of RNAase were obtained, and analytical data on these fractions are given in Table I. Electrophoresis of the monomeric fraction on cellulose acetate strips demonstrated the absence of native RNAase.

Table I. Analytical Data for the Three Gel Filtration Fractions from Amidinated Ribonuclease

Fraction, % of total	Reagent incorpd,[a] moles/mole of protein	Lysine modified,[b] moles/mole of protein	N_ϵ,N_ϵ'-Adipamidinobis-lysine,[c] moles/mole of protein	Enzymic activity,[d] % of native
Monomer, 47	2.3	3.2	1.0	160
Dimer, 29	2.7	4.0	1.2	135
Aggregated, 24	2.8	4.1	1.2	130

[a] Based on radioactivity incorporated. [b] Based on free lysine in acid hydrolysates of dinitrophenylated, deamidinated (with NH_4OH)[11] samples. [c] Amount found in acid hydrolysates, corrected for destruction, using the automatic amino acid analyzer. [d] Activity toward cytidine 2',3'-cyclic phosphate.

The data clearly demonstrate that approximately 60% of the incorporated reagent has reacted monofunctionally. This is perhaps not surprising in view of the known susceptibility of imido esters to hydrolysis.[2,6]

The increased enzymatic activity of the modified RNAase can be attributed either to the chemical modification *per se* or to the stabilization, resulting from the introduction of cross-links, of a superactive conformation of the enzyme. The latter possibility is supported by the fact that modification with methyl hexanoimidate, the corresponding monofunctional imido ester, does not result in increased enzymic activity.

To determine the location of intramolecular cross-links, tryptic digests of performic acid oxidized, amidinated RNAase monomers have been subjected to peptide mapping as described by Anfinsen, *et al.*[9] Autoradiograms of the peptide maps revealed the presence of three major radioactive components, two of which were completely separated from peptides arising from native RNAase.

These two peptides were isolated by preparative paper electrophoresis of 5 mg of trypsin digest, hydrolyzed, and analyzed. The results are given in Table II.

The amino acid composition of peptide 1 is consistent with the presence of a cross-link between lysine residues 31 and 37.[10] A cross-link in this position would result in the release of a peptide composed of residues 11–39 because the peptide bonds involving the amidinated lysine residues are not susceptible to trypsin-catalyzed hydrolysis.[11] Since the maximum distance that the reagent can span is 8.6 A and the minimum distance between the ϵ-amino groups of lysine residues 31 and 37 in a fully extended peptide chain is 12 A, this region of the RNAase molecule must be folded to some extent.

Component 2 appears to be a mixture composed of approximately equimolar quantitites of two peptides, one of which contains residues 1–10 and 34–39 indicating a cross-link between lysine residues 7 and 37, and

(9) C. B. Anfinsen, S. E. G. Åqvist J. P. Cooke, and B. Jönsson, *J. Biol. Chem.*, **234**, 1118 (1959).
(10) For complete amino acid sequence, see D. G. Smyth, W. H. Stein, and S. Moore, *ibid.*, **238**, 227 (1963).
(11) M. L. Ludwig and R. Byrne, *J. Am. Chem. Soc.*, **84**, 4160 (1962).

Table II. Amino Acid Analyses of Peptides 1 and 2

Amino acid	No. of residues: Peptide 1 Found[a]	Peptide 1 Calcd[b]	Peptide 2 Found[a]	Peptide 2 Calcd[c]
Di-Lys[d]	0.8	1	0.7	1
Lys	0	0	2.1	2
Arg	1.6	2	2.0	2
Asp	4.6	5	2.1	2
Thr	2.3	2	3.2	3
Glu	2.3	2	3.5	3
Ala	2.3	2	6.0	6
Leu	0.95	1	1.0	1
Phe	....		1.1	1
Tyr	1.0	1	...	
His	0.9	1	...	
Cysteic	0.8	1	...	
Ser	6.3	7	...	
Met SO_2	2.5	3	...	

[a] The following corrections have been made: (1) destruction of Di-Lys, Thr, and Ser during hydrolysis; (2) lysine due to hydrolysis of di-Lys; (3) Gly and Ala found in chromatography paper. [b] Peptide corresponding to residues 11–39 (31–37 bridge). [c] Equimolar amounts of two peptides corresponding to residues 1–10, 34–39 (7–37 bridge), and 1–7 (monofunctional amidination of Lys 1), respectively. [d] N_ϵ,N_ϵ'-Adipamidinobislysine.

the other residues 1–7, whose appearance could be explained by assuming monofunctional modification of the N-terminal lysine. The formation of a 7–37 cross-link is not surprising in view of previous reports[3c,d] of the formation of a 7–41 cross-link with 1,5-difluoro-2,4-dinitrobenzene.

