tion of the mixture. The solid residue (14.0 g.) was dissolved in acetone and then precipitated in methanol to obtain 5.0 g. of white, powdered polymethyl bromoacrylate. This material contained no phosphorus. The recovery of starting materials either unreacted or as polymethyl bromoacrylate amounted to 89%.

B. In the Presence of Triethyl Phosphite.—A mixture of diethyl hydrogen phosphite (41.4 g., 0.3 mole), methyl 2-bromoacrylate (49.5 g., 0.3 mole) and triethyl phosphite (1.0 g.) was heated on the steam-bath for 5 hr. After standing 3 days, the reaction mixture was distilled. The recovery of starting materials either unreacted or as polymethyl bromoacrylate amounted to 89%.

C. In the Presence of Sodium Diethyl Phosphite.—Diethyl hydrogen phosphite (41.4 g., 0.3 mole) was treated with sodium (0.05 g., 0.002 mole), then added to methyl 2-bromoacrylate (49.5 g., 0.3 mole). The mixture was heated at 75–80° for 4 hr., then distilled. Recovery of diethyl hydrogen phosphite and methyl 2-bromoacrylate amounted to over 95%.

Acknowledgments.—We wish to thank Mr. Marshall Otis and Mr. Cham Canon for their help in interpreting the infrared absorption spectra.

KINGSPORT, TENNESSEE

[CONTRIBUTION FROM THE CARLSBERG LABORATORY]

The Iron Catalysis of Thioglycolate Oxidation by Oxygen

BY HILDEGARD LAMFROM[1] AND SIGURD OLAF NIELSEN

RECEIVED NOVEMBER 9, 1956

The rate of anaerobic bleaching of red ferric thioglycolate in mixed thioglycolate (0.2 *M*)-acetate (0.1 *M*)-phosphate (*M*/15) buffers *p*H 4.6–5.8 at 37° (system I) has been measured spectrophotometrically and shown to follow second-order kinetics, whereas the anaerobic bleaching of the labile blue ferric thioglycolate in 0.07 *M* thioglycolic acid in 60% ethanol at −35° follows first-order kinetics. In the air saturated system I the rate of oxidation, catalyzed by 1.4×10^{-5} *M* iron salt, was measured, as well as the steady-state concentration of red ferric thioglycolate. The oxidation rate was maximal at *p*H 4.9. The iron catalysis of thioglycolate (RSH^-) oxidation by oxygen has sometimes been assumed to proceed as follows: (a) formation of ferrous complex, Fe(II)RS-complex, (b) oxidation: Fe(II)RS-complex + O_2 → Fe(III)RS-complex, and (c) autoöxidation-reduction: Fe(III)RS-complex → Fe(II)RS-complex + $RSSR^{--}$. It is shown that the oxidation rate calculated for this scheme can account for only 1–3% of the experimentally observed oxidation rate. A modification of this reaction scheme involving free radicals is discussed.

Introduction

The oxidation of sulfhydryl compounds has attracted much interest following the discovery of cysteine and its metal-catalyzed oxidation by molecular oxygen.[2] It could be demonstrated that carefully purified samples of cysteine show but slow oxidation,[3] and that iron and copper ions are potent catalysts for the oxidation of a variety of sulfhydryl compounds by oxygen.[4] The nature of this catalysis was exemplified by the behavior of neutral solutions of ferric salt and thioglycolate. The initial red color formed on addition of ferric salt gradually fades, but can be regenerated by shaking the solution with air as long as there is unreacted thioglycolate present.[5] When iron is replaced by cobalt a stable brown cobalti-complex is formed upon aeration.[6] On the basis of these observations a mechanism A has been proposed for the iron-catalyzed oxidation of sulfhydryl compounds, exemplified by cysteine and thioglycolic acid. This scheme involves 3 principal steps[7]: (a) formation of a ferrous complex with the sulfhydryl compound, (b) oxidation of the ferrous complex to a ferric complex by oxygen, and (c) autoöxidation–reduction of the ferric complex forming the corresponding disulfide and ferrous complex.[8]

