A Thesis Entitled

SYNTHETIC OLIGOAMIDES AS PROTEIN MODELS.

Submitted to the University of Glasgow for the degree of Doctor of Philosophy in the Faculty of Science

by

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SECTION 3. Mass Spectral Investigations.

ILLUSTRATIONS.

SUMMARY.

The aspect of protein structure, for which a simple model was sought in the present investigations, was that of their tertiary structure. It is pointed out that there are certain similarities between this organisation, and that to be found in the folding of the polymer chain that has been recorded as being present in polymeric material recrystallised from dilute solution.

A prerequisite for studying the conformational properties of polymers, and more specifically the effects introduced by the inclusion of a variety of groups at the turning points of their fold-chain crystals, is the efficient synthesis of very long monomer units. These monomer units should, for our purposes, be approximately 70 to 80Å in length. The present studies were undertaken in order to devise an efficient and flexible method of synthesising such long molecules.

A stepwise reaction technique has been evolved which is sufficiently flexible for groups of differing size and polarity to be introduced at the ends of the linear oligoamide chains, and the technique refined to the stage where the small scale preparation of high molecular weight oligoamides has become practicable. Using this technique, several new series of linear oligoamides have been synthesised. These series were designed so that investigations might, much later, be carried out into the effects that different terminal groups on the monomers might have on their crystal structure, and also on the solution properties of the polymeric materials derived from them.

Section two of this thesis is concerned with an alternative method of synthesis of specific high molecular weight linear oligoamides, namely through a "Doubling Reaction". It is pointed out that this method has up to now been neglected, largely owing to the low yields obtained when trying to activate, or to protect, just one of two symmetrically placed identical functional groups. After some preliminary work was carried out covering a wide range of separation techniques, an improvement was instituted whereby this difficulty might be overcome.

The doubling reaction technique is thought to be capable of being made more efficient by the use of highly coloured or fluorescent materials, introduced either by the preparation of suitable derivatives at the ends of the constituent parts, or actually within the molecules themselves. This inclusion enables the materials to be synthesised by normal methods and yet to be readily followed in very low concentrations during chromatographic or other separation techniques.

A brief mass spectral investigation of certain of the compounds synthesised in these investigations was undertaken with a view to showing something of the possibilities of such a technique in obtaining a sequential analysis of peptides. From the very brief inspection of the mass spectra obtained (which is all that time would allow) it would seem that, although complex, they are capable of interpretation and also of yielding a sequential analysis.

GENERAL INTRODUCTION.

GENERAL INTRODUCTION.

The term 'Protein Model' is capable of many differing interpretations. Two of the definitions given for the word 'model' are[1]:- 'A representation of structure' and

'Something that accurately resembles something else'. Modern scientific usage has tended to give the word a rather broader meaning of which a suitable definition would seem to be:-

'Something that accurately resembles something else, at least in certain respects.' This gives us a rather better idea of what we shall signify by the term 'protein model'. This broader definition is often used today in such a way that the aspect brought out by the model would be one of function and not merely of structure.

A very simple example would be that of a model ship. In such cases one normally thinks in terms of a small scale representation of the article and such a model can give one a very good idea of the structure of the ship and even, perhaps, of how it works, but at the same time it would be quite useless for carrying the cargoes of the parent ship. This is not the only model that could be taken however. Suppose in the early days of iron ships a shipbuilder was trying to convince an owner of wooden ships that iron ships could indeed float, then he might well have taken as his model a simple hollow iron sphere. He would be justified in his choice of a model system because, if the sphere were of the correct size and weight, it would prove the point that he was trying to make. Such a model ship would in no way look like the full-scale

ship, but it is a model because it represents a structure from one particular point of view.

In just the same way proteins, being such complex structures, are capable of having many different models made, each of which will emphasise certain different aspects of the total protein structure, or function. Nylon could be said to be a protein model in that it is a polyamide, but in that it goes no further it would be a model of very limited applicability. One functional aspect of proteins, that of the specific catalytic activity of enzymes, has found a model recently in the cycloamyloses[2] which have a doughnut kind of structure. They are polysaccharides and it is found that on one side of the torus there are primary hydroxyl groups whereas on the other side of it we find secondary hydroxyl groups. This leaves the centre of the torus with no hydroxyl groups at all. This structure means that the outside portions of the torus are hydrophilic, but the inside is hydrophobic. Bender investigated the rates of hydrolysis of aromatic esters and he found that these simple amyloses mimicked a number of aspects of enzyme activity far more effectively than more complex structures synthesised to resemble proteins. This shows that one can produce a model system for proteins which is totally unlike the parent in just the same way as described in the example of the ship above.

The aspect of proteins that prompted the present investigations was that of their so-called information content[3].

There is a very low information content present in a random sequence of building units and it is also true that an isotactic polymer (one in which the basic unit is the same throughout the whole polymer e.g. polycaprolactam) shows a similarly low information content. Information in the polypeptide occurs as a result of a definite sequence of building units and it is as a result of this information that the amino-acid chain folds up as it does. What seems to be a simple situation is rendered more complex as it is known that several different sequences of amino-acids can still give rise to proteins which have the same physiological functions[4], but, generally speaking, the modern ideas about protein functions are closely linked with the accuracy of the amino-acid sequence[5].

In the present investigations it was decided to use as our model chemically homogenecus compounds since proteins are chemically homogeneous. Each protein molecule must be produced with exactly the same amino-acid sequence as every other molecule of the same protein. This state of affairs is very different from that found in normal polymeric material. In polymers, although the same low molecular weight building units are included, they will, in most cases, be included in the final polymer chains in differing ratios and in all cases the polymerisation reaction will yield materials with a very wide range of molecular weights.

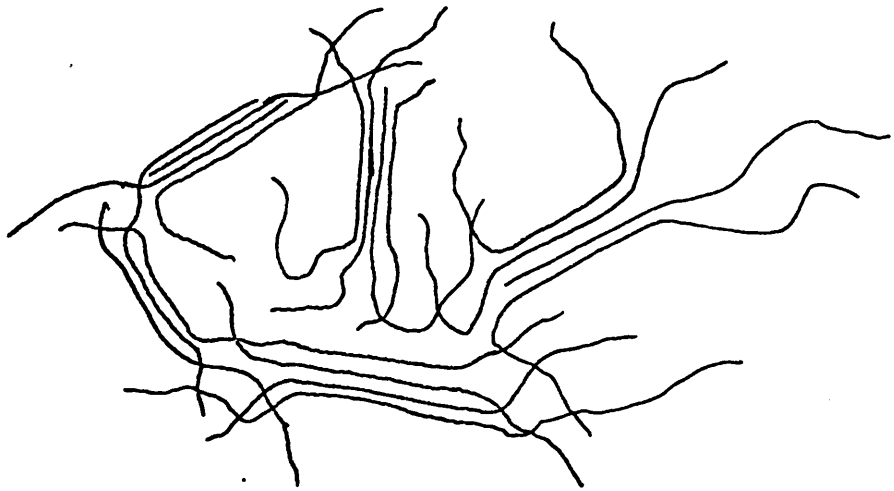
Another fact about proteins that we consider it advisable to retain is their, normally high, molecular weight. It would be far less meaningful to take as our protein model something with a molecular weight of only a few hundred as we wish to obtain information concerning the self-folding properties of long molecules. This factor of molecular weight is important. For example the α-helix forms a very important part of protein structure[6,7] and this helix is known to result from the cumulative effects of a large number of small forces[7,9]. (A similar helix has been observed even in simple hydrocarbon polymers[8].) It can therefore only become a stable structure in molecules of at least intermediate size. The tertiary structure of a protein is almost certainly even more dependent on the cumulative effects of a large number of even weaker interactions.

Polymer Organisation.

Let us now turn our attention to the knowledge that has been obtained concerning the organisation of straightforward polymeric materials.

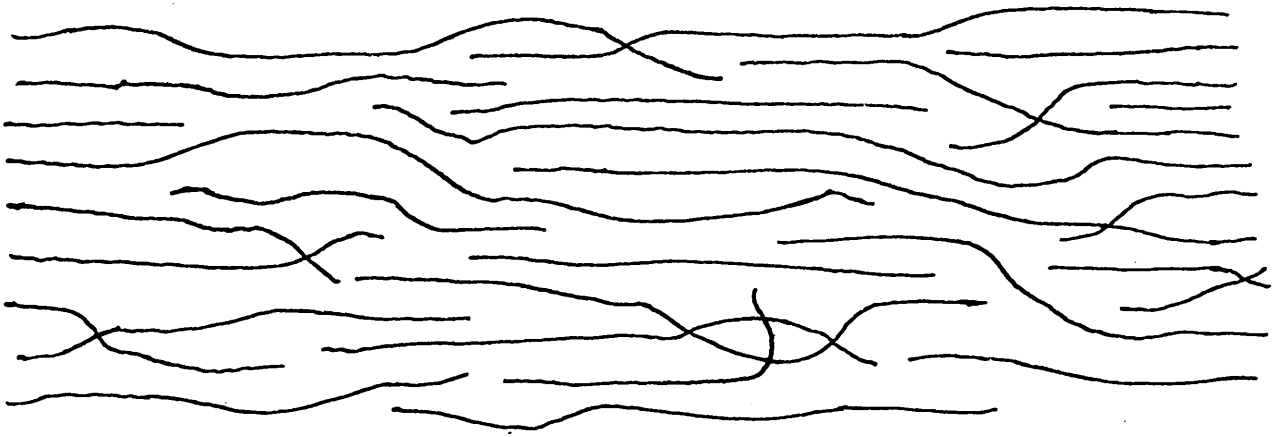
Staudinger carried out a considerable amount of work on cellulose[10] and as a result of his investigations people began to realise that cellulose and certain other naturally occurring materials such as keratin, wool etc. were not single compounds, but rather they were mixtures of compounds which, although homologous in the given materials, yet they differed in their molecular weights, often by very considerable amounts. Such systems they called Polymeric Materials.

Figure 1.



The Fringed Micelle Theory of
Hermann, Gerngross & Ablitz[13].

Figure 2.



The Fringed Micelle Theory
for Drawn Fibres.
Hess & Kiessig[14].

At this time it was considered that polymer chains, being so long, would form the solid material by a completely random intertwining of their chains. This idea was held to be true for a while, till the discovery of spherulites[11] and the work of Bunn[12] rendered such a theory untenable. In 1939 Bunn decided to carry out investigations into the possibilities of stretched polymer fibres showing signs of X-ray diffraction, a characteristic property of crystallinity in a solid. The patterns he obtained were diffuse, but that he obtained any pattern at all was a definite sign of there being some degree of crystallinity within polymeric materials.

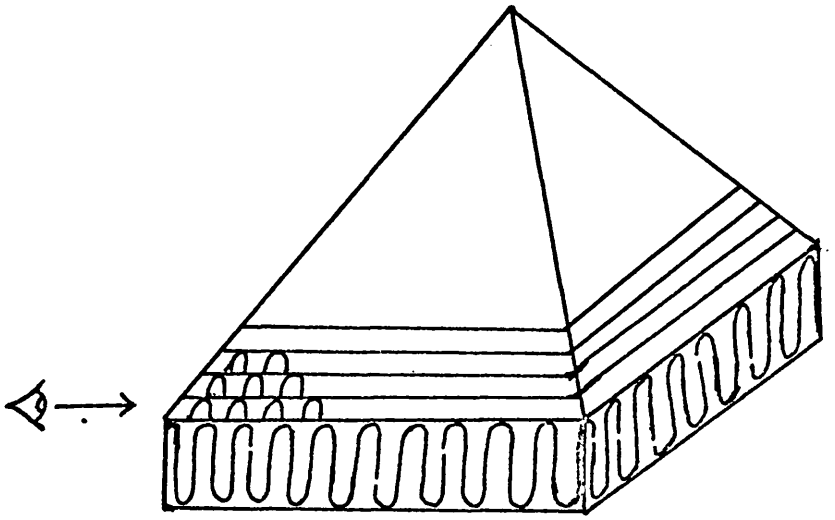
As a result of these discoveries Hermann, Gerngross and Ablitz proposed their Fringed Micelle Theory of polymer structure[13]. In this they proposed (Fig 1) that a polymer, instead of being considered purely as a random arrangement of polymer chains, would more accurately be represented by saying that in certain areas, purely by chance, the polymer chains instead of being completely random, showed sufficient chain alignment to create some kind of local crystal lattice structure. This alignment of the polymer chains will obviously be helped by stretching the fibres, which is what Bunn had done. This idea of stretched fibres increasing the alignment of the polymer chains also leads to the picture proposed by Hess and Kiessig[14] for drawn fibres (Fig 2.)

When we look at the fringed micelle theory of polymer structure it is seen that there are, in effect, two different kinds of region. There are the crystalline areas in a 'sea' of amorphous material and this is why this theory is referred to as the two phase model of polymer structure. The amorphous phase is considered as being a supercooled liquid which, due to the entanglement of the chains, high viscosity of the solid phase etc., cannot achieve crystallinity.

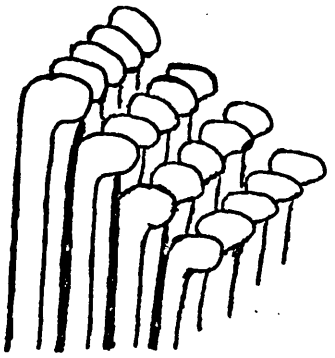
It was not until 1957 that the crystallinity of polymers became really well known and an accepted fact, for it was not until then that Keller[15], Fischer[15] and Till Jr.[15] each independently discovered that polyethylene could readily be crystallised from a dilute solution in xylene, for example. These crystals were found to be very small indeed, but they did posess a quite definite form in which the inclusion of the very long molecules could only be accounted for by assuming that the polymer chains folded back on themselves a great many times and in a very regular fashion. This had to be so since the polymer chains are often of the order of 100,000Å. in length and yet the crystals formed were only about 100Å thick.

Since these discoveries were first made it has been found that there are, indeed, few polymeric substances that do not show some degree of crystallinity and many have been crystallised from dilute solution to form single crystals in just the same way as originally done for polyethylene. Nowadays a wide variety

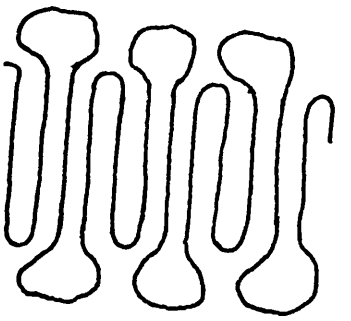
Figure 3.



Folded Chain Theory. Hollow Pyramid Structure.



Looking along the ribbon-plane the pyramidal structure is shown to be caused by regular folding.



Irregular folding is shown to cause a density deficient surface layer.

of solvents are known for the crystallisation of polymers. In all of these single crystals the remarkable thing about them, apart from the fact that they occur at all, is the great similarity of their crystal thicknesses. Generally speaking it lies within the range 60 - 100Å for crystals formed under normal conditions of temperature and pressure, but irrespective of the particular polymer being crystallised.

These discoveries show that the old idea concerning polymer structure must be drastically changed and the Folded-Chain Theory was proposed[16]. This theory considers (Fig 3) that the solid polymer is composed of polymer chains folding back on themselves in a very regular fashion which achieves perfection in single crystals.

This was the first real sign that organisation of huge molecules was not something peculiar to proteins, and there is therefore hope that, if one chooses a suitable model system, then one might indeed obtain information showing the importance of the variety of small forces involved in such organisation.

At this point we find ourselves with two conflicting theories concerning the structure of the solid state of polymers and we must see whether they can in fact be reconciled. Naturally both the fringed micelle and the folded chain theories for polymer structure predict a degree of organisation and yet they are really two extremes of the same picture. In the years that have elapsed since they were proposed an enormous amount of data

has been collected[17] and a lot of work carried out into the various problems that the theories posed.

We now know that there are indications of such folding even in solid polymer formed from the melt, though naturally in such cases the formation of the single crystals tends to be much less perfect. The original folded chain theory[16] was proposed for the single crystals and it explains the lack of perfect crystallinity (100%) by saying that the slight amount of amorphous character arised from lattice defects of various types.

Another observation is that the fold period (and therefore the crystal thickness) varies with the temperature and pressure under which the single crystals were formed and that this fold period can be increased irreversibly by annealing the crystals at temperatures about 10°C below their melting points[19].

The important thing that makes the folded chain so different from the fringed micelle theory is that, according to the latter, the solid material could reasonably be expected to show an extreme variation of structure from an absolutely random arrangement of the molecular chains, up to their complete alignment; when the extended molecular chains will determine crystal thicknesses. The former (folded chain) theory, though it also predicts fully extended chain crystals says that, rather than merely being very unlikely, they have, in fact, a very definite probability of occurring, particularly when one is dealing with materials whose molecules are all of a uniform length. Further reference to this kind of

Figure 4.

The Modern Fringed Micelle Theory.

work will be made later in this introduction. All that needs to be said at the moment is that extended chain crystals have indeed been found[20,21,22,23].

It is now accepted that the old idea concerning the fringed micelle theory is incorrect. It has been replaced by a new concept in which there are still the two phases, a crystalline and an amorphous phase, but in the modern theory it is considered more likely for the chain-folding to occur than for the simple chain alignment of the different polymer chains. Thus it is considered that the two ends of the polymer chain could be independently nucleated and so start to fold, thus preventing the central portion from doing anything else except forming an amorphous region, (See Fig. 4).

Whereas the fringed micelle theory is really quite easy to understand and many analogies can be drawn with common every-day things, the folded chain theory means that we must provide some reasonable explanation, not merely for why the polymer chain folds at all, but also why it folds with such extreme regularity.

Researchers have therefore turned their attentions to trying to obtain some kind of a solution to these questions.

The two concepts that have been proposed for an explanation of these points are based on two rather different attitudes. They are:-

A. The Kinetics of Crystallisation, and

B. The Thermodynamic Stability gained by the chains folding to form such crystals.

Very briefly, (for it is not the purpose of this work to investigate the differences between the theories, nor to try and bring any evaluation of their relative merits,) the Kinetic Theory of Polymer Crystallisation[24] predicts that it is the size of the minimum secondary nucleus on the growing crystal face that determines the thickness of the crystals, but that, after being laid down, the polymer chain can then extend its fold period for a short time and so attain the observed crystal thickness.

The Thermodynamic Theory for Polymer Crystallisation[25], on the other hand, says that the systems with long aligned chains are thermodynamically more stable than are the folded chain structures and it predicts that, as the ends of the polymer chain are laid down in the crystal, they are sufficiently flexible so that the whole chain can take up the position with the minimum free energy density. This position will be decided by taking into consideration all of the interchain and intrachain interactions and will be the thermodynamically most stable one.

A summary of the position would seem to be that as the long chain material crystallises it does so fastest when such crystallisation proceeds by the folding of the chain after fixed lengths have been crystallised, but that on the other hand, in any given crystal, due to interactions, an extended chain would be

more stable than a folded chain and so the crystal will try and take up this form. For a fuller treatment of the rather subtle differences between these two theories the reader is referred to the original literature.

Oligomers.

Reference has been made earlier to work carried out on molecularly uniform species and to the formation of extended chain crystals. This is a very interesting and relatively modern approach to the subject. It had been predicted[31] that if one could produce homogeneous individual oligomers then the mechanical properties, as well as the thermal stability of the compounds, would be increased over those for the random polymers and this prediction has been borne out.

During his investigations in this work Van der Want introduced a new term[26]. This was the word 'Oligomer' which was later defined rather more rigorously by Kern[27] thus:- Oligomers are molecularly homogeneous substances of low molecular weight which are homologous with polymers and differ sufficiently from them in their physical properties to allow their separation into chemically individual compounds.

Another term which has been recently introduced into this work is the word 'Pleinamer'. This is defined by Zahn[28], its originator, thus:- Pleinamers are molecularly homogeneous substances homologous with polymers, but no longer sufficiently different from them in their physical properties to permit separation

into chemically individual compounds. From this definition this latter term would appear to be somewhat vague for it is really determined by a negative quality; that of not being able to separate something. Modern techniques have greatly changed our ideas of what is and what is not separable and such ideas are likely to change even more radically in the next few years with further advances in chromatographic techniques. A rather better definition is given by Keller[22] in which he defines Pleinamers as being oligomers which show long spacings unaffected by molecular weight.

In chemistry one normally speaks of different kinds of uniformity. There is chemical, structural and molecular weight uniformity in oligomeric work. In an oligomeric series one is concerned with chemical uniformity if working with a series of the type on which Zahn and his co-workers carried out certain of their investigations[29], namely linear oligomers of ϵ-amino-n-caproic acid. This is reasonably obvious, but what is perhaps less obvious is that one will also be dealing with chemical uniformity when one synthesises any oligomeric series; for one is then ensuring that the different chemical units in the structure are all present in the same sequence and in the same ratio in all of the molecules made. This is manifestly not the case when one carries out a normal polymerisation reaction. Also in these oligomeric series there will be uniformity of molecular weight, by definition, again in marked contrast to polymers. This only leaves the question

of structural uniformity. Again, where the chemical structure is concerned, one is dealing with uniformity, though this is not necessarily so in the physical sense unless the molecules are in an extended chain single crystal structure.

In the years since the mid-1950's a considerable amount of work has been carried out, notably by Zahn et.al.; Kern et. al.; Fordyce, Lovell and Hibbert; and Rothe on the synthesis of widely differing series of oligoamides, oligoesters, oligoethers and oligourethanes amongst others. The progress in the synthesis of oligomeric series is covered by various review articles[28,30].

The particular investigations concerning this thesis are those of Zahn et. al.[29]. These workers synthesised a series of oligoamides using €-amino-n-caproic acid and also carried out some X-ray work on their products in order to try and determine whether they would show extended chain crystals. This they found to be the case with the lower members of the series, but that above a certain molecular length, the crystal thickness remained more or less constant, provided that they used constant conditions. In their case the constant crystal thickness turned out to be in the region of 70Å, according to the solvent from which the crystals were formed and they found that the transition from fully extended chain crystals to those of constant crystal thickness was a reasonably sharp one.

Baltá Calleja and Keller[22] later carried out a more detailed study of this work, using the same materials, and found that, in the region where there was the transition described above,

there was evidence for saying that the chains preferred to take up a fully extended chain structure at an oblique angle to the crystal axis rather than to be perpendicular to it and slightly folded.

Very similar results have also been obtained by Kern et. al.[23] using their urethane compounds. The situation is, however, not completely unambiguous[22] due to the difficulty of resolving X-ray powder photographs when the relation between molecular orientation and the planes giving rise to the long spacings are unknown.

Despite this slight ambiguity the results seem reliable enough to be able to say that here we have an indication, for the first time, that when one uses chemically uniform materials, then special effecfs can indeed be observed and this lends further weight to the conviction expressed earlier that, in a model system for studying proteins from our point of view, one could best think in terms of chemically uniform species.

Oligomeric Synthesis.

Now our attention must be turned to the particular difficulties concerned with the synthesis of this type of series of compound. The first methods that were tried in efforts to obtain such a series of oligomeric compounds were not ones of synthesis, but rather of the partial degradation of a technical polymer followed by the attempted separation of the variety of materials so obtained[28]. This method is not very widely used,

nor has it ever been notably successful for a variety of reasons. A number of such investigations were attempted, but it was only the first few members of each series of oligomers, both linear and cyclic, that could be obtained, due to the difficulties of separation of the higher members from the technical polymer. This method is also limited by the types of technical polymer available.

It will be remembered that in the definition of the term 'oligomer', reference was made to this very question of separability. This is because, by normal methods of crystallisation, it is very difficult indeed to separate products in such a homologous series when they have molecular weights much in excess of 1,000. Certain modern chromatographic methods, notably gel filtration, are capable of improving on this limitation, but that approach is nowadays more of historical and academic interest than of practical importance.

This difficulty has therefore lead to a concentration of effort on the problems concerned in synthesis from low molecular weight starting materials, though in this case too, unless care is taken with the route used, the question of separation can also arise. The problem in such synthesis then, is not one of chemistry, for it has been amply shown that normal chemical reactions can be used and a wide variety of oligomeric series have been synthesised[28].

The first step in attempts to overcome the difficulty to be experienced in the synthesis of the higher members of the series is to plan the reaction sequences so that the reactions involved give the very highest yields. This requirement concerning high yields is not merely due to the number of individual steps involved in such a synthesis, but also so that as much of the starting materials as possible be removed and converted into products, so easing the subsequent separation. There should also be as few side reactions as possible; again so that materials similar to the desired products are not produced in too great a quantity.

Normally a meticulous purification need only be carried out once with each complete cycle of reactions. It is up to the individual concerned when he considers the most convenient time for this, but when it is carried out it must be thorough; for any inefficiency will have a cumulative effect such that, after a number of cycles, in an extreme case, it would be possible to end up with material that could have been produced far more rapidly and in greater yield using a simple polymerisation reaction.

One of the methods used for confirming the purity of the oligomers produced is by a comparison of the number average and weight average molecular weights[31]. The nearer that this ratio is to unity, then the purer is the material obtained. Other methods used vary from paper chromatography[46] to modern methods of gel filtration[48].

One of the most interesting of the modern methods that have been evolved for the synthesis of huge molecules is that devised by Merrifield[32]. He uses a solid, cross-linked polystyrene polymer as a support for his synthesis. To this special polymer he attaches one compound which will form one end of the final molecule and he then proceeds to carry out a whole sequence of reactions. He is able to continue each reaction to completion for he can wash out all the excess reagent before the next one is added. Finally he removes the completed molecule from its supporting polystyrene. In this way he has synthesised a number of naturally occurring simple proteins and in extremely high yields[32].

This method of synthesis is in very marked contrast to the methods of classical chemistry and, although the problems concerned in obtaining a suitably cross-linked polystyrene polymer as support are very considerable, yet it is a method most worthy of note for it is one step nearer to the methods that nature herself uses to synthesise, with great accuracy, the macromolecules she requires in so pure a form. It also leads one on to speculate about the possibilities of polymers as templates and even of replicating polymers. The work of this thesis, however, is concerned with the more classical methods of synthesis of series of oligomeric compounds designed to try and throw some light on the small forces acting on polymer chains when they organise themselves into single crystals.

One example of the great sensitivity of the crystal to comparatively small disrupting forces is shown by the fact that even a small amount of branching of the polymer chain often means that the polymer cannot be crystallised from dilute solution (to form single crystals). This can be understood because of the spacial requirements of the chain branches, together with their probable intertwining, that makes the organisation required for the production of single crystals quite impossible. What would be of great interest, and be much more subtle, would be to try and determine whether there is any kind of gradation of effects, for example, at the positions where the folds occur. Would, for example, the inclusion of bulky three dimensional groups, at intervals approaching the intervals at which the folds normally occur, force the crystal to fold at these points so as to be able to accommodate the bulky groups? Alternatively, what would be the effect of putting the bulky groups at intervals somewhat greater than the normal fold periods? What would be the corresponding effects for the large, but planar, aromatic system; or of polar or even ionic groups at these positions?

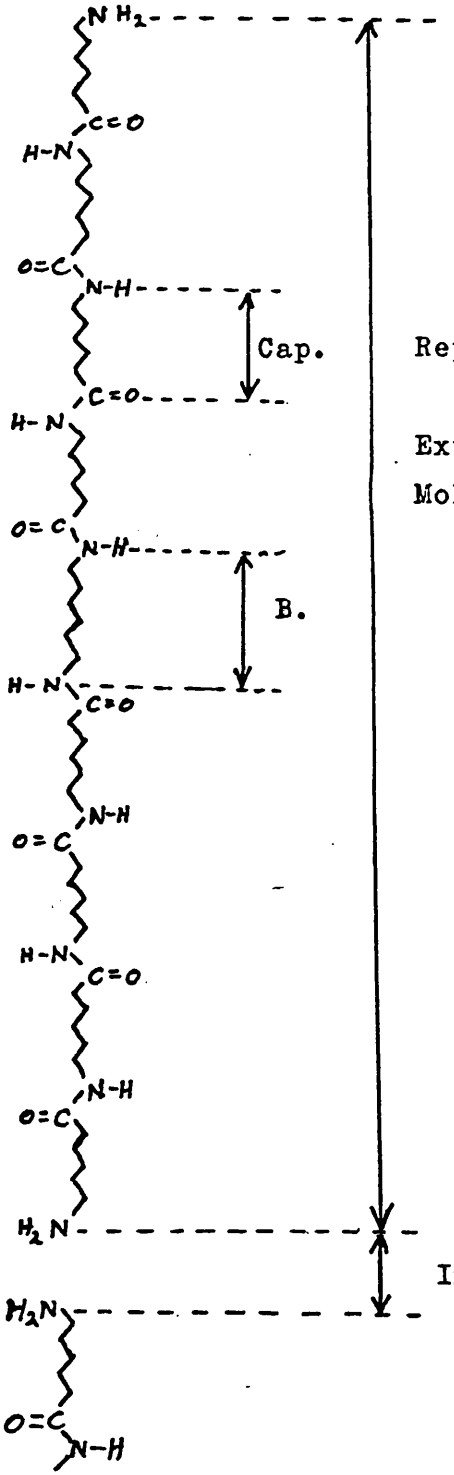
None of these questions has, as yet, received much, if any attention and the present work was undertaken in order to try and devise a method of synthesis of such macromolecules which would be rapid and also adaptable enough to permit modification so as to allow the incorporation of the projected variety of groups

at the critical intervals required.

SECTION ONE.

Figure 5.

Definitions of Terms Used in This Thesis.



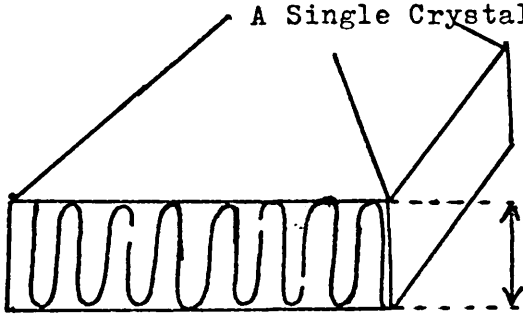
Repeat Length.

=

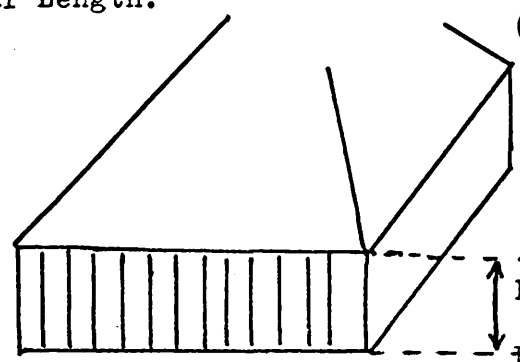
Extended Chain Molecular Length.

Intermolecular Distance.

A Single Crystal (Ideal).



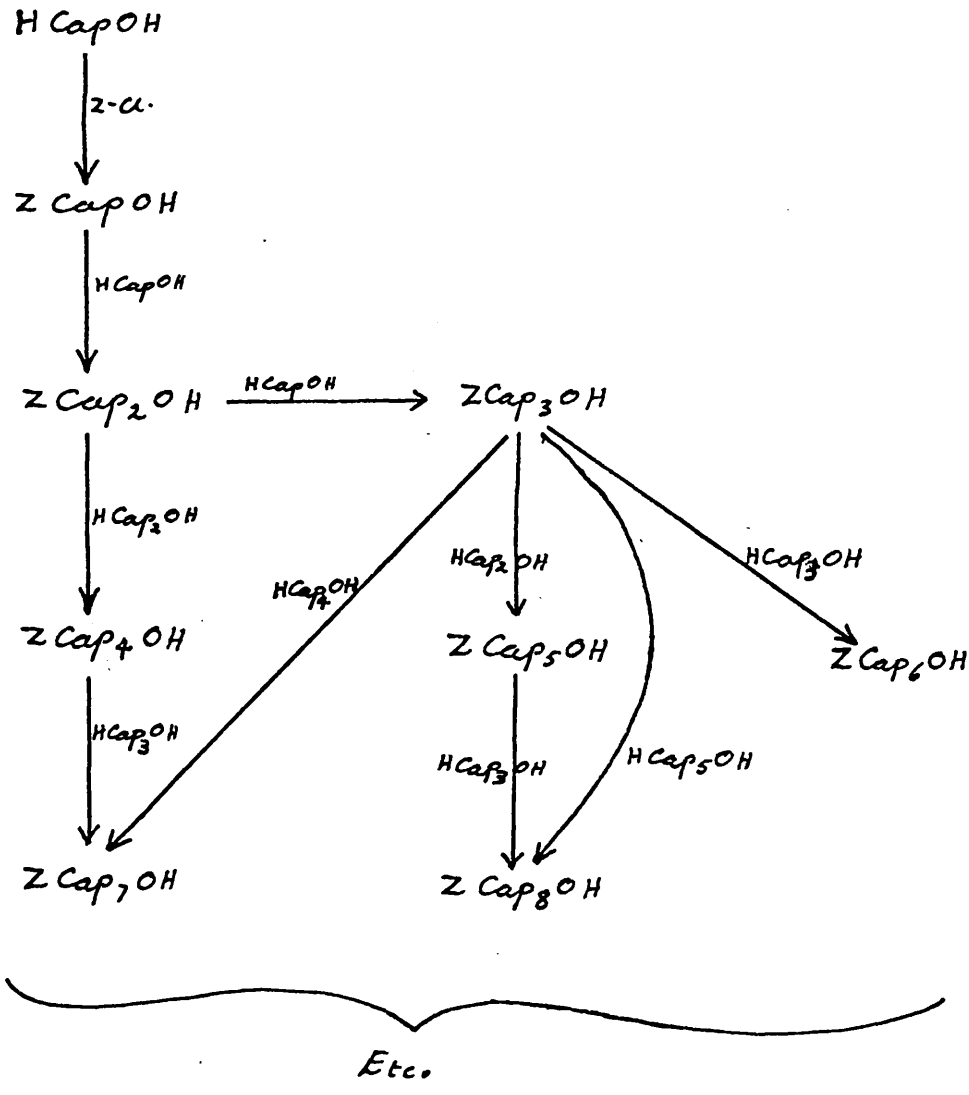
An Extended Chain Crystal (Ideal).



Long Period = Molecular Length + Intermolecular Distance

Figure 6.

Flow Sheet of the Oligocaprolactam Series[29].



DISCUSSION.

Preliminary Observations.

As mentioned in the introduction, Zahn et al. have synthesised a series of oligoamides[29] and used them to ascertain whether their single crystals showed extended chain and folded chain single crystal structures. (A summary of the main terms used is given in Fig. 5.) Keller has also carried out a more detailed study using Zahn's materials[22] and come to the conclusion that the lower members do indeed show extended chain crystals, but that above a certain critical length, a folded chain structure is more likely. In view of the facts to be mentioned below it is of interest to wonder just how extended were the fully extended chains that they observed, but in any case it would not affect the validity of their argument.

