SYNTHETIC STUDIES TOWARDS CEPHAM DERIVATIVES

A THESIS

BY

GERALD DAVID ROSEBERY

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Synthetic Studies Towards Cepham Derivatives

Gerald David Rosebery

Department of Chemistry

McGill University

Montreal, Quebec, Canada

Abstract

The synthesis of an isonitrile that, after an Ugi type condensation, yields an amide that can be removed by mild oxidation and hydrolysis is described. The o-nitrocinnamoyl function was developed as an N-protecting group in peptide synthesis. The synthesis of 2-o-nitrostyryl-4-thiomethylene-5-oxazolone is described. Improved procedures for the synthesis of a number of key intermediates useful in a cepham synthesis were developed. A new, cheap and easy method, suitable for the synthesis of large amounts of D-mannitol diacetonide, is included. The total synthesis of a cepham derivative was accomplished. In addition, several new compounds were prepared and characterized.

Essai sur la synthèse des dérivés céphames

Gerald David Rosebery

Department of Chemistry

McGill University

Montreal, Quebec, Canada

Résumé

On décrit la synthèse d'un isonitrile qui, par une condensation de type Ugi, donne un amide pouvant être enlevé par oxidation douce suivie d'une hydrolyse. Le groupement fonctionnel o-nitrocinnamoyl a été employé pour protéger l'amine dans la synthèse peptidique. On décrit la synthèse de la 2-o-nitrostyryl-4-thiométhylène-5-oxazolone. On a amélioré les procédés de synthèse de plusieurs intermédiaires clefs dans la synthèse de céphames. On a inclus une nouvelle méthode de synthèse de l'acetonide de D-mannitol, simple et avantageuse car facilement transposable sur de grandes quantités. La synthèse totale d'un dérivé céphem a été réalisée. En outre, plusieurs composés nouveaux ont été preparés et caractérisés.

To Nancy

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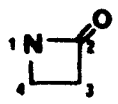
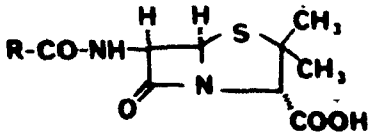
INTRODUCTION

β-Lactams are four-membered heterocyclic compounds of the type (1). Although the functionality was recognized as early as 1907 by Staudinger[1], real interest was only focused on it during the early 1940's when the then new antibiotic penicillin (2) was discovered to contain this ring system.[2]



(1)

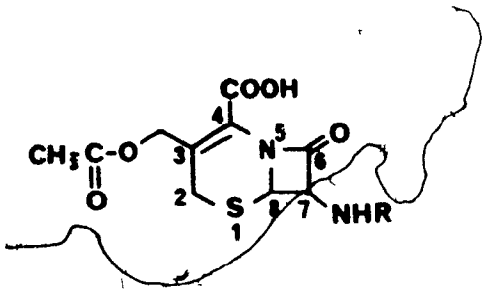


(2)

The structure of penicillin had hardly been elucidated when Brotzu[3] in 1945 discovered what turned out to be another β-lactam producing fungus near a sewage outlet in the sea off Sardinia. Because of inadequate facilities in Sardinia, a culture of the fungus of species Cephalosporium was sent to Sir Howard Florey at Oxford University who had been very active in penicillin research.

Abraham and Newton[4], also at Oxford, carried out a detailed examination of all the antibiotics produced by this cephalosporium sp. In 1955 they isolated from cultures of a mold, cephalosporium acremonium, a new antibiotic substance which they called cephalo-

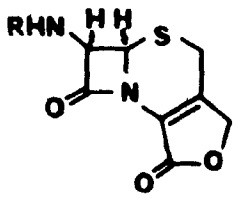
sporin C (3). The structure was determined[5] by the same workers in 1961 and confirmed by Hodgkin and Maslen[6] by means of single crystal X-ray diffraction studies.



(3)

Much work was done between 1940 and 1945 on the chemical synthesis of penicillins but without much success. In the meantime, single step fermentation processes had become sufficiently developed so as to make any multistep chemical synthesis noncompetitive. This was not true, however, for the cephalosporin series of β-lactam antibiotics. While a mutant of the original cephalosporium acremonium strain, which could produce substantial amounts of cephalosporin C, was discovered by the Antibiotics Research Station of the Medical Research Council[7], attempts to induce the biosynthesis of analogs of the antibiotic during fermentation were unsuccessful. Hence, the total synthesis of cephalosporin C and its derivatives seemed eminently worthwhile.

The total synthesis of a derivative of cephalosporin C_c (4a), which has no biological activity, was reported by Heymès, Amiard and Nominé[8] in 1966. Their work was the culmination of attempts by several groups to apply the successful penicillin synthesis of



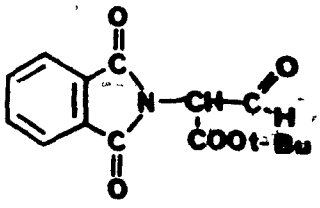
(4)

(4a) R = -CO-(CH$_2$)$_3$-CH(COO$^-$)-NH$_3^+$

(4b) R = -CO-CH$_2$-(thienyl)

Sheehan and Henery-Logan[9] to the synthesis of cephalosporin C$_C$ (4b).

A very important advantage in choosing this unsaturated γ-lactone (4a) as the synthetic goal is that the carboxy and the hydroxy groups in the dihydrothiazine ring are protected and the double bond is immobilized. The key reaction for this synthetic route is condensation of the enamine (6), formed from the aldehyde (5),



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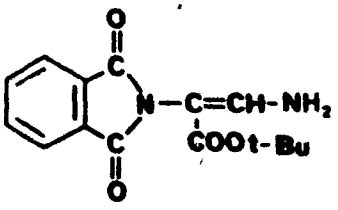
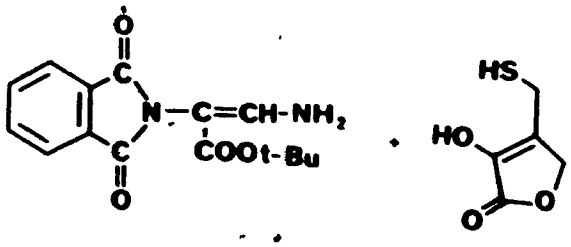


(6)

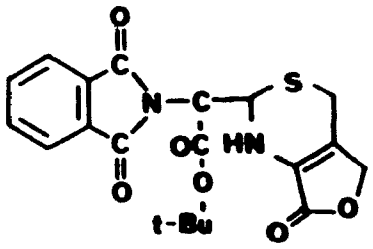
with α-hydroxy-β-thiomethyl butenolide (7) to form two isomers of (8).



(6)

(7)

It is claimed[6] that, upon removal of the N-phthaloyl group in (8), the two isomeric t-butyl esters give, on acid treatment and tritylation, only one amino acid (9).



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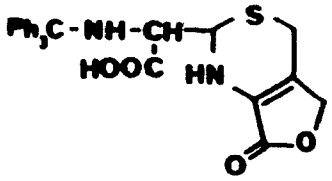
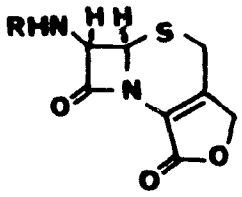


(9)

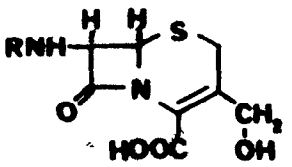
The cyclization of (9) to the β-lactam (10a) proved difficult but was eventually accomplished in 70% yield using dicyclohexylcarbodiimide in tetranitromethane. Detritylation and acylation with 2-thienylacetyl chloride gave a racemic cephalosporin C_c derivative (10b).



(10a) R= -C(Ph)$_3$

(10b) R = -CO-CH$_2$-(thienyl)

The main disadvantage to this approach is that there exists only one low yield method of opening the γ-lactone without hydrolyzing the β-lactam. In 1970 Neidleman and Dolfini[10] managed to obtain a 10% yield of the deacetylcephalothin (11) from the corresponding lactone (10b). This hydroxy-acid could be converted only with great difficulty to cephalothin[11].

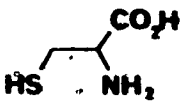


R = -CO-CH$_2$-(thienyl)

(11)

In the light of Neidleman's work[10], the synthesis of the Heymès group can therefore be considered a total synthesis of a racemic

cephalosporin. The only complete stereospecific total synthesis of cephalosporin C and cephalothin was reported by R.B. Woodward[12] in his Nobel prize address in 1965.



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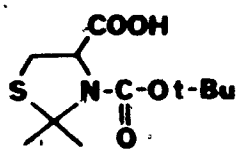
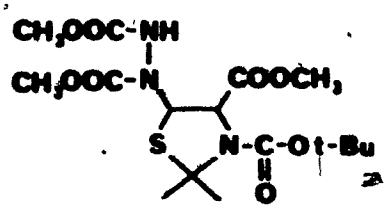


(13)

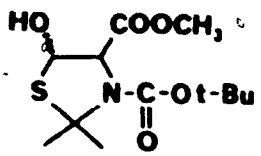
Using L-cysteine (12) as the starting point ensures the correct absolute stereochemistry at the 7-position of cephalosporin C (see diagram 3). The amino group in the β-position to the carboxy group, required for the formation of the β-lactam ring, was introduced stereospecifically in a novel fashion.

L-cysteine (12) was protected with an acetonide function and then reacted with t-butoxy carbonyl chloride to give (13). The acid (13) was esterified with diazomethane and then reacted with dimethyl azodicarboxylate to afford (14).



(14)

Oxidation of (14) with lead tetracetate followed by treatment with sodium acetate in methanol produced the trans hydroxy compound (15). Treatment of (15) with azide followed by reduction with aluminum amalgam in methanol gave the desired cis amino-ester (16). The



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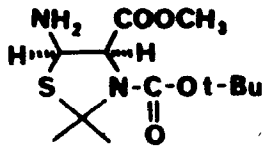
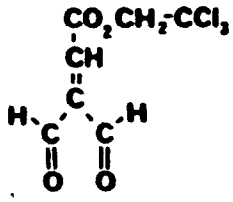


(16)

β-lactam was formed using triisobutyl aluminum and this condensed with preformed dialdehyde (17) to give (18).



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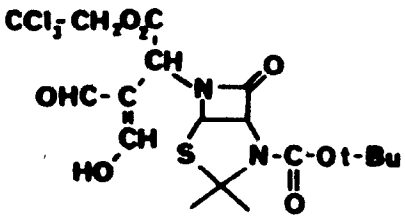
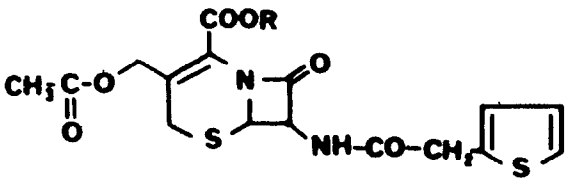


(18)

From (18) the amino-aldehyde (19) was produced by treatment with trifluoracetic acid. Then, acylation of the 7-amino group, reduction of the aldehyde with diborane followed by acetylation of the resulting hydroxy group and isomerization of the double bond in

pyridine gave the ester (20a) which could be converted to the free acid (20b) by reduction with zinc in 90% aqueous acetic acid.



(20a) R = $-CH_2CCl_3$

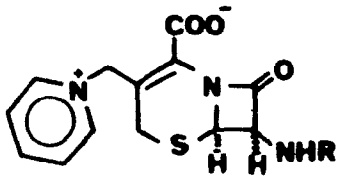
(20b) R = $-H$

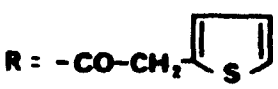
There have been other attempts at the synthesis of cepham derivatives, none of which has been as successful as that reported by Woodward. A number of review articles and monographs[13-24] have been written on the subject and deal in some detail with other approaches and with the chemistry and the biological activity of cephalosporins.

Cephalosporins, like penicillins, are by no means effective against all infectious diseases. The virus diseases such as influenza, measles, chicken pox or mumps are immune to the effects of these drugs. Also, none of the protozoal and parasitic diseases such as malaria, sleeping sickness, dysentry or schistosomiasis respond to cephalosporins. Likewise fungal diseases such as ringworm, athlete's foot, histoplasmosis, or psoriasis, are unaffected. As well, some bacterial diseases like tuberculosis, cholera, brucellosis, bubonic plague, or typhus fever, do not respond. However, the diseases that are controlled by the cephalosporins make an impressive list. Respiratory diseases like pneumonia, scarlet fever, sore throat,

and ear infections all respond to cephalosporin treatment. Also, rheumatic fever, many infections of the urinary tract, infections of wounds, boils, abscesses, gas gangrene, meningitis and tetanus can be controlled with these drugs. Syphillis and gonorrhea are also cured.

There are several clinically available derivatives of cephalosporin C. The Glaxo-Allenbury's Pharmaceutical Company markets cephaloridine (21) (trade name Ceporan). Eli Lilly produces sodium cephalothin (22) (trade name Keflin) and also an orally active derivative cephalexin (23) (trade name Keflex).





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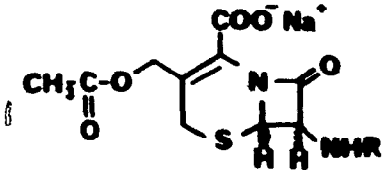

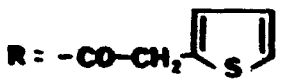

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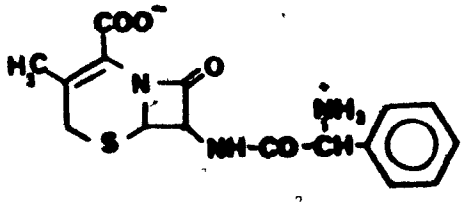
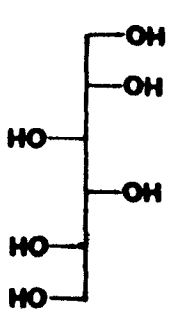


(23)

Cephalosporins and penicillins seem to have similar modes of action, interfering with bacterial cell wall synthesis[22-25]. But cephalosporins have some characteristics that in many cases make them more useful than penicillins. They are, like penicillins, non-toxic,but they are also more acid stable, and more chemical variations are possible. They have good gram-positive and some gram-negative antibacterial activity and are active against penicillin resistant staphylococci. Perhaps the greatest advantage of cephalosporins over penicillins is that they are less liable to cause allergic reactions. The greatest disadvantage, however, is that they are much more expensive than penicillins. It is partially upon this basis that the work in the following chapters was undertaken. The objective in this work, as was that of Rossy[26],was to devise a novel and adaptable scheme for the synthesis of cepham derivatives using inexpensive and readily available starting materials.

Rossy's scheme for the synthesis of a cepham derivative is diagrammed below. D-Mannitol (24) was converted to the diacetonide (25)



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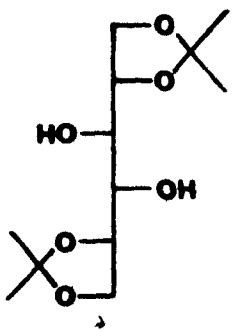
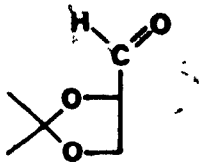


(25)

which was then cleaved with lead tetracetate to glyceraldehyde acetonide[27] (26). Treatment of (26) with formaldehyde and potassium carbonate in aqueous methanol gave the dioxan (27). Pyrolysis



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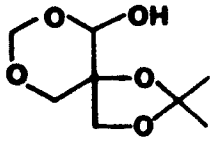
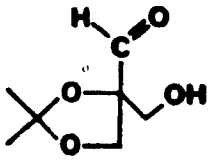


(27)

of (27) under vacuum afforded about a 50% yield of the aldehyde alcohol (28). Immediate reaction of (28) with N-methylethanolamine (29) in ether gave a fair yield of the oxazolidine-alcohol (30).



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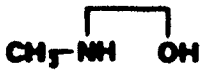

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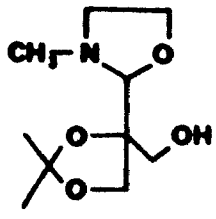
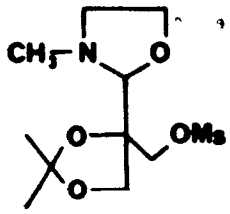


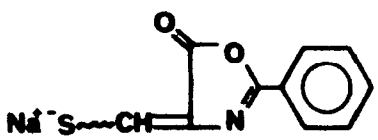
(30)

This could be converted in up to 80% yield to the corresponding crystalline mesylate (31). Condensation of oxazolidine-mesylate (31)



(31)

with the sodium salt of thiol (32) derived from 2-phenyl-4-ethoxy-methylene-5-oxazolone[28] (33) in dimethylsulphoxide at 75°C gave a low yield of the condensed product (34) as a yellow oil.



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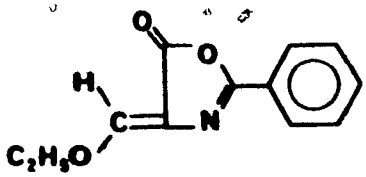

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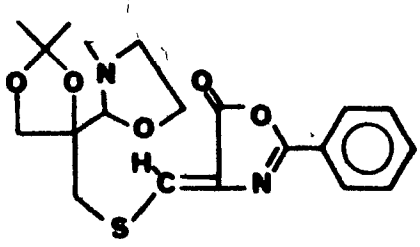
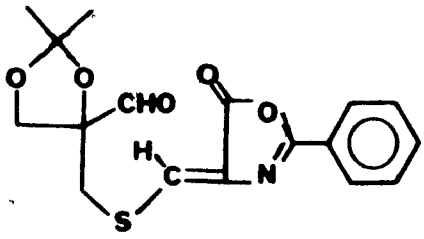


(34)

Hydrolysis of the oxazolidine protecting group was effected with 50% aqueous acetic acid in one hour at room temperature to yield (35) as a pale orange oil. The oxazolone ring in (35) was then opened by treatment with 2N sodium hydroxide for two hours at room temperature to give the aldehyde-acid (36) in low yield. Treatment of the crude acid (36) with ammonia gas in boiling benzene for 24 hours gave the imino-acid (37). Reaction of this crude



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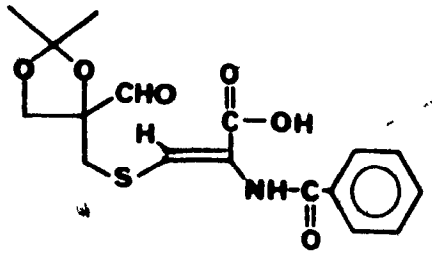

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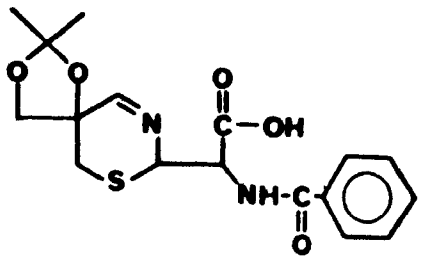
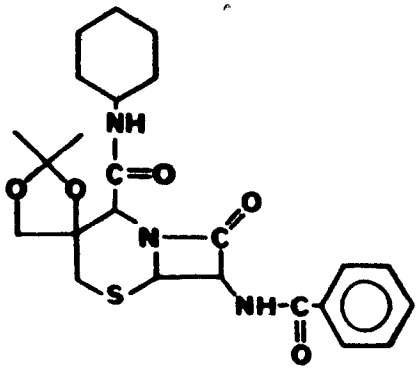


(37)

imino-acid (37) with cyclohexylisonitrile in a two phase system (methylene chloride-carbon tetrachloride/water) afforded a small amount of the impure β-lactam (38), which was characterized only

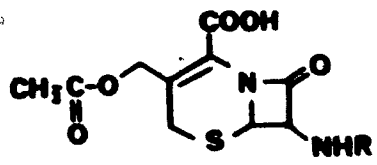


(38)

by its infrared and mass spectrum.

Although Rossy's work was successful in attaining the goal, namely the synthesis of the β-lactam (38), the yield and purity of the material obtained were insufficient for further work. There were many other problems inherent in the synthetic route and these form the basis of the work in the following chapters.

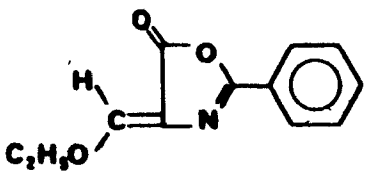
Chapter 1 deals with the problem of the isonitrile. While cyclohexylisonitrile does react with the imino-acid (37) to give (38) it is only a model study. Since cephalosporins (3) have a



(3)

carboxylic acid functionality at the 4-position, not an amide as in (38), it would be very useful if an isonitrile could be developed which would undergo the Ugi reaction and yield an amide that could readily be converted to the desired acid without damaging the remainder of the molecule. Chapter 1 is a summary of our attempts to synthesize some such isonitriles and the methods by which we planned to remove the amide generated in a Ugi reaction. Although such an isonitrile was developed, we decided that our approach would not be an improvement on the cleavage of amides in cephalosporin type compounds using phosphorous pentachloride in methylene chloride that had been published by Fechtig[29] in 1968.

Chapter 2 deals with a problem of a very similar nature. β-Lactam (38) has the benzamide function at the 7-position, which has to be removed at the end of the synthesis. It was originally felt that the oxazolone (33) would only be a model compound that could be modified at a later date so as to provide, when incorporated in the β-lactam (38), an amide that is easily removable. We believed



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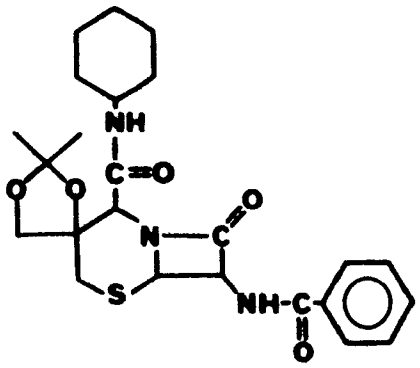
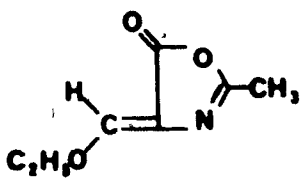


(38)

that the 2-methyl compound (39) could be used in place of (33) to give an acetate at the 7-position. This could be removed stereospecifically using an enzyme such as hog renal acylase[30]. The synthesis of the 2-methyl compound (39) proved, however, to be impossible using known techniques[31]. We then decided to try to prepare a new oxazolone (40) which would give an amide that could easily be removed by hydrogenation under mild conditions. In Chapter 2 the synthesis of the oxazolone (40) is discussed and the



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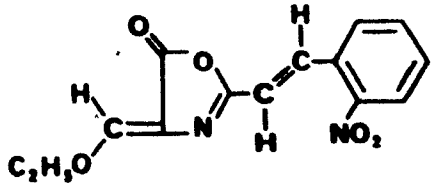
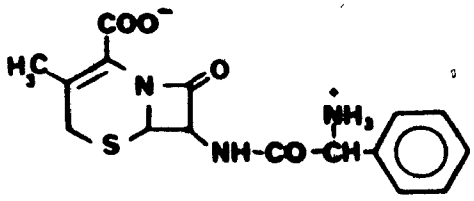


(40)

potential use of the o-nitrocinnamoyl group as a replacement for the benzoyl group at the 7-position of the β-lactam is evaluated. The use of the o-nitrocinnamoyl group as an N-protecting group in peptide synthesis, one that can be removed without racemization of the peptide, is also examined in some detail.

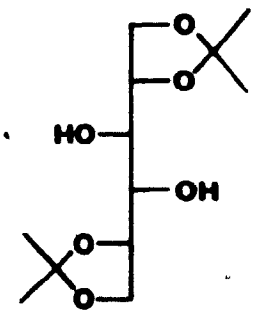
Chapter 3 shows that the methodology developed by Rossy for his synthesis could also be applied to produce 3-methylcephalosporins similar to cephalexin (23). The 3-methyl compounds are orally active making them useful for general application. This study, which was not extensive, was planned to show the flexibility of our technique and was in part successful. The approach was only abandoned when



(23)

the work that is detailed in Chapter 4 became of more immediate interest.

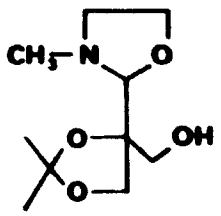
As was pointed out previously, the Rossy synthesis did produce the desired β-lactam but the yields were such that the synthesis could not be used as such to make cephalosporins. Hence it was important that techniques be improved if the whole synthesis was to be completed. Our work in Chapter 4 was aimed at this goal. An improved method for preparing the diacetonide of D-mannitol (25) is



(25)

described. The cleavage reaction to give (26) and the formaldehyde reaction to afford (27) could not be improved but a whole

new technique to give much purer and larger amounts of the key intermediate (30) was developed. The mesylation procedure to give (31) was also improved somewhat. The sodium thiol shown as (32) was originally, in the Rossy synthesis, a mixture of cis and trans thiols.



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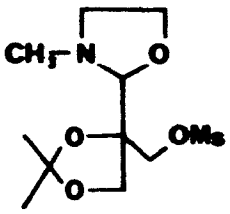

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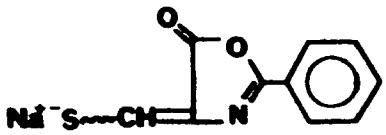

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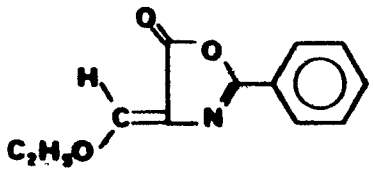
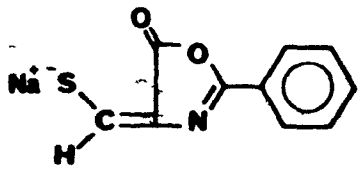


(33)

Each isomer (41)[32] and (42) was prepared separately and the desired isomer (42) could be made crystalline and in excellent yields from (33).