$$Fe(II) + RSH^- \longrightarrow Fe(II)RS\text{-complex}$$

$$Fe(II)RS\text{-complex} + O_2 \longrightarrow Fe(III)RS\text{-complex}$$

$$Fe(III)RS\text{-complex} \longrightarrow Fe(II)RS\text{-complex} + RSSR^{--}$$

A restatement of this principal scheme has recently appeared in the literature.[9] Accurate determinations of the composition[10] and rate of break-down[11] of the red ferric thioglycolate in the alkaline *p*H range have been made. A check of reaction scheme A now seems possible by comparing the observed over-all rate of oxidation by oxygen with the oxidation rate calculated on the basis of A from the rate of break-down of ferric thioglycolate and its steady state concentration in air-saturated solution. Such a comparison is undertaken in the present investigation.

Experimental

Thioglycolic acid (80% Merck puriss.) was purified by vacuum distillation and stored as a 7 *M* aqueous solution in a glass stoppered bottle at 3°. After 4 months of use the disulfide content was estimated spectrophotometrically to be less than 3%. All other chemicals were of analytical grade. Glass distilled water was used throughout. Spectrophotometric measurements were made in a Beckman DU spectrophotometer equipped with a 2 sec. Brown recorder[12] and thermostated cell compartment.

Bleaching of Red Complex.—For measuring the bleaching of the red ferric thioglycolate complex in an oxygen-free system

(1) This work was done during the tenure of a Research Fellowship of the American Heart Association.

(2) E. Baumann, *Hoppe Seylers Z. physiol. Chem.*, **8**, 299 (1883–1884).

(3) O. Warburg and S. Sakuma, *Pflügers Arch. ges. Physiol.*, **200**, 203 (1923).

(4) F. Bernheim and M. L. C. Bernheim, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 174 (1939).

(5) R. Andreasch, *Ber.*, **12**, 1390 (1879).

(6) L. Michaelis and M. P. Schubert, THIS JOURNAL, **52**, 4418 (1930).

(7) M. P. Schubert, *ibid.*, **54**, 4077 (1932). Several similar proposals have appeared in the literature, see ref. 11.

(8) The following abbreviations are used: thioglycolic acid, HRSH and dithiodiglycolic acid HRSSRH.

(9) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952, p. 384.

(10) D. L. Leussing and I. M. Kolthoff, THIS JOURNAL, **75**, 3904 (1953).

(11) D. L. Leussing and L. Newman, *ibid.*, **78**, 552 (1956).

(12) S. O. Nielsen, *Rev. Sci. Instr.*, **26**, 516 (1955).

in the pH range 4.5–5.8 a 30-ml. bifurcated glass tube (optical path 1.46 cm.) was used. Into one arm was introduced 0.100 ml. of 3.1×10^{-4} M ferric ammonium sulfate in 0.01 M HCl. To the other arm the following solutions were added in the order given: 6 ml. of a mixed acetate (0.1 M)-phosphate (M/15) buffer, 0.230 ml. 5.8 M NaOH, 0.200 ml. 7.1 M thioglycolic acid and 0.015 ml. of the iron solution. This small iron addition was made in order to remove residual oxygen. The entire system was immediately cooled in an ice-bath and evacuated with an oil pump, carefully avoiding splashing. After slowly warming the tube to 37° it was equilibrated for 10 minutes in a 37° water-bath, during which time the red color in the buffer branch usually faded almost completely. A blank reading was taken of the thioglycolate solution in the 37° thermostated cell compartment. Immediately thereafter the thioglycolate solution was tipped over into the other arm and back again, and the optical density at 525 mμ followed on the recorder. After completion of an experiment the pH was measured at 25° with a glass electrode–KCl–calomel electrode system standardized against 1/1 Sørensen phosphate buffer pH 6.81 at 25°.

