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PROCEEDINGS OF THE CHEMICAL SOCIETY

MARCH 1963

PUBLICATIONS SURVEY

THE COUNCIL of the Chemical Society has authorised a Publications Survey, the objects of which shall be to study and report on the needs, problems, and possible developments in the publication of chemical information, and to recommend actions, on an informed basis, whereby the pattern of publications can more adequately meet present and future needs of those who publish and those who read, alike; the survey and its recommendations are to be embodied in a report that will be published; the survey to be complete within two years.

Further, the Council has appointed Dr. R. S. Cahn (see p. 98) to conduct this survey, thereby taking advantage of his wide experience in chemistry and unique knowledge of the various aspects of publication. Dr. Cahn has undertaken academic research and teaching in this country and abroad, has experience of industrial research as well as academic, has run an industrial information service, and was an abstractor for many years and ultimately Associate Editor of *British Abstracts*. As Editor to the Chemical Society for the last 14 years he has seen not only the steady climb in volume of original publication but the demise of the main British activity in abstract work. He inaugurated the first major Chemical Journal devoted to current awareness and, as an original member of the Editorial Board of the I.U.P.A.C. Journal "Pure and Applied Chemistry" has become acquainted with the problems of international publications.

The need for such a publications survey is indicated by the fact that for several decades past the volume of chemical publication has doubled in geometrical proportion every 8—10 years. The increase takes the form of larger and more journals of original research, more patents, and larger abstract journals and compendia. Whilst problems resulting for the learned Societies that act as the principal publishers are difficult, those for the user are already almost beyond control. These users include academic and industrial research workers, research directors and man-

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agers, information officers, and teachers; it is only with great difficulty that they can keep abreast of research even in their own fields. The Chemical Society believes that it owes a duty to its Fellows, and to chemists and chemistry in general, to replace the present drifting developments by a rational general policy.

It is rather generally realised that new techniques are required and some work is being done in this direction. There are, however, in Britain few detailed and no correlated data on the complex and inter-related issues of production and use of chemical publications. The survey must provide such data and outline methods whereby the increasing amount of information may be disseminated efficiently to those who need to use it.

The Three Stages in the Dissemination of Information.—To be of value, experimental results and theoretical progress require dissemination, which involves three stages: (*a*) the work must be made easily and permanently accessible in detail; (*b*) the work must be brought to the attention of the relevant working specialist ("current awareness"); and (*c*) the total of work must be correlated and individual items later retrieved.

Publication of Original Research.—The increasing size and cost of journals issued by learned Societies and commercial publishers alike have so far been met by increasing the price, by splitting one large into several smaller journals, or by a page charge. Each method has its own disadvantages. The first two in particular lead to less availability of the work to the individual potential user, thus partially stultifying the very object of publication. In 1962, *Current Chemical Papers*, which deals with "pure" chemistry only, listed 31,101 titles taken from 2415 issues of journals. In its much wider field, *Chemical Abstracts* now reports more than 160,000 published items a year.

Current Awareness.—Originally, the chemist read all original chemical work, later all in his own field only; for long, however, he has relied on abstract journals; but, particularly in the last ten years, even abstract journals have become too large for scanning and too tardy for present competitive conditions, and there has resulted a rapid expansion in review journals and in periodic correlated lists of titles, new compounds, and spectra. In addition, individual industries, firms, and research teams require their own scanning services.

Correlation and Retrieval.—The classical methods of correlation and retrieval involve the abstracting journals and great compilations such as Beilstein and Gmelin. The volume of current research has outstripped these methods. Thus for instance, the last decennial index of *Chemical Abstracts* comprised 19 volumes, and future indexes must be still larger; retrieval of a fact from such indexes by individual search is already difficult and will soon become often well-nigh impossible. Again, the supplement to Beilstein now being issued covers the literature only to 1939; the still more prolific post-war years remain wholly to be included in later supplements. The potentialities of electronic methods are being studied by *Chemical Abstracts* on a large research budget; and similar methods are being studied elsewhere, notably in industry. The potentialities of these and other modern methods for data are obvious; their usefulness for concepts is still to be determined.

Interlocking of the Three Stages.—The three aspects above cannot be considered separately. Current awareness might perhaps be achieved more readily with co-operation of the publishers of original research, so might correlation. The methods used for current awareness, correlation, and retrieval are clearly in the melting pot, and the changes that are inevitable there may well have notable effects on the publication of original research, which however, has problems whose solution may in turn affect the other two aspects. If, for example, an international information service included an adequate copying service, then much of the detail now in journals of original research might be deposited for supply only on demand; one can make a first estimate that this would reduce the size of journals to one half or one quarter; but one cannot yet estimate the effects on the user, and other objections can be envisaged. Journals containing original papers shorn of detail might aid the reader to survey the whole, to find items of particular interest to him, but they might hamper his assessment of the new research. Again realignment from journals dealing with general chemistry to more specialist journals, perhaps organised on an international basis, might benefit the individual in avoiding scatter of related work and provide a more

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rational basis for expansion and for development as a whole, but equally this might be damaging in its restrictive effect. In none of these cases can the conflicting factors yet be weighed; the examples serve to illustrate some of the types of problem that await and require study.

The difficulties, of course, affect all branches of science; but they are particularly acute for chemistry, and it is hoped that any remedies found for chemical publications may incidentally benefit also other disciplines.

Co-ordination and Collaboration.—It must be made clear that there is no intention to duplicate the research being done by *Chemical Abstracts* which is directed specifically to recommend changes in the methods of abstracting and retrieval; it is manifest, however, that research in those fields may have great influence on developments in methods of original publication, and that the Chemical Society's actions in the latter field must be compatible with and may need to be dovetailed into progress in the former.

It is believed that a programme restricted to "pure" chemistry would be inadequate and that, as indicated above, it should embrace chemical publication as a whole, pure and applied, and indeed also aspects of the bordering sciences. The Chemical Society is very glad, therefore, of the interest shown by other learned Societies, DSIR, and ASLIB.

Further, inasmuch as science is international and as conditions in different countries are not all the same, it is earnestly hoped that collaboration will be forthcoming also from organisations and individuals of other countries.

To be of real value such a survey must clearly be based on the maximum amount of evidence. Individuals and organisations having information or comment relating to the objects of the survey are therefore cordially invited to contact the Director of Publications Research, The Chemical Society (Dr. R. S. Cahn).

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PEDLER LECTURE*

Amino-acid Sequences in the Active Centres of Certain Enzymes

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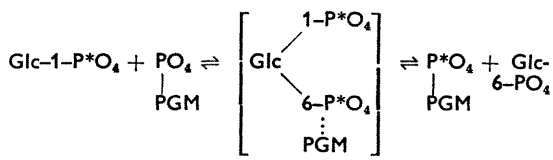
FROM the point of view of the chemist one of the most interesting features of living matter is the large number of high-molecular catalytic protein molecules, which are responsible for controlling and directing the metabolism and chemical reactions that go on in the body. These are the enzymes and the question of what they are like and how they work is one of the fundamental problems we have to solve in order to understand the nature of living matter. There can be little doubt that the catalytic activity of an enzyme depends on its exact chemical structure, so that the problem is intimately connected with protein chemistry.

Presumably an enzyme exercises its catalytic function by coming into contact with its substrate in a specific manner and exerting an appropriate force which will labilise the substrate, bringing about the chemical change. Whereas enzymes are very large molecules the substrates on which they act are frequently small. It thus seems probable that only a part of the enzyme may be involved in combining with the substrate and this part is usually known as the "active centre." It does not necessarily follow that the rest of the molecule is unimportant for activity, but it seems that particular importance should be attached to the active centre and its exact chemical structure. Since enzymes are proteins the active centre must be composed of amino-acid side chains, arranged in a specific three-dimensional array which will depend both on the sequence of amino-acids in the polypeptide chains and on the method of folding of these chains. Frequently side chains forming an active centre may be distantly spaced from one another along the line of the peptide chain but brought into close contact by the folding of the chain. A number of enzymes contain non-protein prosthetic groups which are involved in the catalytic activity. However, these are bound to the protein and suitably activated by amino-acid side-chains so that, in these cases too, the problem of the active centre is essentially the same, namely, the specific arrangement of amino-acid side-chains. One way to determine the structure of an active centre, which may eventually prove to be the only reliable way, is to determine the whole structure of the protein molecule. This is clearly a formidable task and has not yet been accomplished for a single enzyme. The amino-acid sequences of a number of the smaller proteins have now been determined, and the configuration of the chains in myoglobin and haemoglobin has been deduced by the *X*-ray method.[1] Thus there seems to be a possibility that we may have a complete picture of the structure of an enzyme molecule in the not too distant future, and much work is at present in progress in this direction.

In certain cases it has been possible, by using suitable labelling techniques which depend on enzyme activity, to obtain a limited amount of information about parts of the active centres of some enzymes without determining the structure of the whole of the molecule and in this lecture I shall describe some of these techniques with which we have been concerned. Although only a limited picture of amino-acid sequence in a part of the active centre can be drawn, it is at least a beginning and the methods can in some cases be applied to relatively large proteins whose complete structure could probably not be determined by present techniques.

The enzymes to be discussed can all be labelled with ^{32}P in their active centres and advantage has been taken of this to determine amino-acid sequences around the bound ^{32}P.

^{32}P-*Labelled enzymes.*—There are two ways in which enzymes can be labelled with ^{32}P. First, there are enzymes which contain in their active centre a phosphate group that actually enters into the catalysed reaction. Thus phosphoglucomutase (PGM), which catalyses the interconversion of glucose 1-phosphate and glucose 6-phosphate, contains a phosphate group that becomes labelled if the enzyme is incubated with radioactive substrate.[2] The reaction of labelled glucose 1-phosphate with phosphoglucomutase may be summarised as follows:



In this way phosphoglucomutase containing labelled phosphate in its active centre may be pre-

* Delivered before The Chemical Society at The Royal Institution, Albemarle Street, London, W.1, on October 11th, 1962, at The University, Bristol, on November 1st, and at the University, Hull, on November 8th.

[1] Kendrew, Dickerson, Strandberg, Hart, Davies, Phillips, and Shore, *Nature*, 1960, **185**, 422; Perutz, Rossmann, Cullis, Muirhead, Will, and North, *ibid.*, p. 416.

[2] Jagannathan and Luck, *J. Biol. Chem.*, 1949, **179**, 569.

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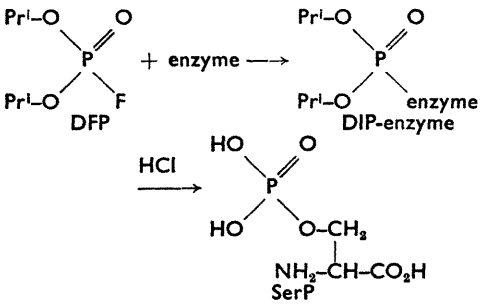
pared. In this case there can be little doubt that the active centre is actually labelled since the fact that the ^{32}P comes from the substrate indicates that this must be the area of the molecule that comes into contact with the substrate and is primarily responsible for the catalytic effect.

Another way in which enzymes can be labelled with ^{32}P is by use of the specific inhibitor di-isopropyl phosphofluoridate (DFP). This compound reacts specifically and stoicheiometrically with a number of hydrolytic enzymes in such a way that they are inactivated when one equivalent of the phosphofluoridate has reacted with them to form the di-isopropylphospho-enzyme (DIP-enzyme). If the DIP-protein is then subjected to acid hydrolysis, serine phosphate (SerP) can be isolated. The reactions are summarised as follows:



Although these enzymes may contain many serine residues, only one reacts with di-isopropyl phosphofluoridate in this way, and it seems likely that this unique serine is in fact a part of the active centre and that its hydroxyl group becomes acylated by the substrate during the formation of the enzyme–substrate complex. If di-isopropyl phosphofluoridate containing radioactive phosphorus (^{32}P) is used, a radioactive enzyme (DI^{32}P-enzyme) is formed in which the label is attached to the active centre, serine.

After acid hydrolysis of both types of ^{32}P-labelled enzyme the radioactivity is found in the form of phosphate and serine phosphate. If, however, hydrolysis is only partial, the radioactivity is found in peptides of serine phosphate and may thus be used as a marker for the isolation and identification of such peptides. Turba and Gundlach,[3] and Schaffer and his colleagues,[4] applied partial acid hydrolysis to DI^{32}P-chymotrypsin and isolated phosphopeptides by means of a Dowex-50 (sulphonic acid) resin. Owing to the strongly acidic nature of the phosphate group these peptides are not adsorbed on the resin, whereas other normal peptides are. The phosphopeptides were then fractionated on ion-exchange columns and in this way Schaffer *et al.*[4] were able to isolate and identify the peptides SerP-Gly, Asp-SerP, Asp-SerP-Gly, and Gly-Asp-SerP-Gly and from this it was concluded that the sequence around the active serine residue in chymotrypsin was Gly-Asp-Ser-Gly. This was amply supported by the work of others, though there was some doubt about some larger peptides from acid hydrolysates. Similar experiments with trypsin indicated that it had the same tetrapeptide sequence.

Partial acid hydrolysis is not very suitable for the determination of longer sequences in this way since, if mild conditions of hydrolysis are used to give longer radioactive peptides, the mixtures produced are extremely complex owing to the non-specific nature of the splitting, and purification is then difficult. Better results can, however, be obtained by using proteolytic enzymes for the hydrolysis and this method has been used extensively by Cohen and his collaborators.[5] Thus, for instance, they hydrolysed DI^{32}P-chymotrypsin with a crude pancreatic extract, causing extensive breakdown of the molecule but yielding only two major radioactive peptides; these were purified by ion-exchange chromatography, ionophoresis, and paper chromatography. The structure of one of these was shown to be Gly-Asp-SerP-Gly-Gly-Pro-Leu.[6] In this case the ^{32}P was still in the form of the di-isopropyl derivative (SerDIP), so that the peptide did not have the strong negative charge characteristic of the phosphate group which had proved useful in isolating the SerP peptides from acid hydrolysates. A more rigorous purification was therefore necessary and the isolation was greatly helped by the fact that there seemed to be very few other peptides as large in the hydrolysate.

Similar studies were carried out on other enzymes and these are summarised in the Table. By using more specific enzymes larger peptide fragments can be isolated: thus the sequence of 15 amino-acids reported by Dixon *et al.*[7] was deduced from the structure of a peptide obtained by the action of trypsin on DI^{32}P-trypsin.

The Use of Radioautograph Techniques.—In connection with attempts to develop techniques for determining amino-acid sequences in larger proteins by isotopic methods we were anxious to develop a method for deducing the sequence around a given radioactive amino-acid. Enzymes of this type labelled

[3] Turba and Gundlach, *Biochem. Z.*, 1955, **327**, 186.
[4] Schaffer, Simet, Harshman, Engle, and Drisko, *J. Biol. Chem.*, 1957, **225**, 197.
[5] Cohen, Oosterbaan, Warringa, and Jansz, *Discuss. Faraday Soc.*, 1955, **20**, 114.
[6] Oosterbaan, Kunst, van Rotterdam, and Cohen, *Biochim. Biophys. Acta*, 1958, **27**, 556.
[7] Dixon, Kauffman, and Neurath, *J. Biol. Chem.*, 1958, **233**, 1373.

