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MODEL PATHWAYS FOR ENZYMATIC OXIDATIVE DEMETHYLATION—I

THE MECHANISM OF THE REACTION OF DIMETHYL SULPHOXIDE WITH ACETIC ANHYDRIDE

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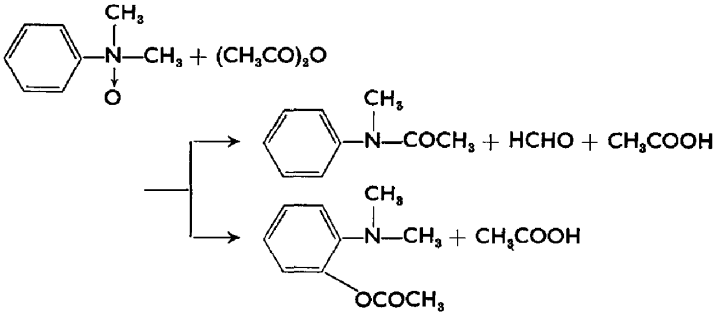
Abstract—The reaction of sulphoxides with at least one methyl or methylene group with carboxylic acid anhydride, called the Pummerer reaction, gives α-acyloxy derivatives of the corresponding sulphide. The mechanism of this reaction was studied by oxygen-18 tracer technique. The reaction between dimethyl sulphoxide and acetic anhydride appears to proceed through an intermolecular rearrangement by nucleophilic attack of acetate anion on methylene carbon atom of the intermediate II. The implications of this reaction and the enzymatic oxidative demethylation are considered in connection with a previous investigation with t-amine N-oxides.

EARLIER publications,[1–3] on the mechanisms of the reactions of the several t-amine N-oxides with acetic anhydride were studied by means of oxygen-18 tracer experiments. Of particular interest is the Polonovski reaction,[4] whereby t-amine oxides with at least one N-methyl group are converted by means of acetic anhydride into s-amines (as an acetyl derivatives) and formaldehyde, and it was suggested that the reaction proceeds by a solvent-caged free radical pair process.[3] Recently, it was shown that



acetylphenylphosphate is also an effective agent for the Polonovski reaction of N,N-dimethylaniline N-oxide yielding *o*-acetoxy-N,N-dimethylaniline under mild condition[5] and, therefore, this reaction can serve as another model for the oxidative demethylation of t-amine oxides in cellular reaction sequences.

Although the demethylation of methionine to homocysteine is an extremely important enzymatic reaction there is no adequate model reaction for this demethylation. It has been known for many years that the "Pummerer Reaction",[6] takes place very readily and that the resulting ester (A) hydrolyses in the presence of water

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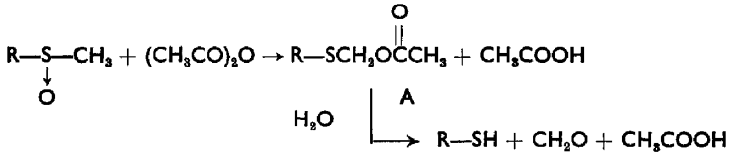
[1] S. Oae, T. Kitao and Y. Kitaoka, *J. Amer. Chem. Soc.* **84**, 3359 (1962).
[2] S. Oae, T. Kitao and Y. Kitaoka, *J. Amer. Chem. Soc.* **84**, 3362 (1962).
[3] S. Oae, T. Kitao and Y. Kitaoka, *J. Amer. Chem. Soc.* **84**, 3366 (1962).
[4] M. Polonovski and M. Polonovski, *Bull. soc. chim.* **41**, 1190 (1927).
[5] S. Oae, T. Kitao, S. Kawamura and Y. Kitaoka, *35th General Meeting of Japanese Biochemical Society* Abstr. p. 93. Tokyo, Oct 30 (1962).
[6] L. Horner and P. Kaiser, *Leibigs Ann.* **626**, 19 (1959).

into mercaptan, formaldehyde and acetic acid. Although several mechanisms of the "Pummerer Reaction" have been suggested,[6–8] only the one, suggested by Bordwell



and Pitt,[7] involving the intermediate II, appears reasonable in view of previous work on similar reactions with t-amine oxides,—the elimination of proton from I would be easy because of the added stabilization through 3d orbital resonance of the resulting carbanion with the adjacent sulphur atom.