Bleaching of Blue Complex.—The bleaching of the labile blue ferric thioglycolate complex in acid solution[13] could only be followed at low temperatures. A special cuvette for use at −35° consisted of a test-tube and a beaker; the test-tube (2.0 cm. diameter) was fixed coaxially in the 600-ml. beaker (8 cm. diameter). The beaker was filled with ethanol and the test-tube with 20 ml. of 1.2×10^{-2} M ferric ammonium sulfate in 60% (v./v.) ethanol, through which pyrogallol purified nitrogen was bubbled for 15 minutes. With the nitrogen tube raised just above the surface of the iron solution both compartments were stirred while the cuvette was cooled to −35° in an ethanol–Dry Ice bath. The cuvette was then removed to room temperature for 2 minutes, immersed for 2 seconds in ethanol at room temperature to prevent interference from condensation on the cold cuvette surface during subsequent measurement. With the wet cuvette placed in the 10-cm. cell compartment, 0.200 ml. of 7.1 M thioglycolic acid was added to the ferric solution on a piston-shaped spatula, which was quickly moved up and down in the solution twice. The change in optical density at 730 mμ was followed for one minute on the recorder. The temperature in the test-tube changed less than 1.5° during 2 minutes. No attempt was made to measure 525 mμ absorption.

Rate of Oxygen Uptake.—The iron-catalyzed oxidation by air of thioglycolate at pH 4.45–6.3 was measured in a Warburg manometric apparatus at 37°. To the main compartment were added 3 ml. of mixed acetate (0.1 M)-phosphate (M/15) buffer, 0.115 ml. of 5.8 M NaOH, and 0.100 ml. of 7.10 M thioglycolic acid, and to the side arm 0.100 ml. of a 4.64×10^{-4} M ferric ammonium sulfate solution in 0.01 M HCl. The composition of the solution, except for the iron concentration, is thus the same as that used in the red ferric thioglycolate bleaching experiments. KOH was added to the center well. After equilibration at 37° for 10 minutes the oxygen uptake was followed 20–30 minutes before and 15–30 minutes after the iron salt was tipped in. In both periods the uptake varied linearly with time. The shaking rate was sufficiently high to ensure air saturation of the solution. The pH was measured at 25° after completion of each experiment. For a series of experiments the same Warburg vessel was used and rinsed only with M HCl and water.

Steady-state Concentration.—The steady-state concentration of the red ferric thioglycolate complex was determined on solutions with identical composition, but 5 times the volume, as those used in the Warburg experiments. The solutions were equilibrated in open 100-ml. erlenmeyer flasks for 15 minutes in the 37° Warburg bath (144 strokes/min.). After pouring the solution into a 5-cm. cuvette the optical density at 525 mμ was observed on the recorder. The initial reading was stable for at least 10 seconds.

Results

Oxygen-free Systems.—The bleaching of red ferric thioglycolate measured spectrophotometrically between pH 4.6 and 5.8 in mixed thioglycolate (0.2 M)–acetate (0.1 M)–phosphate (M/15) buffer (buffer I) follows the second-order rate equation (Fig. 1).

$$d(\Delta D)/dt = -\kappa_1(\Delta D)^2 \text{ or } d(1/\Delta D)/dt = \kappa_1,$$
$$\Delta D = D - D_{blank}$$

To obtain the true rate constant k_1 from the spectrophotometric rate constant κ_1, use is made of the following observations. Almost all ferric iron in thioglycolate solutions pH 8.4–10 is complexed as the mononuclear red ion $FeOH(SCH_2COO)_2^{--}$