The condensation of (31) and (42) was also greatly improved. Better yields with a crystalline product were easily obtained.



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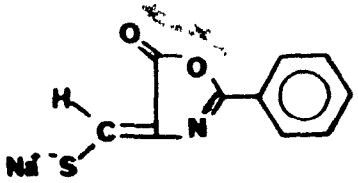

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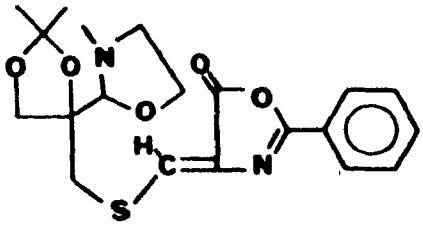

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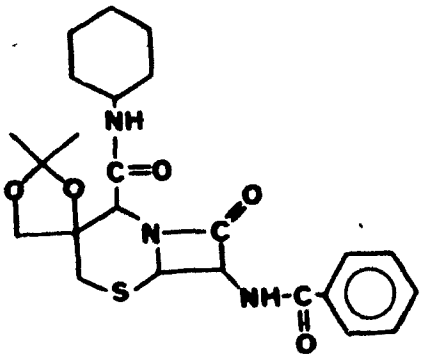


(38)

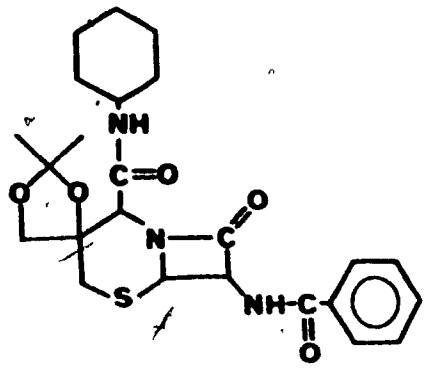
Rossy had the product (34) as a yellow oil and continued with crude products to the β-lactam (38). Most of the intermediates obtained by Rossy as oils of varying purity were isolated as crystalline compounds.

Since the synthesis that we achieved used a different approach after the condensation step that gave (34), this synthesis is treated as a separate entity in Chapter 5.

CHAPTER 1

Synthesis of isonitriles that undergo Ugi-type reactions to yield easily hydrolyzable amides

One of the weaknesses of the synthesis of the β-lactam derivative (38) was that the isonitriles used for the Ugi condensation generated a secondary amide at the 4-position of the thiazine ring.

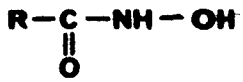


(38)

Since a carboxyl group was desired at this position some method had to be found to convert this amide to the desired acid. There are well known methods for the hydrolysis of secondary amides to the corresponding acids[33] but the severe conditions required for this conversion would also have hydrolyzed the laboriously produced β-lactam. Hence, an isonitrile that yielded an amide that could be hydrolyzed under sufficiently mild conditions such that the β-lactam remained intact was required. We approached this problem in several different ways, each of which I shall discuss separately.

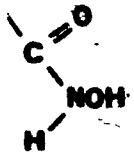
We felt that it might be possible, starting from either hy-

droxylamine or an appropriate derivative, to prepare an isonitrile that, after the Ugi reaction, could readily be turned into a hydroxamic acid of the type (43). Organic hydroxamic acids may be



(43)

classified as oximes[34] with two tautomeric forms (44) and (45) and as such will undergo oxidation with lead tetracetate to yield the corresponding carboxylic acid in a manner analogous to the oxidation of oximes to the corresponding ketones[35].



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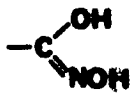
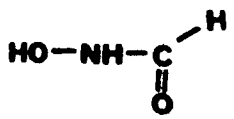


(45)

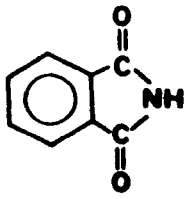
We believed that it would be impossible to convert formhydroxamic acid (46) directly into the corresponding isonitrile without protecting the hydroxyl function. Several O-protecting groups

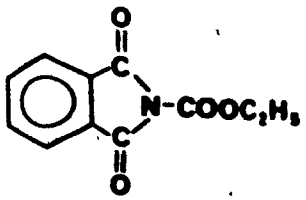


(46)

were evaluated. One of the most easily removed was the tetrahydropyranyl ether group. Hence this was tried first.

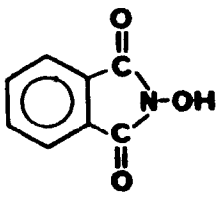
Phthalimide (47) was converted to N-carboethoxyphthalimide (48) using the procedure of Nelbens and Tesser[36]. Reaction of





(47) (48)

(48) with one equivalent of hydroxylamine hydrochloride (neutralized with triethylamine) in hot absolute ethanol[37] gave the N-hydroxyphthalimide (49). The free hydroxy group was then protected as a tetrahydropyranyl ether (50) by treatment of (49) with 1.5 equivalents of dihydropyran and a catalytic amount



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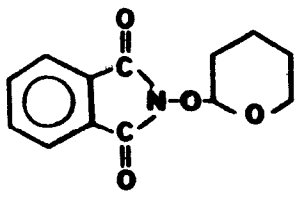
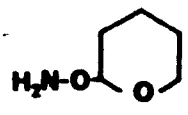


(50)

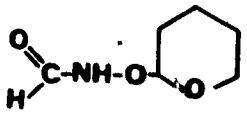
of phosphorus oxychloride in tetrahydrofuran for 3 hours. The O-protected hydroxylamine (51) was then formed[38] by heating (50) with an equivalent amount of hydrazine hydrate in benzene for 3 hours to give a 50% yield of the desired product. This long pro-



(51)

cedure was necessary since numerous attempts to prepare compound (51) by direct reaction of the hydrochloride salt of hydroxylamine with dihydropyran were unsuccessful. Compound (51) could be converted to the N-formyl derivative in almost quantitative yield by treatment with mixed acetic-formic anhydride[39] in pyridine.

However, attempts to transform (52) into the isonitrile (53) using the usual procedures[40,41] were not successful. The reactions were



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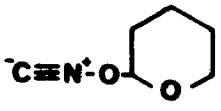
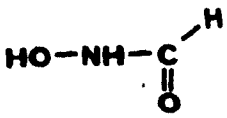


(53)

followed by ir spectroscopy since all isonitriles have a very characteristic band at 2120-2180 cm^{-1}, but no such band was observed in the ir spectrum of the products from either the phosphorus oxychloride procedure[40] or the newer phosgene method[41]. It appeared that the tetrahydropyranyl ether of (52) was too labile to withstand the reaction conditions used. An attempt to prepare (53) from (51) using the dichlorocarbene reaction discovered many years ago[42-44] and recently re-examined[45] was also unsuccessful.

Since the tetrahydropyranyl ether turned out to be unsuitable we decided instead to prepare an O-benzyl derivative that would be stable to the hydrolytic conditions of the isonitrile synthesis but could later be removed readily by hydrogenolysis[46].

The procedure outlined above for the synthesis of (51) cannot be used for the O-benzylation of hydroxylamine. We attempted to prepare N-formyl-O-benzylhydroxylamine (59) by direct benzylation of formhydroxamic acid (46) but this approach was unsuccessful.



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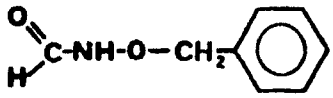
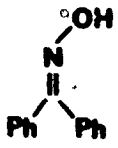


(59)

Hence, the following synthetic route was used. The oxime of benzophenone (54), mp 141-142°C, was prepared by the reaction of hydroxylamine with benzophenone in 95% ethanol[47]. However, it was discovered that the sodium salt of the oxime would not react with benzyl chloride under any of the normal conditions. Therefore, acetone oxime (55) was prepared according to the procedure of Janny[48]. This oxime was



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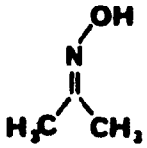
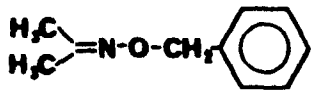


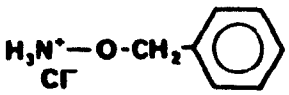
(55)

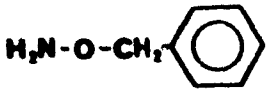
then treated with sodium in absolute ethanol to generate the sodium salt and this was reacted with benzyl chloride at reflux for 2 hours[49]. After filtration and removal of the solvent, the desired product (56) was obtained as a pale yellow oil. Treatment of this yellow oil with



(56)

concentrated hydrochloric acid at 100°C afforded the crystalline hydrochloride salt of O-benzylhydroxylamine (57). The free amine (58) was liberated by treatment of (57) with aqueous sodium

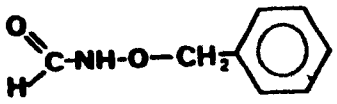




(57) (58)

carbonate followed by extraction with ether. The formylation of (58) to yield the N-formyl derivative (59) proceeded only with difficulty but was eventually accomplished by the very careful addition of the mixed acetic-formic anhydride to a solution of the amine (58) in pyridine. Unfortunately, attempts to transform (59) to the corresponding isonitrile (60) were unsuccessful using the usual procedures[40-45]. An attempt was made to perform the dehydration with p-toluenesulphonyl chloride in pyridine[51], but

this also was nonproductive.



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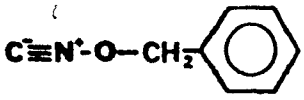
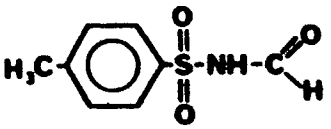
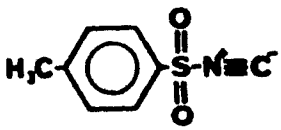


(60)

With these failures we abandoned the idea of using a hydroxylamine derivative and thought that since N-acetyl-p-toluenesulphonamide can be prepared, it may be possible to generate the corresponding N-formyl-p-toluenesulphonamide (61) which could be converted to the isonitrile (62). Any amide derived from (61) would be easily hydrolyzed. To this end we attempted to formylate



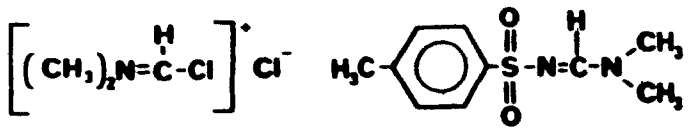
(61)



(62)

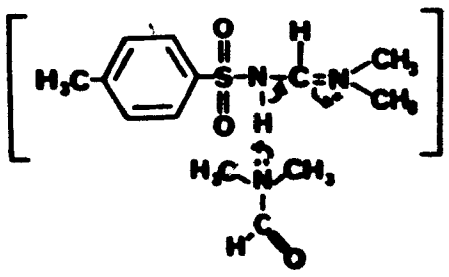
p-toluenesulphonamide to give (61), but the sulphonamide did not react, due to its acidic behaviour, with any of the more common formylating reagents such as formic acid, mixed acetic-formic anhydride or ethyl

formate. An attempt to use the phosgene-N,N-dimethylformamide complex (63) which is a good reagent for difficult formylations[52], gave only the condensation product (64). The condensation product



(63) (64)

(64) proved remarkably stable and could not be transformed to the desired formyl derivative (61). This unusual type of condensation, which is not normally observed with this reagent, can also be attributed to the acidic nature of the amide protons of the sulphonamide. In this case the solvent, N,N-dimethylformamide, was a strong enough base to remove the proton from the intermediate (65) and give (64) before the usual hydrolytic work-up could be carried out.

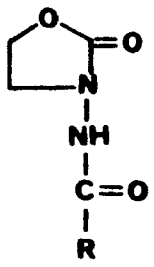


(65)

As in the case of the difficult formylation of O-benzylhy-

droxylamine, we also tried the alternate reaction sequence, that is, the tosylation of formamide. However, formamide and p-toluenesulphonyl chloride could not be made to react even under the forcing conditions of refluxing pyridine. We therefore gave up this approach.

Hydrazones are known to be easily oxidized by lead tetracetate[53-55]. Therefore, we felt that it was possible that acid hydrazides of type (66) could be oxidized with lead tetraacetate to (67). This intermediate (67) under hydrolytic conditions would decompose in the manner shown (68) to give the acid (69) and easily removed volatile components.



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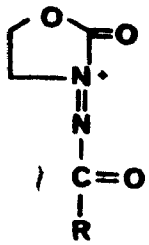

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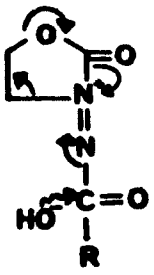

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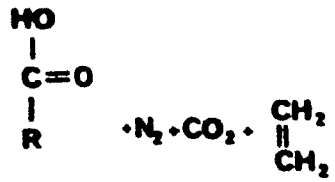


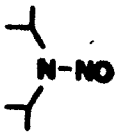
(69)

However, we first desired to prove that an isonitrile of the hydrazide type which would undergo the Ugi reaction could be made. Otherwise, any study on the hydrolysis of compounds of the type (66) to the acid (69) using the oxidation and hydrolysis procedure would not be relevant. A number of syntheses of hydrazide isonitriles have been reported[56,57], but in most cases the yields have been low or unstated. We decided to see if we could prepare a simple hydrazide isonitrile such as (70).

(70)

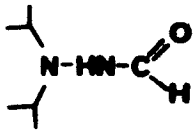
Diisopropyl amine (71) was converted to the N-nitroso compound (72) in quantitative yield by the action of sodium nitrite[58] in a

(71)



(72)

strongly acidic medium. This was then reduced[57] in the presence of zinc dust in 90% aqueous formic acid with mercuric chloride as catalyst to yield directly the N,N-diisopropyl-N'-formyl hydrazine (73) as a crystalline material. It was found that (73) could be converted to the isonitrile (70) in up to 80% yield using the usual phosgene procedure[41]. Reaction of (70) with the Schiff base of β-alanine and



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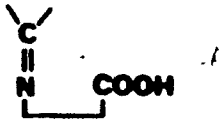
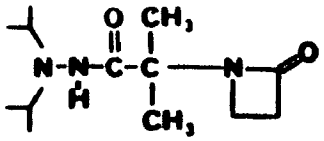


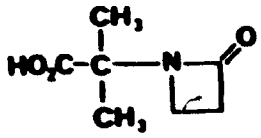
(74)

acetone (74) in dry methanol over a period of 85 hours[59] at room temperature gave the β-lactam (75) as a crystalline compound, mp 91-92°C, after chromatography on alumina. Attempts to hydrolyze this amide



(75)

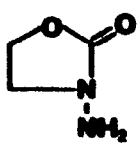
directly to the acid (76) while leaving the β-lactam intact showed us that this hydrazide was no more easily removed than were the secondary amides of Rossy's synthesis. An effort was made to quarternerize the N,N-diisopropyl substituted nitrogen with methyl iodide to



(76)

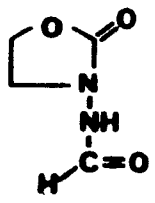
see what affect a quarternary nitrogen would have on the ease of amide hydrolysis, but this was unsuccessful - probably because the nitrogen is too sterically hindered to react with the iodide. There was no reaction even after prolonged heating.

Having shown that the formation of the hydrazide isonitriles was relatively easy and that they do undergo the desired Ugi reaction we endeavored to prepare our initial goal, 3-amino-2-oxazolidinone (77).

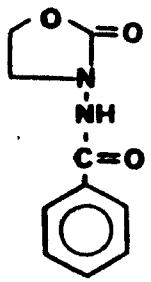


(77)

Initial attempts to condense 2-hydroxyethylhydrazine with phosgene led to complex mixtures which could not be separated. The condensation of the hydrazine with diethyl carbonate[60] in anhydrous methanol with sodium methoxide as catalyst was more successful, yielding (77) as a crystalline material, mp 64-66°C . The compound could be easily formylated using mixed acetic-formic anhydride at 0°C to give a quantitative yield of the N-formyl derivative (78) as an oil. This material was not transformed to the isonitrile as it was felt that simple acylation of the 3-amino group of (77) with an appropriate acid chloride would give us a model upon which to test the oxidation and hydrolysis reactions. Thus (77) was acylated with benzoyl chloride in pyridine to yield the desired hydrazide (79) in 61% yield.



(78)

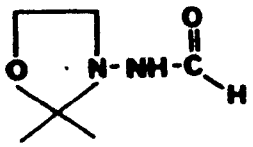


(79)

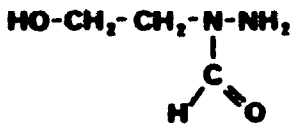
N-benzoyl-3-amino-2-oxazolinone (79) was then treated with one equivalent of lead tetracetate in cold methylene chloride for 30 minutes, and then with 5% aqueous bicarbonate, and the two phases mixed for 24 hours at room temperature by vigorous stirring. After separation of the two phases, acidification of the aqueous layer and extraction with chloroform, up to 83% benzoic acid was recovered. Over a number of

such reactions the yields of benzoic acid ranged from 54% to 83%. To show that the reaction does involve oxidation and hydrolysis of the oxidized intermediate, and not just simple base hydrolysis, the hydrazine (79) was stirred for the same period of time with 5% aqueous bicarbonate solution. Only the starting material was recovered after 24 hours, thus showing that the oxidation and subsequent hydrolysis took place in the desired manner. Details of the oxidation itself were not studied as they were not considered relevant to the study in question.

Other attempts to prepare oxazolidines such as (80) were not successful. Selective N-formylation of the terminal position of 2-hydroxyethylhydrazine was not possible. Only the 2-formyl derivative (81) was ever isolated and this could not be converted to the desired product (80). Attempts to prepare (82) directly led only to



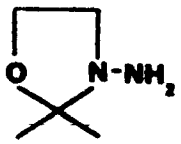
(80)



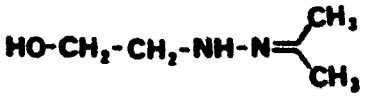
(81)

the hydrazone (83) so this approach was abandoned.

Thus, we were successful in synthesizing a compound

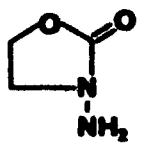


(82)



(83)

(77) that i) could perhaps be converted to an isonitrile and ii) produced an amide that could be readily removed under mild conditions. We felt that, should Fechtig's[29] work on the hydrolysis of amides on cephalosporin type compounds not be applicable to our cepham derivative, then this isonitrile provides an alternative.

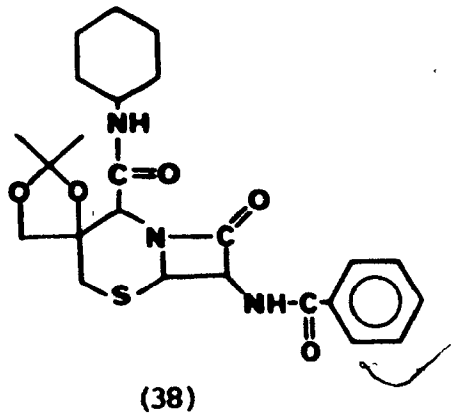


(77)

CHAPTER 2

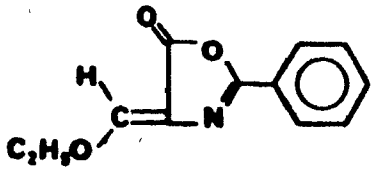
o-Nitrocinnamoyl chloride, an easily removable amine blocking group

One of the problems that had to be faced in the Rossy synthesis was the removal of the benzamide group at the 7-position of β-lactam (38). It is possible to remove such blocking groups



(38)

using Fechtig's procedure[29]. However, we believed that it would be useful if a group, removable under mild conditions, were substituted for the 2-phenyl group in (33) from which the sodium thiol (42) was prepared.



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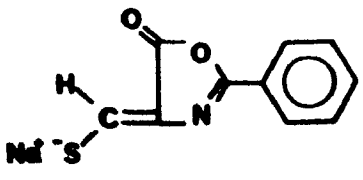
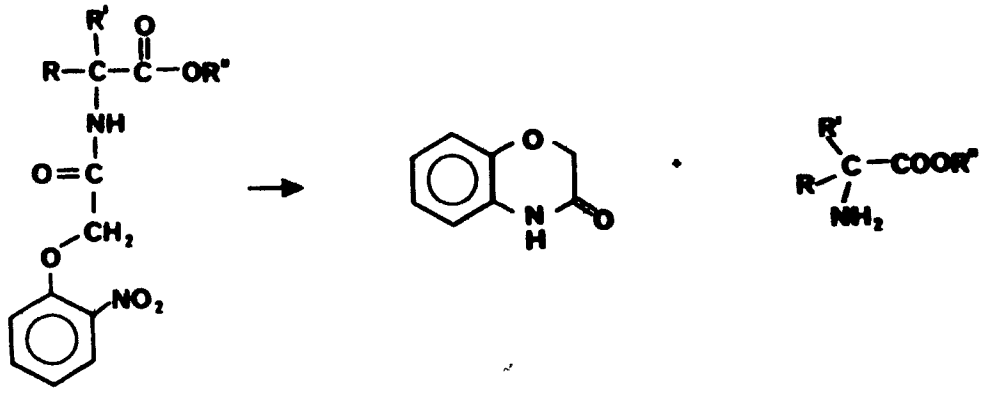


(42)

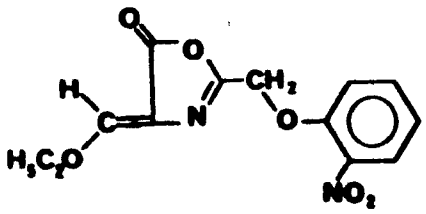
o-Nitrophenoxy acetates (84) are sometimes used as N-blocking groups in peptide syntheses[61]. They are easily removed by hydrogenation and gentle warming to yield the lactam (85) and the free peptide (86). However, the oxazolone (87) with a 2-o-nitrophenoxymethyl substituent cannot be prepared easily, since the desired 4-ethoxymethylene oxazolones can be made readily only when the 2-



(84)

(85)

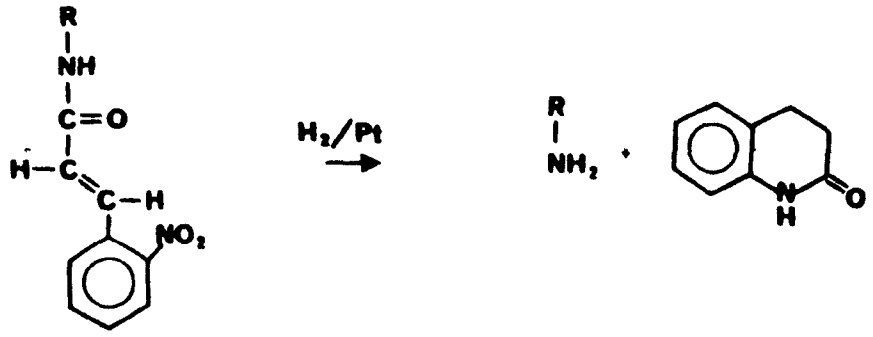
(86)



(87)

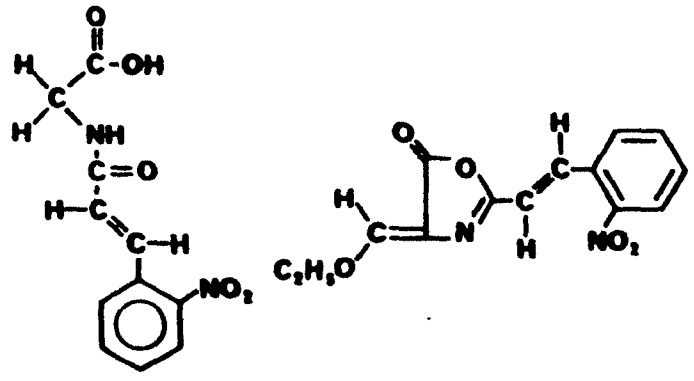
substituent is either aromatic or conjugated to an aromatic ring[2]. A 2-substituent which meets this requirement and which would undergo a cleavage reaction similar to an o-nitrophenoxy acetate is the o-nitrocinnamoyl group. On hydrogenation and warming, an o-nitro-

cinnamoyl protected amine (88) would generate the lactam (89) and release the free amine (90) in a manner analogous to (84)→(86).



(88) (90) (89)

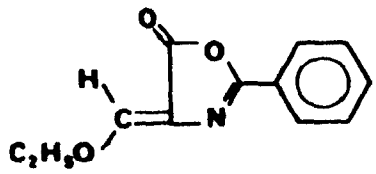
It was possible to prepare oxazolone (92) using the method described for the corresponding p-nitro compound[62]. Glycine was converted to the N-o-nitrocinnamoyl derivative (91) by condensation with o-nitrocinnamoyl chloride prepared from the commercially available acid with thionyl chloride. The result-



(91) (92)

ing acid (91) was refluxed with acetic anhydride and triethyl orthoformate in ethyl acetate for three hours. On evaporation of

the solvents and trituration with cold ethyl acetate, the oxazolone (92) separated as a crystalline product, mp 129-130°C, in about 30% yield. This was comparable to the yield of (33) obtained from hippuric acid (93).



