In view of the widespread use of the method of grinding in 'Nujol' for analytical work in the infra-red region, attention may be directed to the fact that in the case of materials existing in tautomeric forms with small energy differences, radical changes in the infra-red absorption spectrum can be produced by grinding. Prolonged grinding has been recommended[3] as a means of securing random orientation of the crystals in the absorption cells; but the possibility of changes occurring due to tautomeric interconversion should always be borne in mind.

A full account of this work will be published elsewhere. Thanks are due to Dr. R. A. Raphael for his kindness in supplying specimens of synthetic[4] and natural penicillic acid and their analogues, to Mr. L. Beecher for assistance with the experimental work, which was carried out for the Chemical Inspectorate, Ministry of Supply, at the Royal Arsenal, Woolwich, and to the Chief Scientist, Ministry of Supply, for permission to publish.

C. W. MUNDAY

Research and Development Department,
The Distillers Co., Ltd.,
Epsom.

[1] Birkinshaw, J. H., Oxford, A. E., and Raistrick, H., *Biochem. J* **30**, 394 (1936).
[2] Raphael, R. A., *J. Chem. Soc.*, 805 (1947).
[3] Barnes, R. B., Gore, R. C., Williams, E. F., Linsley, S. G., and Petersen, E. M., *Indust. and Eng. Chem. (Anal. Ed.)*, **19**, 620 (1947).
[4] Raphael, R. A., *Nature*, **160**, 261 (1947).

Oxidation of α-Diketones and α-Keto-Acids by Hydrogen Peroxide

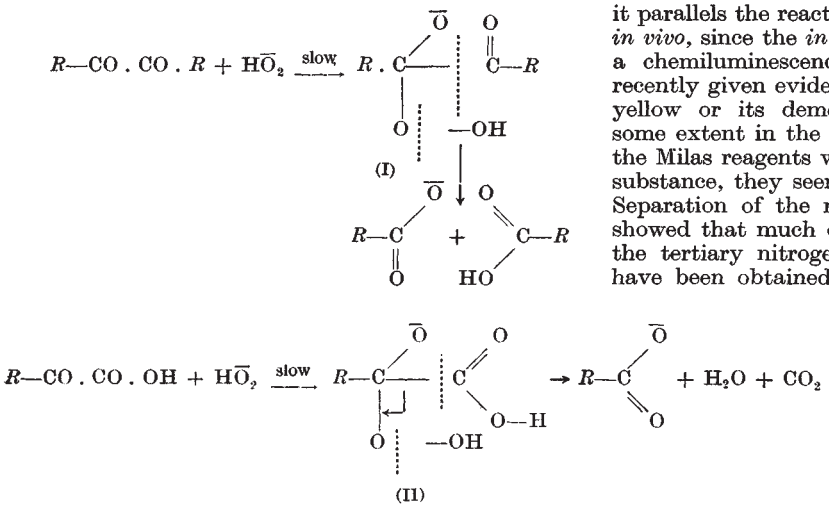
THE oxidation of α-diketones and α-keto-carboxylic acids by hydrogen peroxide involves the breaking of the carbon–carbon bond between the carbonyl carbon and the carbonyl or carboxyl groups[1].

$$R\text{—CO . CO . } R + H_2O_2 \rightarrow 2R\text{—CO . OH};$$

$$R\text{—CO . CO . OH} + H_2O_2 \rightarrow R\text{ . CO . OH} + H_2O + CO_2.$$

This note discusses the mechanism of these reactions in the light of new experimental evidence, and in terms of suggestions I and my co-workers have previously made[2].

The kinetics of these oxidations have been studied in aqueous solutions, and a very marked base catalysis is observed. Such base catalysis is frequently found in the reactions of hydrogen peroxide, and it is considered to indicate a rate-determining attack by the hydroperoxide ion, formed by the ionization of the hydrogen peroxide. The reaction mechanism suggested for the reactions now considered involves the addition of this nucleophilic ion to the carbonyl carbon atom. The conclusion that the addition is rate-determining is in accord with what is known concerning additions of other nucleophilic anions, for example, the cyanide ion, to carbonyl groups.



The kinetics give little information as to the life of the intermediates, I and II, which probably have at most a transient existence.