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in specific positions with ^{32}P seemed to be ideal model systems with which to work out such methods. The studies were thus initiated on chymotrypsin which was known to have the sequence Gly-Asp-Ser-Gly, and it was found to be possible to work out sequences of this type by using radioactive techniques and without depending on the ninhydrin colour reaction as had hitherto been the case. This had several advantages for determining small sequences, especially only peptides present in significant amounts in such a hydrolysate were SerP-Gly, Asp-SerP, Asp-SerP-Gly, and Gly-Asp-SerP-Gly. Moreover, when the various peptides from the ionogram were investigated it was found that several were interconvertible and appeared to have the same structure. This was eventually found to be due to an interconversion of the aspartyl residues that was occurring during the acid hydrolysis. Thus the normal α-aspartyl residue

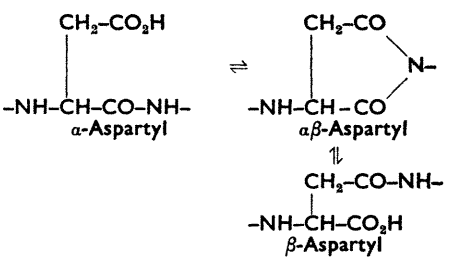
Sequences near the active centres of some enzymes.

DFP-inhibited enzymes		Ref.
Trypsin	$AspNH_2$-Ser-Cys-Glu-Gly-Gly-Asp-*Ser*-Gly-Pro-Val-Cys-Ser-Gly-Lys	7
Chymotrypsin	Gly-Val-Ser-Ser-Cys-Met-Gly-Asp-*Ser*-Gly-Gly-Pro-Leu-Val-Cys-Lys	4, 6, *a*
Elastase	Gly-Asp-*Ser*-Gly	9
Thrombin	Asp-*Ser*-Gly	*b*
Liver aliesterase (horse)	Gly-Glu-*Ser*-Ala-Gly-Gly	11
Pseudocholine esterase	Phe-Gly-Glu-*Ser*-Ala-Gly	*c*
Acetylcholinesterase (electric tissue)	Glu-*Ser*-Ala	10
Subtilisin	Thr-*Ser*-Met-Ala	15
Mold protease (*Aspergillus oryzae*)	Thr-*Ser*-Met-Ala	10
Serum albumin (rabbit, human)	Arg-*Tyr*-Thr-Lys	16
Serum albumin (bovine)	Arg-*Tyr*-Thr-Arg	16
Phospho-enzymes		
Phosphorylase a	Lys-$GluNH_2$-Ileu-*SerP*-Val-Arg	*d*
Phosphoglucomutase	Thr-Ala-*SerP*-His-Asp	13

Refs.: (*a*) Hartley, Proc. 5th Internat. Biochem. Congress, Moscow, Symp. IV, in the press. (*b*) Gladner and Laki, *J. Amer. Chem. Soc.*, 1958, **80**, 1263. (*c*) Jansz, Brons, and Warringa, *Biochem. Biophys. Acta*, 1959, **34**, 573. (*d*) Fisher, Graves, Crittenden, and Krebs, *J. Biol. Chem.*, 1959, **234**, 1698.

in the case of the ^{32}P-enzymes which were then studied in more detail as described below.

The main technical difficulty in this type of work, and indeed in any work on amino-acid sequences, lies in the purification of the peptides. The method most commonly used for fractionating the small SerP peptides obtained from acid hydrolysates was ion-exchange chromatography, which has proved so successful in work on the structure of ribonuclease[8] and other proteins. We had been using high-voltage paper ionophoresis for the separation of peptides and this seemed to offer considerable advantages for the fractionation of serine phosphate peptides from the point of view of simplicity and of resolution. Fig. 1 shows a radioautograph of an ionogram of a partial acid hydrolysate of $DI^{32}P$-chymotrypsin.[9] This shows considerably more components (thirteen) than appear on an ion-exchange column of a similar hydrolysate, suggesting more efficient resolution. However, the large number of bands raised a serious difficulty since Schaffer *et al.*[4] had shown that the in the peptides was being converted in acid to the αβ-ring form which by hydrolysis could give rise to the β-form:



This reaction appears to be a general one and will have to be taken into account in experiments in which partial acid hydrolysis is used.

An ionogram of the type shown in Fig. 1 can be regarded as a very specific characterisation for a given radioactive amino-acid in a particular amino-acid sequence. The position of the bands is deter-

[8] Hirs, Moore, and Stein, *J. Biol. Chem.*, 1960, **235**, 633.
[9] Naughton, Sanger, Hartley, and Shaw, *Biochem. J.*, 1960, **77**, 149.

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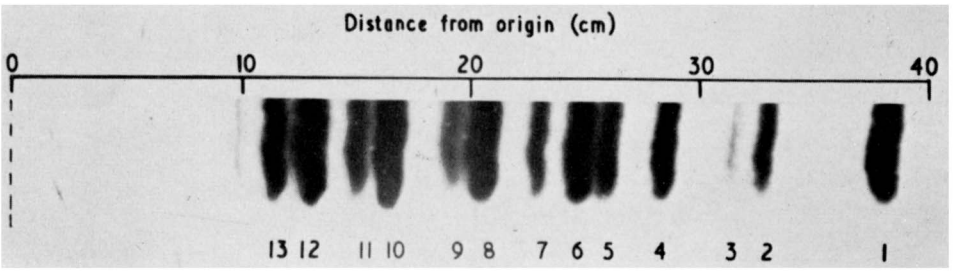

FIG. 1. *Radioautograph of ionogram* (pH 3·5, 40 v/cm., 2·5 hr.) *of partial acid hydrolysate of* DI^{32}P-chymotrypsin.

(Reproduced, by permission, from *Biochem. J.*, 1960, **77**, 149.)

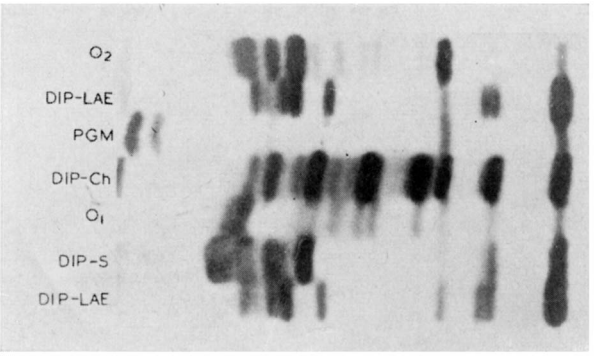

FIG. 2. *Radioautograph of ionogram* (pH 3·5, 40 v/cm., 2 hr.) *of partial acid hydrolysates of various* ^{32}P-labelled proteins; DIP-Ch, DI^{32}P-chymotrypsin; DIP-S, DI^{32}P-subtilisin B., DIP-LAE, DI^{32}P-liver aliesterase; PGM, ^{32}P-phosphoglucomutase; O_1 and O_2, *peptides from chymotryptic hydrolysate of* ^{32}P-labelled ovalbumin. (*D. Shaw, unpublished work.*)

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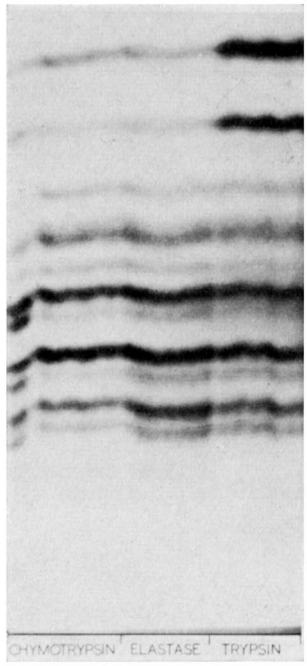

FIG. 3. *Radioautograph of ionogram* (pH 3·5, 40 v/cm., 2 hr.) *of partial acid hydrolysates of* DI^{32}P-derivatives *of chymotrypsin, elastase, and trypsin.* (*The strong fast-moving bands from trypsin are derived from* DF^{32}P, *which contaminated the preparation.*)
(Reproduced, by permission, from *Biochem. J.*, 1960, **77**, 149.)

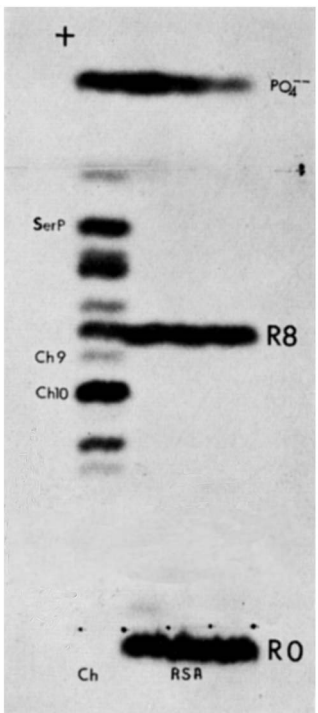

FIG. 7. *Radioautograph of ionogram* (pH 3·5, 40 v/cm., 2 hr.) *of partial acid hydrolysate of* DI^{32}P-derivative *of rabbit-serum albumin* (RSA) *compared with similar hydrolysate of* DI^{32}P-chymotrypsin(Ch).

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mined by the amino-acids that are bound to the SerP residue. Thus the pattern shown in Fig. 1 is characteristic for the sequence Gly-Asp-SerP-Gly. Patterns obtained for other enzymes are shown in Fig. 2. We were interested in the enzyme elastase, which like trypsin and chymotrypsin is a pancreatic proteinase and was found to react with di-isopropyl phosphofluoridate. Fig. 3 shows a radioautograph of an ionogram of a partial acid hydrolysate of DI^{32}P-elastase.[9] This was run on ionophoresis, together with similar hydrolysates of the DIP derivatives of trypsin and chymotrypsin. It can be seen that the patterns obtained from the three enzymes are identical showing that elastase also contains the sequence Gly-Asp-Ser-Gly around its reactive serine residue. In a similar manner it was shown[10] that electric tissue acetylcholinesterase contains the sequence Glu-Ser-Ala since it gives the same pattern as liver aliesterase (Fig. 2), which had already been shown to have this sequence.[11]

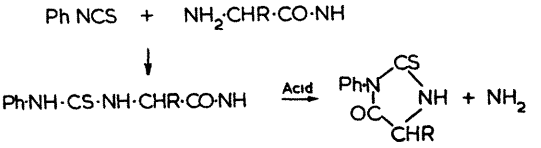
Determination of Sequence by Radioactive Methods.—In the case of phosphoglucomutase preliminary results[12] had suggested a sequence similar to that found in chymotrypsin and trypsin. However, Fig. 2 shows that the pattern of radioactive peptides is entirely different from that obtained with chymotrypsin and also different from that obtained with liver aliesterase or subtilisin. Thus the question arises, how can we determine an amino-acid sequence represented by a pattern which is not the same as that shown by any known sequence?

To the classical organic chemist the correct way to find the structure of an unknown compound is to start by determining its melting point and elementary composition. On the small amounts of material we are working with, this is out of the question and in any case it would not provide much useful information about the structure of a peptide. Thus the protein chemist has come to rely more on spots on chromatograms and on quantitative analyses of amino-acids by the ninhydrin method as an initial step in studying the structure of peptides. Thus one way to determine the sequence around the active serine in a labelled enzyme such as phosphoglucomutase would be to cut out the radioactive bands shown in Fig. 2, elute the material from them, and hydrolyse and identify the amino-acids by paper chromatography or ion-exchange chromatography. This is the method that has been used by Schaffer, Cohen, and others. However, in this case certain difficulties are involved. One is the small amount of material available; but a more serious difficulty is that the radioactive bands are usually not pure, being contaminated by non-radioactive peptides. Thus a partial hydrolysate of a protein may contain several hundred different peptides which may contaminate the few radioactive ones. Normally the serine phosphate peptides may be almost completely separated from the other peptides by virtue of the strong acidic phosphate residue, by ion-exchange chromatography, or by paper ionophoresis at pH 3·5 (as in Fig. 2). In the latter system they migrate towards the anode, whereas most other peptides are neutral or basic at this pH and stay near the origin or move towards the cathode. However, in the case of phosphoglucomutase most of the radioactive peptides are near the origin, because they contain a basic residue as well as the acidic phosphate group, and they are thus heavily contaminated with non-radioactive peptides. It was thus necessary to develop a method for determining the amino-acid sequence of the radioactive peptides that did not depend on the use of the ninhydrin method but used only radioactive techniques. Such techniques would apply only to the few radioactive peptides that can readily be separated from one another and are not affected by the presence of non-radioactive impurities.

The first step in this study was to work out the inter-relations of the various radioactive spots.[13] Samples of each band were eluted and subjected to two treatments:

(*a*) Partial hydrolysis with acid followed by ionophoresis at pH 6·5 in parallel with a sample of the complete hydrolysate, to see which were the breakdown products of each peptide.

(*b*) Treatment with phenyl isothiocyanate by the Edman method, followed by ionophoresis. This method splits off the *N*-terminal residue of the peptide as a phenylthiohydantoin derivative. If serine



phosphate is *N*-terminal the radioactivity would be expected to appear as the thiohydantoin of serine phosphate; however, this is unstable and phosphate is in fact produced. If the serine phosphate is not *N*-terminal, the radioactivity would appear in the peptide from which the *N*-terminal residue was removed. The results with the peptides from phosphoglucomutase are summarised diagrammatically

[10] D. C. Shaw, unpublished work.
[11] Jansz, Posthumus, and Cohen, *Biochim. Biophys. Acta*, 1959, **33**, 396.
[12] Koshland and Erwin, *J. Amer. Chem. Soc.*, 1957, **79**, 2657.
[13] Milstein and Sanger, *Biochem. J.*, 1961, **79**, 456.

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in Fig. 4. In this case ionophoresis was carried out at pH 6·5, since better separation of the peptides is obtained than at pH 3·5 (Fig. 2).

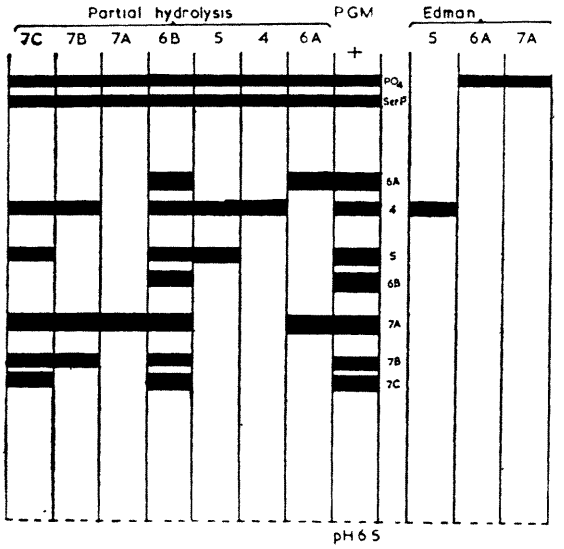

FIG. 4. *Diagram of ionograms* (pH 6·5) *of the products formed from partial acid hydrolysis or the Edman degradation, of radioactive peptides obtained from partial acid hydrolysis of* ^{32}P-PGM. *A hydrolysate of* ^{32}P-PGM *was run in parallel to identify bands* (*column labelled* PGM).

If a dipeptide is subjected to partial acid hydrolysis only two radioactive products, serine phosphate and phosphate, are produced, besides the unchanged dipeptide. Larger peptides would give more breakdown products. There should be two dipeptides containing the serine phosphate residue and these are clearly peptides 4 and 7A (Fig. 4). Peptide 7A when subjected to the Edman degradation gave only phosphate, indicating that serine phosphate is *N*-terminal. There was not sufficient of peptide 4 to subject it to the Edman method, but by difference it must be the dipeptide with serine phosphate *C*-terminal. Peptide 5, on degradation, gave peptide 4, showing it is the tripeptide formed by addition of a residue to the *N*-terminal group of peptide 4. Peptide 6A had *N*-terminal serine phosphate and on partial acid hydrolysis gave peptide 7A as well as phosphate, serine phosphate, and unchanged 6A. Such behaviour would be expected from a tripeptide formed by addition of a residue to the *C*-terminal end of the dipeptide 7A. If the sequence around the serine phosphate is represented as A-B-SerP-X-Y the relations and size of the various radioactive peptides as deduced from the results shown may be summarised as in Fig. 5.