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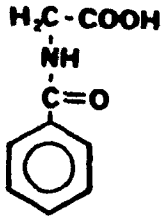
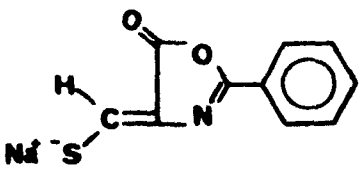
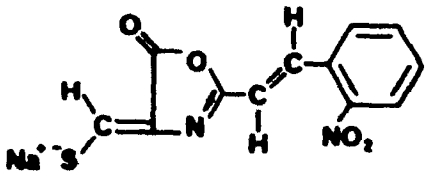


(93)

The sodium thiol (94) was prepared by treatment of (92) with sodium hydrosulphide in methanol in the manner analogous to the formation of sodium thiol (42) from (33).



(42)

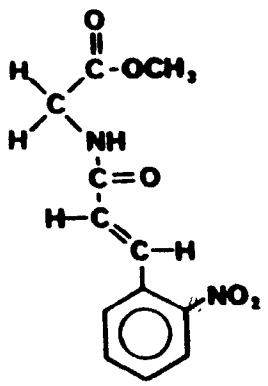


(94)

It remained to be shown, however, that the o-nitrocinnamoyl group would cleave in the same way as o-nitrophenoxy acetates.

When N-o-nitrocinnamoylglycine methyl ester (95) was hydrogen-

ated in ethanol with a platinum oxide catalyst[63], N-o-aminocinnamoylglycine methyl ester (96) precipitated from the reaction mixture. However, when the hydrogenation was carried out on (95) in warm glacial acetic acid, the expected δ-lactam (89) was obtained in excellent yield.



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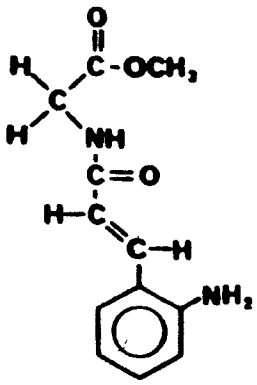
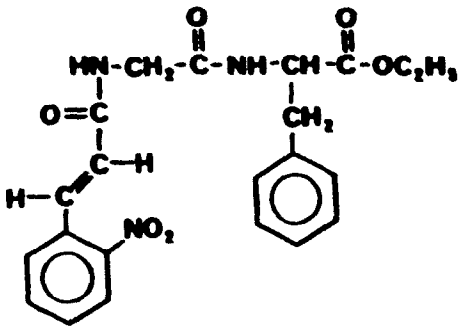


(96)

Since the methyl ester of glycine was too volatile to isolate easily from the hydrogenation reaction and we needed to be sure that the free amino-ester was being released, we decided to prepare some optically active dipeptides to see if i) the free amino-ester could be isolated and ii) no racemization occured.

o-Nitrocinnamoylglycine was successfully coupled with L-phenylalanine ethyl ester using the Sheehan and Hess procedure[64] to give the N-protected dipeptide (97) as a crystalline material, mp 149°C, in 78% yield. Hydrogenation of (97) in warm glacial acetic acid with platinum oxide catalyst gave the lactam (89) and the free peptide (98) in excellent yield and optical purity. The peptide (98) could be easily separated from the lactam (89) by extraction. The specific rotation of the synthetic dipeptide (98) was determined



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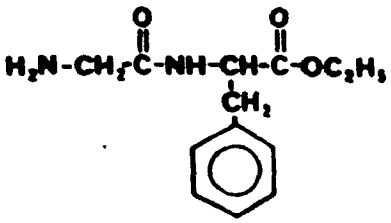
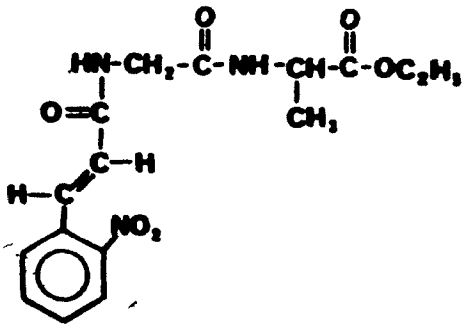


(98)

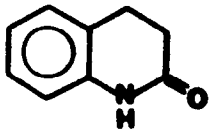
to be $[\alpha]^{25}_{5893}$=11.4° (c=3.80). An authentic sample showed $[\alpha]^{25}_{5893}$= 11.1° (c=2.00). It can be seen that no racemization took place during the hydrogenation reaction.

As a further verification the corresponding N-protected glycyl-L-alanine ethyl ester (99), mp 183-184°C, was prepared in the same



(99)

way[64] and on hydrogenation yielded the lactam (89) and the free peptide (100) also in excellent yield and optical purity. The specific rotation of (100) was determined to be $[\alpha]^{25}_{5893}$=37.3°



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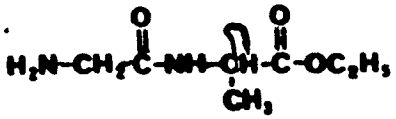
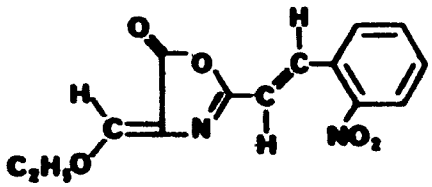


(100)

(c=1.60). An authentic sample showed $[\alpha]_{589}^{25}$=39.4° (c=2.00).

These results demonstrated that the o-nitrocinnamoyl group could be quite useful in peptide syntheses and that it is entirely possible to prepare an oxazolone (92) such that the resulting amide on ring opening can be removed under very mild conditions. This protecting group was not applied to the general Rossy synthesis as it is somewhat expensive and would only be applied once the methodology of β-lactam formation had been thoroughly proven.

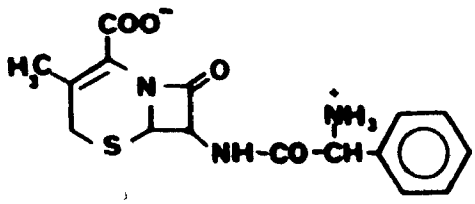


(92)

CHAPTER 3

Preliminary studies towards the synthesis of 3-methylcephalosporins

This study was undertaken to determine if some of the methodology developed by Rossy during the course of the synthesis of cepham (38) could be applied toward the synthesis of a 3-methylcephalosporin derivative such as cephalexin (23).



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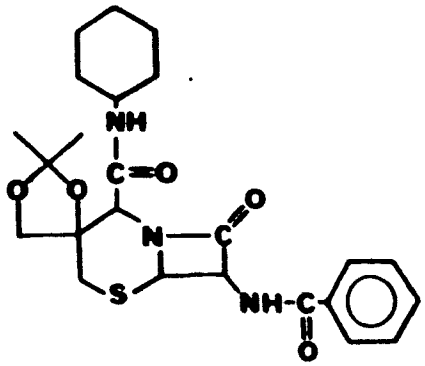
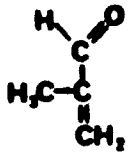


(38)

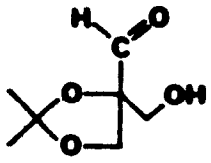
This would require a starting material which would differ from the one used in the synthesis of (38) only in that the "terminal" oxygen function would be missing. The hydroxy group at C-3 would still be necessary in a suitably protected form, in order to introduce the double bond.

Methacrolein (101) was chosen as a compound analogous to (28). It is readily available and inexpensive. It has the required 2-methyl substituent and a double bond that can be substituted so as to give a 2-hydroxy group as desired and, in the 3-position, a good leaving group that can be displaced by the thiol (42) to give

(102) in a manner analogous to the formation of (35).



(101)



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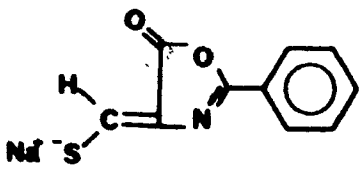
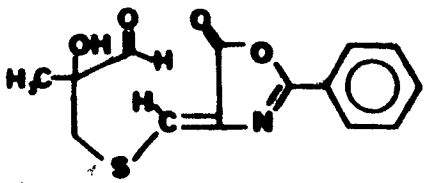
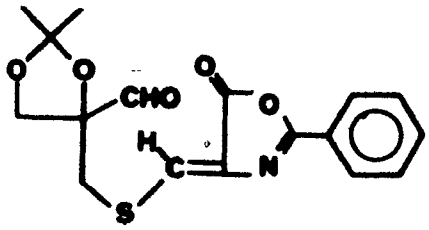


(42)

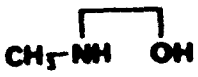


(102)



(35)

Since methacrolein is unstable and polymerizes quickly at room temperature, we felt that it was necessary to first protect the aldehyde before functionalizing the double bond in the desired manner. Attempts to use N-methylethanolamine (29) as a protecting group for methacrolein to give the oxazolidine (103) analogous to (30) were unsuccessful. Mixtures of the 1,1 and 1,4 addition products which could not be separated by the normal means were obtained. Therefore, methacrolein diethyl acetal (104) was prepared



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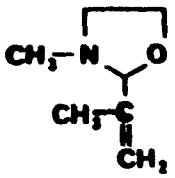
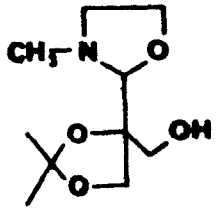
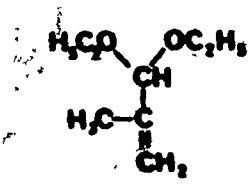


(103)

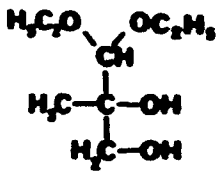


(30)

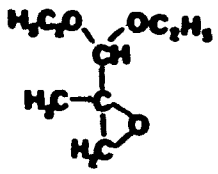


(104)

using triethyl orthoformate in ethanol with ammonium nitrate as catalyst[65]. Attempts to prepare the 2,3 diol (105) from the acetal (104) using either the osmium tetroxide/hydrogen peroxide[66] or aqueous potassium permanganate[67] procedures led only to complex mixtures that could not be separated by distillation. However, we felt that the epoxide (106) might undergo a ring opening reaction with a thiol

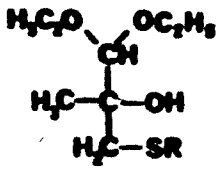


(105)



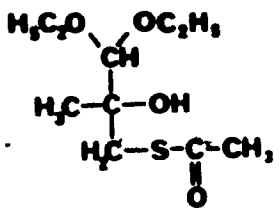
(106)

to give the 2-hydroxy-3-thio derivative (107). Thus the epoxide (106) was prepared using m-chloroperbenzoic acid in methylene chloride[68].

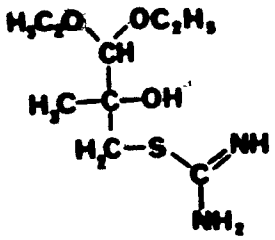


(107)

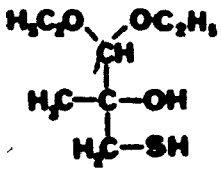
It was found that the resulting epoxide (106) did not react with thioacetic acid to give (108). Reaction with thiourea to give (109), from which the corresponding thiol (110) can be made[69,70], was too slow to be of any interest. Reaction of epoxide (106) with potassium thioacetate gave only complex mixtures. However, the thiol (110) could be isolated in good yield by treatment of (106) with hydrogen sulphide in methanolic sodium hydroxide[71].



(108)



(109)

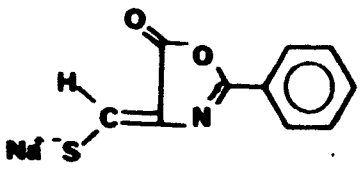


(110)

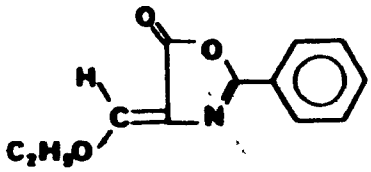
Because the epoxide (106) would not react cleanly with either potassium thioacetate or thiourea we did not attempt its condensation

with sodium thiol (42) according to the Rossy procedure.

Since thiol (42) was prepared by treatment of the ethoxymethyleneoxazolone (33) with sodium hydrosulphide we felt that

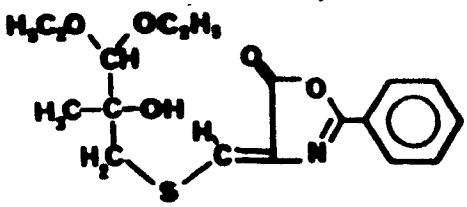


(42)



(33)

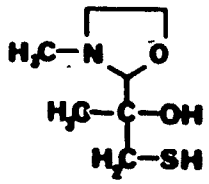
the ethoxy substituent may be displaced by thiol (110) in a like manner. On standing in pyridine for five days at room temperature (110) and (33) condensed in the desired way to give the compound (111) in 75% yield.



(111)

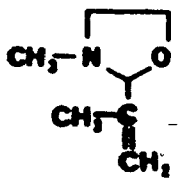
In order to continue with the synthesis, the diethyl acetal had to be removed. We therefore tried to regenerate the aldehyde.

However, it was discovered that the thiomethyleneoxazolone did not survive the strongly acidic conditions necessary to effect the hydrolysis. Although the acetal was hydrolyzed in less than one minute in 90% aqueous trifluoroacetic acid and in 5 hours in 10% trifluoroacetic acid in aqueous acetone the material isolated from these reactions was so impure as to be no further use. These results showed that we needed a more easily removable protecting group such as the N-methyloxazolidine function (112).

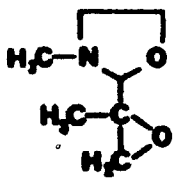


(112)

Since neither the oxazolidine of methacrolein (103) nor that of the corresponding aldehyde-epoxide (113) prepared according to the

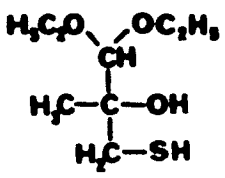


(103)



(113)

procedure of Payne[72] could be synthesized as a pure compound , it was decided to convert the labile thiol (110) into the much less reactive disulphide (114). On warming (110) in dimethylsulphoxide



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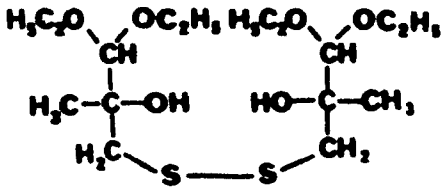
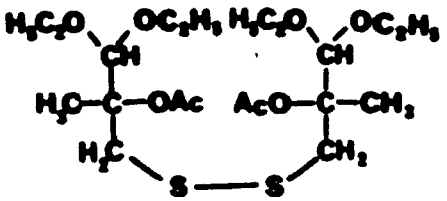


(114)

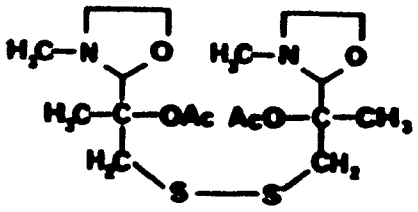
at 85°C for 18 hours[73], the disulphide (114) was isolated in 63% yield.

Its diacetate (115) could be prepared in 92% yield from the disulphide (114) using p-dimethylaminopyridine as catalyst[88]. We proposed to then hydrolyze the acetals in (115), reprotect the



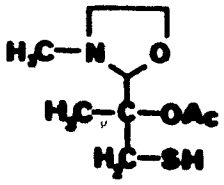
(115)

resulting dialdehyde with N-methylethanolamine to get the dioxazolidine (116), then cleave the disulphide to obtain the desired



(116)

thiol (112a). The work that is detailed in the next chapter became of more immediate interest to us at the time and these studies were discontinued. However, we have shown that, with some modifications, the methodology and intermediates developed by Rossy for his cepham studies could be applicable to the synthesis of a methylcephalosporin. Much work remains to be done and this is discussed in the section on "Proposals for Further Study".



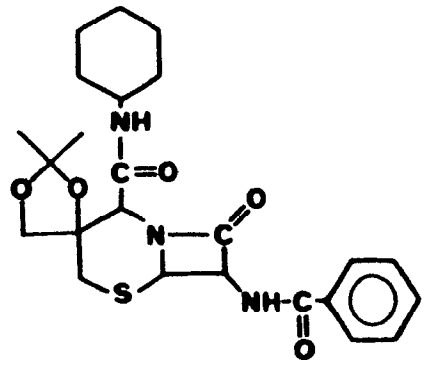
(112a)

CHAPTER 4

Improvements in the synthesis of key intermediates: some new observations

The synthesis of cepham (38) developed by Rossy suffered from the fact that several steps proceeded in relatively low yield, and that some intermediates could not be purified without resorting to chromatography.

In order to proceed with the synthesis, it was imperative to simplify the synthetic sequence so as to make key intermediates easily available.

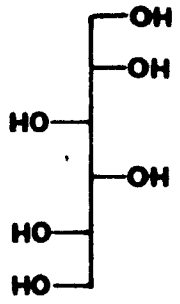


(38)

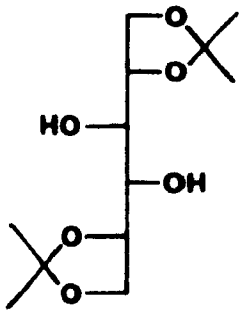
The starting point of the synthesis was D-mannitol (24) from which the diacetonide (25) was prepared using the known procedures[27]. Since this procedure rarely gave as much as a 50% yield and involved large quantities of reagents we felt that an improved method for its preparation was possible. It was on this basis that a study of

the formation of the acetonides of D-mannitol in acidic solutions of acetone and dimethoxypropane was undertaken with Dr. G. Kohan.

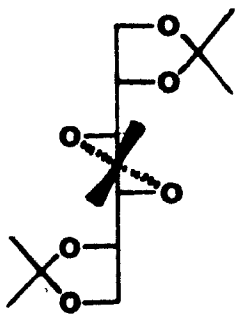
Discouraging initial studies showed that when a suspension of D-mannitol (24) was stirred in a mixture of dimethoxypropane and acetone at room temperature with p-toluenesulphonic acid as catalyst, the triacetonide (117) was formed rapidly. However, it was then discovered that if stirring was continued for longer periods, thin layer chromatography also showed the presence of the desired diacetonide (25). The concentration of diacetonide in the reaction



(24)



(25)

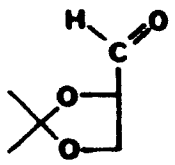


(117)

mixture seemed to reach a maximum after one and a half hours, after which it declined rapidly. After filtration to remove unreacted mannitol, neutralization with potassium carbonate, and removal of solvents, a 50% yield of the mixture of di and triacetonides was isolated. One recrystallization from either hexane or petroleum ether afforded the pure diacetonide (25) in 20% yield. Although the yield of this reaction was lower than that of the Baer and Fisher procedure[27], the shortness of the reaction time and the fewer reagents required, as well as the simple work-up, made this new procedure more suitable for the formation of large quantities of the diacetonide (25).

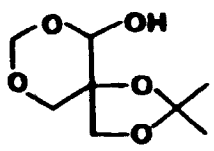
We felt that since the triacetonide (117) was formed initially, the diacetonide may have been formed by an unusual mechanism involving two molecules of the triacetonide (117) and one of mannitol (24) to yield three of the diacetonide (25). However, prolonged stirring of these ratios of triacetonide and mannitol in acidic solutions of dioxan or dimethoxyethane did not lead to the formation of any diacetonide (25).

From the diacetonide (25), glyceraldehyde acetonide (26) was



(26)

prepared by cleavage of the vicinal diol with lead tetraacetate in benzene. Removal of the benzene solvent by distillation led to loss of some product by co-distillation and polymerization. Since the formation of the dioxan (27) took place in an aqueous medium we attempted to perform the cleavage reaction with aqueous periodate. Isolation of the generated glyceraldehyde acetonide (26) then would be unnecessary. Even after prolonged stirring in potassium periodate solutions buffered at various pH values to protect the

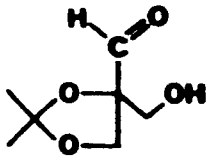


(27)

isopropylidine group, the diacetonide (25) was recovered in nearly quantitative yield. Thus the cleavage reaction and subsequent near quantitative transformation of the distilled glyceraldehyde acetonide (26) to the dioxan (27) could not be improved.

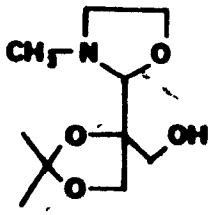
During an attempt to distill the dioxan (27) Rossy discovered that formaldehyde was evolved when the pot temperature exceeded 130°C. When evolution of formaldehyde ceased and the residue was quickly distilled under high vacuum, the distillate was found to be principally 2-hydroxymethylglyceraldehyde acetonide (28).

However, never more than a 50% yield of the hydroxy-aldehyde (28) was obtained from this pyrolysis reaction. The yields of the reaction dropped considerably below that figure when attempts were made to prepare larger amounts. The distillation could not be performed quickly enough to prevent the hydroxy-aldehyde from polymerizing in the distillation pot. Thus an alternative to the pyrolysis had to be found. N-methylethanolamine (29) was used to prepare (30) from the hydroxy-aldehyde (28). We believed that since formaldehyde



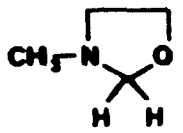
(28)

(29)



(30)

was liberated from the dioxan (27) so easily, the compound might react with N-methylethanolamine (29) directly, to give the oxazolidine-alcohol (30) and N-methyloxazolidine (118). Treatment of the dioxan



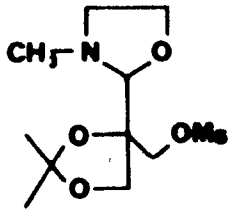
(118)

(27) with 2 equivalents of N-methylethanolamine in refluxing benzene rapidly led to the formation of (30) and (118). Water was azeotroped from the reaction mixture with benzene and, on evaporation of the solvent, N-methyloxazolidine (118) co-distilled. The residue was vacuum distilled to give an 86% yield of the desired oxazolidine-alcohol (30).

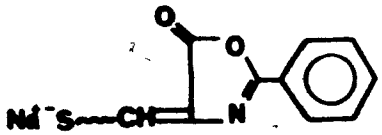
This new procedure had several advantages: i) It permitted the elimination of one step; ii) The step eliminated proceeded at best in 50% yield with the yield decreasing drastically upon scale-up; iii) The formation of a 10% impurity, which previously had to be removed by chromatography, was eliminated.

Formation of the oxazolidine-mesylate (31) from the alcohol (30) required great care. Too rapid additon of the mesyl chloride at -5°C in the original procedure led to severe reductions in the claimed yield of 80%. It was discovered, however, that the rate of mesyl

chloride addition was not so critical when the reaction was carried out at -50°C. At this temperature virtually quantitative yields of the crystalline oxazolidine-mesylate (31) were obtained.

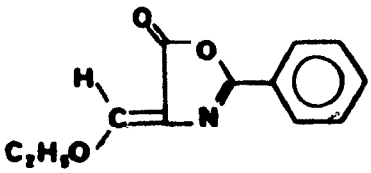


(31)

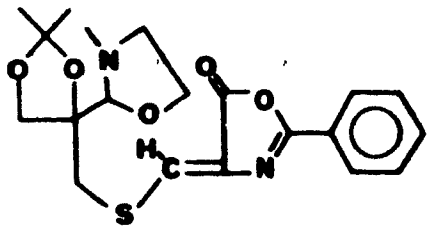


(32)

The next reaction in the Rossy sequence was displacement of the mesylate of (31) with the sodium thiol (32) in dimethylsulphoxide to give the oxazolidine-oxazolone (34). The sodium thiol (32) had been prepared from the ethoxymethyleneoxazolone (33) by treatment with sodium hydrosulphide in methanol for 30 minutes at 5°C. The sodium thiol (32) had been precipitated with ether to give a brown amorphous

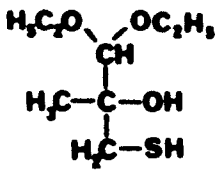


(33)

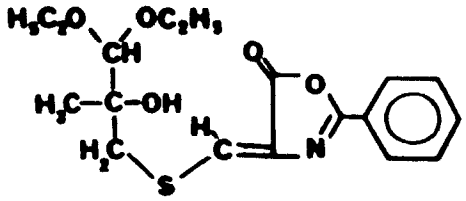


(34)

solid. We were not satisfied with this procedure or the quality of the sodium thiol produced so a study was undertaken to see if the condensation reaction conditions developed in Chapter 3 were applicable to this synthesis. In that case the thiol (110) had been condensed with ethoxymethyleneoxazolone (33) to give the acetal-oxazolone (111) in good yield.