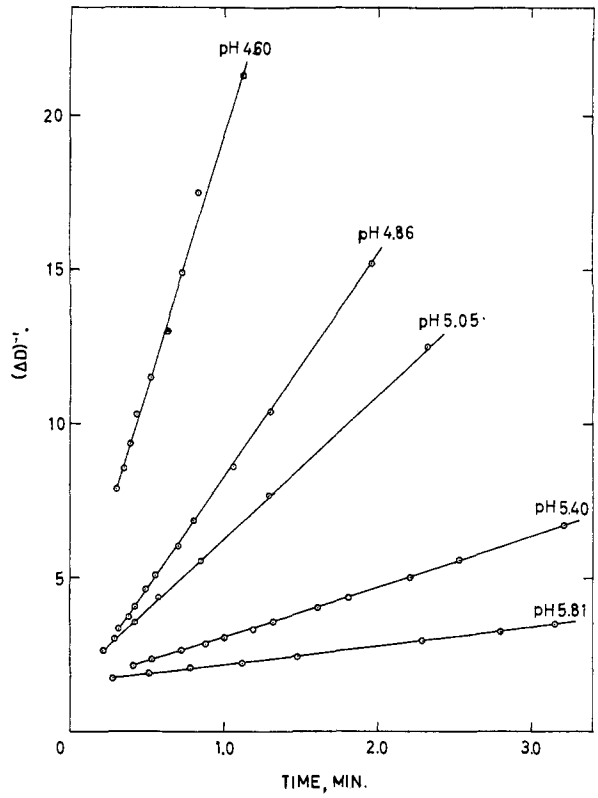


Fig. 1.—Reciprocal optical density at 525 mμ (path l = 1.46 cm., slit 1.0 mm.) *vs.* time for anaerobic bleaching of red ferric thioglycolate at 37° under conditions stated in text.

(II) with an extinction coefficient ($\log_{10}$) 3.73–3.93 $\times 10^3$ M^{-1} cm.$^{-1}$ at 530 mμ (absorption maximum).[10,11] It has been shown in the present investigation that on addition of ferric ammonium sulfate (final concn. 7×10^{-4} M) to air-saturated 0.2 M sodium thioglycolate solution pH 7.00, 2.94 moles of NaOH per mole of added iron are required to readjust the pH to 7.00.[14] The extinction coefficient ϵ at 525 mμ, based on total iron concentration, is 3.83×10^3 M^{-1} cm.$^{-1}$ for 3×10^{-4} M ferric ammonium sulfate in air-saturated buffer I pH 6.3; Beer's law was valid up to at least that iron concentration. Furthermore, no difference could be detected in the relative absorbancies, measured from 400 to 750 mμ, of solutions of ferric ammonium sulfate in air-saturated buffers I, when the pH was changed from 4.6 to 6.3. This leads to the conclusion that from pH 4.6 to 6.3, under the conditions of the present experiment, II is responsible for the visible color of the solutions and that in air-

(13) R. K. Cannan and G. M. Richardson, *Biochem. J.* (*London*), **23**, 1242 (1929).

(14) $FeNH_4(SO_4)_2 + H_2O + 2HSR^- \rightarrow FeOH(RS)_2^{--} + NH_4^+ + 3H^+ + 2SO_4^{--}$.

saturated solutions at pH 6.3 almost all iron is present in the form of II.

The rate constant for the second-order bleaching of II is now given as (Table I)

TABLE I

RATE OF ANAEROBIC BLEACHING OF FERRIC THIOGLYCOLATE; ITS STEADY-STATE CONCENTRATION AND RATE OF IRON-CATALYZED OXYGEN UPTAKE IN AIR-SATURATED BUFFER I AT 37°, $[RSH^-] = 0.215\ M$

	Anaerobic bleaching [Fe(III)] initial = $4.8 \times 10^{-4}\ M$		Oxidation by air [Fe] total = $1.40 \times 10^{-5}\ M$		
pH	Slope from Fig. 1, min.$^{-1}$	2nd-order rate const. k_1, mole^{-1} l. sec.$^{-1}$	Steady-state concn. c_{ss}, μmoles/l.	Rate O_2 uptake	Rate O_2 uptake (calcd.)[a]
				μmoles sec.$^{-1}$ l.$^{-1}$	
4.45				0.58	
4.60	16.25	1510	7.3[c]	.67[c]	0.020
4.86	7.15	665	10.7[c]	.73[c]	.019
5.05	4.70	437	11.9[c]	.70[c]	.015
5.40	1.60	149	13.1[c]	.53[c]	.0064
5.81	0.60	56	13.6[c]	.29[c]	.0026
6.31			13.9	.05	
7.48[b]				.01	
9.07[b]				.05	
10.02[b]				.03	

[a] Calculated on the basis of reaction scheme A and reduction of molecular oxygen to water. [b] Without buffer. [c] Interpolated value.

