 6-phenyl-1,2,4-triazin-5-one group, of compounds with extremely high sugar beet tolerance. Indispensable for this kind of selectivity was a methyl group in the 3-position and a free or reversibly substituted NH_2-group in the 4-position. Substitution in the phenyl ring decreased, but did not destroy, the selectivity although the herbicidal activity was greatly reduced. Therefore the

compound 4-amino-3-methyl-6-phenyl-1,2,4-triazin-5-one (Metamitron) was developed as a selective herbicide for use in sugar beet crops. The very narrow structural requirements for sugar beet tolerance suggested that the 3-methyl (and perhaps the 4-amino group) was involved in a detoxification reaction, which is peculiar to that plant.

Before a herbicide can be marketed extensive research into its effects on the plant being treated, future plants being sowed and its surrounding environment, must be carried out.

Experiments were carried out to determine the effects of Metamitron on weeds in *red beet.*[13] These experiments showed that Metamitron had a high degree of tolerance by red beet to pre-emergence applications and to those made at different post-emergence stages. This was confirmed in the subsequent experiments; in none was there any evidence of adverse effects on yield attributable to phytotoxicity from incorporated pre- and post-emergence treatments.

At the rates used, Metamitron incorporated or applied pre-emergence consistently killed a range of common weeds. On light soil there was no consistent difference in the activity of Metamitron incorporated before drilling as compared with surface drilling after drilling, and even when there was a little rainfall good weed control was obtained. The period of persistence of activity in the soil appeared to be adequate for effective weed control in these experiments. At the same time it appeared unlikely that problems of damage to following crops would arise, even in an abnormally dry summers.

From trials with sugar and fodder beet, it was concluded that early post emergence treatment controls the widest spectrum of weeds. However, it appeared that tolerance develops quite quickly in certain species. When applied to red beet at the early two-leaf stage, some weeds had already

become too large and Metamitron was less effective than when applied pre-emergence. Application of 2.8 kg/ha at this stage following 2.8 kg/ha pre-emergence gave excellent results, and under conditions leading to an extended period of weed emergence such a sequential treatment would have obvious advantages.

Post-emergence activity of Metamitron was considerably increased when an adjuvant oil was present. Experiments showed that the addition of the adjuvant, Actipron, to Metamitron significantly increased the level of weed control in crops. Although slight leaf damage was noticed this did not affect crop growth. The results suggested that the adjuvant could be useful in situations where for one reason or another the weeds have passed the optimum stage of growth for control by Metamitron.

It was concluded that because of the high degree of crop safety and the wide spectrum of weeds killed by both soil and early foliar applications, Metamitron could be a valuable addition to the range of herbicides for use in red beet.

Because of its good solubility in water, Metamitron was quickly absorbed from the soil by the plant. Metamitron was metabolised in beet plants by desamination to desamino-metamitron (Figure 1). Within two weeks, approximately 95% of the root absorbed Metamitron was metabolised in the aerial parts of the sugar beet plants; after enrichment by TLC and isolation, 77% of this amount was identified as desamino-metamitron by TLC, GLC and MS. In the soil, degradation of Metamitron to desamino-metamitron proceeded more slowly than in the beet plant. In addition to the initially predominantly formed desamino-metamitron, slight amounts of a further metabolite of an unknown chemical structure were also discovered in some soils. Both compounds can be determined by GLC.

Desamino-metamitron

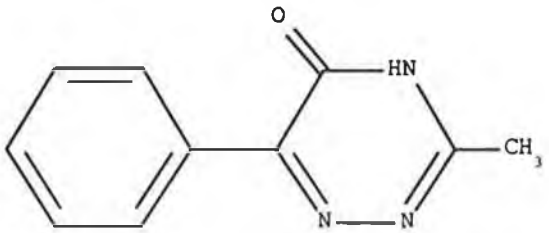


Figure 1

In field experiments, it was shown that the degradation of Metamitron in soils seems to be a predominantly microbial process since no degradation occurred in sterile soils incubated at temperatures ranging between 20 and 27 °C. In the soil, desamino-metamitron was very greatly adsorbed to soil particles.

Another degradation pathway emerged from long-term experiments with ^{14}C Metamitron in soil samples, in which $^{14}CO_2$ were evolved. This was indicative of an opening of the heterocycle in the molecule by micro-organisms. Within 230 days, up to 21% of the applied radioactivity was collected as $^{14}CO_2$. In these experiments, light did not influence the formation of $^{14}CO_2$. The photolytic degradation of Metamitron to desamino-metamitron on the soil surface by sunlight was negligible.

Metamitron, like more than half of the herbicides available, was designed to inhibit photosynthesis of weed plants. However, it was very important that the herbicide does not affect the action of cyanobacteria in the soil. Cyanobacteria are free-living micro-organisms capable of dinitrogen fixation.[14] Their capacity to grow under photoautotrophic conditions is an advantage compared with other free-living diazotrophs which depend on organic substrates. Cyanobacteria can proliferate under favourable conditions in all agricultural areas on the surface layer of the soil. Thus, nitrogen-fixing

cyanobacteria participate in improving the nitrogen budget of the agrochemical ecosystem. Additionally, they are involved in soil fertility, and the biomass of algae provides a substrate for soil micro-organisms.

Herbicides are divided into two groups, "DCMU-type" and "phenol-type" inhibitors. DCMU-type inhibitors are widely used agrochemicals. Directly or indirectly the major proportion of these herbicides after their application enters the surface soil. This, in turn, may adversely affect the development of cyanobacteria. Therefore, it was desirable to know the effect of Metamitron on Nitrogen-fixing cyanobacteria, especially that of photosynthesis inhibitors. The influence of formulated Metamitron, Goltix, on the activity of *Anabaena cylindrica* and *Nostoc muscorum* was studied. The results showed that A. cylindrica was entirely inhibited by goltix while the nitrogenase activity of N. muscorum was unaffected. Delayed inhibitory effects were caused by high concentrations of goltix and these were supposedly due to degradation product(s). In the natural environment other species of cyanobacteria may occur which are able to tolerate photosynthesis-inhibiting herbicides.

The residual life of Metamitron in soil, and hence its biological availability, was very important and was very largely governed by climatic conditions and by soil properties. It is upon these physical factors that the leachability of Metamitron into deep soil layers also depends. Results of studies carried out to investigate the distribution and behaviour of Metamitron (as Goltix) in beet field soils were reported.[15]

The adsorption of Metamitron correlates to both the C-content and the N-content of the soils. The adsorption potential (k) of Metamitron on other beet field soils was determined and k-values at equilibrium concentration ranging from 4 to 11 μg/g soil were found. These

relatively high adsorption values suggest that the leachability of Metamitron in the studied beet field soils would be slight.

Further studies to investigate the leaching of Metamitron in soils showed that despite discontinuous simulated rainfall in the laboratory with amounts many times higher than the natural rainfall amounts, only slight amounts of Metamitron passed through sieved soil columns thirty centimetres high. The moisture level of the soil columns used in these experiments influenced the amount of Metamitron leached out.

The analytical determination of the Metamitron content in soil in long-term experiments produced comparable results in the laboratory and in the field.

Long-term studies on the leaching of Metamitron on natural 1.3 metre soil columns with simulated field rainfall over eighteen months showed that neither Metamitron nor its degradation product, desamino-Metamitron were found in the leachate. On the grounds of the available results from laboratory and field investigations, penetration of Metamitron into the groundwater zone could, therefore, be ruled out.

It has been reported that Metamitron degraded on exposure to sunlight.[16] This report stated that sunlight produced a reaction of Metamitron dissolved in water. This solution became yellow in colour and was accompanied by a decrease in the pH, from 7 to 3. Analysis by GLC-MS indicated the presence of a single compound of molecular mass 187. Its identification was completed by NMR and IR analysis which showed it to be 4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one (desamino-metamitron). However Metamitron was protected by the soil from direct sunlight and as a result desamino-Metamitron would not be present to any great extent due to the effects of sunlight.

Many different methods for the synthesis of Metamitron have been reported using various different raw materials and reagents. As this is an industrial synthetic agrochemical, most of the reported work in this area is in the form of patent applications.

These patents generally stated that Metamitron displayed excellent herbicidal and beet-compatible properties. Thus it provided a method of combating weeds in areas of beet cultivation which comprised applying to their weeds or their habitat, Metamitron alone or in the form of a composition containing Metamitron as the active ingredient in admixture with a solvent or carrier. It was stated that Metamitron showed a considerably higher herbicidal activity than 3-(methoxycarbonylaminophenyl)-N-(3'-methylphenyl)-carbamate whose herbicidal activity was not always entirely satisfactory when using low amounts and concentrations and was substantially better than 3-methylthio-4-amino-5-H-6-phenyl-1,2,4-triazin-5-one which was not tolerated by beet plants, these being the nearest active compounds of the same type of action.

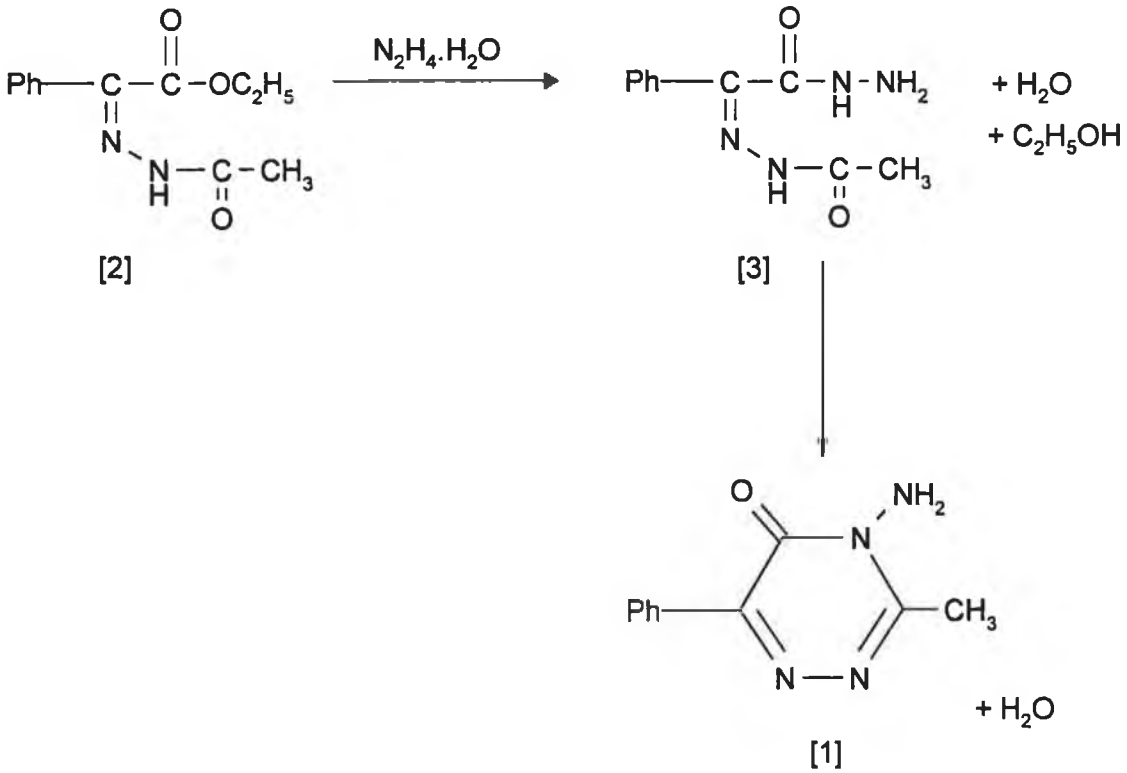
Many experiments were carried out to determine the effect of Metamitron on important bacteria and other soil components, including its leachability into surface drains. The overall evaluation was that Metamitron was an effective herbicide in the treatment of sugar beet crops and is not detrimental to the environment and future crops after application.[17-22]

1.2 Synthesis of Triazines:

The synthesis of Metamitron was described in various patent applications and papers. Similar synthetic routes varied from paper to paper in the concentrations of raw materials used and the conditions under which the reactions were carried out, while alternative methods were also described

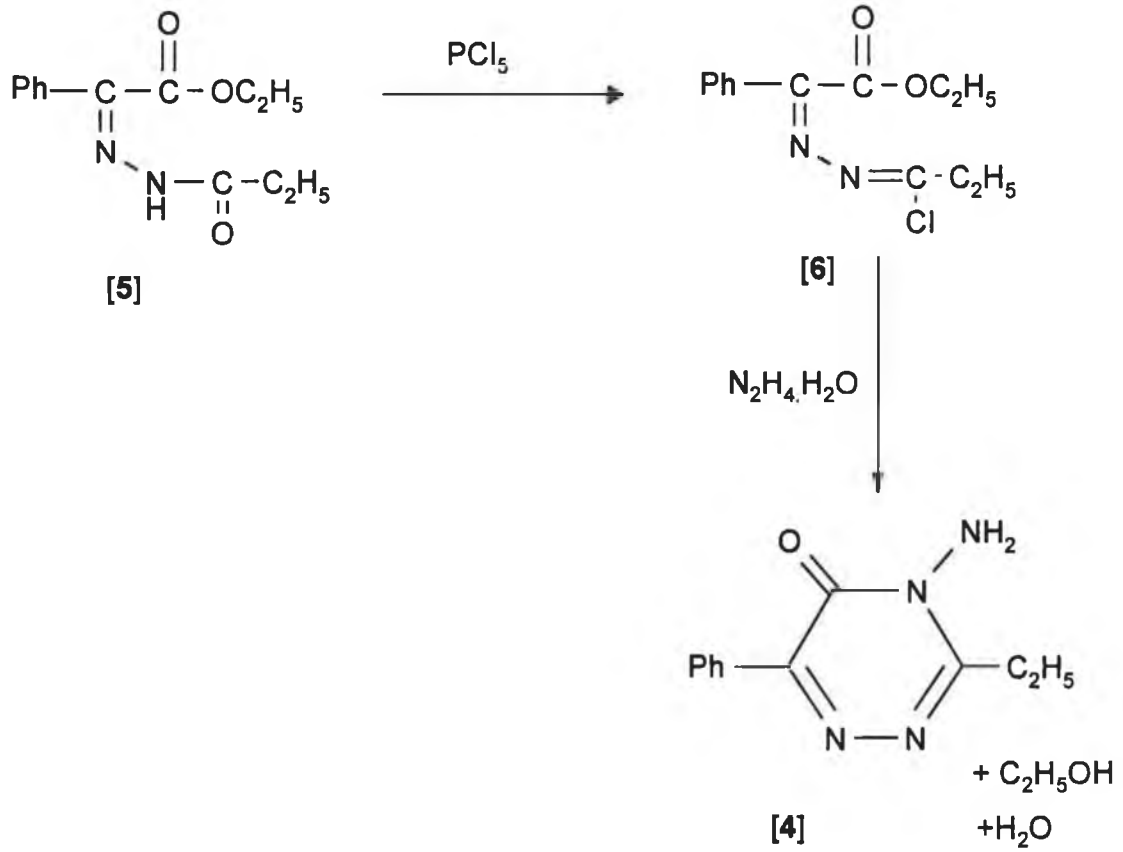
utilising different pathways. These reactions yielded Metamitron in different yields and states of purity.

Metamitron [1] was prepared by the reaction of phenylglyoxylic acid (m)ethyl ester 2-acetylhydrazone [2] with hydrazine hydrate forming the intermediate phenylglyoxylic acid hydrazide 2-acetylhydrazone [3].[10,11,23-27] On heating, this intermediate has been reported to form Metamitron in yields varying from 36 to 62% based on phenylglyoxylic acid methyl ester 2-acetylhydrazone as starting material. The molar ratio of hydrazine hydrate to hydrazone used differed among the various references from 1:1 to 2:1, and a range of different solvents were used for the conversion.

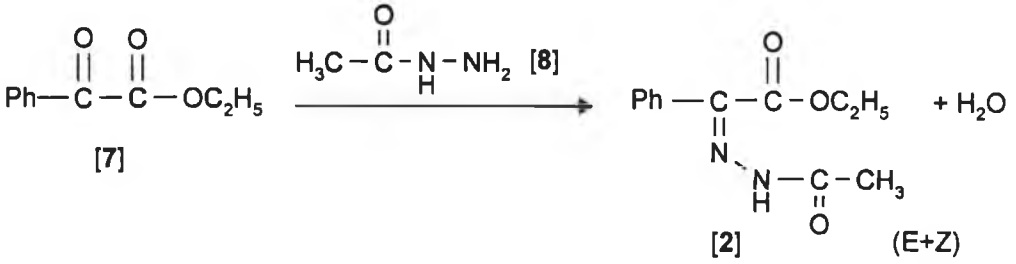


It was suggested that when anhydrous sodium acetate was added to the final stage, an increased yield of Metamitron from phenylglyoxylic acid ethyl ester 2-acetylhydrazone (75%) was obtained.[11]

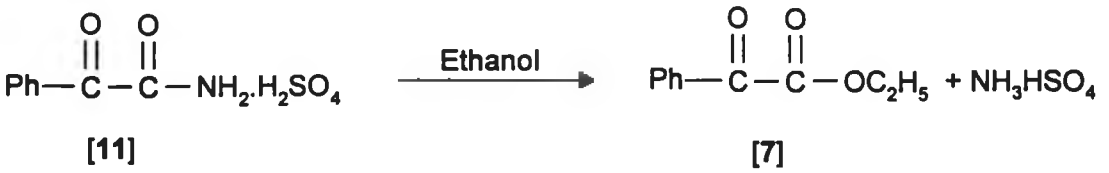
These references also described the synthesis of 4-amino-3-ethyl-6-phenyl-1,2,4-triazin-5-one [**4**] from the hydrazone [**5**] by a 1:1 molar reaction with phosphorus pentachloride forming the intermediate, 1-phenyl-1-ethoxycarbonyl-4-chloro-2,3-diaza-pentadiene [**6**], which was then reacted with hydrazine hydrate forming 4-amino-3-ethyl-6-phenyl-1,2,4-triazin-5-one [**4**].[24,28] The ratio of the pentadiene to hydrazine hydrate between references varied from 1:1 to 1:4.9 giving yields of Metamitron of between 62 and 68%.



The synthesis of phenylglyoxylic acid (m)ethyl ester 2-acetylhydrazone [**2**] from phenylglyoxylic acid (m)ethyl ester [**7**] was reported in some of these references. In most cases it was formed by a direct condensation reaction of the ester with acetylhydrazine [**8**] without the use of a catalyst [24-28] but the use of p-toluene-sulphonic acid, as catalyst, was also reported.[29] The use of a solvent in this reaction was not required as the reaction appeared to proceed to completion just as quickly in the absence of solvent. The reported yields of the hydrazone were approximately 81% when a 1:1 molar ratio of ester and acetylhydrazine was used.



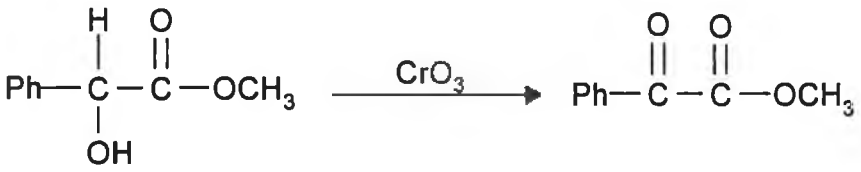
The synthesis of the ester, phenylglyoxylic acid ethyl ester [**7**], has been described in many papers. The starting materials in this synthesis varied from phenylglyoxylonitrile [**9**][25-27,30,37] to mandelic acid methyl ester [**10**].[29] The reactions with phenylglyoxylonitrile involved hydrolysis of the cyano group with dilute hydrochloric/sulphuric acid to form the corresponding amide salt [**11**] followed by alcoholysis of this amide to the corresponding ester (methyl [**12**] or ethyl ester [**7**] depending on the alcohol used, methanol or ethanol). Yields of about 85% for these reactions have been reported.