The base catalysis is observed in the oxidation by hydrogen peroxide of diacetyl and sodium pyruvate, and also of benzil and sodium benzoylformate. Neither of these latter compounds can undergo enolization, and therefore this base catalysis cannot be explained by any mechanism which involves the enolization of the compound.

Mechanisms involving free radicals derived from hydrogen peroxide are also excluded under our experimental conditions. It is found that hydrogen peroxide in the presence of slightly acid ferrous or ferric salts, a recognized source of free hydroxyl radicals[3], does not readily decarboxylate benzoylformic acid. Furthermore, pyruvic acid can actually be prepared by the oxidation of lactic acid with ferrous sulphate and hydrogen peroxide[4].

A detailed account of this work will be published later.

C. A. BUNTON

Sir William Ramsay and
Ralph Forster Laboratories,
University College,
London, W.C.2.
Sept. 18.

[1] Holleman, *Proc. K. Akad. Wetensch. Amsterdam*, **6**, 715 (1904).
[2] Bunton, Minkoff, *et al.*, *Nature*, **161**, 172 (1948).
[3] Haber and Wiess, *Proc. Roy. Soc.*, A, **147**, 333 (1934).
[4] Fenton and Jones, *J. Chem. Soc.*, **77**, 71 (1900).

Demethylation of N : N-Dimethyl-*p*-Amino-azobenzene (Butter Yellow) with Hydrogen Peroxide

THE reaction which butter yellow undergoes with the Milas reagents[1] (hydrogen peroxide in *tert.* butyl alcohol with osmium tetroxide as catalyst) is being studied in detail to determine the extent to which it parallels the reaction occurring with this substance *in vivo*, since the *in vitro* reaction is accompanied by a chemiluminescence[2]. Miller and Miller[3,4] have recently given evidence that hydroxylation of butter yellow or its demethylated derivatives occurs to some extent in the rat, and, as it was expected that the Milas reagents would effect hydroxylation of the substance, they seemed suitable for the comparison. Separation of the reaction products, however, also showed that much of the action had taken place at the tertiary nitrogen atom. The following results have been obtained so far.

0·75 gm. of butter yellow, which had been previously freed from traces of the monomethyl compound and the free base by chromatography using an alumina column and petroleum ether (60–80° C.) as solvent and eluent, was dissolved in 270

ml. of an 8·1 per cent solution of hydrogen peroxide in *tert.* butyl alcohol, and 7 ml. of a 0·5 per cent solution of osmium tetroxide in *tert.* butyl alcohol was added to this. The mixture was maintained at 37° C. for 12 hours, since this was the temperature at which the chemiluminescence observations had been made. The alcohol solution was then diluted to 2 litres with water and extracted with 4 litres of benzene. The benzene extract was concentrated and passed on to an alumina column. After elution of the different zones and repeated chromatography of these, a large number of different eluates was obtained. The materials producing four of these zones were (*a*) 0·11 gm. unchanged butter yellow, (*b*) 0·13 gm. N-methyl-*p*-aminoazobenzene, (*c*) 0·013 gm. *p*-aminoazobenzene, (*d*) 0·013 gm. *p*-nitroazobenzene. The materials were identified by comparison of solubilities and colours in dilute hydrochloric acid, chromatographic behaviour and finally melting-points, with those of samples of known compounds. For the small fractions of *p*-aminoazobenzene and *p*-nitroazobenzene, absorption spectra provided confirmatory evidence. Another small fraction has been obtained which has acid and basic properties and would therefore seem to be a hydroxyl derivative of butter yellow or its demethylated products.

The remainder of the reaction products are still being investigated; but the reactions which take place at the dimethylamino-group seemed worthy of separate note. The removal of alkyl groups from aromatic tertiary amines is not uncommon in *in vivo* reactions; but little is known of the reaction mechanism and the process is simply referred to as 'dealkylation'. Studies with amine oxidase[5,6] show that a number of amines undergo oxidation at the carbon–nitrogen linkage in the presence of this enzyme, and the experiments of Hess *et al.*[7] on cyclic $>N.CH_3$ compounds containing a ketone or aldehyde grouping (derivatives of pyrrolidine and piperidine) afford examples of intramolecular oxidative demethylation. The reaction described above provides an example of oxidative demethylation of an aromatic tertiary amine, effected by hydrogen peroxide.