$$k_1 = d(1/c_{II})/dt = \epsilon d(1/\Delta D)/dt = \epsilon k_1 = 3.83 \times 10^3 \times 1.46 \times (1/60) \times (\text{slope from Fig. 1})\ \text{mole}^{-1}\ \text{l. sec.}^{-1}.$$

The bleaching of blue ferric thioglycolate measured spectrophotometrically follows the first-order rate equation (Fig. 2).

$$d(\Delta D)/dt = -\kappa_2(\Delta D) \text{ or } d \ln (\Delta D)/dt = -\kappa_2, \qquad \Delta D = D - D_{blank}$$

indicating that the true rate equation might also be of the form $dc_{blue}/dt = -k_2 c_{blue}$.

Air-saturated Systems.—The rate of the iron-catalyzed oxidation of thioglycolate by oxygen in buffer I is calculated as the difference between the oxidation rates with and without added iron salt (Table I, column 5). The justification for this procedure will be considered in the discussion. In buffers I the rate of the iron-catalyzed oxidation proceeds at a maximal rate at pH 4.9 in the pH range 4.5–10, although the oxidation rate without added iron starts to increase as the pH approaches 10. Within the limits of experimental error the iron-catalyzed rate of oxidation is proportional to the total concentration of added iron salt at least up to $2 \times 10^{-5}\ M$, as tested in buffer solutions I pH 5.0 and 5.9. The maximum of the oxidation rate *vs.* pH curve is shifted to higher pH with decreasing thioglycolate concentration. In two "oxidation-to-completion" experiments in mixed acetate–phosphate buffers at pH 5.0 and 6.5 and 37° ([Fe] total = $1.48 \times 10^{-4}\ M$, $[RSH^-]$ initial = $1.88 \times 10^{-2}\ M$) the oxygen uptake after 10 hours corresponded to 98 and 102%, respectively, of complete oxidation calculated on the basis of

$$4RSH^- + O_2 \longrightarrow 2RSSR^{--} + 2H_2O \qquad (1)$$

After 20 hours of oxidation the corresponding figures were 104 and 106%, respectively, thus indicating that the predominant oxidation products are the disulfide[6,15] and water. The steady state concentrations of II in the oxidation experiments were calculated from the optical density and the extinction coefficient[16] of II. As seen in Table I the steady-state concentration of II approaches an upper limit with increasing pH.

Discussion

Before the main subject of the present investigation is dealt with, it may be interesting to see how the data obtained for the anaerobic bleaching of II in the pH range 4.6–5.8 fits the rate equation given by Leussing and Newman[11] for bleaching at 25° in NH_4NO_3 buffers pH 8.4–10, ionic strength 0.58. One obtains for the rate extrapolated to the range $p\text{H} < 6.0$

$$dc_{II}/dt = -3.3 \times 10^6 \times \frac{[H^+]}{[RSH^-]}\, c^2_{II} \text{ mole l.}^{-1} \text{ sec.}^{-1} \qquad (2)$$

i.e., for $[H^+] = 10^{-5.81}\ M$ and $[RSH^-] = 0.215\ M$ the rate constant is calculated to be 24 mole^{-1} l.sec.$^{-1}$ at 25° compared to 56 mole^{-1} l.sec.$^{-1}$ (Table I) found in the present investigation at 37° and ionic strength $I \simeq 0.43$. In accordance with (2) a plot of $\log_{10} k_1$ *vs.* pH (Table I) fits approximately a straight line with slope −1.2 indicating the participation of one hydrogen ion per 2 moles of II in the formation of the "transition complex" in the bleaching reaction of II. The deviation of the slope from −1 can be qualitatively accounted for by the increase in ionic strength I from $I \simeq 0.32$ at pH 4.6 to $I \simeq 0.43$ at pH 5.8 if the following reaction mechanism[11] is here assumed to be operative at pH 4.6–5.8

$$Fe(III)OH(RS)_2^{--} + H^+ \rightleftharpoons Fe(III)OHRS + RSH^- \qquad (3)$$

$$Fe(III)OH(RS)_2^{--} + Fe(III)OHRS \rightleftharpoons Fe(III)_2(OH)_2(RS)_3^{--} \qquad (4)$$

$$Fe(III)_2(OH)_2(RS)_3^{--} \longrightarrow Fe(II)RS\text{-complex} + RSSR^{--} \qquad (5)$$