[11] [7]

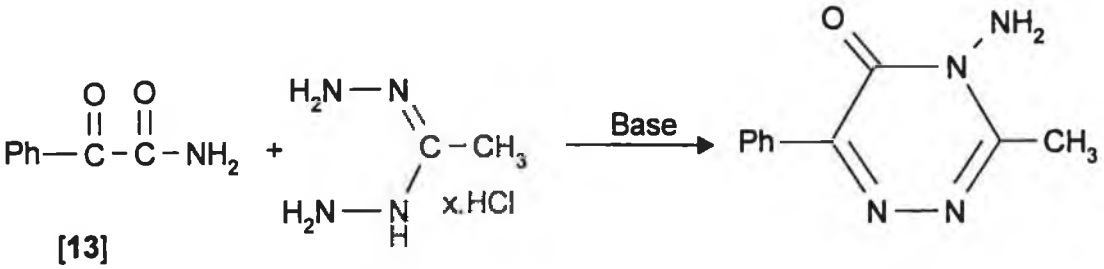
[11] [12]

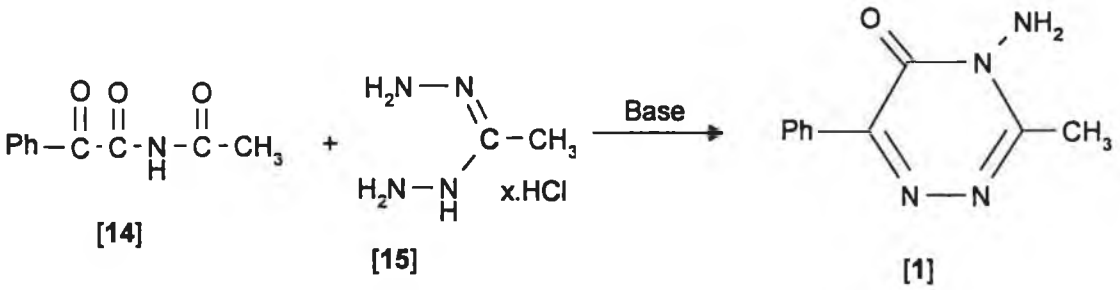
The synthesis of the ester from mandelic acid methyl ester [**10**] involved its oxidation with a 1:1 molar equivalent of chromium trioxide giving an overall yield of the ester [**12**] of approximately 89%.[29]



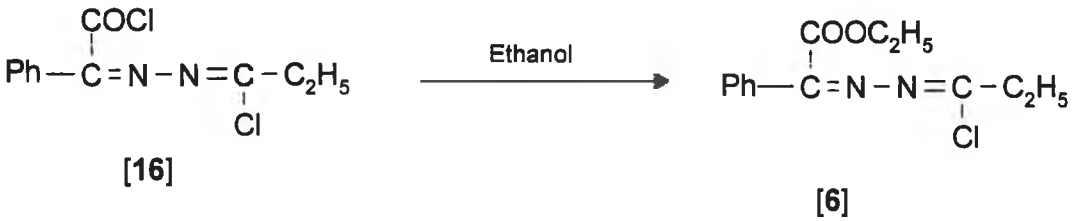
[10] [12]

Other references described the synthesis of Metamitron by a route from phenylglyoxylic acid amide [**13**] or phenylglyoxylic acid-N-acetylamide [**14**].[27,31,32] The initial stage involved the condensation reaction of the amide or acetylamide with acetylhydrazide-hydrazone hydrochloride [**15**] in the presence of the catalyst, p-toluenesulphonic acid. On addition of the base, anhydrous calcium carbonate, cyclisation to Metamitron occurred. The yield of Metamitron in this reference is 89% from both starting materials.

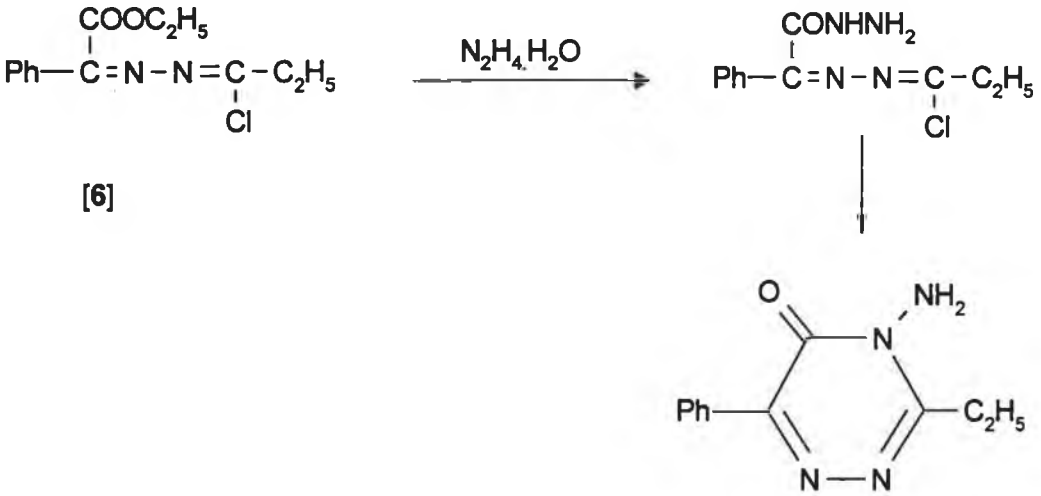




Metamitron was also prepared by reacting diazabutadienes with hydrazine or alkylhydrazine, in a 1:2 molar ratio, in the presence of a base, with an overall yield of 67% obtained.[33] This patent application described the synthesis of Metamitron from the raw material, 1-phenyl-1-chlorocarbonyl-4-chloro-4-ethyl-2,3-diazabutadiene [**16**], by its addition to a solution of ethanol and ethyl acetate. This reaction formed 1-phenyl-1-ethoxycarbonyl-4-chloro-4-ethyl-2,3-diazabutadiene [**6**] as a viscous liquid.

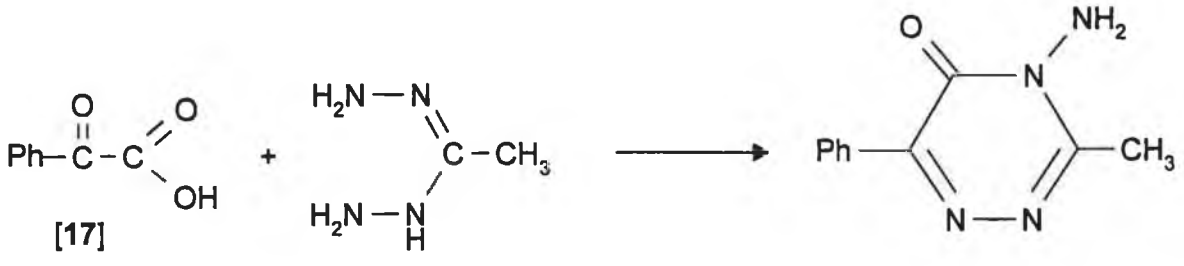


This intermediate **[6]** was then reacted with hydrazine monohydrate in the presence of dimethylformamide forming 4-amino-3-ethyl-6-phenyl-1,2,4-triazin-5-one **[4]** with an overall yield from 1-phenyl-1-chlorocarbonyl-4-chloro-4-ethyl-2,3-diazabutadiene **[16]** of 61%.



[6] [4]

Other reactions involving acetylhydrazide hydrazone [**15**] with 1,2-bifunctional compounds or carboxylic acids [**17**] have been described.[28,32] These reactions formed triazines including Metamitron with yields of approximately 56%.



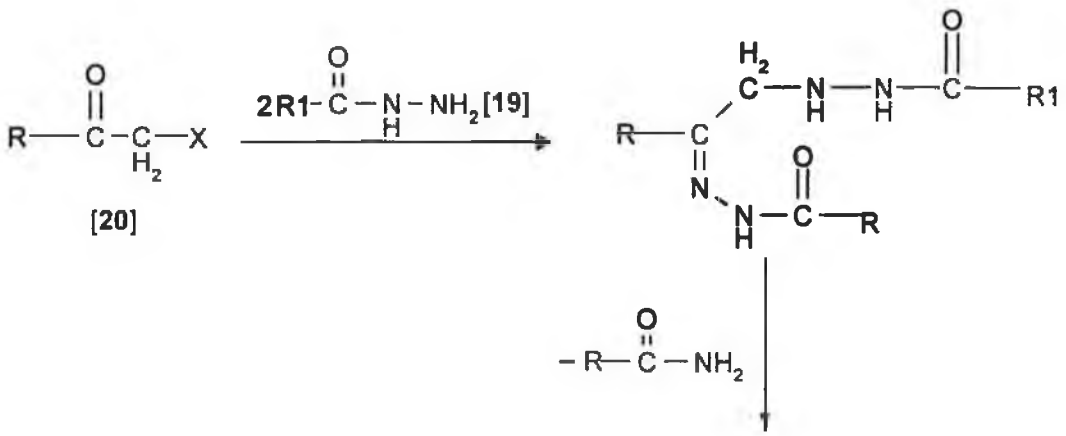
[17] [15] [1]

There are three general methods for the synthesis of aromatic 1,2,4-triazines containing no functional groups. Condensation of acylhydrazides with benzil in acetic acid containing ammonium acetate was used to give 5,6-diphenyl-1,2,4-triazines with various aromatic and heterocyclic groups attached at position 3. A similar method was also applied using a variety of aliphatic and aromatic 1,2-diketones and aliphatic, aromatic and heterocyclic acid hydrazides, but with preliminary isolation of the 1,2-diketone monoacylhydrazones followed by ring closure with alcoholic ammonia under pressure. Monohydrazones of aromatic (but not aliphatic) 1,2-diketones react with formamide, to give 5,6-disubstituted 1,2,4-triazines.

The third general method involved dehydrogenation with potassium dichromate or sulphur of dihydrotriazines of unknown structure, which were prepared by condensation of an α-acylamino-ketone with hydrazine followed by ring closure. 1,2,4-Triazines of this type were also prepared by the oxidation of 3- and 5-hydrazinotriazines with mercuric oxide or cupric salts, by treatment of 3-(benzenesulphonylhydrazino)triazines with alkali and by the reaction of Grignard reagents with 3-chlorotriazines.[34]

A number of 6-aryl- and 3-substituted-6-aryl-1,2,4-triazines **[18]** have been prepared by heating a mixture of an acid hydrazide **[19]** and a haloacetophenone **[20]** (2:1), in ethanol or acetic acid in the presence of molar quantities or a slight excess of sodium acetate.[35]

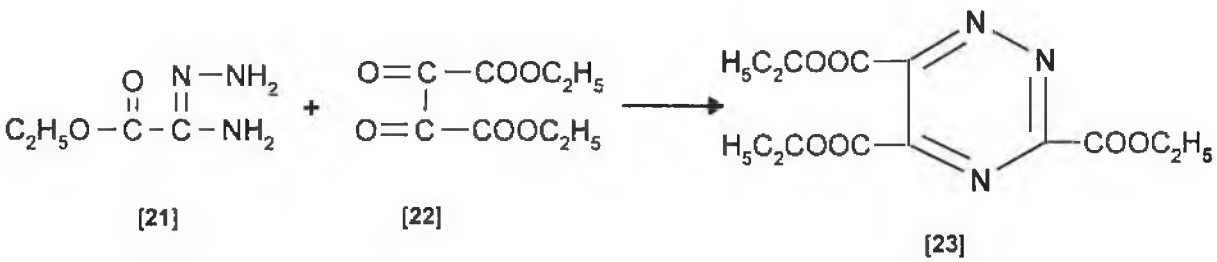
Triazines were also synthesised by the condensation reaction of ethyl oxaloamidrazonate **[21]** with diethyl diketosuccinate **[22]** yielding the triester of 1,2,4-triazine-3,5,6-tricarboxylic acid **[23]**. Hydrolysis to the tricarboxylic acid followed by thermal decarboxylation formed the anhydride of 1,2,4-triazine-5,6-dicarboxylic acid. Similarly, 5,6-diphenyl-1,2,4-triazine-3-carboxylic acid was readily decarboxylated to 5,6-diphenyl-1,2,4-triazine.[36]



[20]

X = -Cl or -Br, R = aryl
R1 = H, alkyl, aryl or heterocyclic

[18]



[21] [22] [23]

DISCUSSION

2.1 General introduction:

The synthesis of 3-methyl-4-amino-6-phenyl-1,2,4-triazin-5-one (Metamitron) (Figure 2), the active ingredient of an agricultural herbicide, and the development of chromatographic systems to accurately monitor each stage of the synthesis was the main aim of this work. Metamitron was required to be synthesised, in a high state of purity (>97%), using relatively inexpensive raw materials in as high a yield as possible.

Metamitron

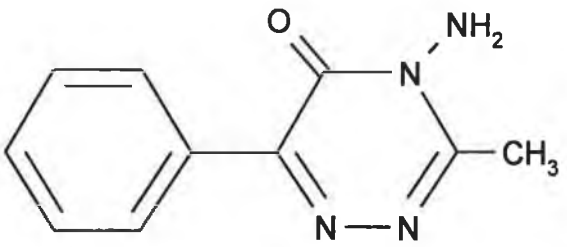


Figure 2

The project also required the isolation of samples of the intermediates of the process, which must then be purified, characterised and their purity determined. These samples would then be used for closer study of the reactions.

The entire process consisted of the five separate stages, which were most commonly described, in the references studied. The initial stage involved the hydration of phenylglyoxylonitrile with dilute sulphuric acid to

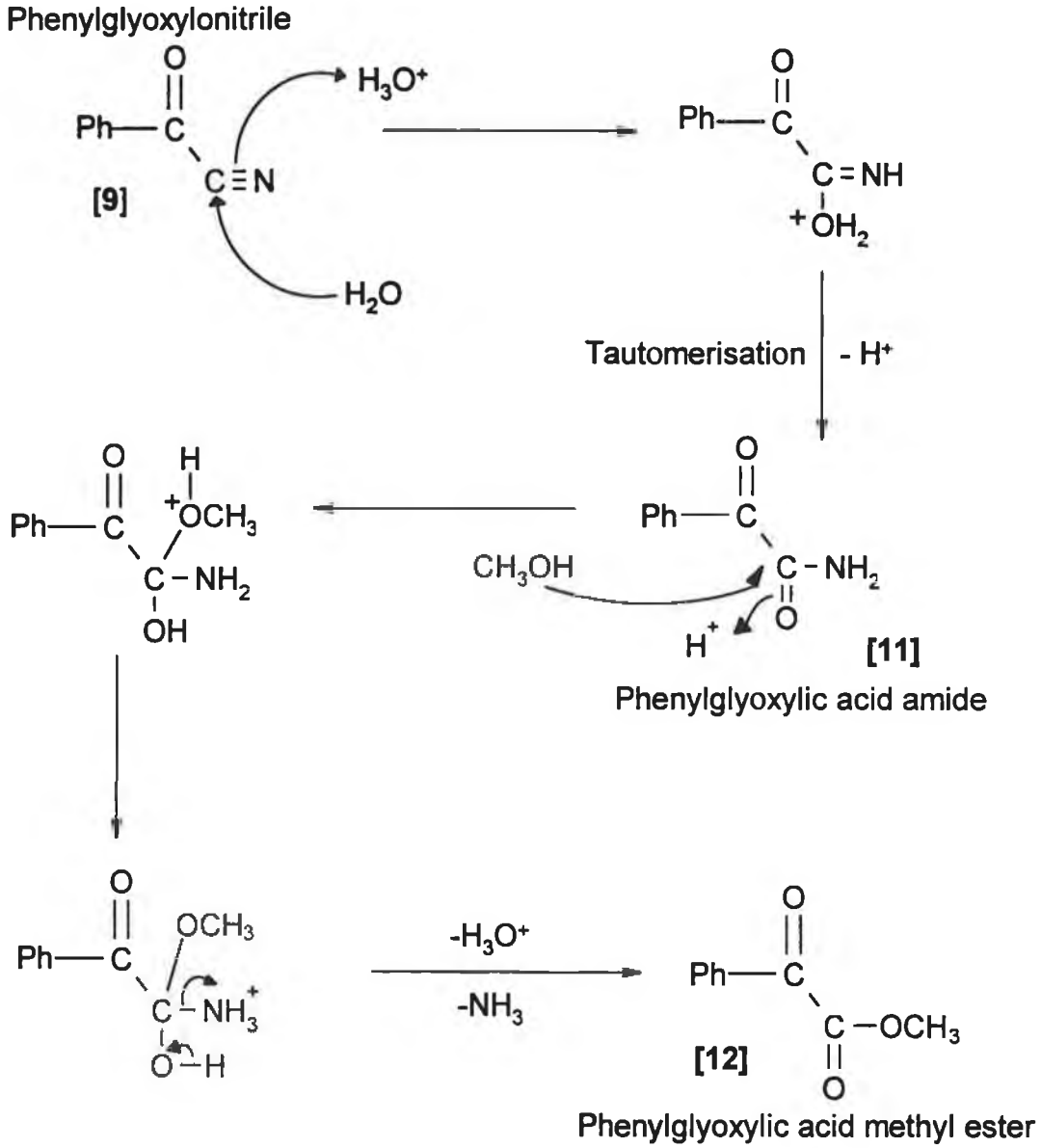
its corresponding amide salt, phenylglyoxylic acid amide, while the next stage involved the methanolysis of this amide to phenylglyoxylic acid methyl ester.[25-27,30,37] The next stage involved the condensation reaction of this ester with acetylhydrazine to form phenylglyoxylic acid methyl ester 2-acetylhydrazone.[24-28] In the next stage hydrazine monohydrate was reacted with this hydrazone derivative forming phenylglyoxylic acid hydrazide 2-acetylhydrazone by displacement of the methoxy group of phenylglyoxylic acid methyl ester 2-acetylhydrazone with hydrazine.[10,11,23-27] The final stage involved the cyclisation of this hydrazide derivative with heat forming 4-amino-3-methyl-6-phenyl-1,2,4-triazin-5-one (Metamitron).[10,11,23-33] One of the aims of this project was to develop synthetic methods which give good yields at each stage of the process.

2.2 Synthesis of phenylglyoxylic acid methyl ester:

2.2.1 Details of the synthesis

The first stage of this process involved the hydration of phenylglyoxylonitrile [**9**] with dilute sulphuric acid to its corresponding amide, phenylglyoxylic acid amide [**11**], in the presence of acetic anhydride and a catalytic amount of sodium bromide, both of which retarded the formation of the more favourable methyl benzoate by preventing cleavage of the cyano functionality from the benzoyl cyanide during the hydration.[37]

The next stage involved the methanolysis of this amide to its corresponding ester, phenylglyoxylic acid methyl ester [**12**], by reaction with methanol (Scheme 1).[37] The product was isolated by distillation yielding phenylglyoxylic acid methyl ester (61%). This ester may be readily purchased and as a result the later stages were deemed to be of more importance.



Scheme 1

2.2.2 Analysis of these reaction stages by HPLC:

Using the HPLC (Section 2.11) method developed for monitoring these steps, it was observed that each had reached completion after 3 hours resulting in an in-situ synthesis of phenylglyoxylic acid methyl ester from phenylglyoxylonitrile after 6 hours (Figures 3 and 4).

Chromatograms of the hydration reaction initially (Figure 3) and the methanolysis reaction at completion (Figure 4)

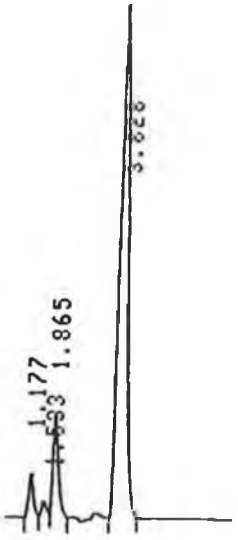


Figure 3

Figure 4

Phenylglyoxylonitrile and phenylglyoxylic acid methyl ester at 3.8 and 3.0 minutes respectively.