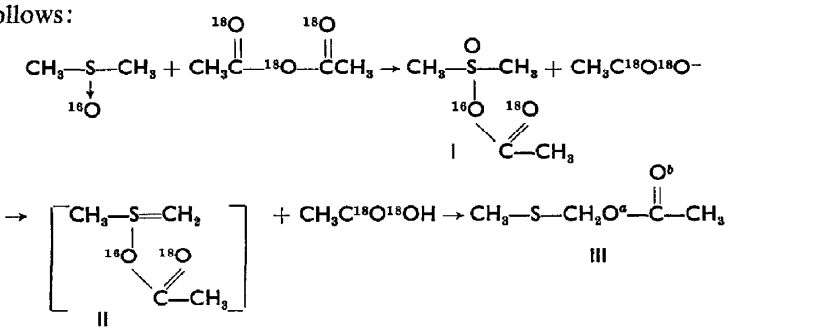
The present paper reports observations on the reaction of dimethyl sulphoxide with acetic anhydride studied by oxygen-18 tracer technique.

Horner *et al.* obtained the acyloxy sulphides either by the reaction of diacylperoxide with sulphides[9] or by the reaction of sulphoxides with carboxylic acid anhydrides;[6] the former usually gives a mixture of the corresponding sulphoxide and the α-acyloxy derivatives while the latter is an excellent preparative method for the acyloxy derivatives of sulphides.

Dimethyl sulphoxide reacts with acetic anhydride in chloroform at 25° for 3 days or in benzene at 80° for 6 hr., yielding α-acetoxymethyl methyl sulphide.

If this compound is refluxed with a small amount of acetic acid in benzene, the acetoxy group is immediately exchanged. In chloroform, the rate of exchange is much slower, but still quite substantial, but if the reation is carried out in ether, there is no or very little exchange. Ether was, therefore, used as solvent throughout this mechanism study. Oxygen-18 labeled acetic anhydride,[1] with all three oxygens were equally enriched by ^{18}O, was reacted with 0·3 moles of dimethyl sulphoxide in ether, yielding α-acetoxymethyl methyl sulphide (about 20%). The resulting oxygen-18 labeled sulphide was purified without isomerization or degradation by gas-phase chromatography using a column packed with fire brick impregnated with high vacuum silicon grease.

The distribution of oxygen-18 according to three different mechanisms involving II is as follows:



Intermolecular nucleophilic attack by $AcO^{\ominus}$	$a = b = (3 \times {}^{18}O + {}^{16}O)/4 = III$
Intramolecular cyclic rearrangement	$a = {}^{18}O$, $b = {}^{16}O$, $III = ({}^{18}O + {}^{16}O)/2$
Free radical cage process	$a = b = ({}^{18}O + {}^{16}O)/2 = III$

[7] F. G. Bordwell and B. M. Pitt, *J. Amer. Chem. Soc.* **77**, 572 (1955).
[8] W. J. Kenney, J. A. Walsh and D. A. Davenport, *J. Amer. Chem. Soc.* **83**, 4019 (1961).
[9] L. Horner and E. Jürgens, *Liebigs Ann.* **602**, 135 (1957).

In the intermolecular mechanism which involves a nucleophilic attack by acetate ion, all the oxygen atoms of both the external acetoxy anion and that formed by the cleavage of the sulphur-oxygen bond would become equivalent by scrambling three ^{18}O-enriched oxygens and one natural, giving rise to an equal value of oxygen-18 for both the ether and carbonyl oxygens of α-acetoxymethyl methyl sulphide. The intramolecular cyclic rearrangement which involves shifts of electron pair in II requires all the excess oxygen-18 in α-acetoxymethyl methyl sulphide to be incorporated in the ether group and the carbonyl oxygen to be natural. The formation of α-acetoxymethyl methyl sulphide via. a "free radical pair" will provide products in which both oxygens of the acetoxy radical are scrambled and the two oxygen atoms contain an average concentration of a natural and an excess oxygen-18.

The analytical values of oxygen-18 for α-acetoxymethyl methyl sulphide are shown in Table 1.

TABLE 1

Compound	Atom% oxygen-18
$CH_3COOCOCH_3$	0·75
$CH_3—S—CH_2OCOCH_3$	0·69
$CH_3S(O)CH_3$	0·20

Inspection of the data excludes both the intramolecular cyclic rearrangement and the radical pair process for this reaction, as both require oxygen-18 values to be 0·48 atom % for the sulphide. Whereas, the intermolecular rearrangement by nucleophilic attack of acetate anion on methylene carbon atom in II, requires the oxygen-18 concentration to be 0·70 atom % for the sulphide.

