The Synthesis of Unsymmetrical Disulfides

by Thomas G. Back

(Submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the M.Sc. degree)

Chemistry Department

McGill University

The widespread natural occurrence and varied biological functions of unsymmetrical disulfides has stimulated much interest in their synthesis. The scope and limitations of existing synthetic routes are discussed, and a new preparative method involving the thiolysis of thiophthalimides is described. This technique was used to generate a wide variety of dialkyl and aralkyl unsymmetrical disulfides in high yields. A new cysteine-containing thiophthalimide was synthesized by the nucleophilic attack of the phthalimide anion on the sulfenyl bromide derived from bromination of N,N'-bis(trifluoroacetyl)-L-cystine dimethylester. Thiolysis of this thiophthalimide with benzyl mercaptan, cysteine, and glutathione provided excellent yields (92-99%) of the corresponding disulfides. The synthesis of the latter two disulfides demonstrates the feasibility of this technique in the preparation of unsymmetrical peptide disulfides.

THE SYNTHESIS OF UNSYMMETRICAL DISULFIDES

by

Thomas George Back

A thesis submitted to the Faculty of
Graduate Studies and Research in partial
fulfillment of the requirements for the
degree of Master of Science

Department of Chemistry
McGill University
Montreal, Quebec.

June 1971

Dedicated to my grandmother,
parents, and sister.

ACKNOWLEDGEMENTS

I wish to thank Peter Currie for the mass spectra.

I extend my appreciation to Dave Ash, Dr. J. Gleason, Dr. W. Van Horn, and Dr. J. Snyder for aid in the synthesis of some of the compounds listed in Table 1.

For many illuminating discussions, I thank Dave Ash, Martin Vines, Dr. J. Snyder, and Dr. J. Gleason.

Above all, I would like to thank Dr. David N. Harpp for his patience and constant encouragement.

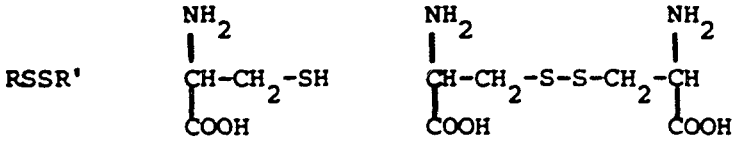
TABLE OF CONTENTS

INTRODUCTION

INTRODUCTION

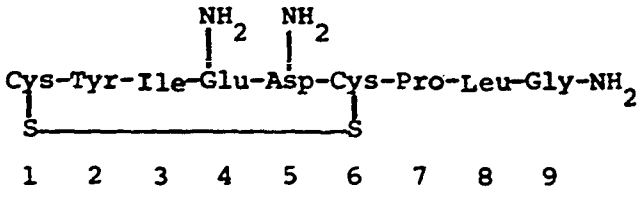
Unsymmetrical disulfides 1 occur widespread in nature where they perform many varied functions. They often possess unique biological properties which render them indispensable to many living organisms, including man. Consequently, this has stimulated much interest in their synthesis.

Perhaps the most important of these compounds are polypeptides containing the amino acid cysteine (2). The thiol groups of these cysteine residues may be joined together to give unsymmetrical derivatives of cystine (3), the disulfide form of cysteine.



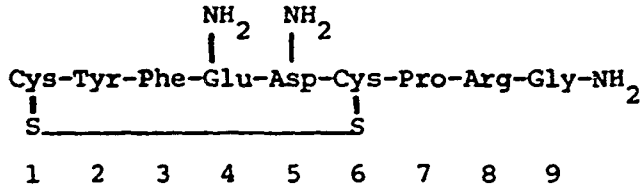
1 2 3

An example of such a compound is the pituitary hormone oxytocin (4), which causes uterine contractions in childbirth. The structure of oxytocin was first deduced by du Vigneaud and co-workers[1], who found it to be a nonapeptide with cysteine in the 1 and 6 positions. These cysteine residues were found to be joined by a disulfide bond, thus making this compound a cyclic, unsymmetrical disulfide.



4

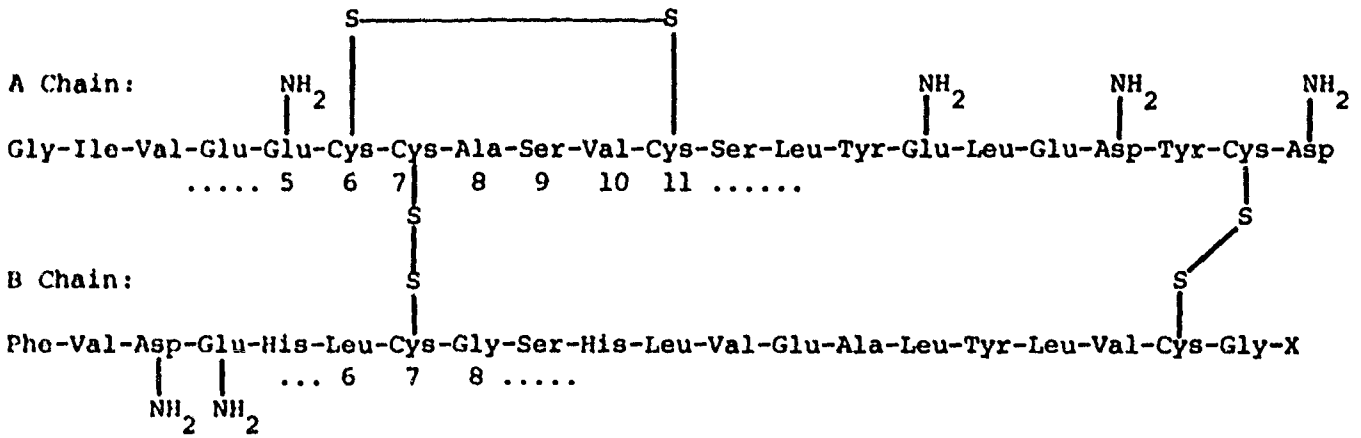
A related pituitary hormone, which possesses antidiuretic activity, is vasopressin (5)[2].



5

The above structure 5 is arginine vasopressin, which occurs in most mammals[3]. A slightly different form, lysine vasopressin, is found in the pig and hippopotamus[3] and differs from 5 in that the arginine residue in position 8 is replaced by lysine.

One of the best known of all unsymmetrical peptide disulfides which occur naturally is the pancreatic hormone insulin (6), which is vital in glucose metabolism. The amino acid sequence of bovine insulin (6) was first established by Sanger[4]. Although slight variations in this sequence occur in different species, insulin is generally composed of fifty-one amino acids occurring in two chains. These chains (A and B) are connected by two disulfide bonds; in addition, a third disulfide

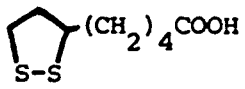


X = Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala

bond occurs between two cysteine residues in the A chain.

Similarly, pancreatic ribonuclease is a complex peptide composed of a single chain of amino acids which is internally cross-linked by four disulfide bonds[5].

A biologically active unsymmetrical disulfide which is not a peptide is α-lipoic acid (7). This is a co-enzyme required by certain types of lactic acid bacteria for the oxidative decarboxylation of pyruvate[6]. In trace amounts it can replace acetate ions in their growth-stimulating role[7]. α-Lipoic acid was first isolated by Reed and co-workers[8] from water-insoluble extracts of beef liver.



7

These few examples of unsymmetrical disulfides suffice to point out the significant role that these compounds play in nature. It thus follows that exploration of their synthetic pathways is of great interest, both to relieve man's dependence on natural sources for their supply, and to further illuminate their many functions.

One of the first methods employed[9] for the synthesis of mixed (unsymmetrical) disulfides was the disporportionation of a mixture of two symmetrical disulfides by means of an alkaline medium containing a thiol catalyst. A mixture of the two starting materials and the corresponding unsymmetrical disulfide was obtained in the expected statistical ratio

of 1:2:1, as shown in eq.I.

$$\text{(eq.I)} \quad \underset{\text{thiol}}{\text{RSSR + R'SSR'}} \xrightarrow{\text{KOH}} \underset{1 \;:\; 2 \;:\; 1}{\text{RSSR + R'SSR + R'SSR'}}$$

The synthetic utility of this method is thus severely restricted, both by the statistical limitation to the yield (maximum 50 mole %), and by the necessity of separating the desired product from its two symmetrical counterparts.

A similar method employed by the same authors[9] involved the oxidation of a mixture of two different thiols, again giving all three disulfides in the 1:2:1 ratio obtained with the disproportionation method. The process is depicted in eq.II.

$$\text{(eq.II)} \quad \text{RSH + R'SH} \xrightarrow{(o)} \underset{1 \;:\; 2 \;:\; 1}{\text{RSSR + RSSR' + R'SSR'}}$$

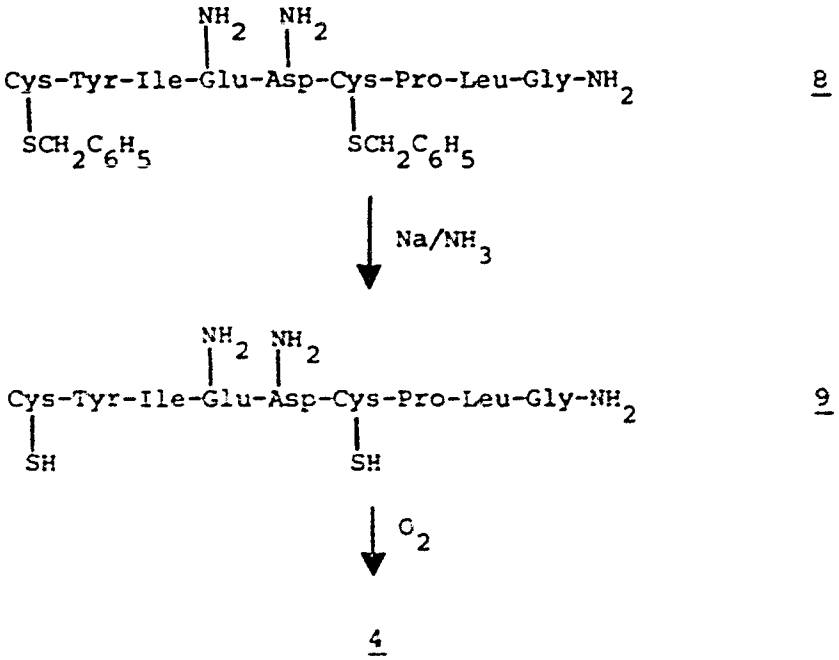
Thus, it can be seen that oxidation of a mixture of two thiols to give an unsymmetrical disulfide is not a practical synthetic method due to the simultaneous formation of the two symmetrical disulfides. However, oxidation of a dithiol may provide a feasible route for producing the corresponding cyclic disulfide, as shown in eq.III. This has provided a major pathway to many peptide disulfides, the precursors of which are usually prepared with blocked thiol groups. Removal of the S-protective groups, followed by oxidation, provides the product disulfide.

(eq.III)

The oxidation itself is usually effected by passing a stream of oxygen or air through an aqueous solution or the dithiol[10]. Refinements of this method have included the use of ferricyanide ion[11] as oxidizing agent, as well as 1,2-diiodoethane[12], which is reduced to ethylene and iodide ion.

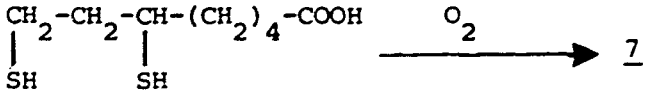
An illustrative example of this general method was the first complete synthesis[13] of oxytocin (4). The S,S'-dibenzyl derivative 8 was treated with sodium in liquid ammonia to give the free dithiol 9, which was converted by aerial oxidation to oxytocin having biological activity comparable to that of the purified natural product. The scheme is illustrated by eq.IV.