The next problem was to identify the unknown residues A, B, X, and Y. Since peptide 7A was neutral on ionophoresis at pH 3·5 (Fig. 2) it was concluded that it contained a basic amino-acid, which must of course be X. Further details about the

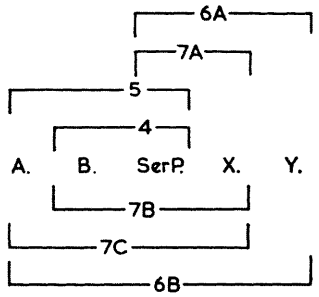

FIG. 5. *Relationships of the* ^{32}P-peptides *obtained from partial acid hydrolysate of* ^{32}P-PGM. (Reproduced, by permission, from *Biochem. J.*, 1961, **79**, 458.)

nature of the various charged groups could be obtained by studying the ionophoretic rates of the various radioactive peptides at different pH values. The results for peptides 6A and 7A are shown in Fig. 6, compared with those for synthetic SerP-Gly. These curves are essentially analogous to titration curves. Comparing the curve for 7A (SerP-X) with that for SerP-Gly it can be seen that, whereas 7A contains an extra basic group at low pH's, this basic group becomes discharged around pH 6—7 so that, at pH 8, peptide 7A has almost the same mobility as SerP-Gly. The only basic amino-acid that ionises in the range 6—7 is histidine, so it could be concluded that X was histidine. By comparing the pH–mobility

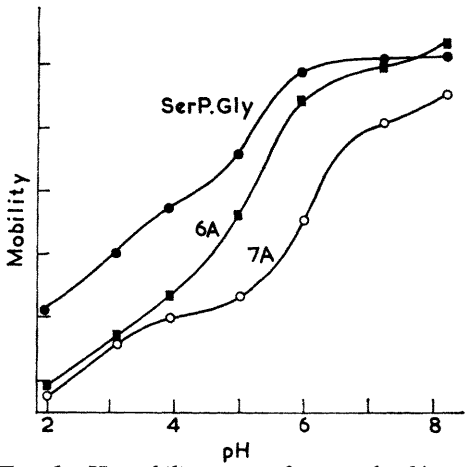

FIG. 6. pH–*mobility curves for peptides* 6A *and* 7A *from* ^{32}P-PGM, *and for* SerP-Gly. *Mobilities were expressed relative to a standard serine phosphate marker.* (Reproduced, by permission, from *Biochem J.*, 1961, **79**, 458.)

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curves of peptides 6A and 7A it can be seen that, whereas at low pH's they are similarly charged, peptide 6A takes on an extra negative charge at pH 4—5. This must be a carboxyl group, indicating that residue Y must be one of the acidic amino-acids (glutamic and aspartic acid). Similarly from the ionophoretic rates of peptides 4 and 5 it could be concluded that residues A and B were neutral amino-acids.

Further identification could be made by carrying out specific chemical reactions on the radioactive peptides and seeing if changes in mobility occurred. Thus histidine residues are uniquely sensitive to photo-oxidation. After photo-oxidation those peptides containing residue X became more acidic whereas other peptides were unaffected. In this way the identification of X as histidine was confirmed. Another useful test was the use of periodate. Only those peptides containing serine or threonine as the *N*-terminal residue react with this reagent to become more acidic. Peptide 5 was attacked whereas 4 was not, showing that acid A is either serine or threonine, but B is not. At this stage the sequence could be reduced to:

$$\left.\begin{matrix}\text{Thr}\\ \text{Ser}\end{matrix}\right\}\text{B–SerP–His}\left\{\begin{matrix}\text{Glu}\\ \text{Asp}\end{matrix}\right.$$

where B was a neutral amino-acid other than serine or threonine.

Clearly these specific reactions are somewhat limited and it seemed more desirable to have a general method that could be used to identify any residue. The most hopeful approach to this problem was by studying the R_F values of the different radioactive peptides on paper chromatography in different solvent systems. It has been suggested by Pardee[14] that the R_F value of a peptide ($R_{F(p)}$) in a given solvent system can be related to the R_F values of the component amino-acids ($R_{F(a)}$) by the following formula:

$$RT \ln[(1/R_{F(p)}) - 1] = (n - 1)A + B + \Sigma RT \ln[(1/R_{F(a)}) - 1]$$

in which **R** is the molar gas constant, T the absolute temperature, and n the number of amino-acid residues in the peptide, and A and B are constants. If for a given solvent system the values of the constants and the R_F value of a peptide with one unknown amino-acid are known, the R_F value of the unknown amino-acid can be deduced. The R_F value of an unknown amino-acid is not in most cases enough to characterise it, but from a combination of R_F data on more than one system this should be possible.

In order to identify residues A and B two solvent systems were studied. The R_F values of the known amino-acids were first determined, then the R_F values of a number of synthetic peptides; from these the values of the constants A and B could be calculated. From the R_F value of peptide 4 (B-SerP) on one system it could be deduced that residue B was either threonine, alanine, or proline. The periodate results had already indicated that it was not threonine. The R_F values of peptide 4 on another system (phenol) on which alanine and proline are very widely separated, was then determined, and from this it could be concluded that residue B was alanine. Similarly, from the R_F values of peptide 5 residue A was shown to be threonine rather than serine. The sequence could then be written as:

$$\text{Thr–Ala–SerP–His–}\left\{\begin{matrix}\text{Glu}\\ \text{Asp}\end{matrix}\right.$$

The efficacy of this method clearly depends on the accuracy of the Pardee formula. It is certainly not absolutely accurate and some peptides may show greater deviations than others, but for most purposes it is probably sufficiently accurate. This approach is likely to improve as more experience is gained both with regard to the known rates of peptides and as to whether to expect deviations from the formula or from any other improved formula that may be developed. In the case of the above sequence it was not possible to decide whether residue Y was aspartic or glutamic acid, and in order to do this and to confirm the above results it was necessary actually to isolate peptides 5 and 6A in a pure form and to identify the amino-acids by the standard method. This purification was greatly helped by the fact that their structure was already almost known, so that the purification was continued until a rational result was obtained. In the case of peptide 6A it was necessary to fractionate it on five different systems, and even then small amounts of impurities were still present. These results confirmed the above results and showed that the sequence was:

Thr–Ala–SerP–His–Asp.

Serum Albumin.—In all cases where di-isopropyl phosphofluoridate has been found to react with the active centre of an enzyme, serine phosphate has been obtained on hydrolysis of the DIP-protein, indicating that the reaction has been with the hydroxyl group of a serine residue. On the basis of early results it was suggested that all DIP-enzymes contained the same sequence (Asp-Ser-Gly) at the reactive site. When, however, horse liver aliesterase was found to have the sequence Glu-Ser-Ala[11] the generalisation had to be modified by saying that the sequences were similar, the serine being bound to an acidic residue through its amino-group and a small neutral one through its carboxyl group.

[14] Pardee, *J. Biol. Chem.*, 1951, **190**, 757.

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Fig. 2 shows the pattern obtained with the bacterial proteinase subtilisin. This is entirely different from the other patterns, showing that this enzyme does not have the sequence Asp-SerP-Gly of chymotrypsin or Glu-SerP-Ala of aliesterase or Ala-SerP-His of phosphoglucomutase. By studying the radioactive peptides by the methods described above, it was shown that the sequence was Thr-Ser-Met-Ala.[15] This is entirely different from those found in the other DFP-inhibited enzymes and indicates that a serine residue in at least three different sequences is capable of reacting with di-isopropyl phosphofluoridate. The most likely explanation of this seems to be that reactivity of the serine is not in fact conditioned by this sequence but rather by other amino-acid side chains which come near the serine residue. Such side chains may be far removed from the serine according to the primary structure along the polypeptide chains but brought near to it in space owing to the secondary and tertiary folding of the chains. Alternatively, it may be that the amino-acids linked to the serine in the chain are concerned in its reactivity and that somewhat different mechanisms are involved in different cases.

It seems that the only thing that the DIP-enzymes have in common is a serine residue. Recent work by D. C. Shaw,[16] however, shows that even this is not entirely true. In an attempt to study new DIP-enzymes, the reaction of the phosphofluoridate with various biological preparations was studied, and an extensive reaction was found with blood. On hydrolysis and ionophoresis of the DI^{32}P-derivative a pattern was obtained which was different from these of the known DIP-enzymes. Further investigation showed that the activity was due to serum albumin. The reaction was considerably slower than with the DFP-inhibited enzymes but was nevertheless virtually specific for a single active site.

Fig. 7 shows the radioautograph obtained with DIP-albumin. It is entirely different from that obtained with other ^{32}P-labelled proteins and in particular no serine phosphate is present. Instead the main end-product of hydrolysis is a band (R8, Fig. 7) which was identified as tyrosine phosphate (TyrP) showing that the phosphofluoridate had reacted with an activated tyrosine residue. The peptides obtained by acid and enzymic hydrolysis of the DI^{32}P-albumin were studied by the methods outlined above and it was concluded that the sequences in rabbit- and human-serum albumin were

A–B–C–D–Arg–Tyr–Thr–Lys

where A, B, C, and D are neutral residues. In bovine material the sequence was Arg-Tyr-Thr-Arg and there were further differences in the neutral residues. These results show the presence of a uniquely reactive tyrosine side-chain in serum albumin, and the question arises whether the reactivity has any physiological significance. It could, of course, be a mere coincidence that a particular centre binding di-isopropyl phosphofluoridate is present. Serum albumin is not normally considered to be an enzyme though it does possess certain unique reactivities. The best known of these is its ability to bind anions and to transport them in the blood, and some preliminary results suggest that the reactive tyrosine residue may in fact be involved in this ion-binding reaction. If indeed this were the case, the presence of two basic side chains near the active tyrosine residue would fit the hypothesis. However, further work is clearly necessary on this problem.

General Conclusions.—In the Table are listed the various sequences that have been found to exist in the region of the reactive site on DFP-inhibited enzymes or serine phosphate enzymes. While there are certain similarities, there are also considerable differences, and no general conclusion seems possible as to what residues are responsible for the activation of the serine. That there are differences is not altogether surprising in view of the different activities of the enzymes and it seems that more significance should be attached to the similarities, as in the case of the pancreatic enzymes and thrombin. One explanation for these similarities is that the sequences are necessary for the biological activity. Another is based on the biological origin of the proteins: thus it may be considered that all three pancreatic proteinases may have evolved from a single protein, which was present in some more primitive organism and controlled by a single gene; as evolution proceded duplication of the gene has occurred and the different enzymes have developed separately, giving rise to molecules with different specificities but retaining the common sequence Gly-Asp-Ser-Gly. In fact, both explanations may be correct. The enzymes may have evolved from a common precursor but have retained the common sequence since this was suitable for the catalytic activity.

The techniques described above, if generally applicable, make it possible to determine an amino-acid sequence around a given labelled amino-acid, and can therefore be used in any enzyme whose active centre can be labelled. It may also be possible to use them more generally for the determination of longer sequences in proteins. For instance, by biological incorporation methods, proteins may be prepared in which a single amino-acid is labelled. If such a protein were digested with an enzyme and the

[15] Sanger and Shaw, *Nature*, 1960, **187**, 872.
[16] Shaw, in the press.

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digest fractionated, peptides would be isolated each containing one or a few residues of the labelled residue and the sequence around each of these residues could be determined. By using a number of labels it might be possible to deduce complete sequences. At present such a method would be more tedious than other techniques available; however, if a routine system could be developed, the possibility of working entirely with paper-fractionation techniques could offer a considerable advantage.

The reaction of di-isopropyl phosphofluoridate has certainly proved to be the most profitable method for determining sequences in active centres; the method is clearly limited to a small group of enzymes and to only a part of the active centres of these enzymes. However, other techniques of labelling are available and have been much less explored.

Certain enzymes contain prosthetic groups that are sufficiently strongly bound to the protein chain for the peptides containing the prosthetic group to be isolated. The classical example of this is the work of Tuppy and Paleus[17] on cytochrome c. Here the hæm group is bound to two cysteine residues. From enzymic hydrolysates it was possible to isolate hæm peptides and by studying their structure the sequence around the "active" cysteine residues was determined. In general, most other prosthetic groups are less firmly bound but it may be possible to apply this approach more generally by the careful use of enzymes under mild conditions.

Many enzymes contain thiol groups in their active centres. These groups are very reactive and offer the possibility of labelling with coloured or radioactive markers. Thus glycerophosphate dehydrogenase contains a unique thiol group which reacts stoicheiometrically with iodoacetate, causing inhibition of the enzyme activity. Harris and Park[18] have used iodo[^{14}C]acetate to label this site and by isolating labelled peptides have determined the sequence around this active centre.

Another approach is suggested by recent work of Grazi *et al.*[19] on the enzyme aldolase. In this case the substrate (dihydroxyacetone phosphate) is bound to an amino-acid group on the enzyme as a Schiff base in the enzyme–substrate complex. By reducing this complex with borohydride it was possible to stabilise this linkage and, after hydrolysis, a compound was isolated in which the substrate was bound by a secondary amino-group to a lysine residue.

The possibility of using bifunctional reagents has also been suggested. In such a reagent one function should resemble the substrate molecule and thus attract the reagent to the active centre, while the other function should be a reactive group such as an acid chloride and thus become attached to groups in the vicinity of the active centre. Clearly there are many possibilities in this field. All enzymes have a unique reactivity which is the basis for their catalytic effect, and as we learn more about these reactivities it may be possible to devise suitable labelling techniques.

[17] Tuppy and Paleus, *Acta Chem. Scand.*, 1955, **9**, 353.
[18] J. I. Harris and J. Park, unpublished work.
[19] Grazi, Rowley, Cheng, Tchola, and Horecker, *Biochem. Biophys. Res. Comm.*, 1962, **9**, 38.

COMMUNICATIONS

A New Route to Thione Esters

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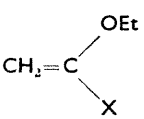
ALKOXYACETYLENES have been used previously to prepare 1-alkoxyvinyl esters of carboxylic (I) and phosphoric acids (II).[1,2] We have investigated the use of ethoxyacetylene with and without mercuric ion as catalyst in the formation of ethoxyvinyl esters of various acids. By reaction of the appropriate thiolic acid with an excess of ethoxyacetylene we have isolated 1-ethoxyvinyl thiolacetate (III; R = Me) (Found C, 49·15; H, 6·7; S, 22·1. $C_6H_{10}O_2S$ requires C, 49·3; H, 6·9; S, 21·9%) and thiolbenzoate (III; R = Ph) (Found C, 64·0; H, 5·5; S, 15·3. $C_{11}H_{12}O_2S$ requires C, 63·45; H, 5·8; S, 15·8%), both having the characteristic double peaks between



(I) X = O·COR
(II) X = O·PO(OR)$_2$
(III) X = S·COR

5·7 and 6·0 μ in the infrared spectrum. (The double peak between 5·7 and 6·0 μ appears to be a characteristic of all the alkoxyvinyl esters and this absorption may be used quantitatively in kinetic studies.[3])

[1] Wasserman and Wharton, *Tetrahedron*, 1958, **3**, 321; *J. Amer. Chem. Soc.*, 1960, **82**, 661; Cohen and Springall, in the press.
[2] Wasserman and Cohen, *J. Amer. Chem. Soc.*, 1960, **82**, 4435.

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These intermediates act as acylating agents in the same way as the enol esters of carboxylic acids (I).[1,3] Thus the thiolbenzoate with aniline gives benzanilide in >70% yield. The other product of these acylations is ethyl thionacetate.

[3] Wasserman and Cohen, unpublished work.
[4] Matsui, *Ber.*, 1909, **42**, 423.

$$CH_2{:}C(OEt){\cdot}S{\cdot}COR + R'{\cdot}NH_2 \rightarrow R'{\cdot}NH{\cdot}COR + CH_3{\cdot}CS{\cdot}OEt$$

The ethyl thionacetate was identified by reaction with ammonia to form thionacetamide and by comparison with an authentic sample.[4]

(*Received, January 28th*, 1963.)