WM. ANDERSON

Research Department,
Glasgow Royal Cancer Hospital.
Sept. 22.

[1] Milas, N. A., and Sussman, S., *J. Amer. Chem. Soc.*, **58**, 1302 (1936).
[2] Anderson, W., *Nature*, **160**, 892 (1947).
[3] Miller, J. A., and Miller, E. C., *Cancer Res.*, **7**, 39 (1947).
[4] Miller, J. A. (data in preparation).
[5] Richter, D., *Biochem. J.*, **31**, 2022 (1937).
[6] Blaschko, H., Richter, D., and Schlossmann, H., *Biochem. J.*, **31**, 2187 (1937).
[7] Hess, K., Merck, F., and Uibrig, Cl., *Ber. deutsch. chem. Gesell.*, **48** 1886 (1915).

Methylation in the Study of Polynucleotides

ALTHOUGH detailed knowledge of the components of pentose nucleic acids is still incomplete, considerable effort over a long period has been directed towards the problem of the mode of union of the constituent nucleosides. It was early recognized that these units are linked through phosphoric acid residues, and the precise manner in which this takes place has been studied more recently by electrometric titration[1].

As regards the groups in the nucleoside radicals which are concerned in the structure of the polynucleotide, direct evidence that carbohydrate hydroxyl groups are involved is scanty. It is now known that all four nucleotides, isolated after mild alkaline hydrolysis of the pentose polynucleotide of yeast, possess phosphoric ester groupings at position 3 of the sugar residue, and the question has been discussed whether di-ester linkages exist between positions 2 and 3 or 3 and 5 of the different nucleosides making up the polynucleotide[2]. Since, however, yields of the nucleotides are always far below expectation, it cannot be assumed that all the nucleosides are esterified in this way, and an open mind must be kept regarding the precise parts played by the carbohydrate radicals and the phosphoric acid residues in the internucleosidic linkages.

It is clear, therefore, that a technique is required whereby uncombined carbohydrate radicals may be counted and identified, in order that a complete picture of the mode of linkage of the carbohydrate radicals may be obtained. Methylation of yeast nucleic acid has been investigated with this end in view, as mentioned by the late Prof. J. Masson Gulland[3]. Since his death, this work has been carried forward and is now briefly reported. Levene records that the use of Purdie's reagent[4] brought about decomposition of nucleotides and nucleosides at a greater rate than methylation. In the absence of experimental details concerning this breakdown, it was decided to attempt again to make use of this reagent for the methylation of the pentose polynucleotide of yeast.

Repeated treatment of a suspension in methanol of yeast nucleic acid purified by the method of Sevag, Lackman and Smolens[5] with methyl iodide and silver oxide resulted in the formation of approximately eight, and no more, methoxyl groups per statistical tetranucleotide, four of which were assumed to be located on the phosphoric acid residues, since they could be hydrolysed readily by boiling aqueous sodium hydroxide. It was found that if methylation was carried out at the boiling point, the product was pale brown in colour, was deficient in nitrogen and contained no methylamino groups. If, however, experiments were conducted with vigorous shaking at 25° C., methylation proceeded satisfactorily, giving a pale straw-coloured hygroscopic powder, soluble in water and methanol, but insoluble in methyl iodide, acetone and ether, and possessing eight methoxyl groups as before and also approximately six methylamino groups per statistical tetranucleotide.

The degree of success attained in these experiments must be judged, for the present purpose, according to the extent to which the state of combination of carbohydrate hydroxyl groups involved in the internucleosidic links has been altered during methylation. If the assumption, for which there is no real justification at present, be made that carbohydrate radicals are involved in all the internucleosidic linkages, the molecular weight of the methylated product is probably the most reliable criterion. Accordingly, molecular weights were determined by the method of Fletcher, Gulland and Jordan[6] before and after methylation, and it was found that the nucleic acid had suffered no appreciable degradation. The values of the molecular weights, estimated from measurements of the diffusion coefficients by making the same assumptions as those made by Fletcher, Gulland and Jordan[6], range from 10,000 to 12,000 for the nucleic acid before methylation, and from 13,000 to 15,000 after treatment. It is considered, therefore, that this method of investigation promises well to