(eq.IV)



Similarly, both optical forms of α-lipoic acid (7) were synthesized[14] by aerial oxidation of the dithiol 10 in aqueous ferric chloride solution (eq.V).

(eq.V)

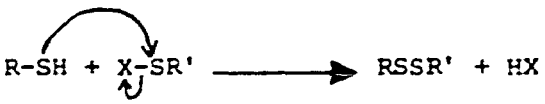


10

Although this method has been used extensively for the synthesis of fairly complex molecules, it suffers from some severe drawbacks. Firstly, the yields obtained are usually quite low (20-30%), and secondly, the products obtained generally require extensive purification.

Most of the methods available in the literature for the synthesis of unsymmetrical disulfides employ nucleophilic attack of thiols on various sulfenyl derivatives, as shown below.

(eq.VI)

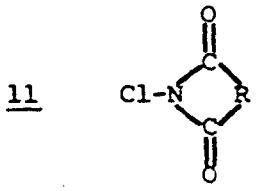


Among the sulfenyl compounds which have been used with varying degrees of success, are sulfenyl halides (X = halide), thiolsulfinates (X = R'S(O)-), thiosulfates (X = $R'SO_3$-), thiolsulfonates (X = $R'SO_2$-), sulfenyl thiocyanates (X = NCS-), sulfenyl hydrazides (X = R_1NH-NR_1-), sulfenyl thioureas (X = NH=C(NH_2)-S-), and thioimides[a] (X = -CO-N-CO-).

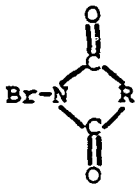
a) The term "sulfenimide" has often been used instead of "thioimide" in the literature.

It should be pointed out that none of these methods suffice for all synthetic situations. Also, the full scope of some of the more recent procedures has not yet been fully explored.

Among the first sulfenyl reagents to be investigated were the sulfenyl chlorides (i.e. X = Cl in eq.VI), of which a large variety abounds in the literature[15, 16]. They are generally prepared by low-temperature chlorinolysis[15] of the corresponding disulfide or mercaptan, or alternately by the action of N-chloro or N-bromoimides 11, 12, on mercaptans[17].



11



12

A simple example of the thiolysis of a sulfenyl chloride to give an unsymmetrical disulfide was provided by Douglass et al.[18], who reacted chloro, dichloro, and trichloromethanesulfenyl chloride (13a, b, and c respectively) with various mercaptans to obtain the corresponding disulfides (eq.VII).

(eq.VII)

13a	$ClCH_2SCl$		$ClCH_2SSR$
b	Cl_2CHSCl	$\xrightarrow{RSH}$	$Cl_2CHSSR + HCl$
c	Cl_3CSCl		Cl_3CSSR

Unfortunately, this method was hampered by instability of the precursor sulfenyl chlorides, and, more seriously, by disproportionation

of some of the products during distillation, thus preventing their isolation in pure form.

Schöberl and co-workers[19] also utilized this method to prepare disulfides of the form $RO_2CCH_2SSR'CO_2H$ by treating sulfenyl chlorides with mercaptocarboxylic acids. Their efforts were rewarded by poor yields (35-53%) of a limited variety of products.

The high reactivity of sulfenyl chlorides usually results in complications such as simultaneous α-chlorination[20] of the sulfenyl chloride during its formation by chlorinolysis, as illustrated by eq.VIII.

(eq.VIII)

$$RCH_2SCl + Cl_2 \longrightarrow RCH_2SCl_3$$

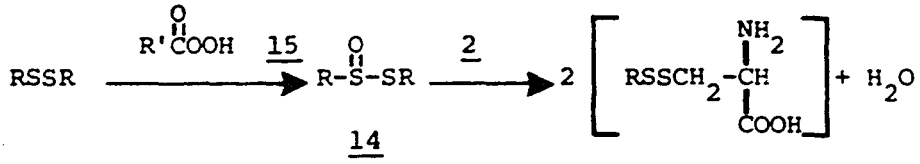
$$RCH_2SCl_3 \longrightarrow RCH(Cl)SCl + HCl$$

Furthermore, the presence of moisture results in the formation of sulfonyl chlorides[21]. Lastly, it is obvious that this method is unacceptable when substituent groups capable of being halogenated exist in the molecule.

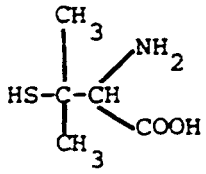
The nucleophilic displacement of sulfenyl bromides or iodides appears to be even less feasible than that of chlorides, as these materials offer even lower stability and greater difficulty in preparation than the latter.

Another leaving group which was investigated was the sulfinate moiety. Small et al.[22] prepared a series of thiolsulfinates <u>14</u> by oxidation of the corresponding disulfides with peracids <u>15</u>. The authors then found that these thiolsulfinates reacted in good yield with

two molar equivalents of cysteine (2) to form mixed disulfides as in eq.IX.

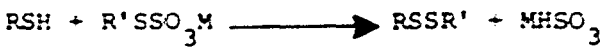
(eq.IX) 

Schöberl and co-workers extended this method to the preparation of disulfides composed of cysteine and another mercaptocarboxylic acid, such as penicillamine (16)[19].

16 

Unfortunately, this method is limited to those cases where the intermediate thiolsulfinate is readily prepared and stable enough to avoid rearrangement to disulfides (RSSR) and thiolsulfonates (RSO_2R). Furthermore, the thiolsulfinate yields reported by Small[22] were generally poor (20-65%), also a factor contributing to the limited use of this method.

A technique which has found broader application is the thiolysis of thiosulfates 17 or Bunte salts 18, as portrayed by eq. X.

(eq.X) 

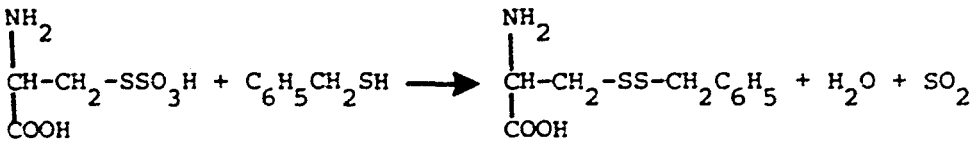
17 M = H

18 M = Na

This method was first reported by Footner and Smiles[23], who treated Bunte salts with sodium mercaptides ($Na^+ \ ^-SR$) at room temperature in alkaline media. They discovered that their products included the two symmetrical disulfides as well as the expected unsymmetrical one. This observation may be explained by the fact that disulfide interchange (disproportionation) occurs rapidly under basic conditions and is catalyzed by traces of thiols[24].

Swan[25] then re-examined the method under weakly acidic conditions, where disulfide interchange occurs more slowly and is inhibited by the presence of thiols[26]. The thiolysis was driven to completion by removing the sulfur dioxide formed with a stream of nitrogen, or alternately, by precipitating sulfite ion as its strontium salt. Reaction of L-cysteine-S-sulfonic acid[b] (19) with benzyl mercaptan (20), as in eq. XI, gave disulfide 21.

(eq.XI)



19 20 21

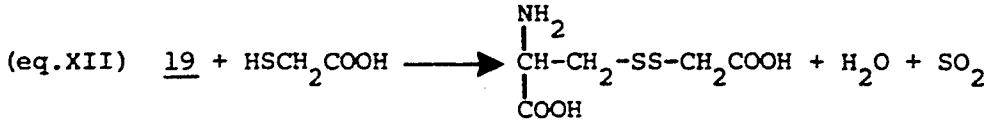
Similarly, thiolysis of 19 with thioglycolic acid (22) and with N-Cbz-L-cysteine (23)[c] gave disulfides 24 and 25 respectively, as shown

---

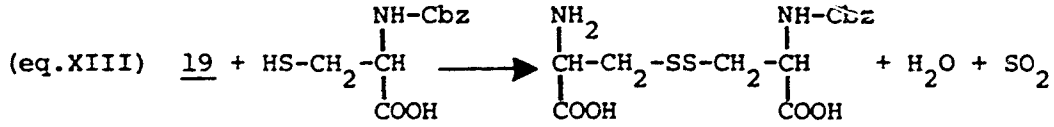
b) Swan referred to compound 19 as "S-sulfo-L-cysteine", but it is more correctly named as a sulfonic acid derivative.

c) Cbz = Carbobenzoxy = $C_6H_5CH_2OC-$ (O)

in eqs.XII and XIII.

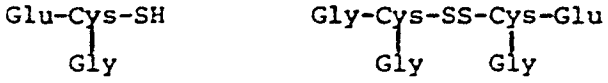
(eq.XII) 

22 24

(eq.XIII) 

23 25

Reaction of 19 with glutathione (26) was less successful as partial rearrangement to cystine (3) and oxidized glutathione (27) was observed.



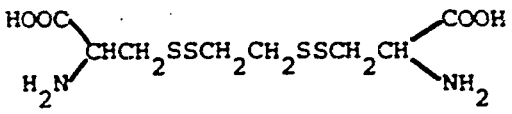
26 27

Disulfides 21 and 25 were also prepared by Stapleton and Swan[27], who used cysteine as the thiol in eq.X, and the thiosulfate derivatives 28 and 29 respectively.

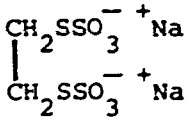
28 29

A practical application of this method was patented by Haefele[28], who used it to modify the structure of some keratin-containing materials, such as human hair and wool. The naturally occurring disulfide bonds of these materials were reduced with mild reducing agents, and the free thiol groups were treated with one or more acids or salts of organic thiosulfates, according to eq.X. The physical and chemical properties of these keratins were drastically modified by the use of various R' groups (eq.X). For example, it was claimed that wool could be made more resistant to shrinkage; in addition, its wettability, resistance to wool moths, and fiber strength could be increased. Human hair could be given improved "lustre" and "feel".

The bisdisulfide 30 was also synthesized by Swan[25] by the treatment of disodium ethylene dithiosulfate (31) with two equivalents of cysteine.



30



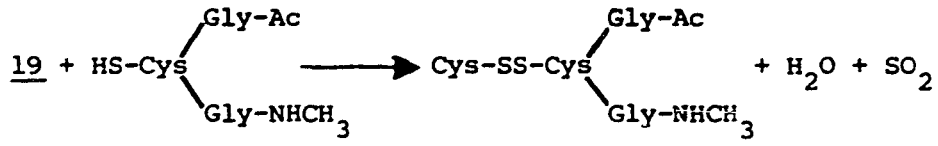
31

Schöberl and Bauer[29] also prepared a series of bisdisulfides similar to 30 by this method, but these authors extended the technique to the treatment of reduced keratin (kerateine) with di-Bunte salts such as 31 to produce unsymmetrical disulfides of the form $RSS(CH_2)_nSSR$.

The effect of this process was to form an extensive lattice from the disulfide cross-linkages between the peptide chains of the natural keratin. The properties of the keratin could thus be modified.

An example of a relatively complex, homogeneous peptide disulfide synthesized by this method was furnished by Zahn and Otten[30]. They treated 19 with acetylglycyl-L-cysteinylglycinemethylamide (32) to give disulfide 33. However, purification of the product by chromatography and electrophoresis was necessary.

(eq.XIV)



32[d] 33

Thus, we see from these examples that thiolysis of thiosulfates provides a synthetic pathway to a variety of unsymmetrical disulfides, including some of moderate complexity. However, the scope of the method is limited by the availability of the precursor thiosulfates and Bunte salts. Although simple Bunte salts are easily prepared from alkyl halides and sodium thiosulfate[31], more complex ones are generally difficult to synthesize. Hence, the range of mixed disulfides which can be obtained through this method is restricted to those having a rather simple moiety (e.g. cysteine) on one side of the disulfide bond. The method usually affords high yields, but care must be taken to avoid conditions favouring disulfide interchange.

---

d) Ac = Acetyl

A somewhat similar synthesis of unsymmetrical disulfides involves the thiolysis of thiolsulfonates[32] 34 as in eq. XV.