Aziridino-derivatives of Carbohydrates

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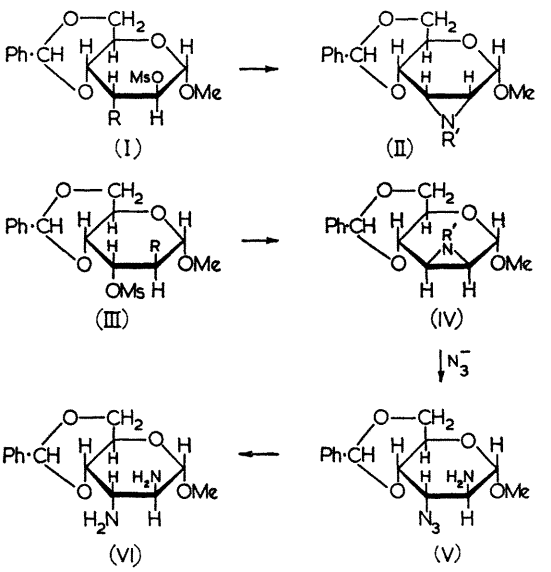
(DEPARTMENT OF ORGANIC CHEMISTRY, THE UNIVERSITY, BRISTOL)

THE preparation of non-toxic aziridines is important because of their possible applications in cancer chemotherapy.[1] Christensen and Goodman[2] have recently outlined the preparation of an aziridino-sugar, namely, methyl 2,3-aziridino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-allopyranoside (II; R′ = H), with m.p. 153—154° and 143—145°. We now report three new methods developed independently in the above two laboratories which have greatly facilitated the preparation of these derivatives. The D-*allo*-isomer (II; R′ = H) has been prepared in Bristol from methyl 3-benzamido-4,6-*O*-benzylidene-3-deoxy-2-*O*-methanesulphonyl-α-D-altropyranoside (I; R = NHBz) by treatment either with lithium aluminium hydride in tetrahydrofuran or hot methanolic sodium methoxide,[3] and in Leicester by treatment of the corresponding 3-azido-derivative[4] (I; R = N_3) with Raney nickel in methanolic hydrazine. The very weakly basic imine (II; R′ = H), m.p. 152—153°, $[\alpha]_D$ + 150° (in $CHCl_3$), was characterised by conversion into the *N*-acetyl and *N*-benzoyl derivative.

The D-*manno*-isomer, m.p. 145—146°, $[\alpha]_D$ + 105° (in $CHCl_3$), was similarly prepared by these methods and gave a crystalline *N*-acetyl and *N*-benzoyl derivative. The benzoate was directly prepared from the amide (III; R = NHBz) by treatment with sodium methoxide at room temperature, demonstrating the ease of imine-ring formation. Treatment of the benzoate (IV; R′ = Bz) with hot methanolic sodium methoxide or lithium aluminium hydride removed the acyl group with the formation of the free base (IV; R′ = H). The loss of the *N*-benzoyl group with lithium aluminium hydride is obscure, but it is noteworthy that the aziridino-*N*-acetyl derivative (IV; R′ = Ac) is similarly converted into the aziridine (IV; R′ = H), albiet in poor yield.

Both the D-*allo*- and the D-*manno*-aziridine were stable to lithium aluminium hydride in contrast to the corresponding epoxides and episulphides. However, ring opening of the D-*manno*-aziridine was effected by the Leicester group using sodium azide in aqueous 2-methoxyethanol, to give the *trans*-diaxial azido-amine (V) which was converted into the syrupy diamine (VI), characterised as the crystalline di-*N*-acetyl derivative. Ring opening has also been achieved by using the thiocyanate ion.



These experiments should give derivatives of amino-sugars, difficultly accessible by conventional methods.

The Leicester studies were supported by the U.S. Army through its European Office, and one of us (D.H.B.) thanks the Department of Scientific and Industrial Research for a Research Studentship.

(*Received, January 15th*, 1963.)

[1] Internat. Cancer Congress, Moscow, *Angew. Chem., Internat. Edn.*, 1962, **1**, 600.
[2] Christensen and Goodman, *J. Amer. Chem. Soc.*, 1960, **82**, 4738.
[3] Cf. Taguchi and Kojima, *J. Amer. Chem. Soc.*, 1959, **81**, 4316.
[4] Guthrie and Murphy, *Chem. and Ind.*, 1962, 1473.

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The Crystal Structure of Trimethyltin Fluoride

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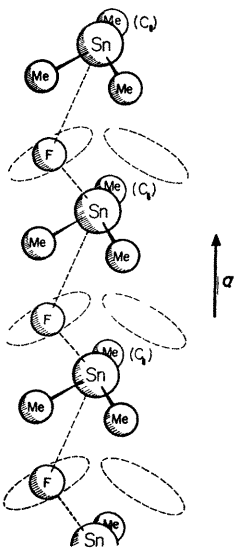
INTEREST in the structures of organo-tin compounds has been stimulated by the recent report of five-co-ordination of the tin atom in the compound Me_3SnCl,Py,[1] and by the suggestion[2] that trimethyltin acetate contains bridging acetato-groups, again giving five co-ordination to the tin atom. It therefore seemed valuable to determine the structure of trimethyltin fluoride, which on the basis of its infrared spectrum[3] has been considered to consist of planar Me_3Sn^+ and F^- ions. A number of unusual structural features have been observed.

Crystals of trimethyltin fluoride, Me_3SnF, are orthorhombic with four molecules in a unit cell of dimensions $a = 4{\cdot}32$, $b = 10{\cdot}85$, $c = 12{\cdot}84$ Å, space group *Pmcn*. The structure was determined by Patterson methods, and refined by three-dimensional Fourier and difference syntheses. R is $0{\cdot}12_7$ for 230 observed reflexions.

The structure consists of chains of trimethyltin groups and fluorine atoms along a, with only weak van der Waals forces between the chains. In the three-dimensional electron-density distribution the tin atom and one of the carbon atoms (C_1) are represented by spherical peaks in the mirror plane at $x = 1/4$, but both of the other carbons (C_2 and C_3) are split into two half-atoms, one on either side of the plane. The electron density at the fluorine atom is even more remarkable, being spread out over two parts of a surface of a sphere; the density is highest in positions above the C_1–Sn–C_2 and C_1–Sn–C_3 angles. One possible interpretation of these rather unusual features is that within any one chain the fluorine atoms are disordered, occupying any position on parts of a spherical surface about $2{\cdot}1_5$ Å from one tin atom, so that Sn–F···Sn is not linear, while the atoms C_2 and C_3 are ordered and displaced from the plane $x = 1/4$ in the opposite direction to the Sn–F bond (see Figure). The disorder of C_2 and C_3 and the further disordering represented by reflexion of a fluorine density in the mirror plane at $x = 3/4$ are a consequence of the chains' being able to align themselves in either direction along a. The considerable amount of disorder may allow interpretations which differ in detail from this description, but one definite conclusion is that the structure is not that of a purely ionic solid; indeed, the non-linear Sn–F···Sn arrangement is a definite indication of some covalent interaction. Moreover, each fluorine atom is not equidistant from the two Sn atoms so that there is some small tendency towards the formation of discrete molecules. The apparent lack of planarity of the trimethyltin groups should produce an infrared absorption at $\sim$ 515 cm.$^{-1}$ due to the Sn–C symmetric stretching vibration. Although this has not been previously reported, it can be observed as a weak band in the spectrum obtained from a very concentrated Nujol mull. Again, in this structure, the tin atom is five-co-ordinate although its stereochemistry is not simple. Infrared spectroscopic



studies of compounds such as Me_3SnClO_4 and Me_3SnBF_4 suggest[4] that their structures consist of planar trimethyltin groups interacting strongly with the anionic group, so as to make the tin atom essentially five-co-ordinate and to destroy the regular tetrahedral symmetry of the anion. The available evidence therefore indicates that five-co-ordination of tin may be common and that the free, planar Me_3Sn^+ ion is rarely encountered.

The authors thank the National Research Council of Canada for financial support and Canadian Industries Limited for a Fellowship (to R.J.O'B.).

(*Received, December 14th*, 1962.)

[1] Beattis, McQuillan, and Rulme, *Chem. and Ind.*, 1962, 1429.
[2] Van der Kerk, Luijten, and Janssen, *Chimia*, 1962, **16**, 10.
[3] Okawara, Webster, and Rochow, *J. Amer. Chem. Soc.*, 1960, **82**, 3287.
[4] Clark and O'Brien, unpublished results.

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Novel Spectra and Some Sterically Uncomplicated Basicities for Metal Halide–Nitrogen Base Equilibria

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Published on 01 January 1963. Downloaded by New York University on 27/10/2014 06:07:02.

PREVIOUS studies[1] of the interaction, in inert solvents, of halides of the non-transition metals (MX_n, *e.g.*, $SbCl_5$, BF_3, $SnCl_4$) with various classes of indicator base (B, *e.g.*, aromatic hydrocarbons, ketones, amines) have been considered to reveal one or both of two distinct spectral effects: (1) effects similar to those produced on protonation of the base and probably due to formation of the species $^+B–MX_n^-$ and (2) effects arising from the presence of the free radical ions B^+ and MX_n^-. Here paramagnetic phenomena are also observed. For amines we now report a new effect, not previously recognised in these contexts, indicative of a weaker type of interaction.

Nitroanilines, in *o*-dichlorobenzene, react reversibly with stannic chloride to give 1:1 complexes (C). A wide basicity range can be covered. Solutions of the complexes have negligible radical character, yet their spectra are not always similar to those of solutions containing the corresponding anilinium ions. A new absorption band is often found between 410 and 480 mμ whose relative intensity to that of the base tends to vary inversely with the latter's strength (see Table). We interpret the new bands as charge-transfer spectra and the complexes as charge-transfer species[2] possessing an appreciable non-bonding component (B, $SnCl_4$) coupled with a usually less appreciable ionic component ($^+B–SnCl_4^-$); for the stronger bases (nos. 1—4) the latter component may be more important, making their spectra more akin to those of the anilinium ions, the charge-transfer band being weak. However, it seems that the extreme of radical-ion formation is only found for similar bases with much stronger Lewis acids,[1c] or perhaps in more polar solvents.

When large reagent concentrations are used coloured complexes are precipitated. For bases nos. 2, 4, and 6 these have a $2B:1SnCl_4$ stoicheiometry, but dissolve in an excess of solvent to give the equilibria noted above.

Values of pK ($K = [C]/[B][SnCl_4]$) are included in the Table. They appear to be the first determined for stannic chloride and, in fact, comprise the bulk of all the available data not concerned with Lewis acids derived from boron. K may be calculated from the stoicheiometry and the changes in absorption of either the base or the complex. K may be determined for successive bases either directly or by stepwise comparison. A comparison of thermodynamic basicities is therefore accomplished.[1d]

Spectral details and dissociation constants at 20° ± 2°.
[B] ≈ 10^{-4}M. [$SnCl_4$] ≈ 10^{-4} to 1M. ϵ = extinction coefficient at band maximum ($\lambda^{max.}$).

No.	Aniline derivative	$10^{-3}\epsilon_B$	$10^{-3}\epsilon_C$	$\lambda_C^{max.}$ (mμ)	$\lambda_B^{max.}$ (mμ)	pK	pK_a (at 25°)
1	3-Nitro	1·81	Small at > 310*		366	—3·60	2·50
2	4-Nitro	15·7	,,		347	—3·18	0·99
3	4-Methyl-2-nitro	7·93	,,		412	—2·50	0·45
4	2-Nitro	5·02	,,		397	—2·30	—0·29
5	2-Chloro-4-nitro	12·6	7·81	425	352	—0·70	—0·94
6	4-Chloro-2-nitro	4·77	1·67	418	410	—0·71	—1·03
7	5-Chloro-2-nitro	5·51	3·2	444	393	—0·34	—1·54
8	2,5-Dichloro-4-nitro	9·26	10·1	410	347	0·15	—1·87
9	*N*-Phenyl-4-nitro	21·6	22·6	476	384	—0·96	—2·48
10	2,6-Dichloro-4-nitro	11·5	16·9	415	350	0·53	—3·02

* Stannic chloride absorption prevented measurements at shorter wavelengths. Anilinium ions absorb below 310 mμ.

Since all previous studies (not subsequently shown suspect[1e]) have either deliberately courted steric effects[3] or have covered only a small basicity range[1d,3] it has not been possible hitherto to state with confidence whether relative basicities of unhindered bases towards metal halides may be expected to parallel their basicities towards protons (*e.g.*, as defined by pK_a determined in aqueous acids). There seem sufficient sterically uncomplicated comparisons available in the Table to show that, although the general sequence remains much as in aqueous solution, relative basicities are often notably altered—and altered more than would be expected from the solvent change.[3,4]

(*Received, January* 23*rd*, 1963.)

[1] (*a*) Aalbersberg, Hoijtink, Mackor, and Weijland, *J.*, 1959, 3055; (*b*) Shuba and Zenchelsky, *J. Amer. Chem. Soc.*, 1960, **82**, 4136; (*c*) Kainer and Hausser, *Chem. Ber.*, 1953, **86**, 1563; (*d*) Moodie, *Chem. and Ind.*, 1961, 1269; (*e*) Manelis, Vinnik, and Chirkov, *Zhur. fiz. Khim.*, 1959, **33**, 1030; see, however, Gerrard and Mooney, *J.*, 1960, 4028.
[2] Mulliken, *J. Amer. Chem. Soc.*, 1952, **74**, 811; Jorgensen. *Acta Chem. Scand.* 1957, **11**, 166.
[3] Brown, *J. Chem. Educ.*, 1959, **36**, 424; McLaughlin, Tamres, Searles, and Nukina, *J. Inorg. Nucl. Chem.*, 1961, **17**, 112.
[4] Satchell, *J.*, 1958, 1916.

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The Crystal Structure of the β-Form of Triglycerides

By KÅRE LARSSON

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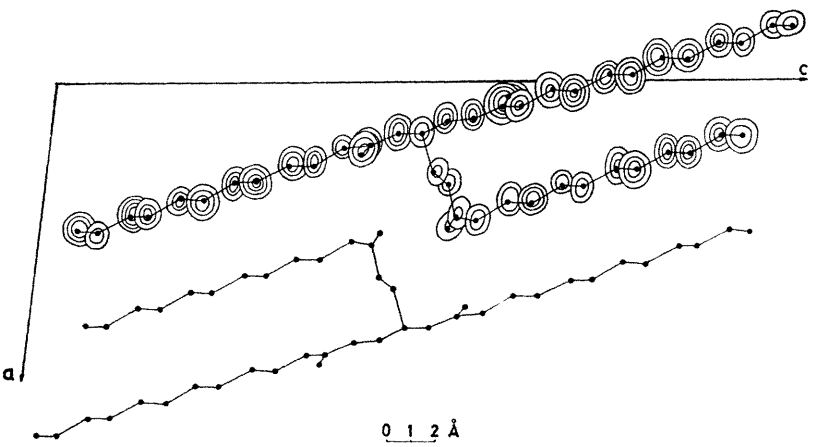
Published on 01 January 1963. Downloaded by New York University on 27/10/2014 06:07:02.

THE stable crystal form of simple fatty-acid triglycerides, the β-form, has been studied by Vand and Bell.[1] They analysed the chain packing thoroughly, but a complete structure determination is necessary to see the shape of the whole molecule, and such knowledge is fundamental for discussions on the solid-state behaviour of triglycerides. The present author has therefore reinvestigated the β-form of trilaurin; experience has shown that this chain length is suitable for obtaining good single-crystals.