It is also in accordance with this scheme that the bleaching of blue ferric thioglycolate follows first-order kinetics, if the complex is assumed to be dinuclear, as originally proposed by Michaelis and Schubert[6] for the corresponding stable Co(III) complex. The formula $Fe_2(OH)_2(RS)_3^{--}$ is therefore tentatively assigned to the blue complex.

While the mechanism of oxidation of thioglycolate by ferric ion is thus believed to be fairly well understood, the same is not true for the mechanism of oxidation of thioglycolate and other sulfhydryl compounds by molecular oxygen in the presence of iron salts. It appears that, *e.g.*, reaction scheme A has never been checked by kinetic analysis. Such a check will now be attempted. The reasoning given below is applicable to all reaction schemes of type A the essential feature of which is that the oxygen oxidation reaction(s) and the autoöxidation–reduction reaction(s) are coupled only by the ferric intermediate in the ground state. This type of reaction mechanism will be referred to below as reaction mechanism (scheme) A.

(15) P. Claesson, *Ber.*, **14**, 409, 412 (1881).

(16) The extinction coefficient in the 5-cm. cell is slightly higher than the value reported above for a 1-cm. cuvette.

The validity of scheme A is checked by comparing the observed uptake with that calculated for the iron-induced oxygen uptake by thioglycolate solutions using data from the anaerobic bleaching of II and its steady-state concentration (c_{st}) in air-saturated solution (Table I). In the last column of Table I is given the iron-induced oxygen uptake calculated as $0.25 \times k_1 \times c^2_{st}$. A comparison between the two last columns of Table I shows that reactions according to scheme A account for only a small fraction of the observed iron-induced oxygen uptake. However, before scheme A is rejected on these grounds a closer examination of the assumptions involved in the calculation is necessary.

The oxygen uptake is calculated on the basis of oxygen being reduced to water. Although hydrogen peroxide has been found in sulfhydryl compound–oxygen reaction mixtures[4] it is a reasonable assumption that it is present only to a minor extent during the oxidation, in view of the approximately stoichiometric oxygen uptake according to (1).

The calculation of the iron-induced oxygen uptake as a differential uptake may give values dependent on the presence of other metals,[17,18] but the values so obtained can still be compared with the calculated oxygen uptake, the influence of trace metals being reflected in c_{st} according to scheme A.

Spectrophotometrically only the presence of II is suggested, although at a lower *p*H the existence of other ferric complexes cannot be completely ruled out. However, even if it is assumed that oxygen is reduced to hydrogen peroxide and that at *p*H 4.6 II constitutes only *ca.* 50% of the total amount of ferric complex present in the steady state (Table I), the calculated oxygen uptake is only raised to $2 \times 2^2 \times 0.020 = 0.16$ μmole sec.$^{-1}$ l.$^{-1}$, compared to 0.67 μmole sec.$^{-1}$ l.$^{-1}$ observed.

As scheme A also in this extreme case fails to account for more than 24% of the observed oxidation rate, it is concluded that reaction mechanisms of type A are not representative of thioglycolate oxidation.

An additional difficulty in maintaining scheme A is the discrepancy between, on the one hand, the observed proportionality between rate of oxygen uptake and concentration of added iron, and on the other hand, the predicted quadratic dependency of the rate on the total iron concentration at *p*H 6.

Only a few preliminary experiments have been carried out to determine the nature of the suggested new reaction mechanism. Two possibilities have been considered, a free radical mechanism, and a slightly modified mechanism A in which reaction (4) of the autoöxidation–reduction of II is rate determining instead of (5). This last modification would not change the form of the rate equation for the bleaching of II, but would explain the low values of the rate of oxygen uptake calculated on the basis of the bleaching of II, if at the same time it is assumed that the initial ferric complex is (blue) $Fe_2(OH)_2(RS)_3^{--}$ This *ad hoc* hypothesis is, however,

(17) Compare J. H. Baxendale, *Advances in Catalysis*, **4**, 31 (1952).

