LETTERS TO THE EDITORS

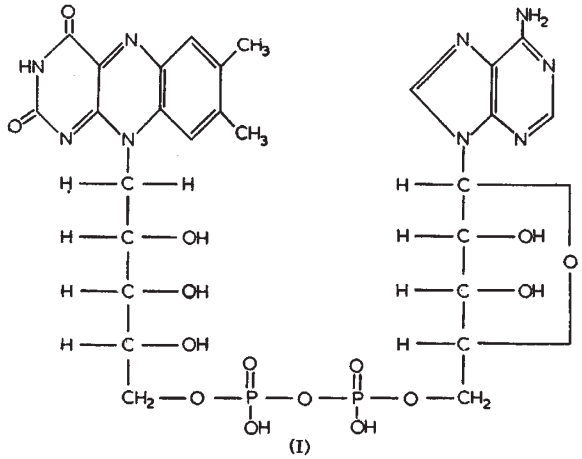
The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications

Total Synthesis of Flavin-adenine-dinucleotide

Flavin-adenine-dinucleotide (FAD) is the co-enzyme of a number of flavoprotein enzymes functioning as oxidation-reduction catalysts in biological systems. Isolated in 1938 by Warburg and Christian[1], it was assigned a probable structure (I) on the basis of available degradative evidence, although its preparation in a pure state has probably been rarely accomplished since its original isolation. Flavin-adenine-dinucleotide is structurally related to several other equally complex coenzymes (for example, coenzymes I and II, uridine-diphosphate-glucose and coenzyme *A*) in that all of them appear to be diesters of pyrophosphoric acid, in which one ester group embraces a pyrimidine or purine nucleoside residue, and indeed the members of the group are usually described loosely as nucleotide coenzymes. No chemical synthesis of any of these coenzymes has hitherto been achieved, although both flavin-adenine-dinucleotide and coenzyme-I have been prepared by enzymic reactions[2].

Over a period of years an extended series of investigations has been undertaken in this laboratory, having as its object the clarification of structural problems in the general field of nucleotide chemistry using the methods of synthetic organic chemistry. These researches have led to the structural elucidation and total synthesis of the ribonucleosides, and the development of a range of flexible methods for phosphorylation and polyphosphorylation of nucleosides[3]. Following the demonstration of the validity of our approach by the synthesis of adenosine triphosphate (ATP)[4], we have devoted increasing attention to the problem of synthesizing unsymmetrical diesters of pyrophosphoric acid of the nucleotide coenzyme type by unambiguous and generally applicable methods. As one of our initial objectives, flavin-adenine-dinucleotide was selected, since not only had riboflavin and adenosine been synthesized, but also we had devised convenient methods for the preparation of riboflavin-5′ phosphate[5] and adenosine-5′ phosphate[6], both of which could be regarded as possible intermediates.

Many attempts were made to apply methods involving disproportionation of polyphosphates and other mixed anhydrides to this problem (notably by Drs. H. S. Forrest and H. S. Mason[7]); but these failed, partly owing to the adverse physical properties of riboflavin and its derivatives, and partly owing to the tendency of the basic catalysts employed to disrupt any flavin-adenine-dinucleotide which might have formed. Arising from this work, however, came an understanding of the behaviour of mixed anhydrides of the oxy-acids of phosphorus, and this provided a novel method for preparing mixed diesters of phosphorous acid which could be applied to the nucleosides. Using it, we were able to synthesize 2′ : 3′-isopropylideneadenosine-5′ benzyl phosphite[8], and from it in turn 2′ : 3′-isopropylideneadenosine-5′ benzyl chlorophosphonate[9]. Condensation of this chlorophosphonate with the monosilver salt of riboflavin-5′ phosphate under carefully controlled conditions yielded the monobenzyl ester of the 2′ : 3′-isopropylidene derivative of (I). Debenzylation, followed by removal of the isopropylidene residue by treatment with acid under conditions which minimized fission of the pyrophosphate linkage, furnished P_1-riboflavin-5′ P_2-adenosine-5′ di-hydrogen pyrophosphate (I), identical with flavin-adenine-dinucleotide from natural sources in chromatographic behaviour (two solvent systems), chemical properties and ultra-violet absorption spectrum. When combined with the flavin-adenine-dinucleotide-free apoenzyme of D-amino-acid oxidase, the synthetic material gave a fully active reconstituted enzyme[10]; in the test solution used the value of 91 per cent was obtained for content of flavin-adenine-dinucleotide as expressed in terms of intensity of light absorption at 450 mμ[1]. We are indebted to Prof. D. Keilin and Dr. E. F. Hartree for carrying out these enzyme experiments.



(I)

This synthesis of flavin-adenine-dinucleotide not only establishes the correctness of structure (I), but it also opens the way to the synthesis of several other nucleotide coenzymes, and experiments in this direction are in progress. Full details of the synthesis here reported will be published elsewhere.

S. M. H. Christie
G. W. Kenner
A. R. Todd

University Chemical Laboratory,
Cambridge. Nov. 6.

[1] *Biochem. Z.*, **298**, 150 (1938).
[2] Shrecker, A. W., and Kornberg, A., *J. Biol. Chem.*, **182**, 795 (1950).
[3] Cf. Review by Kenner, G. W., *Fortschr. Chem. Org. Naturstoffe*, **8** 97 (1951).
[4] Baddiley, J., Michelson, A. M., and Todd, A. R., *J. Chem. Soc.*, 582 (1949).
[5] Forrest, H. S., and Todd, A. R., *J. Chem. Soc.*, 3295 (1950).
[6] Baddiley, J., and Todd, A. R., *J. Chem. Soc.*, 648 (1947).
[7] Forrest, H. S., Mason, H. S., and Todd, A. R., *J. Chem. Soc.*, 2530 (1952).
[8] Corby, N. S., Kenner, G. W., and Todd, A. R., *J. Chem. Soc.*, 3669 (1952).
[9] Kenner, G. W., Todd, A. R., and Weymouth, F. J., *J. Chem. Soc.*, 3675 (1952).
[10] Keilin, D., and Hartree, E. F., *Nature*, **157**, 801 (1946).

Evolution of Animal Fats

Using the theory of evolution as a general background, Hilditch and Lovern[1] have directed attention to the gradual simplification in the fatty-acid composition of animal fats as one proceeds from the lower forms of life to those that are more highly organized. In particular, they have noted the striking