A study at this Institute of long-chain compounds containing different heavy-atoms has shown that a bromine atom can often replace a terminating methyl group isomorphously.[2] The triglyceride of 11-bromoundecanoic acid was therefore prepared and crystals of its β-form gave the following *X*-ray data: $a = 12{\cdot}40 \pm 0{\cdot}10$, $b = 5{\cdot}52 \pm 0{\cdot}05$, $c = 31{\cdot}6 \pm 0{\cdot}3$ Å; $\alpha = 90{\cdot}5° \pm 0{\cdot}6°$, $\beta = 96{\cdot}2° \pm 1°$, $\gamma = 101{\cdot}9° \pm 1°$. The corresponding *X*-ray data for trilaurin were: $a = 12{\cdot}35 \pm 0{\cdot}08$, $b = 5{\cdot}44 \pm 0{\cdot}04$, $c = 31{\cdot}75 \pm 0{\cdot}10$ Å; $\alpha = 94{\cdot}0° \pm 0{\cdot}5°$, $\beta = 96{\cdot}7° \pm 0{\cdot}5°$, $\gamma = 99{\cdot}2° \pm 0{\cdot}5°$. The triclinic unit cell contains two centrosymmetrically related molecules.

The structure for the bromo-glyceride was solved from bromine-phased electron-density projections with bromine positions derived from Patterson projections. Only the (010) projection showed acceptable resolution and three-dimensional refinement was started with the reflexion data of the unsubstituted acid. A composite drawing of the first three-dimensional electron density series is shown in the Figure. The *R*-value was 0·37 for the 680 reflexions used in the summation.

The directions of the chains in the molecule correspond to the proposed "tuning-fork" form with the chains in 1- and 3-position pointing opposite to the chain in 2-position. The molecules are arranged "head-to-head" in double layers. The methyl groups in a chain layer do not lie in one plane, as they do in earlier known long-chain structures, but form terraces (cf. Figure). This is a consequence of the fact that the chains do not possess translational freedom in relation to each other.



The β-form of trilaurin viewed along the b-axis shown as a composite drawing of the three-dimensional electron-density series. Contours are given at intervals of 1eÅ^{-3} *starting with* 2eÅ^{-3}.

The unit-cell dimensions and the *x*- and *z*-co-ordinates of the carbon atoms in the chains are in general accordance with Vand and Bell's results[1] on trilaurin. There is, however, an error in their matrix *s* which gives the transformation between the subcell and main-cell co-ordinates. This is obvious if the main-cell co-ordinates are calculated by using this matrix, as the lateral packing of the chains then becomes irregular. The element denoted s_1^2 should be −0·290 instead of −0·241. Their calculation of the

[1] Vand and Bell, *Acta Cryst.*, 1951, **4**, 465.
[2] Larsson, *Acta Chem. Scand.*, 1962, **16**, 1751.

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subcell dimensions is based on this matrix and therefore contains smaller errors.

The structure is now being refined by anisotropic least-squares methods.

This work is financially supported by a grant from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

(*Received*, *December* 10*th*, 1962.)

Published on 01 January 1963. Downloaded by New York University on 27/10/2014 06:07:02.

The Three-co-ordinate Boron–Nitrogen Four-membered Ring System (1,3-Diaza-2,4-boretane)

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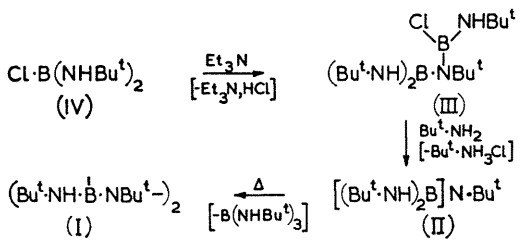
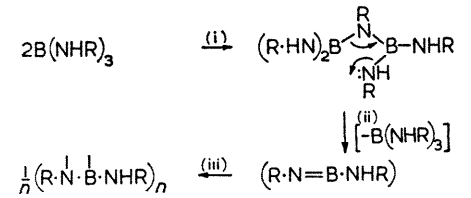
OF the borazynes (XB=NY) and their oligomers, only the cyclic trimers[1] (borazoles) and recently the tetramers[2] (borazocines) have been well characterised; polymeric, presumably linear, derivatives have also been described.[3] We now report the preparation of a cyclic dimer (I), b.p. 72—74°/0·005 mm., n_D^{20} 1·4582, d_4^{20} 0·8666. Typical reactions leading to its formation are indicated below. Compound (IV), b.p. 72—74°/10 mm., n_D^{20} 1·4330, obtained so far in only ~ 90% purity, was made from tris-t-butylaminoborane[4] and boron trichloride; an alternative method of preparing compound (III), b.p. 82—86°/0·02 mm., n_D^{20} 1·4548, d_4^{20} 0·9014, was from the trichloride and t-butylamine; compound (II) had b.p. 98—100°/0·01 mm., m.p. 52—54°, n_D^{20} 1·4630. Compounds (I)—(IV) represent novel classes of boron compounds (see also ref. 5).



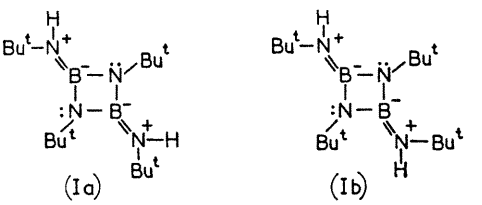
Polyborazynes are invariably formed by elimination reactions from borazenes, XX′B·NYY′, and we regard the mechanism as a three-step process: (i) an intermolecular condensation with formation of a compound having a B–N–B skeleton; (ii) an intramolecular 1,3-nucleophilic rearrangement; and (iii) polymerisation of the borazyne. This is illustrated for the case of a trisaminoborane.

Evidence for structure (I) rests on full elemental analysis, molecular-weight determinations (cryoscopic and ebullioscopic, in benzene), and the mass spectrum.

The diazaboretanes are of interest also because formally they may be regarded as isoconjugate with the cyclobutadienes. The instability of the latter has been explained in terms of molecular-orbital theory.[6] Compound (I) proved to be diamagnetic, implying that delocalisation within the ring is not significant. Indeed we believe that π-bonding is largely exocyclic and that this is responsible, at least in part, for the stability of compound (I).



The evidence is spectroscopic and indicates that compound (I) is a mixture of geometrical isomers (Ia) and (Ib). Thus, the infrared spectrum shows a (poorly resolved) doublet at ~ 3460 cm.$^{-1}$ (NH stretching frequency), and the ^{1}H nuclear magnetic resonance spectrum indicates that the methyl absorption consists of a quartet.



We are grateful to Messrs. R. A. Saunders, A. E. Williams, and Imperial Chemical Industries Limited, Dyestuffs Division, for the mass-spectral data, Mr. P. A. Barfield and Dr. J. Lee for the nuclear-magnetic data, and Mr. N. Paddock for the magnetic measurement.

(*Received*, *November* 28*th*, 1962.)

[1] Cf. Sheldon and Smith, *Quart. Rev.*, 1960, **14**, 200; Mikhailov, *Uspekhi Khim.*, 1960, **29**, 972.
[2] Turner and Warne, *Proc. Chem. Soc.*, 1962, 69.
[3] Burg and Banus, *J. Amer. Chem. Soc.*, 1954, **76**, 3903; Bissot, Campbell, and Parry, *ibid.*, 1958, **80**, 1868; Burch, Gerrard, and Mooney, *J.*, 1962, 2200.
[4] Aubrey and Lappert, *J.*, 1959, 2927.
[5] Nöth, *Z. Naturforsch.*, 1961, **16b**, 618.
[6] Longuet-Higgins and Orgel, *J.*, 1956, 1969.

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The Structure of Elsinochrome A

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Published on 01 January 1963. Downloaded by New York University on 27/10/2014 06:07:02.

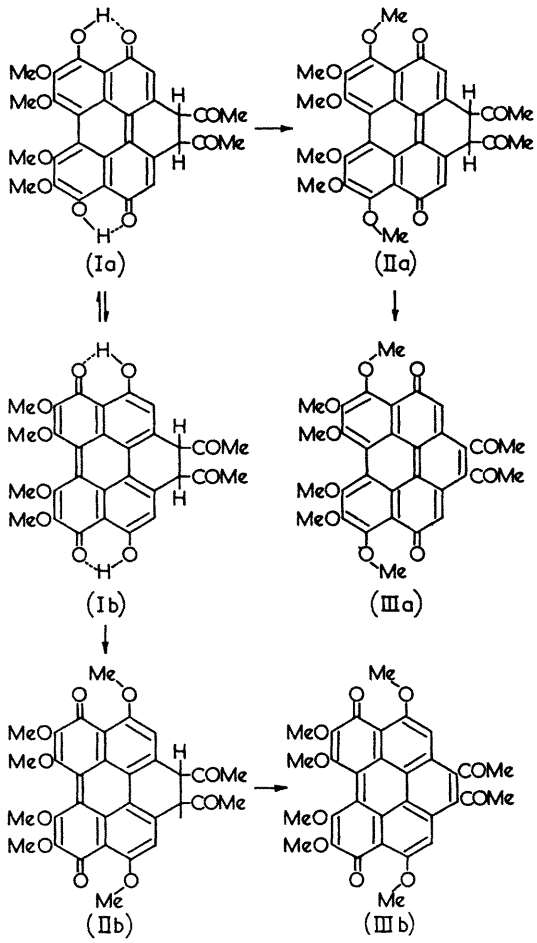
A NUMBER of species of the fungus *Elsinoë* (Ascomycetes), and its conidial stage, *Sphaceloma*, produce mixtures of red pigments with absorption spectra similar to that of erythroaphin *fb*.[1] We propose the name "elsinochromes" for these compounds. Thin-layer chromatography of crude extracts from *E. annonae* showed the presence of at least five related pigments. After separation by chromatography on silica gel, the least polar member of the series, elsinochrome A (I), crystallised from benzene–hexane as dark-red plates, $C_{26}H_{12}O_6(OMe)_4$, m.p. 255°. We propose the rapidly interconverting tautomeric structures (Ia) and (Ib) for this pigment.

Compound (I) shows infrared bands at 1712 and 1623 cm.$^{-1}$ which we assign, respectively, to the two identical methyl ketone groups and the hydrogen-bonded extended quinone system. The presence of the 4,9-dihydroxyperylene-3,10-quinone nucleus is shown by comparison of the absorption spectra of compound (I) [$\lambda_{max.}$, (in $CHCl_3$) 445, 460, 532, 572 mμ] and its leuco-acetate [$\lambda_{max.}$ (in $CHCl_3$) 277, 294, 437, 469, 500 mμ] with those of erythroaphin-*fb*[2] [$\lambda_{max.}$ (in $CHCl_3$) 447, 485, 520, 560, 586 mμ] and its leuco-acetate [$\lambda_{max.}$ 278, 436, 465, 498 mμ].

The strikingly simple nuclear magnetic resonance spectrum of compound (I), with only six single peaks, confirms the presence of two methyl ketone groups (τ 7·95) and shows the presence of two pairs of methoxyl groups (τ 5·68, 6·00), two ring protons (τ 3·48), two alicyclic protons (τ 4·86), and two hydroxyl groups (τ −5·9). The extremely sharp signal from the hydroxyl protons can be explained as a combination signal from the rapidly interconverting tautomers (Ia and Ib). Methylation (dimethyl sulphate and potassium carbonate in acetone) confirms this interpretation by producing the isomeric dimethyl ethers (IIa) [$\nu_{max.}$ 1715, 1632 cm.$^{-1}$; τ (quinonoid) 3·82] and (IIb), [$\nu_{max.}$ 1715, 1632 cm.$^{-1}$; τ (aromatic) 3·10].

In hot polar solvents (*e.g.*, methanol or acetic acid) the ethers (IIa) and (IIb) disproportionate readily into the corresponding dehydro-ethers (IIIa) [$\nu_{max.}$ 1685, 1632 cm.$^{-1}$; τ ($CO{\cdot}CH_3$) 7·36 (quinonoid) 3·75] and (IIIb) [$\nu_{max.}$ 1700, 1630 cm.$^{-1}$; τ ($CO{\cdot}CH_3$) 7·30 (aromatic) 3·3] and quinols. The latter, in methanol, are converted into purple, insoluble products; in acetic anhydride–acetic acid, however, they can be trapped as acetates.

Peaks at τ 3·75, 7·36 and τ 3·25, 7·30 in the nuclear magnetic resonance spectra of the dehydro-compounds (IIIa) and (IIIb), respectively, can best be explained if, in the former, both ring protons and methyl ketone groups are attached to the quinonoid system, while in the latter the same groups are attached to a condensed aromatic system. Conversion of hydroxy-quinone (I) into the methoxy-



quinone (IIa) does not alter the absorption spectrum greatly. However, the isomer (IIb) shows a shift of the main band of the phenol (I) (445 mμ) to 435 mμ (reduced intensity) and disappearance of bands at longer wavelengths. These effects are due to twisting of the extended quinone by the alicyclic group. In the

[1] Weiss, Flon, and Berger, *Arch. Biochem. Biophys.*, 1957, **69**, 311.
[2] Calderbank, Johnson, and Todd, *J.*, 1954, 1285.

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ether (IIa) the ring proton is quinonoid (τ 3·82) while in the isomer (IIb) it is aromatic (τ 3·10). These considerations place the methoxyl groups as shown in (Ia) and (Ib).

Compound (I) is optically active (negative Cotton effect centred at $\sim$ 425 mμ). Dreiding models show crowding of the perylene ring system by substituents at positions 1 and 12 and 6 and 7. This could produce optical isomers which, however, should racemise easily; hence permanent optical activity can best be ascribed to a *trans*-arrangement of groups in the alicyclic portion of the molecule.

On biogenetic grounds, compound (I) could have been formed by dimerisation of an acetate-derived unit related to javanicin (cf. the biosynthesis of hypericin[3]); the necessary removal of carboxyl-derived carbon and introduction of oxygen are unexceptional.

(*Received, January 30th*, 1963.)

[3] Brockman, Pohl, Maier, and Haschad, *Annalen*, 1942, **553**, 1.

Addition of Dichlorocarbene to Norbornylene

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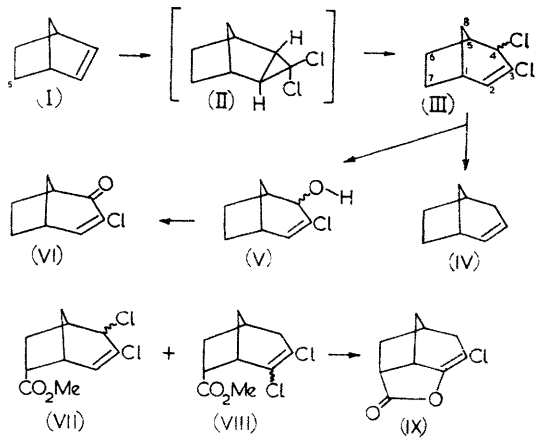
IT is known that dihalogenocarbenes and olefins readily yield *gem*-dihalogenocyclopropanes,[1] that cyclohexene[1] and cycloheptene[2] give very stable adducts, but that dibromo-[3] and dichloro-carbene[2] adducts of cyclopentene undergo ring expansion under mild conditions. We find that the reaction of dichlorocarbene with a strained olefin such as norbornylene yields a rearranged addition product[2] (III).