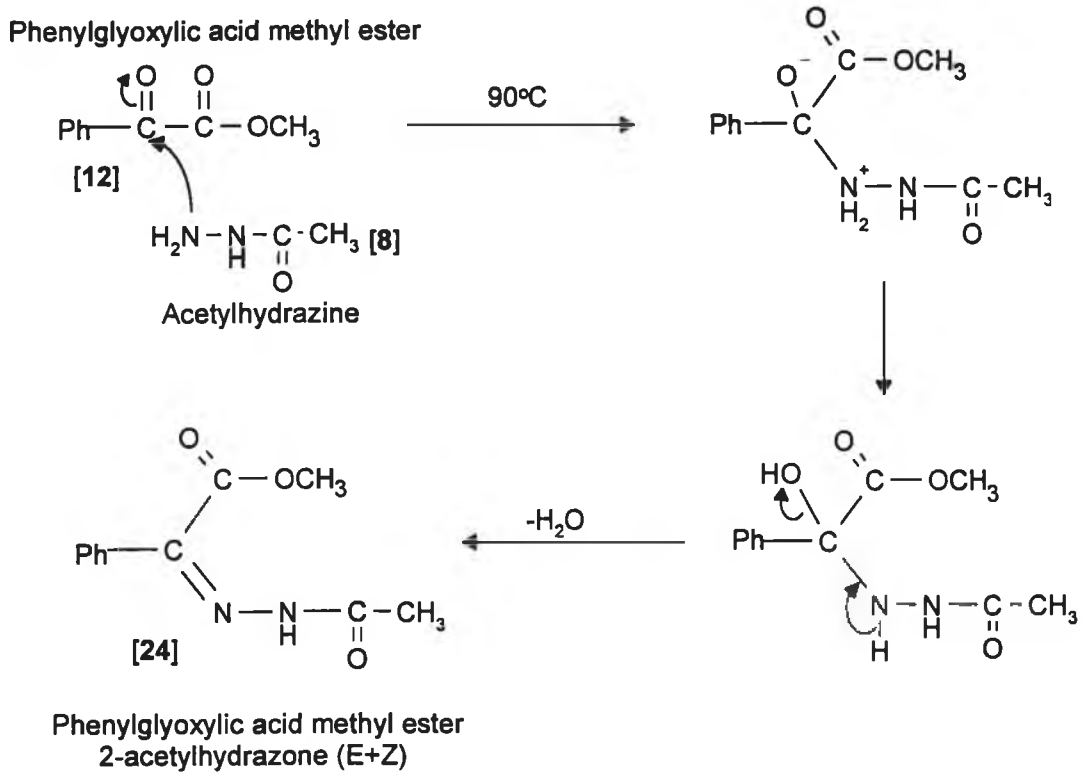
The reaction run time for the hydration was determined by observing the disappearance of the phenylglyoxylonitrile peak while the methanolysis run time was determined by calculating the concentration of the phenylglyoxylic acid methyl ester present in the reaction mixture at regular intervals until the concentration no longer increased with time (phenylglyoxylic acid amide was fully retained on the column and so was not detected by this method).

2.3 Synthesis of phenylglyoxylic acid methyl ester 2-acetylhydrazone:

2.3.1 General:

The next stage involved the condensation reaction of phenylglyoxylic acid methyl ester **[12]** with acetylhydrazine [**8**] to form the corresponding

hydrazone derivative, phenylglyoxylic acid methyl ester 2-acetylhydrazone [24], with water as the by-product of the reaction (Scheme 2).[24-28] These condensation reactions tend to be unusually rapid with equilibrium constants which strongly favour the formation of the hydrazone.



Scheme 2

2.3.2 Initial synthesis of phenylglyoxylic acid methyl ester 2-acetylhydrazone :

This stage was carried out by reacting phenylglyoxylic acid methyl ester with acetylhydrazine (molar ratio of 1:1, as this was the most commonly used ratio in the references studied). The by-product of the reaction (water) was removed by distillation as it formed and the reaction was stopped when no

more water was released (approx. 1 hour). The reaction was monitored by TLC, which showed that the concentration of phenylglyoxylic acid methyl ester no longer decreased upon further heating after this time. These results suggested that an excess of acetylhydrazine was needed for the reaction to proceed to completion.

2.3.3 Optimisation of the hydrazone formation reaction:

Investigation into this reaction by TLC revealed that a 1:1.2 molar ratio of phenylglyoxylic acid methyl ester to acetylhydrazine, along with a run time of 60 minutes, was required for the reaction to proceed to completion. The use of solvents increased the run time of the reaction and did not appear to benefit the reaction process.

2.3.4 Analysis by ^{1}H NMR spectroscopy:

In order to understand more clearly the synthetic processes, the hydrazone forming stage was followed by NMR spectroscopy as no solvent was used, making sampling for spectroscopy straightforward. Samples were taken every 15 minutes, dissolved in deuterated chloroform and proton NMR spectra obtained. The spectrum obtained after 45 minutes showed singlets at 2.0 (CH_3), 4.0 (OCH_3), 8.7 and 11.7 ppm (NH), a pair of singlets at 2.4 and 2.45 (CH_3), another pair of singlets at 3.85 and 3.9 ppm (OCH_3) and a multiplet at approximately 7.5 ppm (arom) (Figure 5 and 6).

Spiking of the sample taken after 45 minutes with pure samples of acetylhydrazine and phenylglyoxylic acid methyl ester verified the presence of these compounds in the sample with the peak at 2.0 from the

^{1}H NMR spectrum of the hydrazone forming reaction mixture after 45 minutes

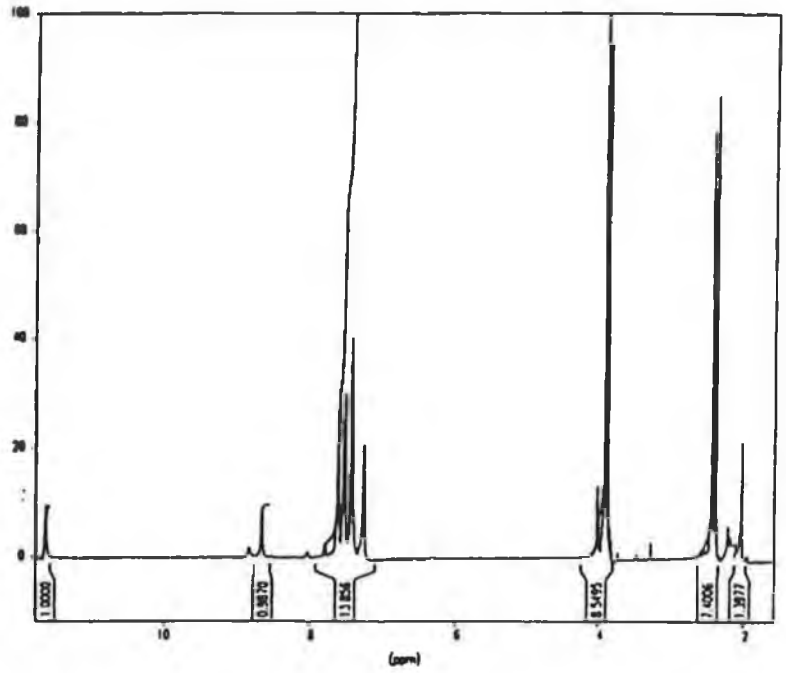


Figure 5

Expanded portion of the above spectrum

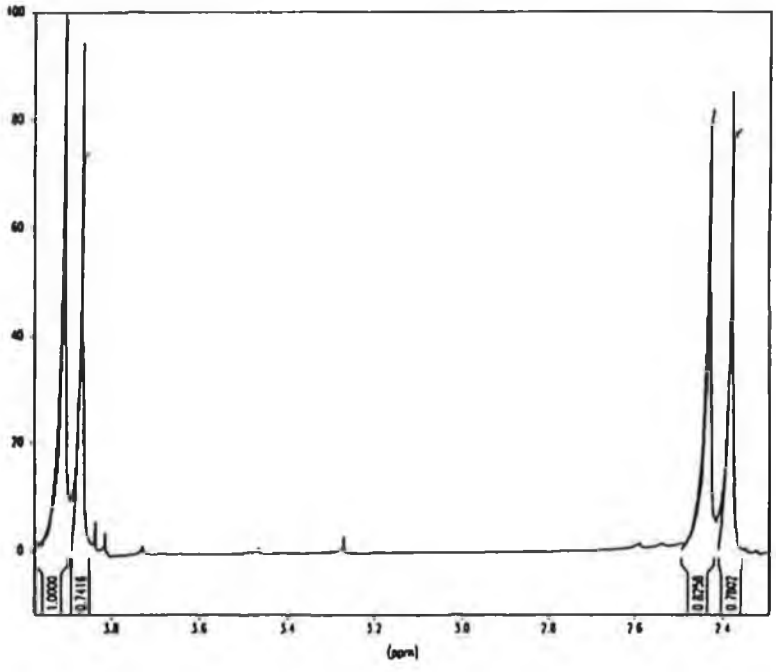


Figure 6

CH_3 group of acetylhydrazine and the peak at 4.0 from the OCH_3 group of phenylglyoxylic acid methyl ester. It was deduced that the pairs of singlets at 2.4 and 2.45, 3.85 and 3.9 ppm, and the singlets at 8.7 and 11.7 ppm were due to the product of the reaction. The spectrum of the reaction mixture after 60 minutes showed that the methoxy peak from the ester group of phenylglyoxylic acid methyl ester had almost disappeared while the peaks at 2.4 and 3.9 ppm in each of the original pairs of singlets began to dominate over those at 2.45 and 3.85 ppm and the peak at 11.7 dominated over that at 8.7 ppm. This situation continued up to 90 minutes and then appeared to remain unchanged upon further heating. These results suggested the presence of two isomeric products in the reaction mixture and that an equilibrium had been established between these products after 90 minutes. As acetylhydrazine was added in excess at the beginning of the reaction the peak at 2.0 ppm from the CH_3 group of acetylhydrazine did not vanish completely (Figure 7 and 8).

The product of this reaction was recrystallised from methanol and a sample of the filtered solid taken. A ^{1}H NMR spectrum was obtained which showed singlets at 2.45, 3.85 and 8.7 ppm (Me, OMe and NH respectively) and multiplets at 7.25 and 7.5 ppm (arom). This solid appeared to be the minor product of the reaction and the ^{1}H NMR spectrum was consistent with the structure of phenylglyoxylic acid methyl ester 2-acetylhydrazone. ^{1}H NMR spectra were obtained for a sample of the reaction mixture and a sample of this mixture spiked with the isolated solid. These spectra confirmed that the isolated solid was the minor product in the mixture. By the process of elimination it appeared from these spectra that the second product of the reaction was also the hydrazone derivative which suggested that this compound was present as two isomers (E- and Z- isomers) and that one isomer was dominant over the other (Figure 9).

^{1}H NMR spectrum of the hydrazone forming reaction mixture after 90 minutes

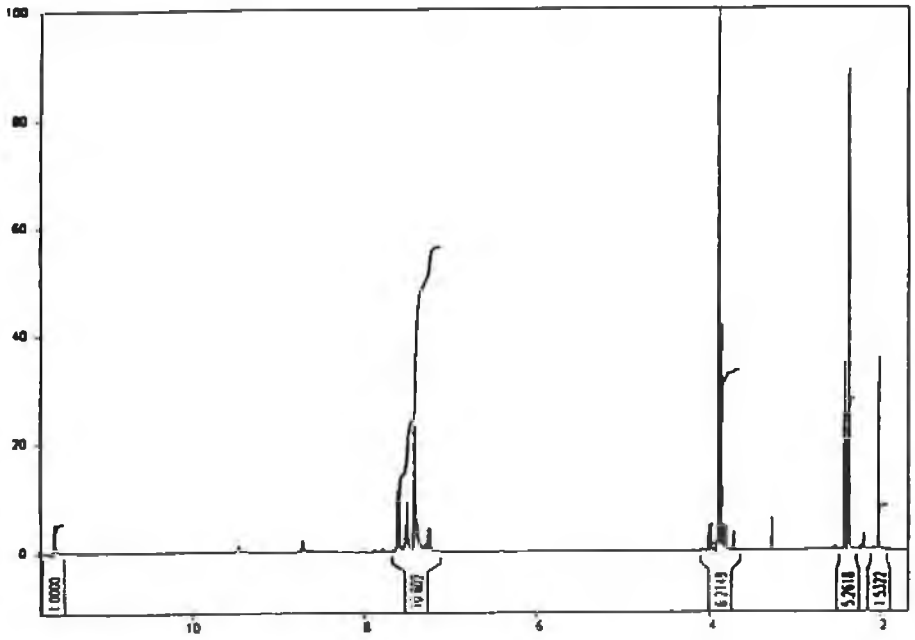


Figure 7

Expanded portion of the above spectrum

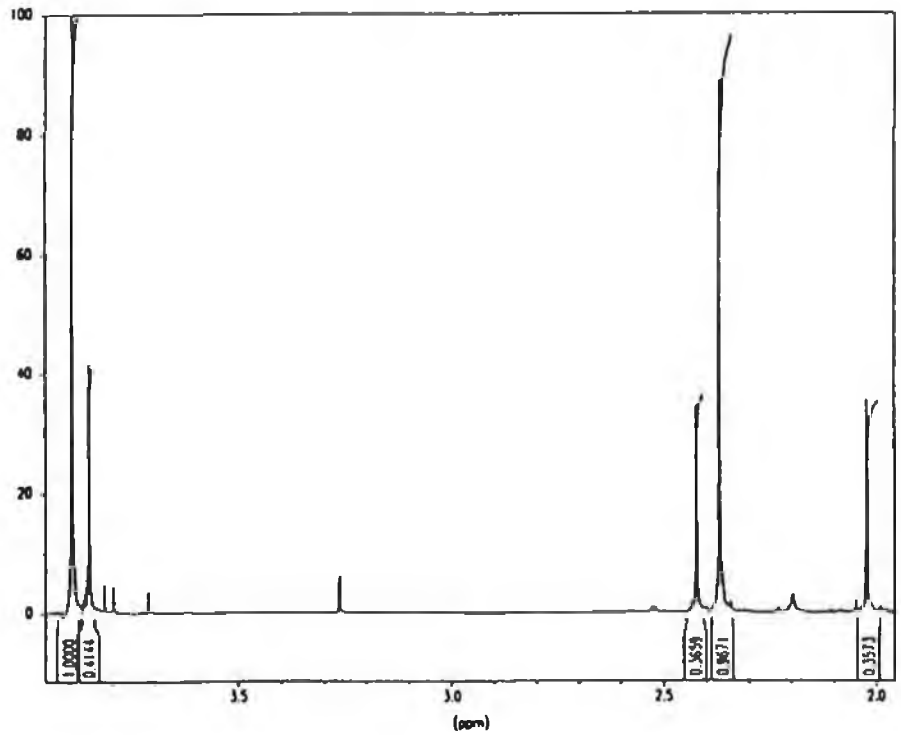


Figure 8

Isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone [24]

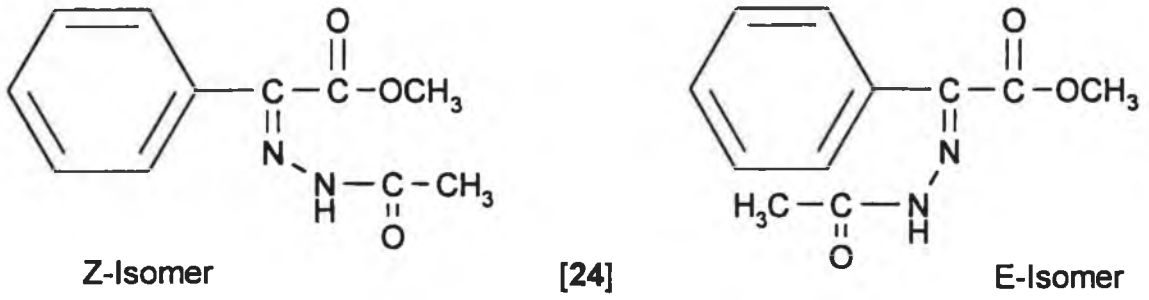


Figure 9

As NMR spectroscopy is not a practical method for monitoring reactions in industry and TLC is not an accurate method for monitoring these reactions (both isomers had similar Rf values), it was decided to develop a practical and accurate system for monitoring these reactions, namely, High Performance Liquid Chromatography (HPLC).

2.3.5 Analysis of the hydrazone forming reaction by HPLC:

This reaction was carried out and monitored by HPLC (Section 2.11) which showed the gradual disappearance of phenylglyoxylic acid methyl ester and a corresponding increase in the concentration of the isomers of the corresponding hydrazone derivative. After 45 minutes the phenylglyoxylic acid methyl ester peak had almost completely disappeared and the peak areas from both isomers were almost equal. Additional acetylhydrazine was added and the reaction conditions maintained for a further 60 minutes but the concentration of phenylglyoxylic acid methyl ester did not change. After 60 minutes one of the isomers (B) began to dominate over the other (A) (peak at 6.6 minutes (B) had a greater area than that at 2.8 minutes (A)). After 90

minutes an equilibrium appeared to be established between the two isomers (the ratio of peak areas did not change upon further heating) (Figure10).

This stage was repeated and at the formation of the equilibrium, a sample was taken from the reaction mixture and run against known weights of the standards of both isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone. These results showed that the reaction mixture contained isomer A (33%) and isomer B (52%) giving an overall yield of 85%.

Chromatogram of the hydrazone forming reaction at equilibrium

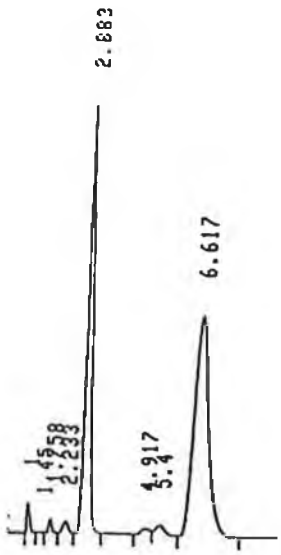


Figure 10

Isomer A and B of the hydrazone and phenylglyoxylic acid methyl ester at 2.9, 6.6 and 5.4 minutes respectively.

2.3.6 Isolation of isomers A and B of phenylglyoxylic acid methyl ester 2-acetylhydrazone:

In order to quantitatively monitor the reaction, samples of known purity of both isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone were

needed. This stage was repeated and monitored by HPLC and when equilibrium had been established the contents of the flask were recrystallised from methanol. HPLC analysis of the isolated solid showed that it consisted of only one of the isomers while ^{1}H and ^{13}C NMR spectra showed no trace of impurities. ^{1}H NMR spectroscopy and HPLC was used to determine its purity (99%) enabling its determination in the reaction mixture by HPLC.

The filtrate was concentrated and the resulting solid analysed by HPLC, which showed the presence of a small amount of the isolated isomer (A) with the remainder consisting of the second isomer (B).

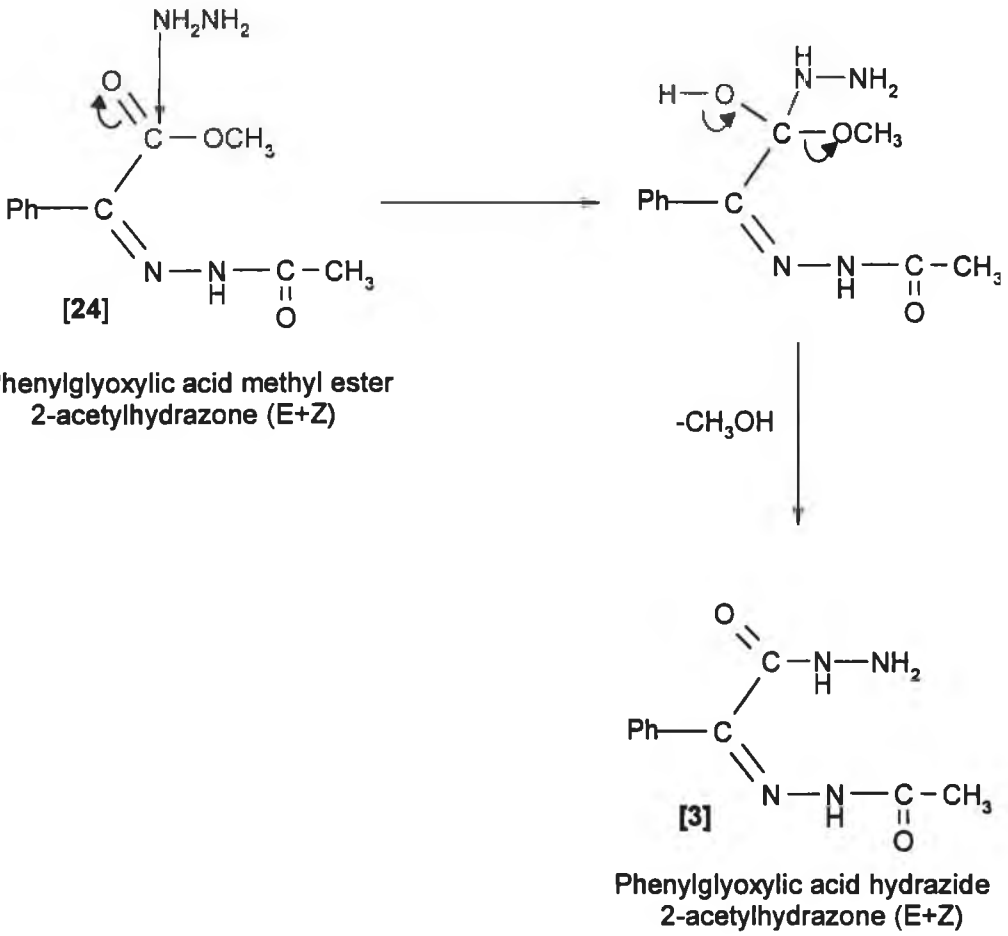
In an attempt to isolate this isomer (B), semi-preparative chromatography was used. The fractions collected were run on HPLC and those comprising only of this isomer (B) were combined and the solvent removed. The solid isolated was analysed by ^{1}H and ^{13}C NMR spectroscopy, which, along with elemental microanalysis and HPLC, confirmed its identity as the second isomer (B) of phenylglyoxylic acid methyl ester 2-acetylhydrazone. Its purity was determined as 99% by ^{1}H NMR spectroscopy and HPLC.