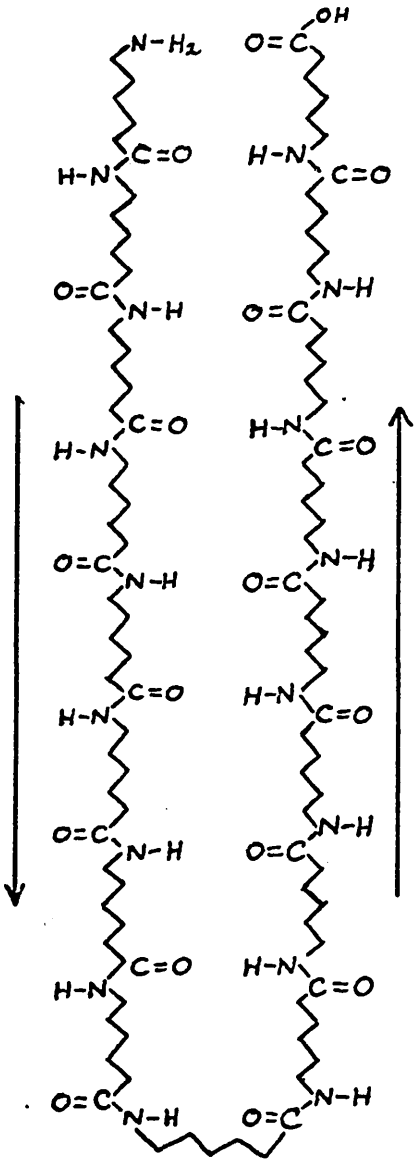
The particular synthetic procedure that Zahn used throughout his work was a stepwise addition of units of ε-amino-n-caproic acid to each other in a head to tail fashion. In order to minimise the difficulties of separation they added on as many multiples of the basic unit at one time as possible. Their route to the macromolecules is shown in Fig. 6. The difficulty, though not stated, was probably that no solvent could be found for the reaction of the amine protected amino acids higher in the oligomeric series than the trimer. Hence why three was the maximum number by which they could extend their chain at any one time.

At the outset of the present investigations it was decided to aim at working on a greatly reduced scale to that of previous workers. Zahn had carried out his synthesis starting with several hundred grammes of material. It was felt that to work on such a scale would be to increase the difficulties concerned with the purification of the final material and it was therefore decided to reduce the scale of operations to that of starting with only a gram or so of material.

Another basic decision was to synthesise series of oligomers containing a di-functional molecule as their central portion. By use of this technique it would be possible to add on two trimer units at one time and it was reasoned that the sought for long molecules should then be much more readily accessible. There was also the important consideration that this reaction scheme would mean the physical properties of the desired products, and of the biproducts, would differ by greater amounts than had been the case with Zahn's system. This, it was expected, would greatly aid the purification of the crude reaction products. As it was intended to use the same amino-acid for the outer portions of our molecules as that which Zahn had used, there were still several alternatives for the functionality of the central portion, but after consideration, it was decided to synthesise oligoamides and therefore the central units used in this work are either dicarboxylic acids or diamines.

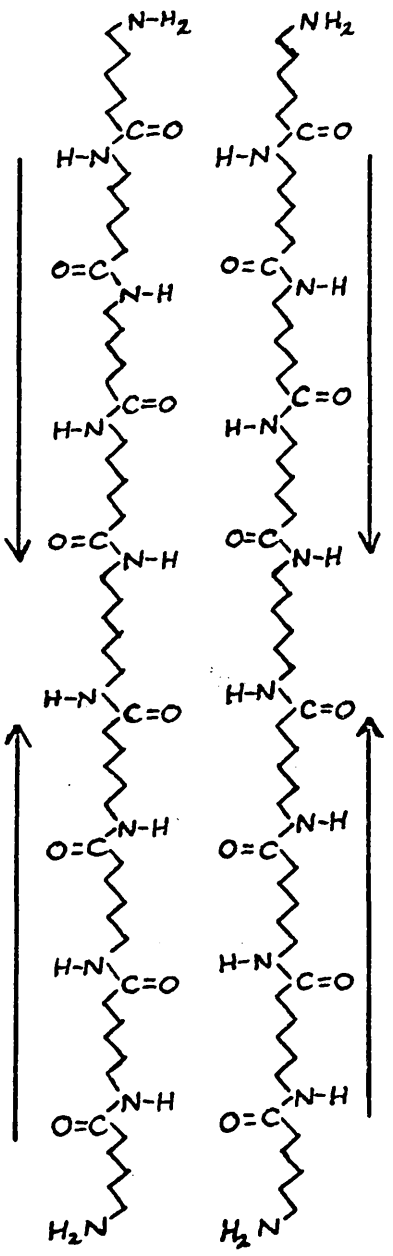
Figure 7.

A.



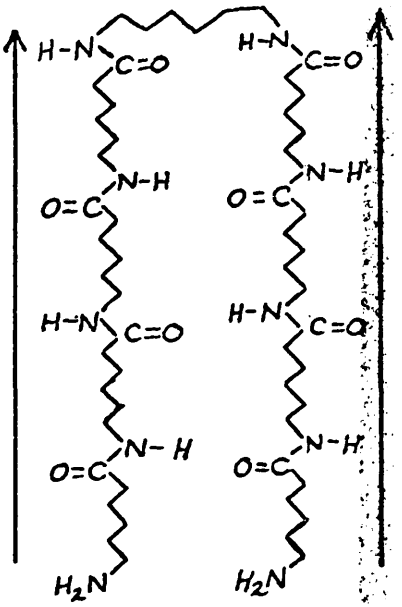
$H[Cap]_n OH.$

B.

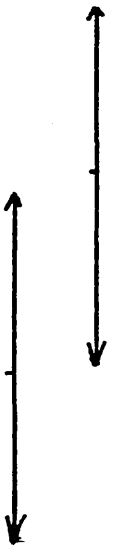


$HCap_4BCap_4H + HCap_4BCap_4H.$

B'.



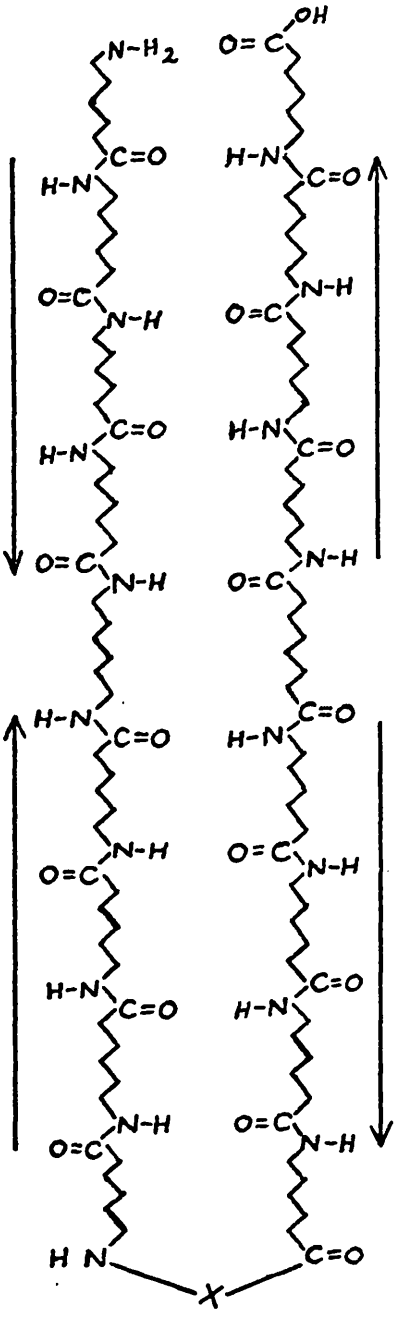
B".



$HCap_4BCap_4H.$

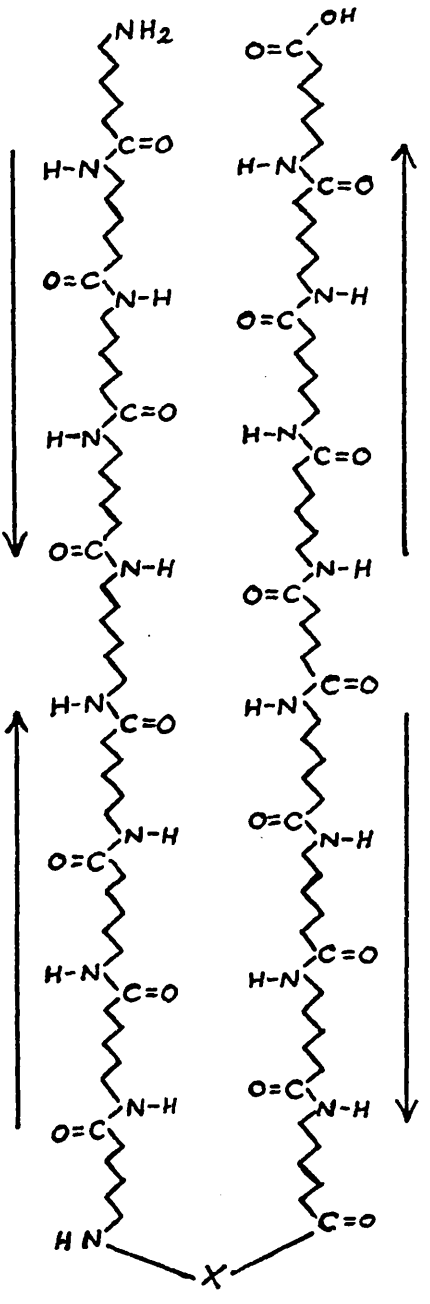
Extended Chain Drawings.

C.



$HCap_4BCap_4H + HOCap_4SebCap_4OH.$

D.



$HCap_4BCap_4H + HOCap_4ACap_4OH.$

There was with this system, however, the possible danger that, once the reaction had occurred at one end of the molecule the resulting mono-condensed material would be sufficiently insoluble to be precipitated out of solution. An even worse condition would arise if the mono-condensed product were to be only partially precipitated from solution, so allowing the reaction to occur for a second time at the other end of the molecule, when that product too would precipitate out of solution. The resulting mixture could be expected to be very difficult to separate. Fortunately however, this difficulty was not found to occur in the series of compounds investigated here.

Having decided on the use of either a dicarboxylic acid or a diamine as the central building units for our series an inspection of the resulting long molecules became necessary in order to determine whether there would be any particular problems unique to working with the resulting systems.

An initial brief inspection of the molecules would seem to indicate very little difference between our systems and the simpler system that Zahn had prepared; however when they are written out in full as shown in Fig. 7, inspection shows several very important differences to be present. (The diagrams in Fig. 7 are not meant to signify the bond angles or conformations, but only the points discussed below.)

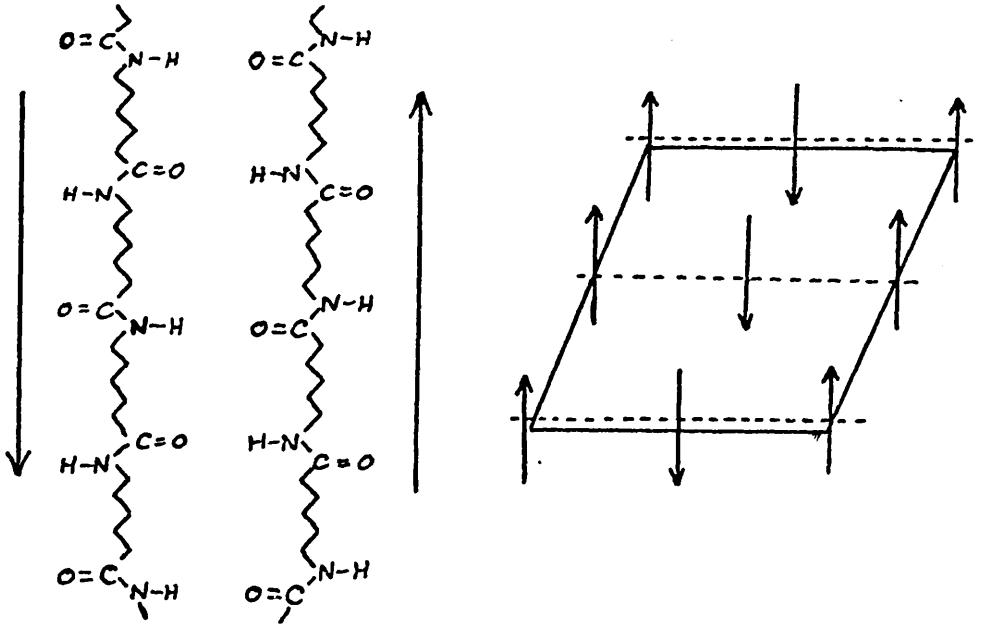
In the first place let us consider the molecules themselves. If we look at the system that Zahn has prepared we can see (Fig. 7A)

that by reading off along the polyamide chain we are reading always in the same sense. If we start with the amino group then next we come to the methylene chain, then to the carbonyl group. After this we return to the aza group, the methylene chain, the carbonyl group and so on for the whole length of the molecular chain. On the other hand when we try to do the same thing with the molecules of the systems we have chosen to study it is seen that there is a reversal in the direction in which we read the chain, which occurs in the middle of the polyamide chain as shown in Fig. 7B. This is readily understood to be a direct result of having used a di-functional building unit as the central portion.

What are the results, if any, of this 'directionality' of the oligomeric chains?

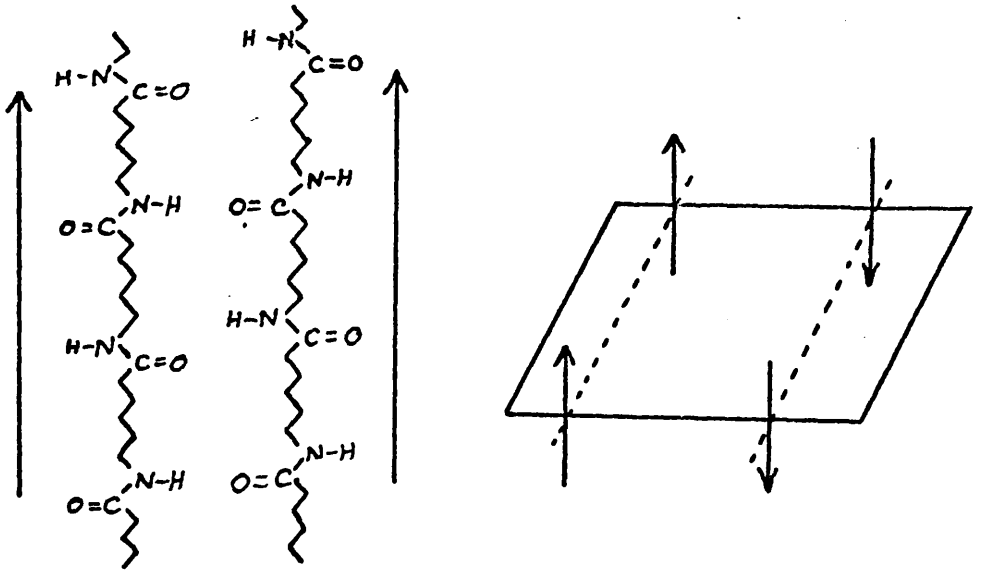
To return to Fig. 7A. As one follows the oligomer chain round the fold, since the chain is always being read in the same direction, all the way down its length; whereas we started by reading down the page, after turning the fold, one is reading up the page. This is shown in the directions of the arrows. It can also be seen that this anti-parallel arrangement of the polyamide chains, as it is called, leads directly to the chance for all of the hydrogen bonds to be the shortest and strongest possible whilst the chain itself is in the fully extended form. Such an antiparallel arrangement of the neighbouring polyamide chains corresponds to the α-nylon structure[33] (Fig. 8).

Figure 8.



The α-Form of Nylon.

Figure 9.



The γ-Form of Nylon.

Now let us turn out attentions to the possibilities open to the chains to be synthesised in this investigation. Here one finds a rather different set of circumstances. In the first place the chains have the possibility either of lining up as shown in Fig. 7B, or they could possibly accommodate a fold in their central portion as shown in Fig. 7B'. In the former alternative it can be seen that the hydrogen bonds, supposing that the chain is still fully extended, must either be longer and therefore weaker, as shown, or else one of the chains could be displaced slightly, either up or down, so as to bring about half of the hydrogen bonds to the shortest and strongest possible position, but at the expense of the rest.

It can also be seen from Fig. 7B' that producing the fold in the centre of the molecule does not remove this dilemma from the system. An examination of the known forms of nylon shows that their systems closely resemble the parallel alignment of the polyamide chains to be found in the γ-nylon structure (Fig. 9).

Vogelsong has reported[33] an alternative way in which the polyamide chains in nylon could align themselves in the so-called γ-phase. He points out that in this phase, if one considers the chains to be fully extended then there is only the opportunity of partial hydrogen bonding, as was pointed out in detail above. Then he goes on to state that it is known that in almost all linear polyamides investigated the hydrogen-bonding is

found to be complete[34]. Vogelsong shows how the polyamide chains, by allowing a slight degree of twist, can take up positions in which, despite their parallel chain alignment, there is the possibility of full hydrogen bonding occurring between neighbouring chains and he also notes that this theory accounts for the observed shortening of the identity period in nylon chains in the γ-form over the α-form.

There is a final form of chain alignment which must not be overlooked. The chains could be displaced linearly to a much greater extent, as shown in Fig 7B". It may seem strange, but the same amount of hydrogen bonding will occur here as in the previously discussed methods of chain alignment, though they are probably the more likely.*

It is felt that since, in the present work, the chains of the pure material would be forced into lying in the manner shown, then they would be forced also into taking up the γ-nylon structure in any single crystals. An exactly similar argument can be found to apply with each of the long chain polyamides synthesised in this section of the work.

A closer examination of this very interesting phenomenon shows the central portion of these macromolecules as being very reasonably considered to be the critical thing in this question of the chain-alignment, for it is theoretically possible to restore the polyamide chains to their α-nylon form by co-polymerising materials, as shown in Fig. 7C. In this case a chain with hexamethylene diamine as its central portion is shown aligned with one containing suberic acid as its central portion and it can be seen that the result is for full hydrogen bonding to occur with fully extended chains. That this would

* See Appendix 2, A special note.

not be the case where the central unit contains many other numbers of methylene groups is shown in Fig. 7D in which the central unit is adipic acid. Such a polymerisation could readily be modified so that some small unit could be incorporated at the ends of one chain. This could be an α-amino-acid in which case we would still have a polyamide, but now we could vary the size and polarity of the functional groups at the chain terminae simply by varying the α-amino-acid incorporated.

The thesis therefore resolved itself into the synthesis of oligoamides containing various di-functional central building units and using the mixed anhydride method for forming the amide links as our starting point. Although one would be incorporating the same outer portions in each of the series, one could try and accommodate as many different kinds of functional group as possible at the chain terminae, both for size and polarity, so that when investigations of their structures are carried out there will be some chance of obtaining useful information about the relative importance of these various effects. It might be an interesting project for an X-ray crystallographer to try and determine the possibilities of these predictions being correct.

Synthetic Procedures.

Zahn and his co-workers had dissolved the amine-protected amino-acid in an inert solvent at -5°C in the presence of a little triethylamine and added ethyl chloroformate, so forming the mixed anhydride. After allowing about twenty minutes for this reaction to go to completion the amine was added, dissolved in dilute aqueous

sodium hydroxide also at -5°C, at the same time being careful to maintain the pH in the range from 8 to 9. The reaction was continued for about three hours, maintaining the pH, but slowly allowing it to regain room temperature before being worked up. The full reaction details are described in the Reaction System A to be found at the start of the experimental description at the end of this section of the work. As it is fairly typical of the conditions used for the mixed anhydride reaction in peptide chemistry it was reasonable to use it as the starting point for our own investigations.

Preliminary work at the start of the synthesis of these compounds showed that there was no suitable solvent for the reaction of $ZCap_4OH$ at -5°C. (The abbreviations used in this work are given in the Table 1.) The material was even found to be insufficiently soluble at -5°C in all mixtures of NN-dimethyl formamide and dimethyl sulphoxide which freeze below this temperature. It was therefore apparent that we would have to be satisfied with the addition of a maximum number of three units at any single condensation reaction.

In the system Zahn and his co-workers were using they were able to obtain good yields with the conditions described in the Reaction System A. (For the sake of convenience I shall continue to refer to these as being the aqueous conditions.) In the systems under investigation in this work, however, it very soon became apparent that these conditions would require

some modification if pure material was to be obtained in reasonable yields.

It can be seen from Table 2 that, although the preparation of ZCapBCapZ using the aqueous conditions gave quite a good yield; with increasing size of molecule, the yields decreased very rapidly. It was also found that use of these conditions gave crude material which was very difficult to purify as well as being rather wasteful. This was because of the similarity of physical properties between the starting materials, the products and some of the biproducts. Since this work was started the advances in gel filtration in particular have been so considerable that nowadays it is likely that such a technique would effect this separation without too much difficulty.

It seemed therefore that investigation of the conditions under which the reaction was being carried out was called for in order to improve on the yields.

It was found that the mixed anhydride, formed by the reaction of the carboxylic acid and ethyl chloroformate, decomposed very rapidly thermally. This was known to be a property of such mixed anhydrides[35], but in the particular mixed anhydrides used in these reactions it was found that even at 0°C their thermal decomposition became so rapid as to be quite prohibitive. Because of these observations, as a precaution for the future, it was decided to lower the reaction temperature to -10°C and to allow a longer reaction time to compensate for this. This alteration

did not, however, bring about the hoped for improvement in the product from the reaction using the aqueous conditions.

If the pH of the solution was decreased it would only reduce the yield, but to increase the pH could reasonably be expected also to bring about an adverse effect because, it was reasoned, there would be competition between the lone pair of electrons on the primary amine and the lone pairs on the hydroxyl ion base for reaction with the mixed anhydride. The mixed anhydrides being used in these syntheses could be particularly vulnerable, if the rate of thermal decomposition is anything to go by. It was this thought that led to the suspicion that it may have been the base used and perhaps also the water molecules which had been the cause of the low yields in this system. This hypothesis received further substantiation when it was observed (Table 3) that the reactions in which the solvent used had been immiscible with water gave acceptable yields, whereas the reactions in which the solvents used were miscible with water were the very ones which gave the depressed yields.

There is in the literature a variation of the reaction conditions for the mixed anhydride method of peptide chemistry in which the amine is dissolved in a tertiary amine and this solution added to the mixed anhydride[36]. Thus we see that the conditions are kept basic, the acidic biproducts can be removed from the reaction mixture as solids, if the right tertiary amine is chosen, and finally, the competition for the reaction with the mixed

anhydride is removed. These conditions, (I shall continue to refer to them as being the anhydrous conditions,) are described in detail in the Reaction System B to be found at the start of the experimental part of this section. It will be seen at once that this system is also just as applicable to the reaction if the amine has to be added as a suspension of it in triethylamine. This is due to the equilibrium which will be set up between the hydrobromide of the amine required for reaction with the mixed anhydride and that of triethylamine.

It is important to note that, since triethylamine normally available contains primary amines, these must be removed before use. In the present work this was done by refluxing the triethylamine with p-toluene sulphonyl chloride for several hours followed by repeated distillation. It was found that this treatment could increase yields by as much as three percent. Triethylamine was chosen as the tertiary base as it was thought to be important to remove the acidic biproducts as solids. It was known that the disadvantage of triethylamine was that it has a greater reactivity towards chloroformates and perhaps mixed anhydrides than the other commonly used tertiary amines which are methyl or ethyl piperidine[35], but it was thought that the advantages obtained by these anhydrous conditions would far outweigh any disadvantage of this kind.

The other precaution that became necessary because of the use of these conditions was the use of carefully dried solvents.

Tetrahydrofuran and NN-dimethyl formamide in particular had to be freshly distilled, just before use, from storage over a suitable drying agent[37]. The normal precautions were taken against moisture during the course of the reactions.

During the above investigations a variety of other methods of peptide chemistry were tried to ascertain whether they would be more suitable than the mixed anhydride method of synthesis for the particular systems being used in this work.

One method which is very widely used is that of dicyclohexylcarbodiimide, but when this method was tried it was found to give only very poor yields. The reasons for this are not understood, but may have been due to moisture in the solvents. This method was not pursued further since the purpose of the investigations was the preparation of the compounds by a good method and the suitable modification of the mixed anhydride method was found.

The method of Weiland and Heinke[38] using phosphorous oxychloride was also tried, but in this case too, the dampness of the solvents proved to be the stumbling block although some drying of the solvents had been carried out prior to their use in these reactions. This method was also discontinued, however, when the suitable modification of the mixed anhydride method was devised.

The first member of the adipic acid series of oligoamides (HOCapACapOH) was synthesised by a second route. In the mixed anhydride method, since the formation of the mixed anhydride is

virtually quantitative, one only needs to add equivalent amounts of ethyl chloroformate and of the carboxylic acid. It was therefore thought that it would be practicable to synthesise this series without the need to protect the carboxylic acid function of the amino-acid to be reacted with the mixed anhydride, by making its methyl ester. Accordingly adipic acid di-mixed anhydride was reacted with ϵ-amino-n-caproic acid dissolved at -5°C in dilute aqueous sodium hydroxide and the reaction left for several hours before being worked up. The final traces of adipic acid required to be sublimed out of the final product, even after several recrystallisations and hence it was found to be rather more troublesome to purify this material than to go the longer way round. This difficulty could reasonably be expected to get greater with the higher members of the series and therefore the longer route was felt to be the more effective.

One method of synthesis, that is of some interest, occurred as a direct result of the work to be described in Section 2 of this thesis. A well known and well tried method of peptide synthesis is that using the so called Active Esters[39] such as the p-nitro phenyl esters. The conditions for this reaction are particularly mild, only requiring the primary amine to be dissolved in excess tertiary amine and added to the solution of the active ester in some inert solvent. It will be noted that these are very similar conditions to those described in the Reaction System B, namely the anhydrous conditions for the mixed anhydride reaction.

As a result of investigations into the synthesis of starting materials marked by highly fluorescent or highly coloured materials, para-hydroxy azo benzene was esterified with N-carbobenzoxy-ϵ-amino-n-caproic acid, using dicyclohexyl-carbodiimide. It had been hoped that the resulting ester would have been of use in the synthesis of a series of oligomers corresponding to $MeOCap_nACap_nOMe$ (see Flow Sheet 4). This would have involved this particular ester link being stable under the the conditions for the reaction system B and to test this requirement some of the ester was dissolved in triethylamine, hexamethylene diamine added and the reaction left at room temperature for several hours. The yield of ZCapBCapZ at the end of this period was almost sixty percent, indicating that the para-azo-phenyl esters are almost as effective as the para-nitro benzyl esters from this point of view. This was rather unfortunate for the use of this ester in the reaction scheme for which it was originally synthesised, but at the same time it is a rather interesting variation on the methods of synthesis of these materials.

Throughout this work the protecting group that was used for the amino-function was the carbobenzoxy group. There have been a large number of different groups used for this purpose in normal peptide chemistry, but these are very adequately discussed elsewhere[39]. The carbobenzoxy group has been found

a particularly useful one. Its great advantages over other groups lie in the ease with which it can be used. Not only is it very readily put onto the particular amino-function, but a wide range of reaction conditions have been worked out by which it can be removed selectively. It can be removed under conditions which will not cleave amide links, ester links, or even cause the racemisation of α-amino acids; again, these are adequately covered elsewhere[39].

The conditions for reacting the carbobenzoxy chloride with the primary amine have been well worked out by Zahn for ε-amino-n-caproic acid[29] and, provided the temperature is maintained below -5°C a good yield is obtained.

The carboxylic acid functional groups were protected by making their methyl esters. The first method tried was the simple one using diazomethane, but this worked only with the lowest member of the series due to the comparative insolubility of the higher oligomers. The other classic method, esterification under acidic conditions, was not used due to the possibilities of hydrolysis of the amide links which, although slow compared with the rate of esterification, would be most undesirable. The method finally chosen was the base catalysed method of Stodola[40]. This uses dimethyl sulphate and a tertiary base, dicyclohexylethylamine, synthesised by the method of Hunig and Kiessel[41]. This method is very mild and gave good yields, particularly if slightly longer reaction times were allowed than those that Stodola quotes in his paper.

The Removal Of The Protecting Group.

The method that Zahn and his co-workers had used to remove the carbobenzoxy group from the amino-nitrogen was catalytic hydrogenation, but they found[29] that as the molecular weight of the material increased, so did the difficulty of removal of the carbobenzoxy group. It was therefore decided to use the much more rapid and convenient method using hydrogen bromide in glacial acetic acid[42]. This proved to be a much more satisfactory method and the full details of this typical reaction are described in the Reaction System C to be found at the start of the experimental description at the end of this section of the work. When using this system it was found to be important that sufficient time be allowed for the reaction to proceed sufficiently, particularly in the present work where relatively small quantities were used, otherwise considerable errors arose in the quantities of reagent effectively added as compared with the calculated quantities. An example of this effect is seen in the preparation of MeOCapSubCapOMe (See Table 3) and to a lesser extent in the next higher homologue.

In these reactions the amounts of ZCapOMe and $ZCap_2OMe$ used were calculated on the basis of a seventy-five percent yield of the amine hydrobromide, but it would appear that in these cases insufficient time was allowed for the effective removal of the protecting group and only a sixty to sixty five percent yield was, in fact obtained. The normal procedure after this experience was to consider the yields to be either sixty five or seventy five

percent, always calculating that one requires an excess of the mixed anhydride reagent for the diamine series and an excess of the amino-group for the dicarboxylic acid series.

The conditions for the hydrolysis of the ester linkages to form the dicarboxylic acids were found to vary very considerably. The lack of solubility of the materials was the principal obstacle, though this was overcome without too much difficulty. At the start of this work it had been hoped there might be a chance of obtaining the hydrolysis of the diester to the corresponding monoacid ester and thereby provide access to a greater variety of long chain materials. (See Section 2.) In all of the systems investigated in this section of the work, however, no sign of such a hydrolysis was observed.

The first method to be tried for obtaining the desired hydrolysis was the use of methanolic barium hydroxide[43]. It has been established that the effectiveness of this method, using the aliphatic dicarboxylic acid dimethyl esters, increases with their molecular weights[44] and that it is not of much use for diesters lower in the homologous series than sebacid acid dimethyl ester. It had been hoped that this might be due to the solubility of the mono-anion in methanol in the case of the lower homologues and that, as our systems have so high a molecular weight, their mono-anions might be sufficiently insoluble to be precipitated. Unfortunately, even use of less than one equivalent of barium hydroxide did not produce any trace of such a hydrolysis; the only product being from the complete hydrolysis to the di-anion.

When the full two equivalents of base were used then this method was found to yield the di-anion very efficiently.

The next homologue ($MeOCap_2ACap_2OMe$) was only very sparingly soluble in methanol, even at reflux temperature, but here again none of the sought-for mono-anionic material was found. It was also found that some sixty percent of the starting material was recovered unchanged.

Due to the lack of solubility and of hydrolysis it was then decided to try a more powerful base and a more polar solvent. A strong (Ca 5N.) solution of sodium hydroxide in ethanol was made up and $MeOCap_2ACap_2OMe$ dissolved in NN-dimethylformamide at about 70°C. This solvent was chosen as it would act as its own indicator when the reaction was complete because of the evolution of volatile amines caused by the hydrolysis of the solvent rather than the amide links of the material being saponified (by a simple bulk effect.) The ethanolic solution of sodium hydroxide was added, very slowly and cautiously and with vigorous stirring, to the solution of the diester and a crystalline precipitate rapidly formed. When, on addition of further base a sudden evolution of volatile bases occurred the reaction was stopped. On a separate occasion, when carrying out the saponification of the corresponding diester $MeOCap_2SubCap_2OMe$, the reaction was allowed to continue for a further half an hour at this point and from Table 3 it is apparent that there is a small, but significant amount of saponification still occurring during this time.

The hydrolysis of these diesters all occurred rapidly and in high yields, but on increasing the molecular weight again, although the materials were still soluble in NN-dimethyl formamide, there was a marked decrease in the rate of saponification of these higher homologues (See Table 3.)

A stronger base or a more polar solvent was therefore again sought and the system chosen was to use a more polar solvent. The solvent chosen was dimethyl sulphoxide at about 80°C, again using sodium hydroxide as the base, but this time dissolved in normal butanol because of the raised temperature. The temperature for this reaction was 80°C so as to increase the rate of saponification and yet not to get too near 100°C at which temperature dimethyl sulphoxide is known to be slowly decomposed thermally[45].

With this system the calculated slight excess of sodium hydroxide in normal butanol was added, all at once, to the material in solution and the reaction stirred very vigorously, but for no longer than two minutes before the solid product was filtered. The extreme rapidity and efficiency of these conditions (Table 3) is in very marked contrast to anything which had been observed before. The increase in the yields is extremely marked and the only reasonable explanation would seem to be that the base carrying out the saponification is not the simple hydroxide ion, but some complex of it, perhaps with the solvent. Whatever the explanation, no difficulty was observed with hydrolyses using these conditions and the yields, particularly considering the extreme

size of the molecules, were very good. Beyond doubt still better yields could be obtained with the highest homologues yet tried, using the same conditions, but insufficient materials and time were available for a detailed investigation along these lines.

The free dicarboxylic acids were obtained from their di-anions by their solution in the minimum volume of some suitable hot solvent followed by its acidification using dilute aqueous hydrochloric acid. On cooling the solution, the solid dicarboxylic acids were obtained and removed by filtration.

Purification Procedures.

Once the preliminary problems had been overcome concerning the reaction conditions there was never any difficulty in finding a suitable solvent for the recrystallisation of the various materials obtained from the reactions. One of the more interesting solvents used was ethyl acetate saturated with water at room temperature. This was found to be a particularly useful solvent for the lowest members of the dicarboxylic acid series of oligoamides and it was also occasionally used in the purification of other materials where difficulty was experienced in removing the triethylammonium salts produced during the reactions.

Wherever NN-dimethyl formamide or dimethyl sulphoxide were used as solvents for recrystallisation it was routine for the excess solvent to be removed from the crystals by washing them two or three times either with ethyl acetate or ethanol. It was found that, particularly with the higher oligomers, the crystals

very frequently appeared in a very fine form, far too fine to allow filtration or centrifugation in a normal fashion. Instead they had to be sedimented at the bottom of the tube by centrifugation and the solvent decanted from them.