(18) Without added iron, ethylenediaminetetraacetate inhibited the oxygen uptake.

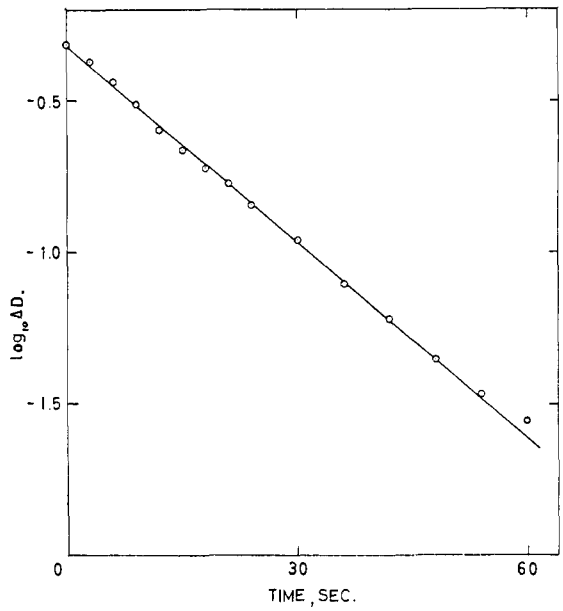


Fig. 2.—Logarithm of optical density at 730 mμ (path approx. 2 cm.) *vs.* time for anaerobic bleaching of blue ferric thioglycolate at approx. −35° under conditions stated in text.

easily disproved in a special rapid-flow apparatus[19] with an additional inlet immediately after the first mixing chamber. On addition of excess sodium acetate buffer *p*H 6 to acid blue ferric thioglycolate solution at 5 and 20°, the change from blue to red is faster (immediate) than the bleaching of the blue color in acid solution in the absence of acetate. This conclusively demonstrates that reaction (4) (right to left) is faster than reaction (5).

In view of the important role assigned to thiyl radicals ·SR′ in many reactions in the liquid phase, a free radical mechanism for the oxidation of thioglycolate by oxygen suggests itself. Such a mechanism could incorporate the catalytic effect of dithiodiglycolic acid[20] and the observed proportionality between rate of oxygen uptake and total iron concentration, both unexplained by scheme A. A free radical mechanism could also explain the induced oxidation by thioglycolic acid of unsaturated compounds as lecithin,[21] styrene[22] and acrylonitrile.

At present the proposal of any detailed scheme would seem immature, but a general outline might be visualized as

$$\text{Fe(II)RS-complex} + O_2(H_2O_2) \longrightarrow \text{Fe(III)RS-complex} + \cdot O_2^-(\cdot OH) \quad (6)$$

$$\cdot O_2^-(\cdot OH) + HSR^- \longrightarrow HO_2^-(H_2O) + \cdot SR^- \quad (7)$$

$$\cdot SR^- + \text{Fe(III)RS-complex} \longrightarrow \text{Fe(II)RS-complex} + RSSR^{--} \quad (8)$$

(19) H. Hartridge and F. J. W. Roughton, *Proc. Roy. Soc.* (*London*), **A104**, 376 (1923).

(20) M. Dixon and H. E. Tunnicliffe *ibid.*, **B94**, 266 (1923).

(21) O. Meyerhof, *Pflügers Arch. ges. Physiol.*, **199**, 531 (1923).

(22) M. S. Kharasch, W. Nudenberg and G. J. Mantell, *J. Org. Chem.*, **16**, 524 (1951).

It appears remarkable that in a free radical mechanism for thioglycolate oxidation the reaction stops at the disulfide. This might, however, be explained by assuming that in reaction (7) all $\cdot O_2^-$ and $\cdot OH$ radicals are trapped before they can react with the ferric complex. In accordance with this it was found that the addition of 0.1 *M* sodium benzoate[23] did not affect the initial rate of oxygen uptake.