Although semi-preparative chromatography enabled isolation of a pure sample of the second isomer (B), the quantity isolated was negligible. Work with recrystallisation from various solvents (methanol, ethanol, butan-1-ol, propan-2-ol, light petroleum (b.p. 40-60 °C), ethyl acetate, acetonitrile and acetone), and mixtures of these enabled a process to be developed which isolated larger quantities of this isomer. The isolated solid was found to be approximately 99% pure as determined by HPLC and ^{1}H NMR spectroscopy.

2.4 Synthesis of phenylglyoxylic acid hydrazide 2-acetylhydrazone:

2.4.1 General:

This stage involved the reaction of phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**] with hydrazine monohydrate which displaced the methoxy group forming phenylglyoxylic acid hydrazide 2-acetylhydrazone [**3**], with methanol and water (from hydrazine monohydrate) as the only by-products of the reaction (Scheme 3).[10,11,23-27]



Scheme 3

2.4.2 Initial synthesis:

The crude product from the previous reaction, containing phenylglyoxylic acid methyl ester 2-acetylhydrazone, was reacted with hydrazine monohydrate (1:1 molar ratio initially as references varied considerably from 1:1 to 1:2), in butan-1-ol, at room temperature. A white precipitate was isolated and analysis by TLC showed that reaction did not appear to proceed beyond 45 minutes while some phenylglyoxylic acid methyl ester 2-acetylhydrazone still remained. Further heating did not alter this situation.

2.4.3 Optimisation of the reaction:

Further investigation of the reaction by TLC showed that a molar ratio of 1:1.2 between phenylglyoxylic acid methyl ester 2-acetylhydrazone and hydrazine monohydrate was needed for the reaction to proceed to completion after 45 minutes. It was also determined that a temperature of 35°C was the optimum temperature for reaction to be complete after 30 minutes.

2.4.4 Analysis of the reaction by HPLC:

With a molar ratio of phenylglyoxylic acid methyl ester 2-acetylhydrazone to hydrazine monohydrate of 1:1, analysis by HPLC (Section 2.11) (Figure 11) showed the rapid decrease of both isomers of the hydrazone. The HPLC traces showed two new peaks which suggested the possible formation of the two isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone (isomers C and D) from the isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone (A and B respectively). Analysis of the isolated precipitate showed that it consisted entirely of one product (isomer D) while analysis of the mother liquor showed it to consist of isomer A of phenylglyoxylic acid methyl ester 2-acetylhydrazone and the products of the reaction (isomers C

and D of phenylglyoxylic acid hydrazide 2-acetylhydrazone). These results also confirmed that a 1:1 molar ratio of phenylglyoxylic acid methyl ester 2-acetylhydrazone and hydrazine monohydrate was not sufficient for the reaction to reach completion. Further investigation confirmed the suggestion from TLC that a 1:1.2 molar ratio was required for the reaction to proceed to completion.

Chromatogram of the hydrazide forming reaction after 15 minutes

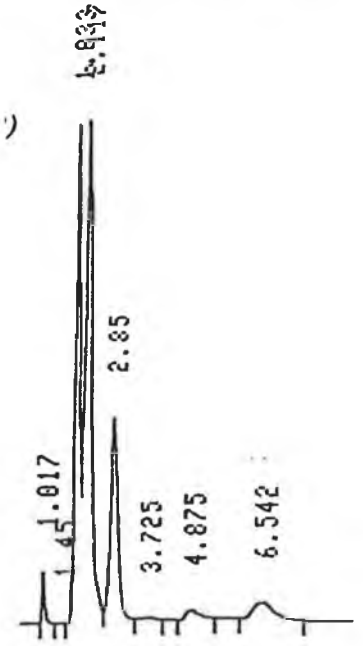


Figure 11

Both products of the reaction and isomers A and B of phenylglyoxylic acid methyl ester 2-acetylhydrazone at 1.8, 2.1, 2.8 and 6.5 minutes respectively.

At the end of the reaction the mixture was analysed using the HPLC conditions which provided better separation between the product(s) of the reaction (presumably the two geometrical isomers, C and D, of phenylglyoxylic acid hydrazide 2-acetylhydrazone) (Section 2.11) (Figure 12). Isomer B of the hydrazone eluted very late (38 minutes) using these

conditions and which were therefore used only on completion of the reaction when they showed that no trace of this isomer remained in the reaction mixture.

Chromatogram of the hydrazide forming reaction after 30 minutes

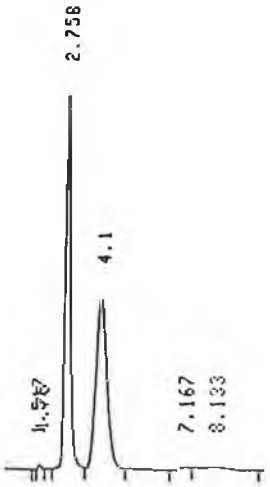


Figure 12

Both products of the reaction at 2.8 and 4.1 minutes respectively.

In an attempt to further increase the yields in the hydrazide and the Metamitron forming stages and to cut costs, the filtrates from both stages were used in subsequent reactions of these stages. However this resulted in the isolation of very impure products and low yields of phenylglyoxylic acid hydrazide 2-acetylhydrazone and Metamitron when purified. The solvents used in these reactions could be easily recovered, purified and reused in subsequent reactions but this may not prove to be economically viable.

2.4.5 Isolation of the products (presumed geometrical isomers) of phenylglyoxylic acid hydrazide 2-acetylhydrazone:

The precipitate formed at the end of the hydrazide forming stage was isolated by filtration and HPLC analysis showed it to consist of a single species (D). IR spectra showed NH_2 and two C=O absorptions at 3300, 1675, 1645 cm^{-1} respectively. ^1H NMR spectra showed singlets at 2.25 (Me), 4.6 (NH_2), 9.9 and 10.65 (N-H) and a group of peaks between 7.4 and 7.65 (arom) ppm, while ^{13}C NMR spectra of the solid showed singlets at 20.48 (Me), 142.61 (C=N), 163.76 (C=O), 172.83 (C=O) and a group of peaks at 127.81, 128.32, 129.43 and 130.54 (arom) ppm. These spectra, along with elemental microanalysis results, confirmed its identity as phenylglyoxylic acid hydrazide 2-acetylhydrazone. ^1H NMR spectroscopy and HPLC were used to determine its purity (98%). This solid could then be used to quantitatively determine the concentration of this product (isomer D) in the reaction mixture by HPLC. The filtrate contained a high concentration of the second product (presumably the other geometrical isomer of the hydrazide, C). This suggested that the entire reaction mixture at the end of the reaction should be carried forward to the next stage as isolation of the precipitate only, for use in the cyclisation stage, would result in the loss of much of the second product (C)

Isolation of the second product (isomer C) by extraction of the crude filtrate, after removal of D, with solvents (chloroform, hexane and light petroleum (b.p. 40-60 °C)) was attempted. The extracts were analysed by HPLC but none of the chromatograms showed any trace of the product (isomer C). Similarly after removal of all solvents from the filtrate, recrystallisation of the residue from various solvents (ethanol, acetonitrile, ethyl acetate and propan-2-ol) also failed to yield a pure sample. Flash

chromatography and semi-preparative chromatography also failed to yield a pure sample.

The individual isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone (A and B) on reaction with hydrazine monohydrate would each presumably yield the corresponding isomer of phenylglyoxylic acid hydrazide 2-acetylhydrazone. To determine whether this is the situation a pure sample of isomer A of the hydrazone was reacted with hydrazine monohydrate at 35 °C. This reaction yielded only one hydrazide (isomer C), as determined by HPLC. Reaction of isomer B of the hydrazone with hydrazine monohydrate yielded the second hydrazide (isomer D), as determined by HPLC. This suggested that the hydrazide derivative was also present as two geometrical isomers and that isomers A and B of the hydrazone derivative had the same geometrical configurations as isomers C and D of the hydrazide derivative respectively.

After the synthesis of isomer A to isomer C was complete, the solvent was removed on the rotary evaporator. Recrystallisation from different solvents (methanol, propan-2-ol, ethanol, acetonitrile and acetone) was attempted and it was found that heating the solid to boiling point in propan-2-ol and filtering the mixture while hot increased its purity. IR spectra showed NH_2 and two C=O absorptions at 3300, 1680, 1645 cm^{-1} respectively. ^1H NMR spectra showed singlets at 2.3 (Me), 4.5 (NH_2), 9.85 and 10.65 (N-H) and an aromatic group of peaks between 7.4 and 7.75 (arom) ppm while ^{13}C NMR spectra showed singlets at 20.45 (Me), 142.64 (C=N), 163.78 (C=O) and 172.74 (C=O) and an aromatic group of peaks at 127.78, 128.60, 129.45 and 130.57 (arom) ppm. These results along with elemental microanalysis results, which were consistent with the elemental composition of the suggested compound, confirmed its identity as phenylglyoxylic acid hydrazide 2-acetylhydrazone, while HPLC analysis

showed it to consist of the second species only confirming the earlier suggestion that the second species was a geometrical isomer of isomer D (isomer C). ^{1}H NMR and HPLC were used to assign a purity of 95% to the solid.

2.5 Synthesis of Metamitron:

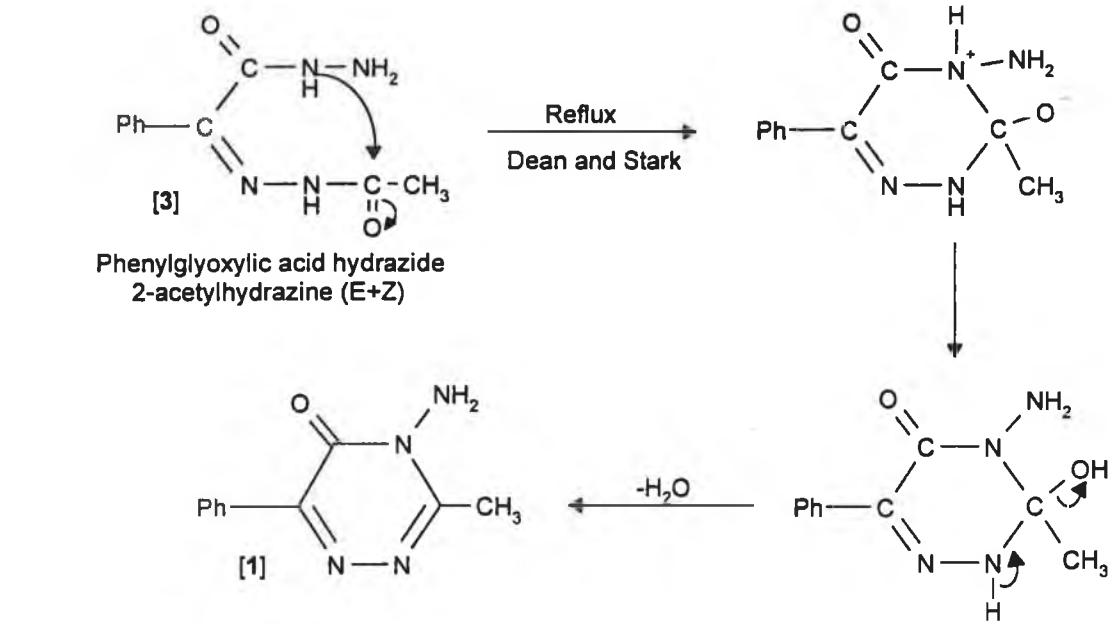
2.5.1 General:

The final stage of the synthesis involved ring closure of phenylglyoxylic acid hydrazide 2-acetylhydrazone to form Metamitron [1] with the other product of the cyclisation (water) being removed as it formed (Scheme 4). The cyclisation process involved the nucleophilic nitrogen of the hydrazide NH group and the electrophilic carbon of the hydrazone C=O group reacting to form a six membered heterocyclic ring. Although the NH_2 group in the hydrazide derivative would be expected to be more nucleophilic than the NH group, which has an electron withdrawing C=O group adjacent to it, the reaction involving the NH group is favoured due to the formation of a stable 6π electron ring.

2.5.2 Initial cyclisation:

The reaction was carried out with the crude reaction mixture from the previous stage by refluxing using a Dean and Stark condenser and was monitored by HPLC (Section 2.11) (Figure 13).[10,11,23-33] The results showed that isomer D of the hydrazide had cyclised fully to Metamitron after 2 hours. However the concentration of isomer C did not appear to change over 10 hours. The isolated product was found to be Metamitron by IR, ^{1}H and ^{13}C NMR spectroscopy, microanalysis and HPLC (Section 2.8).

A yield of 40% from phenylglyoxylic acid hydrazide 2-acetylhydrazone was obtained and as a result further investigation of the process was needed, as this yield would not be economically viable.



Scheme 4

The HPLC results suggested that isomer D was the Z-isomer of phenylglyoxylic acid hydrazide 2-acetylhydrazone (Z-hydrazide) [**3**a] as it readily cyclised to Metamitron [**1**] and isomer C was the E-isomer of the hydrazide derivative (E-hydrazide) [**3**b] which did not isomerise to the Z-isomer and as a result cyclisation to Metamitron did not occur (Figure 14).

Chromatogram shows the reaction mixture after 1 hour

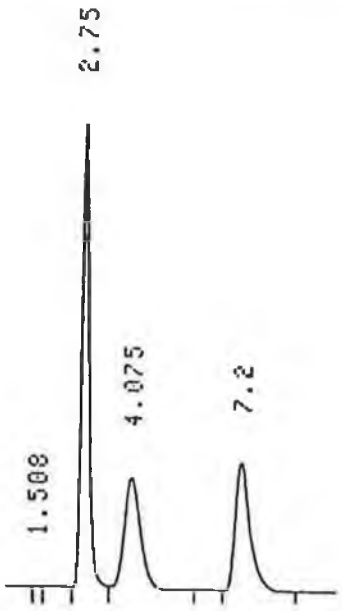


Figure 13

Isomers C and D of phenylglyoxylic acid hydrazide 2-acetylhydrazone and Metamitron at 2.8, 4.1 and 7.2 minutes.

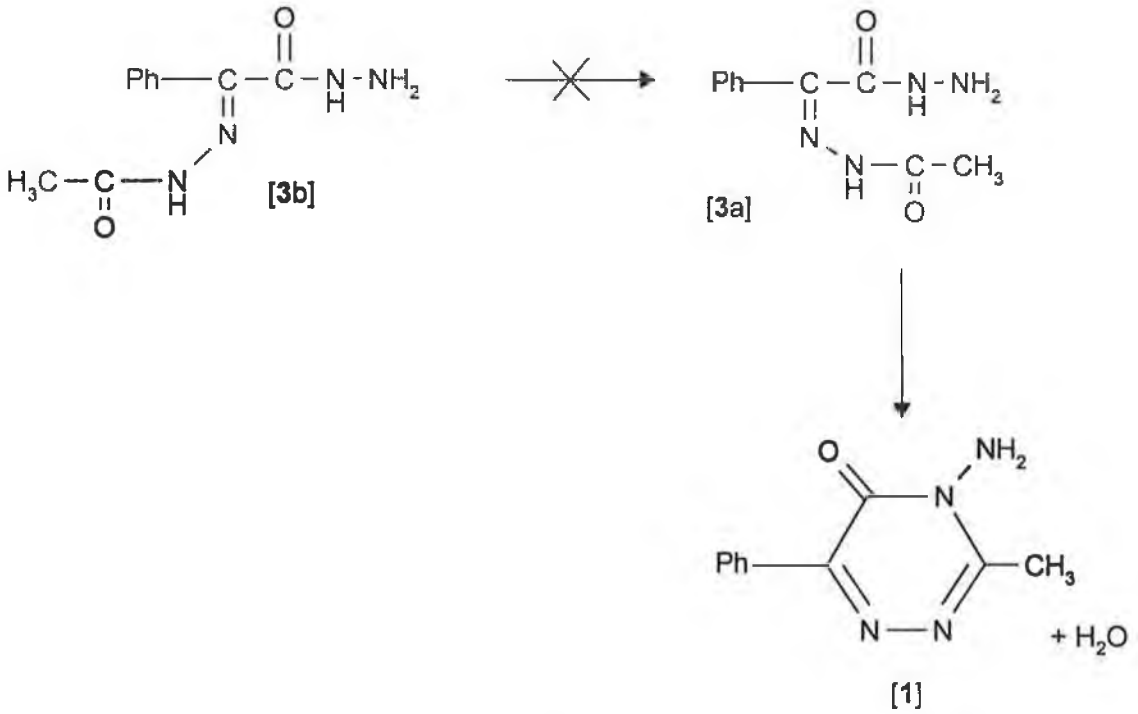
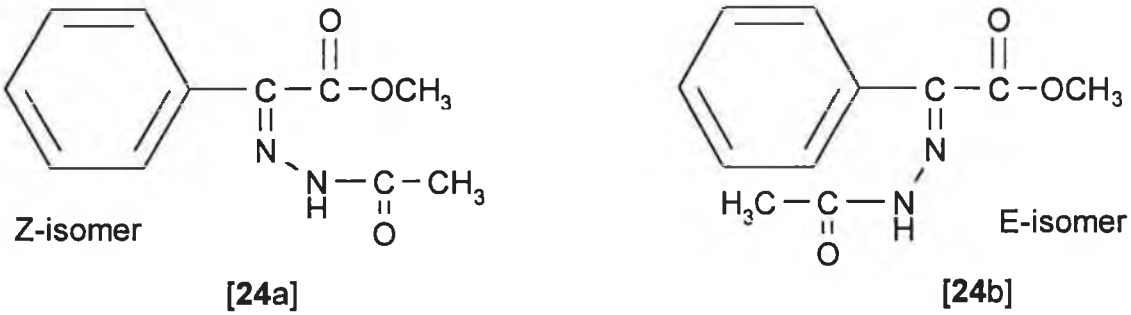


Figure 14

As isomer A of phenylglyoxylic acid methyl ester 2-acetylhydrazone readily formed isomer C of phenylglyoxylic acid hydrazide 2-acetylhydrazone on reaction with hydrazine monohydrate, and with isomer B of the hydrazone derivative forming isomer D of the hydrazide derivative, it appeared that isomers A and B were the E- and Z-isomers respectively of phenylglyoxylic acid methyl ester 2-acetylhydrazone (E-hydrazone and Z-hydrazone) [**24**b and **24**a] (Figure 15).

Isomers A and B of phenylglyoxylic acid methyl ester 2-acetylhydrazone



[**24**a] [**24**b]

Figure 15

Different concentrations of acids and bases were added to the mixture at the end of the hydrazide forming reaction in attempts to induce interconversion of the carbon-nitrogen double bond in the E-isomer. Each reaction was monitored by HPLC but the results showed that isomerisation did not occur.

2.6 Investigation into the lack of isomerisation of the E- to Z-hydrazide:

The amount of water in the filtrate (from the filtration of the hydrazide forming stage end-reaction mixture) was determined using the Karl-Fischer titration method. These results showed that water was present in approximately 1% of the total weight of the solution. The water was removed, using anhydrous magnesium sulphate followed by filtration. The resulting mixture was heated under reflux using a Dean and Stark condenser for 6.5 hours and monitored by HPLC. No change occurred, suggesting that water present did not interfere with the isomerisation process.

Further investigation of the isomerisation process was needed if yields of Metamitron were to be improved. Thermal isomerisations of this kind have been reported.[39] However the lack of isomerisation in the present case suggested the possible presence of a species in the hydrazide forming reaction mixture which hindered the rotation of the E-hydrazide (C) about the carbon-nitrogen double bond to give the corresponding Z-hydrazide (D). That this was the case was shown by cyclisation of a pure sample of the E-hydrazide as starting material. HPLC analysis showed the gradual disappearance of this isomer and the corresponding formation of Metamitron, over 6.5 hours, while a low concentration of the Z-hydrazide was present throughout this time, disappearing at the end of the reaction when the low concentration of the E-hydrazide no longer decreased (Figure 16 and 17).

The identification of this interfering species involved the addition of the reactants used in the process to individual pure samples of the E-hydrazide, in butan-1-ol, to determine their effect, if any, on the isomerisation process. The results showed that added acetylhydrazine or phenylglyoxylic acid methyl ester had no effect on the isomerisation process.