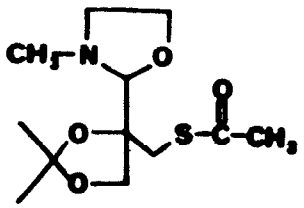


(110)



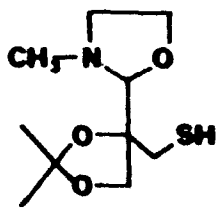
(111)

Treatment of the mesylate (31) with one equivalent of sodium hydrosulphide in methanol at room temperature or under reflux gave only complex mixtures. Attempts to use sodium sulphide to give the sodium thiol directly were also unsuccessful. However, the oxazolidine-thioacetate[74] (119) could be prepared. The mesylate (31)

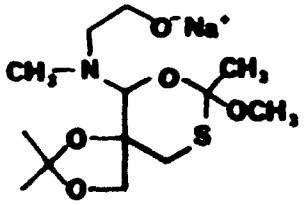


(119)

was refluxed in the presence of excess potassium thioacetate in 2-butanone for 3 hours. Filtration and evaporation of the solvent afforded the pure thioacetate (119) as a yellow oil in quantitative yield. However, attempts to generate the thiol (120) from the thioacetate (119) were not successful. Treatment of the thioacetate (119) with excess methoxide in methanol led to the formation of an intermediate that was more polar than the starting material according to thin layer chromatography but, even after prolonged reaction periods, some of the starting material remained. Neutralization and evaporation of methanol resulted in the recovery of the almost pure starting material, thioacetate (119). It is possible that a stable addition product such as (121) which was in equilibrium with (119) was formed in the reaction mixture.



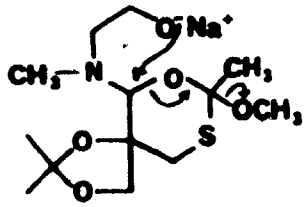
(120)



(121)

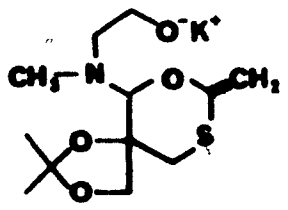
This could then revert to the starting material in work-up as shown, (121a).

To try to circumvent the formation of such an addition product a recently described procedure[75] for the hydrolysis of thioacetates



(121a)

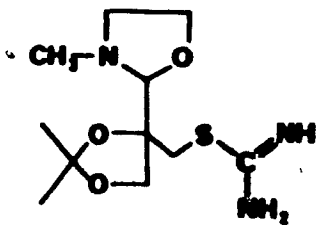
using strong non-nucleophilic bases was employed. However, treatment of the thioacetae (119) with either potassium t-butoxide in t-butanol or lithium triphenylmethane in ether led to entirely analogous results to those described above. In these cases an intermediate of the type (121b) is postulated. In most thioacetates, removal of a pro-



(121b)

ton and subsequent formation of ketene was the hydrolysis mechanism observed[75]. However, attempts to trap the intermediate (121b) by reaction with methyl iodide were unsuccessful.

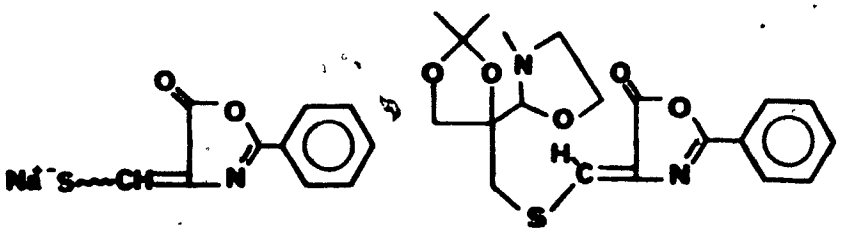
Since the thioacetate (119) could not be hydrolyzed to form the desired thiol (120) and the thiourea derivative (122), from which



(122)

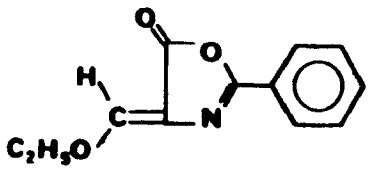
the thiol (120) could perhaps be made, could not be formed cleanly, this approach was dropped.

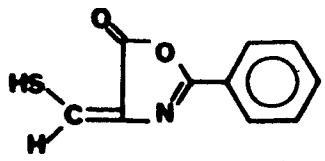
We turned our efforts to improving the method for preparation of the sodium thiol (32) and improving the subsequent condensation reaction to give (34).



(32) (34)

Thiolmethyleneoxazolone (123) could be prepared as follows[32]. A pyridine solution of the ethoxymethyleneoxazolone (33) was

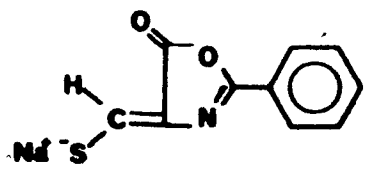


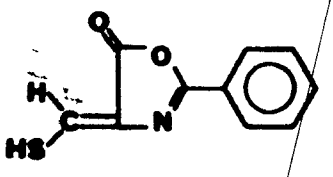


(33) (123)

saturated with hydrogen sulphide and allowed to stand at room temperature for 24 hours. It was then poured into dilute aqueous hydrochloric acid. After filtration, the thiol (123) crystallized from the filtrate in about 10% yield. Extensive efforts were made to improve the yield but were not successful.

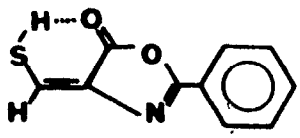
It was then discovered that if the ethoxymethyleneoxazolone (33) was treated with one equivalent of sodium hydrosulphide in methanol for 2 minutes at 0°C and the reaction mixture poured into hot dioxan, the sodium thiol (42) separated as yellow starlike crystals on cooling. The average yield over several runs was 85%. The free thiol (124), prepared from (42) by precipitation from



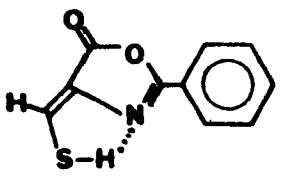


(42) (124)

aqueous solution with acetic acid, proved to be a different isomer from (123). The actual stereochemistry was assigned by spectral means (see following page). cis-Thiol (123) can be drawn (123a) to show the hydrogen bonding between the thiol hydrogen and the oxazolone carbonyl. This reduces the carbonyl stretching frequency of the anhydride type doublet to 1760 cm^{-1} and 1710 cm^{-1}. The C-N double bond streching frequency appears as a shoulder at 1690 cm^{-1}. In (124) the trans-thiol, the hydrogen bonding shown (124a) reduced the C-N double bond frequency to 1660 cm^{-1} while the carbonyl anhydride doublet appears at 1805 cm^{-1} and 1800 cm^{-1}.



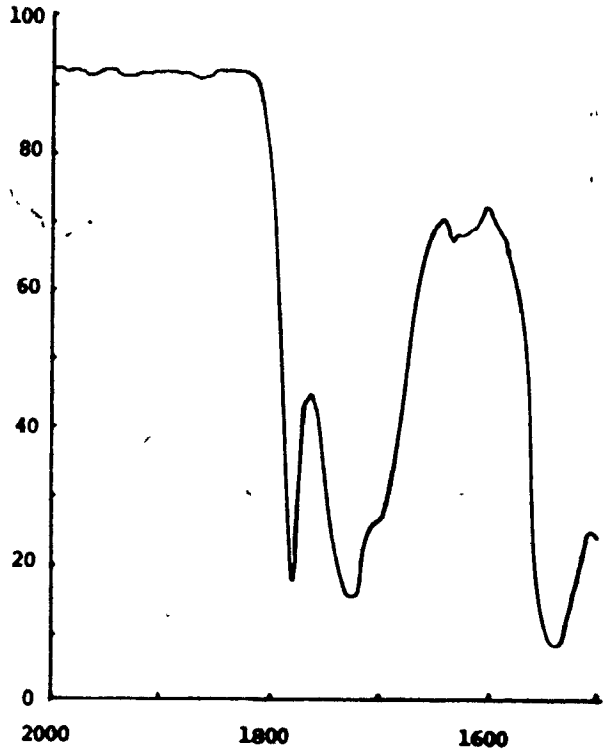
(123a)

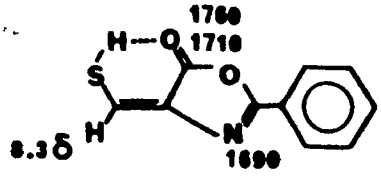


(124a)

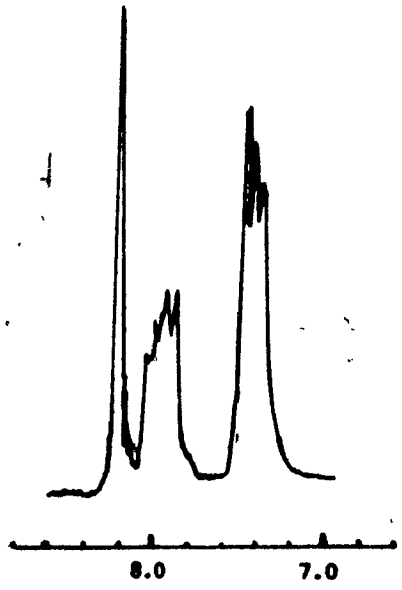
We believe the nmr spectra corroborate this evidence. The relative chemical shifts of cis and trans β-hydrogens in α-β unsaturated carbonyl compounds is well documented[76-78]. Compounds analogous to (123) and (124) have not been studied but general trends indicate our assignments are probably correct.

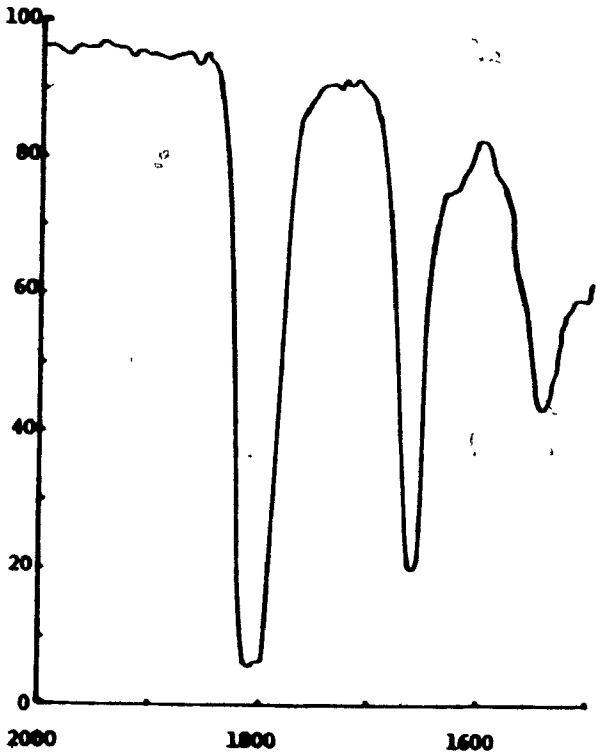
The methylene proton in (124) appears as a singlet at 7.3 δ


100
80
60
40
20
0
2000
1800
1600


1760
1710
1690
8.3δ

(123a)


8.0
7.0


100
80
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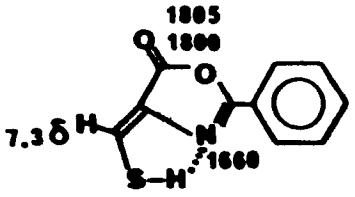
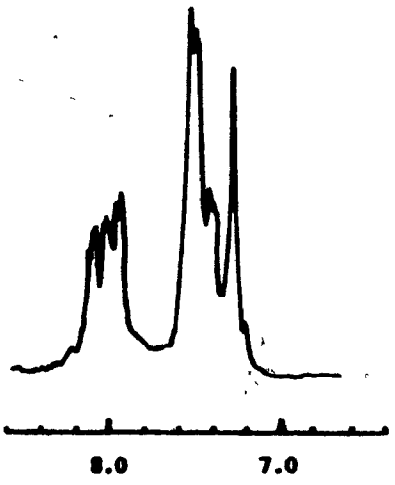
1600


1805
1800
1660
7.3δ

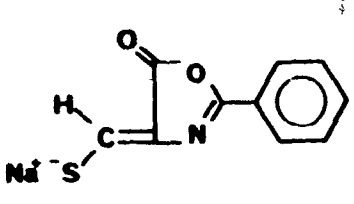
(124a)


8.0
7.0

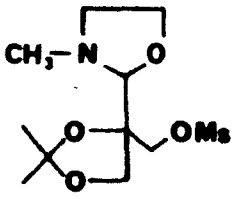
whereas the more deshielded methylene proton in (123), trans to the carbonyl but cis to the nitrogen, is shifted down to 8.3 δ.

The separate preparation of both these isomers allowed us to assign the stereochemistry of (42) with certainty for the first time.

Since the condensation reaction of sodium thiol (42) and mesylate (31) in dimethylsulphoxide was difficult to work up and



(42)

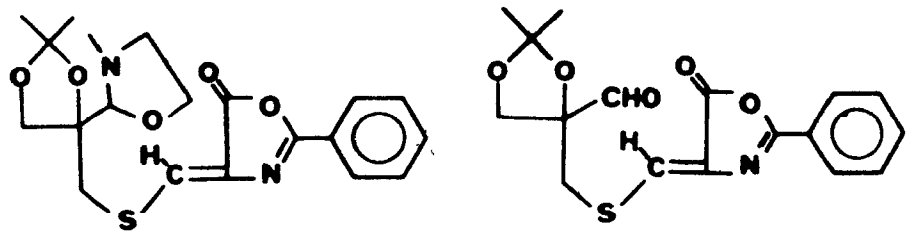


(31)

gave an impure product, we wished, if possible, to avoid this solvent for this reaction. Potassium thioacetate was observed to displace the mesylate (31) cleanly in refluxing 2-butanone in 3 hours. Hence, we attempted to use the same conditions for the condensation of (31) and (42). After only one and a half hours with a 30% excess of the sodium thiol (42), thin layer chromatography showed little or no starting mesylate (31) and one major product with several minor products. The dark coloured reaction mixture was filtered through alumina to remove the coloured impurities and the oxazolidine-oxazolone (34) was isolated as a yellow crystalline material, mp 127°C, in 50-55% yield. This relatively low yield may be attributed to an

attack of the thiol (42) on the labile oxazolidine protecting group of the mesylate (31) as a competing reaction. Such reactions have been observed by Mr. B.Y. Chung using the sodium salt of 2-phenyl-4-hydroxymethylene-5-oxazolone in an analogous displacement attempt.

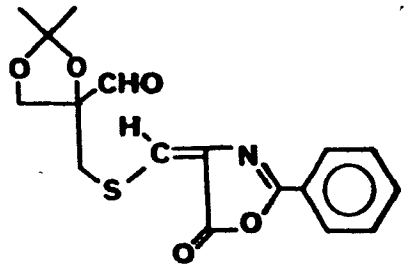
The hydrolysis of the oxazolidine protecting group in (34) to give the corresponding aldehyde (35) was carried out by treatment of



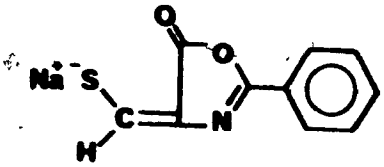
(34) (35)

(34) with 50% aqueous acetic acid for 1 hour at room temperature as before. Dilution of the reaction mixture and extraction with chloroform afforded the crystalline aldehyde (35), mp 80-81°C, in up to 80% yield. Entirely similar results were obtained when the cis sodium thiol (41) was used in place of the trans sodium thiol (42) for the condensation reaction. The aldehyde-oxazolone (35a) was obtained as a white crystalline solid, mp 83-85°C.

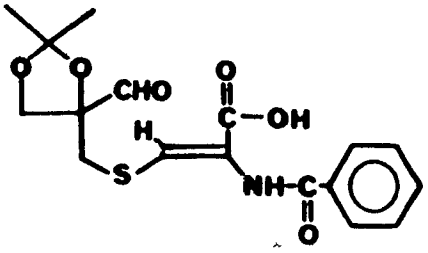
Attempts to convert the aldehyde oxazolone (35) to the corresponding aldehyde acid (36) were less successful. The hydrolysis reaction was followed by ultraviolet spectroscopy.



(35a)



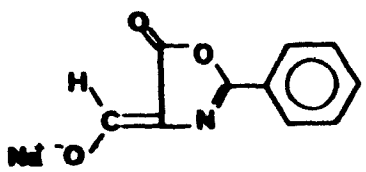
(41)



(36)

The thiomethylene-oxazolone (35) has a characteristic uv absorption at 360 nm. Upon hydrolysis of (35) to the acid-amide (36) the uv spectrum should shift to the absorption characteristic of a benzamide and a β-thio-substituted enone, namely about 225 nm and 285 nm[80]. However, when the hydrolysis was carried out in 2N aqueous sodium hydroxide[81], a rapid shift of the 360 nm absorption to 330 nm was observed after which there was no further change. The 330 nm absorption is characteristic of the hydroxymethyleneoxazolone salts.

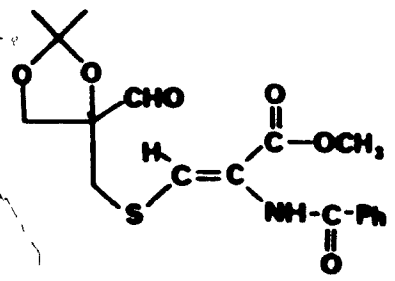
Thus, displacement of the thio substituent of the oxazolone by hydroxide ion must have taken place. This was confirmed when the sodium salt of the hydroxymethyleneoxazolone (125) was observed to precipitate from the reaction mixture. Any amounts of the acid (36) obtained from this reaction were always very small as observed by Rossy[26] in his synthesis. Whereas we obtained a clean crystalline aldehyde (35) from the hydrolysis of (34) the material obtained by Rossy from the same reaction showed no spectral evidence of an aldehyde function. It is believed that the hydrated aldehyde or a cyclic tautomer may have been the material that was converted by base to the acid (36).



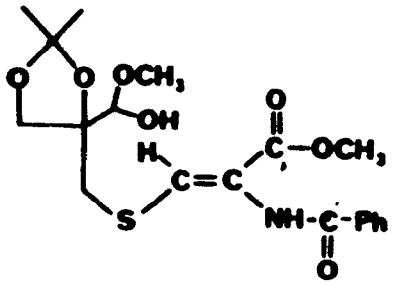
(125)

We felt that it was possible that this problem could be circumvented if a milder base such as aqueous sodium bicarbonate or carbonate were used to open the oxazolone. However, no reaction was observed when a suspension of (35) was stirred for prolonged periods in a solution of sodium bicarbonate or sodium carbonate. On heating these aqueous suspensions or on addition of a co-solvent such as dimethoxyethane, dioxane or tetrahydrofuran to give a

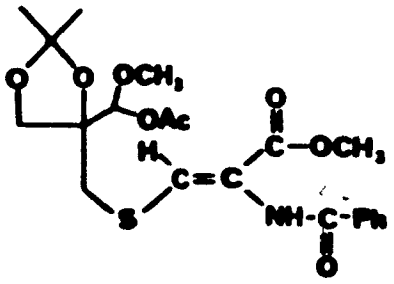
homogeneous solution, results similar to those obtained with aqueous sodium hydroxide were observed. Reaction of (35) with bicarbonate in 50% aqueous methanol led to a different result. In this case the uv spectrum showed the desired 285 nm and 225 nm absorptions. When the reaction mixture was diluted with water and extracted with chloroform to remove neutral impurities before acidification, all the material was observed to extract into the chloroform from the basic aqueous layer. It was identified as a mixture of the methyl ester-aldehyde (126) and the corresponding hemiacetal (127) isolated as the acetate(127a). How the methyl ester is formed in basic aqueous methanol is not understood. The reaction does not take



(126)

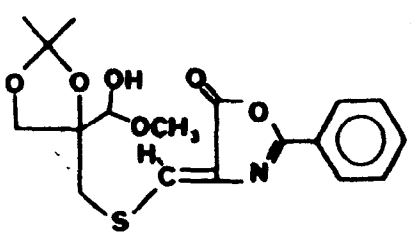


(127)

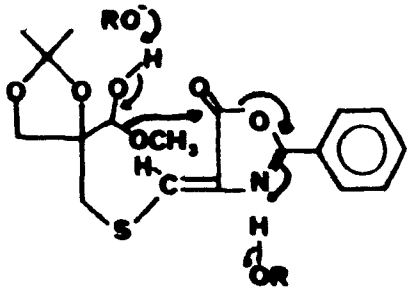


(127a)

place in the absence of a base. The oxazolone of (35) is quite stable in neutral aqueous methanol. It was originally believed that the hemiacetal (128) was formed first, then a base initiated internal methoxy transfer of the type shown, (129), leading to (126) was the

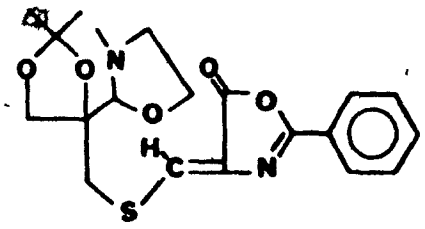


(128)



(129)

mechanism of ester formation. However, it was discovered that the oxazolidine-oxazolone (34) also underwent the same ring opening reaction to give (130). In dry methanol using about 10% triethylamine as catalyst, the ester formation was complete in less than 10 minutes at room temperature on both (34) and (35). Since the



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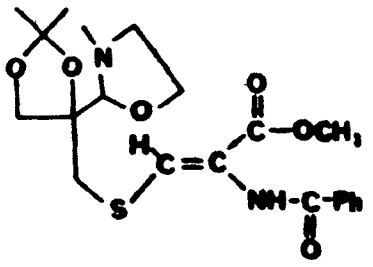
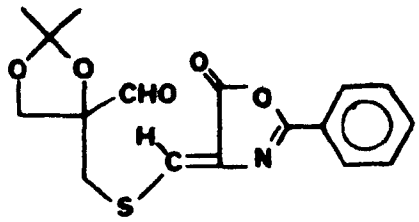


(130)



(35)

proposed hemiacetal formation and methoxy shift is not applicable to (34), some other unknown mechanism must be involved. It was observed that the rate of the reaction was a function of base concentration but a rate study was not undertaken.

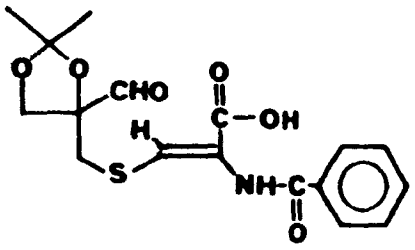
The oxazolidine-ester (130) could be converted to the corresponding aldehyde (126) in quantitative yield using the aqueous acetic acid procedure. This led to an aldehyde free of hemi-acetal (127) and this route became the preferred procedure.

Since we were unable to hydrolyze the aldehyde-oxazolone (35) to the acid (36) in acceptable yield, we decided to develop a synthesis of the cepham (38) using the methyl ester (126) which we were able to prepare in good yield. The synthesis and its variations are described in the next chapter.

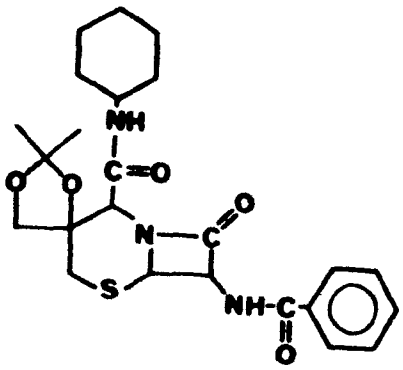
CHAPTER 5

Synthesis of a cepham derivative

The aldehyde-acid (36), which is an essential intermediate in the Rossy synthesis of cepham (38) could not be prepared in a satisfactory manner.

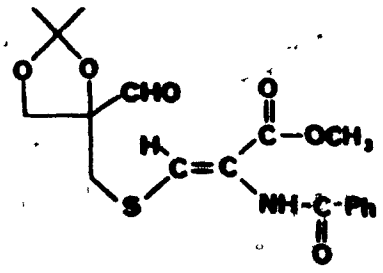


(36)



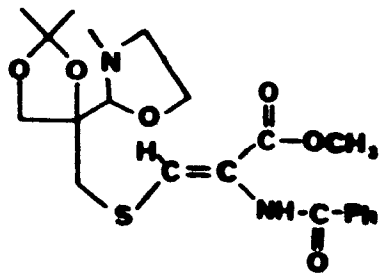
(38)

However, the corresponding methyl ester (126) could be made in good yield and purity. Hence, we attempted to develop an



(126)

alternate synthetic route to the cepham (38) using an ester as a starting point. We wanted to prepare (36) from (126) but because of the many functional groups in (126) we anticipated that selective hydrolysis of the methyl ester might prove difficult. We thus attempted to remove the methyl ester of (126) under the mildest hydrolytic conditions possible. However, after prolonged stirring of the ester (126) in aqueous tetrahydrofuran or aqueous dioxan solutions of bicarbonate or carbonate only starting material was recovered. Treatment of the ester (126) with either excess or one equivalent of sodium hydroxide in aqueous tetrahydrofuran led to the disappearance of starting material according to thin layer chromatography, but the acid-aldehyde (36) could not be isolated. Attempts to generate the acid from the ester using anhydrous lithium iodide in pyridine[89] led only to black tars. Entirely similar results were obtained using the oxazolidine-ester (130).

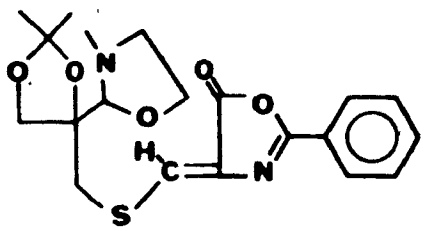


(130)

This led us to see if we could prepare another ester that could be removed more easily. We wanted an ester that could be

removed by either extremely mild hydrolytic conditions or by non-hydrolytic methods.