These experiments demonstrate the feasibility of employing diimido esters as bifunctional protein reagents.

(12) National Institutes of Health Postdoctoral Fellow (1-F2-CA-20, 585-01).

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Rate Law for the Oxidation of Methanol and Ethanol by Peroxydisulfate Ion

Sir:

The oxidations of ethanol[1,2] and methanol[1-3] by

$$RCH_2OH + S_2O_8^{2-} \longrightarrow RCHO + 2H^+ + 2SO_4^{2-} \quad (1)$$
$$R = CH_3, H$$

aqueous peroxydisulfate proceed *via* a radical mechanism. The influence of radical traps (allyl acetate[1,3] and diphenylpicrylhydrazyl[4]) on rates is only consistent with both a chain mechanism and initiation by the unimolecular, homolytic scission step

$$S_2O_8^{2-} \longrightarrow 2SO_4\cdot^- \quad (2)$$

However, it is difficult to explain the dependence upon reactant concentrations in the previously proposed rate

(1) I. M. Kolthoff, E. J. Meehan, and E. M. Carr, *J. Am. Chem. Soc.*, **75**, 1439 (1953).
(2) M. Santappa and L. R. Subbaraman, *Current Sci.* (India), **33**, (7) 208 (1964).
(3) P. D. Bartlett and J. D. Cotman, *J. Am. Chem. Soc.*, **71**, 1419 (1949).
(4) C. E. H. Bawn and D. Margerison, *Trans. Faraday Soc.*, **51**, 925 (1955).

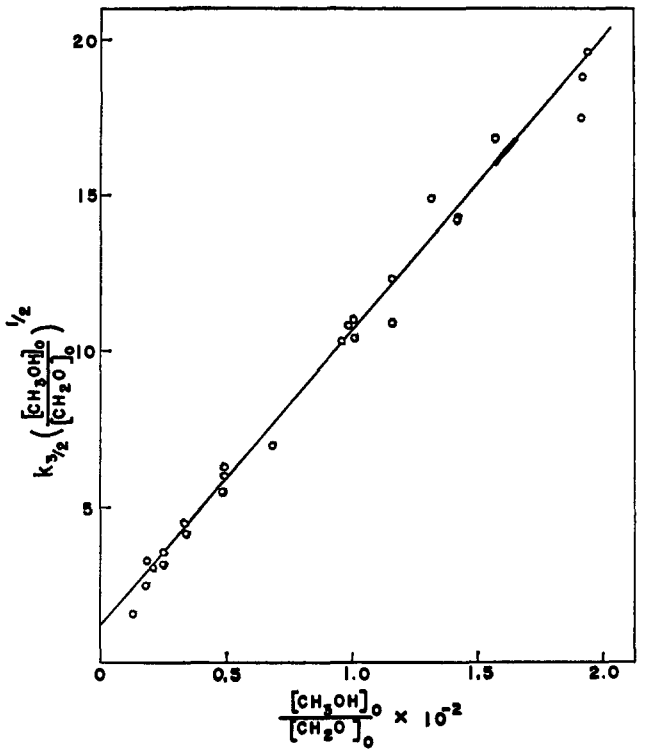

Figure 1. Fit of experimental data to eq 12; $[S_2O_8^{2-}]_0 = 8 \times 10^{-3}$ *M*, temperature 70°.

law

$$\text{rate} = k[S_2O_8^{2-}]^{3/2}[RCH_2OH]^{1/2} \quad (3)$$

without invoking a bimolecular initiation.

To clarify this situation, we have reinvestigated[5] the oxidations and *find that, in the absence of oxygen, both reactions are three-halves order in peroxydisulfate and zero order in alcohol.* There are slight differences between these two alcohols (*e.g.*, in the influence of additives such as cupric ion and oxygen on rate), but both follow the pattern to be elucidated below.