The question of how one knows a compound to be pure is one which acquired great importance in this work. The normal criterion of the analytical figures becomes less reliable with these very high molecular weight compounds (Table 4). From this table it can be seen that if one were to try and synthesise each homologous series by a stepwise technique only ascending by one unit at a time, at each end of the chain, then the point would rapidly be reached where the acceptable error in the analytical figures (normally of less than or equal to 0.3%) is of the same order as, or even greater than the difference between the two homologues. This is why, in this work, great stress was laid on increasing the molecular weights by as great an amount as possible at each sfage. Even in such cases, however, it was found that material was obtained which could give 'correct' analytic figures and yet be some $10^{o}C$ low in its melting point. Thus in general it can be said that in this work the analytical figures can only be taken as being conclusive if they are wrong.

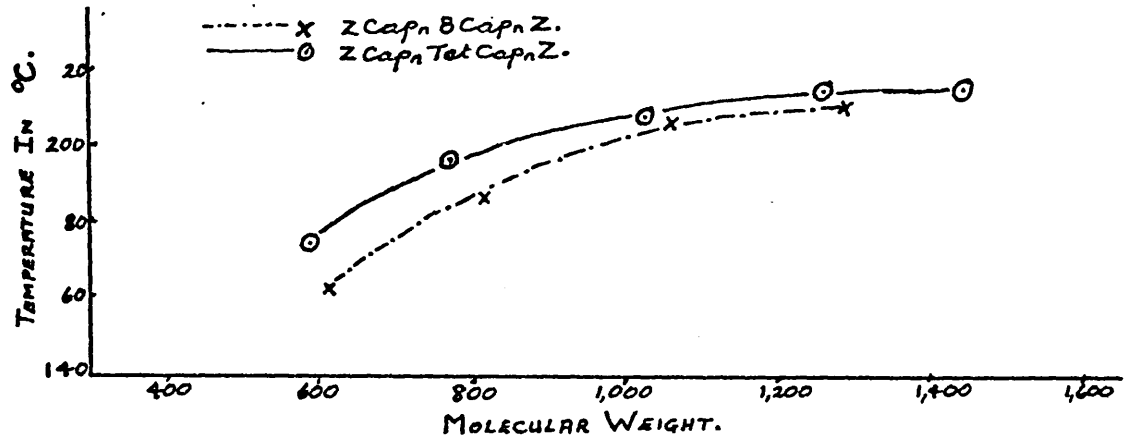
In this work the suplementary method used as a criterion of purity was the melting point of the solid, particularly when taken in conjunction with those of the other members of the series. It has been proved[46,47] that, for a given series of linear

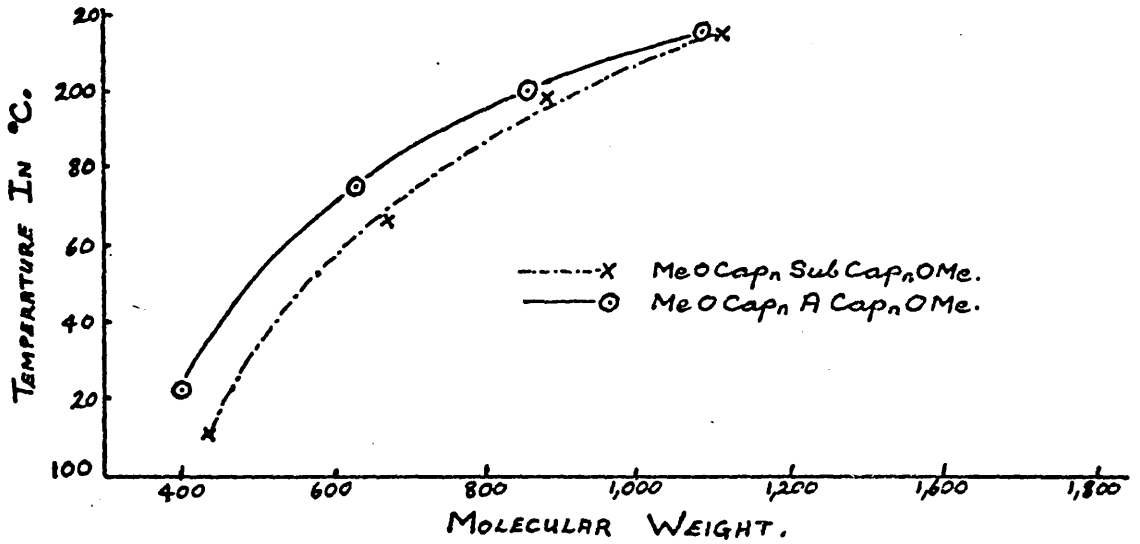
oligoamides, as the chain length increases, so the melting point of the oligomers increases, asymptotically approaching that of the technical polymer. For linear oligoamides one normally finds these melting points to be on a reasonably smooth curve when plotted against the molecular weight, and this can therefore be used as a method of quality control. Again, to be strictly correct, this method does not tell one that all the materials obtained are pure, but only that they are in similar states of purity. Another method of qualitative control is the use of paper chromatography[28], or with the modern advances in gel filtration, this would be a very good method also[47].

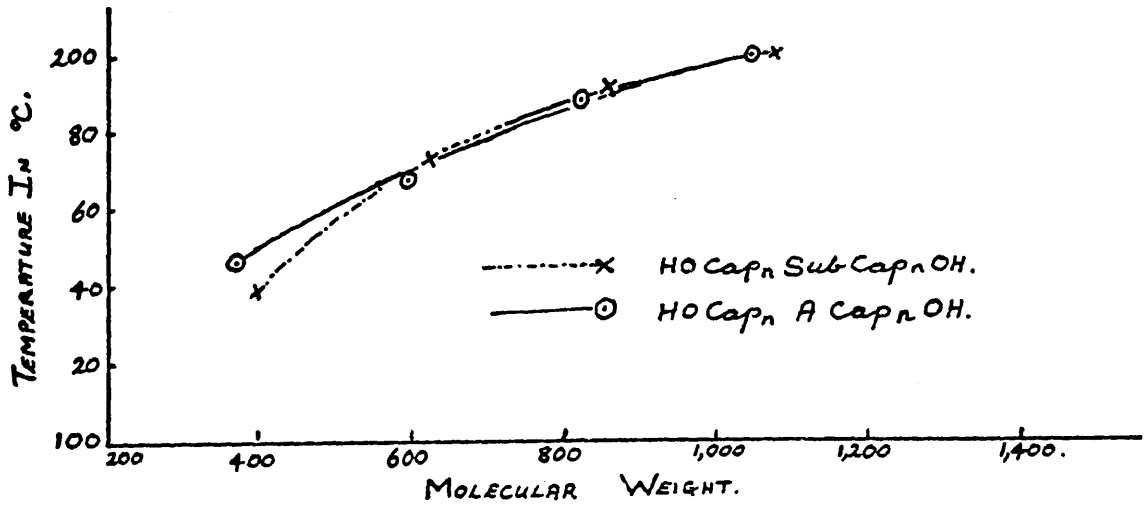
One point concerning the melting points of the individual oligomers should be noted and that is the possibility that one can get different values when one uses different rates of heating of the crystals. This is so because on heating the solid to take its melting point, annealing can occur[20,28,46], but this will take time. Thus, if the temperature is raised rapidly the annealing process cannot occur to any great extent and the melting point obtained will be considerably lower than that obtained by heating the solid only slowly, allowing annealing to occur fully. This relation between the long period (increased by the annealing process) and the melting point of the solid has been investigated by Keller[22] amongst others.

In these investigations the melting points were always taken by heating the solids very slowly and so obtaining the

Figure 10. Melting Point Correlation Curves.







highest possible values. This also ensured that the values obtained were reproducible.

The graphs of the melting points provided by the materials prepared in this section of the work are shown in Fig. 10.

Some investigations were carried out in efforts to obtain suitable crystals so that X-ray crystallographic investigations could be carried out. The caesium salts of two members of the dicarboxylic acid series were prepared by the neutralisation of the parent compounds with caesium hydroxide.

It was not found possible, however, to form crystals of a size suitable for single crystal X-ray study and, as it was felt that no useful additions to present day knowledge could be obtained by the repeating of simple powder photographs, this line of investigations was carried no further.

The full flexibility of this modification of the stepwise reaction system is shown by the final inclusion of the amino-acid tryptophan at the end of the chain in compound VII. This shows one way in which the groups of varying size and polarity can be included in such positions, as required.

One of the more important checks one has that ones products are as pure as can be obtained is given by the synthesis of the same materials by other routes. In the present work this has been done with several of the materials, and where a difference the melting points was obtained, then the higher value has been taken as being the one from the purer material.

During all these investigations, when NN-dimethyl formamide or dimethyl sulphoxide was required for recrystallisation, then it was found necessary to use glass filter paper for the filtrations since normal filter paper was appreciably soluble in these solvents, particularly at elevated temperatures.

Despite the limited checks on purity that were available it is felt that the materials described in this thesis were obtained in as pure a form as could be achieved by the techniques available at the time.

The nomenclature of the compounds presented in this thesis follows that used by Zahn et al. (49).

Instrumentation.

Melting points quoted in this thesis were all taken on a Reichert Kofler Apparatus, and are uncorrected.

Infra-red spectra were recorded on a Unicam SP 200 spectrometer, and were calibrated with the polystyrene 1603 cm^{-1} peak.

Ultra-violet spectra were recorded on a Unicam SP 800A spectrometer.

Thin-layer chromatography plates were prepared using Kieselgel G, prepared after the method of Stahl.

Mass spectra were recorded using an A.E.I. MS 12 instrument.

EXPERIMENTAL.

Reaction System A.

N-Carbobenzoxy-bis- ϵ-Amino-n-Caproic Acid.

To a clear solution of N-carbobenzoxy- ϵ -amino-n-caproic acid (5 gm, 20 mmole) dissolved, at -5°C, in toluene (110 ml) and triethylamine (1.5 gm) was added, dropwise and with stirring, ethyl chloroformate (2.2 gm, 20 mmole) and the reaction stirred for 20 minutes. While maintaining the temperature at -5°C a solution of ϵ-amino-n-caproic acid (2.6 gm, 20 mmole) dissolved in 2N sodium hydroxide (10 ml) was added, dropwise and with vigorous stirring, ensuring that the pH remained in the range 8 to 9. The solution was stirred for about two and a half hours, maintaining the pH in the same range, but slowly allowing the temperature to regain room value.

The partially solidified product was dissolved in water (125 ml) and the aqueous layer washed with ether. The residual ether was boiled off, the cooled solution acidified with concentrated hydrochloric acid and the precipitate allowed to agglomerate for about half an hour before being filtered, dried and recrystallised from hot benzene to yield the pure product.

Reaction System B.

Tetramethylene Diamine-bis-(N-carbobenzoxy- ϵ -amino-n-Caproic Acid) Amide.

Ethyl chloroformate (1.10 gm, 10 mmole) was added dropwise to N-carbobenzoxy- ϵ-amino-n-caproic acid (2.65 gm, 10 mmole)

dissolved at -10°C in toluene (60 ml) and triethylamine (2 ml) and the mixture stirred for about 20 minutes. Maintaining the temperature at -10°C, a mixture of tetramethylene diamine (0.44 gm, 5 mmole) and triethylamine (10 ml) was added to the mixture and the whole reaction stirred for about eight hours, slowly allowing it to regain room temperature. The solid material was filtered and recrystallised from ethanol to yield the pure final product.

Reaction System C.

Methyl-ϵ-amino-n-caproate Hydrobromide.

Into a large conical flask, protected by a good drying tube, was placed methyl-N-carbobenzoxy-ϵ-amino-n-caproate(2.80 gm, 10 mmole) and to this was added an excess of fresh hydrogen bromide in glacial acetic acid (50%w/v) and the drying tube firmly replaced. The flask was left at room temperature, with occasional swirling, over a period of three to four hours, or until the evolution of carbon dioxide had ceased and then into the flask was poured just sufficient sodium dried ether so as considerably to dilute the solution and yet not to cause the precipitation of the product. The total contents of the flask were then poured into a large excess of sodium dried ether and the solid product allowed to setfle. After gently pouring off the supernatent liquid the solid hydrobromide product was washed with several further portions of sodium dried ether in order to remove all traces of the reagent.

As the hydrobromides produced were almost always very hygroscopic, they were used immediately in the reactions.

N-carbobenzoxy-ε-amino-n-Caproic Acid. (I).

Benzyl chloroformate (23 gm, 135 mmole) and 4 N sodium hydroxide (25 ml) were added dropwise over two hours and with vigorous stirring, using a Hirshberg stirrer, maintaining the temperature of the solution between 0°C and -5°C, to a solution of ε-amino-n-caproic acid (13 gm, 100 mmole) in 4 N sodium hydroxide (20 ml). The mixture was stirred vigorously for approximately one hour after the addition had been completed, the solution still being maintained between 0° and -5°C. Water (75 ml) was then added to dissolve the partially dissolved product and the solution washed well with ether.

After the residual ether had been boiled from the aqueous layer, the solution was diluted to 300 ml, cooled to 0°C, and acidified with concentrated hydrochloric acid and the precipitate allowed to agglomerate for about one hour. The precipitate was finally filtered, dried and purified by dissolving it in cold benzene and reprecipitating it by pouring this solution into a large excess of 40/60 pet. ether. The final yield of I was 21.5 gm (80 mmole, 80%) m.p. 53½-54°C. (Lit. 54°C.)

Found C, 63.23; H, 6.93; N, 5.44%

$C_{14}H_{19}NO_4$ requires C, 63.38; H, 7.22; N, 5.28%.

N-carbobenzoxy-bis-ε-amino-n-Caproic Acid. (II).

To the clear solution of N-carbobenzoxy-ε-amino-n-caproic acid (5 gm, 20 mmole) dissolved, at -5°C, in toluene (110 ml) and triethylamine (1.5 ml) was added dropwise ethyl chloroformate

(2.2 gm, 20 mmole) and the mixture stirred for 20 minutes. Still maintaining the temperature at -5°C a solution of ε-amino-n-caproic acid (2.6 gm, 20 mmole) in 2 N sodium hydroxide (10 ml) was added dropwise and with vigorous stirring ensuring, by the addition of further small quantities of sodium hydroxide, if required, that the pH remained in the range from 8 to 9. The solution was stirred for two and a half to three hours maintaining the pH in the same range, but allowing the mixture slowly to regain room temperature.

The partially solidified product was dissolved in water (125 ml) and the aqueous layer washed with ether. The residual ether was boiled off, the cooled solution acidified with concentrated hydrochloric acid and the precipitate formed allowed to agglomerate for half an hour before being filtered, dried and recrystallised from hot benzene to yield II (4.80 gm, 127 mmole 63%) m.p. 105-106°C. (Lit. 106°C).

Found C, 63.39; H, 7.98; N, 7.38%.

$C_{20}H_{30}N_2O_5$ requires C, 63.47; H, 7.99; N, 7.40%.

N-carbobenzoxy-tris-ε-amino-n-Caproic Acid. (III).

To the clear solution of N-carbobenzoxy-bis-ε-amino-n-caproic acid (3.78 gm, 10 mmole), dissolved at -5°C in dry tetrahydrofuran (500 ml) and triethylamine (1.0 gm), ethyl chloroformate (1.08 gm, 10 mmole) was added dropwise and the mixture stirred for about 20 minutes. Still maintaining the temperature at -5°C a solution of ε-amino-n-caproic acid (2.62 gm, 20 mmole) dissolved in 2 N sodium hydroxide (10 ml) was added dropwise, ensuring that the

pH remained in the range between 8 and 9.

The solution was stirred for two and a half to three hours, slowly allowing it to regain room temperature and then it was acidified with concentrated hydrochloric acid and diluted to approximately ten times its original volume. The precipitate formed was filtered and the damp, crude product recrystallised from ethyl acetate which was saturated with water at room temperature to yield III (4.48 gm, 9.1 mmole, 91%) m.p. 133-134°C. (Lit. 133-134°C.)

Found C, 63.60; H, 8.45; N, 8.58%.

$C_{26}H_{41}N_3O_6$ requires C, 63.52; H, 8.41; N, 8.55%.

N-Carbobenzoxy-tetra-ε-amino-n-Caproic Acid.

N-carbobenzoxy-bis-ε-amino-n-caproic acid (2 gm, 5.3 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C, and the solid hydrobromide formed dissolved in the minimum volume of 4 N sodium hydroxide so as to make the resulting solution just basic.

To N-carbobenzoxy-bis-ε-amino-n-caproic acid (1.5 gm, 4 mmole) dissolved at -5°C in dry tetrahydrofuran (50 ml) and triethylamine (1 ml) was slowly added with vigorous stirring, ethyl chloroformate (0.45 gm, 0.4 mmole) and the reaction stirred for about 20 minutes. After this time, being careful to maintain the temperature at -5°C, the solution of the hydrobromide in dilute sodium hydroxide was added slowly, together with sufficient further quantities of 2 N sodium hydroxide to ensure the pH remained within the range from 8 to 9. After the additions had been completed the reaction was

stirred for a further three hours, during which time it was slowly allowed to regain room temperature.

After the reaction had been completed water (50 ml) was added and the mixture filtered. The aqueous layer was extracted with several portions of ether and then, after boiling off the residual ether, acidified and the white precipitate formed filtered off and dried. The solid material was then recrystallised from ethanol to yield pure product (1.93 gm, 0.32 mmole 80% with respect to the carboxylic acid activated.) Its melting point was found to be 152-154°C (Lit. 154-155°C.)

Found C, 63.43; H, 8.56; N, 9.02%.

$C_{32}H_{52}N_4O_7$ requires C, 63.55; H, 8.67; N, 9.26%.

Hexamethylenediamine-bis (N-carbobenzoxy-ϵ-amino-n-Caproic Acid) Amide. (IV).

METHOD 1.

Ethyl chloroformate (2 gm, 19 mmole) was added dropwise to N-carbobenzoxy-ϵ-amino-n-caproic acid (5 gm, 19 mmole) dissolved at -5°C in toluene (110 ml) and triethylamine (1.5 gm) and the mixture stirred for about 20 minutes. Maintaining the temperature at -5°C, a solution of hexamethylene diamine (1.10 gm, 9.5 mmole) in water was slowly added and the reaction stirred for three hours, adding sodium hydroxide if necessary to maintain the pH between 8 and 9, but allowing the solution slowly to regain room temperature. The solid material which had precipitated was filtered and recrystallised from ethanol to yield IV. Further product was

obtained by evaporation of the filtrate to dryness, the extraction of the solid residue with ethanol, and the recrystallisation of this extracted material, from ethanol, to constant melting point. The combined yield of IV was 4.25 gm (7.0 mmole, 70%), m.p. 162-163½°C.

Found C, 66.91; H, 8.04; N, 9.37%.

$C_{34}H_{50}N_4O_6$ requires C, 66.86; H, 8.25; N, 9.17%.

Compound IV. METHOD 2.

To para-hydroxy azo phenyl-N-carbobenzoxy-ε-amino-n-caproate (222 mgm, 0.5 mmole) dissolved at room temperature in toluene, was added hexamethylene diamine (29 mgm, 0.25 mmole) dissolved in triethylamine (10 ml) and the reaction stirred for four hours. After this time the solvent was removed under reduced pressure and the solid material obtained recrystallised from ethanol to yield IV (62 mgm, 0.1 mmole, 40%) m.p. 163-163½. In addition to this product there was also recovered the starting ester material XVI (76 mgm, 0.17 mmole) thus giving a yield relative to the quantity of ester used of 60%.

Compound IV. METHOD 3.

To hexamethylene diamine (440 mgm, 3.8 mmole) dissolved at 0°C in dioxane (25 ml) and acetone (5 ml) with triethylamine (1 ml) was added dicyclohexylcarbodiimide (1.57 gm, 7.6 mmole) and N-carbobenzoxy-ε-amino-n-caproic acid (2.0 gm, 7.6 mmole) dissolved in dioxane (5 ml). The reaction was stirred at 0°C for some two hours and then it was allowed to reach room temperature and continued

at this temperature for 18 hours. The NN'-dicyclohexyl urea formed was filtered off and the solvent removed from the filtrate under reduced pressure. The solid residue showed only a very small amide band in the carbonyl region of its infra-red spectrum. It was concluded that the required condensation reaction had not occurred.

7,14,21,28-Tetra aza-6,13,22,29 Tetra oxo-1,34-di (N-carbobenzoxy)-Amino Tetratriacontane. V.

Ethyl chloroformate (0.88 gm, 8 mmole) was added dropwise to N-carbobenzoxy-bis-ε-amino-n-caproic acid (3.0 gm, 8 mmole) dissolved, at -5°C in dry tetrahydrofuran (50 ml) and triethylamine (2 ml) and the mixture stirred for about 20 minutes. Maintaining the temperature at -5°C a solution of hexamethylene diamine (0.48 gm, 4 mmole) dissolved in water was slowly added and the reaction left stirring for about three hours, with the occasional addition of a few drops of sodium hydroxide solution if necessary in order to maintain the pH of the solution between 8 and 9. After three hours the whole solution was evaporated to dryness under reduced pressure and the whole solid residue repeatedly extracted with ethanol. The product so obtained was recrystallised either from ethanol or from NN-dimethylformamide to yield V (2.1 gm, 2.5 mmole, 63%) of m.p. 185-186½°C.

Found C, 65.79; H, 8.38; N, 10.13%.

$C_{46}H_{72}N_6O_8$ requires C, 66.00; H, 8.66; N, 10.04%.

7,14,21,28,35,42-Hexa aza-6,13,20,29,36,43-Hexa oxo-1,48-di(N-carbobenzoxy)-amino Octatetracontane. VI.

METHOD 1.

Ethyl chloroformate (0.55 gm, 5 mmole) was added dropwise to N-carbobenzoxy-tris-ϵ-amino-n-caproic acid (2.455 gm, 5 mmole) dissolved at -5°C in dry NN-dimethyl formamide (25 ml) and triethylamine (1 ml) and the mixture stirred for about 20 minutes. After this time hexamethylene diamine (0.290 gm, 2.5 mmole), dissolved in a small amount of water, was added to the reaction mixture. The pH of the solution was adjusted so that it always lay in the range from 8 to 9 by the addition, if necessary, of a few drops of 2 N sodium hydroxide solution and then the reaction stirred for about three hours during which time it was allowed slowly to regain room temperature.

The solid product was filtered off and recrystallised from NN-dimethyl formamide, washing the crystals with ethyl acetate before drying to yield VI (1.268 gm, 1.19 mmole, 48%) m.p. 204-205°C.

Found C, 65.31; H, 8.80; N, 10.61%.

$C_{58}H_{94}N_8O_{10}$ requires C, 65.50; H, 8.91; N, 10.54%.

Compound VI. METHOD 2.

Compound IV (ZCapBCapZ) (1.51 gm, 2.66 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C to remove its amine-protecting groups and the solid hydrobromide produced was dissolved in water and sufficient

dilute sodium hydroxide added to render the solution just basic.

To N-carbobenzoxy-bis-ε-amino-n-caproic acid (1.9 gm, 5 mmole) dissolved at -5°C in dry tetrahydrofuran (30 ml) and triethylamine (0.5 ml) was slowly added ethyl chloroformate (0.55 gm, 5 mmole) and the solution stirred for about 20 minutes. After this time the basic solution from above, also at -5°C, was slowly added together with further small quantities of 2 N sodium hydroxide in order to maintain the pH of the solution in the range between 8 and 9, and the reaction stirred for a further four hours, slowly allowing it to regain room temperature. The solid material produced was filtered and recrystallised from ethanol to yield VI (1.13 gm, 1.06 mmole, 40%) where the yield is calculated relative IV. The melting point of VI obtained was 192-193°C, (cf that obtained from Method 1.)

Found C, 65.28; H, 9.13; N, 10.51%.

$C_{58}H_{94}N_8O_{10}$ requires C, 65.51; H, 8.91; N, 10.54%.

7,14,21,28,35,42,49,56-Octa aza-6,13,20,27,36,43,50,57-Octa oxo-1,62-di(N-carbobenzoxy)amino Dohexacontane. VII.

Compound IV (ZCapBCapZ) (1.1 gm, 1.8 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C to remove the carbobenzoxy, amine-protecting groups. The solid hydrobromide produced was suspended in pure triethylamine (10 ml) and kept dry till required.

Ethyl chloroformate (0.3 gm, 2.6 mmole) was added dropwise to N-carbobenzoxy-tris-ε-amino-n-caproic acid (1.23 gm, 2.5 mmole)

dissolved in dry NN-dimethyl formamide (100 ml) and triethylamine (1 ml) at -10°C and the mixture stirred for 20 minutes. To this solution was then slowly added the suspension of the hydrobromide in triethylamine at -10°C that had been obtained above, and the whole reaction then stirred for a further 18 hours, allowing the temperature slowly to regain room temperature.

The solid product was filtered and purified by a fractional crystallisation from ethanol to yield VII (492 mgm, 0.381 mmole, 30%.) m.p. 206-208°C, the yield being calculated relative to the expected yield of hydrobromide from IV.

Found C, 65.06; H, 8.96; N, 10.74%.

$C_{70}H_{116}N_{10}O_{12}$ requires C, 65.19; H, 9.07; N, 10.86%.

Tetramethylene diamine-bis(N-carbobenzoxy -ε-amino-n-Caproic Acid) Amide. VIII.

Ethyl chloroformate (1.10 gm, 10 mmole) was added dropwise to N-carbobenzoxy-ε-amino-n-caproic acid (2.65 gm, 10 mmole) dissolved at -10°C in toluene (110 ml) and triethylamine (2 ml) and the mixture stirred for about 20 minutes.

Maintaining the temperature at -10°C a solution of tetramethylene diamine (0.44 gm, 5 mmole), dissolved in triethylamine (10 ml) was added slowly and the reaction stirred for a further 18 hours, allowing the temperature slowly to rise to the ambient value. The solid product was filtered off and recrystallised from ethanol to yield VIII (2.33 gm, 4 mmole, 80%) m.p. 173-174°C.

The analytical figures for compound VIII were:-

Found C, 66.16; H, 8.03; N, 9.65%.

$C_{32}H_{46}N_4O_6$ requires C, 65.96; H, 7.96; N, 9.61%.

7,14,19,26-Tetra aza-6,13,20,27-Tetra oxo-1,32-di(N-carbobenzoxy)amino Dotriacontane. IX.

Ethyl chloroformate (1.10 gm, 10 mmole) was added dropwise to N-carbobenzoxy-bis-ε-amino-n-caproic acid (3.79 gm, 10 mmole) dissolved in dry tetrahydrofuran at -10°C (50 ml) and triethylamine (2 ml), and the mixture stirred for 20 minutes. To this reaction, maintaining the temperature at -10°C , was added a solution of tetramethylene diamine (0.44 gm, 5 mmole) in triethylamine (10 ml) and the reaction then stirred for a further 18 hours, allowing it slowly to regain room temperature. After this period the solid material precipitated was filtered, and recrystallised from NN-dimethyl formamide, washing the crystals with ethyl acetate before drying them. The final yield obtained of IX was 2.77 gm, (3.45 mmole, 69%) m.p. 196-197°C.

Found C, 65.52; H, 8.37; N, 10.29%.

$C_{44}H_{68}N_6O_8$ reqires C, 65.32; H, 8.47; N, 10.39%.

7,14,21,26,33,40-Hexa aza-6,13,20,27,34,41-Hexa oxo-1,46-di(N-carbobenzoxy)amino Hexatetracontane. X.

Ethyl chloroformate (0.3 gm, 2.7 mmole) was added dropwise to N-carbobenzoxy-tris-ε-amino-n-caproic acid (1.23 gm, 2.5 mmole) dissolved at -10°C in NN-dimethyl formamide (15 ml) and triethylamine

(1 ml) and the reaction stirred for 20 minutes. To the mixture, maintaining the temperature at -10°C, was added a solution of tetramethylene diamine (0.11 gm, 1.25 mmole) dissolved in triethylamine (10 ml) and the reaction stirred for a further 18 hours. The solid material precipitated was filtered and recrystallised from NN-dimethylformamide, washing the crystals with ethyl acetate before drying them to yield X (778 mgm, 0.751 mmole, 60%) m.p. 205-206°C.

Found C, 64.74; H, 8.68; N, 10.99%.

$C_{56}H_{90}N_8O_{10}$ requires C, 64.96; H, 8.76; N, 10.82%.

7,14,21,28,33,40,47,54-Octa aza-6,13,20,27,34,41,48,55-Octa oxo-1,60-di (N-carbobenzoxy) amino Hexacontane. XI.

Compound VIII (ZCapTetCapZ) (1.1 gm, 1.8 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced suspended in triethylamine before being used as quickly as possible.

Ethyl chloroformate (0.3 gm, 2.6 mmole) was added dropwise to N-carbobenzoxy-tris-ε-amino-n-caproic acid (1.23 gm, 2.5 mmole) dissolved at -10°C in dry NN-dimethyl formamide (100 ml) and triethylamine (1 ml) and the reaction stirred vigorously for 20 minutes. Maintaining the temperature at -10°C the hydrobromide obtained above, and suspended in triethylamine, was added slowly and the reaction stirred for a further 18 hours, allowing the temperature slowly to rise to the room value. The solid materials precipitated were

filtered and recrystallised from NN-dimethyl formamide to yield XI (1.180 gm, 0.935 mmole, 52% relative to compound VIII hydrobromide) m.p. 212½-214°C.

Found C, 64.66; H, 8.70; N, 11.05%.

$C_{68}H_{112}N_{10}O_{12}$ requires C, 64.74; H, 8.95; N, 11.10%.

7,14,21,28,35,40,47,54,61,68-Deca aza-6,13,20,27,34,41,48,55,62,69-Deca oxo-1,74-di (N-carbobenzoxy) amino Tetraheptacontane, XII.

Compound IX ($ZCap_2TetCap_2Z$) (1.45 gm, 1.77 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced suspended in pure triethylamine ready to be used as quickly as possible for the next stage.

Ethyl chloroformate (0.3 gm, 2.7 mmole) was added dropwise and with vigorous stirring to N-carbobenzoxy-tris-ε-amino-n-caproic acid (1.23 gm, 2.5 mmole) dissolved at -10°C in dry NN-dimethyl formamide (100 ml) and triethylamine (1 ml) and the reaction continued for 20 minutes. After this time, and still maintaining the temperature at -10°C, the hydrobromide suspension in triethylamine, obtained above, was slowly added and the reaction continued for a further 18 hours, slowly allowing it to regain room temperature.

The solid material precipitated was filtered and recrystallised from ethanol by a fractional crystallisation procedure to yield XII (186 mgm, 0.125 mmole, 10%) m.p. 212-214°C.

Found C, 64.41; H, 9.09; N, 11.36%.

$C_{80}H_{134}N_{12}O_{14}$ requires C, 64.58; H, 9.08; N, 11.30%.

Preparation of Diazomethane.

To ether (300 ml), ethyl digol (45 ml) and 30% sodium hydroxide (60 ml) contained at 0°C in a round bottomed flask equipped with a side arm and cork stopper, was added Nitrosan (18 gm). The temperature of the flask and contents were cautiously raised from 0°C using a warm water bath and the diazomethane (0.07 mole) slowly distilled over in etherial solution, to be condensed and collected in a plain conical flask containing a little dry ether and maintained at 0°C. The etherial solution of diazomethane was prepared only just prior to its use.

Precautions.

Diazomethane is explosive and care should be taken during its preparation and subsequent use to ensure that various agents are not present which will initiate such an explosion. Boiling stones, ground glass apparatus, chipped or cracked apparatus, strong light and jolting of the apparatus should all be avoided as should any attempts at stirring the reaction.

Dicyclohexylethylamine.

To dicyclohexylamine (370 gm, 2.3 mole) heated to 90°C in a round bottomed flask well protected by a drying tube diethyl sulphate (320 gm, 2.08 mole) was added slowly over two hours and the mixture stirred thoroughly for a further 17 hours. After this time a very concentrated aqueous solution of potassium hydroxide (140 gm, 2.5 mole) was added, with stirring, to the flask. The amine layer was separated and the aqueous layer then extracted with

several portions of ether. The combined ether extracts were added to the amine layer and the total etherial solution dried by leaving it over solid potassium hydroxide for about 10 hours. The solution was then separated and fractionally distilled under reduced pressure, using a fine air leak to prevent uneven boiling. The yield of dicyclohexylethylamine obtained was 283 gm, (1.35 mole, 59%) b.p. 145° at 20 mm pressure.

Found C, 80.11; H, 12.94; N, 6.59%.

$C_{14}H_{27}N$ requires C, 80.38; H, 12.92; N, 6.70%.

Methyl-N-carbobenzoxy-ε-amino-n-Caproate. XIII.

METHOD 1.

To N-carbobenzoxy-ε-amino-n-caproic acid (13.2 gm, 50 mmole) dissolved in dry ether was added a slight excess of an etherial solution of diazomethane and the solution left to stand in a well ventilated fume-cupboard overnight. The solvent was then removed under reduced pressure and the liquid ester purified by eluting it down a column of grade IV acid washed Woelm alumina using a ratio of alumina to material of 50:1 and eluting with benzene/ether (9:1). The yield of pure ester XIII was 12.30 gm, (44 mmole, 88%.)

Found C, 64.80; H, 7.75; N, 5.039%.

$C_{15}H_{21}NO_4$ requires C, 64.50; H, 7.58; N, 5.01%.

Compound XIII. METHOD 2.

To N-carbobenzoxy-ε-amino-n-caproic acid (13.2 gm, 50 mmole) dissolved in either acetone or methanol was added freshly distilled

dimethyl sulphate (7.0 gm, 55 mmole) and dicyclohexylethylamine (16 gm, 75 mmole). The flask was heated on an open steam bath for about 2 hours and then left standing at room temperature for a further 18 hours after which the solvent was removed under reduced pressure and the mixture eluted down a column of grade IV acid washed alumina using a ratio of alumina to material of 50:1 and eluting with benzene/ether (9:1) as solvent. The yield of XIII was 12.30 gm,(44 mmole, 88%.)

Methyl N-carbobenzoxy-bis-ε-amino-n-caproate. XIV.

METHOD 1.

To N-carbobenzoxy-bis-ε-amino-n-caproic acid (3.79 gm, 10mmole) dissolved in sodium dried ether/sodium dried dioxane (1:1) was added an excess of an etherial solution of diazomethane and the solution left to stand overnight in a well ventilated fume-cupboard. The solvent was then removed under reduced pressure and the solid ester recrystallised from benzene to yield XIV (3.48 gm, 8.9 mmole, 89%.) m.p. 73-74°C.

Found C, 64.15; H, 8.29; N, 7.02%.

$C_{21}H_{32}N_2O_5$ requires C, 64.26; H, 8.22; N, 7.14%.

Compound XIV. METHOD 2.