We discovered that the oxazolidine-oxazolone (34) would undergo ring opening reactions to give the corresponding ester only with primary alcohols in the presence of a base. Secondary and tertiary alcohols would not react even after prolonged heating. This gave us only a limited number of esters of the type we desired from which to choose. The βββ-trichloroethyl ester (131)



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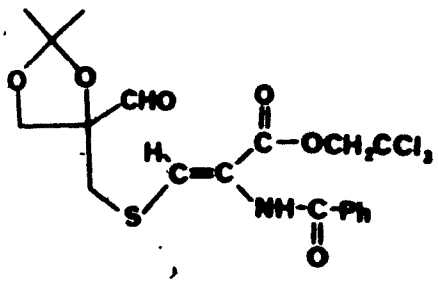
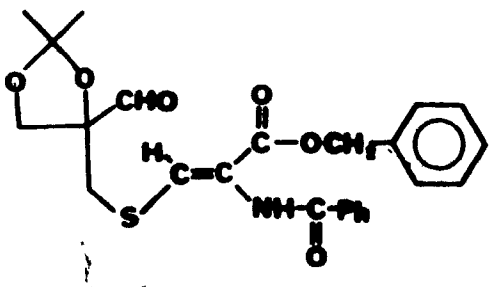


(131)

could be prepared as could the benzyl ester (132) and the p-anisyl ester (133). At this point we wanted to see if we could prepare



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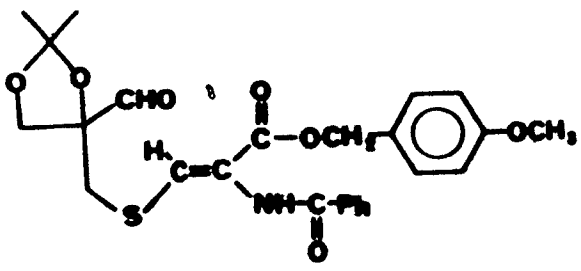
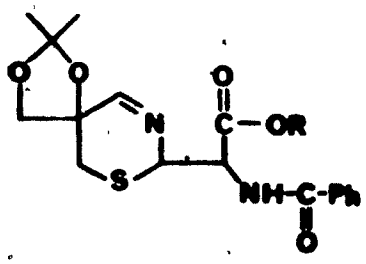


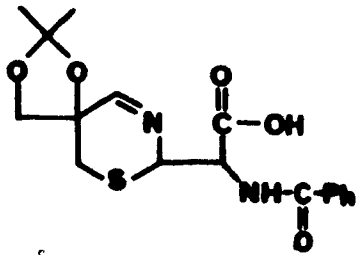
(133)

the corresponding imines (134) from these aldehyde-esters.

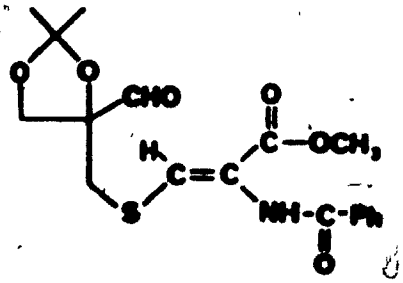
The conditions used by Rossy for the formation of the imine acid (135), bubbling of ammonia into boiling benzene for 24 hours, were considered unnecessarily vigorous for the formation of the corresponding imine-esters (134) in which the cyclization reaction would be expected to take place more easily. Thus, the methyl ester (126) was dissolved in a number of solvents such as ethanol,



(134)

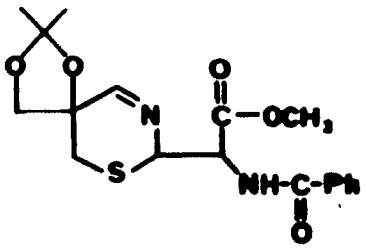


(135)

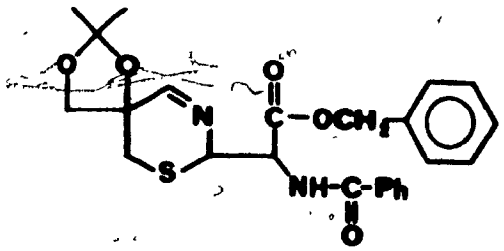


(126)

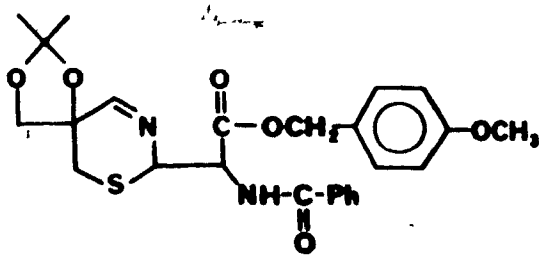
dimethylsulphoxide and dimethoxyethane and the solutions were saturated with ammonia at 0°C and then allowed to stand at room temperature. The change in the uv spectrum as a function of time was used to follow the reaction. The absorption at 285 nm characteristic of the β-thio substituted α,β-unsaturated ester, which would disappear upon ring closure, was observed to decline in all solvents, while the 225 nm benzamide absorption remained unchanged. The 285 nm absorption disappeared completely within 3 hours in dimethoxyethane or ethanol and after 5 hours in dimethylsulphoxide. However, a small absorption at 275 nm which could not be explained, appeared in both ethanol and dimethylsulphoxide but not in the dimethoxyethane reaction. Thin layer chromatography also indicated a cleaner product with the latter solvent. Upon complete evaporation of dimethoxyethane from the reaction, the imine (136) was obtained as a crystalline solid, mp 125-128°C, in 70% yield. In a like manner the benzyl ester-imine (137) and the p-anisyl ester-imine (138)



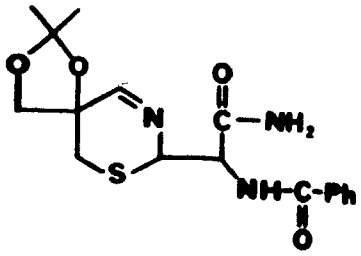
(136)



(137)



(138)

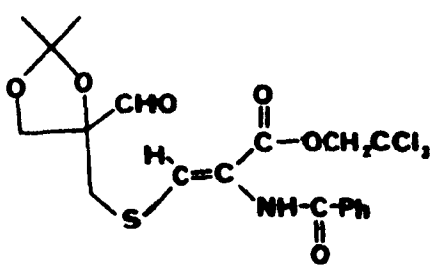


(139)

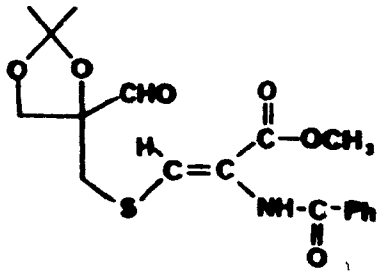
could be prepared as amorphous solids. Attempts to prepare the trichloroethyl ester-imine led only to the formation or what appeared to be amide-imine (139)*. We then undertook a study of the hydrolysis of both the aldehyde-esters and the imine ester. Attempts to use the previously mentioned hydrolysis methods on (136) led chiefly to hydrolysis and decarboxylation. The decarboxylation proceeds presumably after isomerization of the double bond to give a β-imino-acid. Such compounds are known to decarboxylate easily[83]. Attempts to hydrogenolyze the benzyl ester-imine (137) using palladium on charcoal in t-butanol or ethanol were unsuccessful. Only starting material was recovered after 24 hours. Attempts to use the nitrous acid deamination[33] of the amide-imine (137) were also unsuccessful. Treatment of the p-anisyl ester-imine (138) with trifluoroacetic acid led only to complex mixtures in which there appeared to be no major component. We, thus, attempted† to remove the trichloroethyl ester in (131) using zinc in acetic acid and zinc in methanol.

* This reaction was carried out by Mr. S.G. Kim

† With the help of Mr. S.G. Kim



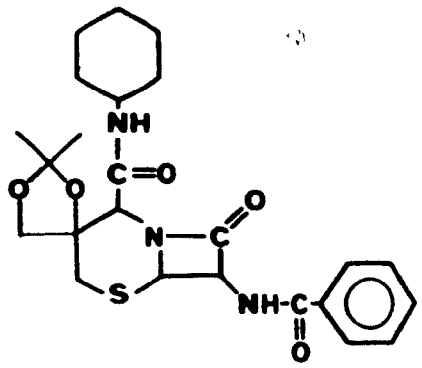
(131)



(126)

In acetic acid a general decomposition of the molecule was observed while in methanol only the methyl ester (126) was isolated.

At this time a new mild procedure for the hydrolysis of methyl esters involving treatment with lithium n-propyl mercaptide in hexamethylphosphoric triamide[84] was brought to our attention[85]. We did not feel that the imine-acid (135) generated from this reaction could be isolated from the hexamethylphosphoric triamide solvent. Hence, we planned to perform the hydrolysis and the subsequent Ugi reaction without isolating the intermediate imine-acid (135). The methyl ester-imine (136) was treated with 2 equivalents of lithium n-propyl mercaptide in hexamethylphosphoric triamide and allowed to stand at room temperature for 1.5 hours. The mixture was then treated with deoxygenated phosphate buffer solution and cyclohexylisonitrile in a mixture of methylene chloride/carbon tetrachloride. The two phases were mixed with vigorous stirring for 12 hours. The crude product was observed to contain a substantial amount of the β-lactam (38) as evidenced by the infrared absorption



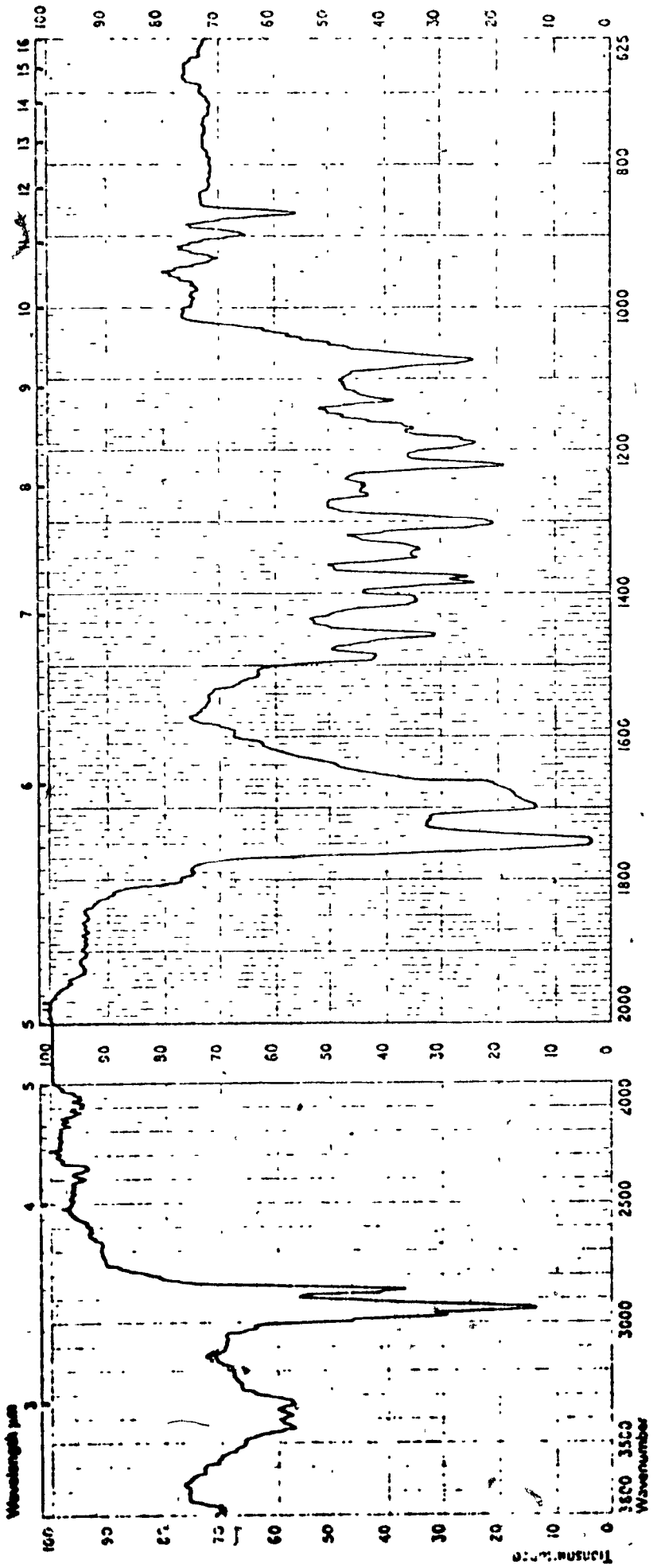
(38)

(1740 - 1750 cm^{-1}). The crude mixture was purified by means of thin layer chromatography. Two bands were removed from the thin layer plates. Both were observed to contain the β-lactam as evidenced by their ir spectra and both showed m/e = 473 (M^+) in their mass spectra. The upper band appeared to be purer, giving a strong β-lactam ir absorption (see following pages for ir and mass spectrum bar graph). Each band represented about a 30% yield.

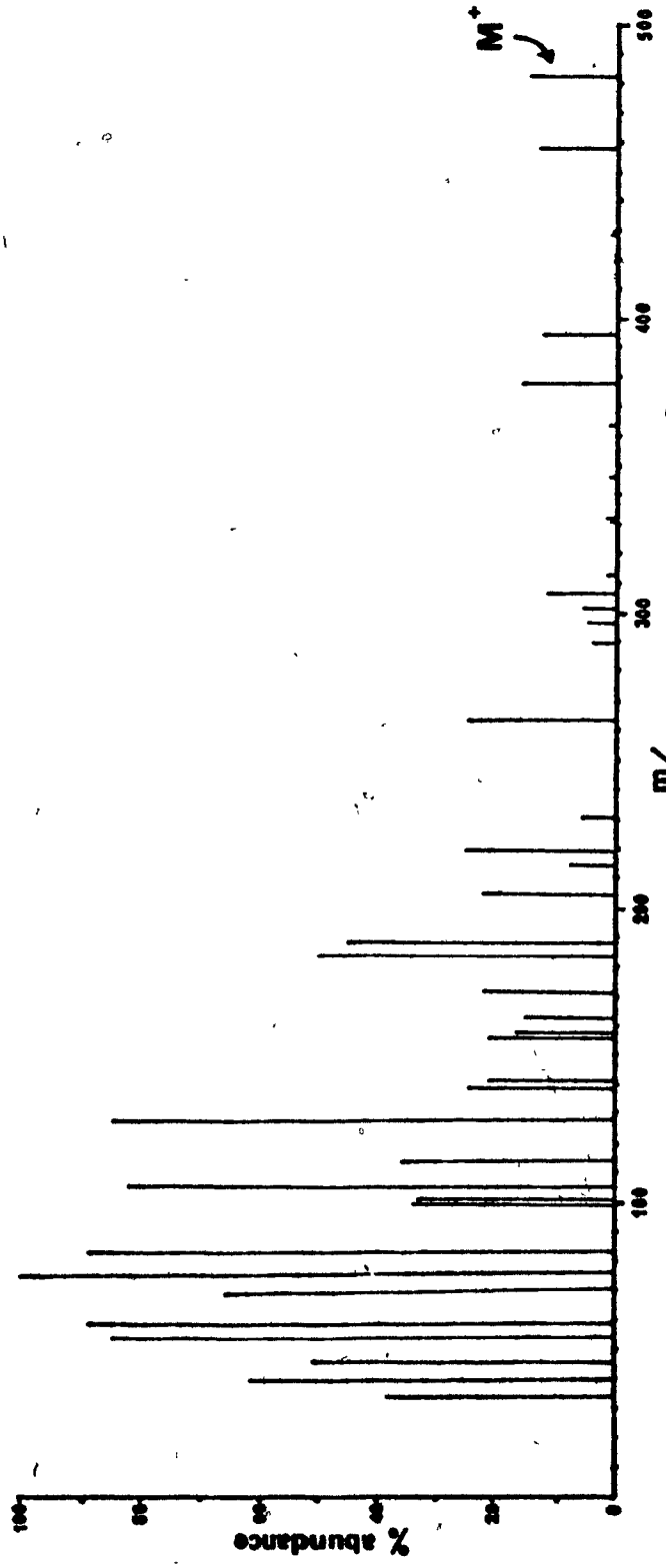
The nmr spectrum of the purer upper band, while of a mixture of two isomers, was consistent with the structure (38).

On attempts to repeat the reaction it was discovered that yields of the β-lactam obtained were very variable. Such factors as temperature, pH of the aqueous phase, and timing were found to be critical. Optimum conditions have not yet been worked out.

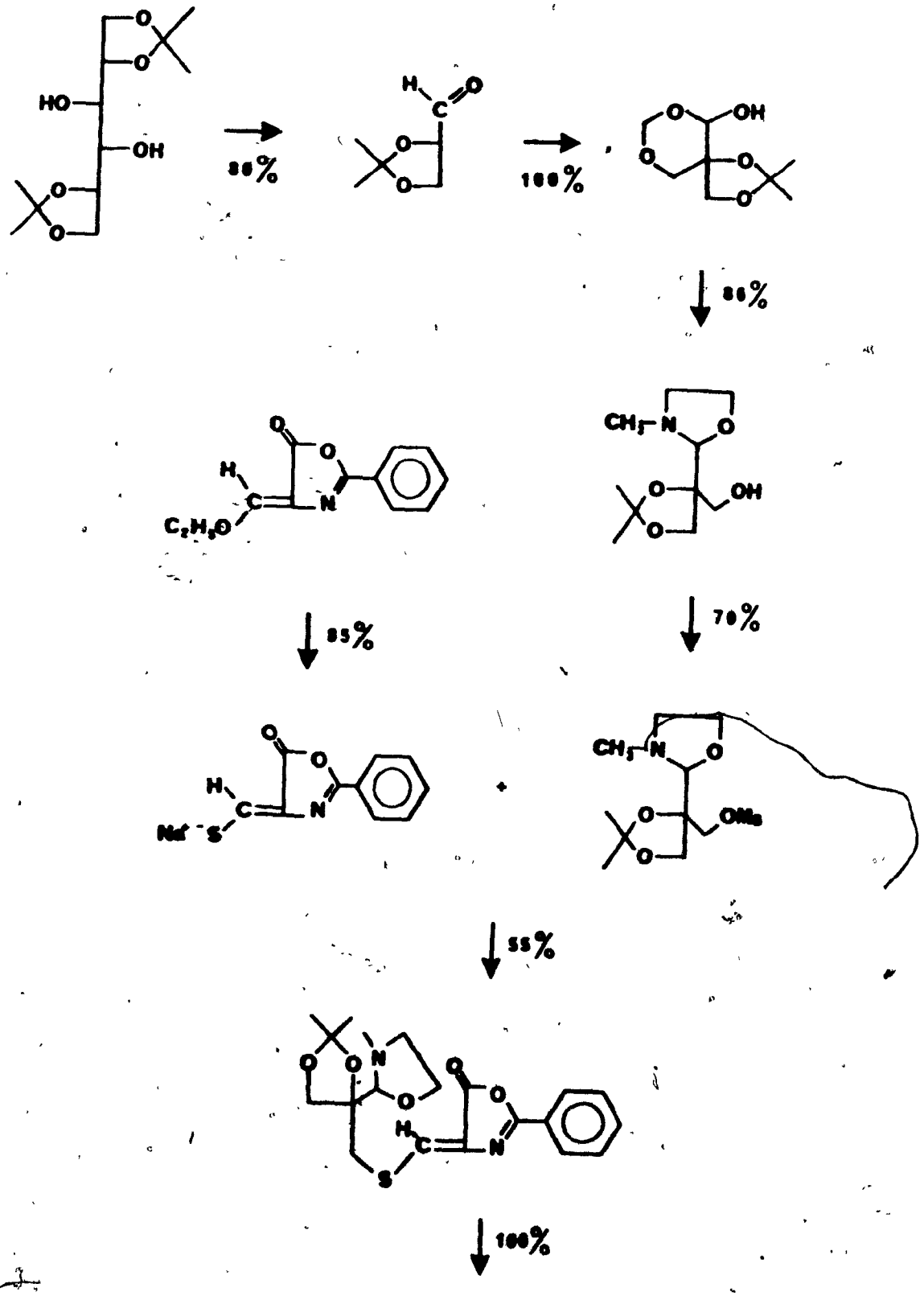
Because of the large number of compounds mentioned in connection with this synthesis the complete synthetic sequence is diagrammed on pages 83 and 84. The overall yield starting from the diacetonide (25) to the imine ester (126) was 18%. Optimization of the final reaction is now being carried out by Mr. S.G. Kim.

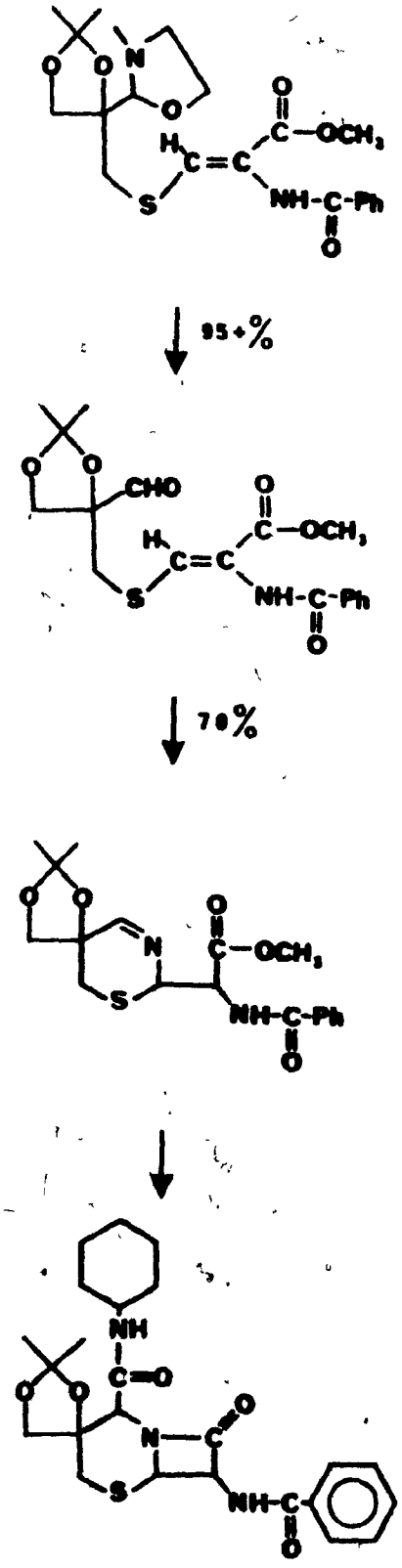


Ir spectrum of β-lactam (38)



Mass spectrum of β-lactam (38)

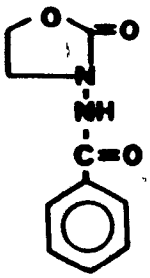

HO
OH
88%
100%
86%
C_2H_5O
85%
CH_3
OH
70%
55%
100%


95+%
70%

Proposals for Further Study

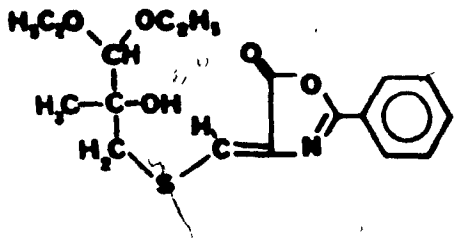
Since the work that has been described in the preceeding chapters is a series of studies aimed at synthesis of cepham derivatives, several new avenues for further study were opened that could not be investigated.

In chapter 1 the oxidation of the N-benzoyl-3-amino-2-oxazolidinone (79) and the subsequent hydrolysis of the oxidation product is described. A study of the mechanism and products of the initial lead tetracetate oxidation as well as the products of the hydrolysis, besides the isolated benzoic acid, should be undertaken.



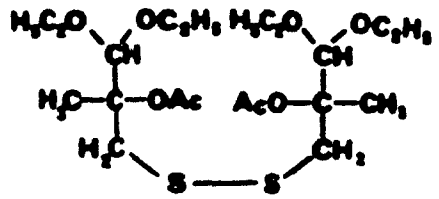
(79)

Chapter 3 is only a preliminary study on the synthesis of a 3-methylcephalosporin and much work remains to be done. The acetal-oxazolone (111) is described but the acetal could not be removed from the molecule without causing a decomposition of the oxazolone. A study to determine if a relatively easily removed acetal such as the ββ-trichloroethyl acetal which can be removed with zinc powder



(111)

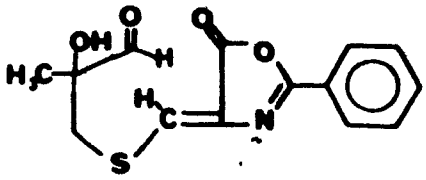
could be undertaken. However, a continuation of the attempt to make use of the easily removed N-methyloxazolidine protecting group after the removal of the acetals in the disulphide (115) is indicated. A



(115)

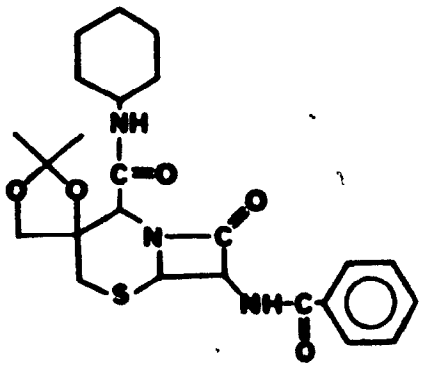
study of the cleavage of the disulphide in the presence of this labile protecting group should also be made. Once the aldehyde-oxazolone (102) has been isolated, a study to determine if the oxazolone ring opening reaction with primary alcohols in the presence of a base observed in chapter 4 is applicable to (102) should be undertaken.