We observed, as had others,[1-3] that the rate increased as initial alcohol concentration increased at constant peroxydisulfate concentration. The influence of aldehyde concentration on rate showed, however, that the product aldehyde, even at low concentrations, had an inhibitory effect. Some data showing this behavior for the ethanol case are presented in Table I. It was found necessary to study the ethanol oxidation at a small but constant aldehyde concentration in order to obtain rate constants independent of initial reactant concentrations. The variation in rate constant with initial aldehyde concentration is shown by the first six entries in the the table. Good three-halves-order (in peroxydisulfate) plots were obtained when the alcohol was in high excess and the aldehyde concentration was constant; this rate dependence on peroxydisulfate is confirmed by the constancy of the three-halves-order constants for the middle six entries in the table. The zero-order dependence on ethanol concentration is indicated by the last six entries. Similar results were obtained for the methanol oxidation, from studies of

(5) Kinetics were followed on a Beckman Model DK-1 spectrophotometer using $K_2S_2O_8$ twice recrystallized from deionized water. Spectral grade MeOH and distilled 95% EtOH were used. The solvent was either unbuffered deionized or laboratory-distilled water.

Table I. Rate Constants as a Function of Reactant Concentrations[a,b]

$[S_2O_8^{2-}]_0 \times 10^3$, *M*[c]	$[EtOH]_0$, *M*[c]	$[CH_3CHO]_0 \times 10^3$, *M*[c]	$k_{3/2}$, $M^{-1/2}$ min^{-1}
9.1	1.2	0.35	8.40
9.1	1.2	1.7	8.17
9.1	1.2	4.0	7.94
9.1	1.2	7.6	6.74
9.1	1.2	15.0	5.67
9.1	1.2	19.0	4.64
9.1	1.6	12.0	5.95
8.4	1.6	12.0	5.80
7.9	1.6	12.0	6.24
7.3	1.6	12.0	6.27
5.4	1.6	12.0	6.49
3.1	1.6	12.0	6.19
8.7	1.0	6.5	7.24
8.7	1.6	6.5	6.97
8.7	2.4	6.5	7.00
8.7	2.8	6.5	7.20
8.7	3.5	6.5	6.54
8.7	4.2	6.5	6.55

[a] All runs at 70° in an N_2 atmosphere. [b] The values of $k_{3/2}$ were obtained from integrated rate plots. [c] The subscript 0 designates initial concentration.

the effects of reactant and additive concentrations upon initial reaction rates. The apparent dependence of rate on alcohol concentration, which had been previously[1-3] attributed to a kinetic order in alcohol concentration, may now be attributed to competition between alcohol and aldehyde for oxidizing radicals[6] (*vide infra*). Our results are, therefore, completely consistent with a unimolecular initiation.

For the initial portion of the reaction, before sufficient aldehyde has accumulated to influence the kinetics, we postulate the following mechanism.

$$S_2O_8^{2-} \xrightarrow{k_1} 2SO_4\cdot^- \quad (4)$$

$$SO_4\cdot^- + RCH_2OH \xrightarrow{k_2} R\dot{C}HOH + H^+ + SO_4^{2-} \quad (5)$$

$$R\dot{C}HOH + S_2O_8^{2-} \xrightarrow{k_3} RCHO + H^+ + SO_4^{2-} + SO_4\cdot^- \quad (6)$$

$$2R\dot{C}HOH \xrightarrow{k_4} \text{products} \quad (7)$$

The sequence 4–7, for long chains,[8] leads on application of the steady-state approximation to the equation

$$\frac{-d[S_2O_8^{2-}]}{dt} = \left(\frac{k_1}{k_4}\right)^{1/2} k_3[S_2O_8^{2-}]^{3/2} \quad (8)$$

The absence of k_2 in eq 8 predicts that the observed rate constants should be insensitive to deuterium substitution at the α carbon. For CD_3OH,[9] using initial three-halves-order rate constants, we find $k_H/k_D = 1.30 \pm 0.10$. The retardation is of the magnitude expected for a secondary isotope effect on k_3 or for an inverse secondary effect on k_4.[10]

As the ratio of alcohol to peroxydisulfate is decreased, the total acid produced has been found to increase be-

(6) Either the sulfate radical ion $SO_4\cdot^-$, or the hydroxyl radical OH·, or both, may be involved in the chain.[7] For simplicity, we shall use the sulfate radical ion.

(7) W. K. Wilmarth and A. Haim in "Peroxide Reaction Mechanisms," J. O. Edwards, Ed., Interscience Division of John Wiley and Sons, Inc., New York, N. Y., 1962, p 222.

(8) At 70° and $[S_2O_8^{2-}]_0 = 1 \times 10^{-2}$ *M*, the chain lengths are approximately 600 for ethanol and 100 for methanol.

(9) CD_3OH was purchased from Merck Sharp and Dohme of Canada and was used without further purification. Mass spectral analysis showed 96 ± 2% isotopic purity.