Chromatograms of the cyclisation reaction mixture after 4 hours (Figure 16) and 6.5 hours (Figure 17)

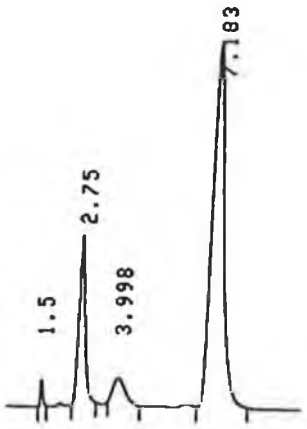


Figure 16

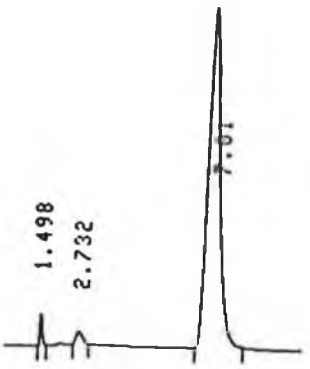


Figure 17

E- and Z-Isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone and Metamitron at 2.7, 4.0 and 7.0 minutes respectively.

However the presence of added hydrazine monohydrate prevented isomerisation from taking place. As an excess of hydrazine monohydrate is used in the hydrazide forming stage it may be present in the reaction mixture during the cyclisation stage.

The reaction mixture during the hydrazide forming stage was analysed for hydrazine monohydrate content using a derivatisation technique (Section 2.13)[40,41] and the concentration of hydrazine monohydrate was found to be approximately the same as the excess added initially.

In an attempt to remove excess hydrazine monohydrate from the mother liquor, an excess of benzaldehyde was added and the resulting mixture heated, under reflux, for 6.5 hours. A sample was analysed by HPLC and the results showed no change in the concentration of the E-hydrazide from the original solution. Other attempts to remove hydrazine monohydrate from the reaction mixture also proved unsuccessful.

2.7 Attempts to increase yield of Metamitron:

2.7.1 By increasing concentration of Z-isomer of phenylglyoxylic acid hydrazide 2-acetylhydrazone :

Previous results showed that the E-hydrazone could readily form the E-hydrazide when reacted with hydrazine monohydrate and parallel conversion occurred for the Z-isomer. As the Z-hydrazide readily cyclised to Metamitron, increasing the concentration of this isomer in the reaction mixture should increase the yield of Metamitron in the cyclisation stage.

The hydrazone forming stage was carried out using methanol as solvent and this reaction was monitored by HPLC. On the establishment of the equilibrium between the E- and Z-hydrazones, the reaction mixture was cooled to room temperature and the precipitate isolated. The filtrate contained a high concentration of Z-hydrazone while the solid consisted of the E-hydrazone only. The filtrate was retained and the solid was added to methanol and refluxed until equilibrium was established. The precipitate was again isolated and the filtrate retained. This process was repeated until no precipitate formed (after establishment of equilibrium) and the filtrates were then combined. This resulted in a solution, which contained a considerably greater concentration of Z- than E-hydrazone. This solution was carried forward to the hydrazide forming stage and it was observed that at the end of this stage the reaction mixture consisted of a corresponding higher concentration of Z-hydrazide, which was then carried forward to the cyclisation stage.

The cyclisation process was monitored by HPLC and the results showed a gradual decrease in the concentration of the Z-hydrazide and the gradual formation of Metamitron. However the concentration of the E-hydrazide also increased. The concentration of Metamitron in the reaction mixture after 6.5 hours was determined by HPLC and the cyclisation was

found to have reached only 50% completion. Further refluxing of the reaction mixture did not alter this situation. Although an increase in the yield of Metamitron was achieved (approx. 10% increase) the method was not practical for industrial scale synthesis.

These results suggested that some conversion of Z- to E-hydrazide occurred at the same time as cyclisation of the Z-hydrazide to Metamitron. However this conversion did not appear to be occurring in the opposite direction due to the presence of hydrazine monohydrate in the reaction mixture, as discussed earlier (Section 2.6).

The procedures for the hydrazone and hydrazide forming reactions were repeated as above by increasing the concentration of the Z-hydrazide. In addition as the cyclisation of the Z-hydrazide to Metamitron readily occurred, lower temperatures were used in this stage in order to try to limit the isomerisation of Z- to E-hydrazide. However the yield of Metamitron was not increased and longer run times were necessary.

2.7.2 By isolation of phenylglyoxylic acid hydrazide 2-acetylhydrazone:

It was decided to develop a system to enable the isolation of the solid hydrazide product in order to eliminate hydrazine monohydrate from it by washing.

Further investigation of the influence of different solvents on the yield of phenylglyoxylic acid hydrazide 2-acetylhydrazone indicated that using a weight of propan-2-ol to crude phenylglyoxylic acid methyl ester 2-acetylhydrazone of 1.1:1 gave the optimum yield of hydrazide. Traces of hydrazine monohydrate were still present in the mixture. The product formed in this way was difficult to manage and so an equal volume of light petroleum (b.p. 40-60 °C) was added and the mixture stirred vigorously until

a manageable suspension formed. After filtration, the solid collected was washed thoroughly with cold propan-2-ol (-10°C) to remove the remaining traces of hydrazine monohydrate. Analysis of the solid by HPLC derivatisation showed no detectable trace of hydrazine monohydrate. An 85% yield of phenylglyoxylic acid hydrazide 2-acetylhydrazone was obtained.

The final stage was carried out on this solid. Almost complete cyclisation of phenylglyoxylic acid hydrazide 2-acetylhydrazone to Metamitron had occurred after 6.5 hours (95%). Using butan-1-ol in 2.1 times the weight of the hydrazide gave the optimum yield of Metamitron. The solid product was washed with cold propan-2-ol and light petroleum (b.p. 40-60 °C) to increase its purity.

2.8 Characterisation of Metamitron:

The Metamitron obtained was characterised using IR, ^{13}C and ^{1}H NMR spectroscopy, microanalysis, HPLC and melting point. The IR spectrum showed a N-H bend at 1600cm^{-1}, C=N, C=O and NH_2 absorptions at 1550, 1675 and 3200 cm^{-1} respectively (Figure 19), consistent with those in the literature reference, as was the melting point range of 165-167 °C (literature value 166.6 °C).[38] The ^{13}C NMR spectrum showed the presence of two C=N groups, a CH_3 and a C=O group at 151.50 and 151.63, 19.04 and 154.84 ppm respectively, with aromatic peaks between 128.26 and 132.60 ppm (Figure 20). The ^{1}H NMR spectrum indicated the presence of CH_3 and NH_2 groups with peaks at 2.7 and 5.2 ppm respectively and an aromatic group with peaks at 7.5 and 8.2 ppm, with integration ratios of 3:2:5 between these groups, consistent with the hydrogen count for CH_3, NH_2 and a phenyl group in Metamitron **[1]**. There also appeared to be an impurity present with a peak at 2.2 ppm, probably due to residual traces of acetone used for washing the

NMR tube, while the peak at 7.39 ppm was due to chloroform (Figure 21). The phenyl hydrogens produced two sets of peaks with integration ratios of 3:2 at 7.5 and 8.2 ppm respectively, consistent with a monosubstituted benzene ring which gave rise to two sets of equivalent hydrogens, one set at carbons 2 and 6 and the second set at carbons 3, 4 and 5 of Metamitron **[1]** (Figure 18).

Metamitron

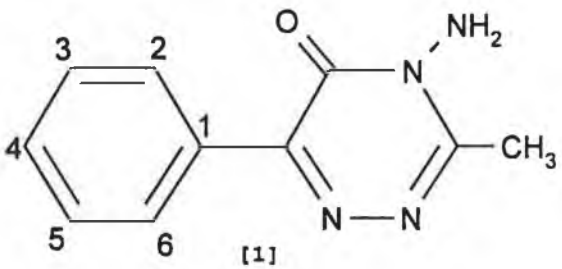


Figure 18

Infra Red spectrum of Metamitron

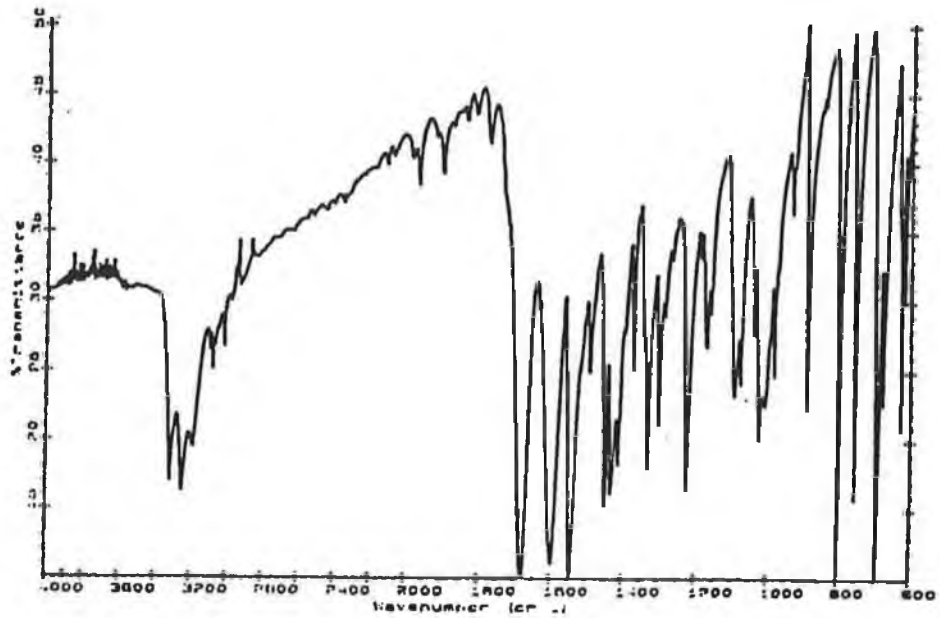


Figure 19

^{13}C NMR spectrum of Metamitron in $CDCl_3$

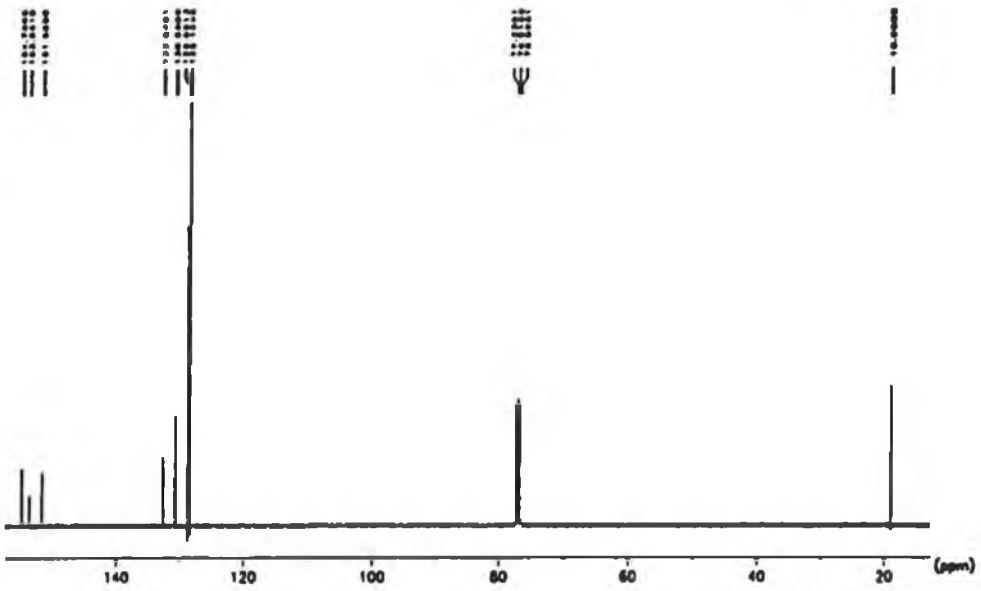
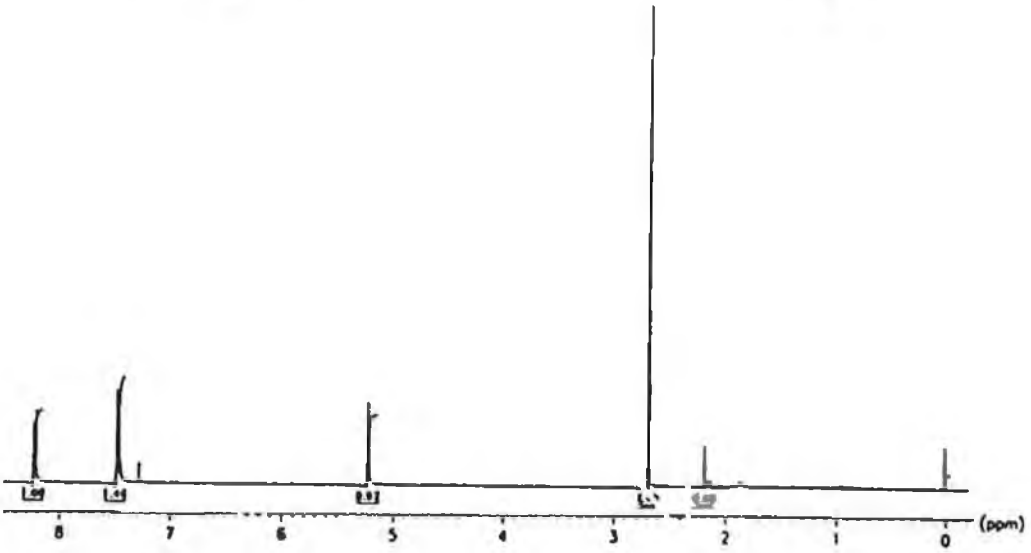


Figure 20

^{1}H NMR spectrum of Metamitron in $CDCl_3$



Chloroform, acetone and TMS peaks at 7.39, 2.2 and 0.0 ppm respectively

Figure 21

2.9 Five batch Analysis of Metamitron:

Five batches of Metamitron were analysed by HPLC, which showed no trace of any of the intermediates. Their purity was determined by HPLC (Section 2.11) which showed that the purity of these batches was as follows:

Batch 1 - 98.3%
2 - 98.4%
3 - 99.4%
4 - 99.2%
5 - 98.4%.

The only significant impurity found was water, which was determined by Karl-Fischer titration. This impurity along with Metamitron accounted for over 100% of the material.

All batches of Metamitron produced were found to have a purity greater than 98% which met the acceptable criteria (>97%). Analytical methods were successfully developed for monitoring each stage of the process and standards of each of the intermediates were isolated for their determination in the reaction mixtures. The yields of Metamitron were sufficient to make the process economically viable. All the criteria set out for this project were met which brought it to a conclusion.

2.10 Development of TLC methods for monitoring the reactions:

The hydrazone forming reaction was be monitored by TLC by dissolving a sample in ethyl acetate and using ethyl acetate and light petroleum (b.p. 40-60 °C) [0.5:9.5] as eluting solvent. The phenylglyoxylic acid methyl ester had a Rf value of 0.5 while the product of the reaction, phenylglyoxylic acid

methyl ester 2-acetylhydrazone, had an Rf value of 0.1, which enabled the reaction progress to be followed.

The hydrazide forming reaction was monitored by TLC using ethyl acetate and light petroleum (b.p. 40-60 °C) [9:1] as eluting solvent. Phenylglyoxylic acid methyl ester 2-acetylhydrazone had an Rf value of 0.7 while the product of the reaction, phenylglyoxylic acid hydrazide 2-acetylhydrazone, had an Rf value of 0.2.

The cyclisation stage was monitored by TLC using ethyl acetate and light petroleum (b.p. 40-60 °C) [9:1] as eluting solvent. The results showed that the product of the reaction (Metamitron) had an Rf value of 0.7 while phenylglyoxylic acid hydrazide 2-acetylhydrazone had an Rf value of 0.2.

2.11 Development of HPLC methods for monitoring the reactions:

It was necessary to develop HPLC methods for monitoring each of the reactions more closely as TLC methods did not separate the isomers of the intermediates. The methods were developed based on a CIPAC method, which comprises of a Lichrosorb RP8 column (25 x 0.46 cm), a UV detector wavelength of 254 nm and a flow rate of 2 ml/min.[38]

A mobile phase consisting of methanol and water (60:40) was used to determine the hydration and methanolysis reactions as this mobile phase gave the best compromise between resolution and peak shape for the reactants and products in these stages (Figure 22 and 23).

Chromatograms of the hydration reaction initially (Figure 22) and the methanolysis reaction at completion (Figure 23)

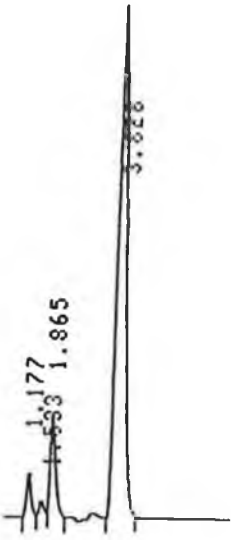


Figure 22

Figure 23

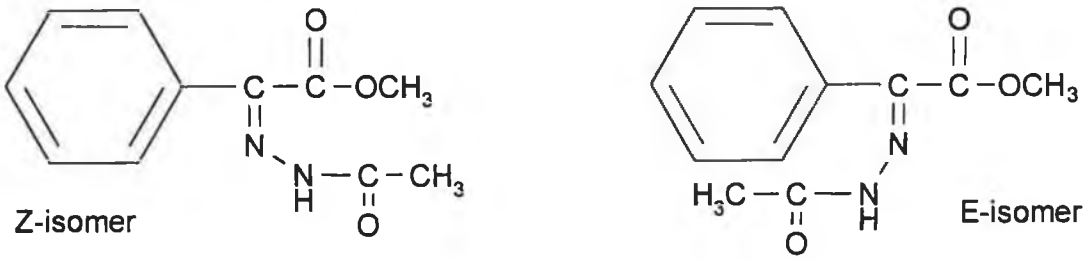
Phenylglyoxylonitrile and phenylglyoxylic acid methyl ester at 3.8 and 3.0 minutes respectively.

The reaction time for the phenylglyoxylic acid amide forming reaction was determined by observing the disappearance of the phenylglyoxylonitrile peak, as the amide was fully retained on the column and therefore not detected, while the phenylglyoxylic acid methyl ester forming reaction run time was determined by calculating the concentration of the phenylglyoxylic acid methyl ester present in the reaction mixture at regular intervals until it no longer increased with time. This was achieved by comparison with a known concentration of an external standard. From this the total weight of ester present in the reaction mixture at any given time was determined.

A HPLC method for monitoring the hydrazone forming reaction was developed. This separated all components of the reaction mixture. A mobile phase consisting of methanol and water (50:50) was found

to be most suitable as the retention times were short, baseline resolution was achieved and peak shape was good. Although the phenylglyoxylic acid methyl ester peak at 4.5 minutes was broad, it was of less diagnostic value than the hydrazone peaks, as its disappearance only was of interest (Figure 24 and 25).

Phenylglyoxylic acid methyl ester 2-acetylhydrazone



[24a] [24b]

Figure 24

The same conditions for monitoring this reaction were used to monitor the hydrazide forming reaction and both sets of isomers were detected (Figure 26). On completion of the reaction a different method was developed to achieve better resolution (Figure 28) between the E- and Z-hydrazides (Figure 27). This method was not used during the reaction as the Z-hydrazone had a long retention time. This new method used a mobile phase consisting of an aqueous sodium dihydrogen phosphate buffer solution (2 g/l) and methanol (70:30).

Chromatogram of the hydrazone forming reaction after 30 minutes

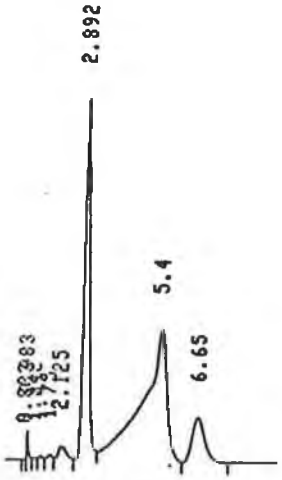


Figure 25

E- and Z-isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone and phenylglyoxylic acid methyl ester peaks at 2.9, 6.65 and 5.4 minutes respectively.