(eq.XV) $$RSH + R'SSO_2R' \longrightarrow RSSR' + R'SO_2H$$

34

This method remained relatively unexplored until Field et al.[33] applied it to the synthesis of certain radioprotective agents. It had been previously reported[34] that compounds containing the 2-aminoethylthio ($\geq NCH_2CH_2S-$) moiety form an important class of agents which protect against the lethal effects of ionizing radiation. In search of unsymmetrical disulfides containing this group, Field made extensive use of the reaction described by eq.XV.

Precursor thiolsulfonates were prepared by oxidation of thiols or disulfides with hydrogen peroxide. Treatment of these compounds with a second thiol gave the product disulfides in good to excellent yield (67-92%). A variety of mixed disulfides was made from thiolsulfonates 35 and 36 and various alkyl and aryl thiols.

$AcNH(CH_2)_2SSO_2(CH_2)_2NHAc$ — 35

$H_2N(CH_2)_2SSO_2(CH_2)_2NH_2 \cdot 2HCl$ — 36

Recently, Field and Giles[35] used this method to synthesize disulfides containing the o-carboxyphenylthio moiety, as this group shows promising latentiating properties for radioprotective thiols. The

synthesis started with o-mercaptobenzoic acid (37), which on oxidation with chlorine, gave thiolsulfonate 38. Reaction with various mercaptans then gave disulfides of the form 39, as shown by eq. XVI.

(eq.XVI)

37 38 39

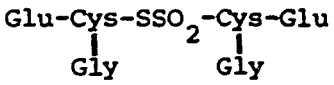
This method has also been applied to the synthesis of peptide disulfides. Eriksson and Eriksson[36] prepared the mixed disulfide of gluathione and cysteine by thiolysis of thiolsulfonate 40 with glutathione (26) in a weakly acidic medium, as depicted in eq. XVII. A high yield (91%) of the product 41 was obtained after purification by ion-exchange chromatography.

(eq.XVII) 26 + Cys-SSO_2-Cys ⟶ Glu-Cys(Gly)-SS-Cys + Cys-SO_2H

40 41

The synthesis of this compound is of particular interest as it has been found to occur naturally in pig and rat livers[37] as well as in extracts of calf-lens[38]. Furthermore, a peptide believed to be 41 was obtained by electrophoresis of liver extracts treated with hepatic carcinogens[39]. This peptide was also detected in rat kidney extracts which had been incubated with glutathione[39].

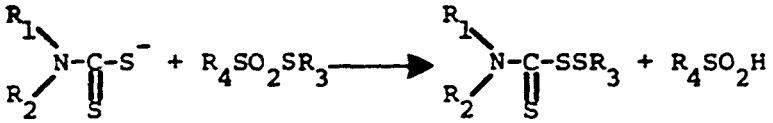
Eriksson[40] has also reported the synthesis of the mixed disulfide of coenzyme A and glutathione by an analogous procedure, starting with the thiolsulfonate derivative of glutathione 42 and coenzyme A, which is commercially available.



42

A variation of this method was reported by Field and Buckman[41], who found that dithiocarbamates 43 react with thiolsulfonates to form unsymmetrical thiocarbamoyl disulfides 44 in 48-88% yield, as shown in eq.XVIII.

(eq. XVIII)



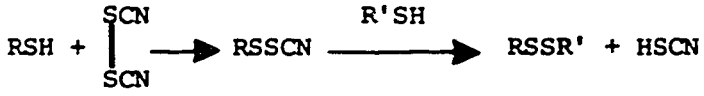
43 44

Hence, we see that the thiolysis of thiolsulfonates provides a convenient synthetic pathway to some important unsymmetrical disulfides. As is the case with the thiosulfate technique however, the scope of this method is limited by the availability of the precursor thiolsulfonates. An added complication often arises from the lability of these materials.

The method which has perhaps been most exploited in the synthesis of peptide disulfides is the thiolysis of sulfenyl thiocyanates 45,

These compounds are generally prepared by treating thiols with thiocyanogen (46) in cold ether solution. Lecher and Wittwer[42] found that nucleophilic displacement of thiocyanic acid (47)would occur upon addition of a second thiol, thus giving an unsymmetrical disulfide (eq.XIX). Several aralkyl disulfides were prepared in this manner, but the only aliphatic thiol to be investigated was ethyl mercaptan.

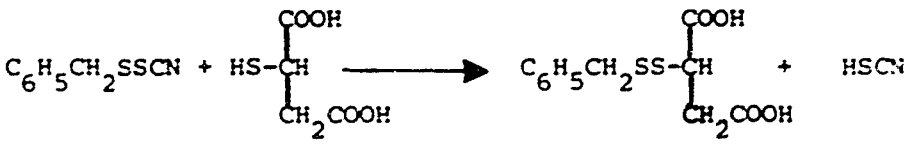
(eq.XIX)



46 45 47

Hiskey and co-workers[43] extended this method to the synthesis of several aliphatic disulfides. The intermediate sulfenyl thiocyanates were used in situ. Yields of 50-70% of a variety of disulfides containing the methoxyl, nitro, carbomethoxy, and carboxy groups were reported. Furthermore, it was found that it was not necessary for the nucleophilic thiol to be ether-soluble. A two-phase reaction of benzylsulfenylthiocyanate (48) with α-mercaptosuccinic acid (49) afforded a 51.5% yield of disulfide 50, as illustrated by eq.XX.

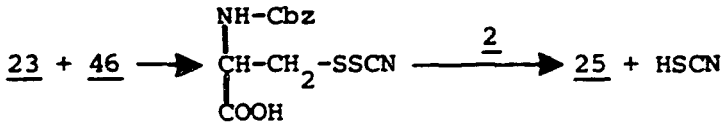
(eq.XX)



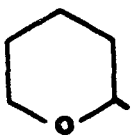
48 49 50

Subsequently, Hiskey and Tucker[44] used this technique to prepare some cysteine-containing disulfides. To avoid interaction between the free amino group of cysteine (2) and thiocyanogen, it was necessary to use N-protected cysteine derivatives. Thus, treatment of N-Cbz-cysteine (23)with thiocyanogen (46), followed by addition of cysteine hydrochloride, gave a 57% yield of disulfide 25, as depicted below.

(eq.XXI)



Also of significance was the observation that certain S-protected compounds would react directly with thiocyanogen and then with a thiol to give unsymmetrical disulfides. The importance of this discovery is that in the final stage of synthesis of a complex peptide, namely S-S bond formation, S-protected thiols may directly form disulfides without the necessity of an additional unblocking step as had been formerly required with methods such as air oxidation. Examples of S-protective groups that were successfully removed by thiocyanogen, and that were extensively used in subsequent syntheses, were the 2-tetrahydropyranyl (51) and the trityl (52) groups.



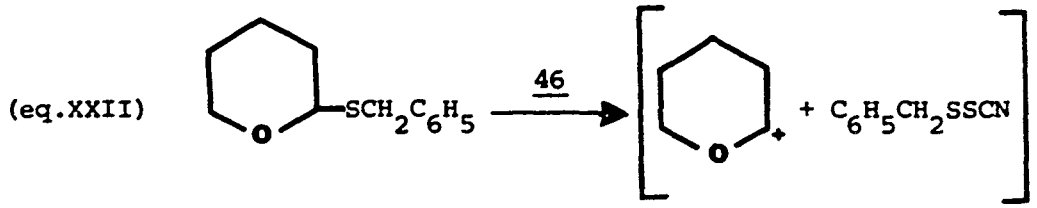
51

$(C_6H_5)_3C$ -

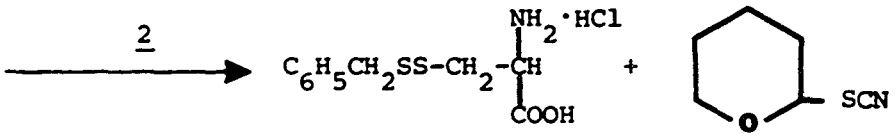
52

For example, benzyl 2-tetrahydropyranyl sulfide (53) was treated with thiocyanogen and then with cysteine hydrochloride to give a 59% yield of disulfide 54, (eq.XXII).

(eq.XXII)



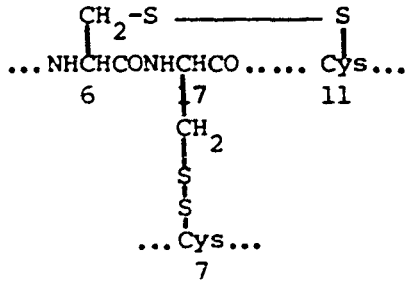
53



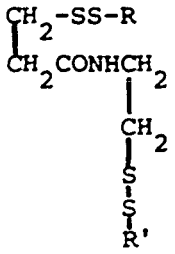
54

A disadvantage of using cysteine derivatives protected as the 2-tetrohydropyranyl thioethers was that these compounds were generally isolated as oils, and were consequently difficult to purify. Hence, Hiskey and Tucker[45] turned their attention to S-trityl derivatives. These compounds were solids which are easily crystallized. However, treatment of the trityl thioethers with thiocyanogen, followed by a thiol, gave lower yields of disulfides than was the case with the 2-tetrahydropyranyl derivatives. This was explained by the decreased electron density around the sulfur atom in the case of the trityl compounds, resulting in lowered nucleophilicity. In view of this theory, it was hoped that yields could be improved by the presence of a Lewis acid. Indeed, it was observed that the yields were increased to ~80% by the presence of zinc chloride in the reaction mixture.

This technique was then applied to the synthesis of bisdisulfides by Hiskey and Harpp[46]. These authors were attempting to produce model compounds resembling the loop 55 in the A chain of the insulin molecule (6) containing the residues 6-11. These residues contain the A chain disulfide bond as well as the point in the A chain at which the disulfide crosslinkage to the 7th residue of the B chain occurs. Hence, successful synthesis of model compounds of the form 56 would give an indication of the feasibility of applying the thiocyanogen method to the synthesis of insulin.



55



56

A series of these model compounds was synthesized from the trityl thioethers 57, according to eq.XXIII. Unfortunately, the use of zinc chloride resulted in the formation of amorphous, zinc-containing precipitates which were not identified. However, when sodium acetate was used instead, yields of 43-74% of bisdisulfides 56 were obtained.

(eq.XXIII) $RSSCH_2CH_2CONHCH_2CH_2SC(C_6H_5)_3 \xrightarrow[NaOAc]{R'SSCN} \underline{55} + (C_6H_5)_3CNCS$

57

Recently, Hiskey et al.[47] have reported the synthesis of a tridisulfide polypeptide which resembles insulin even more closely than bisdisulfides 56.

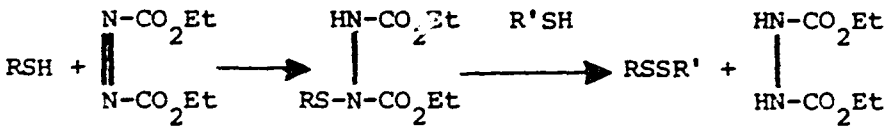
From these examples we see that this method has permitted the synthesis of unsymmetrical peptide disulfides of fair complexity. An important advantage of this method is that disulfide interchange can generally be avoided. Also, it has been demonstrated that the stepwise formation of several disulfide bonds is possible, as protected thiol groups may be unblocked and joined by the use of the thiocyanogen method, even in the presence of previously formed disulfide bonds. However, several disadvantages still remain, as Hiskey has pointed out[48]. First, the thermal lability of the intermediate sulfenylthiocyanates dictates that they be used in situ, without prior isolation and purification. A consequence of their instability is the fact that excellent yields are rarely realized and usually occur in the 50-70% range. Secondly, the reactivity displayed by sulfenylthiocyanates towards basic nitrogen atoms necessitates the use of N-protective groups (e.g. Cbz) on all free amino functions of the peptides involved.

