

consists of three sections in line—the first two are 50 mm inner diameter and 28 cm long and the third 20 mm inner diameter and 30 cm long. These are connected by short lengths of tubing of 8 mm inner diameter and filled with molecular sieve material with a grain size of 0.5–0.8 mm. The second, or 'retention', column was made of a spiral of copper tubing (5 mm inner diameter) with an active length of 160 cm and filled with molecular sieve material of 0.3–0.5 mm grain size, corresponding to a hydrogen capacity of 900 ml. A specially long Dewar vessel was constructed for cooling the first column and this was lowered automatically over a period of about 100 min. Using a 10-l. sample of hydrogen (corresponding to 8 g of water) a final hydrogen sample of 900 ml. was obtained containing 98 per cent of the tritium. The whole process, including reduction of 8 g of water with magnesium at 600° C, took about 3 h.

By increasing the length of the retention column to 4 m it was possible to process 20 l. of hydrogen, which yielded a final fraction of 2 l. containing 96 per cent of the tritium. In all experiments, a concentration of approximately 0.015  $\mu\text{c}$ . tritium/l. of hydrogen was used.

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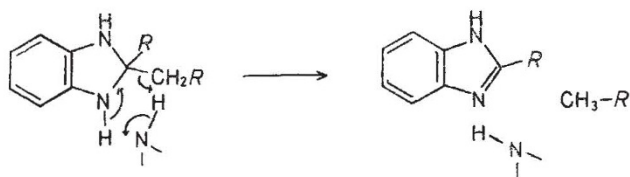
### Mechanism of the *o*-Phenylenediamine Cleavage of Ketones

THE thermal reactions of *o*-phenylenediamine with carbonyl compounds have been investigated for a number of years<sup>1–3</sup>. Recently, Elderfield<sup>4–6</sup> has extensively investigated the cleavage of ketones with this reagent, and has found that the reaction affords 2-alkylbenzimidazoles and alkanes in high yields. Investigations of the scope of the degradation have indicated that the more substituted group is eliminated as the alkane<sup>7–9</sup>. The mechanism of this elimination is not, however, known at the present time.

Initial experiments in this laboratory have indicated that benzyl ketones are more readily cleaved than aliphatic or aromatic ketones. This fact has led us to suggest that the initial intermediate, 2-benzyl-2-alkylbenzimidazoline, either rearranges to 1-benzyl-2-alkylbenzimidazoline which then decomposes to the alkane and benzimidazole or degrades to the final products by means of a bimolecular elimination. In an investigation of the former hypothesis, the second intermediate, 1-benzyl-2-phenylbenzimidazoline, was prepared by reacting *N*-benzyl-*o*-phenylenediamine with benzaldehyde; this compound was then pyrolysed in the usual manner: the benzimidazoline (2.86 g, 0.01 M) was heated in a 'Pyrex' distilling tube. The temperature of the oil bath was raised slowly to 180° C and this temperature was maintained for 12 h. During this time, toluene did not distil from the reaction mixture. After the pyrolysate was allowed to cool, it was washed with methyl alcohol; this treatment gave 1-benzyl-2-phenylbenzimidazole (2.04 g, 72 per cent). It is evident, therefore, that 1-benzyl-2-phenylbenzimidazoline is not an intermediate in the *o*-phenyldiamine reaction.

The latter hypothesis, namely, the bimolecular elimination pathway, was also investigated. This mechanism was suggested because recent investigations<sup>10,11</sup> on the pyrolytic decompositions of esters have indicated a bimolecular pathway, and Elderfield *et al.* have been able to catalyse

the cleavage reactions with both acids and bases<sup>8,9</sup>. The latter result has led us to investigate amines and benzimidazoles as possible catalysts in these reactions. These investigations have shown that the decomposition temperature (initial gas evolution) of 2-benzyl-2-phenylbenzimidazoline can be lowered from 235° C to 200° C by adding small amounts of diphenylamine, benzimidazole, 2-phenylbenzimidazole or 2-methylbenzimidazole to the pyrolysis mixture. A typical experiment is as follows: benzyl phenyl ketone (1.06 g, 0.01 M), and 2-phenylbenzimidazole (0.194 g, 0.001 M) were heated in a 'Pyrex' distilling tube. The temperature of the bath was slowly raised to 195–205° C; at this temperature gas evolution was evident. When the reaction was run in the absence of the benzimidazole, the decomposition temperature was found to be 230–240° C. Based on the catalytic activity of these compounds, the mechanism of this reaction may involve a bimolecular elimination that can be formulated in this manner:



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## BIOCHEMISTRY

### Operon Hypothesis: New Evidence from the 'Constant Proportion' Group of the Emden-Meyerhof Pathway

A NUMBER of examples have now been reported in which a group of enzymes is synthesized in such a manner that the relative activity of each of the enzymes within the group is constant. An important example of this is the 'constant-proportion' group in the Emden-Meyerhof pathway, which has been extensively investigated by Vogell *et al.*<sup>1</sup> and by Pette *et al.*<sup>2</sup>. These workers examined a wide range of materials, extending from baker's yeast to various mammalian tissues, and found that the activity of the five enzymes, triosephosphate isomerase to enolase, formed a pattern which is quite reproducible. They suggest that this indicates a "co-ordinate control of enzyme synthesis" by the cell at the molecular level, a subject which has been the focal point of much recent attention.

A most significant observation, however, which appears entirely to have escaped notice is that the actual pattern reported by Vogell *et al.* in fact represents equimolar amounts of these five enzymes. In Table 1, column 4 shows the calculated 'activity pattern' of equimolar ratios of the enzymes, and column 5 the findings of Pette *et al.* The figures in both columns are expressed as a ratio to the activity of glyceraldehyde phosphate dehydrogenase. Despite the large cumulative errors which may appear in calculations involving several independent measure-