These results are also in accordance with a radical chain mechanism, if one assumes that there is a complete exchange between the acetoxy radical and the acetic acid or acetic anhydride present in the reaction mixture. However, such an exchange reaction appears to be negligible in view of the ^{14}C[10] and ^{18}O tracer investigations.[2,11]

As a possible model pathway of enzymatic oxidative demethylation of methionine, dimethyl sulphoxide was reacted with acetylphenylphosphate yielding α-acetoxymethyl methyl sulphide.[5] Details of the demethylation of dimethyl sulphoxide, N,N-dimethylaniline N-oxide and the results of preliminary experiments with methionine will be reported in forthcoming papers.

EXPERIMENTAL

Oxygen-18 labeled acetic anhydride was prepared by the method previously reported, and contained oxygen-18 concentration of 0·74 atom% ^{18}O. A small portion of this acetic anhydride was converted to acetanilide[1] and was subjected to ^{18}O analysis. The oxygen-18 content of carbonyl oxygen of the acetic anhydride, 0·76 atom% ^{18}O, indicated an equal concentration of oxygen-18 labeled at both carbonyl and ether oxygens of the acetic anhydride, since both values of ^{18}O agreed within experimental error. Here, for the convenience of further calculations the average value, *viz.* 0·75 atom% ^{18}O was adopted.

Oxygen-18 labeled acetic acid was prepared by hydrolysis of the oxygen-18 labeled acetic anhydride (0·74 atom% ^{18}O) with oxygen-18 enriched water (ca. 1·5 atom% ^{18}O) and then distilled, collecting the fraction (0·91 atom% ^{18}O) of b.p. 118°.

The Pummerer reaction of dimethyl sulphoxide with the oxygen-18 labeled acetic anhydride. Following essentially the same procedure used by Horner and Kaiser, a solution of 10 g dimethyl sulphoxide

[10] A. Fry, B. N. Tolbert and M. Calvin, *Trans. Faraday Soc.* **49**, 1444 (1953).
[11] S. Oae, T. Kitao and S. Kawamura, Unpublished work.

and 43 g (3 moles) oxygen-18 labeled acetic anhydride was refluxed (47·5°) for 1·5 days in 32 ml ether. Ether, the resulting acetic acid and an excess acetic anhydride were removed below 40° at 12 mm Hg, and then α-acetoxymethyl methyl sulphide was distilled. The transparent liquid obtained was redistilled, yielding 2·5 g (20%), b.p. 40–42°/12 mm. The I.R. spectrum of this liquid indicated a slight contamination with acetic anhydride.

Purification of the sulphide by vapour-phase chromatography. α-Acetoxymethyl methyl sulphide was further purified using Yanagimoto Model GCG-2 gas chromatograph. A column (5 mm diameter, 1 m in length) was packed with fire brick impregnated with high vacuum silicon grease (10%). Other conditions were: flow rate of carrier gas (nitrogen), 30 ml/min; temp, 70°; retention time for the sulphide, 20 min when 0·04 ml sulphide was injected into the column. Acetic anhydride, its retention time being 8 min, overlapped slightly with the sulphide. To avoid contamination of acetic anhydride, the far delayed portion of the sulphide which showed no characteristic bands of acetic anhydride, was collected and subjected to oxygen-18 analysis.

^{18}O for $CH_3SCH_2OCOCH_3$	0·69 atom% ^{18}O
Tank CO_2	0·20 atom% ^{18}O

Exchange reactions between α-acetoxymethyl methyl sulphide and oxygen-18 labeled acetic acid. Ordinary α-acetoxymethyl methyl sulphide, 1 g and 4·75 g oxygen-18 labeled acetic acid were refluxed in 19 ml benzene for 6 hr. The sulphide was recovered by distillation and purified by vapour phase chromatography. Similar exchanger ections were also performed in chloroform and in ether (48°). Oxygen-18 content of the sulphide recovered on each run was as follows:

	In benzene	In chloroform	In ether
^{18}O for CH_3COOH	0·91	0·91	0·91
^{18}O for $CH_3SCH_2OCOCH_3$	0·90	0·60	0·23
Reaction period	6 hr	1·5 days	1·5 days

Isotopic analysis. Experimental procedure and calculation were similar to the method in the previous paper.[1]

I.R. spectra. All spectra were taken on a Perkin-Elmer Model 221 spectrophotometer using sodium chloride windows in the liquid state of compounds.