Chromatogram shows the hydrazide forming reaction after 10 minutes

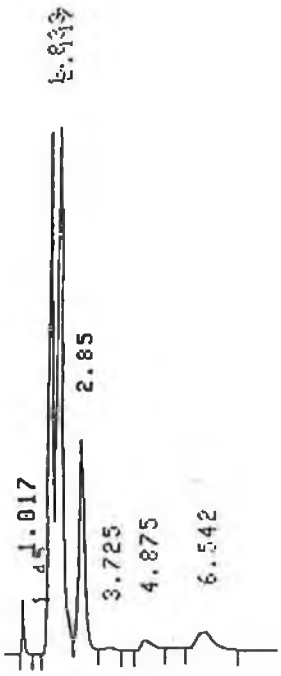
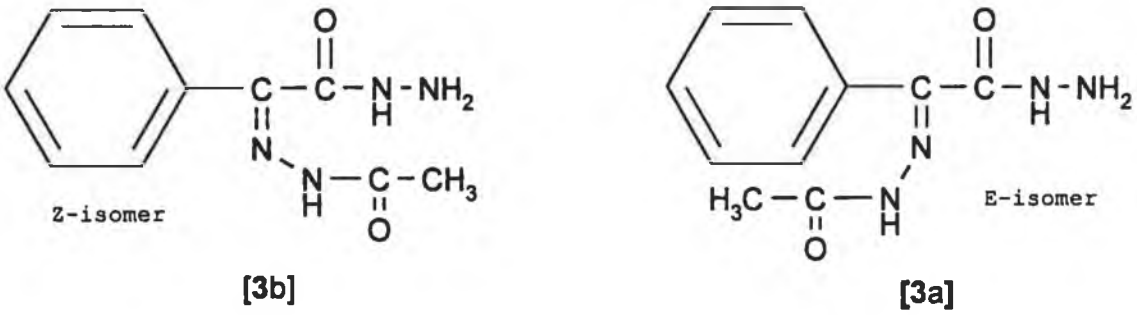


Figure 26

E- and Z-isomers of hydrazide and E- and Z-isomers of hydrazone derivatives at 1.8, 2.1, 2.8 and 6.5 minutes respectively.

Phenylglyoxylic acid hydrazide 2-acetylhydrazone



[3b] [3a]

Figure 27

Chromatogram at the end of the hydrazide forming reaction

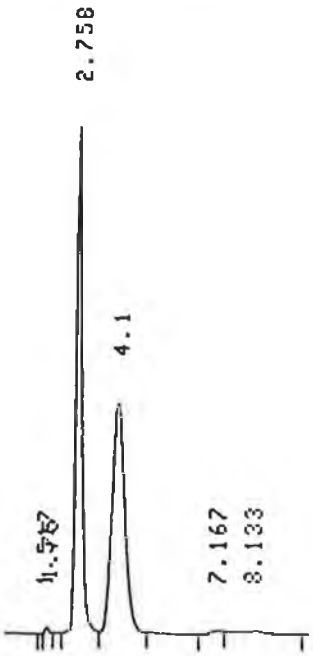


Figure 28

E- and Z-Isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone at 2.7 and 4.1 minutes respectively.

The buffer:methanol (70:30) mobile phase was used to monitor the final cyclisation process to yield Metamitron, as short retention times and good baseline resolution were achieved (Figure 29).[38]

Chromatogram of the cyclisation process after 1 hour

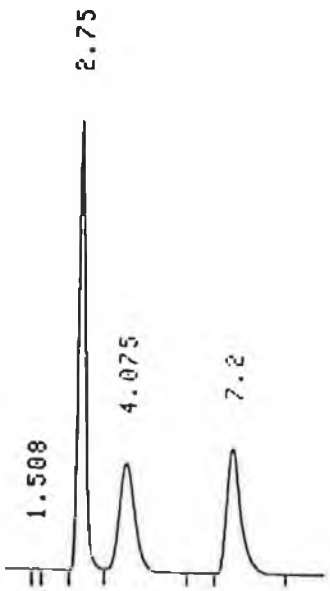
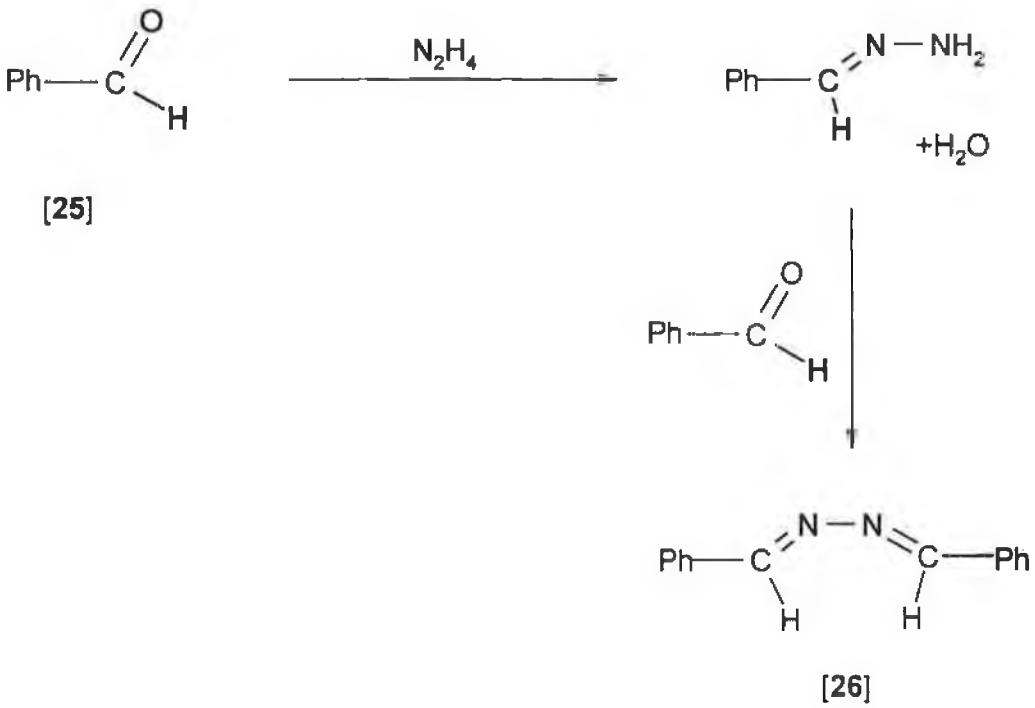


Figure 29

E- and Z-isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone and Metamitron at 2.7, 4.1 and 7.2 minutes respectively.

2.12 Determination of hydrazine monohydrate in the reaction mixture:

The method used for the determination of hydrazine monohydrate in the hydrazide forming reaction mixture involved the derivatisation of hydrazine with benzaldehyde [**25**], forming benzalazine [**26**], followed by extraction into heptane.[40,41] This derivative could then be detected using ultra-violet spectroscopy. This enabled the determination of hydrazine monohydrate in the reaction by comparison with a known quantity of a derivatised standard of hydrazine monohydrate under the same conditions (Figure 30).



Chromatogram of the reaction mixture after derivatisation.

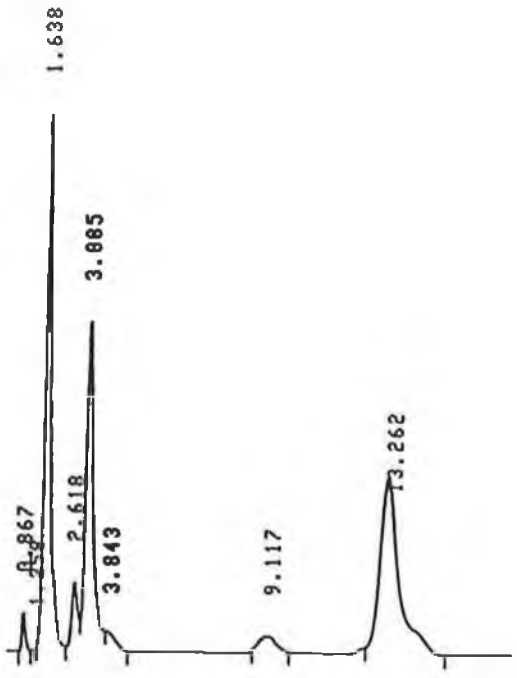


Figure 30

E- and Z-isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone, benzalazine and benzaldehyde at 1.6, 3.8, 9.1 and 13.2 minutes respectively.

2.13 Development of a method for determination of reaction precursors in Metamitron:

It was necessary to develop a method to accurately quantify impurities, which may be present in the synthesised Metamitron. As gas chromatography is an effective method for the determination of small quantities of compounds, it was decided to develop a GC method for analysing Metamitron for any impurities which may be present.

Each set of isomers and Metamitron was dissolved in chloroform and injected (1 μl) onto a Capillary DB5 (30 m x 0.25 mmi.d.) column at temperatures between 140 and 280 °C. The E- and Z-hydrazones were well separated (12.4 and 13.2 minutes respectively) while the E- and Z-hydrazides and Metamitron eluted together (20.8 minutes). The temperature of the column was varied in an attempt to separate these compounds but no separation could be achieved. As this situation is quite uncommon in GC it was deduced that, at the column temperature used, isomerisation and cyclisation of the E- and Z-hydrazides was occurring resulting in only Metamitron being present when the compounds reached the FID. As a result it was concluded that HPLC was the best method available for the determination of impurities in Metamitron.

The determination of the impurities in five batches of synthesised Metamitron was carried out using HPLC. A method was developed which simultaneously separated each of the intermediates and Metamitron. A mobile phase consisting of methanol, acetonitrile and aqueous sodium dihydrogen phosphate buffer (2 g/l) (20:10:70) gave acceptable resolution between each of the components and reasonable peak shapes (Figure 31).

HPLC trace of Metamitron and intermediates

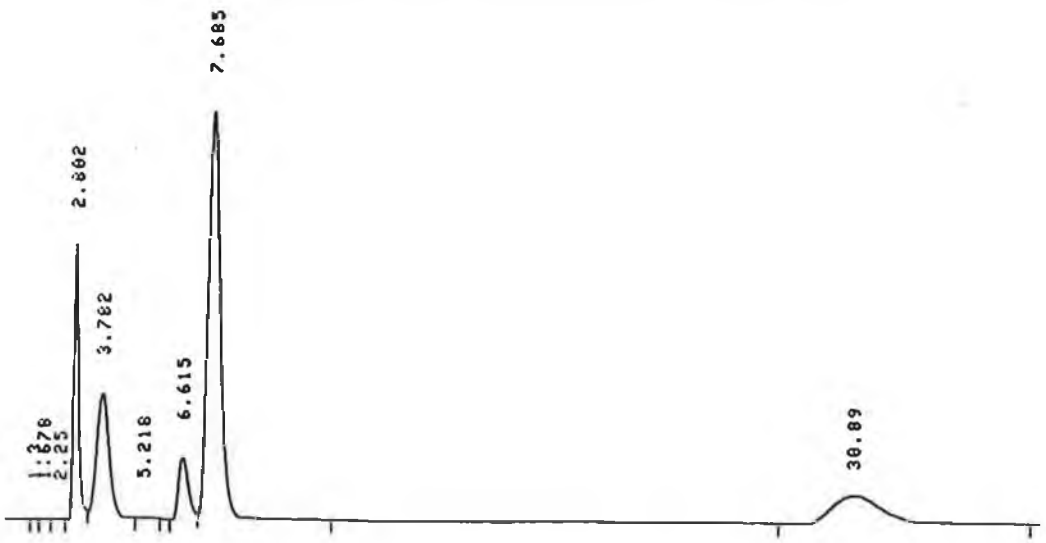


Figure 31

E- and Z-hydrazones, E- and Z-hydrazides and Metamitron at 2.8, 3.8, 7.7, 30.9 and 6.6 minutes respectively.

2.14 Summary of the entire process:

The optimised synthesis of Metamitron from phenylglyoxylonitrile was carried out as follows:

Phenylglyoxylonitrile was hydrated with sulphuric acid (85%) in the presence of acetic anhydride and sodium bromide, at 45 °C, with vigorous stirring for 3 hours. Methanol was then added and the resulting mixture was refluxed with vigorous stirring for 3 hours. The excess methanol was removed and the product, phenylglyoxylic acid methyl ester, was isolated by distillation.

Acetylhydrazine was then reacted with phenylglyoxylic acid methyl ester in a ratio of 1.1:2, at 80 °C, with vigorous stirring for 90 minutes. The water produced by the reaction was removed by distillation, under reduced pressure, as it formed. The product, phenylglyoxylic acid

methyl ester 2-acetylhydrazone, was obtained in an 85% yield (33% Z-isomer, 52% E-isomer).

The phenylglyoxylic acid methyl ester 2-acetylhydrazone was then reacted with hydrazine monohydrate in a ratio of 1:1.2, at 35 °C, with vigorous stirring, in propan-2-ol, for 30 minutes. The product was washed with light petroleum (b.p. 40-60 °C) and cold propan-2-ol which yielded phenylglyoxylic acid hydrazide 2-acetylhydrazone (92%) (55% Z-isomer, 37% E-isomer). The weight of propan-2-ol used was 1.1 times the weight of the hydrazone obtained.

The isolated solid, phenylglyoxylic acid hydrazide 2-acetylhydrazone, was refluxed in butan-1-ol, using a Dean and Stark condenser, for 6.5 hours. The weight of butan-1-ol used was 2.1 times the weight of the hydrazide. The solid formed on cooling was washed with cold propan-2-ol and light petroleum (b.p. 40-60 °C) yielding Metamitron (83%) with a purity of greater than 98% (overall yield: 36% from phenylglyoxylonitrile [**9**], 65% from phenylglyoxylic acid methyl ester [**12**]).

EXPERIMENTAL

3.1 General introduction:

The NMR spectrometer used throughout this work was a Bruker AC-400 instrument. The ^{1}H NMR spectra were measured at a frequency of 400 MHz, while the ^{13}C NMR spectra were measured at a frequency of 100 MHz. The IR spectrometer used was a Nicolet 205 FT-IR instrument and spectra were obtained from 600 to 4000 cm^{-1}. The UV spectrophotometer used was a Hewlett Packard 845 2A diode array UV-VIS instrument and the spectra between 200 and 900 nm obtained. The extinction coefficients (ε) are reported in units of dm^{-3} mol^{-1} cm^{-1}.

The High Performance Liquid Chromatography system used was a Waters 501 HPLC pump, with a Shimadzu SPD-6A UV spectrophotometric detector and a Shimadzu CR68 Chromatopac integrator. The conditions under which the chromatograms were obtained were as follows:

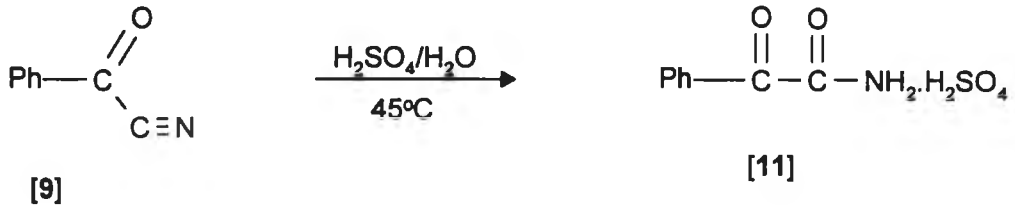
Column: Lichrosorb RP8, 10 μm, 25 x 0.46 cm; Injection volume: $2.0x10^{-2}$ cm^3; Flow rate: 2.0 cm^3/minute; Detector wavelength: 254 nm. The mobile phase was varied to optimise the separation of the individual components in each of the reactions. The buffer solution used consisted of aqueous sodium di-hydrogen phosphate (2 g/l).

Note: A guard column was used throughout this project to protect the analytical column.

Melting point ranges were recorded using a Gallenkamp Melting Point apparatus and are uncorrected. Elemental analyses were carried out by the Microanalytical Laboratory at University College Dublin.

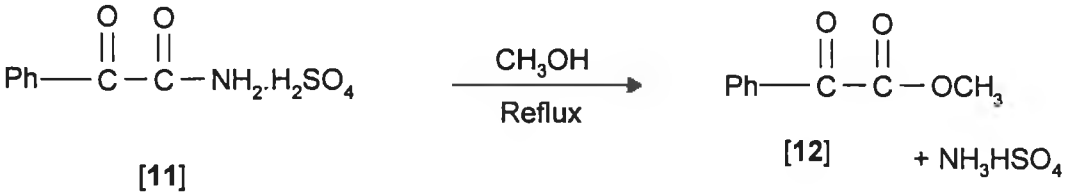
3.2 Synthesis of 4-amino-3-methyl-6-phenyl-1,2,4-triazin-5-one:

3.2.1 Synthesis of phenylglyoxylic acid methyl ester:



[9] [11]

Phenylglyoxylonitrile [**9**] Phenylglyoxylic acid amide [**11**]



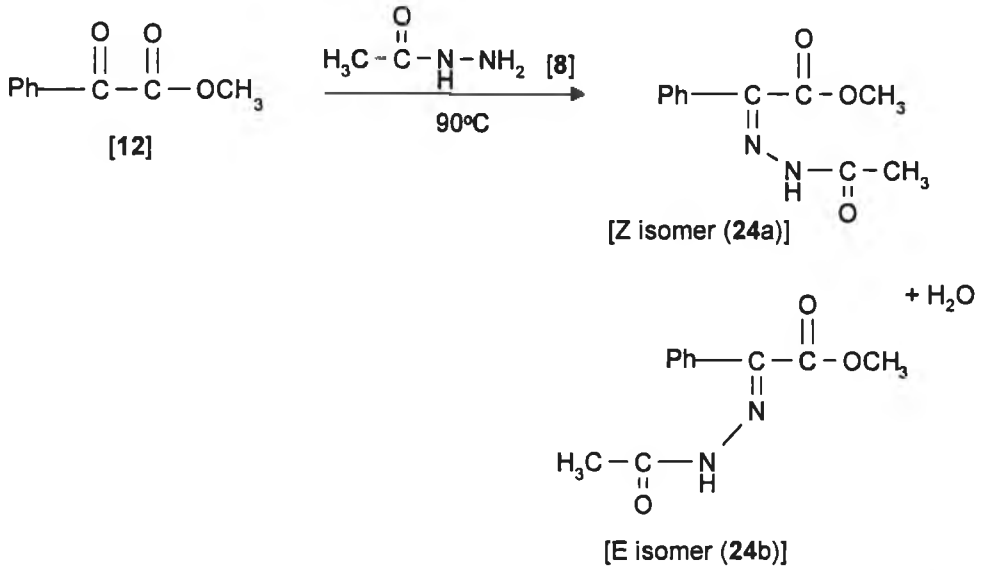
[11] [12]

Phenylglyoxylic acid amide [**11**] Phenylglyoxylic acid methyl ester [**12**]

Sulphuric acid (85%,11.65 cm^3) was placed in a round bottom flask (50 cm^3) fitted with a dropping funnel (50 cm^3) which had been heated to 70 °C. Acetic anhydride (10.40 g, 0.102 mol) was added along with a few grains of sodium bromide. This mixture was then stirred vigorously at room temperature for 5 minutes. Phenylglyoxylonitrile [**9**] (13.1 g, 0.100 mol) was heated to 45 °C and then added slowly to the flask from the heated dropping funnel with continuous stirring and the resulting mixture stirred vigorously at 45 °C for 3 hours. A reflux condenser was attached to the flask and methanol (10 cm^3) was added. The resulting mixture was heated under reflux with vigorous stirring for 3 hours. Excess methanol was removed on the rotary

evaporator, water (20 cm^3) was added and the mixture thoroughly shaken. After allowing to settle for 30 minutes the organic layer was decanted off and transferred to a round bottom flask (50 cm^3) fitted with a vacuum distillation arrangement. The contents of the flask was distilled under reduced pressure (10 mmHg) and a boiling fraction (77 ^oC) yielded phenylglyoxylic acid methyl ester [**12**] (10.1 g, 0.061 mol, 61%) which had a purity of 95%, as determined by HPLC by comparison with a known weight of certified reference material. Comparison of its IR, ^1H NMR and ^{13}C NMR spectra with those of an authentic sample of phenylglyoxylic acid methyl ester confirmed the identity of the product. IR υ_{max} 3300, 1740, 1690 cm^{-1}; ^1H NMR: ($CDCl_3$), δ 3.9 (s, 3H, OMe), 7.45 (t, 2H, arom), 7.60 (t, 1H, arom), 8.0 (d, 2H, arom) ppm; ^{13}C NMR: δ 52.29 (OMe), 128.51 (arom), 129.55 (arom), 131.90 (arom), 134.62 (arom), 163.74 (C=O), 185.77 (C=O) ppm.

3.2.2 Synthesis of phenylglyoxylic acid methyl ester 2-acetylhydrazone:



Phenylglyoxylic acid methyl ester [**12**]

Phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**]

3.2.2.1 Initial reaction:

Acetylhydrazine (5.00 g, 0.068 mol) was reacted with phenylglyoxylic acid methyl ester (11.10 g, 0.068 mol) at 80 °C, with vacuum distillation to remove the water produced. The reaction mixture was stirred vigorously until no more water was being produced by the reaction (approx. 60 minutes). The reaction proceeded to approximately 75% completion.