Adding methyl trichloroacetate[4] to sodium methoxide and norbornylene in pentane at 0° gave a liquid, $C_8H_{10}Cl_2$, b.p. 86—87°/3 mm., n_D^{25} 1·53227, in yields up to 50%. The rearranged structure (III) was proved as follows. The product was unsaturated (permanganate; $\nu_{max.}$ 1624 cm.$^{-1}$). Proton magnetic resonance peaks occurred at τ 8·27 (six CH_2), 7·29 (two bridgehead H), 5·88 (one tertiary allylic H, at a rather low field, because of the chlorine atom bonded to the same carbon atom), and 3·92 (one olefinic H); the expected spin splitting patterns were found for protons 2 and 4, both adjacent to a single hydrogen atom. Sodium in moist methanol[1] reduced the compound to bicyclo[3,2,1]oct-2-ene (IV). As expected for (III), only one chlorine atom was reactive towards dilute aqueous silver nitrate, giving an unsaturated chlorhydrin (V), m.p. 40—42°C, $\nu_{max.}$ 1627 cm.$^{-1}$ (*p*-nitrobenzoate), obtained also by refluxing the adduct in aqueous acetone. The infrared spectrum of a dilute solution (CCl_4) of the chlorhydrin showed a single peak at 3600 cm.$^{-1}$; in concentrated solutions, intermolecular association was comparatively low. These observations suggest an intramolecular interaction between the hydroxyl group and the chlorine atom which could only occur if they are vicinal as in (V). The chlorhydrin (V) was smoothly converted by chromic anhydride in pyridine[5] into a conjugated ketone (VI), ν 1692, 1597 cm.$^{-1}$ (2,4-dinitrophenylhydrazone).

Methyl *endo*-norbornylene-5-carboxylate gave a product whose analytical and spectroscopic properties indicated a mixture of the isomeric compounds (VII) and (VIII); with silver nitrate in water or methanol it yielded a $\gamma\delta$-unsaturated chloro-lactone (IX), m.p. 84—85°, $\nu_{max.}$ 1787 cm.$^{-1}$.



By analogy,[2,3] formation of compound (III) could be explained by the rearrangement of an initial adduct such as (II), but we have not yet been able to prove the existence of this intermediate.

We thank Professor G. Smets, Dr. G. Slinckx, the Fonds National de la Recherche Scientifique, and the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (P.L.) for help and support.

(*Received, December 31st*, 1962.)

[1] Doering and Hofman, *J. Amer. Chem. Soc.*, 1954, **76**, 6162.
[2] Bergman, Abs. Papers 142nd Meeting Amer. Chem. Soc., Atlantic City, N.J., Sept., 1962, p. 790.
[3] Sonnenberg and Winstein, *J. Org. Chem.*, 1962, **27**, 748.
[4] Parham and Schweizer, *J. Org. Chem.*, 1959, **24**, 1733.
[5] Poos, Arth, Beyler, and Sarett, *J. Amer. Chem. Soc.*, 1953, **75**, 422.

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Kinetics of Cross-linking Reactions in Solids

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Published on 01 January 1963. Downloaded by New York University on 27/10/2014 06:07:02.

WHEN poly(vinylidene chloride), $[-CH_2{\cdot}CCl_2-]_n$, is pyrolysed in an inert atmosphere at about 170°C, decomposition tends to a limit corresponding to the loss of about 50% of the hydrogen chloride, although over long periods a slow subsequent reaction can be detected. If the temperature is raised, say by 40°, rapid decomposition sets in again only to reach another limit around 65% of reaction. Further increases in temperature lead to a series of steps in the graph of hydrogen chloride loss against time, and the reaction is not completed in a reasonable time until a temperature of at least 700° is reached[1-3] (Fig. 1). The first stage of reaction, corresponding to the loss of 50% of the hydrogen chloride is an accurately first-order process[3] and is thought[1] to be the elimination of HCl between adjacent carbon atoms to form a vinyl chain, $[-CH{=}CCl-]_n$. Further loss probably occurs by cross-linking which may involve a Diels–Alder mechanism.

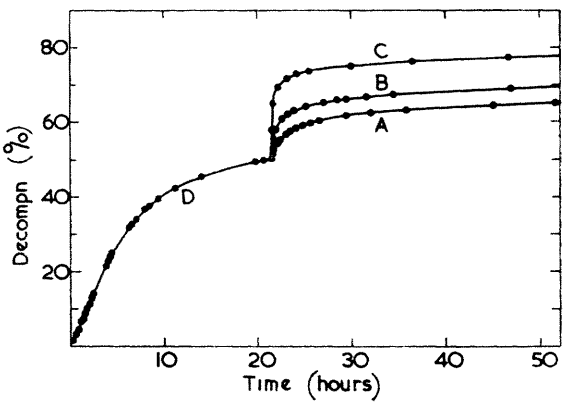

FIG. 1. *Percentage decomposition of poly(vinylidene chloride) as function of time:* 50% *decomposition reached at* (*D*) 175°, *then three samples of this material further decomposed at* (*A*) 211·5°, (*B*) 227·5°, *and* (*C*) 295·7°.

Dacey and Cadenhead[2] suggested that the behaviour shown in Fig. 1 might be explained by a shift in the equilibrium position of the cross-linking reaction with temperature. This seems unlikely and we put forward an alternative explanation[3] which assumed a rapid change in the activation energy of the cross-linking reaction with extent of reaction. We have now carried out experiments to test our hypothesis. We assume, for simplicity, that the cross-linking reaction is of the first order in vinyl units and that the free energy of activation ($\Delta G^{\ddagger}$) increases linearly with the number of cross-links already formed. If the reaction can be divided cleanly into two stages then, when the second stage has proceeded to a fractional extent, ξ, the number of remaining vinyl units is proportional to $(1 - \xi)$ and the number of cross-links to ξ. The rate equation is then

$$d\xi/dt = A(1 - \xi)\exp[-\{\Delta G_0^{\ddagger} + \alpha\xi\}/RT]. \quad (1)$$

A graph of $\log\left\{\left(\frac{d\xi}{dt}\right)\Big/(1 - \xi)\right\}$ against ξ should be linear with a slope of $\alpha/2{\cdot}303RT$. A typical graph of our results is shown in Fig. 2; $d\xi/dt$ was obtained by seven-point numerical differentiation.[4] The dominating effect of the change of activation free energy with extent of reaction makes it impossible to test the order of the reaction: equally good lines are obtained by assuming second- or third-order kinetics. Nor will other details of the mechanism, such as the possible need in the condensation for the simultaneous interaction of two adjacent vinyl groups on

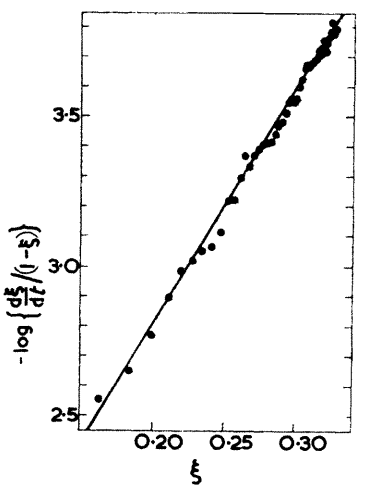

FIG. 2. *Graph of log* $\{(d\xi/dt)/(1 - \xi)$ *against* ξ *for second stage of reaction at* 227·5°.

[1] Winslow, Baker, and Yager, Proc. 1st and 2nd Buffalo Conference on Carbon, Buffalo, N.Y., 1956, p. 93; Winslow, Matreyek, and Yager, "Industrial Carbon and Graphite," Soc. Chem. Ind., London, 1958, p. 190.
[2] Dacey and Cadenhead, Proc. 4th Buffalo Conference on Carbon, Buffalo, N.Y., 1960, p. 315.
[3] Everett, Redman, Miles, and Davies, 4th Internat. Conference of Coal Science, Le Touquet, 1961; to be published in *Fuel*.
[4] Comrie, in Chamber's "Shorter Six-Figure Mathematical Tables," W. and R. Chambers, Ltd., 1961, p. 349.

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one chain with a vinyl group on another chain be detectable in the overall kinetics.

Precise determination of the slope $d\xi/dt$ is difficult, and a better test of eqn. (1) is to compare its integrated form

$$Bt = \int_{\alpha/RT}^{\infty} \frac{e^{-x}dx}{x} - \int_{\alpha(1-\xi)/RT}^{\infty} \frac{e^{-x}dx}{x}, \qquad (2)$$

where $B = A \exp\{-(\Delta G_0^{\ddagger} + \alpha)/RT\}$, with the actual experimental results. The integrals are standard exponential integrals whose values are tabulated.[5] For a given value of α, Bt can be calculated as a function of ξ and this curve should superimpose on the experimental plot of ξ against t when the abscissa is divided by B. Fig. 3 shows this comparison, with $\alpha = 1{\cdot}79 \times 10^4$ cal.mole^{-1} and $B = 8{\cdot}77 \times 10^{-10}$ min.$^{-1}$, for the experiment in which polymer decomposed to 50% at 175° was subsequently heated to 227°. Eqn. (2) represents the experimental data very closely. The above value of α implies that the activation free energy changes by 17·9 kcal. between $\xi = 0$ and $\xi = 1$. Analysis of the experiments at other temperatures is needed to separate this into changes in the energy and entropy of activation.

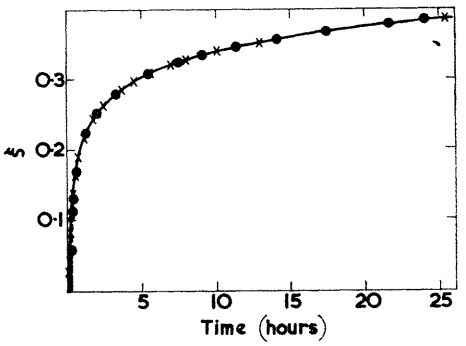


FIG. 3. *Comparison of experiment with curve calculated from eqn.* (2) *for second stage of reaction at* 227·5°. *Crosses, experimental; closed circles, calculated.*

It is suggested that the above method of analysis may be applicable to other cross-linking reactions in solids, and in particular to the graphitisation of carbon.

(*Received, January* 30*th*, 1963.)

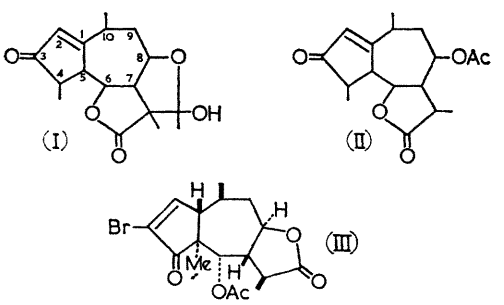
[5] Jahnke and Emde, "Tables of Functions," Dover Publns. Ltd., N.Y., 4th edn., 1945, p. 1; these tables had to be extended by series summation to cover the range needed in this analysis.

The Structure of Bromoisotenulin

By D. ROGERS and MAZHAR-UL-HAQUE

(CHEMISTRY DEPARTMENT, IMPERIAL COLLEGE, LONDON, S.W.7)

TENULIN, which was isolated by Clark[1] from various *Helenium* species and studied by Barton and de Mayo,[2] was assigned formula (I), that of isotenulin being (II), but nuclear magnetic resonance studies[3] have thrown doubt on these formulæ. The correct constitutions and relative stereochemistry have now been determined by an *X*-ray study of bromoisotenulin[4] (III).



The carbon skeleton is biogenetically abnormal, a methyl group having migrated from position 4 to 5; both five-membered rings are *trans*-fused to the seven-membered ring; non-bonded repulsions between the angular methyl (α to C-5) and the α-hydrogens on C-8 and C-10 cause the molecule to be appreciably folded. A Dreiding model shows this interaction clearly and readily folds into the form found.

Bromoisotenulin ($C_{17}H_{21}BrO_5$) crystallises in the monoclinic system with 4 molecules in a cell of dimensions, $a = 8{\cdot}75$, $b = 23{\cdot}15$, $c = 10{\cdot}28$ Å, $\beta = 121°$; the space group being $P2_1$, the asymmetric unit comprises two crystallographically unrelated molecules. Some 2300 independent reflextions were measured visually, and the positions of the two sets

[1] Clark, *J. Amer. Chem. Soc.*, 1939, **61**, 1836; 1940, **62**, 597.
[2] Barton and de Mayo, *J.*, 1956, 142.
[3] Herz, Watanabe, Miyazaki, and Kishida, *J. Amer. Chem, Soc.*, 1962, **84**, 2601.
[4] Clark, *J. Amer. Chem. Soc.*, 1939, **61**, 1840.

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of bromine atoms, deduced from the three-dimensional Patterson function, were such as to avoid phase ambiguity. The first electron-density synthesis revealed all 44 carbon and oxygen atoms. Refinement, not yet complete, has reduced R to 0·21 for all observed reflexions, and shows satisfactory agreement between bond lengths in the two molecules and identical stereochemistry. We publish this Communication at this stage partly because of the concordance between the two unrelated molecules in this study, and partly because of a recent chemical study by Herz *et al.*[5] which, though devoid of stereochemistry, accords with our findings.

We are indebted for assistance and support to Professor D. H. R. Barton, to Dr. J. T. Pinhey, to Woolwich Polytechnic, to the British Council, and to the Department of Scientific and Industrial Research.

(*Received, December 21st*, 1962.)

[5] Herz, Rohde, Rabindran, Jayaraman, and Viswanathan, *J. Amer. Chem. Soc.*, 1962, **84**, 3857.

The Trifluoroacetyl Radical

By B. G. TUCKER and E. WHITTLE

(THE CHEMISTRY DEPARTMENT, UNIVERSITY COLLEGE, CARDIFF)

THE acetyl radical is well known as an intermediate in the photolysis of acetone, but the analogous trifluoroacetyl radical has not been detected in the photolysis of hexafluoroacetone; the primary reaction is thought to be:

$$CF_3{\cdot}CO{\cdot}CF_3 + h\nu \rightarrow 2CF_3 + CO \quad . \; . \; . \; (1)$$

If reaction (1) is correct, we should expect the photolysis of a mixture of hexafluoroacetone and bromine to yield only trifluoromethyl bromide and carbon monoxide in the ratio 2:1, provided that the trifluoromethyl radicals react rapidly with bromine. The results of such photolyses are shown in the Table. The main conclusions are (1) the ratio CF_3Br/CO is greater than 2 and there is very little carbon monoxide at the lower temperatures, and (2) the products contain trifluoroacetyl bromide which was identified and estimated by means of its infrared spectrum.

These observations strongly suggest that the primary process is

$$CF_3{\cdot}CO{\cdot}CF_3 + h\nu \rightarrow CF_3{\cdot}CO + CF_3 \quad . (2)$$

followed by

$$CF_3{\cdot}CO \rightarrow CF_3 + CO \quad . \; . \; . \; (3)$$

In the presence of bromine, the trifluoroacetyl radicals may react to give trifluoroacetyl bromide, particularly at lower temperatures. It can be shown that

$$R(CF_3{\cdot}COBr) = R(CF_3{\cdot}Br) - 2R(CO) \quad . (4)$$

where R denotes rate of formation. In the Table, we compare $R(CF_3{\cdot}COBr)$, calculated by using equation (4), with the observed values; the agreement is reasonable as the trifluoroacetyl bromide analyses were not of high accuracy.

Apparently the trifluoroacetyl radical exists, even at 200°, and the failure to detect hexafluorobiacetyl from the photolysis of hexafluoroacetone alone is probably because of the instability of the compound. If the hexafluoroacetone is photolysed in the presence of nitric oxide, the only products are trifluoronitrosomethane and carbon monoxide in the ratio 2:1;[1] presumably the trifluoroacetyl radical does not react with nitric oxide.

Temp. (°K.)	Products (10^{-12} molecules cm.$^{-3}$ sec.$^{-1}$)			
	CO	CF_3Br	$(CF_3{\cdot}COBr)_{exp.}$	$(CF_3{\cdot}COBr)_{calc.}$
294	0·076	0·71	0·42	0·56
357	0·162	1·57	1·15	1·25
385	0·405	1·91	1·06	1·10
449	1·54	3·99	0·82	0·91
524	2·59	5·08	0·44	0

$P(Br_2)_{av.}$ = 5 mm.; $P(HFA)_{av.}$ = 22 mm. Photolysis times, 30—250 min.

We thank D.S.I.R. for a grant (to B.G.T.).

(*Received, February 8th*, 1963.)

[1] Charles and Whittle, unpublished results.