Several other preparations of unsymmetrical disulfides via S_N2 reactions of sulfenyl derivatives have been reported in the literature. These have been quite recent and their full scope has not yet been explored.

One such method was reported by Mukaiyama and Takahashi[49], who reacted diethyl azodicarboxylate (58) with various alkyl and aryl thiols to give the adduct 59. This adduct was then reacted with a second thiol to give the corresponding mixed disulfide and diethyl

hydrazodicarboxylate (60) as in eq. XXIV. Yields of 75-90% were reported.

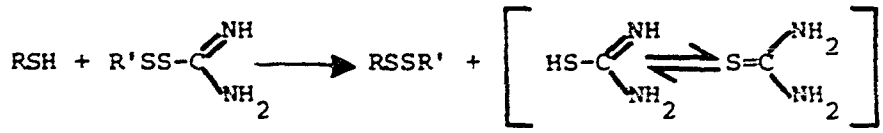
(eq.XXIV)



58[e] 59 60

Another method was reported by Sirakawa et al.[50], who treated S-alkylthioisothioureas 61 with thiols to give the corresponding unsymmetrical disulfide (eq.XXV). The precursors 61 were in turn prepared by the action of hydrogen peroxide on aqueous solutions of thiols and thioureas 62. The latter compounds are regenerated in the thiolysis step.

(eq.XXV)



61 62

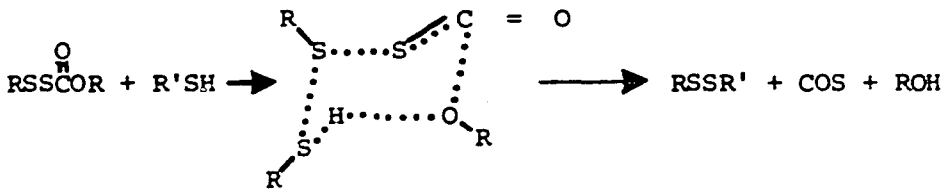
It was also recently found[51,52] that an excellent synthetic pathway to unsymmetrical disulfides involves the thiolysis of thioimides. The method was found to be rapid, clean, and provided the product disulfides in high yields. The advantages,limitations, and experimental techniques of this method will be discussed in detail in the following sections of this thesis.

---

e) Et = Ethyl

Very recently, Brois and co-workers[53] reported an alternative to the S_N2 reactions of various sulfenyl derivatives as a means of obtaining mixed disulfides. They reported that the thiol-mediated fragmentation of sulfenyl thiocarbonates 63 gives excellent yields of unsymmetrical disulfides via the proposed intermediate (64), as depicted below.

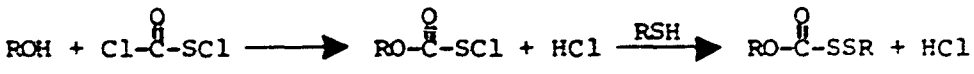
(eq.XXVI)



63 64

The authors report that the precursor sulfenyl thiocarbonates are prepared in excellent yield via the reaction of thiols with carboalkoxysulfenyl chlorides 65. The latter are in turn prepared from chlorocarbonylsulfenyl chloride (66) and alcohols. The process is portrayed below.

(eq.XXVII)



66 65

Although the authors claim no complicating side reactions and facility in preparing the starting materials (via eq. XXVII), the full scope of this method remains to be seen.

RESULTS

AND

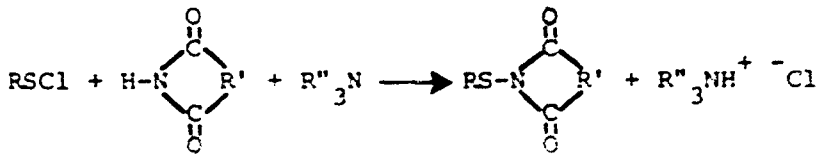
DISCUSSION

RESULTS AND DISCUSSION

As has been pointed out in the previous section, most techniques for the synthesis of unsymmetrical disulfides employ nucleophilic attack of a thiol on a sulfenyl derivative as illustrated by eq. VI. Most of these methods suffer from complications caused by the instability of the sulfenyl precursors, as well as from interactions of these reactive compounds with various substituent groups. However, it was found that thioimides 67 are sulfenyl derivatives which are generally stable, easily prepared crystalline solids.

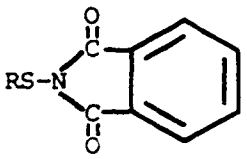
Behforouz and Kerwood[54] reported the preparation of thioimides *via* the corresponding sulfenyl chlorides, which in turn were prepared by the chlorinolysis of disulfides or thiols. Treatment of the sulfenyl chloride with the desired imide 68 gave yields of over 90% in the case of thiophthalimides 69 and 75-87% for thiosuccinimides 70. The by-product hydrogen chloride was removed by the presence of a tertiary amine. The reaction is illustrated by eq. XXVIII.

(eq. XXVIII)

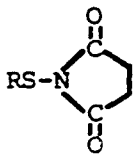


68 67

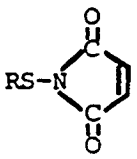
An alternate method of preparation of thioimides was described by Büchel and Conte[55], who treated disulfides with the desired N-bromoimide 12. Yields of ∿90% were reported.



69



70



71

Hence, stability, ease of purification, and convenient preparations of thioimides recommend these compounds as precursors of unsymmetrical disulfides. Then, Boustany and Sullivan[51], and Harpp et al.[52] independently reported the successful thiolysis of these compounds to give mixed disulfides. The former authors reacted thiophthalimides 69, thiosuccinimides 70, and thiomaleimides 71 with various thiols, as in eq. XXIX, to give yields of 82-96% of a number of dialkyl and aralkyl mixed disulfides. The reactions were performed both neat and in a variety of solvents at temperatures ranging from 0 to 100°. Inert solvents were generally preferred as precipitation of the imide occurred, thus helping to drive the reaction to completion.

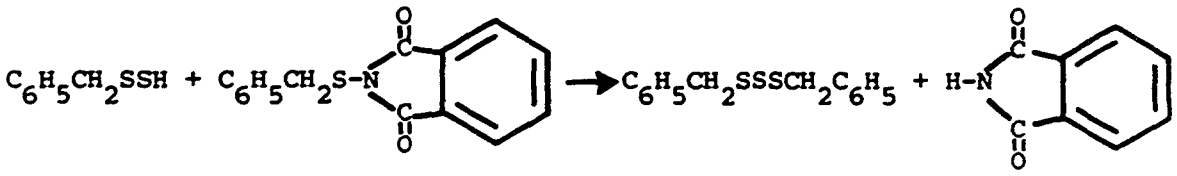
(eq.XXIX) 67 + R"SH ⟶ RSSR" + 68

In our laboratory, attention was confined to the use of thiophthalimides 69, which on treatment with thiols, generated a wide variety

of aralkyl and dialkyl disulfides in yields ranging from 71 to 92%. These compounds are listed in Table 1. The absence of symmetrical disulfides (from disproportionation) in the products was established by means of tlc or vpc. It should be pointed out that the homogeneity of unsymmetrical disulfides often cannot be verified by means of ir or nmr spectroscopy, as the spectra of the product disulfides are usually identical to those of mixtures of their symmetrical counterparts. Only in the case of products 54, 75 and 76 were traces of symmetrical disulfides (ca. 2%) discovered. However, efforts to obtain diaryl disulfides were unsuccessful. Attempts to prepare p-fluorophenyl p-tolyl (78) and phenyl p-tolyl (79)disulfides resulted in each case in a mixture of all three possible disulfides in ratios of 1:2:1 and 1:5:1 respectively. This may be rationalized if it is borne in mind that we have the simultaneous presence of a nucleophilic thiol and a diaryl disulfide in which each half acts as an effective leaving group. In other words, the greater the stability of the mercaptide anions formed by cleavage of a given disulfide, the more rapidly disulfide interchange will occur. Since the aryl group of an aryl mercaptide ion permits the delocalization of the negative charge, these anions will possess greater stability than those derived from alkyl disulfides.

The method described by eq.XXIX also permitted synthesis of trisulfide 80 in 98% yield. It was obtained by addition of benzyl hydrodisulfide (81) to a solution of benzyl thiophthalimide (82) in benzene at room temperature. Phthalimide (83) was recovered as a side product, as shown in eq. XXX.

(eq.XXX)



81 82 80 83

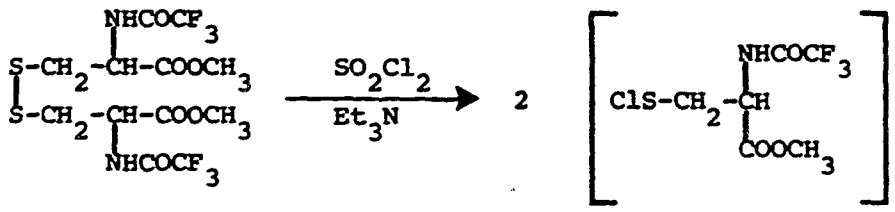
Some important physical constants of compounds 54 and 72 - 80, as well as the experimental conditions under which they were made, are summarized in Table I.

The structures of these compounds were found to be consistent with their ir, nmr, and mass spectra. Elemental analyses of new compounds are listed in Table II.

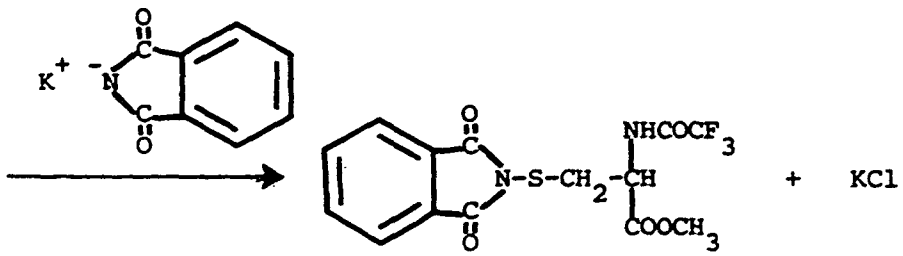
The success of the thiophthalimide method in the preparation of the simple peptides S-benzylthio-L-cysteine hydrochloride (54) and S-benzylthioglutathione (77) prompted attempts to synthesize a cysteine-containing thiophthalimide. Thiolysis of such a compound (via eq.XXIX) with cysteine or cysteine derivatives would then provide unsymmetrical disulfides having both sides of the disulfide bond joined to cysteine-containing residues.

It was hoped that chlorinolysis of disulfide 84 would give the sulfenyl chloride 85, which could then be treated with the phthalimide anion to give thiophthalimide 86, as shown below.

(eq.XXXI)



84 85



86

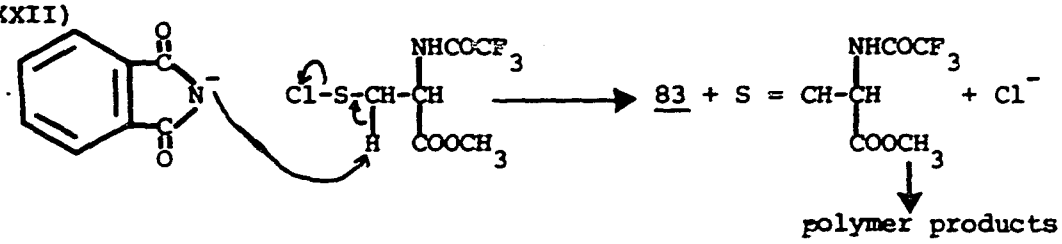
It was necessary to start with an N-protected cystine derivative to avoid interaction between the starting material and the sulfenyl chloride on the formation of the latter. The carboxylic acid group was also blocked to avoid abstraction of the acid proton by the phthalimide anion. Compound 84 satisfies both these requirements. Furthermore, it is easily prepared in 95% yield by the method of Harpp and Gleason[56].