To N-carbobenzoxy-bis-ε-amino-n-caproic acid (7.6 gm, 20mmole) dissolved in methanol was added a freshly distilled quantity of dimethyl sulphate (2.8 gm, 22 mmole) and dicyclohexylethylamine (8.0 gm, 35 mmole) and the mixture heated on an open steam-bath

for 2 hours. The solvent was then removed under reduced pressure and the crude reaction product eluted down a column of grade IV acid washed alumina using a ratio of alumina to material of 50:1 and eluting with benzene/ether (9:1) as solvent. The yield of XIV was 6.2 gm, (15.9 mmole, 80%.) m.p. 73-74°C.

Methyl N-carbobenzoxy-tris-ε-amino-n-Caproate. XV.

To N-carbobenzoxy-tris-ε-amino-n-caproic acid (2.5 gm, 5 mmole) dissolved in acetone was added dimethyl sulphate (0.70 gm, 5.5 mmole) and dicyclohexylethylamine (2.1 gm, 10 mmole) and the mixture heated on an open steam-bath for about 2 hours. The reaction was then left for 18 hours at room temperature before the solvent was removed under reduced pressure and the crude reaction product eluted down a column of grade IV acid washed alumina using a ratio of alumina to material of 50:1 and benzene/ether (9:1) as solvent. The final yield of XV was 2.15 gm, (4.25 mmole, 85%) m.p. 124-125°C.

Found C, 64.15; H, 8.63; N, 8.60%.

$C_{27}H_{43}N_3O_6$ requires C, 64.13; H, 8.57; N, 8.31%.

Para hydroxy azo Phenyl N-carbobenzoxy-ε-amino-n-Caproate. XVI.

To N-carbobenzoxy-ε-amino-n-caproic acid (2.65 gm, 10 mmole) dissolved in anhydrous ethyl acetate (40 ml) was added para hydroxy azo benzene (2.4 gm, 12 mmole). The calculated amount of dicyclohexylcarbodiimide (2.06 gm, 10 mmole) was added at 0°C and after maintaining the temperature of the solution at this value for half an hour it was slowly allowed to regain room temperature and the reaction stirred for a further 18 hours. The NN'-dicyclohexyl

urea which had separated out was filtered off and washed with small quantities of dry ethyl acetate. The filtrate and combined washings were then heated and the solvent removed under reduced pressure and the solid material obtained dissolved in chloroform and eluted down a column of grade III acid washed alumina using a ratio of alumina to material of 100:1 and eluting with chloroform throughout. The required ester was the first coloured material off the column and yielded XVI (2.68 gm, 6 mmole, 60%) m.p. 97-98°C.

Found C, 70.11; H, 6.14; N, 9.35%.

$C_{26}H_{27}N_3O_4$ requires C, 70.10; H, 6.11; N, 9.43%.

Adipic Acid-bis-(Methyl-ε-amino-n-Caproate) Amide. XVII.

METHOD 1.

Methyl N-carbobenzoxy-ε-amino-n-caproate (2.80 gm, 10 mmole) was treated with hydrogen bromide and glacial acetic acid as described in the reaction system C and the solid hydrobromide obtained suspended in triethylamine ready for use as soon as possible afterwards.

Into the same flask was added adipic acid (0.732 gm, 5 mmole), dry NN-dimethyl formamide and sufficient triethylamine to make it in a reasonable excess over the 20 mmole required for neutralisation in the reaction. The flask was then cooled to -5°C and the whole reaction stirred. To the flask was then added, over a period of one minute, phosphorus oxychloride (1.535 gm, 10 mmole) and very dry triethylamine (0.2 gm, 20 mmole) in very dry NN-dimethyl formamide and the reaction stirred for a further 2 hours.

After two hours the mixture was filtered and to the solution of the precipitated triethylammonium salts was added water (10 ml). The solvent was then removed under vacuum, 10 ml of water added and the solution extracted three times with ethyl acetate and the combined extracts washed with water and sodium bicarbonate till no further effervescence occurred. The ethyl acetate solution was finally washed till the washings were neutral, dried over sodium sulphate, filtered and then evaporated to dryness. The white solid product was recrystallised from ethyl acetate to yield XVII (60 mgm, 0.15 mmole, 3%) m.p. 122-122½°C.

Found C, 60.29; H, 8.94; N, 7.256%.

$C_{20}H_{36}N_2O_6$ requires C, 59.98; H, 9.06; N, 6.99%.

Compound XVII. METHOD 2.

Methyl N-carbobenzoxy-ε-amino-n-caproate (3.8 gm, 13.6 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced dissolved in dilute sodium hydroxide till the resulting solution had a pH in the range between 8 and 9.

Ethyl chloroformate (1.2 gm, 12 mmole) was added dropwise and with stirring to a solution, at -5°C, of adipic acid (0.73 gm, 5 mmole) in tetrahydrofuran (30 ml) and triethylamine (1 ml) and the reaction stirred for about 20 minutes. The basic solution from above was then slowly added, maintaining the temperature at -5°C and the pH in the range between 8 and 9 and the reaction was then

stirred for a further 3 hours, slowly allowing it to regain room temperature. The solid material precipitated during the course of this reaction was then filtered, the solvent removed from the filtrate under reduced pressure and the total solid material recrystallised from ethyl acetate to yield XVII (1.025 gm, 25.6 mmole, 51%) m.p. 121½-122°C.

Compound XVII. METHOD 3.

Methyl N-carbobenzoxy-ε-amino-n-caproate (7.5 gm, 27 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide obtained suspended in triethylamine (10 ml) ready for use as soon afterwards as possible.

Ethyl chloroformate (2.4 gm, 22 mmole) was added dropwise to adipic acid (1.46 gm, 10 mmole) dissolved at -10°C in dry tetrahydrofuran (50 ml) and triethylamine (1 ml) and the mixture stirred for about 20 minutes. The suspension of the hydrobromide in triethylamine obtained above was then slowly added, maintaining the temperature at -10°C and the reaction stirred for a further 18 hours, slowly allowing it to regain room temperature. The product and triethylammonium salts precipitated were filtered and recrystallised from ethyl acetate to yield XVII (2.8 gm, 7.0 mmole, 70%) m.p. 122-122½°C.

Found C, 59.91; H, 9.08; N, 7.19%.

$C_{20}H_{36}N_2O_6$ requires C, 59.98; H, 9.06; N, 6.99%.

7,14,19,26-Tetra oxo-6,13,20,27-Tetra aza-Dotriacontane-1,32 Dicarboxylic acid Dimethyl ester. XVIII.

Methyl N-carbobenzoxy-bis-ϵ-amino-n-caproate (2.0 gm, 50 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced suspended in triethylamine (10 ml) ready for use as soon afterwards as possible.

Ethyl chloroformate (430 mgm, 4 mmole) was added dropwise and with stirring, to a solution of adipic acid (290 mgm, 2 mmole) dissolved at -10°C in dry tetrahydrofuran (50 ml) and triethylamine (1 ml) and the reaction stirred for 20 minutes. To this mixture was then added the suspension of hydrobromide in triethylamine obtained above, maintaining the temperature at -10°C, and the whole reaction stirred for a further 18 hours, allowing it slowly to regain room temperature.

The solid material precipitated was filtered off and recrystallised from ethanol to yield XVIII (0.856 gm, 1.36 mmole, 68%) m.p. 174-175°C.

Found C, 61.09; H, 9.04; N, 8.758%.

$C_{32}H_{58}N_4O_8$ requires C, 61.32; H, 9.33; N, 8.94%.

7,14,21,26,33,40-Hexa oxo-6,13,20,27,34,41-Hexa aza-Hexatetracontane-1,46-Dicarboxylic acid Dimethyl Ester. XIX.

METHOD 1.

Methyl N-carbobenzoxy-tris-ϵ-amino-n-caproate (330 mgm, 0.65 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide obtained dissolved in just sufficient dilute aqueous sodium hydroxide to render the solution basic, with a pH in the range between 8 and 9.

To adipic acid (29 mgm, 0.2 mmole) dissolved at -5°C in tetrahydrofuran (20 ml) and triethylamine (2 drops) was added ethyl chloroformate (45 mgm, 0.4 mmole) and the reaction stirred for 20 minutes. Maintaining the temperature of the solution at -5°C, the basic solution obtained above was slowly added, also maintaining the pH in the range 8 to 9 by the addition of small quantities of dilute aqueous sodium hydroxide if necessary. The reaction was then stirred for a further 3 hours , slowly allowing it to regain room temperature. The solid material precipitated during the course of the reaction was filtered and the filtrate heated to remove the solvent under reduced pressure. The total solid material obtained was recrystallised from ethanol to yield XIX (90 mgm, 0.155 mmole, 53%) m.p. 200-201°C.

Compound XIX. METHOD 2.

Methyl N-carbobenzoxy-tris-ϵ-amino-n-caproate (1.6 gm, 3.2 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide

produced suspended in triethylamine (10 ml) ready for use as soon afterwards as possible.

Ethyl chloroformate (230 mgm, 2 mmole) was added dropwise and with stirring to a solution at -10°C of adipic acid (146 mgm, 1 mmole) in dry tetrahydrofuran (50 ml) and triethylamine (1 ml) and the mixture stirred for about 20 minutes. To this mixture was then added the suspension of the hydrobromide in triethylamine obtained above, maintaining the temperature at -10°C, and the whole mixture stirred for a further 18 hours, during which time it was slowly allowed to regain room temperature.

The solid material precipitated during the course of the reaction was filtered off and recrystallised from ethanol to yield XIX (618 mgm, 0.725 mmole, 72%.) m.p. 200-201°C.

Found C, 61.77; H, 9.24; N, 9.85%.

$C_{44}H_{80}N_6O_{10}$ requires C, 61.93; H, 9.45; N, 9.85%.

7,14,27,28,33,40,47,54-Octa oxo-6,13,20,27,34,41,48,55-Octa aza-Hexacontane-1,60 Dicarboxylic Acid Dimethyl Ester. XX.

Methyl N-carbobenzoxy-tris-ϵ-amino-n-caproate (400 mgm, 0.79 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced suspended in triethylamine (10 ml) ready for use as soon as possible afterwards.

Ethyl chloroformate (66 mgm, 0.6 mmole) was added, with stirring, to a solution at -10°C of compound XXV (HOCapACapOH) (93 mgm, 0.25 mmole) in NN-dimethyl formamide (50 ml) and

triethylamine (2 ml) and the reaction stirred for 20 minutes. The suspension of the hydrobromide in triethylamine obtained above was then added, maintaining the temperature at -10°C. The reaction was then stirred for a further 18 hours, allowing it slowly to regain room temperature.

The solid material precipitated during the course of the reaction was filtered and recrystallised from ethanol to yield XX (149.8 mgm, 0.139 mmole, 56%) m.p. 214-215½°C.

Found C, 62.07; H, 9.46; N, 10.68%.

$C_{56}H_{102}N_8O_{12}$ requires C, 62.31; H, 9.53; N, 10.38%.

Suberic Acid-bis-(Methyl-ε-amino-n-Caproate) Amide. XXI.

Methyl N-carbobenzoxy-ε-amino-n-caproate (3.7 gm, 13.5 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced suspended in triethylamine (10 ml) ready for use as soon afterwards as possible.

To suberic acid (871 mgm, 5 mmole) dissolved at -10°C in dry tetrahydrofuran (25 ml) and triethylamine (1 ml) was added dropwise and with stirring, ethyl chloroformate (1.2 gm, 11 mmole) and the reaction stirred for 20 minutes. The hydrobromide suspension in triethylamine obtained above was then added, maintaining the temperature at -10°C and the reaction continued for a further 18 hours, allowing it slowly to regain room temperature.

The solid material precipitated during the course of the reaction was filtered off, the filtrate heated to remove the solvent under reduced pressure, and the total solid material obtained

recrystallised from ethyl acetate to yield XXI (1.023 gm, 2.4 mmole, 48%) m.p. 110½-113°C.

Found C, 61.87; H, 9.29; N, 6.66%.

$C_{22}H_{40}H_2O_6$ requires C, 61.66; H, 9.41; N, 6.54%.

7,14,21,28-Tetra oxo-6,13,22,29-Tetra aza-Tetratriacontane-1,34-Dicarboxylic Acid Dimethyl Ester. XXII.

Methyl N-carbobenzoxy-bis-ε-amino-n-caproate (2.6 gm, 7 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced suspended in triethylamine (10 ml) ready for use as soon afterwards as possible.

To suberic acid (436 mgm, 2.5 mmole) dissolved at -10°C in dry tetrahydfofuran (15 ml) and triethylamine (1 ml) was added dropwise and with stirring, ethyl chloroformate (550 mgm, 5 mmole) and the mixture stirred for about 20 minutes. After this the suspension of the hydrobromide in triethylamine obtained above was added, maintaining the temperature at -10°C, and the reaction continued for a further 18 hours, allowing it slowly to regain room temperature.

The solid material precipitated during the course of the reaction was filtered off and the solvent removed from the filtrate under reduced pressure. The total solid material thus obtained was recrystallised from ethanol to yield XXII (997 mgm, 1.52 mmole, 61%) m.p. 166-167°C.

Found C, 62.26; H, 9.29; N, 8.68%.

$C_{34}H_{62}N_4O_8$ requires C, 62.36; H, 9.54; N, 8.56%.

7,14,21,28,35,42-Hexa oxo-6,13,20,29,36,43-Hexa aza-Octatetracontane-1,48-Dicarboxylic Acid Dimethyl Ester. XXIII.

Methyl N-carbobenzoxy-tris-ε-amino-n-caproate (1.9 gm, 4 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide produced suspended in triethylamine (10 ml) ready for use as soon afterwards as possible.

To suberic acid (174 mgm, 1 mmole) dissolved at -10°C in dry tetrahydrofuran (10 ml) and triethylamine (1 ml) was added ethyl chloroformate (220 mgm, 2 mmole) and the mixture stirred for 20 minutes. The suspension of the hydrobromide in triethylamine obtained above was then added, care being taken to maintain the temperature at -10°C. The whole reaction was then stirred for a further 18 hours, slowly allowing the temperature to regain room temperature.

The solid material precipitated during the course of the reaction was filtered, the solvent removed, under reduced pressure, from the filtrate and the total solid material recrystallised from ethanol to yield XXIII (608 mgm, 0.69 mmole, 69%.) m.p. 197½-198½°C.

Found C, 62.99; H, 9.45; N, 9.66%.

$C_{46}H_{84}N_6O_{10}$ requires C, 62.70; H, 9.59; N, 9.54%.

7,14,21,28,35,42,49,56-Octa oxo-6,13,20,27,36,43,50,57-Octa aza-Dohexacontane—1,62-Dicarboxylic Acid Dimethyl Ester. XXIV.

Methyl N-carbobenzoxy-tris-ε-amino-n-caproate (850 mgm, 2 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C and the solid hydrobromide obtained suspended in pure triethylamine ready for use as soon as possible afterwards.

To compound XXIX (HOCapSubCapOH) (200 mgm, 0.5 mmole) dissolved at -10°C in dry NN-dimethyl formamide (100 ml) and triethylamine (1 ml) was added, dropwise and with stirring, ethyl chloroformate (110 mgm, 1 mmole) and the mixture stirred for 20 minutes. The hydrobromide suspension in triethylamine obtained above was then slowly added, maintaining the temperature at -10°C and the whole reaction then stirred for a further 18 hours.

The solid material precipitated during the course of the reaction was filtered, the solvent was removed from the filtrate under reduced pressure and the total solid material obtained recrystallised from NN-dimethyl formamide, washing the crystals with ethyl acetate before drying them to yield XXIV (376 mgm, 0.34 mmole, 68%) m.p. 213-215°C.

Found C, 63.30; H, 9.66; N, 10.16%.

$C_{58}H_{106}N_8O_{10}$ requires C, 62.90; H, 9.65; N, 10.12%.

Adipic Acid-bis(-ε-amino-n-Caproic Acid) Amide. XXV.

METHOD 1.

Compound XVII (MeOCapACapOMe) (450 mgm, 1.12 mmole) was added to a conical flask containing a solution of barium hydroxide in anhydrous methanol (30ml of a 0.1 N solution) and the flask tightly stoppered. The flask was left at room temperature, with occasional swirling, for 5 days to allow the reaction to go to completion, and then the contents filtered.

The solid barium salt was dissolved in water, the solution reddered acidic by the addition of dilute hydrochloric acid, and the solution extracted several times with ethyl acetate. The combined extracts were washed with a little water, dried over anhydrous sodium sulphate, filtered, and the solution evaporated to dryness. The solid product was recrystallised from ethyl acetate, which had been saturated with water at room temperature, to yield XXV (350 mgm, 0.94 mmole, 84%) m.p. 146-147°C.

Found C, 58.06; H, 8.85; N, 7.404%.

$C_{18}H_{32}N_2O_6$ requires C, 58.05; H, 8.66; N, 7.52%.

Compound XXV. METHOD 2.

Adipic acid (0.73 gm, 5 mmole) was dissolved at -5°C in tetrahydrofuran (25 ml) and triethylamine (0.5 ml) and ethyl chloroformate (1.10 gm, 10 mmole) slowly added dropwise and with stirring and the reaction left for 20 minutes. After this time, ε-amino-n-caproic acid (1.319 gm, 10 mmole) was added,

dissolved in water, and the reaction, being maintained at -5°C, was stirred for a further two hours, after which it was slowly allowed to regain room temperature while being stirred for a further period of 4 hours.

On removal of the solvent under reduced pressure a white crystalline residue remained which was recrystallised from ethyl acetate, saturated with water at room temperature. The final traces of adipic acid required to be removed by sublimation under high vacuum at 100°C to yield, finally, XXV (0.64 gm, 0.177 mmole, 35%) m.p. 146°C.

7,14,19,26-Tetra oxo-6,13,20,27-Tetra aza—Dotriacontane-1,32-Dicarboxylic Acid. XXVI.

METHOD 1.

$MeOCap_2ACap_2OMe$ (Compound XVIII) (209 mgm, 0.33 mmole) was dissolved in anhydrous methanol under reflux and a solution of barium hydroxide in anhydrous methanol (10 ml of a 0.1 N solution) added. The top of the condenser was protected by an efficient drying tube. The reaction was left stirring under reflux for 12 hours before being cooled, and the solid barium salt filtered.

The salt was dissolved in a little hot water and the solution acidified with dilute aqueous hydrochloric acid. On cooling solid product was obtained which was recrystallised from a mixture of ethanol and ethyl acetate to yield XXVI (36 mgm, 0.06 mmole 18%) m.p. 166-168°C.

The original methanolic solution filtrate, on removal of

the solvent under reduced pressure, and the subsequent recrystallisation of the solid from ethanol yielded starting material (compound XVIII) (116 mgm, 55%).

Compound XXVI. METHOD 2.

To compound XVIII ($MeOCap_2ACap_2OMe$) (209 mgm, 0.33 mmole) dissolved at 70°C in NN-dimethyl formamide, a solution of sodium hydroxide in ethanol (ca. 5 N) was very slowly added dropwise and with vigorous stirring. A crystalline precipitate appeared and eventually the further addition of base resulted in the immediate release of volatile amines.

At this point the flask was cooled to room temperature and the solids formed filtered off and washed with a little warm ethanol to remove any excess base present. The solid was then dissolved in water, the hot solution acidified with dilute hydrochloric acid and allowed to cool. The desired product crystallised out, was filtered off and recrystallised from a mixture of ethanol and ethyl acetate to yield XXVI (138 mgm, 0.23 mmole, 70%) m.p. 166-168°C.

Found C, 60.60; H, 8.78; N, 9.417%.

$C_{30}H_{54}N_4O_8$ requires C, 60.18; H, 9.09; N, 9.36%.

7,14,21,26,33,40-Hexa oxo-6,13,20,27,34,41-Hexa aza-Hexatetracontane-1,46-Dicarboxylic Acid. XXVII.

METHOD 1.

To compound XIX ($MeOCap_3ACap_3OMe$) (250 mgm, 0.29 mmole) dissolved in NN-dimethyl formamide at 70°C, sodium hydroxide dissolved in ethanol (ca. 5 N) was added very slowly dropwise and with vigorous stirring. A crystalline precipitate appeared and the addition was continued till, on further addition of base there was merely the sudden evolution of volatile bases.

The flask was then cooled and its contents filtered and the solid materials washed with a little warm ethanol. The white sodium salt was suspended in dimethyl sulphoxide at 80°C, acidified with dilute hydrochloric acid and poured into a mixture of carbon tetrachloride and water. The product formed was filtered off and recrystallised from NN-dimethyl formamide to yield XXVII (83 mgm, 0.1 mmole, 35%) m.p. 187-189°C.

Found C, 61.41; H, 9.29; N, 10.30%.

$C_{42}H_{76}N_6O_{10}$ requires C, 61.14; H, 9.28; N, 10.19%.

Compound XXVII. METHOD 2.

To compound XIX ($MeOCap_3ACap_3OMe$) (250 mgm, 0.29 mmole) dissolved at 80°C in dimethyl sulphoxide was added, all at once, and with vigorous stirring, a slight excess of a concentrated (ca. 5 N) solution of sodium hydroxide in normal butanol and the heating and stirring continued for no longer than 2 minutes before

the contents of the flask were filtered under suction and the sodium salt suspended in dimethyl sulphoxide at 80°C. This suspension was acidified with dilute hydrochloric acid and the heating continued until all of the sodium salt had dissolved in the acidified dimethyl sulphoxide. After allowing this solution to cool, the solid product was removed by filtration and recrystallised from NN-dimethyl formamide to yield XXVII (197 mgm, 0.239 mmole, 82%) m.p. 188-189°C.

7,14,21,28,33,40,47,54-Octa oxo-6,13,20,27,34,41,48,55-Octa aza-Hexacontane-1,60-Dicarboxylic Acid. XXVIII.

To compound XX ($MeOCap_4ACap_4OMe$) (400 mgm, 0.37 mmole) dissolved in dimethyl sulphoxide at 65°C, a strong (ca. 5 N) solution of sodium hydroxide in normal butanol was added all at once and in a slight excess, and the reaction stirred very vigorously for 2 minutes.

The contents of the flask were filtered under suction and the sodium salt suspended in NN-dimethyl formamide at 80°C. To this was added dilute hydrochloric acid and the heating continued until the complete solution of the sodium salt had occurred. After cooling, the solid product was filtered and recrystallised from NN-dimethyl formamide to yield XXVIII (198 mgm, 0.188 mmole 65%) m.p. 200-201°C.

Found C, 61.24; H, 9.43; N, 10.67%.

$C_{54}H_{98}N_8O_{12}$ requires C, 61.69; H, 9.40; N, 10.66%.

Suberic Acid-bis-(ε-amino-n-Caproic Acid) Amide. XXIX.

Compound XXI (MeOCapSubCapOMe) (428 mgm, 1 mmole) was added to a conical flask containing a 0.1 N solution of barium hydroxide in anhydrous methanol (50 ml) and the flask tightly stoppered. The flask was left for 5 days with occasional swirling after which time the solid salt precipitated was filtered off and the filtrate heated to remove the solvent under reduced pressure. The total solid material obtained was dissolved in hot water and the solution acidified with dilute hydrochloric acid. On cooling the solid product obtained was filtered off and recrystallised from ethyl acetate, which had been saturated with water at room temperature, to yield XXIX (312 mgm, 0.78 mmole, 78%) m.p. 138-140°C.

Found C, 59.91; H, 9.07; N, 7.15%.

$C_{20}H_{36}N_2O_6$ requires C, 59.98; H, 9.06; N, 6.99%.

7,14,21,28-Tetra oxo-6,13,22,29-Tetra aza-Tetratriacontane-1,34-Dicarboxylic Acid. XXX.

To compound XXII ($MeOCap_2SubCap_2OMe$) (218 mgm, 0.33 mmole) dissolved in NN-dimethyl formamide at 100°C a strong (ca. 5 N) solution of sodium hydroxide in ethanol was added very slowly and with vigorous stirring until on further addition of base, there was merely the sudden release of volatile amines. At this point the solution was heated and stirred for a further 30 minutes before being filtered and the solid sodium salt obtained washed with a little warm ethanol to remove any excess base or solvent and the

filtrate heated under reduced pressure to remove the solvent.

The combined solid materials were dissolved in hot water and the solution acidified with dilute hydrochloric acid. The solid product obtained by cooling this solution was recrystallised from ethanol to yield XXX (162 mgm, 0.258 mmole, 74%)
m.p. 173-174°C.

Found C, 61.37; H, 9.06; N, 9.12%.

$C_{32}H_{58}N_4O_8$ requires C, 61.32; H, 9.33; N, 8.94%.

7,14,21,28,35,42-Hexa oxo-6,13,20,29,36,43-Hexa aza-Octatetracontane-1,48-Dicarboxylic Acid. XXXI.

METHOD 1.

Th compound XXIII ($MeOCap_5SubCap_3OMe$) (220 mgm, 0.25 mmole) dissolved in NN-dimethyl formamide at 80°C was added, very slowly, a concentrated (ca. 5 N) solution of sodium hydroxide in ethanol, and the solution stirred very vigorously. The addition of the base was continued till with further addition, only the evolution of volatile amines occurred. The heating was continued, after this, for a further half Hour before the flask was cooled and filtered. The solvent was removed from the filtrate under reduced pressure and the combined solids suspended in dimethyl sulphoxide at 80°C. This was acidified with dilute hydrochloric acid and the heating continued till the solution was complete. It was then poured into an excess of carbon tetrachloride and the solid material obtained filtered, dried and recrystallised from NN-dimethyl formamide,

washing the crystals with ethyl acetate to remove the final traces of solvent, before drying to yield XXXI (68 mgm, 0.08 mmole, 30%) m.p. 192-193°C.

Found C, 61.77; H, 9.56; N, 10.07%.

$C_{44}H_{80}N_6O_{10}$ requires C, 61.94; H, 9.45; N, 9.85%.

Compound XXXI. METHOD 2.

To compound XXIII ($MeOCap_3SubCap_3OMe$) (370 mgm, 0.42 mmole) dissolved at 65°C in dimethyl sulphoxide was added, all at once, and with vigorous stirring, a slight excess of a concentrated (ca. 5 N) solution of sodium hydroxide in normal butanol and the reaction allowed to continue for 2 minutes before the solution was filtered.

The solid sodium salt produced was suspended in dimethyl sulphoxide at 80°C and this acidified with dilute hydrochloric acid. The heating was continued till the solution of the materials was complete and then the mixture cooled. The solid product was filtered and recrystallised from NN-dimethyl formamide, washing the crystals with ethyl acetate to remove the final traces of solvent, before drying them to yield XXXI (282 mgm, 0.33 mmole, 80%) m.p. 192-193°C.

7,14,21,28,35,42,49,56-Octa oxo-6.13,20,27,36,43,50,57-Octa aza-Dohexacontane-1,62-Dicarboxylic Acid. XXXII.

To compound XXIV ($MeOCap_4SubCap_4OMe$) (450 mgm, 0.4 mmole) dissolved in dimethyl sulphoxide at 65°C was added, all at once and with very vigorous stirring, a slight excess of a concentrated

(ca. 5 N) solution of sodium hydroxide in normal butanol and after stirring for only 2 minutes, the solution was filtered. The sodium salt was suspended in an acidified mixture of water and NN-dimethyl formamide and the mixture heated till solution was completed. On cooling this solution the product crystallised out and it was filtered off and recrystallised from NN-dimethyl formamide, the crystals being washed with ethyl acetate before drying, to yield XXXII (248 mgm, 0.23 mmole, 58%) m.p. 200-201°C.

Found C, 62.27; H, 9.69; N, 10.30%.

$C_{54}H_{98}N_8O_{12}$ requires C, 62.31; H, 9.53; N, 10.38%.

Caesium Hydroxide.

An aqueous solution of caesium hydroxide was eluted down a column of an Amberlite resin in the -OH form. The elution, with water, was continued slowly and a 0.05 N solution of caesium hydroxide obtained.

The Caesium Salts of Compounds XVIII and XIX.

Compound XVIII (50 mgm, 0.084 mmole) was dissolved in hot water and the calculated quantity of the aqueous solution of caesium hydroxide added. The solution was concentrated under reduced pressure and attempts made to form crystals of a size suitable for use in X-ray studies.

Compound XIX (100 mgm, 0.121 mmole) was dissolved in hot water and the calculated quantity of the aqueous solution of caesium hydroxide added. The solution was concentrated under reduced pressure and attempts made to form crystals of a size suitable for use in X-ray studies.

3,10,17,24,31,38,45,52,59,66-Deca Aza-2,9,16,23,30,39,46,53,60,67-Deca Oxo-1,68-Di-(Indolyl-3'-Methyl)-1,68-Di-(N-Carbobenzoxy)-Amino-Octa Hexacontane. XXXV.

Compound XI ($ZCap_4TetCap_4Z$) (98 mgm, 0.75 mmole) was treated with hydrogen bromide in glacial acetic acid as described in the reaction system C, to remove the carbobenzoxy amine-protecting groups. The solid hydrobromide produced was suspended in pure triethylamine (10 ml) and kept dry till it was required.

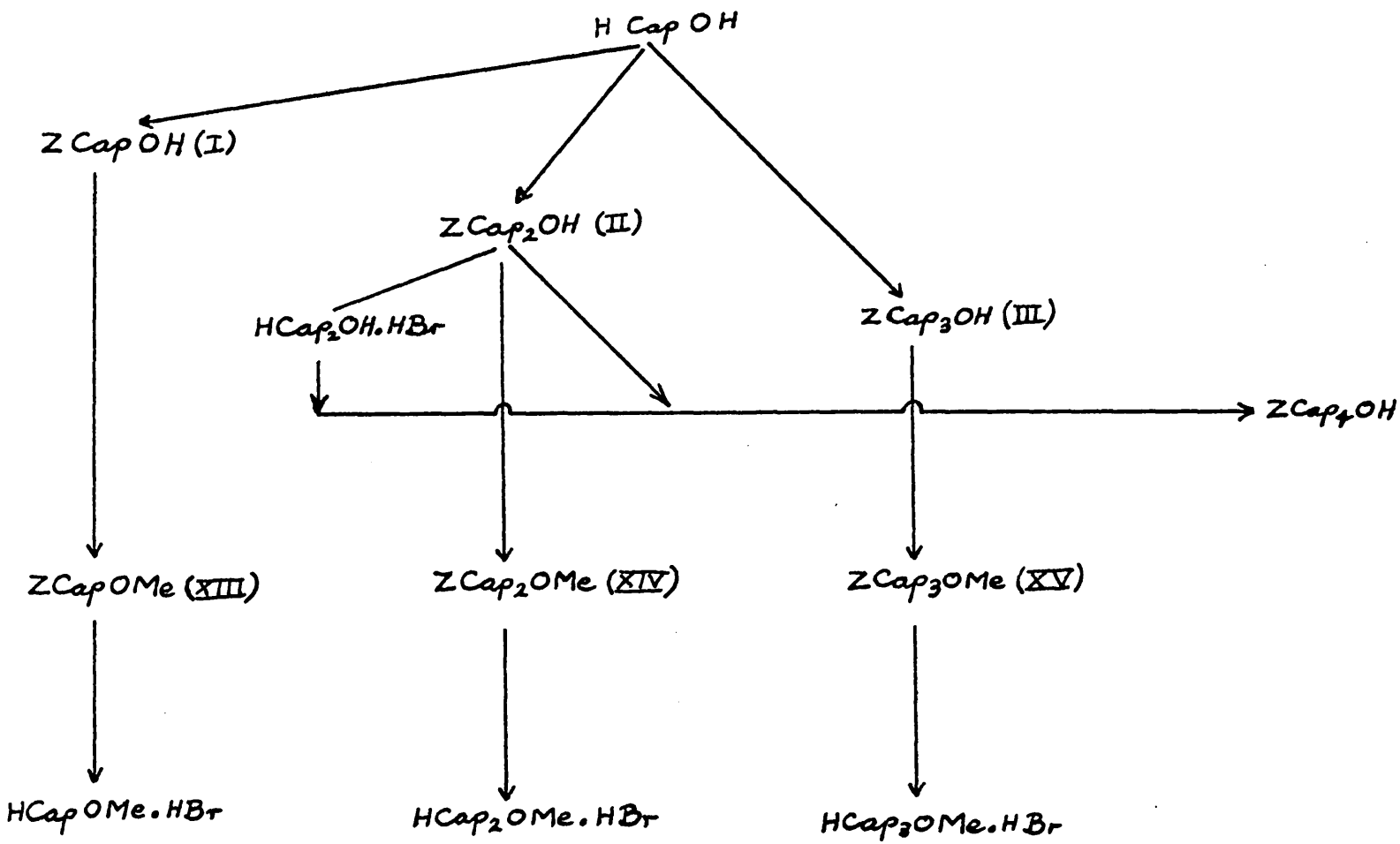
Ethyl chloroformate (11 mgm, 0.1 mmole) was added dropwise to N-carbobenzoxy tryptophan (31.5 mgm, 0.1 mmole) dissolved, at -10°C, in dry tetrahydrofuran (50 ml) and triethylamine (1 ml) and the mixture stirred for about 20 minutes.

To this mixture was then slowly added the suspension of the hydrobromide in triethylamine obtained above, the temperature of the solution being maintained at -10°C. The reaction was then continued for a further 18 hours, slowly allowing it to regain room temperature.