The mechanism of this ring opening reaction to give an ester from the corresponding oxazolone should be studied in some detail.



(102)

Its rate was observed to be a function of base concentration. Since the reaction is used in the synthetic sequence to produce the β-lactam (38) knowledge of its mechanism would be extremely useful.



(38)

Contributions to Knowledge

A new isonitrile that, after undergoing an Ugi type condensation yields an amide that can be removed under mild conditions is described.

A new N-protecting group for peptide synthesis, one that can be removed by hydrogenation with no racemization of the peptide, was developed.

A new synthetic route to a cepham derivative making use of known intermediates was accomplished. Also many improvements on the synthesis of these intermediates were made.

Several new compounds were prepared and characterized.

EXPERIMENTAL

Melting points were determined on a Gallenkamp block and are uncorrected. Mass spectra were obtained on an AEI-MS-902 mass spectrometer at 70ev using a direct insertion probe. Nmr spectra were recorded on Varian T-60, A-60, and HA-100 spectrometers using tetramethylsilane as an internal standard. Doublets, triplets, and quartets in the nmr spectral data were recorded as the centre of the peaks and multiplets as their range of absorption. Ir spectra were obtained on a Unicam SP-1000 and a Perkin-Elmer PE-257 infrared spectrophotometer. Uv spectra were recorded using a Unicam SP-800 spectrophotometer. Optical rotations were measured with a Perkin-Elmer PE-141 automatic polarimeter. Analytical thin layer chromatography was performed on silica gel coated plastic plates (Eastman Kodak) and on a preparative scale on silica gel (Merck $UV_{254,366}$) coated glass plates. Woelm alumina (neutral) and silica gel were used for column chromatography. Microanalyses were carried out by C. Daessle, Montreal. All chemicals are "Reagent Grade" unless otherwise specified.

EXPERIMENTAL - CHAPTER 1

Preparation of N-carboethoxyphthalimide (48)

Phthalimide [500 g] was dissolved in a mixture of dimethylformamide [1800 ml] and triethylamine [500 ml]. The solution was cooled to 10°C with an ice-water bath. Ethylchloroformate [350 ml] was added over a period of 45 minutes with mechanical stirring during which time the temperature of the solution rose to 25°C. After the addition was completed the reaction mixture was allowed to stand at 0°C overnight then poured into cold water [9000 ml] with good mechanical stirring. The precipitated product was removed by vacuum filtration, washed well with water and allowed to air dry.

Yield: 650 g (87%) , mp 78-79°C (lit[36]: 80°C)

Ir ($CHCl_3$): 1815/1785 (O=C-N-C=O), 1735 (C=O), 1615, 1480, 1375, 1330, 1110, 1090, 1030 cm^{-1}

Nmr (DMSO - d_6): δ 1.5(t,3), 4.6(q,2), 8.1(s,4)

Preparation of N-hydroxyphthalimide (49)

Hydroxylamine hydrochloride [10.8 g, 0.155 mole] was dissolved in hot (70°C) absolute ethanol [150 ml] and triethylamine [31.4 g, 0.310 mole] was added. N-carboethoxyphthalimide (48) [34.0 g, 0.155 mole] was added quickly with vigorous stirring. The resulting solution was then cooled in ice-water to room temperature and poured into

dilute hydrochloric acid [800 ml containing 25 ml conc. HCl] with good mechanical stirring. The yellow-brown solution turned colourless on contact with water and a white precipitate was formed. The product was removed by vacuum filtration, washed well with water and dried in vacuo at 65°C.

Yield: 17 g (68%) , mp 225-227°C (lit[37]: 230°C)

Ir ($CHCl_3$): 3400 (broad OH), 1765/1735 (O=C-N-C=O), 1610, 1380, 1290, 1110, 1090 cm^{-1}

Nmr ($CDCl_3$): δ 7.7(s,4), 8.0(s,1)

Preparation of tetrahydropyranyl ether of N-hydroxyphthalimide (50)

N-Hydroxyphthalimide (49) [10.0 g, 0.061 mole] was dissolved in tetrahydrofuran [200 ml] at room temperature. Dihydropyran [11 ml, 0.120 mole] and phosphorus oxychloride [5 drops] was added in one portion. The mixture was allowed to stand at room temperature for 3 hours, then evaporated to dryness under reduced pressure. The product crystallized spontaneously on complete evaporation of the solvent. Petroleum ether (30° - 60°C) [50 ml] was added and the product removed by vacuum filtration and dried in vacuo at 65°C.

Yield: 13.0 g (85%) , mp 120-122°C (lit[38]: 123°C)

Ir ($CHCl_3$): 2970/2880 (CH), 1785/ 1745 (O=C-N-C=O), 1610, 1380, 1310, 1145, 1120, 970 cm^{-1}

Nmr ($CDCl_3$): δ 1.7 (m,6), 3.3-3.8 (m,2), 5.4 (broad s,1), 7.8 (s,4)

Preparation of O-tetrahydropyranylhydroxylamine (51)

The tetrahydropyranyl ether of N-hydroxyphthalimide (50) [140.0 g] was dissolved in benzene [1400 ml]. An 85% solution of hydrazine hydrate [160 g] was added and the solution refluxed for 3 hours. The precipitate was removed by filtration and the filtrate distilled. The fraction boiling at 83° - 86°C @ 19 mm was collected.

Yield: 18 g (27%)

Ir (film): 3310 (NH), 2940/2870 (CH), 1590, 1205, 1040, 970 cm^{-1}

Nmr ($CDCl_3$): δ 1.65 (m,6), 3.3-4.3 (m,2), 4.7 (m,1), 5.55(s,2,NH_2)

Preparation of N-formyl-O-tetrahydropyranylhydroxylamine (52)

O-Tetrahydropyranylhydroxylamine (51) [10 g] was dissolved in dry pyridine [40 ml] and the solution cooled to 0°C in an ice-water bath. Mixed acetic-formic anhydride [22.5 g] was added over a period of 10 minutes with stirring. The solution was allowed to stand at room temperature overnight and the pyridine and excess anhydride were then removed under reduced pressure. Three portions of toluene [3 x 20 ml] were used to azeotrope out last traces of reagents. The compound was used without further purification.

Yield: 11.4 g (100%)

Ir (film): 3220 (NH), 2960/2900 (CH), 1685 (C=O), 1380, 1210, 1120, 1040 cm^{-1}

Nmr ($CDCl_3$): δ 1.65 (m,6), 3.3-4.3 (m,2), 5.05 (broad s,1), 8.1 (d,1,NH), 9.6 (d,1,O=C-H)

Preparation of benzophenone oxime (54)

Benzophenone [106 g] was dissolved in 95% ethanol [200 ml]. To this mixture was added, in one portion, a solution of hydroxylamine hydrochloride [59 g] in water [40 ml]. Powdered NaOH [110 g] was then added in small portions and the mixture swirled after each addition. After the addition of the NaOH had been completed the mixture was refluxed for 5 minutes and then cooled and poured slowly with vigorous stirring in cold dilute HCl [4000 ml containing 300 ml conc. HCl]. The oxime crystallized and was removed by vacuum filtration, washed well with water and dried in vacuo.

Yield: 97.5 g (85%) , mp 141-142°C (lit: 144°C)

Ir ($CHCl_3$): 3250 (OH), 1460, 1320, 1160, 990 cm^{-1}

Preparation of Acetone Oxime (55)

This compound was prepared using the procedure of Janny[46].

Sodium hydroxide [120 g] was dissolved in methanol [500 ml] and hydroxylamine hydrochloride [105 g] was added to the ice-cooled solution. Acetone [125 ml] was then added in one portion and the mixture refluxed for 2 hours. The solution was cooled to 0°C and concentrated HCl [130 ml] was added carefully. Part of the methanol [400 ml] was then removed by evaporation in vacuo and the residue was extracted three times with ether [3 x 100 ml]. The combined extracts were dried with sodium sulphate, filtered and reduced by

evaporation in vacuo to 100 ml. On slow cooling to -20°C the product crystallized and was removed by vacuum filtration.

Yield: 79 g (78%) , mp 58-59°C (lit[48]: 60-61°C)

Ir ($CHCl_3$): 3280 (OH), 3000/2960/2920 (CH), 1680 (C=N), 1450, 1380, 1075, 920 cm^{-1}

Nmr ($CDCl_3$): δ 1.9 (s,6), 8.7 (broad s,1)

Preparation of O-benzylacetoxime (56)

Acetoxime (55) [10 g] was dissolved in absolute ethanol [100 ml] containing dissolved sodium metal [3.15 g]. Benzyl chloride [15.5 ml] was then added in one portion and the mixture refluxed for 2 hours. The mixture was then cooled to room temperature and water [150 ml] was added. The aqueous solution was then extracted with 3 portions of ether [3 x 100 ml]. The combined extracts were dried with sodium sulphate, filtered and evaporated in vacuo. The residue was a yellow viscous oil. It was not purified but used as such for subsequent reactions.

Ir (film): 3060/ 3040/ 2930 (CH), 1670 (C=N), 1590, 1460, 1370, 1190, 1070, 990 cm^{-1}

Nmr ($CDCl_3$): δ 1.85 (d,6), 5.1 (s,2), 7.3 (s,5).

Preparation of O-benzylhydroxylamine (58)

The crude O-benzylacetoxime (56) from the previous reaction was treated with concentrated HCl [100 ml] and this mixture heated

on a steam bath for 2 hours while nitrogen was passed through the solution to remove acetone. The mixture was then cooled in ice, and water [100 ml] was added. A solution of 20% sodium carbonate was added slowly until the solution was alkaline. The mixture was then extracted with 3 portions of ether [3 x 100 ml]. The combined extracts were dried with sodium sulphate and the ether evaporated in vacuo. The residue was distilled in vacuo and the fraction boiling at 101-102°C @ 8.5 mm was collected.

Yield: 6.2 g (37% based on acetoxime)

Ir (film): 3305 (NH), 3060/3020/2905/2860 (CH), 1590, 1460, 1370, 1215, 1200, 1005 cm^{-1}

Nmr ($CDCl_3$): δ 4.6 (s,2,CH_2), 5.2 (s,2,NH_2), 7.3 (s,5)

Preparation of N-formyl-O-benzylhydroxylamine (59)

O-Benzylhydroxylamine (58) [2 g] was dissolved in pyridine [15 ml]. The solution was cooled to 0°C and mixed acetic-formic anhydride [3 ml] was added in 0.5 ml portions at 1 minute intervals. When the addition was complete the mixture was allowed to come to room temperature and stand overnight. The solution was then poured into dilute hydrochloric acid [100 ml, containing 16 ml conc. HCl]. The aqueous solution was extracted 3 times with ether [3 x 50 ml]. The extracts were washed twice with water [2 x 50 ml], and once with 5% sodium bicarbonate [50 ml]. The extracts were combined, dried with sodium sulphate and the ether evaporated in vacuo. The residue was vacuum distilled and the fraction boiling at 150-152°C @ 8 mm was collected.

Yield: 1.72 g (69%)

Ir (film): 3450 - 3300 (NH), 3030/2980 (CH), 1675 (C=O), 1460, 1360, 1090, 1050, 1030 cm^{-1}

Nmr ($CDCl_3$): δ 5.1 (s,2), 7.45 (s,5), 9.1 (broad s,1,NH), 9.6 (s,1,H-C=O)

Attempted formylation of p-toluenesulphonamide with phosgene - dimethylformamide complex

The phosgene - DMF complex[52] [1 g] was dissolved in dry dimethylformamide [10 ml]. p-Toluenesulphonamide [1 g] was added in one portion. The mixture turned pale yellow and warmed slightly. It was stirred at room temperature for 30 minutes and then poured into ice-water. The product precipitated and was removed by vacuum filtration, washed well with water and dried in vacuo at 50°C.

mp 131-132°C (from chloroform)

Ir ($CHCl_3$): 3050/2950 (CH), 1635 (C=N), 1350, 1305, 1155, 1095, 910 cm^{-1}

Nmr ($CDCl_3$): δ 2.45 (s,3,CH_3), 3.10-3.25 (d,6,H_3C-N-CH_3), 7.65 (ABq,4,J=9Hz,aromatic), 8.3 (s,1,N=C-H)

Analysis: Calculated for $C_{10}H_{14}N_2O_2S$: C, 53.10; H, 6.20; N, 12.38; S, 14.15. Found: C, 52.96; H, 6.33; N, 12.17; S, 13.89

Preparation of N-nitrosodiisopropylamine (72)

Diisopropylamine [280 ml] was cooled in ice-water and concentrated

hydrochloric acid [200 ml] was added very slowly. The resulting solution was then heated to 70°C and a solution of sodium nitrite [170 g] in water [400 ml] was added over a period of 1 hour with good stirring. The solution was stirred for a further 2 hours at 70°C and then cooled in ice-water to room temperature. The mixture was transferred to a 2000 ml separatory funnel and extracted 3 times with ether [3 x 300 ml]. The ether extracts were combined, dried with sodium sulphate, filtered, and the ether evaporated in vacuo. The residue was a pale yellow oil which was not further purified.

Yield: 260 g (100%)

Ir (film): 2990/2940/2880 (CH), 1470, 1450, 1385 /1375, (H_3C-C<u>H</u>-CH_3), 1320, 1240, 1230, 1075 cm^{-1}

Nmr ($CDCl_3$): δ 0.95 - 1.10 (d,12), 3.15 (m,2)

Preparation of N,N-diisopropyl-N'-formyl hydrazine (73)

N-Nitrosodiisopropylamine (72) [260 g] was dissolved in 90% aqueous formic acid [2000 ml]. Mercuric chloride [4 g] was added and the mixture stirred until it dissolved. Zinc dust [480 g] was then added in small portions over a period of 6 hours to the vigorously stirred mixture. The temperature was maintained at 40-45°C by cooling in a water bath. After the addition of the zinc dust had been completed the mixture was stirred overnight at room temperature and then filtered and the residue washed with three portions of 90% formic acid [3 x 200 ml]. The filtrate was refluxed for 2 hours and the formic acid was then removed by distillation at

ambient pressure (oil bath to 200°C). The residue was allowed to cool and water [250 ml] was added and solid sodium carbonate added slowly until effervescence ceased. The mixture was transferred to a 2000 ml separatory funnel and extracted with 3 portions of ether [3 x 300 ml]. The ether extracts were combined, dried with sodium sulphate and evaporated to dryness in vacuo. The product crystallized spontaneously on complete evaporation of the solvent. Hexane [200 ml] was added, the product was removed by vacuum filtration, washed with hexane and allowed to dry.

Yield: 170.5 g (60% based on diisopropylamine) , mp 58-61°C (from ether)

Ir ($CHCl_3$): 3310 (NH), 3000/2890 (CH), 1695 (C=O), 1470, 1390/ 1370 ($H_3C-\underline{C}H-CH_3$), 1305, 1145, 1130 cm^{-1}

Nmr ($CDCl_3$): δ 0.95 - 1.10 (d,12), 3.15 (m,2), 8.2 - 8.3 (d,1,NH), 9.2 - 9.3 (d,1,H-C=O)

Analysis: Calculated for $C_7H_{14}N_2O$: C, 58.29; H, 11.18; N, 19.42;
Found: C, 58.10; H, 10.80; N, 19.33

Preparation of N-isonitrilodiisopropylamine (70)

N,N-Diisopropyl-N'-formyl hydrazine (73) [14.4 g] was dissolved in a mixture of methylene chloride [40 ml] and triethylamine [50 ml]. A solution of phosgene [14.8 g] in methylene chloride was added over a period of 10 minutes with vigorous stirring and ice cooling. The mixture was stirred for a further 30 minutes at 0°C and then 20% sodium carbonate solution [140 ml] was added. The layers were separated in a separatory funnel and the organic layer dried with

potassium carbonate [10 g]. The mixture was filtered and the methylene chloride and triethylamine evaporated in vacuo. The residue was vacuum distilled at the lowest possible temperature (to avoid decomposition) (CAUTION: fume hood) and the fraction boiling at 52-58°C @ 9 mm collected.

Yield: 10.0 g (79%)

Ir (film): 3000/2990 (CH), 2200 (C≡N), 1480, 1390/1375 (H_3C-CH-CH_3), 1340, 1230, 1180, 1145, 1095, 1010 cm^{-1}

Nmr ($CDCl_3$) δ 0.95 - 1.10 (d,12), 3.15 (m,2)

No analysis was performed as the compound is volatile and extremely toxic.

Preparation of β-lactam (75)

N-Isonitrilodiisopropylamine [7.35 g] was dissolved in dry methanol [100 ml] containing acetone [10 ml]. Finely powdered β-alanine [5.2 g] was then added and the mixture stirred at room temperature for 85 hours. The solution was then evaporated to dryness in vacuo. Chloroform [50 ml] was added and the flask shaken vigorously for several minutes. The mixture was then filtered to remove chloroform insoluble material and the yellow coloured filtrate passed through a column of alumina [100 g,Woelm, Act. 1]. The column was eluted with chloroform and all eluant collected until the yellow band reached the bottom of the column. The product crystallized upon evaporation of the solvent. Hexane [20 ml] was added and the product removed by vacuum filtration.

Yield: 3.0 g (20%, recrystallized from hexane), mp 78-81°C

Ir (KBr): 3230 (NH), 2970/2940 (CH), 1740 (lactam), 1680 (HN-C=O), 1470, 1400, 1390, 1250, 760 cm^{-1}

Nmr ($CDCl_3$): δ 1.0 - 1.1 (d,12), 1.65 (s,6), 2.8 - 3.5 (m,4,AA'BB' system $N-CH_2-CH_2-C=O$), 7.75 (broad s,1,NH)

Analysis: Calculated for $C_{13}H_{23}N_3O_2$: C, 61.18; H, 9.88; N, 16.47

Found: C, 61.15; H, 10.13; N, 16.27

Preparation of 3-amino-2-oxazolidinone (77)

The procedure of Gever[60] was followed.

2-Hydroxyethyl hydrazine [7.7 g], diethyl carbonate [15 ml] and methanol [5 ml] in which had been dissolved sodium [0.3 g] were combined in a flask to which was attached a Vigreux column and distillation head. The solution was heated on a steam bath and the methanol and ethanol was allowed to distill. A total of 14.2 ml (expected 16.7 ml) was collected after 1.5 hours. The mixture was cooled, absolute ethanol [10 ml] added and the mixture filtered. The filtrate was reduced to 5 ml by evaporation in vacuo and then chilled to -20°C. The crystalline material was removed by vacuum filtration and washed with cold absolute ethanol [20 ml]. It was recrystallized from absolute ethanol.

Yield: 4.6 g (47%) , mp 64-66°C (lit[60]: 68-69°C)

Ir (KBr): 3440 (NH), 1770 (C=O), 1490, 1440, 1285, 1230, 1125, 1040 cm^{-1}

Nmr (D_2O) : δ 3.6 - 4.0 (m,2,part of AA'BB' system $O-CH_2-CH_2-N$), 4.3 - 4.7 (m,2,part of AA'BB' system $O-CH_2-CH_2-N$), 4.75 (s,2,NH_2)

Preparation of N-formyl-3-amino-2-oxazolidinone (78)

3-Amino-2-oxazolidinone (77) [1 g] was added to mixed acetic-formic anhydride [10 ml] at 0°C. The solution was allowed to stand at <5°C for 1 hour. The excess acetic-formic anhydride was then removed under high vacuum at 25°C. The product was obtained as a colourless oil.

Yield: 1.29 g (100%)

Ir (film): 3380 (NH), 3000/2930 (CH), 1775 (O-CO-N), 1700 (H-C=O), 1490, 1420, 1390, 1230, 1150, 1030 cm^{-1}

Nmr (D_2O): δ 3.7 - 4.1 (m,2, part of AA'BB' system of O-CH_2-CH_2-N), 4.4 - 4.8 (m,3, part of AA'BB' system of O-CH_2-CH_2-N, NH)

Analysis: Calculated for $C_4H_6N_2O_3$: C, 36.95; H, 4.62; N, 21.56

Found: C, 37.13; H, 4.88; N, 21.21

Preparation of N-benzoyl-3-amino-2-oxazolidinone (79)

3-Amino-2-oxazolidinone (77) [0.63 g] was dissolved in dry pyridine [5 ml] and cooled to <5°C in an ice-water bath. A solution of benzoyl chloride [0.94 g] in dry pyridine [5 ml] was added over a period of 30 minutes. After the addition was complete the mixture was stirred for 10 minutes at <5°C and then for 30 minutes at room temperature. The solution was then dripped very slowly onto ice [200 g] and the ice was allowed to melt. The crystalline product was removed by vacuum filtration, washed well with cold water and dried in vacuo. The product was recrystallized from ethanol [5 ml].

Yield: 0.77 g (61%) , mp 175-176°C (lit: 178-180°C)

Ir (KBr) : 3300 (NH), 1770 (O-CO-N), 1680 (N-C=O), 1530, 1490, 1425, 1320, 1265, 1225, 1125, 1030, 975, 910 cm^{-1}

Nmr (DMSO - d_6) : δ 3.7 - 4.1 (m,2,part of AA'BB' system of O-CH_2-CH_2-N), 4.4 - 4.8 (m,2,part of AA'BB' system of O-CH_2-CH_2-N), 7.5 -8.2 (m,5,aromatic), 11.3 (s,1,NH)

Analysis: Calculated for $C_{10}H_{10}N_2O_3$: C, 58.25; H, 4.85; N, 13.59

Found: C, 57.97; H, 4.69; N, 13.59

Oxidation and hydrolysis of N-benzoyl-3-amino-2-oxazolidinone (79)

The oxidation method of Iffland[53] was used.

N-Benzoyl-3-amino-2-oxazolidinone (79) [145 mg] was suspended in methylene chloride [5 ml]. The stirred mixture was cooled to 0°C in an ice-water bath and 90% lead tetracetate [380 mg, 1.1 equiv.] was added in small portions over a period of 30 minutes. The resulting solution was allowed to stir at room temperature for 30 minutes. Then 5% sodium bicarbonate solution [13 ml] was added and the mixture stirred vigorously so as to mix the two phases for 20 hours at room temperature. The mixture was then transferred to a separatory funnel and the two phases separated. The aqueous layer was acidified with concentrated HCl to pH 2 and extracted with three portions of chloroform [3 x 10 ml]. The combined extracts were dried with sodium sulphate, filtered and evaporated to dryness, to yield crystalline benzoic acid.

Yield: 60 mg (83%) , mp 118-119°C undepressed upon admixture with an authentic sample of benzoic acid.

CHAPTER 2 - EXPERIMENTAL

Preparation of N-o-nitrocinnamoyl glycine (91)

Thionyl chloride [60 ml] was added to o-nitrocinnamic acid [16 g] and the mixture refluxed for 13 hours. Excess thionyl chloride was evaporated in vacuo at <40°C to yield a crystalline acid chloride.

Glycine [6.2 g] was dissolved in 2N aqueous sodium carbonate [42 ml] and the crude acid chloride added in small portions with vigorous stirring. After each addition of acid chloride sufficient 2N sodium carbonate solution was added to the mixture to return the mixture to pH 10. A beige coloured precipitate was formed during the addition of the acid chloride. When the addition of all the acid chloride was completed the mixture was allowed to stir vigorously for a further 4 hours, then cooled to 0°C and filtered. The product was washed three times with cold water [3 x 15 ml] and then recrystallized from water [30 ml]. The recrystallized material was dissolved in water [100 ml] and concentrated HCl [6.2 ml] in water [10 ml] was added with stirring. The free acid crystallized and was removed by vacuum filtration, washed well with cold water and recrystallized from ethanol.

Yield: 11.0 g (53%) , mp 182-185°C

Ir (KBr): 3390 / 3320 (NH), 3100 (very broad CO_2H), 1745 (C=O), 1660 (N-C=O), 1615, 1530, 1345, 1230, 1045, 980 cm^{-1}

Nmr (DMSO - d_6): δ 3.9 (d,2,N-CH_2-C=O), 7.3(ABq,2,J=16Hz,trans

H-C=C-H), 7.4 - 8.2 (m,4), 8.5 (t,1,NH)

Analysis: Calculated for $C_{11}H_{10}N_2O_4$: C, 53.49; H, 4.47; N, 12.50 Found: C, 53.61; H, 4.57; N, 12.79

Preparation of 2-o-nitrostyryl-4-ethoxymethylene -5-oxazolone (92)

N-o-Nitrocinnamoyl glycine [2 g] was added to a mixture of acetic anhydride [8 ml], triethyl orthoformate [4 ml] and dry ethyl acetate [20 ml]. The mixture was refluxed for 3 hours during which time it turned deep red-brown in colour. The solution was evaporated in vacuo to about 3 ml and ethyl acetate [10 ml] was added and the mixture cooled to -20°C. The product crystallized, was removed by vacuum filtration, washed with cold ethyl acetate [2-3 ml] and allowed to dry.