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Photolytic Generation of Aromatic Radical-anions: Electron-spin Resonance Studies

By P. B. AYSCOUGH and F. P. SARGENT
(DEPARTMENT OF PHYSICAL CHEMISTRY, THE UNIVERSITY, LEEDS, 2)

NUMEROUS observations of the electron-spin resonance spectra of the radical-anions of aromatic hydrocarbons, ketones, and nitro-compounds have been reported recently. Preparative methods include (1) reduction by an alkali metal in an ethereal solvent,[1-3] (2) electrochemical reduction in both aprotic and protic solvents,[3-5] and (3) reduction by zinc or glucose in alkaline solutions. The use of ethanol, methanol, or water as solvent in the latter procedure has the advantage of reducing the line width and permitting more detailed interpretation of the spectra, some new features of which are reported elsewhere.[6] Here we report that these same species can be produced in high yield by ultraviolet irradiation of aromatic nitro-compounds or ketones in concentrated sodium ethoxide, methoxide, or hydroxide solutions. This provides an even more convenient method of preparation, especially where kinetic studies are to be undertaken, since the rate of reduction and the concentration are readily controlled by means of the light intensity.

All the compounds studied (twenty nitro-compounds and six ketones) have strong absorption bands in the ultraviolet region, corresponding to n–π^* or π–π^* transitions. No electron-spin resonance spectra are observed at room temperature in the absence of ultraviolet irradiation. During photolysis the concentration of radical-anions rises to a maximum in 10—30 minutes for most of the nitro-compounds but in a few seconds only in the case of benzophenone. When the light source is removed the fall in concentration follows a similar time-scale (compare ref. 7). Reducing the temperature increases the net rate of formation of radical-anions, increases the limiting concentration, and decreases the rate of decay. Removal of oxygen from the solution has a similar effect. The electron-spin resonance spectra are identical with those observed during chemical or electrochemical reduction in the same solvents and are attributed to the primary radical-anion in each case. We therefore presume that we are observing the transfer of an electron from the alkoxide or hydroxide ion to the vacant orbital of the electronically excited aromatic compound, *e.g.*:

$$Ph{\cdot}NO_2^* + OR^- \rightarrow Ph{\cdot}NO_2^- + {\cdot}OR$$
$$Ph_2CO^* + OR^- \rightarrow Ph_2CO^- + {\cdot}OR$$

The thermally activated reaction of nitrobenzene with alkoxide ions, which can be observed above 60°, gives the same species but increases rapidly in rate with temperature and is therefore easily distinguished. Similar effects are observed on using higher alcohols, though thermal reactions become increasingly important and the spectra are not so well resolved.

Irradiations were carried out inside the cavity of a Varian V-4500 EPR spectrometer, with light from a 250-w medium-pressure mercury lamp from which infrared radiation had been removed by means of a water filter.

We are grateful to D.S.I.R. for financial assistance.
(*Received, January 25th*, 1963.)

[1] Ward, *J. Chem. Phys.*, 1959, **30**, 852; 1960, **32**, 410; 1962, **36**, 1405.
[2] Atherton and Weissman, *J. Amer. Chem. Soc.*, 1961, **83**, 1330.
[3] Ayscough and Wilson, *Proc. Chem. Soc.*, 1962, 16.
[4] Geske and Maki, *J. Amer. Chem. Soc.*, 1960, **82**, 2671; 1961, **83**, 1852.
[5] Piette, Ludwig, and Adams, *Analyt. Chem.*, 1962, **34**, 916.
[6] Ayscough, Sargent, and Wilson, in the press.
[7] Porter and Wilkinson, *Trans. Faraday Soc.*, 1961, **57**, 1686.

Direct Preparation of Some Functional Fluoroaromatic Compounds

By G. M. BROOKE, R. D. CHAMBERS, J. HEYES, and W. K. R. MUSGRAVE
(THE DURHAM COLLEGES IN THE UNIVERSITY OF DURHAM)

OF the routes to hexafluorobenzene,[1] the only one suitable for large-scale work requires fluorination of benzene with cobalt trifluoride and dehydrofluorination of the mixed products to polyfluorocyclohexadienes. These dienes have been defluorinated by passage over a heated iron gauze, giving hexafluorobenzene and polyfluorobenzenes.[2]

We now report the first simple preparation of fully substituted chlorofluorobenzenes, as well as hexafluorobenzene, by a method which can be used on a

[1] Johncock, Mobbs, and Musgrave, *Ind. Eng. Chem.*, Process Design and Development, 1962, **1**, 267; Banks, Birchall, Haszeldine, Simm, Sutcliffe, and Umfreville, *Proc. Chem. Soc.*, 1962, 281; and references therein.
[2] Gething, Patrick, Tatlow, Banks, Barbour, and Tipping, *Nature*, 1959, **183**, 586; Gething, Patrick, Stacey, and Tatlow, *ibid.*, p. 588; Coe, Patrick, and Tatlow, *Tetrahedron*, 1960, **9**, 240.

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large scale. Passage of a three-molar proportion of fluorine diluted with nitrogen through a stirred slurry of hexachlorobenzene in 1,1,2-trichlorotrifluoroethane at room temperature gave a mixture of perchlorofluorocyclohexanes of average composition $C_6Cl_6F_6$, *i.e.*, $C_6Cl_xF_{12-x}$ where $x = 4$—7, in high yield, whereas only small amounts of materials $C_6Cl_6F_6$ and $C_6Cl_6F_4$ were isolated previously from a similar reaction (under different experimental conditions).[3] Dehalogenation of the mixture of perchlorofluorocyclohexanes by passage over hot iron gave hexafluorobenzene, chloropentafluorobenzene, and dichlorotetrafluorobenzenes in high overall yield.

$$C_6Cl_6 + 3F_2 \xrightarrow{CCl_2F\cdot CClF_2} [C_6Cl_6F_6] \;(>90\%) \xrightarrow[330^\circ]{Fe}$$

C_6F_6	22%	64·5% (for the dehalogenation)
C_6ClF_5	27%	
$C_6Cl_2F_4$	12·5%	
$C_6Cl_3F_3$	3%	

The method may be adjusted to give a larger proportion of hexafluorobenzene by further fluorination of the mixture of perchlorofluorocyclohexanes at higher temperatures before dehalogenation.

Variable results were obtained by the iron-gauze technique but remarkably consistent dehalogenation was effected by using a cobalt fluoride reactor[4] in which the cobalt fluoride had been replaced by iron filings. The filings were discarded when spent (after the passage of over 1 kg. of material), so avoiding the uncertain process of regeneration.

We have shown that chlorofluorobenzenes are useful functional compounds, *e.g.*, chloropentafluorobenzene formed a Grignard reagent in diethyl ether when activated by ethylene dibromide and gave 67% of pentafluorobenzene on hydrolysis. Reaction of the Grignard reagent with methylmercury(II) iodide gave 50% of pentafluorophenylmethylmercury and carbonation gave 41% of pentafluorobenzoic acid after preforming the reagent in diethylether and then changing the solvent to tetrahydrofuran before the passage of carbon dioxide, therefore showing similar reactivity to pentafluorophenylmagnesium bromide.[5] When formation of the Grignard reagent in tetrahydrofuran was attempted, high molecular weight fluoro aromatic material was obtained in high yield which did not melt below 360°.

We thank the Imperial Smelting Corporation for a maintenance grant (to J.H.) and the Imperial Chemical Industries Limited for the loan of a fluorine cell.

(*Received, January 16th*, 1963.)

[3] Bigelow and Pearson, *J. Amer. Chem. Soc.*, 1934, **56**, 2773.
[4] Barbour, Barlow, and Tatlow, *J. Appl. Chem.*, 1952, **2**, 127.
[5] Chambers, Coates, Livingstone, and Musgrave, *J.*, 1962, 4367; Harper and Tamborski, *Chem. and Ind.*, 1962, 1824.

Electron Spin Resonance Spectrum of the XeF Radical

By W. E. FALCONER and J. R. MORTON

(DIVISION OF APPLIED CHEMISTRY, NATIONAL RESEARCH COUNCIL, OTTAWA 2, CANADA)

BARTLETT's recent report[1] of the first stable xenon compound, xenon hexafluoroplatinate, has revived interest in the chemistry of the inert gases.[2,3] We here report the existence of a xenon-containing radical, XeF, the electron spin resonance spectrum of which has been obtained at 77°K.

The crystalline nature of xenon tetrafluoride[2] suggested the possibility of stabilising in the lattice xenon-containing paramagnetic fragments which could be studied by electron spin resonance. Xenon tetrafluoride was prepared as recently described[2] and a small single crystal was grown by sublimation. This had the form of an elongated hexagonal plate with prismatic ends; it was irradiated at 77°K with a 5 Megarad dose of 1·3 MeV ^{60}Co γ-rays. The irradiated crystal was blue and exhibited a powerful paramagnetic resonance spectrum when examined at 77°K in the cavity of a Varian V-4500 spectrometer. The spectra were attributed to the species XeF, and the spectrum obtained with the in-plane transverse axis of the crystal parallel to the magnetic field of the spectrometer is reproduced in the Figure. The two strongest lines in the spectrum are due to XeF radicals containing xenon isotopes of zero spin;* they are separated by the hyperfine interaction of the ^{19}F nucleus ($i = 1/2$). Hyperfine interaction with xenon nuclei ^{129}Xe and ^{131}Xe ($i = 1/2$ and $3/2$, respectively) is also apparent, although there is some

* The symbol ^{132}Xe is used to represent all the zero-spin isotopes which together constitute 52·4% of naturally occurring xenon, and have the even mass numbers 124 to 136, inclusive.

[1] Bartlett, *Proc. Chem. Soc.*, 1962, 218.
[2] Claassen, Selig, and Malm, *J. Amer. Chem. Soc.*, 1962, **84**, 3593; Chernick, Claassen, Fields, Hyman, Malm, Manning, Matheson, Quarterman, Schreiner, Selig, Sheft, Siegel, Sloth, Stein, Studier, Weeks, and Zirin, *Science*, 1962, **138**, 136.
[3] Weeks, Chernick, and Matheson, *J. Amer. Chem. Soc.*, 1962, **84**, 4612.

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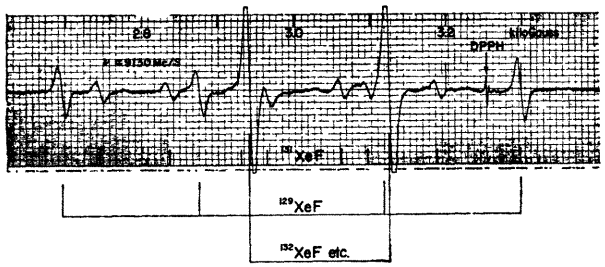
overlapping of the lines from the different isotopic species. The relative intensities of the three groups of lines are consistent with the known isotopic distribution in xenon. Moreover, the ^{129}Xe splitting exceeds that of ^{131}Xe, in accord with their relative magnetogyric ratios.

crystal. The lack of centrosymmetry apparent in the above spectrum, and the deviation from the free-spin g-value suggest that second-order terms in the Hamiltonian are important.[4] The application of second-order theory requires data, as yet unobtained, from spectra for other crystal orientations and therefore precise hyperfine interaction constants and g-values cannot at present be given. However, from the above spectrum approximate hyperfine splittings for the three nuclei ^{19}F, ^{129}Xe, and ^{131}Xe are respectively 180, 425, and 125 Gauss.



Electron spin resonance spectrum of XeF *in a γ-irradiated single crystal of* XeF_4 *at* 77°K.

The spectra were virtually isotropic provided the magnetic field explored a plane perpendicular to the longitudinal axis of the crystal but were otherwise highly anisotropic in the magnetic-field direction. These observations imply that the Xe–F bond is probably parallel to the longitudinal axis of the

(*Received, January* 14*th*, 1963.)

[4] Horsfield, Morton, and Whiffen, *Mol. Phys.*, 1961, **4**, 475; Chantry, Horsfield, Morton, Rowlands, and Whiffen, *ibid.*, 1962, **5**, 233.

Preferred Formation of the *cis*-Olefin in Bimolecular Elimination

By J. ZÁVADA and J. SICHER

(INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY, CZECHOSLOVAK ACADEMY OF SCIENCE, PRAGUE)

IN bimolecular eliminations compounds of the type (I) generally give an olefin mixture in which the *trans*-isomer (II) predominates.[1] We now report several cases of elimination from 'onium compounds (I; R = Pr^n, R′ = Bu^n; X = Me_3N^+ or Me_2S^+) which preferentially give the *cis*-olefin (II). The reaction conditions and results are tabulated.

(I) $R{\cdot}CH_2{\cdot}CHX{\cdot}R'$ $R{\cdot}CH{=}CH{\cdot}R'$ (II)

It is recognised that the mechanism of bimolecular elimination on simple open-chain 'onium salts may range from the fully coupled E_2 processes (transition-state conformations A or B) to E_1cb-like processes (transition state conformations C and D). Banthorpe, Hughes, and Ingold[2] concluded, on the basis of theoretical considerations, that, for 'onium salts, an E_1cb-like mechanism should be more favoured in

Reaction of $C_3H_7{\cdot}CH_2{\cdot}CHX{\cdot}C_4H_9$

X	Me_3N^+			Me_2S^+	
Expt.	1	2	3	4	5
Base/Solvent	Bu^tO^-–Bu^tOH	EtO^-–EtOH	MeO^-–MeOH	Bu^tO^-–Bu^tOH	EtO^-–EtOH
cis-(II) (%)*	26	74	81	9	64

* Percentage of *cis*-(II) in the *cis–trans*-mixture, determined by vapour-phase chromatography on a silver nitrate–triethylene glycol column at 40° by Mr. L. Baštář. The faster-moving isomer was shown to have the *trans*-configuration, in agreement with expectation.

[1] Reviews: Ingold, *Proc. Chem. Soc.*, 1962, 265; Bunnett, *Angew. Chem.*, 1962, **74**, 731.
[2] Banthorpe, Hughes, and Ingold, *J.*, 1960, 4054; we are indebted to Sir Christopher Ingold for a manuscript of this paper before its publication.

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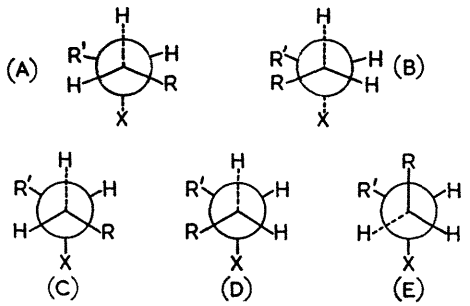
comparison with the fully coupled E_2 mechanism in ethanol than in t-butyl alcohol, and, in any one solvent, more for ammonium than for sulphonium compounds. Since this is the order in which the percentage of the *cis*-isomer increases in our experiments (*i.e.*, expt. 1–2–3; 4–5; 5–2; 4–1) the preferred formation of the *cis*-olefin can be regarded as a consequence of an E_1cb-like mechanism.

A fully coupled E_2 reaction should give preferentially the *trans*-olefin (eclipsing between R and R′ being smaller in A than in B). If the transition states C and D, in which the leaving groups are antiperiplanar, represented the only alternatives for an E_1cb-like process, then this mechanism would—for the same reason as with the fully coupled E_2 reaction—again give preferentially the *trans*-isomer. It is hence unlikely that the reaction in experiments 2, 3, and 5 proceeds to a major extent by way of the transition states C and D. A reaction path proceeding by way of transition state E, which could lead to the *cis*-isomer, must therefore be considered.



In E the non-bonded interactions are undoubtedly smaller than in C or D, the bulky 'onium group here being flanked by hydrogen atoms.* The choice of transition state E would hence indicate that in E_1cb-like processes advantages accruing from antiperiplanarity are no longer necessarily decisive; in our reactions the molecule apparently sacrifices the advantages of antiperiplanarity (cf. C, D) for a sterically less encumbered transition state such as E.