Disulfide 84 was treated with sulfuryl chloride in the presence of a small amount of triethylamine, and the resulting solution of 85 was treated with the phthalimide anion. However, significant quantities of phthalimide (83) were isolated, indicating proton abstraction from the sulfenyl chloride intermediate. The remaining product was an oil which

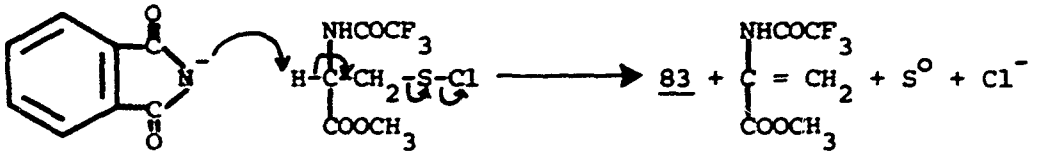
could not be induced to crystallize. Vpc and tlc showed this oil to be a mixture of several components, the separation of which was not attempted. The use of various solvents ranging in polarity from carbon tetrachloride to N,N-dimethylformamide (DMF) was attempted, but in each case phthalimide was isolated.

Danehy and Kreuz[57] have pointed out that although abstraction of protons both α and β to the disulfide bond has been postulated for aliphatic disulfides in basic media, no one mechanistic scheme offers a complete explanation. However, similar processes may occur in the case of the sulfenyl chloride 85. Two such schemes showing the results of α and β abstraction are proposed in eqs. XXXII and XXXIII respectively.

(eq.XXXII)

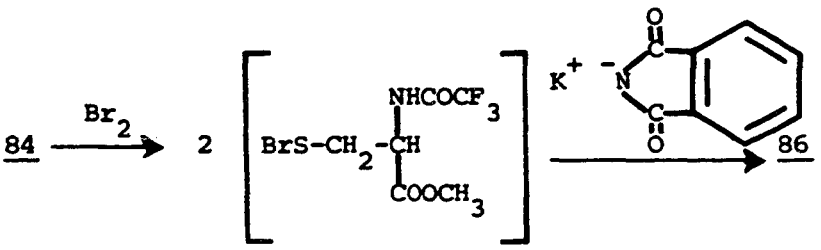


(eq.XXXIII)



It thus appeared that the nucleophilic displacement of chloride ion by phthalimide anion was competing against the more predominant proton abstraction reaction. Thus, it was hoped that an analogous process to that of eq.XXXI using sulfenyl bromide 87 would prove more effective.

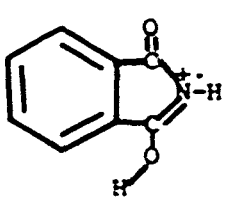
Bromide ion, being a superior leaving group to chloride, might enhance the rate of the nucleophilic displacement. This reasoning was rewarded by realizing a 65% yield of thiophthalimide 86. The product was obtained by first brominating disulfide 84 at 0° *in situ*, and then treating the resulting sulfenyl bromide with the phthalimide anion (eq. XXXIV).

(eq.XXXIV) 

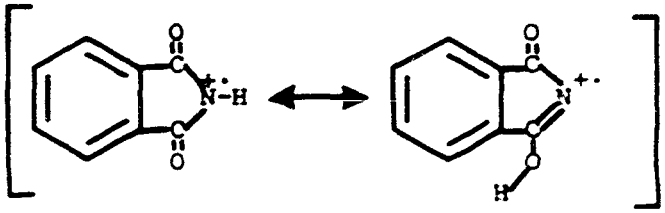
87

Although 87 was used directly without isolation, evidence for its formation derives from nmr data. The methylene absorption of 87 in trifluoroacetic acid solution is shifted 0.3 ppm downfield relative to that of 84 in the same medium. This appears reasonable since the methylene absorption of chloride 85 is found[58] 0.5 ppm downfield from that of 84.

The mass spectrum of thiophthalimide 86 showed intense peaks at m/e 148 and 147, probably due to the formation of the fragments 88 and 89 respectively.



88

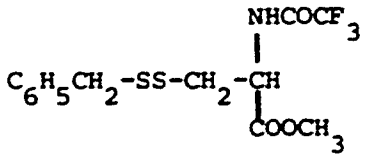


89

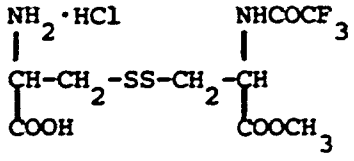
An extremely faint parent peak was observed at m/e 376. It has previously been reported[59] that thiophthalimides display intense peaks at m/e 148, 147, 130, 104, and 76. This is in agreement with the spectrum of 86, in which these peaks have relative intensities of 17, 95, 3, 76, and 100% respectively. The complete spectrum is shown in Fig. I.

The nmr spectrum of 86 is shown in Fig.V and is consistent with its structure. The chemical shifts and assignments are listed in Table III.

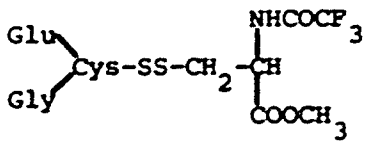
It was subsequently found that thiophthalimide 86 provided an excellent synthetic route (eq. XXIX) to some new unsymmetrical disulfides, in two of which both sides of the S-S linkage are joined to glutathione or cysteine residues. Thiolysis of 86 with benzyl mercaptan (20), cysteine hydrochloride monohydrate (2), and glutathione (26) gave excellent yields (92-99%) of the corresponding disulfides 90, 91, and 92 respectively.



90



91



92

Absence of the corresponding symmetrical disulfides in the products was established by tlc, except in the case of 92, where traces were discovered. Two unidentified trace impurities revealed by tlc in disulfide 91 were also observed in the precursor thiol. The structures of compounds 90 - 92 were consistent with their ir, nmr, and mass spectra. The results of their elemental analyses appear in Table II.

The mass spectrum of 90 showed a parent peak at m/e 353, having a relative intensity of 18%, as well as a base peak at m/e 91, due to the tropylium ion (93). All three disulfides showed cleavage of both the disulfide bond and the C-S bond on the side of the blocked cysteine residue, as evidenced by strong peaks at m/e 230 and 198 respectively. The latter peaks have also been observed for disulfide 84[56]. The complete mass spectra of compounds 90 - 92 are shown in Figs. II - IV respectively.

93 

The nmr spectrum of 90 is shown in Fig. VI. The amide proton is not observed, probably due to masking by the aromatic peak. The chemical shifts as well as their assignments are listed in Table III.

The nmr spectrum of 91 was recorded both in trifluoroacetic acid solution with an internal TMS standard, and in 2.5 N trifluoroacetic acid in D_2O using an external TMS standard. The latter solution afforded superior resolution and the resulting spectrum appears in Fig. VII, while the former solution permitted greater accuracy in the measurement of

chemical shifts. These, along with their assignments, appear in Table III.

The nmr spectrum of 92 was taken in trifluoroacetic acid solution with an internal TMS standard. The spectrum is almost identical to that of the cysteine glutathione mixed disulfide (41) as reported by Eriksson and Eriksson[36]. In addition to the absorptions shown by 41, disulfide 92 shows a singlet absorption due to the methyl ester group at 4.18 ppm, and the broad peak from the amide proton at 8.42 ppm. The complete spectrum is shown in Fig. VIII.

It has been shown that hydrolysis of the trifluoroacylamide bond occurs at pH greater than 10, and that some N-trifluoroacetyl derivatives of amino acids are rapidly hydrolyzed at pH~12[60]. Mild alkaline conditions also serve to hydrolyze esters[61]. However, alkaline cleavage of the S-N bond of thiophthalimides[54], as well as base-promoted disulfide interchange[24], is also well documented in the literature. Thus, it was feared that attempts to remove the trifluoroacetyl and methyl ester protective groups from thiophthalimide 86 and disulfide 90 would be complicated by the simultaneous cleavage of the S-N and S-S bonds respectively. These fears were justified when it was found that treatment of 86 with 0.01 N NaOH at 5° for 0.5 hr. gave 69% phthalimide (83). Reaction of 90 with 1 N NaOH under similar conditions gave 27% benzyl disulfide (72).

Thus, it is clear that the thiolysis of thiophthalimides provides a rapid, clean synthetic route to unsymmetrical disulfides. High yields are generally obtained, and disulfide interchange is minimal, except in the case of diaryl disulfides. The fact that the precursor

thiophthalimides are generally stable and easy to prepare and purify makes this method particularly attractive when it is kept in mind that other methods involving thiolysis of sulfenyl derivatives employ unstable starting materials of limited availability. The method has been successfully applied to the synthesis of the simple cysteine-containing peptides 54, 77, and 90 - 92. Unfortunately, the formation of a cysteine-containing thiophthalimide requires the protection of its amino and carboxylic acid functions. So far, it has not been possible to avoid incorporation of these protective groups into the product disulfides. Hence, the possibility of using similar thiophthalimides with selectively removable amino and carboxylic acid protective groups in peptide synthesis provides an interesting subject for future study.

EXPERIMENTAL

EXPERIMENTAL

Melting points were taken on a Gallenkamp block and are uncorrected. Optical rotations were measured on a Perkin Elmer Model 141 Automatic Polarimeter. Elemental analyses were performed by Organic Micro-analyses, Montreal. Infrared spectra were recorded on a Perkin Elmer 257 Grating Spectrometer. Mass spectra were obtained on an AEI-MS-902 instrument, and nmr spectra were recorded on a Varian T-60 spectrometer.

Compounds 54 and 72 - 80 were all prepared by treating the thiophthalimide with an equimolar amount of the corresponding thiol in an appropriate solvent. The reaction conditions, yields, and melting or boiling points are tabulated in Table I. Since the procedures for obtaining these compounds were essentially identical, only one will be described in detail.

Isopropyl p-tolyl disulfide (75).

A mixture of 1.12 g (9.0 mmol) of p-toluenethiol and 2.00 g (9.0mmol) of isopropylthiophthalimide was refluxed 17 hrs. in 35 ml of benzene. At the end of this time, 1.18 g (89%) of phthalimide (83) crystallized and was filtered; mp 230-240°, (lit.[62] 238°). The filtrate was evaporated in vacuo, giving 1.57 g (88%) of a pale yellow oil. Vpc showed traces of the symmetrical disulfides, which were almost completely removed by distillation; bp 76-80°/0.010 mm, (lit.[65] 93-94°/0.1 mm).

N-Trifluoroacetyl-S-phthalimido-L-cysteine methyl ester (86).

A. Attempted Preparation via the Sulfenyl chloride 85

To a mixture of 4.30 g (9.3 mmol) of disulfide 84 and a few drops of triethylamine in 20 ml of methylene chloride, was added 1.26 g (9.3 mmol) of freshly distilled sulfuryl chloride in 5 ml of methylene chloride. After stirring for 0.5 hr., the clear, red solution was added to 100 ml of DMF containing 3.44 g (18.6 mmol) of the potassium derivative of phthalimide. Anhydrous conditions were maintained throughout the experiment. After stirring for 1.5 hr., the solution was evaporated to dryness and the residue was taken up in carbon tetrachloride. Insoluble material was filtered out and recrystallized from ethanol/water to give 1.00 g (37%) of phthalimide; mp 230-235°. The ir of this material was identical to that of a genuine sample of phthalimide. The filtrate was evaporated to give a dark oil which could not be induced to crystallize. Vpc and tlc revealed the presence of several components in this material.