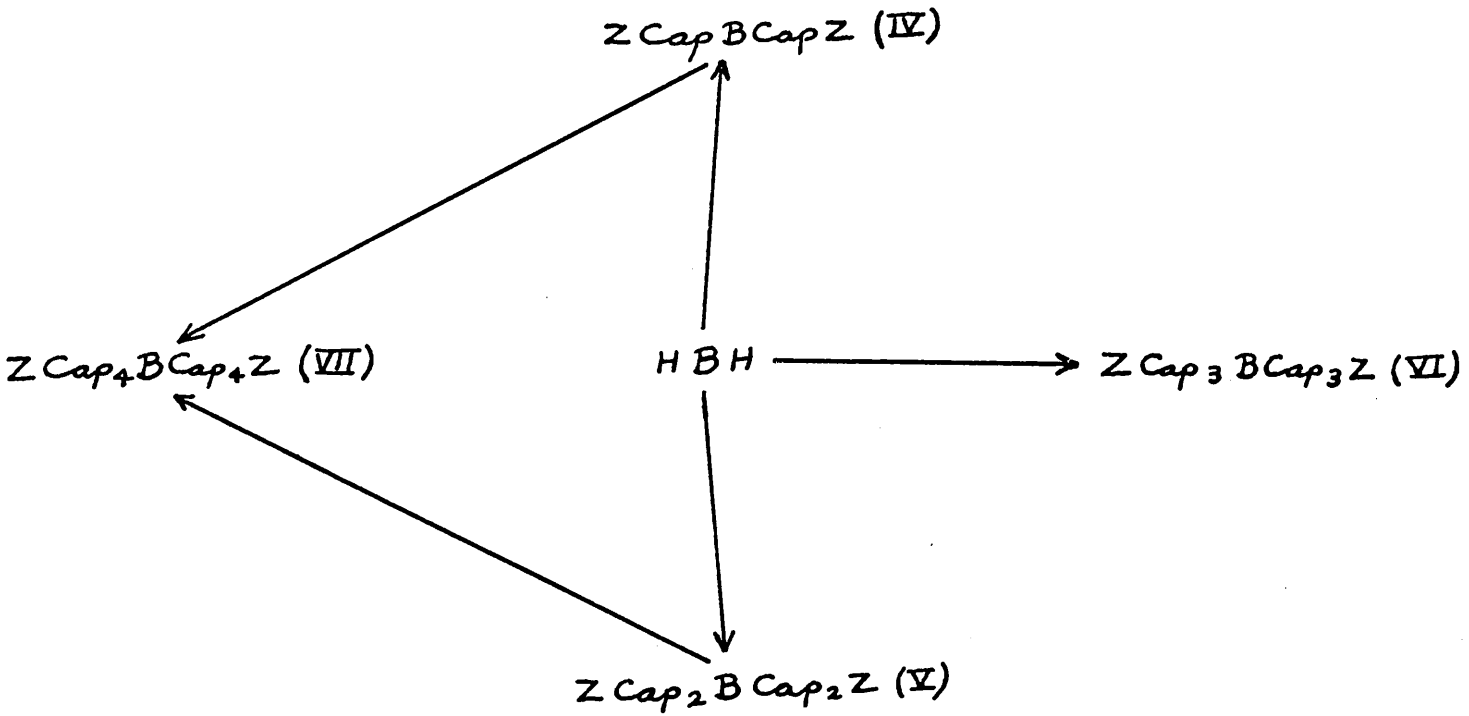
The solid produced during the course of the reaction was filtered, the solvent removed from the filtrated hy heating it under reduced pressure, and the total solid material thus obtained recrystallised from ethanol.

It has not yet been possible to obtain material which is thought to be pure enough to give analytical figures consistent with those required, but the infra-red spectrum of the material is consistant with that expected. The highest melting point so far obtained is 212-213°C.

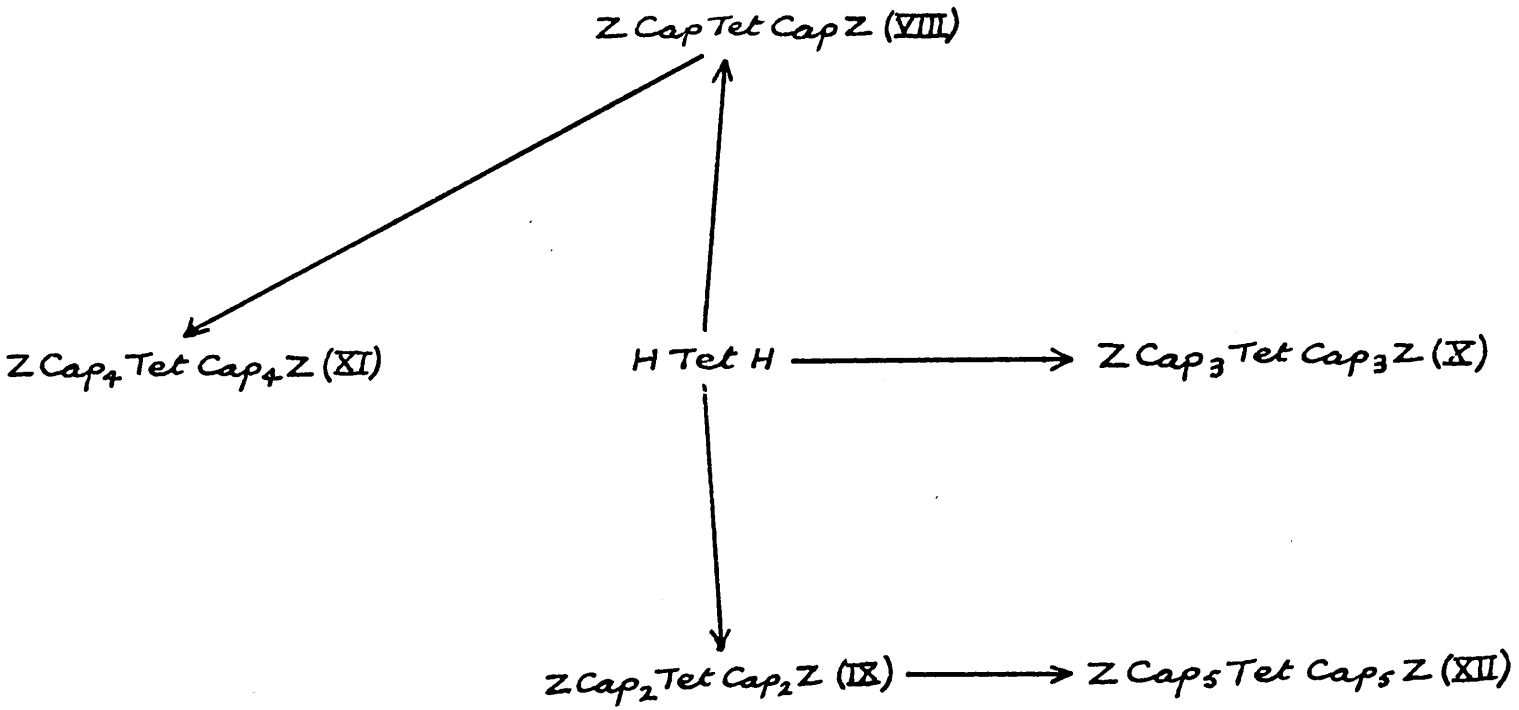
Flow Sheet One.



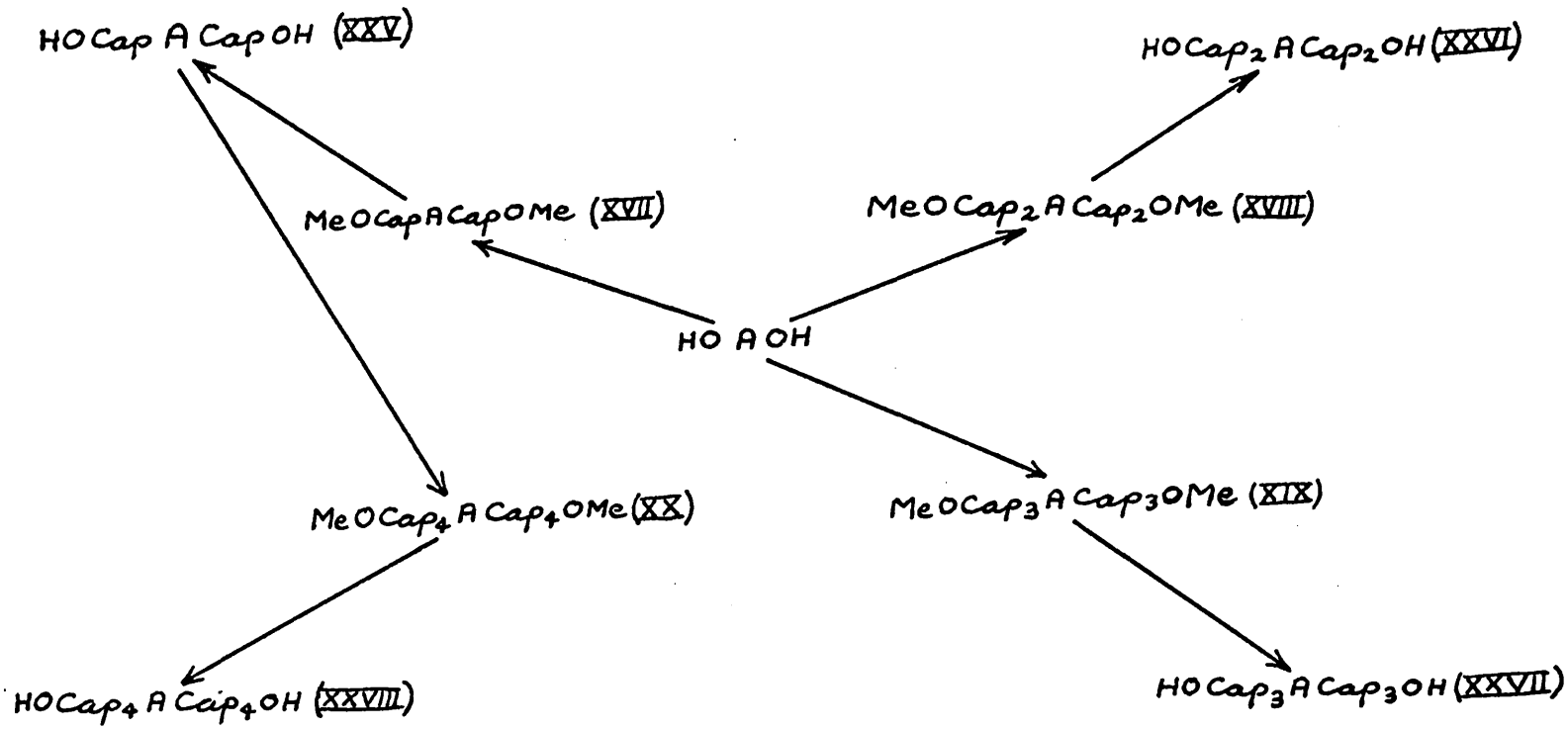
Flow Sheet Two.



Flow Sheet Three.



Flow Sheet Four.



An identical flow sheet can be produced for the series of linear oligoamides starting with Suberic Acid.

Table 1. Abbreviations Used.

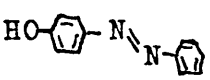
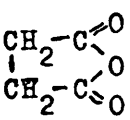
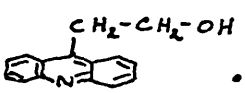
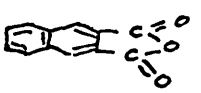
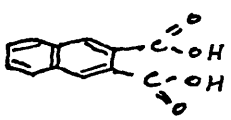
Compound. Full Name.	Abbreviated Form.	Formula.
Carbobenzoxy Chloride.	Z-Cl.	C_6H_5OCOCl.
ϵ-amino-n-Caproic Acid.	H-Cap-OH.	$NH_2(CH_2)_5COOH$.
Hexamethylene Diamine.	H-B-H.	$NH_2(CH_2)_6NH_2$.
Tetramethylene Diamine.	H-Tet-H.	$NH_2(CH_2)_4NH_2$.
Adipic Acid.	HO-A-OH.	$HOOC(CH_2)_4COOH$.
Suberic Acid.	HO-Sub-OH.	$HOOC(CH_2)_6COOH$.
Para-Hydroxy-Azo Benzene.	$H-_{p\text{-}OH}Azo$.	
Succinic Anhydride.	SucO	
Succinic Acid.	HO-Suc-OH.	$HOOC(CH_2)_2COOH$.
5-(1'-Hydroxy-Ethyl) Acridine.	$H\text{-}O(CH_2)_2\text{-}5\text{-}Acr$.	
Naphthalene-2,3- Dicarboxylic Acid Anhydride.	NapO	
Naphthalene-2,3- Dicarboxylic Acid.	HO-Nap-OH.	

Table 1. Cont^{d}.

Compound Number.	Full Name.	Abbreviated Form.	Formula.
I.	N-carbobenzoxy-ϵ-amino-n-Caproic Acid.	ZCapOH.	$C_6H_5CH_2OCONH(CH_2)_5COOH$.
II.	N-carbobenzoxy-bis-ϵ-amino-n-Caproic Acid.	$ZCap_2OH$.	$C_6H_5CH_2OCO[NH(CH_2)_5CO]_2OH$.
III.	N-carbobenzoxy-tris-ϵ-amino-n-Caproic Acid.	$ZCap_3OH$.	$C_6H_5CH_2OCO[NH(CH_2)_5CO]_3OH$.
IV.	Hexamethylene diamine-bis-(N-carbobenzoxy-ϵ-amino-n-Caproic Acid) Amide.	ZCapBCapZ.	$C_6H_5CH_2OCONH(CH_2)_5CONH(CH_2)_6NHCO(CH_2)_5NH.OCOCH_2C_6H_5$.
V.	7,14,21,28-Tetra aza-6,13,22,29-tetra oxo-1,34-di-(N-carbobenzoxy) amino-tetratriacontane.	$ZCap_2BCap_2Z$.	$C_6H_5CH_2OCO.[NH(CH_2)_5CO]_2NH(CH_2)_6NH[CO(CH_2)_5NH]_2OCOCH_2C_6H_5$.
VI.	7,14,21,28,35,42-Hexa aza-6,13,20,29,36,43-hexa oxo-1,48-di-(N-carbobenzoxy) amino-octatetracontane.	$ZCap_3BCap_3Z$.	$C_6H_5CH_2OCO.[NH(CH_2)_5CO]_3NH(CH_2)_6NH[CO(CH_2)_5NH]_3OCOCH_2C_6H_5$.
VII.	7,14,21,28,35,42,49,56-Octa aza-6,13,20,27,36,43,50,57-octa oxo-1,62-di(N-carbobenzoxy) amino-do hexacontane.	$ZCap_4BCap_4Z$.	$C_6H_5CH_2OCO.[NH(CH_2)_5CO]_4NH(CH_2)_6NH[CO(CH_2)_5NH]_4OCOCH_2C_6H_5$.
VIII.	Tetramethylene diamine-bis-(N-carbobenzoxy-ϵ-amino-n-Caproic Acid) Amide.	ZCapTetCapZ.	$C_6H_5CH_2OCO.\ NH(CH_2)_5CO\ NH(CH_2)_4NH\ CO(CH_2)_5NH\ OCOCH_2C_6H_5$.
IX.	7,14,19,26-Tetra aza-6,13,20,27-tetra oxo-1,32-di-(N-carbobenzoxy) amino- do triacontane.	$ZCap_2TetCap_2Z$.	$C_6H_5CH_2OCO.[NH(CH_2)_5CO]_2NH(CH_2)_4NH[CO(CH_2)_5NH]_2OCOCH_2C_6H_5$.
X.	7,14,21,26,33,40-Hexa aza-6,13,20,27,34,41-hexa oxo-1,46-di-(N-carbobenzoxy) amino-hexatetracontane.	$ZCap_3TetCap_3Z$.	$C_6H_5CH_2OCO.[NH(CH_2)_5CO]_3NH(CH_2)_4NH[CO(CH_2)_5NH]_3OCOCH_2C_6H_5$.

Table I. Cont^{d}..

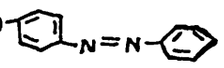
Compound Number.	Full Name.	Abbreviated Form.	Formula.
XI.	7,14,21,28,33,40,47,54-Octa aza-6,13,20,27,34,41,48,55-octa oxo-1,60-di-(N-carbobenzoxy) amino hexacontane.	$ZCap_4TetCap_4Z$.	$C_6H_5CH_2OCO[NH(CH_2)_5CO]_4NH(CH_2)NH[CO(CH_2)_5NH]_4OCOCH_2C_6H_5$.
XII.	7,14,21,28,35,40,47,54,61,68-Deca aza-6,13,20,27,34,41,48,55,62,69-deca oxo-1,74-di-(N-carbobenzoxy) amino tetraheptacontane.	$ZCap_5TetCap_5Z$.	$C_6H_5CH_2OCO[NH(CH_2)_5CO]_5NH(CH_2)NH[CO(CH_2)_5NH]_5OCOCH_2C_6H_5$.
XIII.	Methyl N-carbobenzoxy-ϵ-amino-n-caproate.	ZCapOMe.	$C_6H_5CH_2OCO.NH(CH_2)_5CO.OMe$.
XIV.	Methyl N-carbobenzoxy-bis-ϵ-amino-n-caproate.	$ZCap_2OMe$.	$C_6H_5CH_2OCO[NH(CH_2)_5CO]_2OMe$.
XV.	Methyl N-carbobenzoxy-tris-ϵ-amino-n-caproate.	$ZCap_3OMe$.	$C_6H_5CH_2OCO.[NH(CH_2)_5CO]_3OMe$.
XVI.	p-Hydroxy azo phenyl-N-carbobenzoxy-ϵ-amino-n-caproate.	$ZCapO_{p\text{-}OH}Az$.	$C_6H_5CH_2OCO.NH(CH_2)_5COO$- 
XVII.	Adipic acid-bis(methyl-ϵ-amino-n-caproate) Amide.	MeOCapACapOMe.	$MeO.CO(CH_2)_5NH.CO(CH_2)_4CO.NH(CH_2)_5CO.OMe$
XVIII.	7,14,19,26-Tetra oxo-6,13,20,27-tetra aza dotriacontane-1,32-dicarboxylic acid dimethyl ester.	$MeOCap_2ACap_2OMe$.	$MeO[CO(CH_2)_5NH]_2CO(CH_2)_4CO[NH(CH_2)_5CO]_2OMe$.
XIX.	7,14,21,26,33,40-Hexa oxo-6,13,20,27,34,41-hexa aza-hexatetracontane-1,46-dicarboxylic acid dimethyl ester.	$MeOCap_3ACap_3OMe$.	$MeO[CO(CH_2)_5NH]_3CO(CH_2)_4CO[NH(CH_2)_5CO]_3OMe$.
XX.	7,14,21,28,33,40,47,54-Octa oxo-6,13,20,27,34,41,48,55-octa aza-hexacontane-1,60-dicarboxylic acid dimethyl ester.	$MeOCap_4ACap_4OMe$.	$MeO[CO(CH_2)_5NH]_4CO(CH_2)_4CO[NH(CH_2)_5CO]_4OMe$.

Table 1. Cont^{d}..

Compound Number.	Full Name.	Abbreviated Form.	Formula.
XXI.	Suberic acid-bis-(methyl-ϵ-amino-n-caproate) Amide.	MeOCapSubCapOMe.	$MeO.CO(CH_2)_5NH.CO(CH_2)_6CO.NH(CH_2)_5CO.OMe.$
XXII.	7,14,21,28-Tetra oxo-6,13,22,29-tetra aza tetratriacontane-1,34-dicarboxylic acid dimethyl ester.	$MeOCap_2SubCap_2OMe.$	$MeO\left[CO(CH_2)_5NH\right]_2CO(CH_2)_6CO\left[NH(CH_2)_5CO\right]_2OMe.$
XXIII.	7,14,21,28,35,42-Hexa oxo-6,13,20,29,36,43-hexa aza-octatetracontane-1,48-dicarboxylic acid dimethyl ester.	$MeOCap_3SubCap_3OMe.$	$MeO\left[CO(CH_2)_5NH\right]_3CO(CH_2)_6CO\left[NH(CH_2)_5CO\right]_3OMe.$
XXIV.	7,14,21,28,35,42,49,56-Octa oxo-6,13,20,27,36,43,50,57-octa aza-dohexacontane-1,62-dicarboxylic acid dimethyl ester.	$MeOCap_4SubCap_4OMe.$	$MeO\left[CO(CH_2)_5NH\right]_4CO(CH_2)_6CO\left[NH(CH_2)_5CO\right]_4OMe.$
XXV.	Adipic acid-bis-(-ϵ-amino-n-caproic acid) amide.	HOCapACapOH.	$HO.CO(CH_2)_5NH.CO(CH_2)_4CO.NH(CH_2)_5CO.OH.$
XXVI.	7,14,19,26-Tetra oxo-6,13,20,27-tetra aza-do triacontane-1,32-dicarboxylic acid.	$HOCap_2ACap_2OH.$	$HO\left[CO(CH_2)_5NH\right]_2CO(CH_2)_4CO\left[NH(CH_2)_5CO\right]_2OH.$
XXVII.	7,14,21,26,33,40-Hexa oxo-6,13,20,27,34,41-hexa aza-hexatetracontane-1,46-dicarboxylic acid.	$HOCap_3ACap_3OH.$	$HO\left[CO(CH_2)_5NH\right]_3CO(CH_2)_4CO\left[NH(CH_2)_5CO\right]_3OH.$
XXVIII.	7,14,21,28,33,40,47,54-Octa oxo-6,13,20,27,34,41,48,55-octa aza-hexacontane-1,60-dicarboxylic acid.	$HOCap_4ACap_4OH.$	$HO\left[CO(CH_2)_5NH\right]_4CO(CH_2)_4CO\left[NH(CH_2)_5CO\right]_4OH.$
XXIX.	Suberic acid-bis-(-ϵ-amino-n-caproic acid) amide.	HOCapSubCapOH.	$HO.CO(CH_2)_5NH.CO(CH_2)_6CO.NH(CH_2)_5CO.OH.$
XXX.	7,14,21,28-Tetra oxo-6,13,22,29-tetra aza-tetratriacontane-1,34-dicarboxylic acid.	$HOCap_2SubCap_2OH.$	$HO\left[CO(CH_2)_5NH\right]_2CO(CH_2)_6CO\left[NH(CH_2)_5CO\right]_2OH.$

Table I. Cont^{d}..

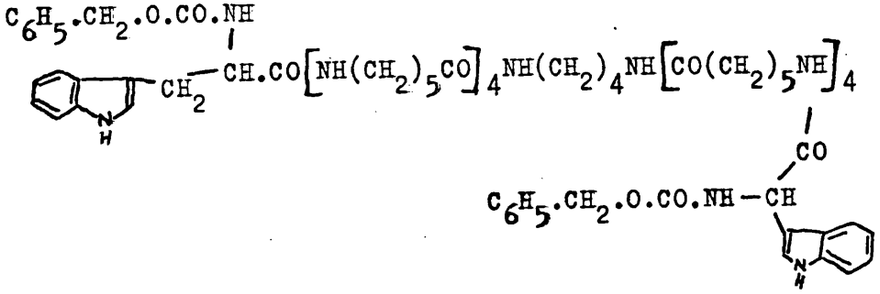
Compound Number.	Full Name.	Abbreviated Form.	Formula.
XXXI.	7,14,21,28,35,42-Hexa oxo-6,13,20,29,36,43-hexa aza-octatetracontane-1,48-dicarboxylic acid.	$HOCap_3SubCap_3OH$.	$HO\left[CO(CH_2)_5NH\right]_3CO(CH_2)_6CO\left[NH(CH_2)_5CO\right]_3OH$.
XXXII.	7,14,21,28,35,42,49,56-Octa oxo-6,13,20,27,36,43,50,57-octa aza-do hexacontane-1,62-dicarboxylic acid.	$HOCap_4SubCap_4OH$.	$HO\left[CO(CH_2)_5NH\right]_4CO(CH_2)_6CO\left[NH(CH_2)_5CO\right]_4OH$.
XXXIII.	Hexamethylene Diamine-Bis-(Methyl Hydrogen Adipate) Mono Amide.	MeO[AB]AOMe	$CH_3O.CO.(CH_2)_4.CO.NH(CH_2)_6NH.CO.(CH_2)_4.COOCH_3$
XXXIV.	Hexamethylene Diamine-Bis(Adipic Acid) Mono Amide.	HO[AB]AOH	$HO.CO.(CH_2)_4.CO.NH(CH_2)_6NH.CO.(CH_2)_4.COOH$.
XXXV.	3,10,17,24,31,38,45,52,59,66-Deca Aza-2,9,16,23,30,39,46,53,60,67-Deca Oxo-1,68-Di-(Indolyl-3'-Methyl)-1,68-Di-(N-Carbobenzoxy)-Amino-Octa Hexacontane.	$Z.TRY.Cap_4TetCap_4TRY.Z.$	

Table 2. Yields Obtained During Syntheses.

Compound. \ Method Used.	Mixed Anhydride. (Aqueous.)	Mixed Anhydride. (Anhydrous.)	Other Methods.
ZCapBCapZ.	70%.		Active Ester. 60%.
$ZCap_2BCap_2Z$.	63%.		
$ZCap_3BCap_3Z$.	Method 1. 48%. Method 2. 40%.		
$ZCap_4BCap_4Z$.		30%	
ZCapTetCapZ.		80%.	
$ZCap_2TetCap_2Z$.		69%.	
$ZCap_3TetCap_3Z$.		60%.	
$ZCap_4TetCap_4Z$.		52%.	
$ZCap_5TetCap_5Z$.		10%.	
MeOCapACapOMe.	55%.	70%.	$POCl_3$ Method 3%
$MeOCap_2ACap_2OMe$.		68%.	
$MeOCap_3ACap_3OMe$.	53%.	72%.	
$MeOCap_4ACap_4OMe$.		56%.	
MeOCapSubCapOMe.		48%.	
$MeOCap_2SubCap_2OMe$.		61%.	
$MeOCap_3SubCap_3OMe$.		69%.	
$MeOCap_4SubCap_4OMe$.		68%.	

Table 3.

Yields of Hydrolysis Reactions.

Conditions / Compound. Used.	Anhydrous $Ba(OH)_2$ MeOH.	NaOH(Ethanolic) and DMF.	NaOH/^{n}BuOH. in DMSO.
HOCapACapOH.	84%.		
$HOCap_2ACap_2OH$.	18%.	70%.	
$HOCap_3ACap_3OH$.		35%.	82%.
$HOCap_4ACap_4OH$.			65%.
HOCapSubCapOH	78%.		
$HOCap_2SubCap_2OH$.		74%.	
$HOCap_3SubCap_3OH$.		30%.	80%.
$HOCap_4SubCap_4OH$.			58%.

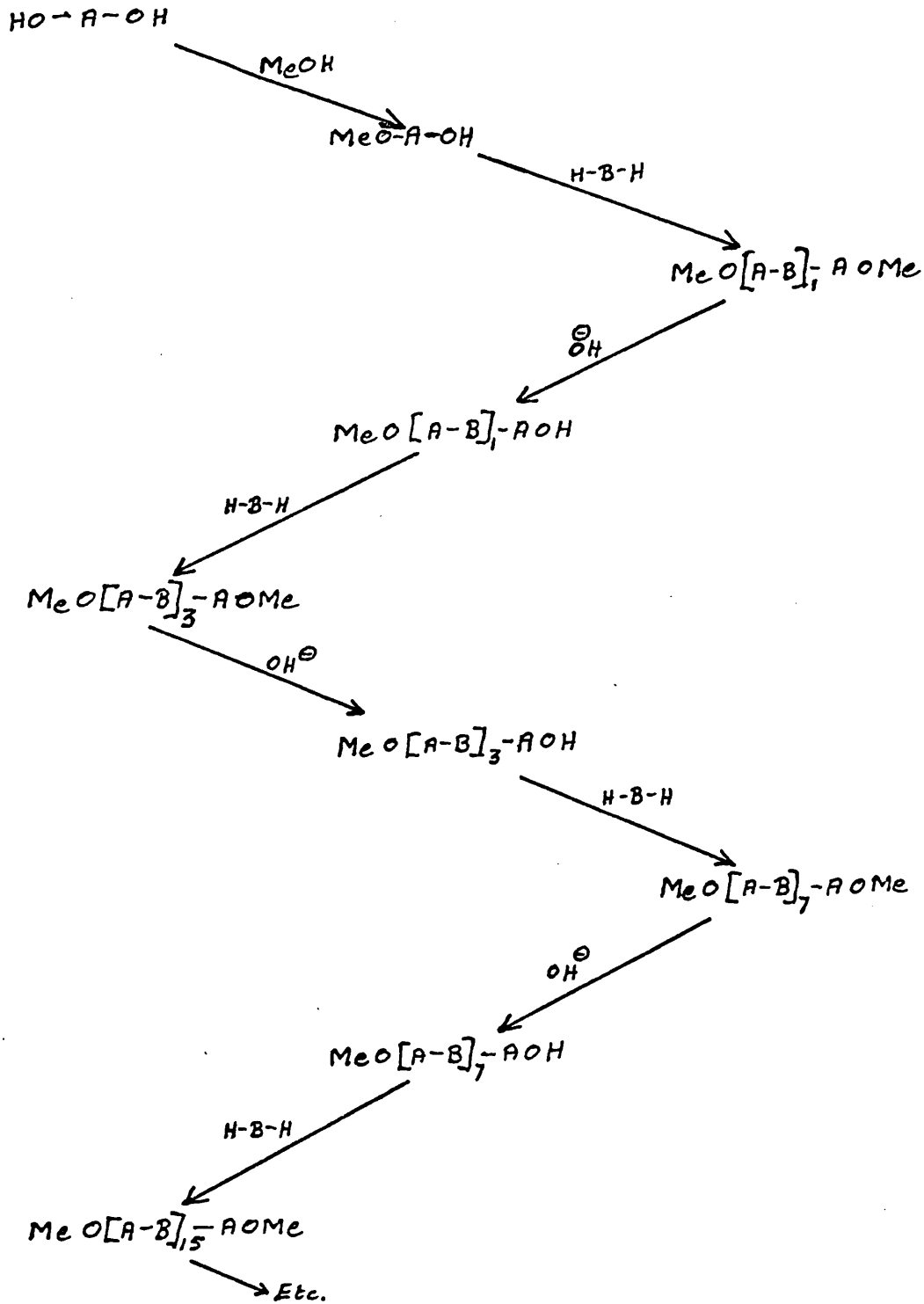
Table 4. Analytical Composition of The Oligoamides (Calculated.)

Compound.	Carbon.	Hydrogen.	Nitrogen.
ZCapBCapZ.	66.86	8.25	9.17
$ZCap_2BCap_2Z$.	66.00	8.66	10.04
$ZCap_3BCap_3Z$.	65.51	8.91	10.54
$ZCap_4BCap_4Z$.	65.19	9.07	10.86
ZCapTetCapZ.	65.96	7.96	9.61
$ZCap_2TetCap_2Z$.	65.32	8.47	10.39
$ZCap_3TetCap_3Z$.	64.96	8,76	10.82
$ZCap_4TetCap_4Z$.	64.74	8.95	11.10
$ZCap_5TetCap_5Z$.	64.58	9.08	11.30
MeOCapACapOMe.	59.98	9.06	6.99
$MeOCap_2ACap_2OMe$.	61.32	9.33	8.94
$MeOCap_3ACap_3OMe$.	61.93	9.45	9.85
$MeOCap_4ACap_4OMe$.	62.31	9.53	10.38
MeOCapSubCapOMe.	61.66	9.41	6.54
$MeOCap_2SubCap_2OMe$.	62.36	9.54	8.56
$MeOCap_3SubCap_3OMe$.	62.70	9.59	9.54
$MeOCap_4SubCap_4OMe$.	62.90	9.65	10.12
HOCapACapOH.	58.05	8.66	7.52
$HOCap_2ACap_2OH$.	60.18	9.09	9.36
$HOCap_3ACap_3OH$.	61.14	9.28	10.19
$HOCap_4ACap_4OH$.	61.69	9.40	10.66
HOCapSubCapOH.	59.98	9.06	6.99
$HOCap_2SubCap_2OH$.	61.32	9.33	8.94
$HOCap_3SubCap_3OH$.	61.94	9.45	9.85
$HOCap_4SubCap_4OH$.	62.31	9.53	10.38

SECTION TWO.

Figure 11.

Doubling Reaction System Flow Sheet.



INTRODUCTION.

The work of the previous section was concerned with the synthesis of large linear oligoamides by the stepwise addition of the maximum number of building units as possible at one time, but this is not the only, nor indeed is it the most rapid method of precise synthesis of huge molecules. By far the most rapid method is by application of what has been called The Doubling Reaction Technique[49,50].

Taking our systems as an example, one way in which this technique could be used would involve the formation of the mono-ester of the chosen dicarboxylic acid followed by the condensation of this material with a suitable diamine, so producing a large symmetrical diester. This diester then requires to be hydrolysed to the corresponding half acid ester so that the condensation may then be repeated with another portion of the same diamine, thus yielding another symmetrical diester with fully alternating acid and basic building units in its chain. (This reaction scheme is shown in Fig.11) As can be seen, by use of this method one rather more than doubles the size of the molecule with the completion of each cycle.
An example of the extreme rapidity with which one could, at least in theory, attain the very longest molecules would be the following:- Suppose one were following this reaction scheme and using only adipic acid and hexamethylene diamine, then after the completion of only 8 cycles one could have reached a molecular weight of almost 57,500 and a corresponding extended chain molecular length of 4,300Å..

A molecule of such a size is well within the range of a great many proteins and, from the point of view of the understanding being sought in this thesis, would therefore be of great interest.

The snag with this method, and the one which has prevented it from being far more widely adopted, is the notorious difficulty encountered either when trying to protect or activate just one of two symmetrically placed, and identical, functional groups. It will be seen that the molecules resulting from any doubling reaction scheme are bound, by the very nature of the method, to be symmetrical. It is the very low yields always obtained from such a protection or activation reaction, when symmetrical difunctional systems are involved, that has led to the neglect of this system, as compared with that of the much more straightforward stepwise technique.

In their review on the synthesis of oligomers[28] Zahn and Gleitsmann mention what work has been done on the synthesis of the oligoesters between terephthalic acid and ethylene glycol by themselves, with various co-workers,[50,51], as well as that from terephthalic acid and 1,4-bis(hydroxymethyl)cyclohexane[52]. The corresponding work of Cowell on oligoamides[53] is also mentioned along with various other investigations carried out in Aachen[49b, 54], also on oligoamides, but very little work has been carried out apart from this. In none of these investigations was the basic difficulty of low yields overcome, though Zahn et al. did achieve molecular weights in excess of 1,000 with some of their oligomeric series[50,51]. It was decided, therefore, that a new

approach could usefully be investigated in the hope that it might throw more light on the problems involved and, perhaps, show a way of getting round them.

At the start of this work some time was spent in trying to determine whether one could follow the hydrolysis of the diesters to the corresponding half acid esters, on the very small scale, by use of the various chromatographic techniques, or by electrophoresis. A wide range of visualising agents were tried[55], but no really effective method was found, and the investigations had, temporarily, to be suspended in favour of the stepwise procedure which has been discussed in Section 1.

As a result of the work carried out in the first section it was realised that this comparative failure of the dyeing reagents was due to the very poor solubility of the materials in the solvents used. This meant that the concentrations of material on the particular medium were so small that the colour produced by the visualising agents would frequently, in its turn, be too low to be seen against the background.

What was required therefore was a much more sensitive method of rendering these low concentrations of material visible, or otherwise detectable. This problem had to be overcome before a successful method could be found for applying the doubling reaction technique on the very small scale as was desired.

The solution of this problem turned out to be, in theory at least, relatively simple. Dyes have been in use in chromatography

for quite a long time, but so far their use has only been pursued along one narrow line. Dyes are normally used by spraying them on to the plate after it has been developed by elution with the required solvent system. In this way the materials are rendered visible, as the dye used sticks to the material rather than to the chromatographic medium. Fluorescence has been used in exactly the same way. It has also very occasionally been used when the materials are known selectively to absorb ultra-violet light, in which case they will show up dark against the fluorescent background. There has, however been very little work carried out into the idea of actually attaching the coloured material on to the molecules being investigated.