Yield: 0.80 g (31%) , mp 129-130°C

Ir ($CHCl_3$): 1780 (C=O), 1680 (C=N), 1630, 1360, 1310, 980, 910 cm^{-1}

Nmr (DMSO - d_6): δ 1.25 (t,3), 4.55 (q,2), 7.5 (ABq,2,J=17Hz,trans H-C=C-H), 7.6 - 8.4 (m,5,aromatic and C=CH-OC_2H_5)

Analysis: Calculated for $C_{14}H_{12}N_2O_5$: C, 58.35; H, 4.16; N, 9.72 Found: C, 58.68; H, 4.28; N, 9.46

Preparation of 2-o-nitrostyryl-4-thiomethylene-5-oxazolone sodium salt (94) from (92)

2-o-Nitrostyryl-4-ethoxymethylene-5-oxazolone (92) [0.5 g] was

suspended in ice-cooled dry methanol [5 ml]. Sodium hydrosulphide [98 mg, 1 equiv.] was then added to the stirred solution and the suspended solid went into solution almost immediately. The red-brown solution was stirred for 30 minutes at 0°C then dripped very slowly into ice-cooled, vigorously stirred, anhydrous ether [50 ml]. The sodium thiol precipitated as a brown solid. The product was removed by vacuum filtration, washed well with ether, and allowed to dry.

Yield: 0.45 g (87%)

Ir (KBr): 1760 (C=O), 1690 (C=N), 1630, 1330, 1180, 980, 910 cm^{-1}

Nmr (D_2O): δ 7.5 (ABq,2,J=16Hz,trans HC=CH), 7.6 - 8.4 (m,4, aromatic), 8.8 (s,1,C=CH)

Analysis: Calculated for $C_{12}H_7N_2O_4SNa$: C, 48.32; H, 2.35; S, 10.74 . Found: C, 48.58; H, 2.18; S, 10.41

Preparation of N-o-nitrocinnamoyl glycine methyl ester (95)

N-o-Nitrocinnamoyl glycine [2 g] was suspended in dry methanol [100 ml] and the stirred suspension cooled in an ice-water bath. Anhydrous HCl gas was passed into the mixture until the suspended solid dissolved. The solution was then stirred at 0°C for 4 hours and then concentrated to about 5 ml by evaporation in vacuo. The solu-

tion was cooled and triturated with anhydrous ether [25 ml] until the product crystallized. The product was removed by vacuum filtration, washed with ether [10 ml] and allowed to dry.

Yield: 1.6 g (76%) , mp 159-160°C

Ir ($CHCl_3$): 3430 (NH), 1760 (O-C=O), 1680 (N-C=O), 1640, 1610, 1530, 1380, 1360 cm^{-1}

Nmr (DMSO - d_6): δ 3.8 (s,3), 4.2 (d,2,$N-CH_2-C=O$), 7.5 (ABq,2, J=16Hz,trans H-C=C-H), 7.8 - 8.4 (m,4,aromatic), 9.1 (t,1,NH)

Mass spectrum (70 eV): m/e 238 (M^+)

Hydrogenation of (95) to yield N-o-aminocinnamoyl glycine methyl ester (96)

N-o-Nitrocinnamoyl glycine [1 g] was dissolved in absolute ethanol [50 ml] warmed to 50°C. Platinum oxide [50 mg] was added and the mixture hydrogenated for 1 hour at 50°C on a Parr apparatus at 60 lb/in^2 hydrogen pressure. The mixture was then heated to dissolve the precipitated product and filtered hot to remove catalyst. On cooling to room temperature the product crystallized, was removed by vacuum filtration and washed with cold absolute ethanol [10 ml].

Yield; 0.65 g (69%) , mp 140-143°C

Ir (KBr): 3450 /3300 (NH), 1760 (O-C=O), 1685 (N-C=O), 1645, 1520, 1290, 1050, 975 cm^{-1}

Nmr (DMSO - d_6): δ 3.9 (s,3,$O-CH_3$), 4.05 (broad s,2,NH_2),

4.15 (d,2,N-CH_2-C=O), 7.5 (ABq,2,J=10Hz,trans H-C=C-H), 7.2 - 8.0 (m,4,aromatic), 9.0 (t,1,NH)

Analysis: Calculated for $C_{12}H_{14}N_2O_3$: C, 61.54; H, 5.98; N, 11.97

Found: C, 61.29; H, 6.13; N, 12.07

Hydrogenation of (95) to yield lactam (89)

N-o-Nitrocinnamoyl glycine methyl ester (95) [1 g] was dissolved in glacial acetic acid [25 ml] and platinum oxide [50 mg] was added. The mixture was hydrogenated at 40°C for 1 hour on a Parr apparatus at 60 lb/in^2 hydrogen pressure. The mixture was then filtered to remove catalyst and concentrated to 2 ml by evaporation in vacuo. Ethyl acetate [25 ml] was added, the solution transferred to a separatory funnel and then extracted with ice-cold 2N sodium carbonate solution [25 ml]. The aqueous extract was back extracted with ethyl acetate [15 ml]. The organic layers were combined, dried with sodium sulphate and concentrated to 5 ml by evaporation in vacuo. The residue was triturated with a mixture of ethyl ether [10 ml] and petroleum ether (30-60°C) [15 ml] until the product began to crystallize. The mixture was cooled at -20°C until crystallization was complete. The product was removed by vacuum filtration and allowed to dry.

Yield: 0.53 g (86%) recrystallized from ether , mp 152-154°C

Ir (KBr): 3200 (NH), 1690 (C=O), 1600, 1500, 1440, 1390, 1290, 1250, 1205, 1040 cm^{-1}

Nmr (DMSO - d_6): δ 2.2 - 3.1 (m,4,AA'BB' system of -CH_2-CH_2-C=O),

6.8 - 7.5 (m,4, aromatic), 10.6 (broad s,1,NH)

Analysis: Calculated for C_9H_9NO : C, 73.50; H, 6.12; N, 9.52

Found: C, 73.60; H, 6.17; N, 9.71

Preparation of N-o-nitrocinnamoyl-glycyl-L-phenylalanine ethyl ester (97)

The procedure of Sheehan and Hess[64] was used.

N-o-Nitrocinnamoyl glycine [1 g] was suspended in tetrahydrofuran [20 ml]. Dicyclohexylcarbodiimide [0.90 g,1.1 equivalent] was added and the mixture allowed to stir at room temperature for 15 minutes. L-phenylalanine ethyl ester [0.85 g] in tetrahydrofuran [25 ml] was then added and the mixture allowed to stir at room temperature for 4 hours. Acetic acid [0.1 ml] was added to destroy excess dicyclohexylcarbodiimide, the mixture allowed to stand 15 minutes and then filtered to remove the dicyclohexylurea. The filtrate was evaporated to dryness in vacuo and the residue taken up in a minimum amount of boiling ethanol (maintained at the boiling point on a steam bath). The mixture was then placed in the freezer at -20°C overnight. The crystalline product was removed by vacuum filtration, washed with cold ethanol and allowed to dry.

Yield: 1.33 g (78%) , mp 149°C, $[\alpha]_{589}^{25}$=5.5° (c=1.80)

Ir ($CHCl_3$): 3410 / 3310 (NH), 1740 (O-C=O), 1690 / 1670 (N-C=O), 1635, 1525, 1350, 1295, 1095, 970 cm^{-1}

Nmr (DMSO - d_6): δ 1.1 (t,3), 3.05 (d,2,Ph-CH_2-), 4.0 (d,2, N-CH_2-C=O), 4.1 (q,2,O-CH_2-CH_3), 4.7 (m,1,N-CH-C=O), 7.25 (s,5,phenyl), 7.4 (ABq,2,J=16Hz,trans H-C=C-H),

7.4 - 8.4 (m,5,aromatic and O=C-NH-CH-), 9.3 (t,1, O=C-NH-CH_2)

Analysis: Calculated for $C_{22}H_{23}N_3O_6$: C, 62.16; H, 5.45; N, 9.88
Found: C, 61.87; H, 5.51; N, 9.84

Hydrogenation of N-o-nitrocinnamoyl-glycyl-L-phenylalanine ethyl ester (97) to give glycyl-L-phenylalanine ethyl ester hydrochloride (98)

Ester (97) [200 mg] was dissolved in glacial acetic acid [30 ml] and platinum oxide [25 mg] was added. The mixture was hydrogenated on a Parr apparatus at 55°C for 1 hour at 60 lb/in^2 hydrogen pressure. The mixture was then filtered and the filtrate evaporated to dryness in vacuo. The residue was taken up in methanol [5 ml] and 1N hydrochloric acid [15 ml] was added. The solution was transferred to a separatory funnel and extracted with ethyl acetate three times [3 x 15 ml] to remove δ-lactam (89). The aqueous solution was evaporated to dryness and the product crystallized spontaneously. It was recrystallized from ethanol [2 ml].

Yield: 109 mg (82%), mp 135-138 (lit[86]: 139-140°C) undepressed by mixed mp with an authentic sample. In ethanol $[\alpha]_{589}^{25}$=11.4° (c=3.80) (Authentic sample $[\alpha]_{589}^{25}$=11.1° (c=2.00))

Ir ($CHCl_3$): 3500 - 3200 (broad NH), 3000/ 2940 (CH), 1730 (O-C=O), 1680 (N-C=O), 1530, 1450, 1385, 1330, 1130, 1100, 950 cm^{-1}

Nmr ($CDCl_3$) : δ 1.05 (t,3,O-CH_2-CH_3), 3.0 (d,2,CH-CH_2-Ph), 3.6 - 4.4 (m,4,O-CH_2-CH_3 and N-CH_2-C=O), 4.75 (m,1,CH-CH_2-Ph), 7.0-7.6 (broad s,8,aromatic and NH_3^+), 9.2 (d,1,O=C-NH-CH)

Preparation of N-o-nitrocinnamoyl-glycyl-L-alanine ethyl ester (99)

N-o-Nitrocinnamoyl glycine [1 g] was suspended in tetrahydrofuran [20 ml] and dicyclohexylcarbodiimide [0.80 g] was added and the mixture stirred at room temperature for 30 minutes. L-Alanine ethyl ester [0.36 g] was added and the mixture stirred at room temperature for a further 4 hours. The mixture was then treated with glacial acetic acid [0.05 ml] to destroy excess dicyclohexylcarbodiimide and allowed to stand for 15 minutes. The mixture was then filtered and concentrated to about 2 ml by evaporation in vacuo. On slight cooling the product crystallized, was removed by vacuum filtration and washed with ether. It was recrystallized from ethanol [5 ml].

Yield: 0.40 g (26.5%) , mp 183-184°C , $[\alpha]_{5893}^{25} = -33.6°$ (c=1.20)

Ir ($CHCl_3$): 3420/ 3320 (NH), 1742 (O-C=O), 1685 /1675 (N-C=O), 1635, 1535, 1460, 1385, 1360, 1300, 1165, 970 cm^{-1}

Nmr (DMSO - d_6): δ 1.25 (t,3,O-CH_2-CH_3), 1.35 (d,3,CH-CH_3), 3.9 - 4.8 (m,5,O-CH_2-CH_3, NH-CH_2-C=O and NH-CH-CH_3), 7.5 (ABq,2,J=16Hz,trans H-C=C-H), 7.7 - 8.4 (m,4, aromatic), 8.8 (d,1,NH-CH-CO), 9.1 (t,1,NH-CH_2-CO)

Analysis: Calculated for $C_{16}H_{19}N_3O_6$: C, 55.01; H, 5.48; N, 12.03

Found: C, 55.20; H, 5.74; N, 12.33

Hydrogenation of (99) to give glycyl-L-alanine ethyl ester hydrochloride 100

The same procedure outlined above for the hydrogenation of (97) was used.

Yield: (72%), mp 169-170°C (lit[87]: 177°C) In ethanol $[\alpha]_{5893}^{25}$=37.3° (c=1.60) (authentic sample $[\alpha]_{5893}^{25}$=39.4° (c=2.00)

Ir ($CHCl_3$): 3310 (broad NH), 1740 (O-C=O), 1685 (N-C=O), 1570, 1385, 1360, 1140, 910 cm^{-1}

Nmr (DMSO - d_6): δ 1.3 (t,3,O-CH_2-CH_3), 1.4 (d,3,CH-CH_3), 3.7 (broad s,2,NH_3^+-CH_2-C=O), 3.9 - 4.7 (m,4,O-CH_2-CH_3 and NH-CH-CH_3), 8.5 (broad s,3,NH_3^+), 9.1 (d,1,CO-NH-CH)

EXPERIMENTAL - CHAPTER 3

Preparation of methacrolein diethyl acetal (104)

The procedure for acrolein diethyl acetal[65] was used.

Methacrolein [61.5 g, 0.79 mole] was dissolved in triethyl orthoformate [130 ml, 0.79 mole]. A warm solution (40°C) of ammonium nitrate [3 g] in absolute ethanol [50 ml] was added in one portion. The mixture was stirred at room temperature for 24 hours. and then filtered to remove precipitated ammonium nitrate. Anhydrous sodium carbonate [4 g] was added and the mixture vacuum distilled. The fraction boiling at 71-72°C @ 80 mm was collected.

Yield: 74 g (65%)

Nmr (neat): δ 1.15 (t,6), 1.7 (d,3), 3.1 - 3.9 (m,4,O-$\underline{CH_2}$-CH_3), 4.75 (s,1,RO-$\underline{CH}$-OR), 4.9 - 5.3 (complex m,2,C=$\underline{CH_2}$)

Preparation of Epoxide (106)

Methacrolein diethyl acetal [7.2 g] was dissolved in methylene chloride [75 ml] in a 250 ml flask equipped with a reflux condenser. m-Chloroperbenzoic acid (85%) [13 g] in methylene chloride [125 ml] was added in one portion. The mixture heated up spontaneously and remained warm for 1 hour. The solution was stirred for a further 5 hours at room temperature then transferred to a separatory funnel and the organic layer washed with 10% sodium sulphite solution until the starch-iodide test was negative. The organic layer was then

extracted with 5% sodium bicarbonate solution until the aqueous washings were basic. The organic layer was dried with sodium sulphate and the methylene chloride removed by evaporation in vacuo at <20°C. The residue was distilled at ambient pressure and the fraction boiling at 168-171°C collected.

Yield: 5.7 g (72%)

Ir (film): 2990 / 2950 / 2890 (CH), 1465, 1385, 1325, 1122, 1085, 1070 cm^{-1}

Nmr ($CDCl_3$): δ 1.3 (2t,6,O-CH_2-CH_3), 1.4 (s,3), 2.75 (ABq,2, J=5Hz,O-CH_2-C), 3.4 - 4.0 (m,4,O-CH_2-CH_3), 4.25 (s,1, RO-CH-OR)

Preparation of thiol (110)

The procedure of Goodman, Benitez, and Baber[71] was used.

Hydrogen sulphide was passed for 1.5 hours into an ice-cooled solution of sodium hydroxide [11.2 g] in methanol [100 ml]. While a steady stream of hydrogen sulphide was maintained, the epoxide (106) [20 g] in methanol [200 ml] was added to the ice-cold mixture over a period of 30 minutes. The reaction mixture was then allowed to stir for 1 hour at 0°C and 17 hours at room temperature. The solution was then poured into ice-water [1000 ml] and the pH was reduced to 2 with 5N sulphuric acid. The aqueous mixture was extracted three times with methylene chloride [3 x 300 ml]. The extracts were combined, washed with 5% sodium bicarbonate [100 ml] and dried with sodium sulphate. The methylene chloride was removed by evaporation

in vacuo and the residue distilled. The fraction boiling at 100-103°C @ 10 mm was collected.

Yield: 20.0 g (82%)

Ir (film): 3500 (broad OH), 2990/ 2940/ 2880 (CH), 2580 (SH), 1450, 1380, 1320, 1170, 1115, 1065, 1030 cm^{-1}

Nmr ($CDCl_3$): δ 1.35 (s,t,9,O-CH_2-CH_3 and O-C-CH_3), 1.65 (t,1,SH), 2.65 (s,1,OH), 2.85 (q,2,HS-CH_2), 3.5 - 4.2 (m,4, O-CH_2-CH_3), 4.55 (s,1,RO-CH-OR)

Analysis: Calculated for $C_8H_{16}O_3S$: C, 49.47; H, 9.34; S, 16.48 .
Found: C, 49.31; H, 9.37; S, 16.19

Preparation of acetal-oxazolone (111)

Thiol (110) [5 g] and 2-phenyl-4-ethoxymethylene-5-oxazolone [5 g] were dissolved in dry pyridine [50 ml] and the mixture allowed to stand at room temperature for 5 days. The pyridine was then removed by evaporation in vacuo and the residue taken up in chloroform [50 ml]. Decolourizing carbon [4 g] was added and the mixture boiled for 20 minutes. It was then filtered and the chloroform removed by evaporation in vacuo. The residue was a yellow oil which crystallized on standing. It was recrystallized from hexane to give pale yellow needles.

Yield: 7.1 g (75%) , mp 85-88°C

Ir ($CHCl_3$): 3500 (broad OH), 3040 /2980 /2940 (CH), 1785 (C=O), 1640 (C=N), 1455, 1330, 1110, 1070, 995 cm^{-1}

Nmr ($CDCl_3$): δ 1.30 (t,6,CH_2-CH_3), 1.35 (s,3,C-CH_3), 2.75 (broad s, 1,OH), 3.4 (ABq,2,J=7Hz,CH_2-S), 3.5 - 4.2 (m,4,O-CH_2-CH_3), 4.5 (s,1,RO-CH-OR), 7.4 - 8.4 (m,6,aromatic and C=CH)

Analysis: Calculated for $C_{18}H_{23}NO_5S$: C, 59.18; H, 6.30; N, 7.67; S, 8.76 Found: C, 59.31; H, 6.01; N, 7.83; S, 9.04

Preparation of disulphide (114)

Thiol (110) [1.94 g] was dissolved in dry dimethylsulphoxide [5 g] and the solution heated at 85°C for 18 hours. The mixture was then cooled, poured into ice-water [50 ml] and this aqueous mixture extracted three times with ether [3 x 50 ml]. Each of the extracts was back extracted 5 times with water [5 x 15 ml] and twice with ice cold sodium hydroxide (10%) [2 x 15 ml]. The ether extracts were combined, dried with sodium sulphate and evaporated to dryness in vacuo. The residue was a yellow oil (one spot on t.l.c.).

Yield: 1.22 g (63%)

Ir (film): 3450 (broad OH), 2980 /2940/ 2880 (CH), 1450, 1380, 1320, 1115, 1070 cm^{-1}

Nmr ($CDCl_3$): δ 1.30 (s,t,18,CH_2-CH_3 and C-CH_3), 2.6 (d,2,OH), 3.15 (ABq,4,J=12Hz,H_2C-S-S-CH_2),3.4 - 4.0 (m,8,O-CH_2-CH_3), 4.35 (s,2,RO-CH-OR)

Analysis: Calculated for $C_{16}H_{34}O_6S_2$: C, 49.74; H, 8.81; S, 16.58 Found: C, 49.61; H, 8.72; S, 16.43

Preparation of the diacetate (115)

The disulphide (114) [0.50 g] was dissolved in a mixture of acetic anhydride [0.82 ml] and triethylamine [0.82 ml]. p-Dimethylamino-

pyridine [10 mg] was added and the mixture allowed to stand 18 hours at room temperature. The mixture was then poured into ice cold dilute hydrochloric acid [50 ml containing 5 ml conc HCl] and allowed to stir for 30 minutes at 0°C. The aqueous mixture was then extracted 3 times with ether [3 x 50 ml] and the extracts back extracted twice with ice cold 5% sodium bicarbonate [2 x 25 ml], twice with ice cold 5% hydrochloric acid [2 x 25 ml] and once with water [25 ml]. The extracts were combined, dried with sodium sulphate, filtered, and evaporated to dryness in vacuo. The residue was a pale yellow oil (one spot on t.l.c.) and was not further purified.

Yield: 0.56 g (92%)

Ir (film): 2980 /2940 /2880 (CH), 1745 (C=O), 1375, 1365, 1240, 1195, 1120, 1070, 1030 cm^{-1}

Nmr ($CDCl_3$): δ 1.2 (t,12,CH_2-CH_3), 1.5 (s,6,C-CH_3), 2.1 (s,6, O=C-CH_3), 3.4 - 4.1 (m,12,O-CH_2-CH_3 and H_2C-S), 4.95 (s,2,RO-CH-OR)

EXPERIMENTAL - CHAPTER 4

Preparation of mannitol diacetonide (25)

D-Mannitol [200 g] was suspended in a mixture of acetone [1500 ml] and dimethoxypropane [180 g]. Toluene-4-sulphonic acid, monohydrate [0.6 g] was added and the suspension stirred at room temperature for 90 minutes. Unreacted mannitol [90 - 100 g] was removed by vacuum filtration and the filtrate shaken with anhydrous potassium carbonate [50 g] until it was colourless. The potassium carbonate was removed by filtration and the filtrate evaporated to dryness in vacuo. The semi-solid residue was transferred to an Erlenmeyer flask containing hexane [3500 ml]. The mixture was then heated to boiling with good stirring until almost all the solid had dissolved (about 20 minutes). The mixture was allowed to settle for 5 minutes, decanted, and allowed to crystallize at room temperature. The product was removed by vacuum filtration, washed well with cold hexane and allowed to dry.

Yield: 28 g (20%) , mp 119-121°C (lit[27] 119°C)

Preparation of glyceraldehyde acetonide (26)

The procedure of Baer and Fischer[27] was followed.

The product was a colourless oil and distilled at 55-58°C @ 22 mm (lit[27]: 35-42°C @ 8-11 mm).

~~Yield~~: 80% (lit[27] 79.3%)

Ir (film): 3450 (OH), 1725 (C=O), 1260, 1220, 1080 (C-O) cm^{-1}

Nmr ($CDCl_3$): δ 1.25 (d,6), 3.9 (m,2), 4.2 (m,1), 9.7 (d,1,H-C=O)

Preparation of dioxan (27)

Freshly distilled glyceraldehyde acetonide (26) [18 g] was added in one portion to an ice cooled stirred solution of anhydrous potassium carbonate [18 g] in a mixture of methanol [200 ml] and water [100 ml]. Formaldehyde solution (40%) [50 ml] was then added and the mixture allowed to stand overnight at room temperature. The solution was concentrated in vacuo at <40°C to a volume of 100 ml. This residue was then extracted three times with methylene chloride [3 x 50 ml]. The combined extracts were dried with sodium sulphate [5 g] and evaporated to dryness. The material was used without further purification.

Yield: 25.1 g (95%) , mp 88-89°C (lit[26]: 89.5-91°C)

Ir (KBr) : 3400 (OH), 1380, 1220, 1160, 1090, 1080, 1000 cm^{-1}

Nmr ($CDCl_3$): δ 1.5 (s,6), 3.9(ABq,2,J=10Hz,CH_2-O-C[CH_3]$_2$),5.1 (s,1), 4.0 - 6.0 (broad,1,OH)

Mass spectrum (70eV): m/e 190 (M^+)

Preparation of oxazolidine-alcohol (30)

The dioxan (27) [25 g] was dissolved in benzene [500 ml] and N-methylethanolamine [20.7 g, 2.1 equivalents] was added. The mixture was heated until the benzene distilled slowly. The distillate was cloudy. After 350 ml of benzene had been collected (about two

and a half hours) the distillate contained no more water and the benzene was removed by evaporation in vacuo. The residue was distilled under high vacuum and the fraction boiling at 100-103°C @ 0.5 mm was collected.

Yield: 24.9 g (86%)

Ir (film): 3600 - 3300 (OH), 2810 ($N-CH_3$), 1470, 1380, 1260, 1225, 1150, 1080, 1070, 1050 cm^{-1}

Nmr ($CDCl_3$): δ 1.45(s,6), 2.60 (s,3,$N-CH_3$), 2.7(m,1,part of AA' BB' system, $N-C\underline{H}_2-CH_2-O$), 3.3 (m,1,$N-C\underline{H}_2-CH_2-O$), 4.0 (m,8,part of AA'BB' system $N-CH_2-C\underline{H}_2-O$, $N-C\underline{H}-O$, AB systems of $C\underline{H}_2OH$ and $C\underline{H}_2-O-C$ and $O\underline{H}$)

Mass spectrum (70 eV): m/e 217 (M^+)

Preparation of oxazolidine-mesylate (31)

The oxazolidine-alcohol (30) [27.6 g] was dissolved in a mixture of methylene chloride [250 ml] and triethylamine [19.4 g, 1.5 equivalents]. The solution was cooled to -50°C with dry ice-acetone and freshly distilled mesyl chloride [16.2 g,1.1 equiv.] in methylene chloride [50 ml] added dropwise over a period of 2 hours with good stirring. The mixture was then poured while still cold into ice water [300 ml] in a separatory funnel. The organic layer was washed twice with ice water [2 x 100 ml], dried with sodium sulphate, and evaporated to dryness in vacuo. The residue crystallized on complete evaporation of the solvent.