B. Preparation via the Sulfenyl bromide 87.

To a suspension of 4.60 g (0.01 mol) of disulfide 84 in 30 ml of 1,2-dichloroethane (DCE) at 0°, was added 4.80 g (0.03 mol) of bromine in 15 ml of DCE. After stirring 2-3 mins., the cloudy, red solution was rapidly added to a similarly cooled suspension of 3.70 g (0.02 mol) of the potassium derivative of phthalimide in 45 ml of DCE. Anhydrous conditions were maintained throughout the experiment. After stirring at 0° for 10 mins., the suspension was stirred an additional 1.5 hr. at

ambient temperature. Insoluble material was then filtered, giving 2.39 g (100%) of KBr. The filtrate was evaporated in vacuo to give an orange solid, which on recrystallization from methanol/water gave 4.87 g (65%) of white needles; mp 121-123°. A second recrystallization gave a sample of analytical purity; mp 125-126°, $[\alpha]_D^{22}$ = +54.4°, (c 0.226, CCl_4); ir (KBr) 3260 cm^{-1}, 1730, 1690, 1540, 1270, 1180, 1150, 1040.

N-Trifluoroacetyl-S-benzylthio-L-cysteine methyl ester (90).

A solution of 1.00 g (2.7 mmol) of 86 and 0.33 g (2.7 mmol) of 20 in 10 ml of ethyl acetate was refluxed 24 hrs. On subsequent cooling, phthalimide crystallized and was filtered. The solvent was removed in vacuo and the residue was taken up in 5 ml of carbon tetrachloride, additional phthalimide being obtained; total yield: 0.38 g (96%); mp 234-235°. The filtrate was again evaporated in vacuo, giving a clear oil which crystallized on cooling to give 0.92 g (97%) of a pale yellow solid; mp 38-40°; $[\alpha]_D^{22}$ = +39.7°, (c 0.363, $CHCl_3$); ir (KBr) 3300 cm^{-1}, 1740, 1700, 1540, 1300, 1200, 1180, 1160.

N-Trifluoroacetyl-S-cysteinyl-L-cysteine methyl ester hydrochloride (91).

A solution of 0.233 g (1.33 mmol) of L-(+)-cysteine hydrochloride monohydrate, and 0.500 g (1.33 mmol) of 86 in 10 ml of ethanol was refluxed for 2 hrs. On cooling, phthalimide crystallized and was

filtered. The filtrate was evaporated to 2-3 ml and 20 ml of water were added, giving an additional 0.006 g of phthalimide on cooling; total yield: 0.179 g (91%); mp 234-237°. The filtrate was then evaporated in vacuo to give a white, solid foam, which was dried to constant weight under vacuum. Yield: 0.512 g (99%); mp 151-153° dec.; $[\alpha]_D^{22}$ = -142.4°, (c 0.433, 1 N HCl); ir (KBr) 3700-2400 cm^{-1} (broad), 1800-1680, 1570, 1200 (broad).

N-Trifluoroacetyl-S-glutathionyl-L-cysteine methyl ester (92).

A solution of 0.408 g (1.33 mmol) of 26 and 0.500 g (1.33 mmol) of 86 in 20 ml of ethanol/water, 50:50 (v/v), was refluxed for 2 hrs. After cooling to room temperature for 8 hrs., 0.187 g (95%) of phthalimide crystallized and was filtered; mp 228-232°. The solvent was then evaporated to 10 ml, and 10 ml of water were added. On cooling overnight, an additional 0.049 g of precipitate formed. Tlc (silica-gel, C_6H_6/Et_2O 5:2) showed this second crop to be composed of phthalimide and the symmetrical disulfide 84. The filtrate was evaporated in vacuo and dried to constant weight, giving 0.659 g (92%) of a white, solid foam; mp 173° dec.; $[\alpha]_D^{22}$ = -103.0°, (c 0.463, 1 N HCl); ir (KBr) 3700-2400 cm^{-1} (broad), 1720, 1650, 1540, 1200 (broad). Tlc (cellulose, BuOH/HOAc/H_2O 12:3:5) showed the presence of a trace impurity of lower mobility than 92, attributable to a small quantity of the symmetrical disulfide 27.

Hydrolysis of 86.

To 500 ml of 0.01 N NaOH at 5° was added a solution of 0.376 g (1 mmol) of 86 in 5 ml dioxane. After stirring for 0.5 hr. at 5°, the solution was acidified to pH~6 by the addition of 1 N HCl. A precipitate of 0.101 g (69%) of phthalimide formed; mp 225-231°. Tlc (silica-gel, C_6H_6/Et_2O 5:2) showed a major component having the same mobility as phthalimide and two minor components of lower mobility.

Hydrolysis of 90.

To a solution of 0.117 g (0.331mmol) of 90 in a few drops of methanol was added 3 ml of 1 N NaOH previously cooled to 5°. After stirring at this temperature for 0.5 hr., the milky solution was acidified to pH~6 by the addition of 1 N HCl. The resulting precipitate was filtered, washed with water, dried, and washed well with ether. Evaporation of the ether washings gave 0.011 g (27%) of benzyl disulfide (72); mp 65-67° (lit.[63] 71.5°). Tlc (silica-gel, C_6H_6/Et_2O 5:2 and cellulose, BuOH/HOAc/H_2O 12:3:5) showed the ether-insoluble residue to be a mixture of at least four components.

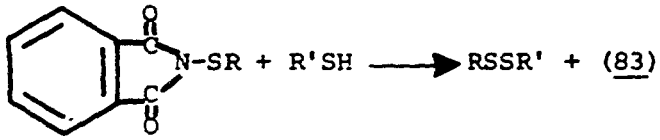
TABLES

AND

FIGURES

TABLE I

Preparation of Disulfides and Trisulfides



Compound	R	R'	% yield	React.[a] Time (hr)	bp or (mp)	lit bp or (mp)
(72)	$C_6H_5CH_2$	$C_6H_5CH_2$	86	20	(70.5-71)	(71.5)[63]
(73)	$C_6H_5CH_2$	$p\text{-}BrC_6H_4CH_2$	80	4	(49-52)	(54-55)[64]
(74)	C_6H_5	$CH_3(CH_2)_2$	71	18	87-93/.1 mm	-
(75)	$(CH_3)_2CH$	$p\text{-}CH_3C_6H_4$	88	17	76-80/.01 mm	93-94/.1[65] mm
(76)	$CH_3O_2CCH_2$	C_6H_{11}	91	12	94-98/.005 mm	-
(54)	$C_6H_5CH_2$	L-Cys·HCl	89	16[b]	(178 dec.)	(175-180 dec.)[66]
(77)	$C_6H_5CH_2$	Glu, Gly, Cys	92	1[b]	(206-207 dec.)	-
(78)	$p\text{-}CH_3C_6H_4$	$p\text{-}FC_6H_4$	--	1[c]	-	-
(79)	C_6H_5	$p\text{-}CH_3C_6H_4$	--	2[d,e]	-	-
(80)	$C_6H_5CH_2$	$C_6H_5CH_2S$	98	20[d]	(47-48.5)	(47)[67]

a) Refluxing benzene was used as solvent unless otherwise noted.

b) Refluxing ethanol was solvent.

c) Mixture of disulfides was obtained (1:2:1).

d) Benzene at room temperature was the solvent.

e) Mixture of disulfides was obtained (1:5:1).

TABLE II

Elemental Analyses of New Compounds

Compound	% Calculated				% Found			
	C	H	N	S	C	H	N	S
(74)	58.64	6.56	-	34.79	58.55	6.42	-	35.02
(76)	49.05	7.32	-	29.10	49.30	7.03	-	28.95
(86)	44.68	2.95	7.45	8.52	44.66	2.99	7.54	8.57
(90)	44.18	3.99	3.96	18.15	43.78	4.21	4.13	17.92
(91)	27.94	3.65	7.24	16.58	28.24	4.17	7.33	16.89
(92)	35.82	4.32	10.44	11.95	35.81	4.43	10.34	12.23

TABLE III

Chemical Shifts of Compounds 86, 90 and 91

Compound	Shift[c]	Description[d]	Assignment
	3.38	2H, t crude	$-CH_2-$
	3.63	3H, s	$-OCH_3$
(86)[a]	4.92	1H, m	-CH
	7.76	4H, s	aromatic
	8.33	1H, d	-NH-
	2.90	2H, d	$-CH_2-$
	3.83	3H, s	$-OCH_3$
(90)[b]	3.99	2H, s	$-CH_2C_6H_5$
	4.83	1H, m	-CH
	7.40	5H, s	aromatic
	3.62	4H, d crude	$-CH_2-$
	4.13	3H, s	$-OCH_3$
(91)[a]	5.00	1H, m	-CH
	5.35	1H, m	-CH
	7.93	3H, s	$-NH_3^+ \ ^-Cl$
	8.33	1H, s	-NH-

a) Solvent: trifluoroacetic acid.

b) Solvent: CCl_4.

c) Measured in ppm from internal TMS.

d) s-singlet, d-doublet, t-triplet, m-multiplet.

Fig.I

The mass spectrum of N-Trifluoroacetyl-S-phthalimido-L-cysteine methyl ester (86)

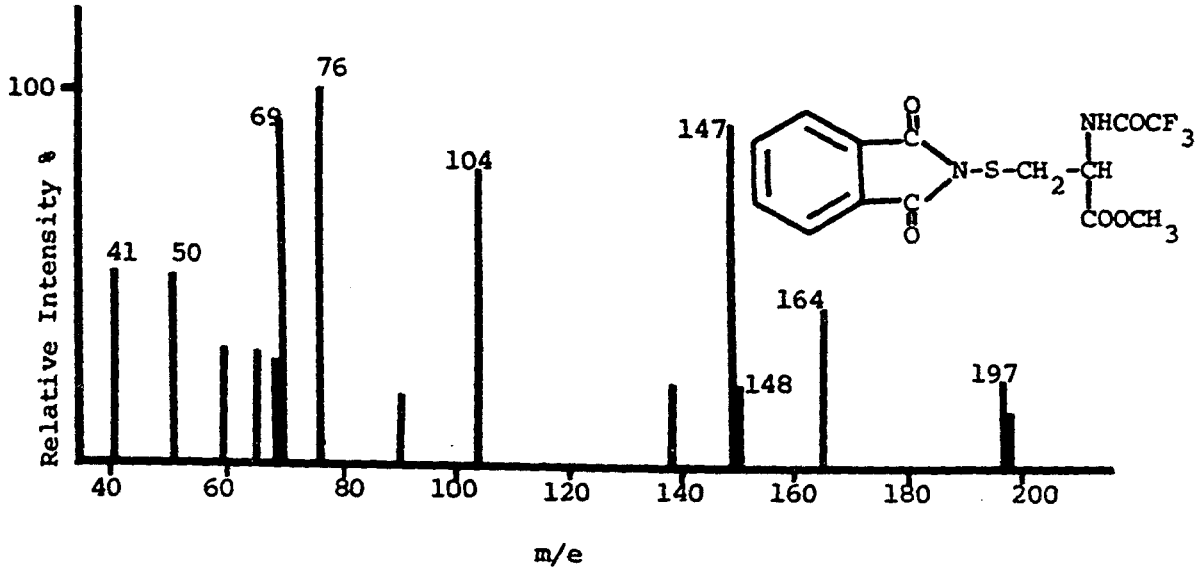


Fig.II

The mass spectrum of N-Trifluoroacetyl-S-benzylthio-L-cysteine methyl ester (90)