3.2.2.2 Optimised reaction:

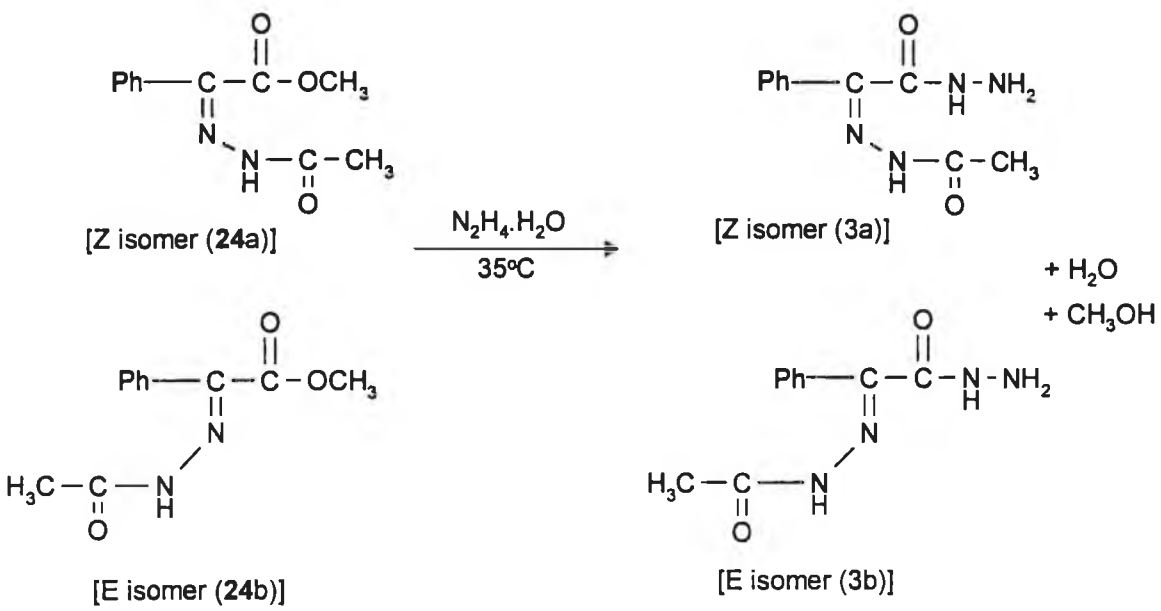
Acetylhydrazine [**8**] (5.00 g, 0.068 mol) was weighed into a two neck round bottom flask (100 cm^3), fitted with a small vacuum distillation arrangement. Phenylglyoxylic acid methyl ester [**12**] (10.1 g, 0.061 mol) was poured into the flask and the temperature increased to 80 °C under reduced pressure (approx. 10 mmHg) with vigorous stirring. The water produced by the reaction was removed via the distillation arrangement as it formed. After 90 minutes the vacuum distillation arrangement was removed and the reaction mixture consisted of phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**], (13.5 g crude product; 0.052 mol, 85% pure), (52% Z-isomer, 33% E-isomer), as determined by HPLC by comparison with known concentrations of isolated isomers of known purity.

3.2.3 Synthesis of phenylglyoxylic acid hydrazide 2-acetylhydrazone:

3.2.3.1 Initial reaction:

Butanol (30 cm^3) was added to the crude sample of phenylglyoxylic acid methyl ester 2-acetylhydrazone (11.5 g, 0.052 mol) at room temperature. Hydrazine monohydrate (2.60 g, 0.052 mol) was added and the resulting mixture stirred vigorously until all solids had dissolved. This solution was stirred for a further 45 minutes when a white precipitate formed. The reaction

proceeded to approximately 80% completion and the entire mixture was carried forward to the next stage.



Phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**]

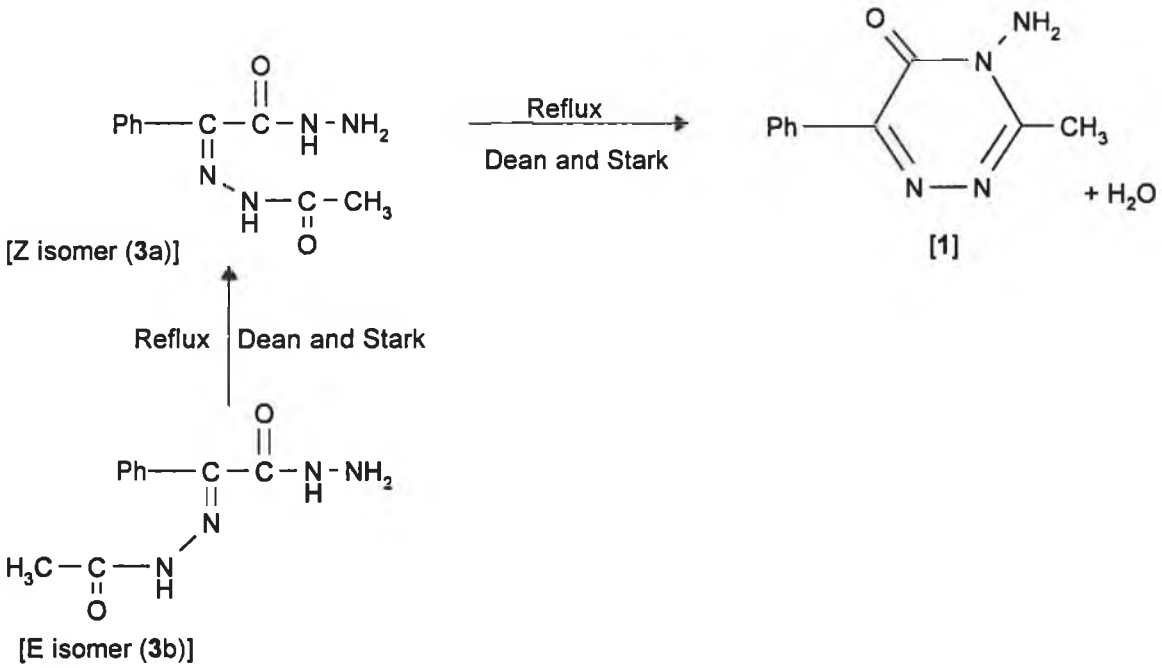
Phenylglyoxylic acid hydrazide 2-acetylhydrazone [**3**]

3.2.3.2 Optimised reaction:

Propan-2-ol (20 cm^3) was added with vigorous stirring to the round bottom flask containing the mixture of isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**], (11.5 g, 0.052 mol), at 80 °C, produced in the last stage. The temperature of the resulting suspension was decreased to 35 °C and hydrazine monohydrate (3.14 g, 0.063 mol) was added to the flask via a dropping funnel (50 cm^3) over a period of 1 minute, while the temperature and vigorous stirring were maintained. After approximately 5 minutes the reaction mixture became clear and then after approximately 20 minutes it became a suspension again. After 30 minutes the reaction was cooled to room

temperature and light petroleum (b.p. 40-60 °C; 20 cm^3) was added. The resulting mixture was stirred vigorously for a further 15 minutes and filtered under reduced pressure. The solid was washed with cold propan-2-ol (20 cm^3) followed by light petroleum (b.p. 40-60 °C; 50 cm^3), then air dried under vacuum for 30 minutes and yielded phenylglyoxylic acid hydrazide 2-acetylhydrazone [3], (10.6 g, 0.046 mol, 92% yield) (55% Z-isomer, 37% E-isomer) and a purity of 95%, as determined by HPLC by comparison with known concentrations of isolated isomers of known purity.

3.2.4 Synthesis of 4-amino-3-methyl-6-phenyl-1,2,4-triazin-5-one [Metamitron]:



Phenylglyoxylic acid hydrazide 2-acetylhydrazone [3]

Metamitron [1]

3.2.4.1 Initial reaction:

The reaction mixture from the hydrazide forming stage was heated under reflux. The cyclisation process proceeded readily for the first 2 hours and then appeared to proceed no further. The cyclisation to Metamitron proceeded to approximately 40% completion.

3.2.4.2 Optimised reaction:

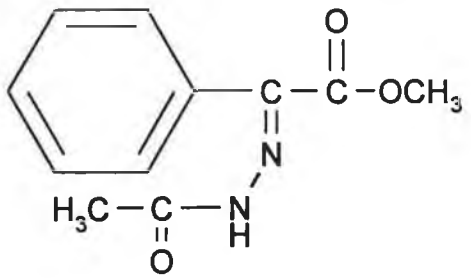
The isolated solid of phenylglyoxylic acid hydrazide 2-acetylhydrazone [**3**] (10.6 g, 0.046 mol) was placed into a two neck round bottom flask (100 cm^3) fitted with a Dean and Stark condenser. Butan-1-ol (25 cm^3) was added and the resulting mixture was heated under reflux for 6.5 hours. The reaction mixture was allowed to cool to room temperature overnight and the precipitate was filtered off under vacuum, washed with cold propan-2-ol (20 cm^3) followed by light petroleum (b.p. 40-60 °C; 50 cm^3) and dried in an oven at 60 °C overnight yielding Metamitron [**1**] (8.0 g; 0.040 mol, 84% yield) and a purity of 97%, as determined by HPLC by comparison with known weights of certified standards of Metamitron (overall yield of 36% from phenylglyoxylonitrile [**9**], 65% from phenylglyoxylic acid methyl ester [**12**]).

3.3 Isolation of pure samples of the intermediates of the synthesis:

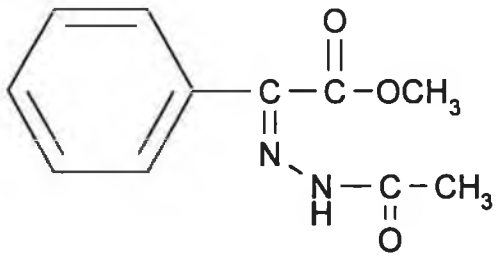
3.3.1 Isolation of the E-isomer of phenylglyoxylic acid methyl ester 2-acetylhydrazone (24b):

This isomer was isolated by placing the cooled reaction mixture (10.00 g, crude, 0.039 mol) of phenylglyoxylic acid methyl ester 2-acetylhydrazone in a round bottom flask (50 cm^3) fitted with a reflux condenser. Methanol (20 cm^3) was added and the mixture was then heated under reflux with vigorous

stirring until all solids had dissolved. The solution was cooled to room temperature and the precipitate filtered off under reduced pressure, washed with light petroleum (b.p. 40-60 °C; 20 cm^3) and air-dried under vacuum for 30 minutes. The resultant solid (2.5 g, 0.011 mol) was found to be approximately 99% pure, as determined by HPLC and ^1H NMR spectroscopy. M.p. = 65-67 °C; IR υ_{max} 3300, 1720, 1700 cm^{-1}; ^1H NMR: ($CDCl_3$), δ 2.45 (s, 3H, Me), 3.85 (s, 3H, OMe), 7.25 (m, 2H, arom), 7.5 (m, 3H, arom), 8.7 (s, 1H, N-H) ppm; ^{13}C NMR: δ 20.32 (Me), 52.81 (OMe), 128.08 (arom), 128.53 (arom), 129.55 (arom), 130.37 (arom), 140.99 (C=N), 163.81 (C=O), 173.14 (C=O) ppm; Calculated for $C_{11}H_{12}N_2O_3$: C, 59.99%; H, 5.49%; N, 12.72%; Found: C, 59.86%; H, 5.62%; N, 12.38%; UV (methanol): λ_{max} 287 nm (ε=2528).



E-Isomer [24b]



Z-Isomer [24a]

3.3.2 Isolation of the Z-isomer of phenylglyoxylic acid methyl ester 2-acetylhydrazone [24a]:

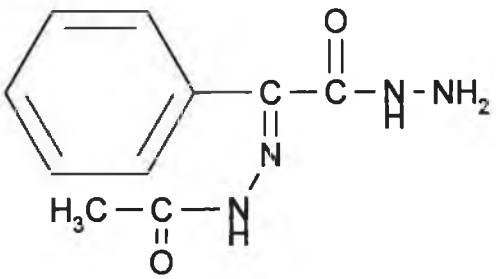
The filtrate and washings from the isolation of the E-isomer of phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24b**] were combined in a conical flask (100 cm^3) and placed in a freezer at -10 °C overnight. The resulting precipitate was filtered cold and the isolated solid transferred to a round bottom flask (50 cm^3). Propan-2-ol (10 cm^3) was added and the

mixture heated with vigorous stirring until all solids had dissolved. The solution was cooled to room temperature and placed in the freezer at -10 °C overnight. The resulting precipitate was filtered under reduced pressure and the solid washed with light petroleum (b.p. 40-60 °C; 30 cm^3) and air-dried under vacuum for 30 minutes. The resultant solid was found to be approximately 99% pure as determined by HPLC and ^1H NMR spectroscopy. M.p. = 155-158 ^oC; IR υ_{max} 3300, 1720, 1700 cm^{-1}; ^1H NMR: (DMSO-d_6), δ 2.4 (s, 3H, Me), 3.9 (s, 3H, OMe), 7.3 (m, 2H, arom), 7.6 (m, 3H, arom), 11.7 (s, 1H, N-H) ppm; ^{13}C NMR: δ 20.34 (Me), 52.84 (OMe), 128.09 (arom), 128.50 (arom), 129.56 (arom), 130.39 (arom), 141.00 (C=N), 163.81 (C=O), 173.16 (C=O) ppm; Calculated for $C_{11}H_{12}N_2O_3$: C, 59.99%; H, 5.49%; N, 12.72%; Found: C, 59.89%; H, 5.47%; N, 12.72%; UV (methanol): λ_{max} 291 nm (ε=5661).

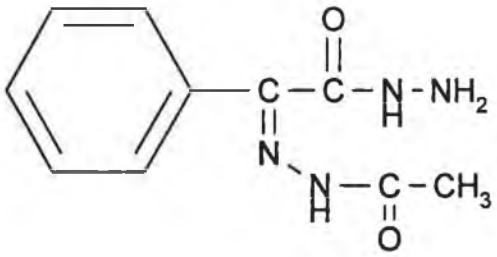
3.3.3 Synthesis of the E-isomer of phenylglyoxylic acid hydrazide 2-acetylhydrazone [3b]:

The E-isomer of phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**b]; (10.00 g, 0.045 mol) was placed in a round bottom flask (50 cm^3) fitted with a dropping funnel (50 cm^3). Propan-2-ol (20 cm^3) was added and the mixture stirred vigorously as the temperature was raised to 40 °C. Hydrazine monohydrate (2.73 g, 0.055 mol) was added slowly from the dropping funnel to the reaction mixture over a period of 1 minute with vigorous stirring and the temperature maintained. These conditions were maintained for a further 20 minutes and the reaction mixture was then cooled to room temperature, the precipitate filtered off under reduced pressure and transferred to a conical flask (50 cm^3). Propan-2-ol (10 cm^3) was added and the mixture heated to 80 °C with constant shaking of the flask. The insoluble product was filtered off

under reduced pressure, washed with light petroleum (b.p. 40-60 °C; 20 cm^3) and air dried under vacuum for 60 minutes. The resulting solid (8.1 g, 0.035 mol, 78% yield) was approximately 95% pure as determined by HPLC and ^{1}H NMR spectroscopy. M.p.=143-147 ^oC; I.R. υ_{max} 3300, 1680, 1645 cm^{-1}; ^{1}H NMR: (DMSO-d_6), δ 2.3 (s, 3H, Me), 4.5 (s, 2H, NH_2), 7.4 (m, 2H, arom), 7.75 (m, 3H, arom), 9.85 (s, 1H, N-H), 10.65 (s, 1H, N-H) ppm; ^{13}C NMR: δ 20.45 (Me), 127.78 (arom), 128.60 (arom), 129.45 (arom), 130.57 (arom), 142.64 (C=N), 163.78 (C=O), 172.74 (C=O) ppm; Calculated for $C_{10}H_{12}N_4O_2$: C, 54.54%; H, 5.49%; N, 25.44%; Found: C, 54.64%; H, 5.64%; N, 24.84%;UV (methanol): λ_{max} 286 nm (ε=2137).



E-Isomer [3b]



Z-Isomer [3a]

3.3.4 Synthesis of the Z-isomer of phenylglyoxylic acid hydrazide 2-acetylhydrazone [3a]:

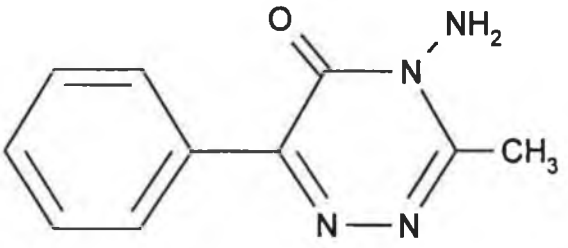
The Z-isomer of phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**a], (10.00 g, 0.045 mol) was placed in a round bottom flask (50 cm^3) fitted with a dropping funnel (50 cm^3). Butan-1-ol (20 cm^3) was added to the flask and the mixture heated to 40 °C with vigorous stirring. Hydrazine monohydrate (2.73 g, 0.055 mol) was added slowly from the dropping funnel over a period of 1 minute with vigorous stirring and the conditions maintained for a further

20 minutes. The reaction mixture was then cooled to room temperature and the precipitate filtered off under reduced pressure, washed with light petroleum (40-60 °C; 20 cm^3) and air dried under vacuum for 30 minutes. The resultant solid (8.5 g, 0.037 mol, 81% yield) was approximately 95% pure, as determined by HPLC and ^1H NMR spectroscopy. M.p.=157-158 ^oC; IR υ_{max} 3300, 1675, 1645 cm^{-1}; ^1H NMR: (DMSO-d_6), δ 2.25 (s, 3H, Me), 4.6 (NH_2), 7.4 (m, 2H, arom), 7.65 (m, 3H, arom), 9.9 (s, 1H, N-H), 10.65 (s, 1H, N-H) ppm; ^{13}C NMR: 20.48 (Me), 127.81 (arom), 128.32 (arom), 129.43 (arom), 130.54 (arom), 142.61 (C=N), 163.76 (C=O), 172.83 (C=O) ppm; Calculated for $C_{10}H_{12}N_4O_2$: C, 54.54%; H, 5.49%; N, 25.44%; Found: C, 54.60%; H, 5.50%; N, 25.72%; UV (methanol): λ_{max} 289 nm (ε=8404).

3.3.5 Synthesis of 4-amino-3-methyl-6-phenyl-1, 2, 4-triazin-5-one [1]:

The mixture of isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone [**3**], (Section 3.2.3.2) (10.60 g, 0.046 mol) was transferred to a two neck round bottom flask (50 cm^3) fitted with a Dean and Stark condenser. Butan-1-ol (25 cm^3) was added and the mixture stirred vigorously under reflux for 6.5 hours. The Dean and Stark arrangement was dismantled and the reaction mixture allowed to cool to room temperature overnight. The precipitate was filtered off under reduced pressure, washed with light petroleum (b.p. 40-60 ° C; 30 cm^3) and air-dried under vacuum for 30 minutes. This solid (8.1 g) was recrystallised from chloroform (20 cm^3), filtered and air-dried under vacuum yielding Metamitron (7.5 g, 0.037 mol, 80% yield) which had a purity of 99% as determined by HPLC on comparison with a certified reference standard of Metamitron. M.p.=165-167 ^oC (Literature reference melting point 166.6 °C);[38] IR υ_{max} 3200, 1675, 1600, 1550 cm^{-1}; ^1H NMR: ($CDCl_3$), δ 2.7 (s, 3H, Me), 5.2 (s, 2H, NH_2), 7.5 (m, 3H, arom), 8.2 (m, 2H, arom); ^{13}C

NMR: δ 19.04 (Me), 128.26 (arom), 128.62 (arom), 130.62 (arom), 132.60 (arom), 151.63 and 153.50 (C=N) and 154.84 (C=O) ppm; Calculated for $C_{10}H_{10}N_4O$: C, 59.40%; H, 4.99%; N, 27.71%; Found: C, 59.80%; H, 4.99%; N, 28.01%; UV (methanol): λ_{max} 312 nm (ε=4959).



Metamitron [1]

3.4 HPLC methods for monitoring reactions:

The phenylglyoxlic acid amide forming reaction involving the hydration of phenylglyoxylonitrile [**9**] to phenylglyoxylic acid amide [**11**] was monitored by HPLC using methanol and water (60:40) as mobile phase.