Yield: (crude) 37.6 g (100%). Recrystallized from petroleum ether (60-80°C) [750 ml]. Yield: 26.3 g (70%) , mp 83-84°C

Ir (CCl_4): 2820 (CH), 1475, 1380 (OMs), 1220, 1180, 1080, 1010 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (s,6), 2.5 (s,3,N-CH_3), 2.4 - 2.8 (m,1), 3.0 (s,3,SO_2-CH_3), 3.0 -3.4 (m,1), 3.6 - 4.0 (m,2,O-$\underline{CH_2}$-CH_2-N), 4.05 (s,1,N-CH-O), 4.3 (ABq,2,J=11Hz,$[H_3C]_2$C-O-$\underline{CH_2}$-C)

Preparation of thioacetate (119) from mesylate (31)

Mesylate (31) [5 g] was dissolved in 2-butanone [100 ml]. Recrystallized (from water) potassium thioacetate [3 g] was added and the stirred suspension refluxed for 3 hours. The mixture was cooled, filtered and evaporated to dryness in vacuo. The residue was taken up in methylene chloride [100 ml]. The solution was washed twice with ice water [2 x 40 ml], dried with sodium sulphate and evaporated to dryness in vacuo. The residue was a pale yellow oil (one spot on t.l.c.) and was not further purified.

Yield: 4.6 g (99%)

Ir (film): 3000 2960 2900 2810 (CH), 1700 (C=O), 1470, 1380, 1220, 1145, 1070 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (d,6,C-$[CH_3]_2$), 2.35 (s,3,O=C-CH_3), 2.5 (s,3), 2.4 - 2.8 (m,1), 3.0 - 3.3 (m,1), 3.3 (s,2,CH_2-S), 3.6 - 4.3 (m,5).

Preparation of 2-phenyl-4-thiomethylene-5-oxazolone (123)

2-Phenyl-4-ethoxymethylene-5-oxazolone (33) [5 g] was dissolved in dry pyridine [25 ml] in a pressure bottle. The solution was cooled to 0°C in an ice-water bath and hydrogen sulphide passed into the mixture for 10 minutes. The bottle was then sealed and allowed to stand at room temperature for 24 hours. The mixture was then poured into ice cold, vigorously stirred, dilute hydrochloric acid [100 ml containing 30 ml conc. HCl]. This aqueous mixture was vacuum filtered as rapidly as possible. The product crystallized from the filtrate on standing. It was removed by vacuum filtration, washed well with cold water and dried in vacuo.

Yield: 0.47 g (10%) , mp 176°C (lit[32]: 175-176°C)

Ir ($CHCl_3$): 3080 (CH), 2480 (SH), 1760/ 1710 (C=O), 1690 (C=N), 1540, 1450, 1260, 1060 cm^{-1}

Nmr ($CDCl_3$): δ 7.3 - 8.2 (m,5,aromatic), 8.25 (s,1,C=CH)

The sodium salt (41) was prepared as follows. The thiol (123) [200 mg] was dissolved in absolute ethanol [2 ml]. A solution of sodium [22.5 mg, 1 equiv.] in abeolute ethanol [2 ml] was added. The mixture was dripped slowly into ice cold vigorously stirred ether [50 ml]. The sodium salt precipitated, was removed by vacuum filtration, washed with ether and dried in vacuo.

Yield: 190 mg (80%)

Ir (KBr): 1790 / 1780 (C=O), 1410, 1370, 1250, 1200, 1160, 1080 cm^{-1}

Nmr (D_2O): δ 7.4 - 8.2 (m,5,aromatic), 9.30 (s,1,C=CH)

Preparation of 2-phenyl-4-thiomethylene-5-oxazolone sodium salt (42)

To a stirred ice-cooled suspension of 2-phenyl-4-ethoxy-methylene-5-oxazolone (33) [5 g] in methanol [10 ml] was added sodium hydrosulphide [1.3 g,1 equiv.]. The mixture turned red and the undissolved material went into solution almost immediately. The solution was stirred for 3 minutes at 0°C and then poured into dioxan [80 ml] heated to 70°C. The clear red solution was cooled in an ice bath until precipitation of the bright yellow sodium thiol was complete (about 1 hour). The product was removed by vacuum filtration, washed twice with dioxan [2 x 20 ml], three times with diethyl ether [3 x 50 ml] and dried in vacuo.

Yield: 4.9 g (95%) , mp 245-247°C (decomp.)

Nmr (D_2O): δ 7.35 - 8.1 (m,5,aromatic), 9.14 (s,1,C=CH)

Analysis: Calculated for $C_{10}H_6NO_2SNa$: C, 52.86; H, 2.64; N, 6.16

Found: C, 52.41; H, 2.83; N, 6.32

Preparation of thiol (124) from (42)

Sodium thiol (42) [1.0 g] was dissolved in water [10 ml] and glacial acetic acid [2 ml] added. The product precipitated and was removed by vacuum filtration, washed well with water and dried in vacuo.

Yield: 0.90 g (100%) , mp 180-181°C

Ir ($CHCl_3$): 3080 (CH), 2460 (SH), 1805/ 1795 (C=O), 1660 (C=N), 1545, 1455, 1260, 1065 cm^{-1}

Nmr ($CDCl_3$): δ 7.3 (s,1,C=CH), 7.3 - 8.2 (m,5,aromatic)

Uv (ethanol) : 405 nm (ε = 22,300)

Mass spectrum (70 eV): m/e 205 (M^+)

Preparation of 4-((((2,2dimethyl-4-(3-methyloxazolidine-2-yl)-dioxolan-4-yl)methyl)thio)methylene)-2-phenyl-2-oxazolin-5-one (34)

To a solution of mesylate (31) [1.2 g] in dry 2-butanone [30 ml] was added sodium thiol (42) [1.2 g, 1.3 equiv]. This mixture was refluxed for 1.5 hours, allowed to cool and filtered. The dark brown filtrate was evaporated to dryness in vacuo. The residue was taken up in chloroform [2 ml] and ether [2 ml] was added. The mixture was filtered and then passed through a column of alumina [10 g, Woelm, Act. 1]. Chloroform-ether (1:1) was used to elute the column. A total of 50 ml of eluant was collected and evaporated to dryness in vacuo. The pale orange residue was taken up in ether [4 ml] and cooled at -20°C until crystallization was complete. The product was removed by vacuum filtration, washed with cold ether [5 ml] and allowed to dry.

Yield: 0.82 g (50%) , mp 127°C

Ir (KBr): 1780 (C=O), 1633 (C=N), 1590, 1560, 1500, 1460, 1385, 1375, 1335, 1300, 1175, 1075, 1055, 858, 850 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (s,6), 2.5 (s,3,N-CH_3), 2.4 - 2.8 (m,1), 3.0 - 4.0 (m,7), 4.1 (s,1,N-CH-O), 7.3 - 8.3 (m,6, C=CH and aromatic)

Mass spectrum (70 eV): m/e = 404 (M^+)

Uv (methanol): 361 nm (ε = 45,600)

Analysis: Calculated for $C_{20}H_{24}N_2O_5S$: C, 59.41; H, 5.94; N, 6.93; S, 7.92 . Found: C, 59.21; H, 5.88; N, 7.18; S, 7.89

Preparation of 2,2-dimethyl-4-((((5-oxo-2-phenyl-2-oxazolidine-4-ylidene)methyl)thio)methyl)-1,3-dioxolane-4-carboxaldehyde (35)

Oxazolidine (34) [0.90 g] was dissolved in 50% aqueous acetic acid [10 ml]. The mixture was allowed to stand at room temperature for 1 hour,then diluted with water [40 ml] and extracted three times with chloroform [3 x 50 ml]. The extracts were washed twice with cold water [2 x 50 ml], combined, dried with sodium sulphate and evaporated to dryness in vacuo. The residue was taken up in ether [5 ml] and scratched to initiate crystallization. The mixture was allowed to stand at -20°C until crystallization was complete (about 5 hours). The product was then removed by vacuum filtration, washed with cold ether [5 ml], and allowed to dry.

Yield: 0.61 g (80%) recrystallized from hexane-benzene (1:1)

mp 80-81°C

Ir (KBr): 1780 (C=O), 1740 (H-C=O), 1630 (C=N), 1590, 1560, 1460, 1385, 1380, 1330, 1265, 1177, 1075, 1055, 1000, 870 cm^{-1}

Nmr ($CDCl_3$): δ 1.5 (d,6), 3.5 (s,2,CH_2-S), 4.2(ABq,2,J=10Hz, O-CH_2-C-O), 7.2 - 8.2 (m,6,aromatic and C=CH), 8.9 (s,1,H-C=O)

Mass spectrum (70eV): m/e = 347 (M^+)

Uv (methanol) : 361 nm (ε = 42,800)

Analysis: Calculated for $C_{17}H_{17}NO_5S$: C, 58.79; H, 4.93; N, 4.03; S, 9.23 . Found: C, 59.03; H , 4.82; N, 4.23; S, 9.25

Preparation of 2-benzamido-3-(((2,2-dimethyl-4-(3-methyl-oxazolidine-2-yl)-1,3-dioxolan-2-yl)methyl)thio)acrylic acid methyl ester (130) from (34)

The oxazolidine-oxazolone (34) [0.50 g] was suspended in methanol [5 ml]. Triethylamine [0.5 ml] was added and the mixture allowed to stir at room temperature for 1 hour. The methanol and triethylamine were than removed by evaporation in vacuo and the residue pumped under high vacuum for 24 hours to remove the last traces of solvent.

Yield: 0.54 g (100%)

Ir (CCl_4): 3420 (NH), 3000/ 2970/ 2910/ 2830 (CH), 1710 (C=O), 1690,(N-C=O), 1490, 1480, 1450, 1390, 1380, 1345, 1275, 1080 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (s,6), 2.5 (s,3,N-CH_3), 2.4 - 2.9 (m,1), 3.0 - 3.4 (m,3,$\underline{H_2}C$-S and part of N-$C\underline{H_2}$-CH_2-O), 3.6 - 4.0 (m,4), 3.8 (s,3,-OCH_3), 4.1 (s,1,N-CH-O), 7.3 - 8.0 (m,7,aromatic,C=CH and NH)

Uv (MeOH): 287 nm (ε = 17,000),226 nm (ε = 13,000)

Mass spectrum (70 eV): m/e = 436 (M^+)

Analysis: Calculated for $C_{21}H_{28}N_2O_6S$: C, 57.80; H, 6.42; N, 6.42; S, 7.34 . Found: C, 57.63; H, 6.70; N, 6.33; S, 7.62

Preparation of 2-benzamido-3(((4-formyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)thio)acrylic acid methyl ester (126) from (130)

The oxazolidine-ester (130) [227 mg] was dissolved in dimethoxyethane [1 ml]. To this solution 50% aqueous acetic acid [10 ml] was added and the mixture allowed to stand at room temperature for 1 hour. Water [50 ml] was added and the mixture extracted three times with chloroform [3 x 50 ml]. The extracts were back washed twice with water [2 x 50 ml], combined, dried with sodium sulphate, and evaporated to dryness in vacuo to yield a foam.

Yield: 195 mg (100%)

Ir (KBr): 3310 (NH), 1735 (O-C=O), 1720 (H-C=O), 1670 (N-C=O), 1605, 1520, 1490, 1440, 1390, 1380, 1340, 1250, 1070 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (s,6), 3.1 (ABq,2,J=15Hz, CH_2-S), 3.7 (s,3,OCH_3), 4.05 (ABq,2,J=10Hz,H_2C-O), 7.2 - 8.0 (m,7,aromatic,C=CH and NH), 8.9 (s,1,H-C=O)

Mass spectrum (70 eV): m/e = 379 (M^+)

Analysis: Calculated for $C_{18}H_{21}NO_6S$: C, 56.99; H, 5.54; N, 3.69; S, 8.44 . Found: C, 57.21; H, 5.81; N, 3.89; S, 8.17

Preparation of the hemiacetal acetate (127) from (35)

The aldehyde-oxazolone (35) [140 mg] was suspended in methanol [5 ml] and triethylamine [0.5 ml] was added. The mixture was allowed to stir for 1 hour at room temperature and then reduced to about 1 ml by evaporation in vacuo. Acetic anhydride [5 ml] and

pyridine [5 ml] was added and the mixture allowed to stand at room temperature overnight. The mixture was poured into ice water[100 ml], allowed to stir at 0°C for a half hour and then extracted three times with ether [3 x 50 ml]. The extracts were washed twice with ice cold 10% hydrochloric acid [2 x 25 ml], twice with ice cold 5% sodium bicarbonate [2 x 25 ml] and once with water [50 ml]. The extracts were then combined, dried with sodium sulphate and evaporated to dryness in vacuo. The residue was a colourless oil.

Yield: 160 mg (88%)

Ir (CCl_4): 3430 (NH), 3010/ 2970 (CH), 1750 (H_3C-C=O), 1720 (C=O), 1685 (N-C=O), 1620, 1490, 1460, 1390, 1380, 1340, 1250, 1080 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (s,6), 2.0 (s,3,Ac), 3.0 (ABq,2,J=14Hz,H_2C-S), 3.2 (s,3,OCH_3), 3.5 (s,3,CO-OCH_3), 3.7 (ABq,2,J=10Hz), 5.5 (d,1,O-CH-O), 6.7 - 7.5 (m,7,aromatic,C=CH and NH)

Mass spectrum (70 eV): m/e = 453 (M^+), m/e = 410 (M^+ - 43, H-C-C=O)

Preparation of aldehyde-oxazolone (35a) from mesylate (31) and (41)

This compound was prepared in the same way as (35) from (31) and (42).

Yield: 35% (based on (31)), mp 83-85°C

Ir (KBr): 3010/ 2960/ 2900/ 2840 (CH), 1765/ 1760 (O-C=O), 1730 (H-C=O), 1640 (C=N), 1480, 1465, 1395, 1385, 1220, 1110, 1080, 1065 cm^{-1}

Nmr ($CDCl_3$): δ 1.55 (d,6), 4.25 (ABq,2,J=10Hz,O-CH_2-C), 4.65 (s,2,H_2C-S), 7.3 - 8.2 (m,5,aromatic), 8.25 (s,1,C=CH), 9.9 (s,1,H-C=O)

EXPERIMENTAL - CHAPTER 5

Preparation of 2-benzamido-3-(((4-formyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)thio)acrylic acid βββ-trichloroethyl ester (131) from (34)

The oxazolidine-oxazolone (34) [0.50 g] was supended in βββ-trichloroethanol [5 ml]. Triethylamine [0.5 ml] was added and the mixture stirred at room temperature for 3 hours. The excess trichloroethanol and triethylamine were then removed by distillation under high vacuum (oil bath at <100°C). The residue was taken up in 50% aqueous acetic acid [10 ml] and allowed to stand for 1 hour at room temperature. Water [50 ml] was added and the mixture extracted 3 times with chloroform [3 x 50 ml]. The extracts were back washed twice with water [2 x 50 ml], combined, dried with sodium sulphate and evaporated to dryness in vacuo.

Yield: 0.64 g (104%, contaminated with trichloroethanol)

Ir ($CHCl_3$): 3440 (NH), 1770 (O-C=O), 1740 (H-C=O), 1680 (N-C=O), 1610, 1590, 1430, 1400, 1390, 1385, 1280, 1170, 1080 cm^{-1}

Nmr ($CDCl_3$): δ 1.5 (d,6), 3.3 (ABq,2,J=13Hz,CH_2-S), 4.2 (ABq,2, J=10Hz,O-CH_2-C), 4.95 (s,2,CH_2-CCl_3), 7.4 - 8.1 (m,7, aromatic,C=CH,NH), 9.95 (s,1,H-C=O)

Preparation of 2-benzamido-3-(((4-formyl-2,2-dimethyl-1,3-dioxan-4-yl)methyl)thio)acrylic acid p-methoxybenzyl ester (133) from (34)

This ester was prepared in the same way as the trichloroethyl

ester (131).

Ir ($CHCl_3$): 3400 (NH), 3080/ 3050/ 2980/ 2960/ 2890 (CH), 1720 (H-C=O), 1705 (C=O), 1690 (N-C=O), 1485, 1465, 1385, 1375, 1330, 1270, 1080 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (s,6), 3.1 (ABq,2,J=16Hz,H_2C-S), 3.8 (s,3,OCH_3), 4.0 (ABq,2,J=9Hz,OCH_3-C-O), 5.25 (s,2,H_2C-Ph), 7.2 - 8.4 (m,11,aromatic,C=CH, and NH), 9.8 (s,1,H-C=O)

Uv (dimethoxyethane) : 287 nm (ε = 24,500)

Mass spectrum : m/e = 485 (M^+)

Preparation of 2-benzamido-3-(((4-formyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)thio) acrylic acid benzyl ester (132) from (34)

This ester was prepared in the same way as the trichloroethyl ester (131).

Spectra on following page.

Ir (CCl_4): 3420 (NH), 3080/ 3040/ 2990/ 2960/ 2890 (CH), 1720 (H-C=O), 1710 (C=O), 1690 (N-C=O), 1485, 1470, 1385, 1375, 1335, 1270, 1080 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (s,6), 3.1 (ABq,2,J=16Hz,H_2C-S), 4.0 (ABq,2, J=9Hz,O-H_2C-C-O), 5.25 (s,2,H_2C-Ph), 7.2 - 8.4 (m,12, aromatic, C=CH and NH), 9.8 (s,1,H-C=O)

Uv (ether) : 286 nm (ε = 26,000), 268 (ε = 22,000), 264 (ε = 22,000), 258 (ε = 21,700)

Mass spectrum: m/e = 455 (M^+)

Preparation of α-benzamido-2,2-dimethyl-1,3-dioxa-7-thia-9-aza (spiro)[4,5]dec-9-ene-8-acetic acid methyl ester (136) from (126)

The aldehyde-ester (126) [1 g] was dissolved in dimethoxy-ethane [100 ml] in a 250 ml round bottom flask. The solution was cooled in ice water and ammonia gas was passed into the solution for 10 minutes. The flask was then well stoppered and allowed to stand at room temperature for 3 hours. The mixture was evaporated to dryness in vacuo, the residue taken up in ether [5 ml] and after scratching to initiate crystallization allowed to stand at -20°C for 5 hours. The white crystalline product was removed by vacuum filtration, washed well with ether [5 ml] and allowed to dry.

Yield: 0.65 g (70%) , mp 125-128°C

Ir (KBr): 3340 (NH), 2980/ 2960/ 2940/ 2870 (CH), 1750 (C=O), 1660 (C=N), 1650 (N-C=O), 1525, 1495, 1390, 1380, 1360, 1265, 1230, 1175, 1160, 1075, 860 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (d,6), 2.75 (ABq,2,J=10Hz,H_2C-S), 3.80 (s,3, OCH_3), 3.8 - 4.4 (m,3,O-CH_2-C), 4.9 - 5.4 (m,2,N-CH-S and CO-CH-NH), 6.8 - 8.0 (m,7,aromatic,NH and H-C=N)

Uv (methanol): 224 nm (ε= 15,100), shoulder at 260 - 275 nm

Mass spectrum (70eV): m/e = 378 (M^+)

Analysis: Calculated for $C_{18}H_{22}N_2O_5S$: C, 57.14; H,5.82; N, 7.41; S, 8.47 . Found: C, 56.88; H, 6.01; N, 7.25; S, 8.17

Preparation of α-benzamido-2,2-dimethyl-1,3-dioxa-7-thia-9-aza (spiro)[4,5]dec-9-ene-8-acetic acid benzyl ester (137) from (132)

The benzyl-ester (132) [1 g] was dissolved in dimethoxyethane [100 ml] in a 250 ml round bottom flask. The solution was cooled to 0°C in an ice water bath and ammonia gas was passed into the solution for 10 minutes. The flask was then well stoppered and allowed to stand at room temperature for 3 hours. The mixture was then evaporated to dryness in vacuo and the residue taken up in ether [5 ml]. This solution was then dripped slowly into ice cold vigorously stirred petroleum ether (60-80°C) [100 ml]. The imine product precipitated as a beige powdery material. It was removed by vacuum filtration, washed with petroleum ether [20 ml] and allowed to dry.

Yield: 0.45 g (45%)

Ir (KBr): 3320 (NH), 3060 /2980 /2940 /2890 (CH), 1755 (C=O),

1670 (N-C=O), 1530, 1490, 1385, 1375, 1260, 1215, 1160, 1070 cm^{-1}

Nmr ($CDCl_3$): δ 1.35 (s,6), 2.5 - 3.0 (m,2,CH_2-S), 3.5 - 4.4 (m,2, O-CH_2-C), 4.8 - 5.4 (m,4,CH_2-Ph, N-C<u>H</u>-S, and CO-C<u>H</u>-N), 7.2 - 8.0 (m,12,aromatic,N<u>H</u> and H-C=N)

UV (methanol): 227 nm (ε = 20,000), shoulder at 260 - 275 nm

Mass spectrum (70eV): m/e = 454 (M^+)

Analysis: Calculated for $C_{24}H_{26}N_2O_5S$: C, 63.44; H, 5.73; N, 6.17 . Found: C, 63.68; H, 5.38; N, 6.43

<u>Preparation of α-benzamido-2,2-dimethyl-1,3-dioxa-7-thia-9-aza (spiro)[4,5]dec-9-ene-8-acetic acid p-methoxybenzyl ester (138) from (133)</u>

The p-methoxybenzyl ester (133) [1 g] was dissolved in dimethoxyethane [100 ml] in a 250 ml round bottom flask. The solution was cooled to 0°C in an ice water bath and ammonia gas was passed into the solution for 10 minutes. The flask was then well stoppered and allowed to stand at room temperature for 3 hours. The mixture was then evaporated to dryness in vacuo and the residue taken up in ether [5 ml]. This solution was then dripped slowly into ice cold, vigorously stirred petroleum ether (60-80°C) [100 ml]. The imine product precipitated as a beige powdery material. It was removed by vacuum filtration, washed with petroleum ether [20 ml] and allowed to dry.

Yield: 0.54 g (55%)

Ir (KBr): 3420 - 3330 (NH), 3040/ 2980/ 2930/ 2880 (CH), 1750 (C=O), 1680 (N-C=O), 1530, 1490, 1385, 1375, 1320, 1265, 1210, 1170, 1070 cm^{-1}

Nmr ($CDCl_3$): δ 1.35 (s,6), 2.5 - 3.0 (m,2) , 3.5 - 4.6 (m,2, O-CH_2-C), 3.8 (s,3,OCH_3), 4.8 - 5.3 (m,4,CH_2-Ph, N-CH-S and CO-CH-N), 7.2 - 8.0 (m,11,aromatic, NH and H-C=N)

Uv (ethanol): 226 nm (ε = 23,000), shoulder at 260 - 275 nm

Analysis: Calculated for $C_{25}H_{28}N_2O_6S$: C, 61.97; H, 5.83; N, 5.78 . Found: C, 61.69; H, 5.91; N, 6.03

Isolation of the α-benzamido-2,2-dimethyl-1,3-dioxa-7-thia-9-aza spiro[4,5]dec-9-ene-8-acetamide (139) from treatment of (131) with ammonia

Treatment of the trichloroethyl ester (131) with ammonia in the same way as (126) led to the precipitation of the amide (139) from the dimethoxyethane solvent.

Ir (KBr): 3420/ 3320/ 3280 (NH), 1670/ 1650 (N-C=O), 1530, 1490, 1390, 1380, 1325, 1260, 1220, 1160, 1080, 990, 865 cm^{-1}

Nmr (DMSO - d_6): δ 1.4 (s,6), 2.8 (m,2,CH_2-S), 3.8 - 4.2 (m,2, O-CH_2-C), 4.5 - 5.0 (m,2), 7.2 - 8.4 (m,9, aromatic, NH_2, NH, H-C=N)

Preparation of 7-benzamido-N-cyclohexyl-2',2'-dimethyl-spiro(cepham-3,4'-(5,4)dioxolane)-4-carboxamide (38) from (136)

The following was performed under N_2 at room temperature

The imine-ester (136) [100 mg] was dissolved in hexamethylphosphoric triamide (HMPT) [0.5 ml]. Lithium n-propylmercaptide (0.55M in HMPT[64]) [1.0 ml, 2 equiv.] was added. The mixture was allowed to stand at room temperature for one and a half hours, then a phosphate buffer solution (pH 7) [10 ml, deoxygenated] was added. The mixture was allowed to stir for 2 minutes, then cyclohexylisonitrile [100 mg] in methylene chloride-carbon tetrachloride (1:1) [10 ml] was added and the two phases mixed with vigorous stirring for 12 hours. The two phases were then separated and the organic layer washed with ice water 5 times [5 x 15 ml], with ice cold 0.5N hydrochloric acid 3 times [3 x 15 ml], and with ice cold sodium bicarbonate twice (2 x 15 ml]. The organic layer was then dried with sodium sulphate and evaporated to dryness in vacuo. Crude yield: 138 mg (110%) contaminated with N-formylcyclohexylamine. The β-lactam was purified by thin layer chromatography on silica gel plates (1.0 mm plates, CCl_4/dimethoxyethane = 8/2, R_f= 0.85). Yield: 38 mg (30%)

Ir (CCl_4): 3420/ 3380/ 3340 (NH), 2970/ 2940/ 2860 (CH), 1740 (lactam), 1690/ 1680 (N-C=O), 1460, 1410, 1385, 1375, 1300, 1220, 1190, 1075 cm^{-1}

Nmr ($CDCl_3$): δ 1.4 (broad d,16), 2.3 - 3.1 (m,3,CH_2-S, and C<u>H</u>-NH-CO), 3.3 - 4.3 (m,3,O-CH_2-C and CO-C<u>H</u>-N),

7.2 - 8.0 (m,7,aromatic,NH)

Mass spectrum (70eV): m/e = 473 (M^+)

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