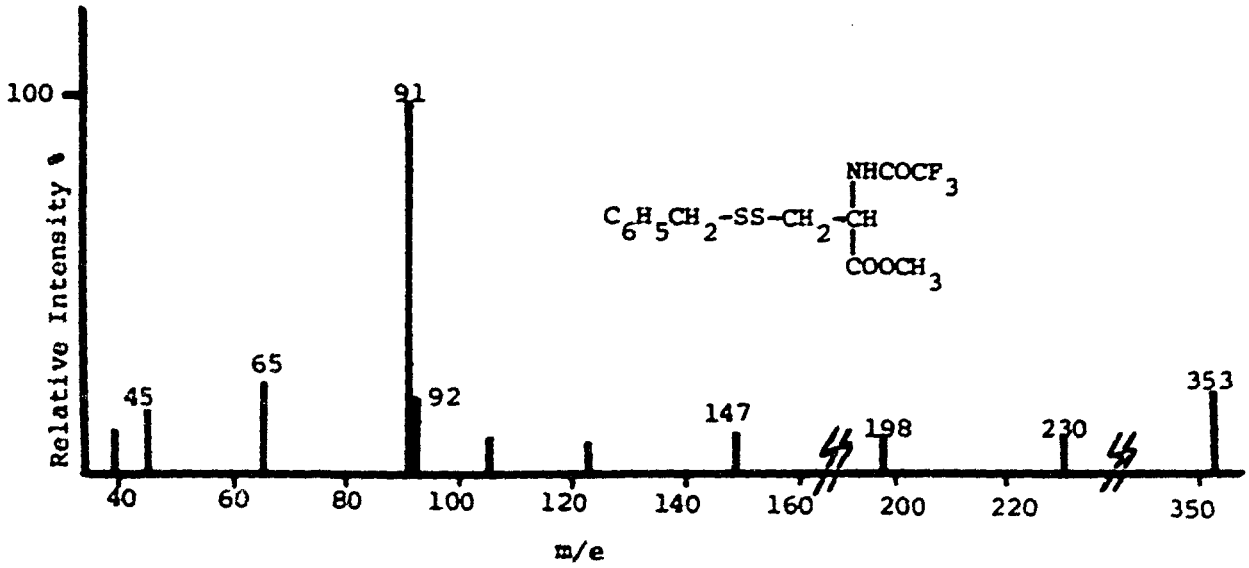


Fig.III

The Mass Spectrum of N-Trifluoroacetyl-S-cysteinyl-L-cysteine methyl ester·HCl (91)

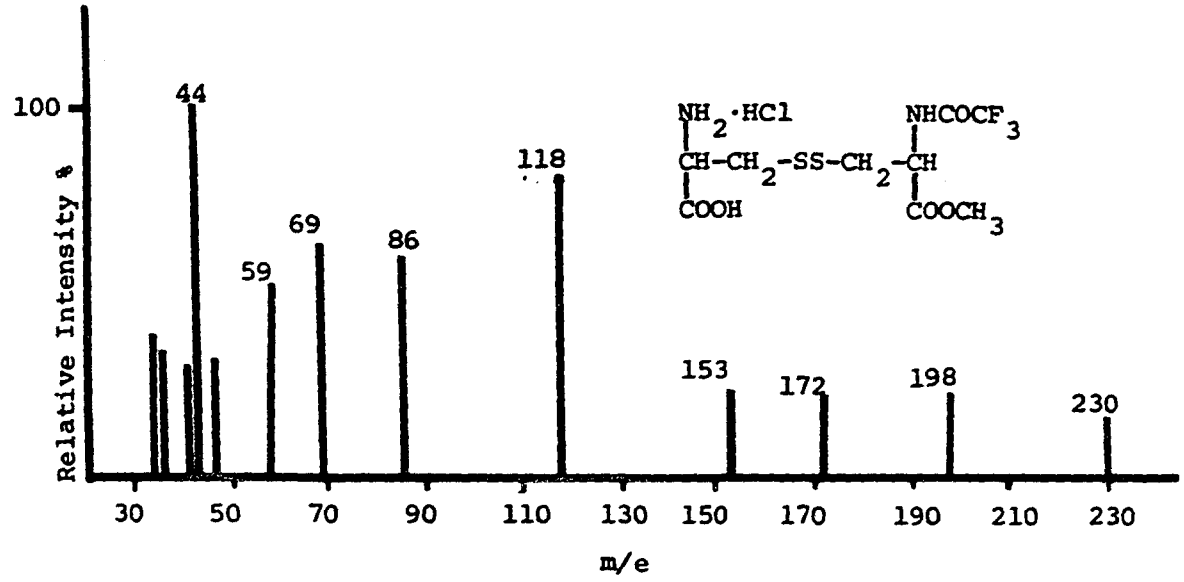


Fig. IV

The Mass Spectrum of N-Trifluoroacetyl-S-glutathionyl-L-cysteine methyl ester (92)

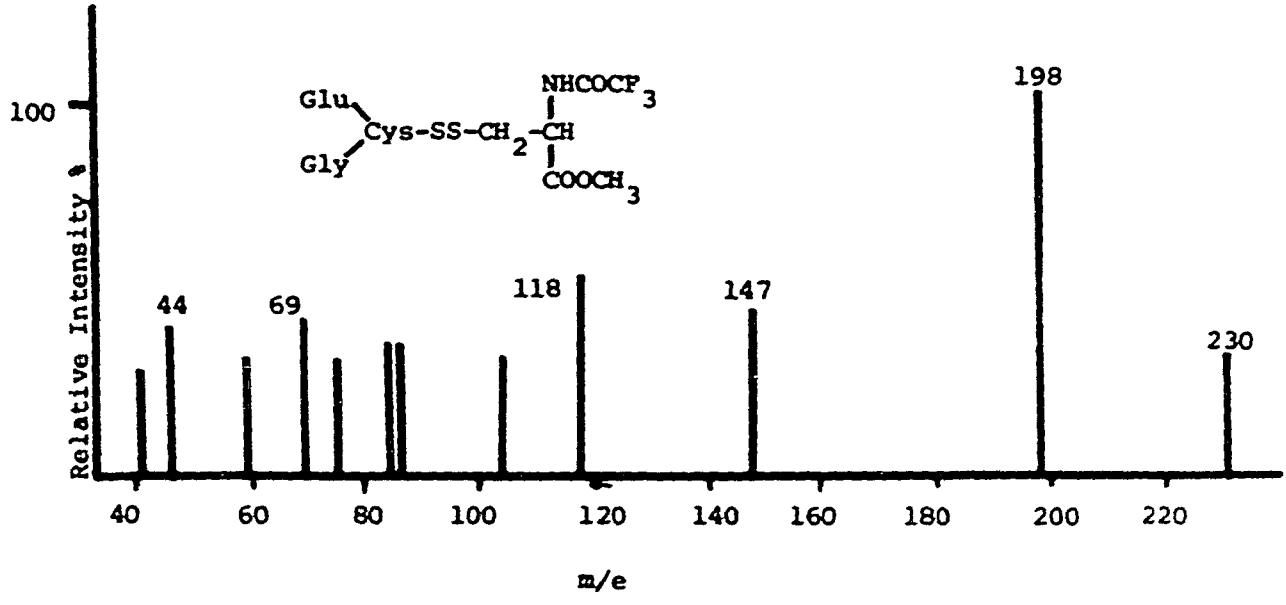


Fig. V

The Nmr Spectrum of N-Trifluoroacetyl-S-phthalimido-L-cysteine methyl ester (86)

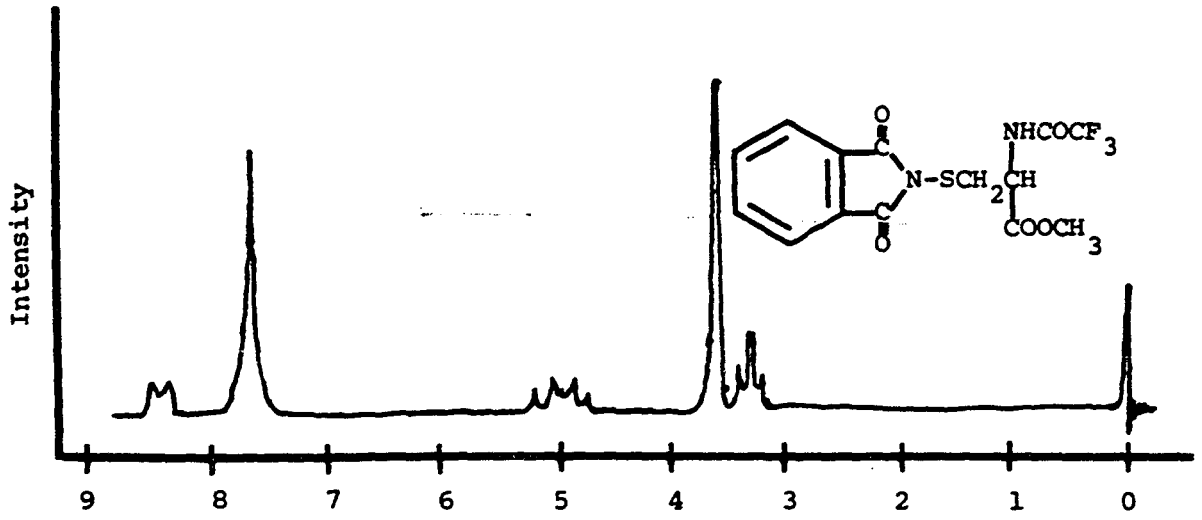


Fig. VI

The Nmr Spectrum of N-Trifluoroacetyl-S-benzylthio-L-cysteine methyl ester (90)

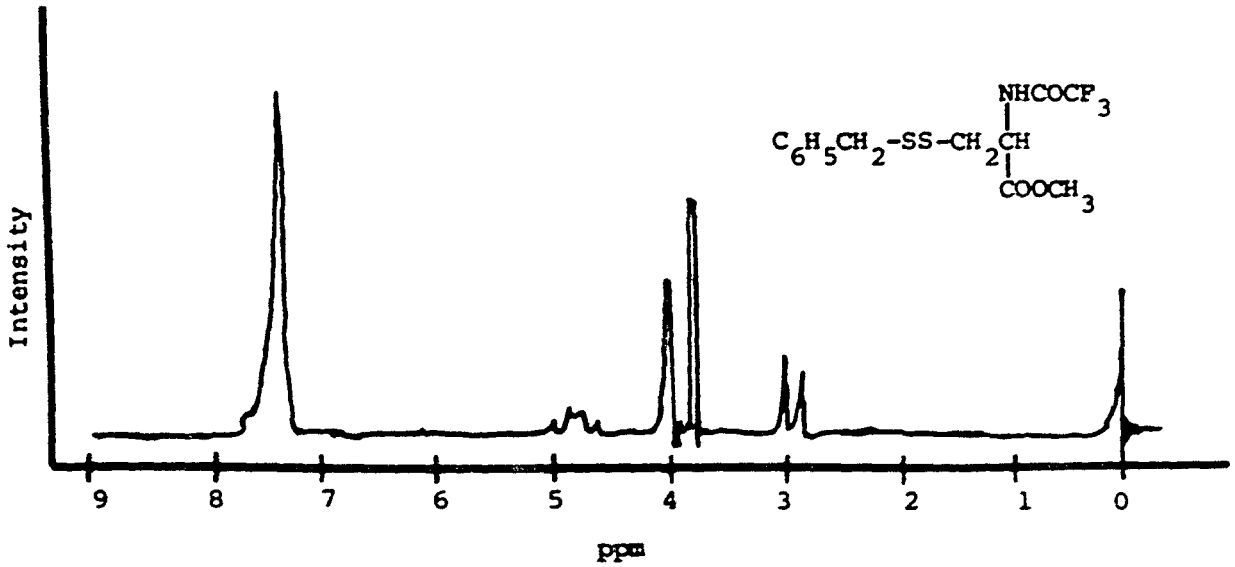


Fig. VII

The Nmr Spectrum of N-Trifluoroacetyl-S-cysteinyl-L-cysteine methyl ester·HCl (91)

