Isotopic Tracer Elements and Stereochemistry

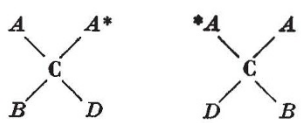
THE interpretations which Ogston[1] has placed on certain tracer experiments on metabolic processes have aroused widespread interest in the role of tracer elements in stereochemistry. Ogston's concept has recently been substantiated in the case of citric acid by the experiments of Potter and Heidelberger[2]. It has implications, however, which go beyond the specific biochemical experiments cited by Ogston.

A general rule has been proposed[3] which may be useful in tracer experiments with asymmetric reagents, whether or not these reagents are enzymes. The following rule will apply to any *partial asymmetric synthesis* or decomposition.

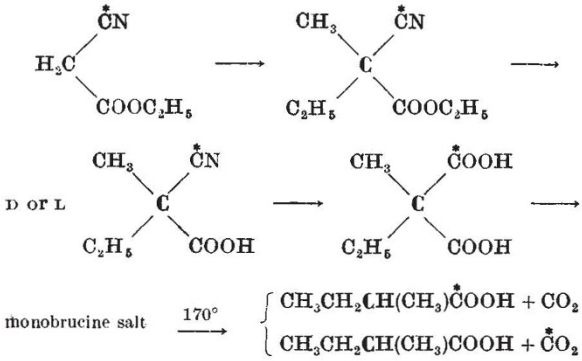
In a molecule which has a plane of symmetry or a point of symmetry, if one of the atoms which does not lie in any plane or point of symmetry is replaced by an isotopic atom, the molecule becomes asymmetric with respect to the labelled atom. In any reaction with an asymmetric reagent, this labelled atom (or group) may react at a rate which is different from that of its counterpart through the plane or point of symmetry, and the difference in rates will be expressed in the distribution of the isotope in the products.

This asymmetric behaviour would be superimposed on any difference in the rates of reaction which would result from the different masses of the isotopic atoms. Stereoisomerism which leads to such asymmetric behaviour of labelled atoms or groups in otherwise symmetric molecules might be designated 'isotopic pseudoasymmetry', since it is analogous to the pseudoasymmetry which results from optical isomeric groups. Isotopic pseudoasymmetric molecules could be produced either by enzymatic reactions or by chemical synthesis, which would necessarily involve the resolution of structurally asymmetric intermediates.

The special case of asymmetry about a carbon atom is of particular interest. Consider the tetrahedral carbon atom carrying two structurally similar groups, A, and a third and fourth group, B and D, respectively, which are dissimilar. If one (or both) of the similar A groups is involved in reaction with an asymmetric reagent, the carbon atom with the two similar groups will become asymmetric and the intermediate complex will behave as a diastereoisomer. Then the A groups are no longer equivalent and may undergo a subsequent reaction at different rates, or one may react almost exclusively in an enzymatic reaction as proposed by Ogston[1]. The asymmetric behaviour of the A groups would be detected if one of them were labelled by an isotopic atom. In this case the two isotopic stereoisomers can be represented as follows:



Tracer techniques are being used here in a reinvestigation of the work of Marckwald[4] on the partial asymmetric synthesis of 2-methylbutyric acid from ethylmethylmalonic acid in order to test the validity of the proposed rule in a non-enzymatic system. (For an excellent discussion of the classic work of Marckwald, see Ritchie[5].) For example, the following series of reactions are being investigated:



Marckwald demonstrated that heating the L-brucine salt of ethylmethylmalonic acid to 170° decarboxylates the acid to give optically active 2-methylbutyric acid, 55 per cent lævorotatory and 45 per cent dextrorotatory. It would be expected that the distribution of the isotopic atom would be affected to a similar extent. The results of these experiments will be forthcoming shortly.

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[1] Ogston, *Nature*, **162**, 963 (1948).
[2] Potter and Heidelberger (in the press).
[3] Thesis submitted by Philip E. Wilcox to the Graduate School of the University of Wisconsin in partial fulfilment of the requirements for the degree of doctor of philosophy.
[4] Marckwald, *Ber.*, **37**, 349 (1904).
[5] Ritchie, "Asymmetric Synthesis and Asymmetric Induction", 17 (Oxford Univ. Press, London, 1933).

Action of Beta Amylase on Amylose

IN a recent paper, Hopkins, Jelinek and Harrison[1] have studied the hydrolysis of potato amylose to maltose with the enzyme, beta amylase. These workers found that the hydrolysis proceeds at a continually diminishing rate and that it is never a first-order reaction as has been claimed by some workers[2,3]. From a study of the products at various stages of the hydrolysis, the theory advanced to explain the facts is that the longer chains of amylose are hydrolysed at a faster rate than short chains and that the longer chains are attacked first. This would account for a continually diminishing rate of hydrolysis as the reaction proceeds and as the length of the chains gradually becomes shorter. From data presented in a recent paper[4], and from other considerations which were briefly mentioned at that time, we have presented a rather different view to explain similar observations.

We are agreed that the reaction is not of zero order, except under certain limiting conditions, and that the rate-constant decreases somewhat even when the equation for a first-order reaction is applied to the hydrolysis as ordinarily performed at a low ratio of enzyme to amylose. We have noted[5], however, that the hydrolysis approaches a first-order reaction under certain conditions, particularly in the case of the hydrolysis of a highly purified corn amylose sample, actually a corn amylose subfraction, which we know to have a DP_n of the order of 225 glucose units[6]. The whole corn amylose fraction (butanol