This idea is far from new. Strain[56] and Williams[56] have mentioned these very possibilities as long ago as the 1940's and towards the end of that decade Baker et al. carried out a little work on the preparation of suitably coloured or fluorescent derivatives of alcohols[57]. Virtually no other similar investigations have been carried out however, and people have tended to stick to the other methods of either positive or negative adsorption on to the materials concerned, once the chromatograms have been run. It was felt it should be possible to prepare some suitable derivatives of our materials so that they could be detected, during chromatography, in their coloured forms.

As the materials concerned in this section of the work all

have ester links at the ends of their chains, it was felt there was a reasonable chance of obtaining highly coloured materials containing suitable alcoholic functions so that the simple methyl esters first used in these syntheses could be replaced by the ester of the material with the dye.

The first class of highly coloured materials investigated, the phenolic azo dyes, proved to yield esters far too reactive towards primary amines. A brief investigation of coloured materials containing the benyl alcohol function showed that their esters too were too reactive and therefore dyes were sought posessing alcoholic functions which would render their esters more like those of normal aliphatic chemistry.

A survey of a Colour Index[58] showed that there was a relatively modern class of dyes, called the Disperse Dyes, which had 5-(1'-Hydroxy-Ethyl) functions. The structures of a few of these dyes looked as if they might well be useful in the present investigations. and small samples of the commercial products were obtained.

An alternative method of obtaining suitable materials was the synthesis of compounds containing the required 1'-hydroxy-ethyl group, (or even higher homologues of it), and so long as these materials were either highly coloured or highly fluorescent then they could be used in this work as substitutes for the simple methyl esters. 5-(1'-Hydroxy-Ethyl) acridine was synthesised from diphenylamine and investigations carried out into its use with this system.

Finally, the idea of the use of fluorescent materials was carried a stage further and investigations were started into the use of such compounds as the basic building units of a series of linear oligomers, and the synthesis of a new series was begun.

DISCUSSION.

Methyl hydrogen adipate was synthesised by a well known method[59], condensed with hexamethylene diamine using the mixed anhydride method to form the amide links, and purified. The next stage in the synthesis, as outlined in Fig. 11, required the hydrolysis of this diester to the corresponding half acid ester. The method chosen for carrying out this hydrolysis was the use of an anhydrous methanolic solution of barium hydroxide[43]. Throughout the work less than one equivalent of base was used, but at first the reaction times were varied from a few days down to periods of only a few hours. On all these occasions it was found that although the required hydrolysis occurred, there was always a proportion of the hydrolysis to the corresponding dicarboxylic acid occurring as well. The proportion of the products was found to vary, but the optimum period for the hydrolysis seemed to be about 18 hours, of which two hours were under reflux. The separation of this mixture was obtained in a variety of ways.

The acidification of the total solid material precipitated during the hydrolysis, and its subsequent recrystallisation was found to be a very poor technique in this case since only small amounts of the required half acid ester were present in the mixture. This meant that the technique was very wasteful of material.

The next technique to be tried was that of chromatography using an alumina column. Grade V neutral Woelm alumina was used

with a ratio of alumina to material of only 30:1. The hydrolysis reaction was stopped by the acidification of the methanolic solution as well as the precipitated solids and the solvent was then removed under reduced pressure. The total solid material thus obtained was dissolved in ethyl acetate and eluted down the column using a mixture of ethyl acetate/methanol (19:1) as the solvent. The first material removed was the starting material, and, after a considerable period of elution, the required half acid ester was obtained. The corresponding dicarboxylic acid could not be removed from the column without increasing the polarity of the solvent to such an extent as to cause the simultaneous breakdown of the column itself. Although insufficient of the half acid ester material was obtained for recrystallisation to provide analytical figures, the infra-red spectrum of the material was consistent with that expected for the product.

These results indicated that a suitable reaction system might indeed be found whereby the doubling reaction system could be made to give reasonable yields of the required materials, though at the expense of the time to be taken over each cycle. For example it should be possible to add a sufficient amount of base so that only a small amount of hydrolysis would occur. If the reaction were then to be stopped at this stage, i.e. before much of the dianion formed by the subsequent hydrolysis of the required product had also been formed, then one could reasonably expect to separate the desired half acid ester from the starting diester material.

This starting material could then be repeatedly recycled so gradually building up large quantities of the half acid ester. The losses in this scheme would therefore depend entirely on just how much hydrolysis to the dicarboxylic acid one was prepared to tolerate and how much time one was prepared to spend on the step. It was recognised that this would be a rather tedious procedure, but it was also felt that the inherent importance of this doubling reaction scheme was such that it was worthy of some detailed investigations. There was also the point that, just as one can automate a variety of processes, once such a reaction scheme had been well worked out then it too would be capable of being automated. Accordingly it was decided to try and find a rather more satisfactory method of separating the products of the hydrolysis, having particular regard to the speed with which the separation could be effected.

Separation on a column of silicic acid yielded almost exactly the same results as the alumina column, except that the solvent required was slightly less polar, only pure ethyl acetate being required at first, but this technique too was discarded owing to the very long times required for running such a column.

The possibilities of ion exchange chromatography were investigated using DEAE Sephadex, but this method also had to be considered as being unsuitable owing to the slowness with which one could carry out the required separation. The method was also found to be rather less satisfactory than an alumina column

from the point of view of the low capacity of the column and the tendency for the materials to follow one another rather closely.

These general conclusions were found to hold both for ion exchange chromatography using the anionic material precipitated during the hydrolysis reaction, and for chromatography using the acidified material. The low solubility of these materials in water was another factor against the use of this technique.

The next technique to be investigated briefly was that of electrophoresis. It was recognised that this was likely to be slow, but there was the possible advantage that it could be continued overnight without requiring attention, thus making the technique a useful one. The most satisfactory conditions found were the use of a buffer of pH 9.1[60] and a potential gradient of 8 volts/cm., but the separation obtained was not found to be particularly efficient due to considerable tailing of the migrating materials. The speed of migration was found to be a reasonable one, but owing to the low capacity of the method and the poor separations obtained, this technique too was considered to be unsuitable.

The final technique to be investigated was that of reverse phase chromatography. Using this method, the starting material, being a diester, would be expected to be the final compound removed from the column and this is a disadvantage inherent in the method. Before trying a full scale column, therefore, preliminary work was carried out using thin layer reverse phase chromatography.

Thin layer Kieselgel G plates were prepared and dried. The hydrocarbon phase was obtained by very gently dipping the plates into a 10% solution of nujol in ethyl acetate and allowing them to dry again. The material was applied to the plate in the normal way and the plates eluted. A wide spectrum of solvent mixtures was investigated varying from pure water through to a mixture of water and methanol (1:3). The expected inversion in the order in which the material moved on the plate, as compared with the normal Kieselgel plate, was in fact not observed. This could possibly have been due to insufficient of the hydrocarbon phase being on the plate, or more likely to the very low solubility of any of the materials in it. The fact that there was indeed sufficient nujol on the plates was shown by eluting a mixture that was known to reverse, on one of the plates and it showed the expected order of spots.

These investigations were repeated using, this time, castor oil, which is another very commonly used hydrocarbon phase. Results exactly corresponding to those obtained when nujol was the hydrocarbon phase were obtained in this case too, however a rather better separation was observed. Because of this failure of either hydrocarbon phase to bring about the desired reversal it was concluded that it would be pointless to try a full-scale column and therefore this technique too had to be discarded.

Visualisation Problems.

Nothing has so far been said regarding the methods used to render visible the materials on the plates etc.. A wide variety of different sprays were tried throughout this work* both when following the columns and during the work on electrophoresis and reverse phase chromatography. The spray that was the most widely useful and certainly the most adaptable was a commercial solution of Universal Indicator dispensed as a fine spray. This had the overwhelming advantage that it not only disclosed the positions of the spots on the supports, but it also gave their pH's and therefore one knew which material one was looking at. The next most useful spray was a solution of Rhodamine B in ethanol, but as the excess reagent had to be removed from the medium this was a little inconvenient. It did, however, give limited results whereas most failed even to give clear indications of the positions of the materials on the medium, above the background colourations produced. This, it was realised later, was due to the very low concentrations of the material on the support meaning that the colouration produced could have been so low that it was below the threshold level for its being observed.

On re-examining the system being used for the hydrolysis of the diester to its half acid ester it was felt that there was a considerable waste of material in being forced to follow the separations by thin layer chromatography. This led to a consideration of whether or not the separation described above could be effected

* See Appendix 1.

in the manner described above, (i.e. most likely using a straightforward alumina column,) but without this waste.

All of the systems investigated in this section of the work possess ester links at the ends of their chains. It was felt, therefore, that some highly coloured or fluorescent material could be used with great advantage so that the simple methyl esters used throughout the investigations described above could be replaced with coloured ones. The inclusion of such a property within the molecules would mean that they could be followed throughout the whole of the reaction scheme. It would greatly alleviate the difficulty of repeatedly running columns in the separation procedures and there would be the further advantage that there would not be the need to use precious material in order to follow the compounds that were coming off the columns.

The first materials to be studied with regard to their suitability for this purpose were the azo-dyes, formed by coupling a diazonium salt with a phenol in basic conditions. The simplest of these, para-hydroxy azo benzene, was readily prepared from aniline and phenol and obtained in a pure form by a simple elution down a grade III acid washed Woelm alumina column, using chloroform as the solvent. Although not very intensly coloured it was decided to test the overall suitability of this class of compound on a known series of compounds. Accordingly this phenolic dye was esterified with N-carbobenzoxy-ϵ-amino-n-caproic acid, using

dicyclohexylcarbodiimide[61], and the ester produced purified by elution down an alumina column as described for the purification of the original dye. A similar esterification attempt, using identical conditions, with the more highly coloured Sudan II (see table 5) failed to yield much ester. This might be due to the possibility of strong intramolecular hydrogen bonding which can be seen in this structure.

Once obtained in a pure form, the ester with para-hydroxy azo benzene was tested to ascertain whether it would be stable under the reaction conditions it would be required to tolerate, for it was recognised that there was some similarity of structure between this ester and the so called 'active esters' - namely the nitro-phenyl and the para-nitro benzyl esters. The manner in which this was done was described in detail in Section 1, and its reactivity at the ester link was found to be quite considerable, though not quite as reactive as the nitro phenyl esters.

The next class of esters that were very briefly examined were the benzyl esters. These are well known to be very labile, but it was hoped that this might be put to good effect once the ester had been formed. Once more it was realised that there would be the danger of an 'active ester' being produced, but it was also likely that this danger could be avoided if one used the meta-azo dye benzyl ester. As the meta substituted starting materials would require preparation, it was decided to carry out a series of preliminary investigations on the corresponding para-

substituted materials, which were more readily available.

Adipic acid was, accordingly, esterified with para-nitro benzyl bromide[62] and the ester purified. The reduction of the aromatic nitro group was achieved by use of aqueous paladium charcoal and sodium borohydride[63], but it was found that, on diazotisation of the crude product and on coupling it with β-naphthol, the same dye was obtained as that obtained from para-toluidine. This indicated that the cleavage of the ester link had occurred as well as the required reduction of the nitro-group.

This same undesired result was obtained even under the much milder conditions using aqueous iron filings and a little ferric chloride[64]. These latter conditions were also used for the reduction of the aromatic nitro group in para-nitro benzyl bromide, but here too the desired reduction occurred along with the reduction of the benzyl bromide to the methyl group. Due to the lack of success with these methods it was decided to look for a rather less reactive type of ester and therefore the corresponding reactions on the meta-substituted materials was not attempted. One final attempt was however made to make use of benzyl esters, in the systems for which they were originally intended.

It was decided to attempt the reduction of a very highly coloured compound possessing a benzoic acid functionality. It is known that phenolphthalein can be reduced under the action of lithium aluminium hydride[65] and so it was decided to attempt

the reduction of Rhodamine B (see table 5) using a similar technique. This reduction was achieved, though in poor yield due to a fairly short reaction time, and the resulting alcohol purified by eluting it down a column of grade III acid washed alumina with chloroform. Although an analysis was not carried out, the infra-red spectrum was consistent with that for the alcohol expected.

Unfortunately the alcohol obtained by this reduction proved to be too insoluble in organic solvents to react to any reasonable degree to form the required ester. As a result of this, the class of benzyl esters had to be discarded.

These failures caused attention to be turned to the coloured materials containing at leasf one further methylene group between the benzene ring and the hydroxyl group. There are a few commercial dyes known which contain the 1'-hydroxy-ethyl functional group and some small samples of a few of these were obtained, (see table 5). Unfortunately, as well as the required coloured materials, these technical dyes also included several other coloured materials as well as various amounts of colourless materials. These latter materials were readily removed, but it has not yet been possible to obtain the required coloured materials in a sufficiently high state of purify, despite repeated columning of the crude dyes using a variety of columns.

It was therefore decided to investigate the possibilities of synthesising a fluorescent compound containing the required

functionality. Accordingly the synthesis of 5-(1'-hydroxy-ethyl) acridine was undertaken from diphenylamine. The diphenylamine was first reacted with acetic acid according to the method of Bernthsen[66] and the crude 5-methyl acridine obtained purified by an improved method. Instead of repeatedly recrystallising the material, it was eluted down an alumina column and the pure material collected. This material was then reacted with formaldehyde[67] to form the desired hydroxy-ethyl functional group, the crude material again being purified by chromatography rather than the previously used technique of recrystallisation.

One of the most convenient methods for obtaining a half acid ester of a dicarboxylic acid is by the reaction of an alcohol with the anhydride of the required dicarboxylic acid, provided that this is available. Quite often it is found that this method leads to a mixture of products, but in this case it was found that the reaction proceeded in high yield to the single desired product. This compound, after purification, was condensed with hexamethylene diamine, using the mixed anhydride method, anhydrous conditions; and the symmetrical diester product purified.

Throughout all of these reactions it was found that the materials could readily be followed by use of thin layer chromatography, or the materials themselves readily followed on a column under ultra-violet light. It was therefore decided to investigate the further possibilities of following the hydrolysis

reaction on this material using the technique described at the start of this section.

At the same time as the investigations into the possibilities of 5-(1'-hydroxy-ethyl) acridine, an investigation was carried out into the possibilities of using a fluorescent anhydride as a starting material in a series of oligoamides. Naphthalene-2,3-dicarboxylic acid anhydride was condensed with hexamethylene diamine and found to give a very high yield of the expected dicarboxylic acid. It was found, however that this molecule readily lost water, even upon recrystallisation from NN-dimethyl formamide, yielding the corresponding di-imide.

EXPERIMENTAL.

Methyl Hydrogen Adipate.

Adipic acid (87 gm, 0.596 mole), methanol (25 ml, 0.625mole) and concentrated hydrochloric acid (7.5 ml) were cautiously heated together till the mixture became homogeneous, after which it was refluxed for a further 8 hours before being fractionally distilled. For the distillation under reduced pressure a six-pear fractionating column was used together with a very fine air leak. The final yield of methyl hydrogen adipate was 33.81 gm, (0.212 mole, 35.5%) b.p. 151-154°C at 6 mm. pressure.

Found C, 52.38; H, 7.75%.

$C_7H_{12}O_4$ requires C, 52.49; H, 7.55%.

Hexamethylene Diamine Bis (Methyl Hydrogen Adipate) Mono Amide.

XXXIII.

Ethyl chloroformate (0.70 gm, 6.4 mmole) was added slowly and with stirring to a solution, at -5°C, of methyl hydrogen adipate (1 gm, 6.25 mmole) in toluene (44 ml) and triethylamine (0.6 gm) and the reaction stirred for about 20 minutes. After this, hexamethylene diamine (0.35 gm, 3 mmole), dissolved in 2N sodium hydroxide solution (4 ml), and also at -5°C, was added to the reaction which was then stirred for a further two and a half hours, maintaining the pH between 8 and 9 by the addition of further small quantities of sodium hydroxide solution if necessary, but slowly allowing it to regain room temperature. The solvent was then removed under

reduced pressure, yielding a white solid which was then extracted several times with hot benzene. The solid material thus obtained was recrystallised from benzene to yield XXXIII (912 mgm, 2.28 mmole, 76%) m.p. 130-131°C (Lit. 132°C).

Found C, 59.80; H, 8.78; N, 7.09%.

$C_{20}H_{36}N_2O_6$ requires C, 59.98; H, 9.06; N, 6.99%.

Hydrolysis and Separations of XXXIII To The Corresponding Half Acid Ester.

XXXIII (1.002 gm, 2.5 mmole) was dissolved in the minimum volume of anhydrous methanol (ca. 1 ml) at 60°C and to this solution was added, slowly and with stirring, a solution of anhydrous barium hydroxide in anhydrous methanol (2.5 ml of a 1 N solution) and the flask and condenser protected against moisture by an efficient drying tube. The reaction was continued at this temperature for 18 hours, with occasional swirling, after which the reaction was stopped by rendering the contents of the flask just acid to litmus by the cautious addition of small quantities of dilute hydrochloric acid. The solvent was then removed under reduced pressure and the white solid obtained repeatedly extracted with a mixture of ethyl acetate and methanol (19:1). This solution was then eluted down a column of grade V neutral alumina using a ratio of alumina to material of 30:1. The solvent system for the chromatography was the same as that used in the extraction of the materials from the residue. The diester starting material was readily removed from the column

and recrystallised to yield XXXIII (614 mgm, 1.5 mmole, 60%) m.p. 128-129°C (Lit. 132°C).

On prolonged elution with the same solvent mixture, a small quantity of material was obtained (20 mgm, 2%) which had the required triple peak in the carbonyl region of its infra-red spectrum. It melted, however, over a wide range and several attempts at recrystallisation were not successful in yielding pure half acid ester. Although analytical figures were, therefore, not obtained the overall infra-red spectrum of this material was consistent with that for the expected product.

The corresponding dicarboxylic acid, formed by the complete hydrolysis of the diester, was never removed from the alumina column. Increasing the polarity of the solvent system being used to elute the column was found to lead only to the break-down of the column itself.

The hydrolysis was repeated several times to try and find conditions which would lead to a satisfactory proportion of the required half acid ester, without too much of the subsequent hydrolysis to the dicarboxylic acid. The most satisfactory conditions obtained were as described above, except that after no more than 2 hours the temperature was reduced to room temperature. Some improvement was also observed if the quantity of base was reduced slightly.

Other efforts to secure a satisfactory method of separation are described below.

The acidified material obtained above was eluted down a column of silicic acid. The ratio of silica to material was 30:1, The starting material was removed using ethyl acetate as the solvent, but the half acid ester required the same solvent mixture as had been required for the alumina column. There was therefore no particular advantage in using such a column.

An ion exchange column of DEAE Sephadex was prepared in the hydrogen form. On to this column was put an aqueous solution of the barium salts precipitated during the course of the hydrolysis reaction, and the column eluted with water. The materials were found to be readily removed ffom the column, but there was a very poor separation. The capacity of the column was also very low.

On repeating the ion exchange column, but this time using the same acidified material as for the two previous column media, it was found that there was no particular advantage over the previous method.

Electrophoresis was investigated using a potential gradient of 8 volts/cm, giving a current of 4 milliamps and a migration speed for the acidic component of 0.5 inches/hour. A variety of buffer solutions were tried, but even the most satisfactory one, of pH 9.1, gave a considerable degree of so-called tailing of the materials.

Reverse phase thin layer chromatography was investigated using Kieselgel G (silica) plates prepared in the normal way.

These plates were gently dipped into a solution of nujol in ethyl acetate (10%) and allowed to dry. The acidified material described above was spotted on to these plates in the normal fashion and the plates were then developed. A wide variety of solvents were used varying from pure water to a mixture of water and methanol (1:3). The results are summarised in the table below.

Hydrocarbon Phase. \ % of Methanol.		0	10	20	30	40	50	75
Nujol.	R_f of Diester.	0.00	0.00	0.00	0.15	0.21	0.45	0.80
	R_f of Half acid Ester.	0.00	0.00	0.00	0.00	0.00	0.05	0.08
Castor Oil.	R_f of Diester.	0.10	0.14	0.20	0.52	0.68	0.90	
	R_f of Half acid Ester.	0.00	0.00	0.02	0.05	0.07	0.10	

This table shows that the expected change in the order in which the materials ran on the thin layer plates was not observed. The reverse phase experiments were therefore repeated, this time using castor oil as the hydrocarbon phase. These results are also to be found summarised in the above table and indicate that here too the expected inversion in the order in which the compounds ran on the plates did not materialise. The results therefore indicated that such a reverse phase column would be no more efficient than would a perfectly normal column.

Hexamethylene Diamine Bis (Adipic Acid) Mono Amide. XXXIV.

Ethyl chloroformate (0.70 gm, 6.4 mmole) was added, slowly and with stirring, to a solution at -5°C of methyl hydrogen adipate (1 gm, 6.25 mmole) in toluene (44 ml) and triethylamine (0.6 gm) and the reaction stirred for about 20 minutes. Hexamethylene diamine (0.35 gm, 3 mmole) dissolved at -5°C in 2 N sodium hydroxide (4 ml) was then added to the reaction flask and the whole mixture stirred for a further two and a half hours, slowly allowing the temperature to rise to the room value, but being careful to maintain the pH between 8 and 9 by the cautious addition of further small quantities of dilute sodium hydroxide solution, if necessary.

At this stage the aqueous layer was acidified with dilute hydrochloric acid and the reaction heated to 60°C for a further hour after which all the solvent was removed under reduced pressure and the total solid material thus obtained recrystallised from small portions of water. The final traces of adipic acid required to be removed by sublimation at reduced pressure. The final yield obtained of XXXIV was 0.41 gm (1.1 mmole, 37%) m.p. 187-189°C. (Lit. 188-189°C.)

Found C, 57.98; H, 8.54; N, 7.53%.

$C_{18}H_{32}N_2O_6$ requires C, 58.05; H, 8.66; N, 7.52%.

Para-Hydroxy Azo Benzene.

Aniline (6.2 gm, 0.66 mole) was diazotised by dissolving it in dilute hydrochloric acid, reducing the temperature of the

solution to 0°C and slowly adding to it a solution of sodium nitrite in cold water. After allowing 30 minutes for the reaction to go to completion the resulting diazonium salt solution was added slowly and with stirring to an alkaline solution of β-naphthol in water, also at 0°C. After a few minutes the resulting solution was rendered acid to litmus by the addition of dilute hydrochloric acid, and then repeatedly extracted with chloroform. The combined chloroform extracts were washed with a little water, dried over calcium sulphate and reduced in volume so that the solution could be conveniently purified by elution down a column of grade III acid washed alumina. The ratio of alumina to expected product was 100:1. The solvent used throughout the chromatography was chloroform and the final yield of the pure yellow dye was 10.23 gm, (0.555 mole, 85%).

Although analytical figures were not obtained, the infra-red spectrum of the material was consistent with that expected for the product.

p-Hydroxy Azo Phenyl —N-carbobenzoxy-ε-Amino-n-Caproate. XVI.

To a solution of N-carbobenzoxy-ε-amino-n-caproic acid (5.30 gm, 20 mmole) in dry ethyl acetate (40 ml) was added para-hydroxy azo benzene (4.8 gm, 24 mmole) and the temperature of the solution reduced to 0°C. The calculated amount of dicyclohexylcarbodiimide (4.12 gm, 20 mmole) was added, and the reaction stirred for 45 minutes, the flask being protected by an

efficient drying tube. After this, the reaction was continued for a further 18 hours, the temperature slowly being allowed to rise to the room value.

The NN'-dicyclohexyl urea precipitated during the course of the reaction, was filtered off and washed with further small quantities of dry ethyl acetate to remove any traces of product from it. The combined ethyl acetate washings, with the ethyl acetate solution, were then heated under reduced pressure to remove all of the solvent and the solid residue then dissolved in the minimum volume of chloroform.

The solution was then eluted down a column of grade III acid washed alumina using a ratio of alumina to solid material of 100:1 and chloroform as the solvent. The pure ester was finally recrystallised from benzene to yield XVI (5.36 gm, 12 mmole, 60%) m.p. 97-98°C.

Found C, 70.11; H, 6.14; N, 9.35%.

$C_{26}H_{27}N_3O_4$ requires C, 70.10; H, 6.11; N, 9.43%.

Determination of the Stability of the Phenolic Ester Link in XVI.

This investigation has been described in detail in Section 1, in the preparation of Compound IV Method 2.

Attempted Esterification of N-Carbobenzoxy-ε-Amino-n-Caproic Acid With Sudan II.

To a solution of N-carbobenzoxy-ε-amino-n-caproic acid (5.3 gm, 20 mmole) in dry ethyl acetate (60 ml) was added Sudan II

(6.65 gm, 24 mmole) and the temperature of the solution reduced to 0°C. The calculated quantity of dicyclohexylcarbodiimide (4.12 gm, 20 mmole) was added and the reaction stirred at 0°C for 45 minutes after which the reaction was continued for a further 18 hours, during which time the temperature was slowly allowed to regain room value.

There was only a very small quantity of NN'-dicyclohexyl urea formed during the course of this reaction time, but the contents of the flask were filtered and the filtrate heated under reduced pressure to remove the solvent. The solid residue obtained was dissolved in the minimum volume of cold chloroform and eluted down a column of grade III acid washed alumina using a ratio of alumina to expected product of 100:1. Only a very small quantity of material was obtained having the required absorption in the carbonyl region of its infra-red spectrum and this material was not investigated further owing to the proven reactivity of the ester link.

Adipic Acid Di-para-Nitro Benzyl Ester.

To a solution of adipic acid (1.46 gm, 10 mmole) and triethylamine (2.02 gm, 20 mmole) dissolved in ethyl acetate (350 ml) was added para-nitro benzyl bromide (4.34 gm, 20 mmole) and the reaction refluxed for 4 hours. The reaction mixture was then cooled and the triethylamine hydrobromide formed during the course of the reaction was filtered off. The solvent was removed from the filtrate under reduced pressure and the solid residue obtained extracted with

several portions of hot benzene. The benzene extracts were combined and the solvent removed under reduced pressure. The solid obtained was then dissolved in the minimum volume of chloroform and this solution eluted down a column of grade III acid washed alumina using a ratio of alumina to solid material of 100:1, eluting with chloroform throughout the separation. The solid diester obtained was recrystallised from a mixture of benzene and 60/80 light petrol to yield 2.61 gm, (6.3 mmole, 63%) m.p. 106-107°C. (Lit. 106°C.)

Reduction of Adipic Acid Di-p-Nitro Benzyl Ester.

METHOD 1.

Paladium charcoal (50 mgm of 10%) was suspended in water (10 ml) and sodium borohydride (0.78 gm, 20 mmole) in water (15 ml) added. A slow stream of nitrogen was bubbled through the mixture and a solution of the adipic acid di-p-nitro benzyl ester (2.08 gm, 5 mmole) in aqueous tetrahydfofuran added, the addition being spread over about 5 minutes. The order in which these processes are carried out is important and care should be taken that this order is followed, otherwise only poor results are obtained. After being left for about 20 minutes at room temperature the solution was cautiously acidified with dilute hydrochloric acid so as to destroy the excess sodium borohydride, and the mixture immediately filtered. The resulting acidic solution was reduced in temperature to 0°C so that it could be diazotised immediately.

To this cooled solution was added a solution at 0°C of sodium nitrite in water and the reaction allowed to continue for about 15 minutes. The resulting solution of the diazonium salt was added to a solution, also at 0°C, of β-naphthol in dilute sodium hydroxide and the azo dye thus produced was filtered and dried. The dye was purified by dissolving it in the minimum volume of cold benzene and eluting it down a column of grade III acid washed alumina, using the very high ratio of alumina to solid material of 1,000:1, and eluting with benzene throughout the separation.

The material obtained was recrystallised from benzene to a constant melting point and proved to be the same azo dye as that obtained by the simple diazotisation of p-toluidine, followed by the coupling of the diazonium salt with β-naphthol. The yield of the materials from the column was 1.785 gm (6.8 mmole, 3.4 m equiv, 68%) m.p. 132-133°C.

Found C, 77.86; H, 5.35; N, 10.90%.

$C_{17}H_{14}N_2O$ requires C, 77.87; H, 5.38; N, 10.68%.

METHOD 2.

A mixture of adipic acid di p-nitro benzyl ester (416 mgm, 1 mmole) iron filings (630 mgm) and water (0.4 ml) was heated for about 10 minutes over a steam bath, shaking the mixture very thoroughly. After this period fresh ferric chloride (6.5 mgm) was added in the solid form and the whole mixture shaken and heated for at least one hour, after which time it was allowed to cool, was

filtered and acidified. This acidified solution was then immediately diazotised by cooling it to 0°C and adding to it a solution of sodium nitrite in water, also at 0°C. After some 10 minutes the resulting solution was added, with stirring, to a solution at 0°C of β-naphthol in dilute sodium hydroxide. The dye obtained was filtered, dried, and recrystallised from 60/80 light petrol to a constant melting point of 132-133°C. From these properties it was thought to be the same dye as obtained directly from the diazotisation and coupling of p-toluidine. A mixed melting point was carried out which melted at 131-133°C, proving that it was in fact the same dye.

Reduction of para-Nitro Benzyl Bromide.

A mixture of p-nitro benzyl bromide (2.16 gm, 0.1 mole) iron filings (3.3 gm) and water (2 ml) was heated for about 10 minutes on a steam bath, shaking very thoroughly. After this period, fresh ferric chloride was added in the solid form (35 mgm) and the whole mixture heated and shaken for a further one hour at least. The mixture was then cooled, filtered and the filtrate acidified.

This acidified solution was immediately diazotised by reducing its temperature to 0°C, and adding a solution, at 0°C, of sodium nitrite in water. After only about 10 minutes this solution was poured into a solution, at 0°C, of β-naphthol in sodium hydroxide and the resulting dye filtered, dfied and recrystallised from 60/80 light petrol to a constant melting point of 132-133°C. A mixed

melting point with the azo dye between p-toluidine and β-naphthol proved it to be identical with this dye.

The Azo Dye Between β-Naphthol and para-Toluidine.

p-Toluidine (10.76 gm, 0.1 mole) was diazotised by dissolving it in dilute hydrochloric acid at 0°C and slowly adding to it a slight excess of an aqueous solution of sodium nitrite, also at 0°C. After about 30 minutes, the resulting solution was slowly added to a solution at 0°C of β-naphthol (14.5 gm, 0.1 mole) in dilute sodium hydroxide. After a few minutes the precipitate formed was filtered, dried and recrystallised from 80/100 light petrol to yield the required dye (23.7 gm, 0.09 mole, 90%) m.p. 132-133°C.

Found C, 77.91; H, 5.15; N, 10.65%.

$C_{17}H_{14}N_2O$ requires C, 77.84; H, 5.38; N, 10.68%.

Reduction of Rhodamine B.

Into a round bottomed flask equipped with a reflux condenser and a nitrogen inlet, was added sodium dried tetrahydrofuran (500 ml) and lithium aluminium hydride (0.8 gm), and the condenser protected by an efficient drying tube. After allowing time for the lithium aluminium hydride to react with any remaining traces of water in the solvent, rhodamine B (4.1 gm, 8.625 mmole) was added to the flask and the temperature raised till the solvent refluxed gently. The reaction was then continued for 24 hours under reflux and with stirring.

After this time the excess lithium aluminium hydride was very cautiously destroyed by the slow addition of small quantities of aqueous sodium carbonate solution. After acidification the solvent was removed under reduced pressure and the crude solid obtained, dissolved in chloroform and eluted down a column of grade III acid washed alumina, a ratio of alumina to material of 50:1 being used. On gradually increasing the polarity of the solvent used for the elution, by the addition of small quantities of ethyl acetate, a highly coloured material was obtained. Although analytical figures were not obtained, the infra-red spectrum of this material was consistent with that expected for the reduction product. The yield obtained was 430 mgm (0.95 mmole, 11%) m.p. 125°C.

Attempted Esterification of Rhodamine B Reduction Product.

N-carbobenzoxy-ε-amino-n-caproic acid (66.5 mgm, 0.25 mmole) was dissolved in dry ethyl acetate (500 ml), the alcohol (116 mgm, 0.25 mmole), obtained by the reduction of Rhodamine B, added to it and the mixture stirred till the solution was complete. The calculated quantity of dicyclohexylcarbodiimide (52 mgm, 0.25 mmole) was then added and the reaction stirred for three days at room temperature with the flask protected by an efficient drying tube.

The NN'-dicyclohexyl urea formed during the course of the reaction was filtered off and the solvent removed from the filtrate by heating it under reduced pressure. The solid material thus obtained was dissolved in chloroform and the solution eluted down

a column of grade III acid washed alumina, using a ratio of alumina to material of 50:1. On increasing the polarity of the solvent used by the addition of small portions of ethyl acetate, it was found that only the starting material was obtained, there having been no product formed during the reaction time.

Attempted Purification Of The Commercial Dye Samples.

Samples of C_1 Disperse Orange 5; C_1 Disperse Red 1; C_1 Disperse Red 13; and of C_1 Disperse Violet 12 were obtained in their commercial forms. It was found relatively simple to remove the non-coloured components from these samples by selective solubility in various organic solvents, normally the coloured components being soluble in organic solvents like chloroform and ethyl acetate, whereas the other materials were not.