Samples (approx. 50 mg) were removed from the reaction mixture, transferred to volumetric flasks (10 cm^3), made up to volume with mobile phase, and placed in the ultrasonic bath for 2 minutes. On allowing to stand for 5 minutes, these solutions were then injected onto the column and the resulting chromatograms showed that phenylglyoxylonitrile eluted at 3.8 minutes (phenylglyoxlic acid amide is fully retained by the column). The reaction was stopped when the phenylglyoxylonitrile peak disappeared (Figure 32).

The methanolysis reaction involving the reaction between phenylglyoxylic acid amide [**11**] and methanol was monitored by HPLC using methanol and water (60:40) as mobile phase.

Samples (approx. 50 mg) were removed from the reaction mixture, transferred to volumetric flasks (10 cm^3), made up to volume with mobile phase and placed in an ultrasonic bath for 2 minutes. On allowing to stand for five minutes the solutions were then injected onto the column and the chromatograms obtained showed the formation of phenylglyoxylic acid methyl ester [**12**] which eluted at 3 minutes (Figure 33).

Chromatogram of the reaction mixture initially.

(1)

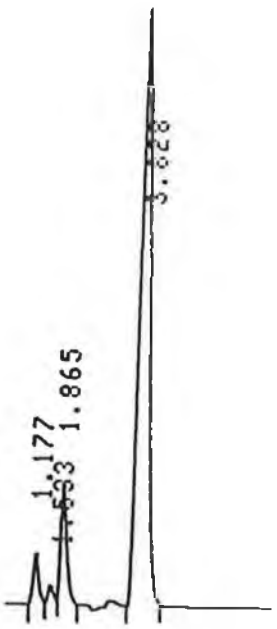


Figure 32

Phenylglyoxylonitrile peak at 3.8 minutes.

This reaction was determined quantitatively by injecting a standard of pure phenylglyoxylic acid methyl ester (approx 25 mg in 10 cm^3 of mobile phase) which showed that the reaction mixture contained phenylglyoxylic acid methyl ester which when isolated had a purity of 98% as determined by HPLC.

Chromatogram of the reaction mixture after 3 hours.

(2)

Figure 33

Phenylglyoxylic acid methyl ester peak at 3.0 minutes.

The reaction between phenylglyoxylic acid methyl ester [**12**] and acetylhydrazine [**8**] was monitored by HPLC using methanol and water (50:50) as mobile phase. Samples (approx. 50 mg) were removed from the reaction vessel, placed in volumetric flasks (25 cm^3), made up to volume with methanol and placed in an ultrasonic bath for 2 minutes. After allowing to stand for 5 minutes, these solutions were injected onto the column and the chromatograms obtained showed the formation of the E- and Z-isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**b and **24**a] at 2.9 and 6.6 minutes respectively and the broad peak of phenylglyoxylic acid methyl ester at 5.4 minutes after 30 minutes (Figure 34).

After 60 minutes phenylglyoxylic acid methyl ester was almost completely consumed in the reaction and both of the isomers were present in high concentrations. The reaction mixture was heated for a further 30 minutes in order for equilibrium to be established (Figure 34).

The product of this reaction was determined quantitatively by injection of standards of both Z- and E-isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone, which showed that the equilibrium reaction mixture contained phenylglyoxylic acid methyl ester 2-acetylhydrazone with the reaction proceeding to 85% completion. The remainder of the mixture consisted of some unreacted phenylglyoxylic acid methyl ester (5.4 minutes) and some unreacted acetylhydrazine (which is not detected by this system).

Note: The peak at 1.0 minute was due to methanol used in making up the solutions.

The reaction between phenylglyoxylic acid methyl ester 2-acetylhydrazone [**24**] and hydrazine monohydrate was monitored by HPLC using methanol and buffer (30:70) as mobile phase.[38] Samples (approx. 25 mg) of the reaction mixture were taken, transferred to volumetric flasks (10 cm^3), made up to volume with methanol and placed in an ultrasonic bath for 2 minutes. On allowing to stand for 5 minutes these solutions were then run on the HPLC system and the resulting chromatograms showed the rapid formation of the E- and Z-isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone [**3**b and **3**a] after only 10 minutes which have retention times of 1.8 and 2.1 minutes respectively (Figure 35).

After 30 minutes no trace of the Z-isomer of phenylglyoxylic acid methyl ester 2-acetylhydrazone (6.6 minutes) remained while the E-isomer (2.9 minutes) had been almost completely consumed by the reaction. The reaction mixture consisted of phenylglyoxylic acid hydrazide 2-acetylhydrazone, propan-2-ol (4.9 minutes) and unreacted hydrazine monohydrate (not detected by this method) with the reaction proceeding to 95% completion. The peak at one minute again results from the presence of methanol used in making up the solutions (Figure 35).

Chromatograms of the reaction after 30 minutes *(3)*, after 1 hour *(4)* and after 90 minutes *(5)*.

(3) (4) (5)

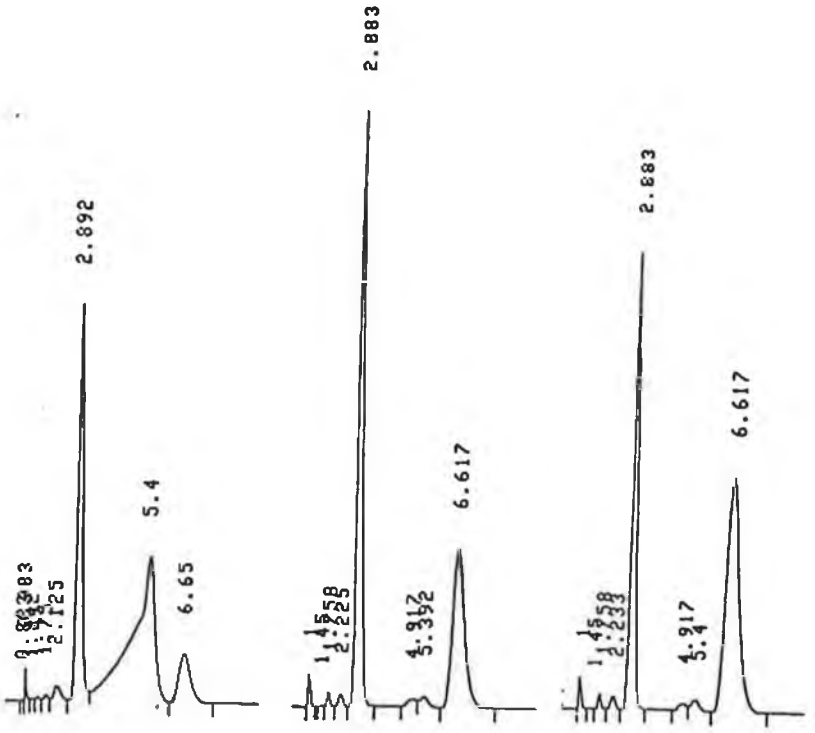


Figure 34

E- and Z-isomers of phenylglyoxylic acid methyl ester 2-acetylhydrazone and phenylglyoxylic acid methyl ester at 2.8, 6.6 and 5.4 minutes respectively.

The isolated solid was found to consist of Z-isomer (55%) and E-isomer (37%) by HPLC (Figure 35).

The cyclisation of phenylglyoxylic acid hydrazide 2-acetylhydrazone [**3**] to Metamitron [**1**] was monitored by HPLC using methanol:buffer (30:70) as mobile phase.[38] Samples (approx. 75 mg) were removed from the reaction vessel, transferred to volumetric flasks (10 cm^3), made up to volume with methanol and ultrasonicated for 2 minutes. After standing for 5 minutes these solutions were then injected onto the column and

Chromatograms of the reaction after 10 minutes *(6)*, after 30 minutes *(7)* and the isolated solid after filtration *(8)*.

(6) (7) (8)

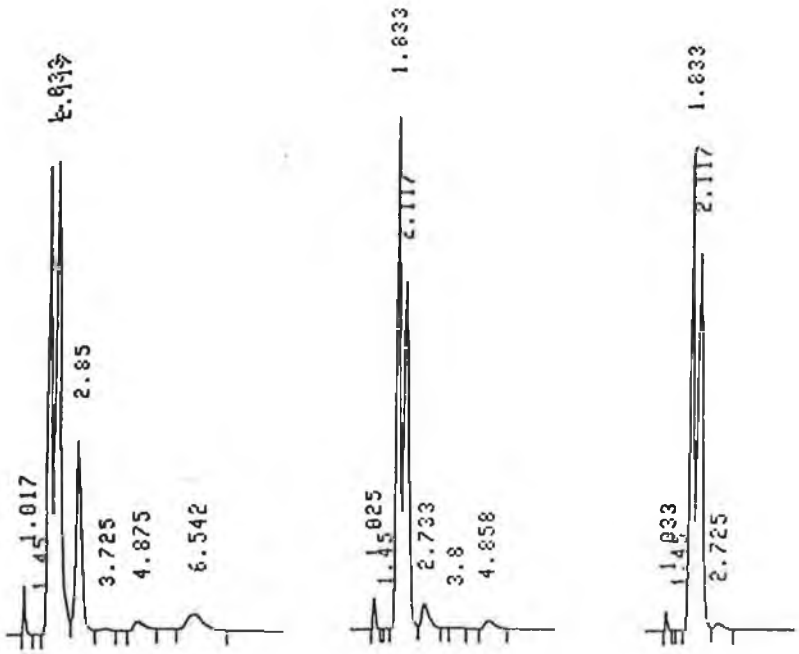


Figure 35

E- and Z-Isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone, E- and Z-isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone at 1.8, 2.1, 2.8 and 6.5 minutes respectively.

the resulting chromatograms showed the gradual formation of Metamitron (7.1 minutes) and the slow decline in the concentrations of the E- and Z-isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone (2.8 and 4.1 minutes respectively) (Figure 36).

After 6.5 hours the reaction mixture consisted of Metamitron (7.0 minutes) and traces of the E- and Z-isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone with the reaction proceeding to 86% completion as determined by HPLC. Methanol elutes at 1.5 minutes (Figure 37).

The isolated solid was found to be 97% pure as determined by HPLC by comparison with a certified standard of Metamitron (Figure 37).

Chromatograms of the reaction mixture initially *(9)* and after 1 hour *(10)*.

(9) (10)

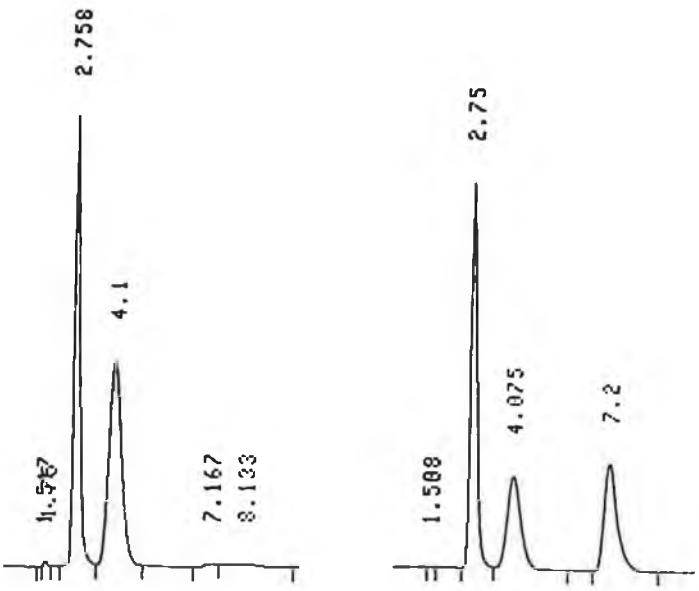


Figure 36

E- and Z-isomers of phenylglyoxylic acid hydrazide 2-acetylhydrazone and Metamitron at 2.8, 4.1 and 7.2 minutes respectively.

Chromatograms of the reaction mixture after 6.5 hours *(14)* and the isolated Metamitron after filtration *(15)*.

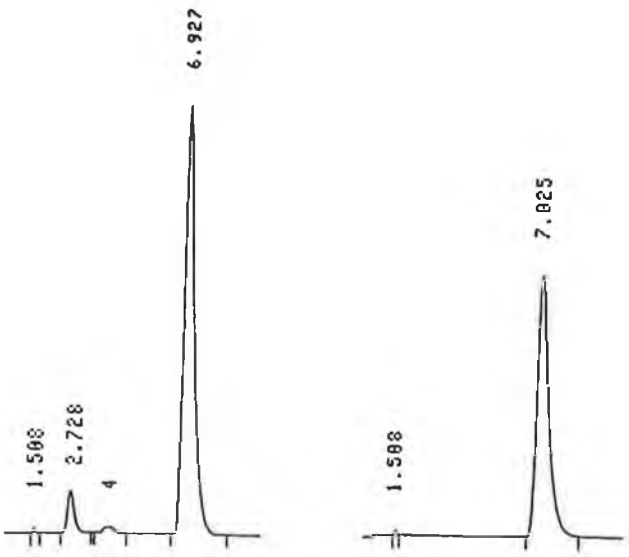


Figure 37

E-isomer of phenylglyoxylic acid hydrazide 2-acetylhydrazone and Metamitron at 2.8 and 7.0 minutes respectively.

3.5 Standard curve for Metamitron:

A standard curve for Metamitron was plotted by dissolving different weights of a certified standard in methanol in a volumetric flask (10 cm^3) which were then injected, in duplicate, onto the HPLC system using the CIPAC method for the determination of Metamitron.[38]

The resulting curve drawn from the resulting data was extrapolated through the origin giving a squared linear regression of 0.9988 between the concentrations of 0 and 22.4 mg/cm^3 enabling accurate determination of Metamitron in solution below 22.4 mg/cm^3 (Figure 38).

The standard curve for Metamitron over the range of 0 to 22.4 mg/cm^3.

Meta Std (mg/ml)	Peak Area
1.4	2291244
6.1	8102142
10.5	13726096
16.4	22186616
22.4	30769980

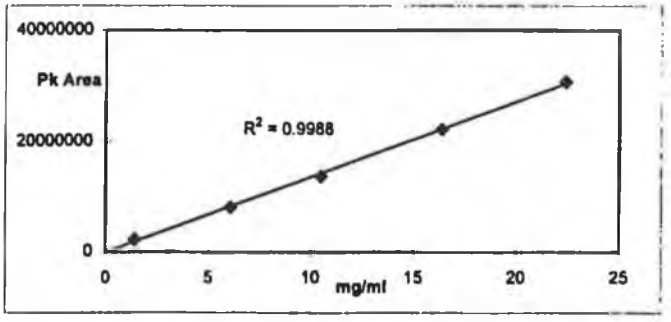


Figure 38

REFERENCES

[1] K.M. Buchell, *Chemistry of pesticides*, Wiley, Stuttgart, 1977, 322-392.

[2] W.W. Fletcher and R.C. Kirkwood, *Herbicides and Plant Growth Regulators*, Granada Publishing Ltd., London, 1982.

[3] D.H. Hutson and T.R. Roberts, 'Herbicides', in *Progress in Pesticide Biochemistry and Toxicology,* Wiley, Chichester, 1987, **6**.

[4] J.R. Corbett, K. Wright and A.C. Baillie, *The Biochemical Mode of Action of Pesticides,* 2nd Edn, Academic Press, London and New York, 1984.

[5] W. Draber, K. Dickore, K.H. Buchel, and A. Trebst, *Naturwissenschaften*, 1968, **55**, 446.

[6] W. Draber, K.H. Buchel, K. Dickore, A. Trebst and E. Pistorius, *Progress in Photosynthesis Research,* 1969, **3,** 1789.

[7] R.J. Cremlyn, *Agrochemicals, Preparation and Mode of Action*, Wiley, Chichester, 1981, 217-270.

[8] L. Eue, K. Westphal, K. Dickore, W. Meiser, *Proc. Europ. Weed Res. Council*, 1968, **1**, 125.

[9] L. Eue, H. Tietz, *Pflanzenschutznachrichten Bayer*, 1970, **23**, 213.

[10] K. Dickore, W. Draber, L. Eue, Bayer AG, *Ger. Patent Appl.,* 2 107 757/1971 (*Chem. Abstr., 77(4):164 767w).*

[11] W. Draber, K. Dickore, H. Timmler, Bayer AG, *Ger. Patent Appl.,* 2 138 031/1971 (*Chem. Abstr., 78(19):124 639v).*

[12] R.R. Schmidt, W. Draber, L. Eue and H. Timmler, *Pestic. Sci.*, 1975, **6**, 239-244.

[13] H.A. Roberts, W. Bond and M.E. Ricketts, *Proceedings of British Crop Protection Conference*-Weeds, 1976, 441-447.

[14] D. Gadkari, *Biol. Fertil. Soils*, 1987, **3**, 171-177.

[15] H.J. Garczyk, *Proceedings of British Crop Protection Conference*-Weeds, 1976, 619-626.

[16] C. Olmedo, L. Deban, E. Barrado, Y. Castrillejo and L. Herrero, *Electrochemical Acta,* 1994, **39**, 2237-2241.

[17] R. Allen and A. Walker, *Pesticide Science,* 1988, **22**, 297-305.

[18] U. Traub-Eberhard, K.P. Henschel, W. Kordel and W. Klein, *Pesticide Science,* 1995, **43**, 121-129.

[19] L. Cox, J. Hermison, J. Cornejo and M. Mansour, *Proceedings of the fifth International Workshop on Environmental behaviour of pesticides and regulatory aspects*, 1994, 248-251.

[20] E.E. Elwy, M. Osman, T. Abdel Rahman and I.M. Ismail, *Can. J. Bot.,* 1989, **67**, 737-741.

[21] F. Heimbach, J. Berndt and W. Pflueger, *Aquatic mesocosm studies in ecotoxicological risk assessment,* 1994, 303-320.

[22] R.R. Schmidt, W. Draber, L. Eue and H. Timmler, *Pesticide Science,* 1975, **6**, 239-244.

[23] H. Timmler, W. Draber, Bayer A.G., *Ger. Patent Appl.* 23 64 474/1973 (*Chem. Abstr., 83(15):131642x).*

[24] W. Draber, H. Timmler, K. Dickore, R.R. Schmidt, L. Eue, Bayer A.G., *Ger. Patent Appl.* 2 224 161/1972 (*Chem. Abstr., 80(9):48042u).*

[25] K. Findeisen, W. Draber, H. Schwarz, Bayer A.G., *Ger. Patent Appl.* 25 28 211/1975 (*Chem. Abstr., 87(1):5666c).*

[26] R. van Schoonbeek, Bayer A.G., *Belg. Patent Appl.* 834.346/1976 (*Chem. Abstr., 87(17):134742y).*

[27] *Brevet Francais* BF 22 56 163/1974.

[28] W. Draber, H. Timmler, K. Dickore and W. Donner, *Liebigs Ann. Chem.*, 1976, **12**, 2206-2221.

[29] H. Schwarz, H. Kratzer, R. Singer, Bayer A.G., *Ger. Patent Appl.* 28 27 977/1978 (*Chem. Abstr., 92(25):215082f).*

[30] W. Lunkenheimer, A. Wittig, W. Draber, H. Timmler, Bayer A.G., *Ger. Patent Appl.* 27 08 189/1977 (*Chem. Abstr., 90(3):22622q).*

[31] T. Schmidt, H. Timmler, G. Bonse, H. Blank, G. Marzolph, Bayer A.G., *Eur. Patent Appl.* 0 056 938/1982 (*Chem. Abstr., 98(9):72137w).*

[32] H. Neunhoeffer, G. Kohler and H.J. Degen, *Liebigs Ann. Chem.*, 1985, **1**, 78-89.

[33] H. Timmler, W. Draber, Bayer A.G., *British Patent Appl.* 1447337/1974 (*Chem. Abstr., 91(19):157472g).*

[34] C.M. Atkinson and H.D. Cossey, *J. Chem. Soc.*, 1962, 1805-1811.

[35] T.V. Saraswathi and V.R. Srinivasan, *Tetrahedron Letters,* 1971, 2315-2316.

[36] W.W. Paudler and J.M. Barton, *J. Org. Chem.,* 1966, **31**, 1720-1722.

[37] J.M. Photis, *Tetrahedron Letters*, 1980, **21**, 3539-3540.

[38] A. Martijn and W. Dobrat, *CIPAC Handbook*, 1985, Vol. D, 124-129.

[39] S. Patai, Wiley, *The chemistry of the carbon-nitrogen double bond,* 1970, 364-408.

[40] W. Wehner, *Pharmazie*, 1995, **50** (6), 436-437.

[41] P.E. Jackson, B. Kahler, *J. High Resolut. Chromatogr.*, 1992, **15** (9), 620-621.