Acknowledgment.—The authors wish to express their very sincere thanks to Professor K. Linderstrøm-Lang for valuable advice and encouragement.

(23) G. Stein and J. Weiss, *Nature*, **166**, 1104 (1950).

COPENHAGEN, DENMARK

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF VIRGINIA]

Some Thionophosphate and Phosphoroamidate Derivatives of Adenosine and Certain Steroids[1]

BY MANFRED E. WOLFF AND ALFRED BURGER

RECEIVED DECEMBER 13, 1956

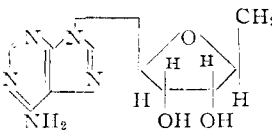
Syntheses of *O,O*-diethyl thionophosphate esters of adenosine, methyl 2,3-isopropylideneribofuranoside, cholesterol, ergosterol and estrone and of two phosphoroamidate derivatives of adenosine are described. These compounds have been prepared as potential antagonists to the corresponding phosphorylated metabolites. Potassium *t*-butoxide smoothly effects the condensation of these complex hydroxy derivatives with diethyl thionophosphorochloridate.

A considerable number of biochemically important substances are phosphorylated prior to being metabolized. The phosphate group in many of the resulting esters seems to bind the substrates to enzyme systems since it frequently remains unchanged in subsequent metabolic conversions. Structural alterations of such phosphate groups may be expected to produce potential inhibitors of enzymes associated with synthetic and catabolic pathways of the corresponding normal metabolites. In previous articles,[2-4] phosphonate derivatives of carbohydrates and of analogs of nucleotides have been described. This paper deals with the synthesis of thionophosphate and phosphoroamidate derivatives.

Thionophosphate esters, $(RO)_3PS$, are known to differ in several respects from the corresponding phosphates. For example, they are much less susceptible to nucleophilic substitution and undergo transformations to thiolophosphates which approximate the isomerization of phosphites to phosphonates.[5] Such differences should affect the biological reactions of thionophosphates even though some of them can be oxidized to the *O*-phosphate esters.[6,7] Tertiary phosphoroamidates could be toxic *per se* and are known to be oxidized enzymatically to toxic *N*-oxides in some instances.[8,9] These products inhibit enzyme systems by phosphorylations similar to those postulated for the attack of organic phosphates on cholinesterases.[10]

The *O,O*-diethyl thionophosphorylation of the alcoholic and phenolic hydroxyl groups of the metabolites used was carried out with diethyl thionophosphorochloridate. According to previous reports, phenolic compounds react more rapidly with this substance in aqueous alkaline solution than in non-polar solvents.[11] A model experiment with a complex primary alcohol, 5-(7-theophyllinyl)-pentanol-1,[2] demonstrated that thionophosphorylation could not be achieved in the presence of such a weak base as pyridine or of sodium carbonate and copper powder in anhydrous benzene. It was concluded that a strong base was required to metalate the hydroxyl groups in order to facilitate the reaction with diethyl thionophosphorochloridate. Potassium *t*-butoxide was chosen because it is soluble in ionizing solvents such as *t*-butyl alcohol and too hindered sterically to react rapidly with the thionophosphorylating agent. The reactions went well under these conditions as described in the Experimental part. The six *O,O*-diethyl thionophosphates obtained are listed in Table I.

By the same general method using potassium *t*-butoxide, 2′,3′-isopropylideneadenosine[12,13] was treated with ethyl phosphorodiethylamidochloridate, and the isopropylidene group of the resulting ester was hydrolyzed with dilute sulfuric acid, yielding *O*-ethyl *O*-(5′-adenosyl phosphorodiethylamidate (II). The corresponding *O*-phenyl *O*-(2′,3′-isopropylidene-5′-adenosyl phosphorodieth-



I, R = $PS(OC_2H_5)_2$
II, R = $PO(OC_2H_5)N(C_2H_5)_2$

(1) This work was supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) J. R. Parikh and A. Burger, THIS JOURNAL, **77**, 2386 (1955).

(3) B. S. Griffin and A. Burger, *ibid.*, **78**, 2336 (1956).

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