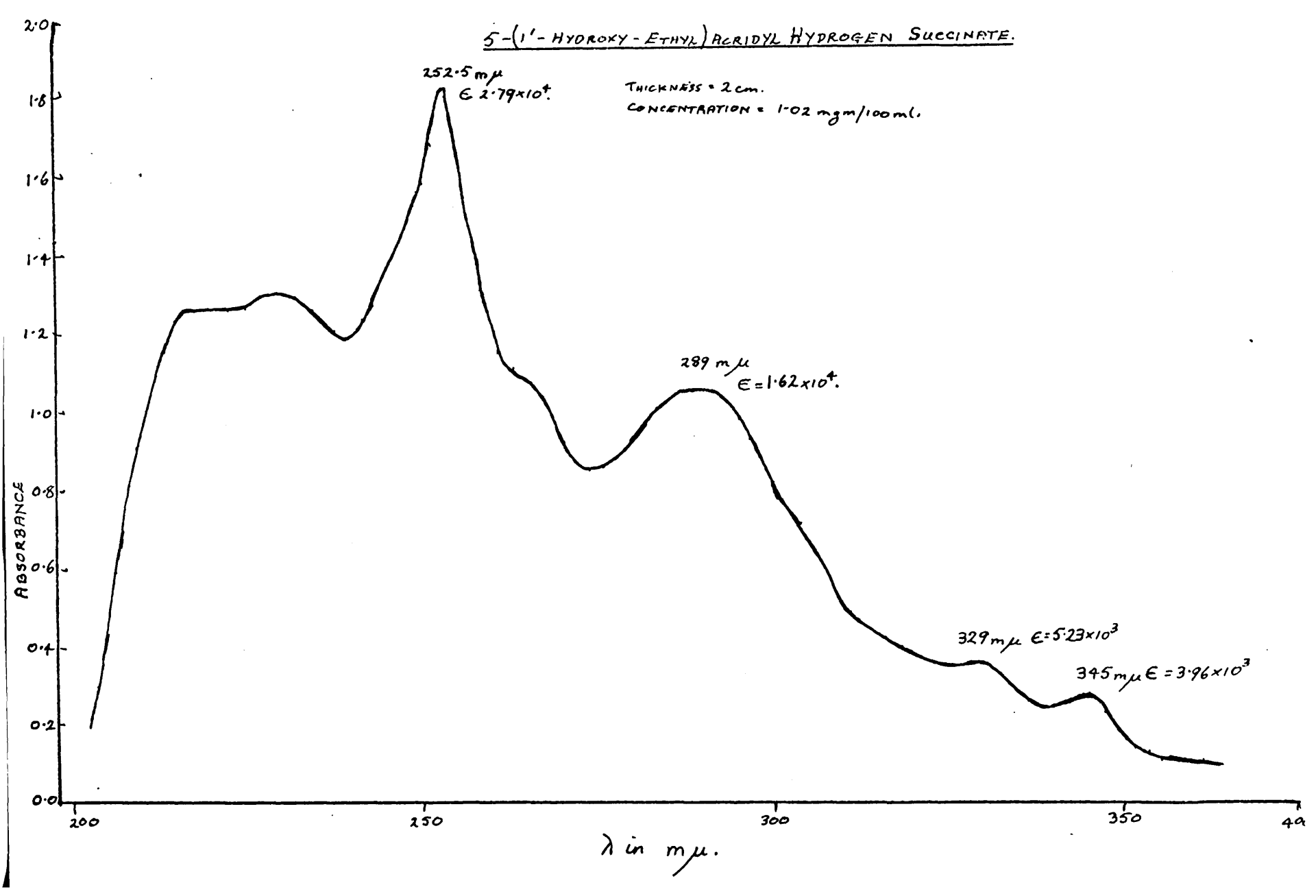
The resulting solutions contained mixtures of coloured materials and so each dye was dissolved in a suitable solvent, such as chloroform, and very carefully eluted down an alumina column. It was found best to use a very high ratio of alumina to material, normally in the region of 500:1, and the best grade was found to be grade II acid washed, or neutral, alumina.

5-Methyl Acridine.

Diphenylamine (50 gm, 30 mmole), Zinc Chloride (8.5 gm) and glacial acetic acid (30 ml, 50 mmole) were refluxed together for 14 hours. The resulting black tar was treated with concentrated sulphuric acid until all of the excess zinc chloride had been destroyed, as signified by the cessation of the evolution of hydrogen chloride, and this solution was then poured into boiling water (2-1) and allowed to stand for a period of not less than 2 hours, during which time it was stirred occasionally. The water was then decanted off and the resin re-extracted with several further portions of boiling water till the water extract no longer gave a turbid solution when treated with ammonia.

The combined aqueous extracts were then treated with 0.880 ammonia till the solution was basic to litmus, when a yellow precipitate appeared. This precipitate was filtered off and dried.

The crude 5-methyl acridine was purified by elution down a column of grade II neutral alumina using a ratio of alumina to material of 30:1 and a mixture of light petrol and benzene (1:1) as the solvent. After the impurities had been removed from the column, the polarity of the solvent was increased to pure benzene so that pure 5-methyl acridine was obtained and recrystallised from benzene to yield 2.62 gm, (13.6 mmole, 45%) m.p. 111-112°C. (Lit. 114°C.)


5-(1'-HYDROXY-ETHYL) ACRIDYL HYDROGEN SUCCINATE.
252·5 mμ
ε 2·79×10⁴.
THICKNESS = 2 cm.
CONCENTRATION = 1·02 mgm/100 ml.
289 mμ
ε = 1·62×10⁴.
329 mμ ε = 5·23×10³
345 mμ ε = 3·96×10³
ABSORBANCE
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0·0
200
250
300
350
λ in mμ.

5-(1'-Hydroxy-Ethyl)-Acridine.

5-Methyl acridine (2.6 gm, 13.6 mmole), and formaldehyde solution (3 ml of 20% solution) were heated together on a steam-bath for 4 hours and left standing overnight.

The solvent was then removed under reduced pressure, and the crude material obtained eluted down a column of grade V neutral alumina, using a ratio of alumina to material of 50:1, and benzene as the solvent at the start so as to remove the impurities. The polarity of the solvent was very slowly increased to benzene and ether (9:1) when the desired product was removed and recrystallised (with some difficulty) from benzene to yield 5-(1'-hydroxy-ethyl)acridine (2.03 gm, 9.11 mmole. 67%) m.p. 112-113°C. (Lit. 114°C).

5-(1'-Hydroxy-Ethyl) acridyl Hydrogen Succinate.

Succinic anhydride (526 mgm, 5.26 mmole) and 5-(1'-hydroxy-ethyl) acridine (527 mgm, 2.37 mmole) were dissolved in benzene and the solution refluxed. A few drops of freshly redistilled pyridine were added to the solution, which was then refluxed for a further 24 hours. The solvent was removed under reduced pressure and the yellow solid obtained recrystallised from a mixture of water and ethanol (1:1) to yield the required product (391 mgm, 1.21 mmole, 51%) m.p. 146½-147½°C.

Found C, 71.05; H, 5.47; N, 4.15%.

$C_{19}H_{17}NO_4$ requires C, 70.58; H, 5.30; N, 4.33%.

Hexamethylene Diamine-bis-(5-[1'-Hydroxy-ethyl]-acridyl Hydrogen Succinate) Mono-amide.

5-(1'Hydroxy-ethyl)-acridyl hydrogen succinate (208 mgm, 0.65 mmole) was dissolved, at -10°C, in dry tetrahydrofuran (50 ml) and triethylamine (2 ml) and ethyl chloroformate (75 mgm, 0.68 mmole) added to the solution which was then stirred for 20 minutes. After this, hexamethylene diamine (38 mgm, 0.32 mmole) dissolved in dry tetrahydrofuran, was added and the reaction continued, with stirring, for a further 18 hours, the temperature slowly being allowed to regain the ambient value.

The solid material produced during the course of the reaction was filtered off and the solvent removed from the filtrate under reduced pressure.

A yellow resinous material resulted which solidified only very slowly and it has not yet been found possible to obtain a solvent suitable for its recrystallisation. Analytical figures have thus not been obtained, but the infra-red spectrum of the material is consistent with that expected for the product.

Hexamethylene Diamine-bis-Naphthalene-2,3-Dicarboxylic Acid Mono Amide.

Naphthalene-2,3-dicarboxylic acid anhydride (1.98 gm, 10 mmole) was dissolved in toluene at 100°C and hexamethylene diamine (0.58 gm, 5 mmole) added. The reaction was stirred for a further 3 hours before the solvent was removed under reduced pressure and the white solid material thus obtained, recrystallised.

It was found that the material lost molecules of water very readily. On recrystallisation from NN-dimethyl formamide it was found that the initial product lost two molecules of water in an intra-molecular elimination so forming the di-imide, m.p. 280-1½°C

Found C, 76.07; H, 5.63; N, 6.18%.

$C_{30}H_{24}N_2O_4$ requires C, 75.62; H, 5.08; N, 5.88%.

In an attempt to recrystallise the parent compound, ethanol was tried, but even these conditions resulted in the elimination of a single molecule of water, forming the mono-imide.

Found C,72.59 ; H, 5.36; N, 5.53%.

$C_{30}H_{26}N_2O_5$ requires C, 72.86; H, 5.30; N, 5.66%.

Although analytical figures for the parent di-amide have not yet been obtained, the infra-red spectrum of the material obtained direct from the reaction was consistent with that for the expected product. The yield of material was 1.88 gm, (3.67 mmole, 73%)

Flow Sheet Five.

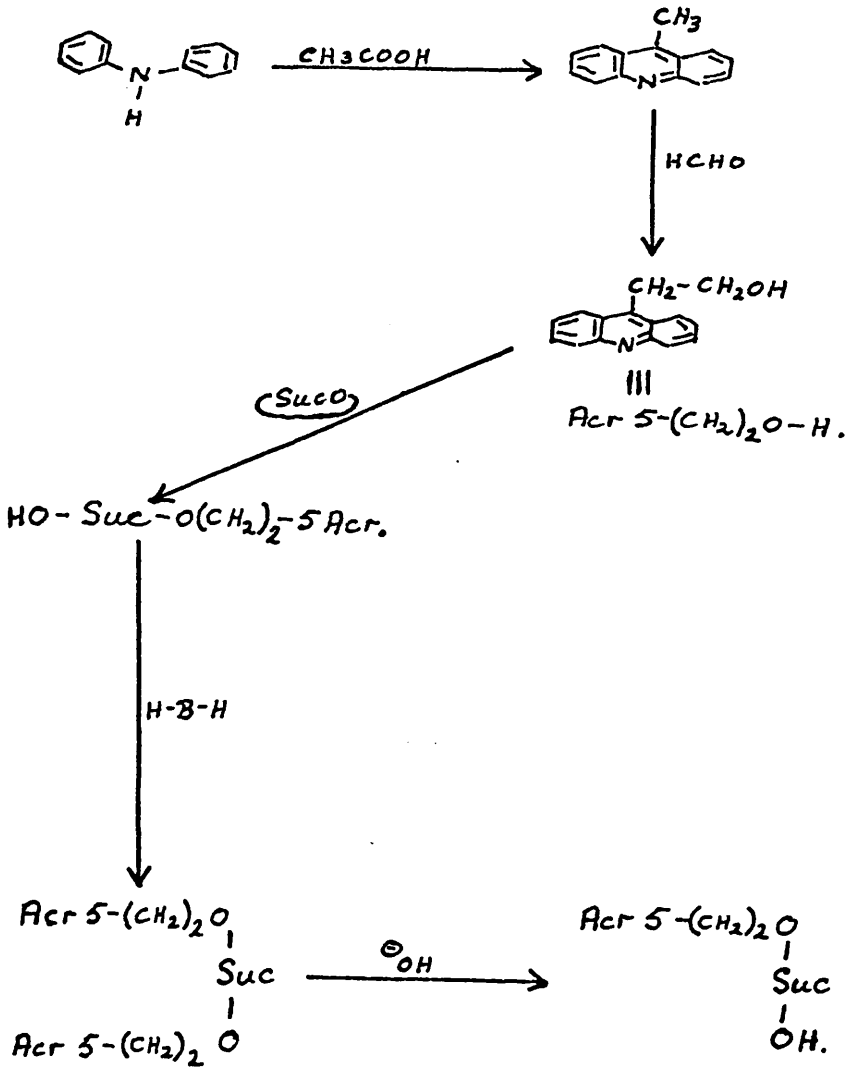
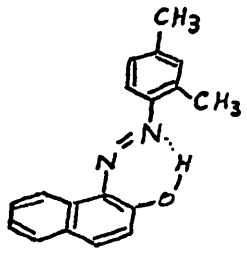
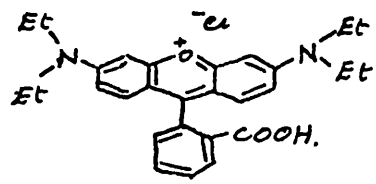
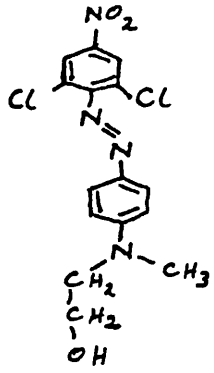
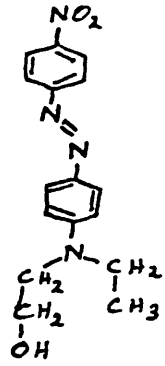
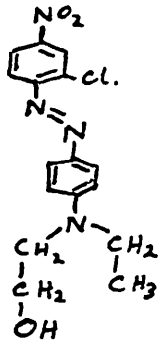
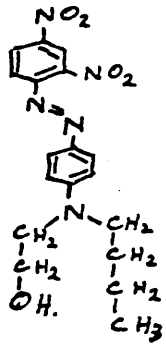


Table 5.

The Commercial Dyes Investigated.

Sudan II. 	Rhodamine B. 
C_1 Disperse Orange 5. 	C_1 Disperse Red 1. 
C_1 Disperse Red 13. 	C_1 Disperse Violet 12. 

SECTION THREE.

INTRODUCTION.

Mass spectral examination of organic compounds has been under investigation for a number of years. It is now reaching the stage where it is being systematised so that it can be used much more widely in the future as a tool of analytical chemistry.

More recently a certain amount of work has appeared in the literature concerning the possibilities of mass spectral investigations of peptides leading to a sequential analysis of the materials[68]. The detailed discussion of the breakdown of peptides in the mass-spectrometer will not be mentioned here since there are various excellent reviews of the subject in the literature[69].

In efforts to obtain sequential analyses of peptides, Bieman et al. have gone so far as to produce the mass spectra of some simple peptides as well as of all of the most common amino-acids. They were all run as their ethyl esters and it was found that these compounds show a number of characteristic break-down patterns[70].

What is to be looked for in a compound whose mass spectrum one desires? In the first place it must be obtainable in a really pure form. In peptide chemistry this is quite a difficulty, but this very fact is one of the reasons why mass spectrometry is an interesting technique, for it only requires tiny quantities of pure material for its successful prosecution. There are chances that small quantities of material can be obtained, whereas to obtain the larger quantities required for other methods of sequential analysis

often makes such methods prohibitively difficult. It is necessary to obtain really pure materials as otherwise one would be quite unable to distinguish the peaks in the mass spectrum that were derived from the material, and those derived from the impurities present.

In the second place the compound we are trying to analyse must be sufficiently volatile to give a mass spectrum. Peptides and amino-acids themselves are not normally volatile since they are often in their Zwitterion form. A lot of the work to date has been carried out into investigating suitable derivatives in which this ionic character is destroyed and the volatility increased. Various derivatives that have been found to be well suited for this purpose are to be found in the literature[68, 70].

If one now looks at peptides then one sees the size of the problems concerned in obtaining a sequential analysis of such materials. Peptides are composed of a number of different amino-acids arranged in chains, each amino-acid being joined to its neighbours by amide links. The peptide can split, in the mass spectrometer, at any of these constituent amide links, and also in any order. This naturally means that the mass spectrum one obtains will increase in its complexity in an almost exponential fashion as the complexity of the peptide increases. It is in the elucidation of the sequence of the amino-acids in the peptide chain from the mass spectrum it yields that one finds that the enormity of the problem arises.

Suppose that one were dealing with a simple compound A-B-C-D-E, then in the mass spectrometer it could break down so as to present one with the species $A\text{-}B\text{-}C\text{-}D\text{-}E^+$; $A\text{-}B\text{-}C\text{-}D^+$; $A\text{-}B\text{-}C^+$; $A\text{-}B^+$; and A^+ at the very least. Thus the resulting spectrum will be more complex than might, at first sight, be thought. In peptides, however, one is dealing with a still more complex situation, for each of the constituent amino-acids also has a characteristic break-down pattern in the mass spectrum.

So far, as mentioned above, the mass spectra of certain simple peptides have been determined, and from these results there would seem to be the strong possibility that one may indeed be able to elucidate the sequence of amino-acids in peptides from their mass spectra. It would, however, also seem likely that some kind of computer technique will be required before such a sequential analysis could be carried out for proteins. The computer would require to be programmed so as to try out all of the various losses from the parent (molecular) ion that are present and to try and match these with the ones thought to be 'reasonable' from our knowledge of the limited number of different α-amino-acids present in that particular protein. It would be required to reject anything that did not fall into this category. This would leave one with a rather simpler problem for one would obtain a whole series of fragments, large and small, of single amino-acids and of amino-acids in sequence; it would be like a rather complex jig-saw puzzle.

There are bound to be certain proteins which would 'break' the rules one had written into the computer programme and these would therefore have to be worked out by 'hand' so to speak.

Another use of such computer techniques would be in the positive identification of whole proteins from their mass spectra since, under constant conditions, the mass spectra obtained are their 'finger prints'.

DISCUSSION.

In these preliminary investigations, the first spectra to be run were those of the $ZCap_nOMe$ series of compounds (n = 1-3). These spectra were run first in order to try and determine the suitability of both the main series of compounds. It was possible to do this since this series contains both the amine-protecting group and the ester function.

It was found that the first member of this particular series, being a liquid, was quite volatile and no difficulty was experienced in obtaining a good spectrum. When the next homologue was tried, however it was found to be very much less volatile. This meant that the temperature of the source had to be increased before a suitable spectrum could be obtained. When the third member of this series was tried, it was found that the source had to be heated still further and that the spectrum obtained lacked any peaks at higher values of m/e than about 400, far less than the molecular weight of the parent compound. Investigation of the spectrum showed that it was the carbobenzoxy group which had, evidently, been removed thermally.

Although, because of this fact, it looked as if the presence of the carbobenzoxy groups would render the $ZCap_nBCap_nZ$ and $ZCap_nTetCap_nZ$ series of compounds less suitable for a mass-spectral examination a brief investigation was instituted. It was soon found that even the lowest member of each of these series was relatively non-volatile. This meant that the temperature of

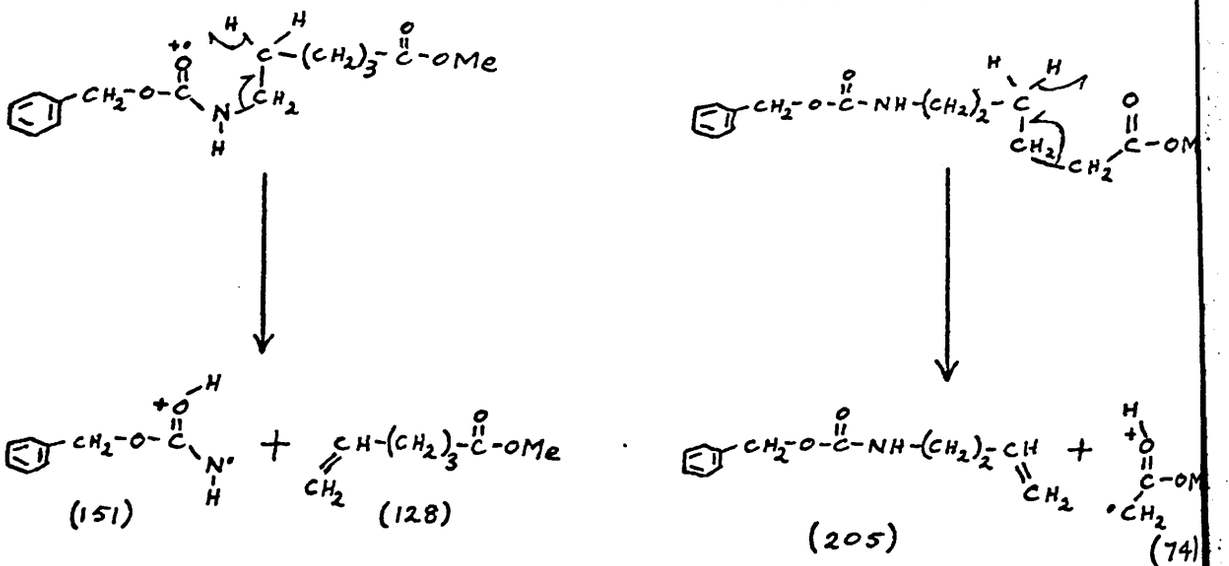
the source had to be increased and when a spectrum was obtained it was found to contain peaks of a significant size up to many times the molecular weight of the parent compounds. This could only be explained by assuming that the molecules had thermally decomposed more or less on the source and had therefore been able to polymerise prior to passing into the ion chamber. The spectrum that was obtained would therefore be very largely from this polymeric material and therefore impossible to interpret.

Attention was therefore turned to the possibilities presented by the diester series. There was a greater probability of these series providing useful information since esters are derivatives that are commonly used in mass spectroscopy. The volatility of this series was greater than that of the blocked diamine series. The fact that esters are useful for increasing the volatility of compounds is well known. The volatility of these series too, was however also found to decrease sharply with increasing molecular weight. The first member of each series was successfully run, but the higher members of both series have so far proved to be too non-volatile to provide satisfactory mass spectra, though efforts to obtain these are being continued.

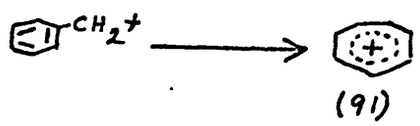
An investigation of the fragmentation patterns obtained from the lowest member of the diester series, when taken in conjunction with those for the $ZCap_nOMe$ series, brings one to the conclusion that certain quite simple patterns are recurring.

Fig. 12.

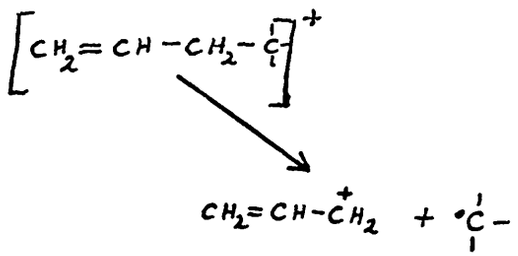
The McLafferty Rearrangement.



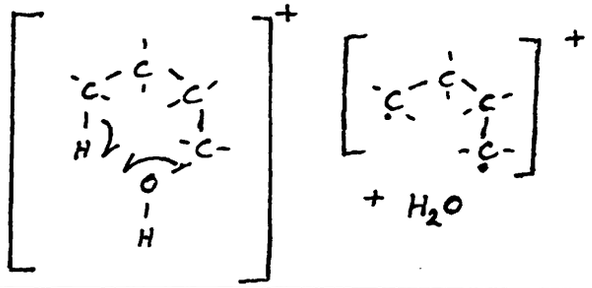
The Tropilium Ion.



Fragmentation By a Double Bond

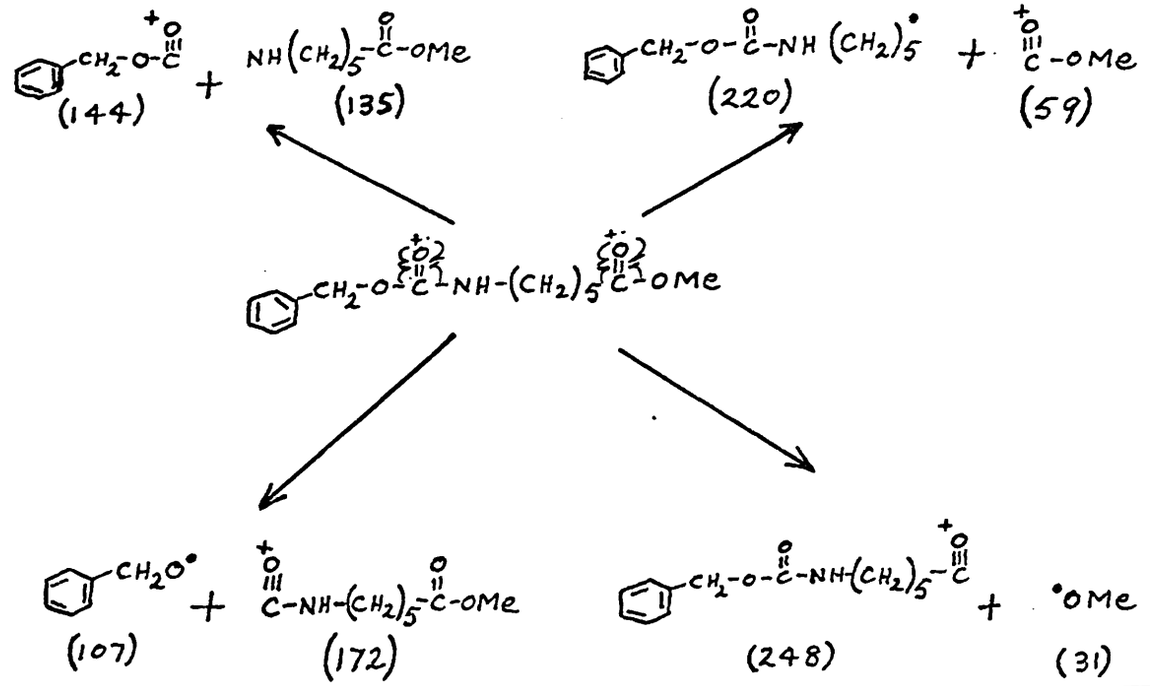


The loss of Water.

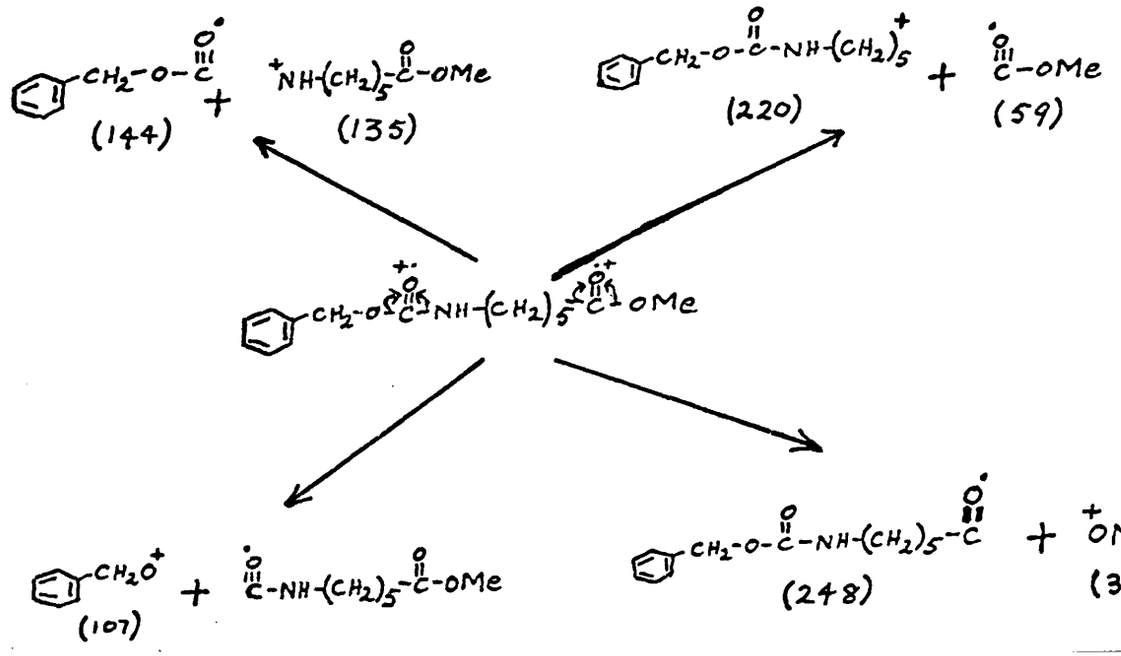


Some Mass Spectral Fragmentations.

Some fragmentations at Carbonyls.



Some fragmentations at Carbonyls.



All of the fragmentation patterns so far established are seen to follow certain well established lines which are well tabulated in the various standard text-books on Organic Mass Spectroscopy. Some of the more important routes found in these break-downs are very briefly summarised in Fig. 12.

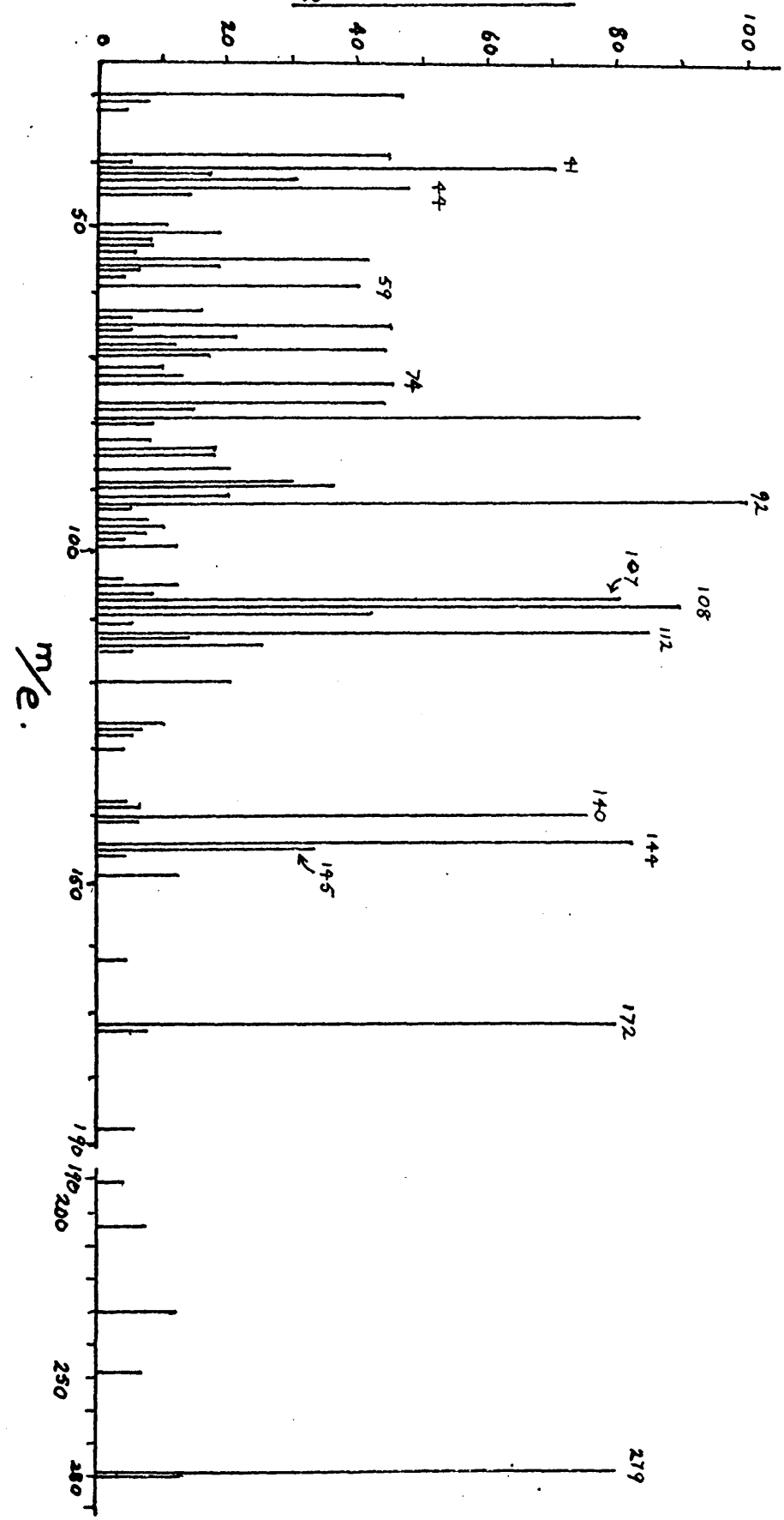
From the brief examination, which is all that time has allowed, it can be seen that the predominant fragmentations occur as a result of the various possibilities, within these linear systems, for the McLafferty Rearrangement. It can also be seen that there is a systematic loss of the various fragments, resulting from the cleavage of the ester and amide links present. From the tabulated results it is also seen that, although the cleavage of the outermost ester link leads to a reasonably abundant series of peaks, as one proceeds to the carbonyles more remote from the ends of the chain, then so the abundance of the peaks diminishes. It is as a result of this that the requirement for a good spectrometer, giving a very low background, arises. If this condition is not fulfilled then it will not be possible to take the smaller peaks into account and therefore one will not gather anything like the same amount of information from the spectra obtained.

A number of the transformations postulated in the fragmentations presented here are supported by the metastable peaks to be found in the various spectra. Other conclusions as to the reliability of certain of these transformations rely rather more

on the fact that the same transformation is to be found in the mass spectrum of one of the other members of the series. This is where the use of the two diester series becomes obvious, for it greatly aids the solution of the problems concerned in the interpretation of the spectra.

It can be seen that there is an abundant (parent + 1) ion. This is well known to occur with esters.

As the mechanisms, by which many of the more common fragmentations occur, have been listed, the explanations of the various mass spectra have been recorded in a tabular form.


% of Base Peak.
0
20
40
60
80
100
41
44
50
59
74
92
100
107
108
112
140
144
145
150
172
180
190
200
250
279
m/e.

Z Cap O Me.

Table 6. ZCapOMe.

m/e	% of Base.	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.
30	46	72	10	109	42	172	80		
31	7	73	13	110	-	173	8		
		74	45	111	5				
39	44			112	85	188	6		
40	5	77	44	113	14				
41	70	78	15	114	25	191	4		
42	17	79	83	115	5				
43	30	80	8	116	-	204	7		
44	47			117	3	205	2.4		
45	14	83	8			206	2.4		
		84	18	120	20				
50	10	85	18	121	3	219	2		
51	28	86	-						
52	8	87	20	126	10	229	0.4		
53	8	88	-	127	7	230	12		
54	5	89	30	128	5				
55	41	90	36	129	3	247	1.2		
56	18	91	20	130	4	248	6		
57	6	92	100			249	0.8		
58	4	93	5	138	4				
59	40	94	-	139	6	279	80		
		95	8	140	75	280	13		
63	16	96	10	141	6				
64	5	97	7						
65	45	98	4	144	82				
66	5	99	12	145	33				
67	21			146	4				
68	12	104	4						
69	44	105	12	149	12				
70	17	106	8						
		107	80	162	4				
		108							
				165	0.4				

Table 6 Cont^{d}. ZCap.OMe.

The Metastable Peaks.

Metastable Peak.	Transition Occurring.	Calculated Value.
213.2	248 ⟶ 230 + 18	213.3
110.3	144 ⟶ 126 + 18	110.3
106.1	279 ⟶ 172 + 107	106.0
87.2	144 ⟶ 112 + 32	87.1
74.3	279 ⟶ 144 + 135	74.3
57.8	144 ⟶ 91 + 53	57.5
42.5	74 ⟶ 56 + 18	42.4
25.0	74 ⟶ 43 + 31	25.0
24.4	69 ⟶ 41 + 28	24.4

ZCap.OMe Spectrum Interpretation.

m/e	Interpretation given.
279.	Parent ion. (P)
248.	P - 31. Loss of $^{\bullet}OMe$
230.	Loss of a molecule of water from 248. (Supported by a metastable ion peak.)
205.	$C_6H_5.CH_2.O.CO.NH(CH_2)_2CH=CH_2$.
172.	P - 107. Loss of $C_6H_5.CH_2.O^{\bullet}$ (Supported by a metastable ion peak.)
144.	P - 135. Loss of $C_6H_5.CH_2.O.CO^{\bullet}$ (Supported by a metastable ion peak.)
128.	$CH_2.O.CO.NH(CH_2)_2CH=CH_2$ derived from 205 peak ion.
126.	Loss of H_2O from 144 peak ion. (Supported by a metastable ion peak.)
114.	$O.CO.NH(CH_2)_2CH=CH_2$ from the 128 peak ion.
113.	Loss of $NH(CH_2)_5CO^{\bullet}$ from various ions e.g. from 248 ion
112.	Loss of $2H_2O$ from 144 peak ion. (Supported by a metastable ion peak.)
98.	$CO.NH(CH_2)_2CH=CH_2$ from the 114 peak ion.
91.	The tropilium ion, derived from various ions.(144)
77.	$C_6H_5^+$ ion.
74.	The $CH_3.CO.OMe$ ion.