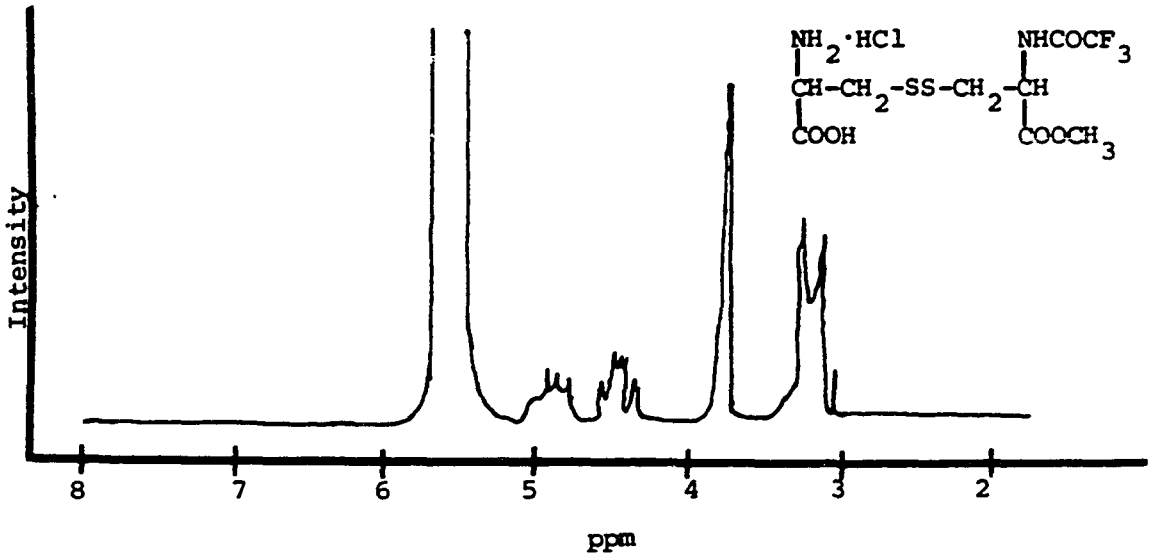
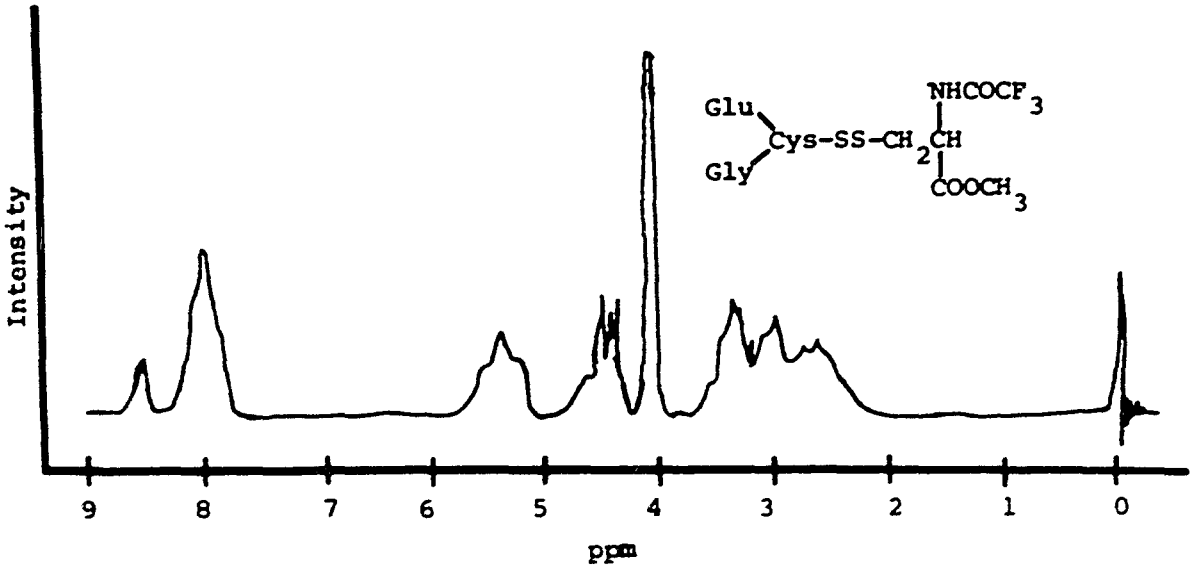


Fig. VIII

The Nmr Spectrum of N-Trifluoroacetyl-S-glutathionyl-L-cysteine methyl ester (92)



REFERENCES

REFERENCES

1. V. du Vigneaud, C. Ressler, and S. Trippett; J. Biol. Chem., 205, 949, (1953).

2. V. du Vigneaud, H.C. Lawler, and E.A. Popenoe; J. Am. Chem. Soc., 75, 4880, (1953).

3. Progress in Medicinal Chemistry, Vol. 4, Ch. 4, (H.D. Law), ed. G.P. Ellis and G.B. West; Butterworth & Co., London, (1965).

4. F. Sanger; Insulin, Brit. Med. Bull., 16, 183, (1960).

5. The Chemical Foundations of Molecular Biology, by R.F. Steiner; D. Van Nostrand Co. Inc., Princeton, N.J., (1965).

6. D.J. O'Kane and I.C. Gunsalus; J. Bact., 54, 20, (1947).

7. E.E. Snell, E.L. Tatum, and W.H. Peterson; J. Bact., 33, 207, (1937).

8. L.J. Reed, B.G. DeBusk, I.C. Gunsalus, and C.S. Hornberger; Science, 114, 93, (1951).

9. D.T. McAllan, T.V. Cullum, R.A. Dean, and F.A. Fidler; J. Am. Chem. Soc., 73, 3627, (1951).

10. D. Jarvis, H.N. Rydon, and J.A. Schofield; J. Chem. Soc., 1752, 1961.

11. D.B. Hope and V. du Vigneaud; J. Biol. Chem., 237, 3146, (1962).

12. F. Weygand and G. Zumach; Z. Naturf., 17b, 807, (1962).

13. V. du Vigneaud, C. Ressler, J.M. Swan, C.W. Roberts, P.G. Katsoyannis, and S. Gordon; J. Am. Chem. Soc., 75, 4879, (1953).

14. E. Walton, A.F. Wagner, F.W. Bachelor, L.H. Peterson, F.W. Holly, and K. Folkers; J. Am. Chem. Soc., 77, 5144, (1955).

15. N. Kharasch, S.J. Potempa, and H.L. Wehrmeister; Chem. Rev., 39, 269, (1946).

16. Organo-Sulfur Compounds, Vol 1, ed. N. Kharasch; Pergamon Press, (1961).

    a) I.B. Douglass, P. 350-360.

    b) F.A. Drahowzal, P. 361-374.

    c) N. Kharasch, P. 375-396.

17. H. Emde, German Patent 804572, (1951); via Chem. Abstr., 46, 529, (1952).

18. I.B. Douglass, F.T. Martin, and R. Addor; J. Org. Chem., 16, 1297, (1951).

19. A. Schöberl, H. Tausent, and H. Grafje; Angew. Chem., 68, 213(1956).

20. I.B. Douglass, K.R. Brower, and F.T. Martin; J. Am. Chem. Soc., 74, 5770, (1952).

21. Organic Chemistry of Sulfur, by C.M. Suter; John Wiley & Sons, N.Y., (1944).

22. L.D. Small, J.H. Bailey, C.J. Cavallito; J. Am. Chem. Soc., 69, 1710, (1947).

23. H. Footner and S, Smiles; J. Chem. Soc., 127, 2887, (1925).

24. A. Parker and N. Kharasch; Chem. Rev., 59, 583, (1959).

25. J.M. Swan; Nature, 180, 643, (1957).

26. R.E. Benesch and R. Benesch; J. Am. Chem. Soc., 80, 1666, (1958).

27. I.W. Stapleton and J.M. Swan; Aust. J. Chem., 15, 570, (1962).

28. J.W. Haefele, U.S. Patent 2,615,782, (1952); via Chem. Abstr., 47, 2502, (1953).

29. A. Schöberl and G. Bauer; Angew. Chem., 69, 478, (1957).

30. H. Zahn and H.G. Otten; Liebigs Ann., 653, 139, (1962).

31. H.E. Westlake and G. Dougherty; J. Am. Chem. Soc., 63, 658, (1941).

32. Methoden der Organischen Chemie, Vol. 9, P. 72, (A. Schöberl and A. Wagner), ed. E. Muller; Houben-Weyl, Stuttgart, (1955).

33. L. Field, T.C. Owen, R.C. Crenshaw,,and A.W. Bryan; J. Am. Chem. Soc., 83, 4414, (1961).

34. A. Pihl and L. Eldjarn; Pharmacol. Rev., 10, 437, (1958).

35. L. Field and P.M. Giles; J. Org. Chem., 36, 309, (1971).

36. B. Eriksson and S.A. Eriksson; Acta Chem. Scand., 21, 1304, (1967).

37. R. Plaquet, G. Biserte, and P. Boulanger; Bull. Soc. Chim. Biol., 44, 301, (1962).

38. D.H. Calam and S.G. Waley; Biochem. J., 93, 526, (1964).

39. W.J.P. Neish and A. Rylett; Biochem. Pharmacol., 12, 913, (1963).

40. B. Eriksson; Acta Chem. Scand., 20, 1178, (1966).

41. L. Field and J.D. Buckmann; J. Org. Chem., 33, 3865, (1968).

42. H. Lecher and M. Wittwer; Ber., 55b, 1474, (1922).

43. R.G. Hiskey, F.I. Carroll, R.M. Babb, J.O. Bledsoe, R.T. Puckett and B.W. Roberts; J. Org. Chem., 26, 1152, (1961).

44. R.G. Hiskey and W.P. Tucker; J. Am. Chem. Soc., 84, 4789, (1962).

45. R.G. Hiskey and W.P. Tucker; J. Am. Chem. Soc., 84, 4794, (1962).

46. R.G. Hiskey and D.N. Harpp; J. Am. Chem. Soc., 87, 3965, (1965).

47. R.G. Hiskey, R.L. Smith, A.M. Thomas, J.T. Sparrow, and W.C. Jones Jr.; Colloq. Int. Centre Nat. Rech. Sci., No. 175, 209, (1968).

48. R.G. Hiskey and B.F. Ward; J. Org. Chem., 35, 1118, (1970).

49. T. Mukaiyama and K. Takahashi; Tetrahedron Lett., 5907, 1968.

50. K. Sirakawa, O. Aki, T. Tsujikawa, and T. Tsuda; Chem. Pharm. Bull., 18, 235, (1970).

51. K.S. Boustany and A.B. Sullivan; Tetrahedron Lett., 3547, 1970.

52. D.N. Harpp, D.K. Ash, T.G. Back, J.G. Gleason, B.A. Orwig, W.F. Van Horn, and J.P. Snyder; Tetrahedron Lett., 3551, 1970.

53. S.J. Brois, J.F. Pilot, and H.W. Barnum; J. Am. Chem. Soc., 92, 7629, (1970).

54. M. Behforouz and J.E. Kerwood; J. Org. Chem., 34, 51, (1969).

55. K.H. Büchel and A. Conte; Ber., 100, 1248, (1967).

56. D.N. Harpp and J.G. Gleason; J. Org. Chem., 36, 73, (1971).

57. J.P. Danehy and J.A. Kreuz; J. Am. Chem. Soc., 83, 1109, (1961).

58. P. Mathiaparanam; PhD Thesis, McGill University, (1970).

59. B.A. Orwig; MSc Thesis, McGill University, (1971).

60. E. Schallenberg and M. Calvin; J. Am. Chem. Soc., 77, 2779, (1955).

61. Basic Principles of Organic Chemistry, by J.D. Roberts and M.C. Caserio; Ch. 16; W.A. Benjamin Inc., N.Y., (1964).

62. Handbook of Chemistry and Physics, 47th Ed.; The Chemical Rubber Co., Cleveland, Ohio.

63. F.M. McMillan and J.A. King; J. Am. Chem. Soc., 70, 4143, (1948).

64. J.G. Gleason; PhD Thesis, McGill University, (1970).

65. J.L. Kice and E.H. Morkved; J. Am. Chem. Soc., 86, 2270, (1964).

66. H. Bretschneider and W. Klotzer; Monatsh. Chem., 81, 589, (1950).

67. S. Hayashi, M. Furukawa, J. Yamamoto, and K. Hamamura; Chem. Pharm. Bull., 15, 1310, (1967).