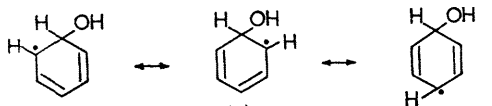
(*Received, January 11th*, 1963.)

* Ukaji and Bonham (*J. Amer. Chem. Soc.*, 1962, **84**, 3631) recently found by electron refraction that for s-butyl chloride there is essentially no difference in energy between the conformers of the type C and D (R = R′ = Me, X = Cl); it seems very unlikely that this would be the case where X is the bulky (and solvated) 'onium group.

An Intermediate in Homolytic Aromatic Substitution

By W. T. DIXON and R. O. C. NORMAN
(DYSON PERRINS LABORATORY, OXFORD UNIVERSITY)

BENZENE reacts with titanous ion and hydrogen peroxide, to give phenol and biphenyl. We here report evidence that the resonance-stabilised radical (I) is an intermediate.



(I)

Warm, acidified aqueous solutions of titanous ion and hydrogen peroxide, each saturated with benzene, were allowed to react in a flow system less than 0·02 sec. before entering a cell in the cavity of a 100 kc./sec. Varian V4500 electron spin resonance spectrometer. The resulting spectrum (Figure) replaced the signal attributed to the hydroxyl radical which we have observed in the absence of benzene.[1] The reconstruction shown is based on coupling with two single protons (coupling constants, 36·0 and 13·4 gauss) (± 5%) and two pairs of protons (coupling constants, 9·3 and 2·9 gauss) (± 5%). This is consistent with the adduct (I) in which coupling would be expected with the six protons on carbon atoms but not with that on oxygen.[2]

When phenol was used in place of benzene, the formation of the phenoxy-radical was confirmed by its spectrum[3] (coupling constants: *o*-H, 6·4; *m*-H, 1·7; *p*-H, 9·7 gauss). This is almost identical in form with half the spectrum ascribed to radical (I), as expected from examination of the appropriate canonical structures, and leads to the assignment of three of the coupling constants for radical (I) (9·3, 2·9, and 13·4 gauss) to *o*-, *m*-, and *p*-protons, respectively. The smaller couplings for the phenoxy-radical arise from the additional possibility of delocalisation of the unpaired electron on to the oxygen atom. The largest splitting observed for radical (I) is evidently due to the proton on sp^3-carbon, which is favourably situated for interaction with the π-electron system in which the unpaired electron is found.

[1] Dixon and Norman, *Nature*, 1962, **196**, 891.
[2] Ingram, "Free Radicals as Studied by Electron Spin Resonance," Butterworths Scientific Publications, London, 1958, p. 174.
[3] Stone and Waters, *Proc. Chem. Soc.*, 1962, 253.

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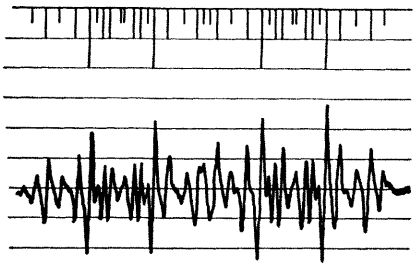
Our assignments for radical (I) receive further support from the similarity of the coupling constants to those of the related radical, cyclohexadienyl (CH_2, 50; *o*-H, 10·6; *m*-H, 2·6; *p*-H, 10·6 gauss).[4]



Spectrum of the intermediate, and reconstruction based on the coupling constants given in the text.

The formation of radical (I) is consistent with the absence of a hydrogen isotope effect in the formation of both phenol and biphenyl when benzene is oxidised with Fenton's reagent, which behaves similarly to the system of Ti^{3+} and H_2O_2.[5] Finally, the radical (I) is analogous to the intermediate postulated in the phenylation of benzenoid compounds on the basis of kinetic isotope effect measurements[6] and product analysis.[7]

One of us (W.T.D.) thanks the D.S.I.R. for a maintenance grant.

(*Received, December 20th*, 1962.)

[4] Fischer, *J. Chem. Phys.*, 1962, **37**, 1094.
[5] Lindsay Smith, and Norman, unpublished observations.
[6] Convery and Price, *J. Amer. Chem. Soc.*, 1958, **80**, 4101; Chang Shih, Hey, and Williams, *J.*, 1959, 1871.
[7] DeTar and Long, *J. Amer. Chem. Soc.*, 1958, **80**, 4742.

NEWS AND ANNOUNCEMENTS

Editorial Appointments.—*Dr. R. S. Cahn*, the Society's Editor since 1949, has been appointed to the newly created post of Director of Publications Research with the task of conducting a Survey of Chemical Publications (see page 73). *Dr. L. C. Cross*, Deputy Editor, has been appointed Editor as from September 1st, 1963, when Dr. Cahn's new post will become a full-time appointment. *Mr. G. P. Pollard* has been appointed Senior Assistant Editor and will take up his duties on April 17th. *Dr. N. A. Keen* was appointed as an additional Assistant Editor from January 1st, 1963.

Library.—The Library will close for the Easter Holiday from 9 p.m., Thursday, April 11th, until 9.30 a.m., Wednesday, April 17th, 1963.

Local Representatives.—The Council has approved the following changes of Local Representatives:

Birmingham	.. *Dr. E. J. Forbes* in place of *Dr. A. B. Foster*
Bristol	.. *Dr. W. D. Ollis* in place of *Dr. R. Parsons*
Cardiff	.. *Dr. J. H. Thomas* in place of *Dr. A. R. Pinder*
Durham	.. *Dr. H. M. M. Shearer* in place of *Dr. F. Glockling*
Northern Ireland	.. *Dr. H. G. Heal* in place of *Dr. M. F. Grundon*
Tees-side	.. *Dr. L. A. Duncanson* in place of *Dr. I. J. Faulkner*

The Corday-Morgan Medal and Prize.—The Council of the Chemical Society has awarded the Corday-Morgan Medal and Prize to *Professor Franz Sondheimer*, Professor and Head of the Organic Chemistry Department at the Weizmann Institute of Science, Rehovoth, in consideration of his contribution to the chemistry of natural products, including his notable synthesis of the steroidal sapogenins and his studies of the synthesis and properties of unsaturated macrocyclic compounds. The award is made in respect of the year 1961.

This Award, consisting of a Silver Medal and a monetary Prize, is made annually to the chemist of either sex and of British Nationality who, in the judgement of the Council of the Chemical Society, has published during the year in question, and in the immediately preceding five years, the most meritorious contribution to experimental chemistry, and who has not, at the date of publication, attained the age of thirty-six years.

Copies of the rules governing the Award may be obtained from the General Secretary of the Society. Applications or recommendations in respect of the Award for the year 1962 must be received not later than December 31st, 1963, and applications for the Award for 1963 are due before the end of 1964.

Election of New Fellows.—289 Candidates were elected to the Fellowship in February, 1963.

Deaths.—We regret to announce the deaths of the following: *Dr. F. M. Brewer* (11.2.63), Reader in Inorganic Chemistry at the University of Oxford; *Professor A. Nasini* (21.1.63), Director of the Chemical Institute of the University of Turin; and *Dr. S. W. Smith* (30.1.63), formerly Chief Assayer at the Royal Mint.

Aspects of Molecular Dissymmetry.—A Chemical Society Symposium on this subject will be held in the

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afternoon and early evening of Thursday, March 19th, 1964, at Battersea College of Technology, London, S.W.11. As the topic is close to the life-long interests of the late Dr. J. Kenyon, for many years Head of the Chemistry Department in the College, this meeting has been chosen as the occasion for the unveiling of a memorial plaque as part of the tribute of his many friends to his work and inspiration. Full details will be published later.

International Symposia, etc.—A Eurochemic Symposium on Nuclear Fuel Reprocessing will be held in Brussels on April 23rd—26th, 1963. Further enquiries should be addressed to O.E.C.D. European Nuclear Energy Agency, 38 boulevard Suchet, Paris 16eme.

A Congress on "Immediate Separation and Chromatography" will be held in Milan on June 14—16th, 1963. Further details can be obtained from the Secretary of Societa Italiana per lo studio delle sostanze grass, via Lauro, 3, Milan.

An International Conference on Magnetism will be held in Nottingham on September 7—11th, 1964. Further enquiries should be addressed to Mr. N. Clarke, The Institute of Physics and The Physical Society, 47 Belgrave Square, London, S.W.1.

Joint British Committee for Vacuum Science and Technology.—A news bulletin has recently been issued by the Committee giving details of forthcoming meetings and other matters of interest to those concerned with vacuum science and technology. For the time being the bulletin is available, free of charge, from the Secretary, Joint British Committee for Vacuum Science and Technology, 47 Belgrave Square, London, S.W.1.

Personal.—*Dr. P. G. Ashmore* has been appointed to the newly created Chair in Chemistry in the Faculty of Technology at Manchester College of Science and Technology.

Dr. J. Chatt is being released from Imperial Chemical Industries Ltd. at the request of the Agricultural Research Council, to take charge of a team to work on the fundamental chemistry and other aspects of the biological fixation of nitrogen.

Dr. S. K. Deb, formerly with the National Research Council, Ottawa, is now a Research Chemist with the Central Research Division of the American Cyanamid Company, Stamford, Connecticut.

Dr. K. Folkers has been elected President of the Stanford Research Institute.

Dr. G. G. Freeman, Head of Silicones Research, Nobel Division, Imperial Chemical Industries Ltd., since 1954, has retired.

Mr. J. H. Greaves, formerly of Younghusband Stephens and Co. Ltd., has joined Proprietary Perfumes Ltd., as Head of the Analytical Laboratory.

Mr. I. Greenfield, formerly of F. W. Berk and Company, Limited, has been appointed Managing Director of Cayford Technical Service and Cayford Chemicals Limited.

Dr. R. Hurst, Director of the Dounreay Experimental Reactor Establishment of the Atomic Energy Authority has been appointed Director of Research of British Ship Research Association.

Mr. B. S. Jackson, Chief Chemist of Building Chemicals Division of Evode, Limited, has been appointed to the Board of Evomastics Limited.

Dr. R. A. Y. Jones, of the University of Sheffield, has been appointed Lecturer in the School of Chemistry in the University of East Anglia as from September 30th, 1963.

Dr. R. A. Mitchell has joined the staff of the Department of Physiological Chemistry, University of Minnesota Medical School, Minnesota.

Dr. F. H. C. Stewart, formerly of Weizmann Institute of Science, Israel, is now with the Division of Protein Chemistry, C.S.I.R.O. Wool Research Laboratories, Parkville, Victoria, Australia.

Mr. W. F. A. Thorpe, a Director of Authur Holden and Sons Ltd., has been elected Vice-Chairman of the Surface Coating Resin Manufacturers' Association.

Lord Todd has been pre-elected Master of Christ's College in succession to Professor B. W. Downs, whose term of office expires on July 11th, 1963.

Mr. A. H. Waddington has been appointed Consultant to the Patterson Engineering Company Limited on all chemical and bacteriological matters from May 1st, when he relinquishes the post of Chief Chemist.

Dr. W. Wilson, formerly Research Manager of B.I.P. Chemicals Limited, Oldbury, is now Director of Research and Development of CIBA (A.R.L.) Limited.

Mr. J. Wright, formerly of Alkali Division, Imperial Chemical Industries Ltd., is now Senior Research Chemist, Carreras Ltd., Basildon, Essex.

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FORTHCOMING SCIENTIFIC MEETINGS

London

Thursday, May 9th, at 6 p.m.
Hugo Müller Lecture, "The Biogenesis of Phenolic Alkaloids," by Professor D. H. R. Barton, D.Sc., F.R.S., to be given in the Lecture Theatre, The Royal Institution, Albemarle Street, W.1.

Birmingham

Friday, May 10th, at 4.30 p.m.
Hugo Müller Lecture, "The Biogenesis of Phenolic Alkaloids," by Professor D. H. R. Barton, D.Sc., F.R.S. Joint Meeting with the University Chemical Society, to be held in the Chemistry Department, The University.

Liverpool

Thursday, May 9th, at 5 p.m.
Lecture, "Magnetism and Stereochemistry of First Row Transition Elements," by Professor J. Lewis, Ph.D., D.Sc. Joint Meeting with the University Chemical Society, to be held in the Donnan Laboratories, Chemistry Department, The University.

Manchester

Thursday, April 25th, at 10 a.m.
Symposium, "Chemical Product Development." Joint Meeting with the Royal Institute of Chemistry, the Society of Chemical Industry, and the Institute of Petroleum, to be held in Theatre R/C9, Renold Building, Manchester College of Science and Technology.

Thursday, May 2nd, at 6.30 p.m.
Hugo Müller Lecture, "The Biogenesis of Phenolic Alkaloids," by Professor D. H. R. Barton, D.Sc., F.R.S., to be given in Room F1, Manchester College of Science and Technology.

North Wales

Thursday, May 9th, at 5.45 p.m.
Lecture, "Some Problems Experienced in the Manufacture of Pure Beryllium," by J. A. Dukes. Joint Meeting with the University College of North Wales Chemical Society, to be held in the Chemistry Department, University College, Bangor.

Reading

Tuesday, May 7th, at 5.45 p.m.
Lecture, "Simple and Complex Metal Nitrates and Nitrites," by Professor C. C. Addison, D.Sc., F.R.I.C. Joint Meeting with the Royal Institute of Chemistry and University Chemical Society, to be held in the Large Chemistry Lecture Theatre, The University.

St. Andrews

(Joint Meetings with the University Chemical Society, to be held in the Chemistry Department, St. Salvator's College.)

Friday, April 19th, at 5.15 p.m.
Lecture, "Surface Radiochemistry," by Dr. S. J. Thomson.

Friday, April 26th, at 5.15 p.m.
Lecture, "Structure, Stereochemistry, and Biosynthesis," by Professor A. R. Battersby, Ph.D.

Southampton

Wednesday, April 3rd, at 7 p.m.
Lecture, "Recent Trends in the Study and Utilisation of Coal," by A. R. Middleton. To be given at the Portsmouth College of Technology.

ADDITIONS TO THE LIBRARY

Scientific books and collectors. J. L. Thornton and R. I. J. Tully. 2nd edn. Pp. 406. Library Association. London. 1962.

Index to reviews, symposia volumes, and monographs in organic chemistry for the period 1940—1960. Edited and compiled by N. Kharasch, W. Wolf, and E. C. P. Harrison. Pp. 345. Pergamon Press. Oxford. 1962.

Theory and applications of ultraviolet spectroscopy. H. H. Jaffe and M. Orchin. Pp. 624. J. Wiley and Sons. New York. 1962.

The interpretation of NMR spectra. K. B. Wiberg and B. J. Nist. Pp. 593. Benjamin. New York. 1962.

Mass spectrometry: organic chemical applications. K. Biemann. Pp. 370. McGraw-Hill. New York. 1962.

Organic chemical crystallography. A. I. Kitaigorodskii. Pp. 541. Consultants Bureau. New York. 1961.

Introduction to ligand field theory. C. J. Ballhausen. Pp. 298. McGraw-Hill. New York. 1962.

The irreducible tensor method for molecular symmetry groups. J. S. Griffith. Pp. 134. Prentice-Hall International. London. 1962.

Chemical thermodynamics. J. A. V. Butler. 5th edn. Pp. 601. MacMillan. London. 1962.

Reaction heats and bond strengths. C. T. Mortimer. Pp. 230. Pergamon Press. Oxford. 1962.

Azeotropic data—II. Compiled by L. H. Horsley. (A.C.S. Advances in Chemistry Series No. 35.) Pp. 100. American Chemical Society. Washington. 1962. (Presented by the publisher.)

Chemistry of combustion reactions. G. J. Minkoff and C. F. H. Tipper. Pp. 393. Butterworths Scientific Publications Ltd. London. 1962.

Autoxidation and antioxidants. Edited by W. O. Lundberg. Vol. 2. Interscience Publishers Inc. New York. 1962. (Presented by the publisher.)

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