(10) S. Seltzer, private communication.

yond the amount predicted by eq 1. This indicates that the product aldehydes are able to effectively compete with the alcohols for the sulfate radical ion. The former enter into the chain and are subsequently oxidized to the corresponding carboxylic acids which can be titrated along with the strong acid HSO_4^-. On the basis of these facts, we postulate the additional steps

$$RCHO + SO_4\cdot^- \xrightarrow{k_9} R\dot{C}O + H^+ + SO_4^{2-} \quad (9)$$

$$R\dot{C}O + S_2O_8^{2-} \xrightarrow[H_2O]{k_6} RCOOH + H^+ + SO_4^{2-} + SO_4\cdot^- \quad (10)$$

$$R\dot{C}O + R\dot{C}HOH \xrightarrow{k_7} \text{products} \quad (11)$$

When the steady-state approximation is applied to the sequence of steps 4–6 and 9–11, one obtains the equation

$$\frac{-d[S_2O_8^{2-}]}{dt} = \left(\frac{k_1k_3k_6}{k_7}\right)^{1/2}[S_2O_8^{2-}]^{3/2}\left[\left(\frac{k_2}{k_5}\frac{[RCH_2OH]_0}{[RCHO]_0}\right)^{1/2} + \left(\frac{k_5}{k_2}\frac{[RCHO]_0}{[RCH_2OH]_0}\right)^{1/2}\right] \quad (12)$$

Two conditions are necessary for inhibition: (1) the aldehyde must react at a comparable or faster rate than the alcohol with $SO_4\cdot^-$; and (2) the radical derived from the aldehyde must participate in chain termination. According to (12), a plot of $k_{3/2}([RCH_2OH]_0/[RCHO]_0)^{1/2}$ *vs.* $[RCH_2OH]_0/[RCHO]_0$, where $k_{3/2}$ is the initial three-halves-order rate constant, should be linear. The points of Figure 1 (R = H) fit a straight line (correlation coefficient = 0.994) and are thus consistent with the model. The ratio of intercept to slope of Figure 1 gives $k_5/k_2 = 11$ which agrees with condition 1 above.

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Conjugated Dienes as Quenchers for Excited Singlet States of Aromatic Hydrocarbons

The figures below were inadvertently omitted from the Communication to the Editor that was published on p 3665 of the August 5, 1966 issue.

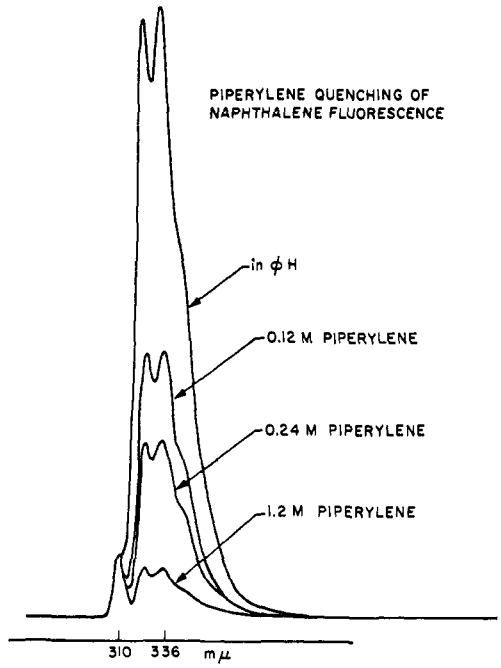

Figure 1. Quenching of the fluorescence of naphthalene by piperylene.

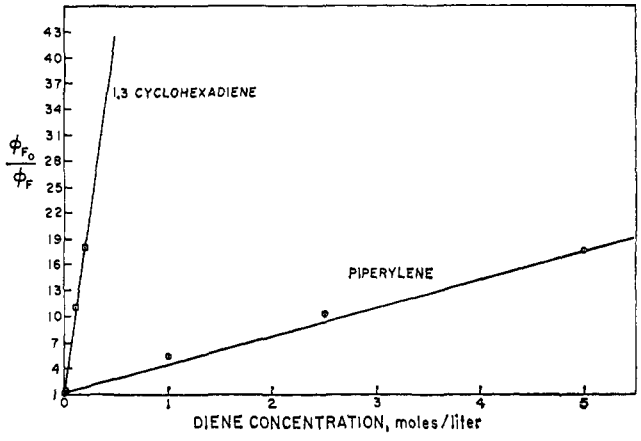

Figure 2. Quenching of 1-methylnaphthalene fluorescence by 1,3-cyclohexadiene and piperylene, ϕ_{f_0}/ϕ_f *vs.* diene concentration.

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