ZCap.OMe spectrum interpretation. cont^{d}.

m/e	Interpretation given.
70.	$NH.(CH_2)_2CH=CH_2$ from the 98 peak.
69.	$CH_2=CH.CH_2.C\equiv O^+$ ion.
59.	The $CH_3O.C\equiv O^+$ ion.
56.	The CH≡COMe ion derived from the 74 peak ion. (Supported by a metastable ion peak.)
44.	$\cdot CH_2.CH_2.NH_2^+$ ion.
43.	The $CH_3.C\equiv O^+$ from 74 peak ion. (Supported by a metastable ion peak.)
41.	Loss of $CH_2=CH-CH_2^+$ from 69 peak ion. (Supported by a metastable ion peak.)
31.	The $^+OCH_3$. ion.

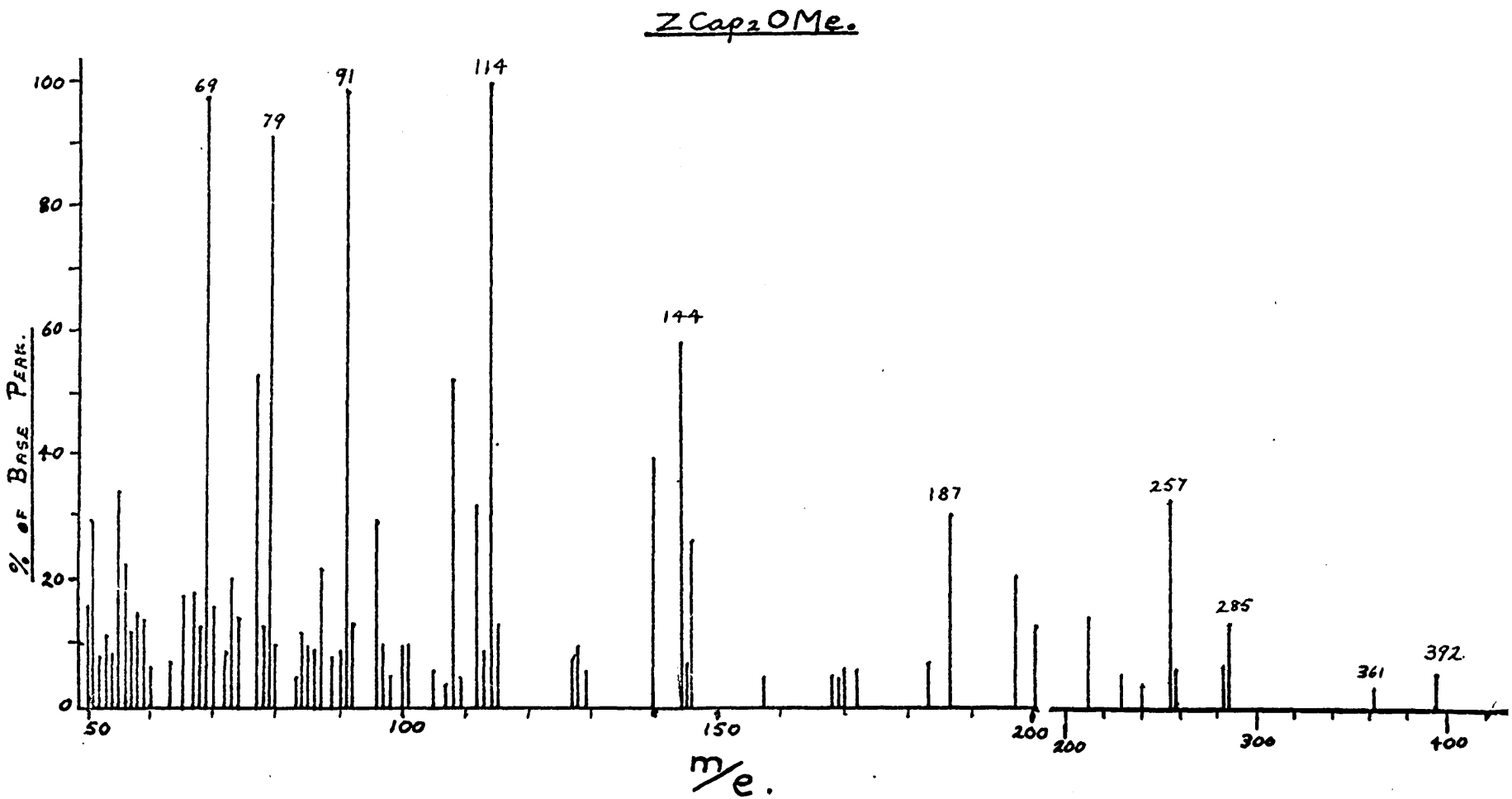

ZCap2OMe.
% of Base Peak.
100
80
60
40
20
0
69
79
91
114
144
187
257
285
361
392.
50
100
150
200
200
300
400
m/e.

Table 7. $ZCap_2OMe$

m/e	% of Base	m/e	% of Base	m/e	% of Base	m/e	% of Base	m/e	% of Base
30	100	72	9	112	32	201	2	392	5
31	8	73	20	113	9			393	1.4
32	14	74	14	114	100	210	1		
				115	13	211	15		
38	6	77	53			212	2		
39	33	78	13	127	8	213	0.6		
40	5	79	92	128	10	214	3		
41	94	80	10	129	6	225	2		
42	16					226	1		
43	21	83	5	140	40	227	0.8		
44	41	84	12			228	1		
45	7	85	10	144	58	229	4		
		86	9	145	7	230	1		
50	15	87	22	146	27				
51	30	88	-			239	4		
52	8	89	8	157	5	240	1.2		
53	11	90	9			241	0.5		
54	8	91	99	168	5	242	1		
55	34	92	13	169	5				
56	23			170	6	257	33		
57	12	96	30	171	1.5	258	6		
58	15	97	10	172	6				
59	14	98	5			284	6		
60	6	99	-	183	7	285	13		
		100	10	184	1.4	286	2		
63	7	101	10						
64	-			187	31	317	0.6		
65	18	105	6	188	1.5	318	-		
66	-	106	-			319	0.6		
67	18	107	4	197	21				
68	13	108	52	198	1.5	361	3		
69	98	109	5	199	-	362	0.8		
70	16			200	11				

Table 7 Cont^{d}. $ZCap_2OMe$.

The Metastable Peaks.

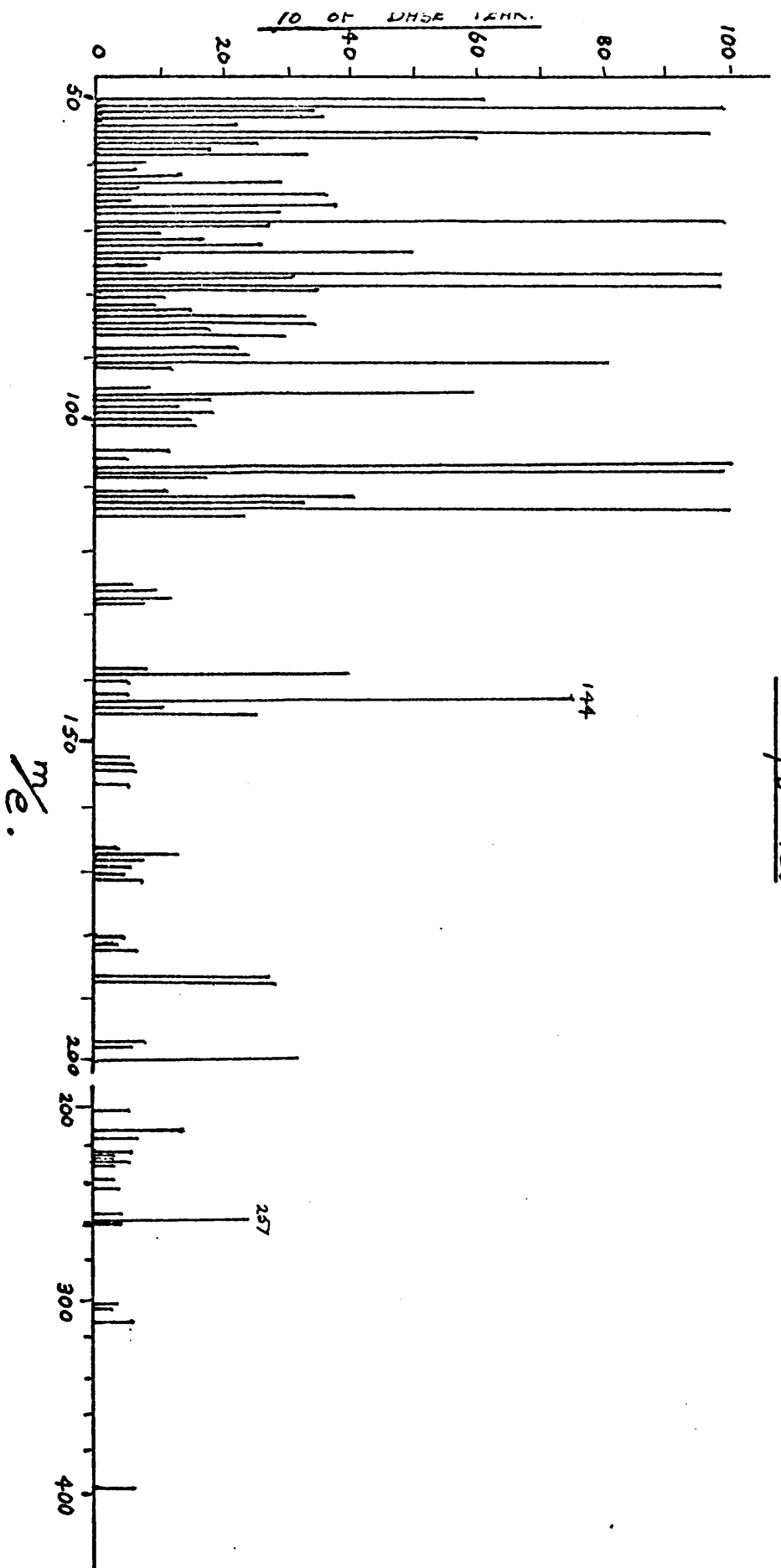
Metastable Peak.	Transition Occurring.	Calculated Value.
222.3	257 ⟶ 239 + 18	222.3
213.4	248 ⟶ 230 + 18	213.3
207.3	392 ⟶ 285 + 107	207.2
168.5	392 ⟶ 257 + 135	168.5
110.1	144 ⟶ 126 + 18	110.3
87.2	144 ⟶ 112 + 32	87.1
80.8	257 ⟶ 144 + 113	80.6
69.5	144 ⟶ 100 + 44	69.4
57.8	144 ⟶ 91 + 53	57.5
24.4	69 ⟶ 41 + 28	24.4

ZCap$_2$OMe Spectrum Interpretation.

m/e	Interpretation given.
392.	Parent ion. (P).
361.	P - 31. Loss of ·OMe.
285.	P - 107. Loss of $C_6H_5.CH_2.O\cdot$ (Supported by a metastable ion peak.)
257.	P - 135. Loss of $C_6H_5.CH_2.O.CO.$ (Supported by a metastable ion peak.)
248.	The $C_6H_5.CH_2.O.CO.NH(CH_2)_5^+$ ion.
239.	Loss of H_2O from the 257 peak ion. (Supported by a metastable ion peak.)
230.	Loss of H_2O from the 248 peak ion. (Supported by a metastable ion peak.)
211.	The $^+O{=}C.NH(CH_2)_5.CO.NH(CH_2)_2CH{=}CH_2$ ion.
144.	Loss of $C_6H_5.CH_2.O.CO.NH(CH_2)_5CO.$ from the 257 ion. (Supported by a metastable ion peak.)
126.	Loss of H_2O from the 144 peak ion. (Supported by a metastable ion peak.)
114.	The $^+O.CO.NH(CH_2)_2CH{=}CH_2$ ion.
113.	The $+NH(CH_2)_5CO$ ion produced by fragmentation.
112.	Loss of $2H_2O$ from the 144 peak ion. (Supported by a metastable ion peak.)

ZCap$_2$OMe spectrum interpretation, cont^d.

m/e	Interpretation given.
100.	Loss of CO_2 from the 144 peak ion. (Supported by a metastable ion peak.)
91.	The tropilium ion e.g. from the 144 peak ion. (Supported by a metastable ion peak.)
87.	The $^+[\dot{C}H_2.O.CO.NH.CH_2^{\cdot}]$ ion.
85.	The $[NH(CH_2)_5]^+$ ion produced by fragmentation and rearrangement.
77.	The $C_6H_5^+$ ion.
74.	The $[CH_3CO.OMe]^+$ ion.
69.	The $CH_2=CH-CH_2-C\equiv O^+$ ion.
59.	The $CH_3O.C\equiv O^+$ ion.
43.	The $CH_3C\equiv O^+$ ion.
41.	The $[CH_2=CH-CH_2]^+$ ion from the 69 peak ion. (Supported by a metastable ion peak.)
31.	The ^+OMe ion.


% OF BASE PEAK.
0
20
40
60
80
100
50
100
150
200
200
300
400
m/e.
144
257

2 Cap3 OMe.

Table 8. $ZCap_3OMe$.

m/e	% of Base.	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.
50	61	82	9	126	6	197	7	269	0.6
51	99	83	15	127	10	198	4	270	1
52	35	84	33	128	12	199	-	271	0.5
53	36	85	34	129	8	200	31		
54	23	86	18			201	6	283	1.6
55	97	87	30	139	8			284	1.8
56	60	88	-	140	40	211	14	285	2.9
57	26	89	23	141	5	212	2.3		
58	18	90	24	142	-	213	1.5	296	1.8
59	34	91	81	143	5	214	7	297	0.8
60	8	92	12	144	75	215	3		
61	7			145	10			300	1.6
62	14	95	8	146	25	225	6	301	0.4
63	30	96	60			226	3		
64	7	97	18	153	5	227	3	310	4
65	37	98	13	154	6	228	6	311	1.5
66	6	99	18	155	6	229	3	312	-
67	38	100	15	156	-			313	3
68	29	101	16	157	5	238	2	314	0.9
69	99					239	3		
70	27	105	12	167	3	240	1.5	322	0.7
71	10	106	5	168	13	241	1.6	323	-
72	17	107	100	169	7	242	4	324	6
73	26	108	99	170	5			325	1.5
74	50	109	17	171	4	252	1.9	326	2
75	10	110	-	172	7	253	4		
76	8	111	11			254	1.6	338	1.2
77	99	112	41	181	4	255	0.7		
78	41	113	33	182	3	256	0.6	341	1.2
79	99	114	100	183	6	257	24	342	1.2
80	35	115	24			258	4		
81	11			187	27	259	2.5		
				188	28				

Table 8. Cont^{d}. $\underline{ZCap_3OMe.}$

m/e	% of Base.								
353	0.9								
354	0.6								
355	1.4								
366	1.4								
367	0.5								
368	0.6								
369	0.5								
397	6								
452	0.1								
453	-								
454	0.2								
455	0.1								
456	0.1								

ZCap$_3$OMe Spectrum Interpretation.

The spectrum of this material can be interpreted in exactly the same manner as was done with the two lower homologues. In this case, however, the highest m/e observed is that for the (parent minus 107) ion. There are then traces of the ions corresponding to losses of 135 and 181 from the parent. The next large peak is the one of mass 285. This corresponds to the ion :- $C_6H_5.CH_2.O.CO.NH(CH_2)_5CO.NH(CH_2)_5^+$ This is a fragmentation exactly analogous to one found in the spectra of the lower homologues.

Similarly almost all of the rest of the spectrum can be related directly to those just previously interpreted. The one major difference is that there is far less fragmentation from the ester end of the molecule, but this is obviously what would be expected because of the initial thermal break down of the carbobenzoxy group.

MeO Cap Sub Cap OMe.

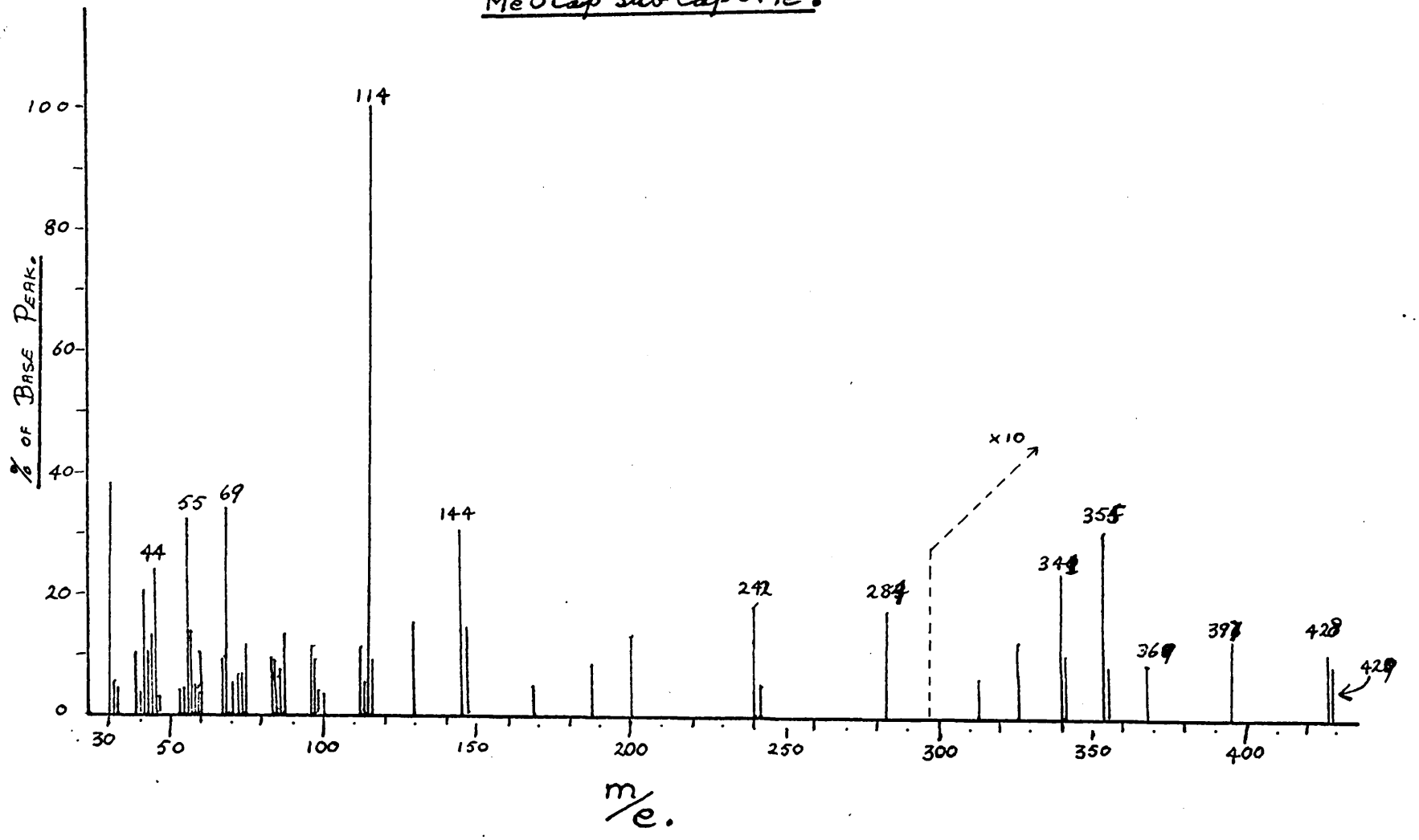


Table 10. MeOCapSubCapOMe.

m/e	% of Base	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.
30	37	81	3	144	30	242	18	323	0.3
31	5	82	2	145	3	243	4.5		
32	4	83	8	146	14			327	1.2
		84	8			252	2.2	328	0.38
39	10	85	6	156	2	253	0.5	329	0.06
40	3	86	7			254	0.2		
41	20	87	13	168	5	255	0.5	337	0.11
42	10					256	1.1		
43	12	93	3	172	2	257	2.7	341	2.3
44	23					258	0.5	342	0.95
45	3	96	11	182	1.3			343	0.13
		97	9			281	1.17		
53	4	98	4	187	8	282	0.23	353	0.13
54	4	99	-	188	1.4	283	0.7	354	0.1
55	32	100	3			284	17	355	3
56	13			196	2.5	285	3.4	356	0.82
57	4	110	2	197	0.5	286	0.5	357	0.1
58	4	111	4	198	0.6				
59	10	112	11	199	0.2	295	0.15	368	0.11
60	2	113	5	200	13	296	0.10	369	0.75
		114	100	201	1.6			370	0.24
67	9	115	9			299	0.13	371	0.04
68	9			210	3	300	-	372	0.05
69	34	126	3			301	0.22		
70	5	127	3	214	3.5			384	0.04
71	-	128	3	215	0.9	309	0.28	386	0.22
72	6	129	15			310	0.15	387	0.15
73	6	130	1	223	1.2				
74	11					313	0.2	396	0.27
		138	4	228	1.4	314	0.57	397	1.1
79	3	139	4	229	2.3	315	0.2	398	0.21
		140	4					399	0.07
								400	0.04

Table 10 Cont^{d}. MeOCapSubCapOMe.

m/e	% of Base.
426	0.05
428	1.0
429	0.75
410	0.12

MeOCap.Sub.CapOMe Spectrum Interpretation.

Because of the greater amount of symmetry within this molecule it is to greater advantage to tabulate the results so that this symmetry is emphasised.

m/e	Interpretation given.
428.	Parent ion.
397.	P - 31. Loss of OMe.
369.	P - 59. Loss of COOMe.
299.	P - 129. Loss of $(CH_2)_5COOMe$.
284.	P - 144. Loss of CapOMe.
256.	P - 172. Loss of CO.CapOMe.
172.	P - 256. Loss of $(CH_2)_6CO.CapOMe$.
144.	The $MeOCap^+$ ion.
129.	The $MeOCO.(CH_2)_5^+$ ion.
59.	The $MeOCO^+$ ion.
31.	The MeO^+ ion.
354.	P - 74. The McLafferty on the first carbonyl.
323.	Loss of 31 from the 354 ion.
295.	Loss of 59 from the 354 ion.

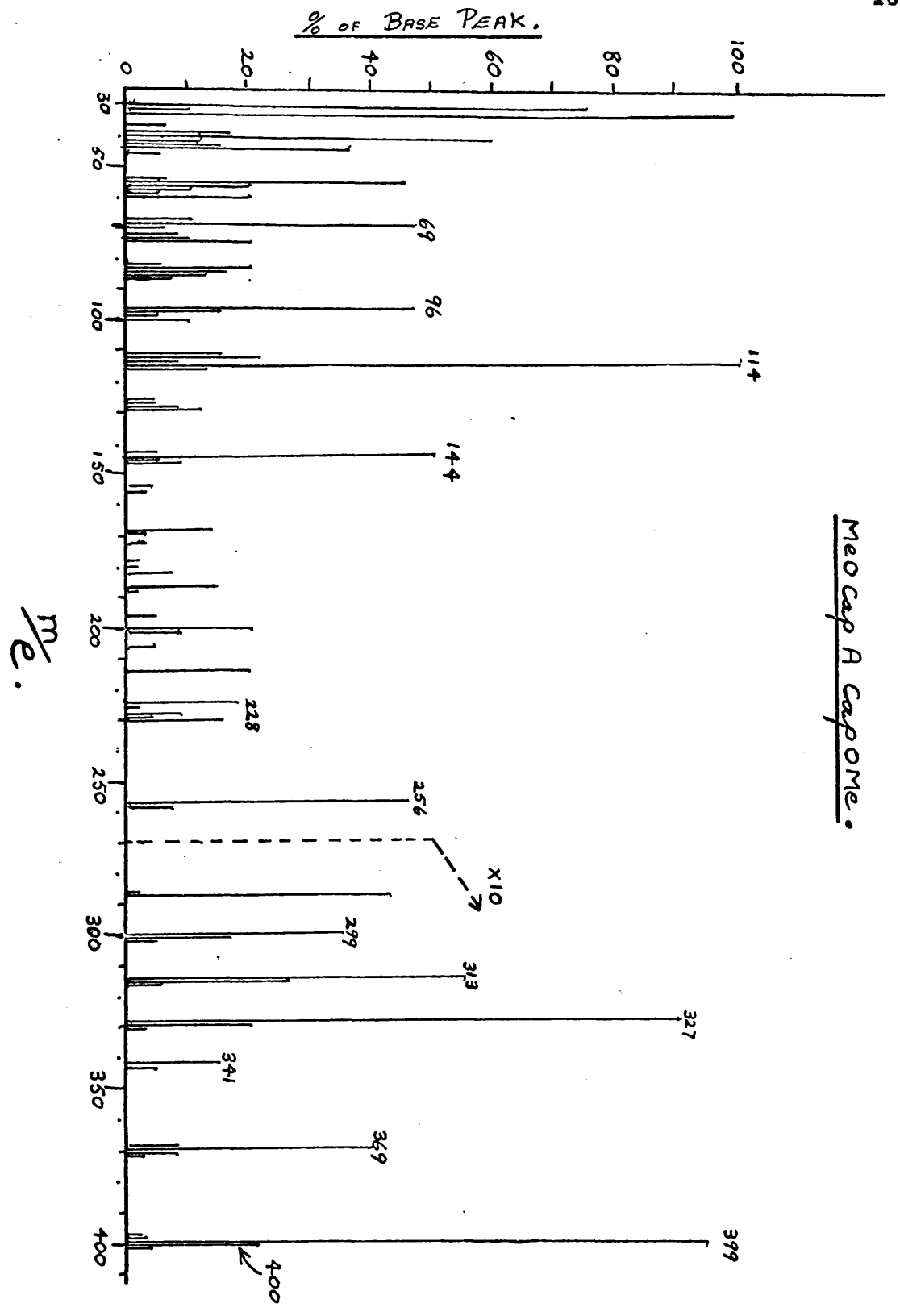
MeOCap.SubCap.OMe. Spectrum Interpretation Cont^{d}..

$^{m}/_{e}$	Interpretation given.
225.	Loss of 129 from the 354 ion.
210.	Loss of 144 from the 354 ion.
182.	Loss of 172 from the 354 ion.
98.	The CH_2=CH-$(CH_2)_2$NH.CO$^+$ ion.
70.	The CH_2=CH-$(CH_2)_2$NH$^+$ ion.
41.	The CH_2=CH-CH_2^+ ion.
74.	The $CH_3COOCH_3^+$ peak.
59.	The CO.OMe$^+$ ion.
31.	The OMe$^+$ ion.
313.	Loss of 41 from the 354 ion.
282.	Loss of 41 from the 323 ion.
254.	Loss of 41 from the 295 ion.
184.	Loss of 41 from the 225 ion.
169.	Loss of 41 from the 210 ion.
141.	Loss of 41 from the 182 ion.
57.	Loss of 41 from the 98 ion.

MeOCap.SubCap.OMe Spectrum Interpretation. Cont^{d}..

$^{m}/_{e}$	Interpretation given.
215.	A McLafferty on the second carbonyl in the chain.
213.	The CH_2=CH-$(CH_2)_3$.CO.NH$(CH_2)_5$COOMe^{+} ion.
182.	Loss of 31 from the 213 ion.
154.	Loss of 59 from the 213 ion.
84.	Loss of 129 from the 213 ion.
69.	Loss of 144 from the 213 ion.
41.	Loss of 172 from the 213 ion.
172.	Loss of 41 from the 213 ion.
141.	Loss of 41 from the 182 ion.
113.	Loss of 41 from the 154 ion.
43.	Loss of 41 from the 84 ion.

Similarly regular losses can be drawn up and matched with peaks occurring in the mass spectrum, when one considers the possibilities presented by carrying out a second McLafferty rearrangement on the species resulting from the first one.



MeO Cap A Capome.

Table 9. MeOCapACapOMe.

m/e	% of Base.	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.	m/e	% of Base.
30	75	82	5	154	4	224	17	341	1.5
31	10	83	20	155	-	225	2.5	342	0.5
32	99	84	16	156	3	226	0.6		
		85	13	157	1	227	3	368	0.8
36	5	86	7			228	8	369	3.9
		87	20	168	14	229	4	370	0.8
39	16			169	3	230	1.5	371	0.3
40	11	96	47					372	0.2
41	59	97	15	172	3	254	0.5		
42	12	98	5			255	1	397	0.2
43	15	99	-	178	2	256	43	398	0.3
44	36	100	10	179	-	257	7	399	9.5
45	5	101	0.4	180	2	258	1	400	2.1
				181	-	259	0.2	401	0.4
53	5	111	15	182	7				
54	6	112	21			285	0.2		
55	45	113	8	187	14	286	4.3		
56	20	114	100	188	1.5	287	0.1		
57	10	115	13			288	-		
58	5			196	4				
59	20	126	4	197	-	295	0.4		
		127	4	198	1				
67	10	128	8	199	1	299	3.5		
68	10	129	12	200	20	300	1.7		
69	47			201	9	301	0.4		
70	6	140	8	202	1				
71	-	141	3			313	5.5		
72	8	142	2	206	4	314	2.6		
73	10	143	5			315	0.6		
74	20	144	50	212	0.5				
		145	5	213	1	327	9		
		146	9	214	20	328	2		
				215	2	329	0.3		

MeOCap.ACap.OMe Spectrum Interpretation.

A tabulation of the full interpretation of the mass spectrum of this compound would be superfluous since it corresponds exactly with that of the preceding compound (MeOCap.SubCap.OMe). It should be noted however that the presence of the two spectra greatly aided in the solution of the problems since a direct comparison was possible. The comparison has lead to the analysis of these compounds being as complete as it has been.

Thus, from this work, it would seem that the principle peaks in these mass spectra can be explained in terms of fairly simple mechanisms. This lends support for the ideas presently being discussed concerning the possibilities of a sequential analysis of peptides, using this technique.

Table 11.

Mass Spectrometer Data.

Compound.	Molecular Weight.	Source Temperature (Ambient + X)
ZCap.OMe.	279	70°C.
$ZCap_2OMe$.	392.5	160°C.
$ZCap_3OMe$.	397.	200°C.
MeOCap.A.Cap.OMe	400.5	210°C.
MeOCap.Sub.CapOMe.	428.5	250°C.

APPENDIX 1.

Dyeing Reagents Used in This Thesis.

1. Ceric Sulphate. (General Reagent.)

Ceric ammonium sulphate (1 gm) was suspended in boiling water (20 ml) and concentrated sulphuric acid slowly added until solution was complete. The cooled solution was sprayed on to the chromatoplate which was then heated at 150°C for about a quarter of an hour.

2. Perchloric Acid. (General Reagent.)

This was sprayed directly on to the chromatoplate which was then heated at 150°C for about a quarter of an hour.

3. Iodine. (A Widely Applicable Reagent.)

The plates were allowed to stand in a vessel containing solid iodine which was allowed to sublime on to the plate. A quarter of an hour was normally sufficient for the materials to become visible.

4. Copper Acetate-Potassium Ferrocyanide. (According to Kaufmann.)

(For Carboxylic Acids.)

Solution 1.

A saturated solution of copper acetate was made up in water and 1 ml of this solution diluted to 25 ml with water.

Solution 2.

A fresh solution of potassium ferrocyanide in water (1.5%).

Procedure.

The chromatoplate was saturated with solution 1 for 45 minutes

then the excess reagent was very gently removed. The chromatoplate was then sprayed with solution 2.

5. Rhodamine B. (For carboxylic acids.)

Rhodamine B (150 mgm) was dissolved in absolute alcohol (10 ml). The chromatoplate was sprayed with this solution and after a short while the excess reagent was washed off by very gently dipping it, for a short while, in 50% ethanol. The materials became visible under ultra-violet light.

6. Bromocresol Green. (For carboxylic acids.)

Commercial bromocresol green solution was taken and to it added eight drops of 30% sodium hydroxide for every 100 ml. of solution. The materials should have become visible by a change in colour from blue to green.

7. Bromophenol Blue-Citric Acid. (For carboxylic acids.)

Bromophenol blue (50 mgm) and citric acid (20 mgm) were dissolved in water (10 ml.).

8. Chlorine-Potassium Iodide-Starch. (Carbobenzoxy amino-acid esters.)

The chromatoplate was first exposed to chlorine gas for about half an hour, with the normal precautions, followed by the removal of the excess chlorine by allowing it to stand in the air for a few minutes. The chromatoplate was finally sprayed with a standard solution of potassium iodide/starch.

9. Hydroxylamine-Ferric Chloride. (For all esters.)

Solution 1.

Equal volumes of a 12.5% solution of sodium hydroxide and a 5% solution of hydroxylamine in water.

Solution 2.

Acetic acid.

Solution 3.

A fresh solution of ferric chloride in water (10%).

The chromatoplate was sprayed with solution 1, allowed to stand for 10 minutes at 100°C and then sprayed with solutions 2 and 3 consecutively.

10. Universal Indicator. (Fairly General Reagent.)

A commercial solution of the indicator was sprayed directly on to the chromatogram. Sometimes this could be followed by allowing the chromatogram to come in contact with ammonia vapour for a few seconds when the materials could be made to stand out more from the background.

APPENDIX 2.

This form of the chain-alignment is comparable with the known biological case of base pairing of nucleic acids.

Khorana has recently discussed his extensive use of this property, as it plays a very important part in his novel synthesis of a nucleic acid[71].

CONCLUSION.

A stepwise reaction technique has been evolved which is sufficiently flexible to allow the inclusion of a wide variety of groups of varying size and polarity and at the intervals designed by the researcher. It has also been designed to be suitable for the synthesis of such materials on a small scale. These points have been proved by the synthesis of a number of series of linear oligoamides with varying functionality at the ends of their chains.

A doubling reaction technique has also been investigated and shown to be capable of improvement by the use of highly coloured or fluorescent derivatives of the components. Investigations have shown the feasibility of such a system in the synthesis of specific linear oligomers.

The preliminary mass spectrometric investigations which have been carried out have shown something of the possibilities of a sequential analysis of peptides, using this technique.

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