

tives which react with isocyanates,²⁰ the reaction may offer a convenient method to follow reactions of phosphorus compounds in which phosphoryl oxygen is being added or removed. Applications to several problems of this type are under study.

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Hydrolysis and Autoxidation of N-Benzoylleucomethylene Blue

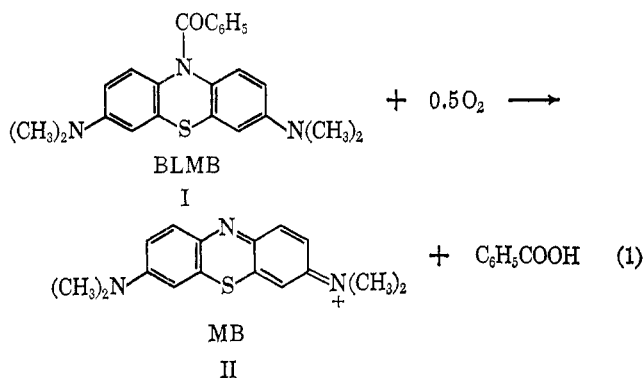
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Hydrolysis of N-benzoylleucomethylene blue in 0.5 M sulfuric acid is a first-order reaction ($k_{50} = 29.3 \times 10^{-7} \text{ sec}^{-1}$), which is unaffected by, and independent of, the concurrent autoxidative development of methylene blue. N-Benzoylleucomethylene blue in aqueous sulfuric acid reacts with oxygen in a 1:0.5 molar ratio to form methylene blue and benzoic acid. After an induction period, the methylene blue formation follows first-order kinetics ($k_{50} = 30.0 \times 10^{-7} \text{ sec}^{-1}$). The rate constant for color production is independent of substrate concentration and oxygen pressure as well as of added methylene blue, benzoic acid, sodium chloride, mannitol, or ethanol. Color production becomes faster when the acid concentration is increased. *p*-Nitrobenzoylleucomethylene blue autoxidizes more slowly, *p*-methoxybenzoylleucomethylene blue more rapidly, than unsubstituted N-benzoylleucomethylene blue. All the data for autoxidation are consistent with a slow hydrolysis of N-benzoylleucomethylene blue to leucomethylene blue, followed by a more rapid oxidation of leucomethylene blue to methylene blue. The available data offer no support for participation of hydrogen peroxide in the oxidative stages. Several oxidants other than oxygen can convert N-benzoylleucomethylene blue to methylene blue without preliminary hydrolysis.

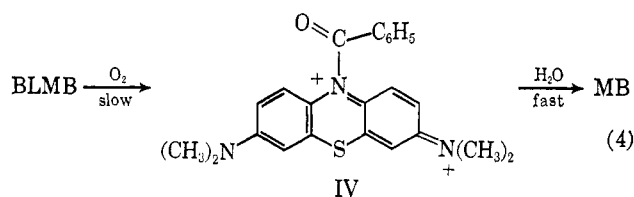
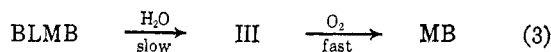
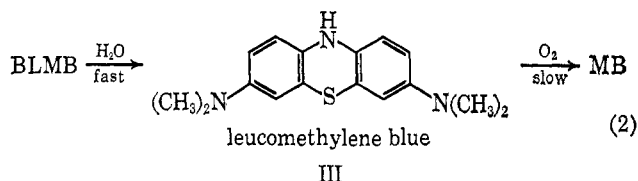
A colorless, acid solution of N-benzoylleucomethylene blue (BLMB, I) becomes blue on exposure to air. We have examined this process both qualitatively and quantitatively and are now reporting the results. Equation 1 summarizes the over-all process. Benzoic acid is formed, as shown by its isolation. The color is that of methylene blue (MB, II), which may be isolated as the solid perchlorate salt and also identified by its absorption spectrum. Oxygen is required; no color develops in its absence.



The stoichiometry of eq 1 was arrived at by finding the BLMB:methylene blue and the oxygen:methylene blue ratios. The former, determined by comparing the amount of BLMB starting material with the maximum intensity of color produced, is 1:1. The latter, determined by following oxygen absorption manometrically and color formation spectrophotometrically, is 1:2.

We considered three sequences for the conversion of BLMB (I) to methylene blue (II). Equation 2 shows how BLMB might first be hydrolyzed to leucomethylene blue (III) in a relatively fast step, which is followed by a rate-determining reaction of leucomethylene blue with oxygen. Equation 3 has the same sequence but with hydrolysis slow and autoxidation fast. Equation 4 has oxygen reacting directly with BLMB in a

rate-determining step to form an intermediate such as benzoylmethylene blue (IV), which would be expected to hydrolyze very rapidly to give the observed prod-



ucts.¹ Comparison of the hydrolysis of BLMB in the presence and absence of air, as well as comparison of the rates of BLMB hydrolysis and methylene blue production, enabled a choice to be made between these three alternatives.

Hydrolysis of BLMB.—Hydrolysis of BLMB in 0.5 M sulfuric acid at 50° was followed with the help of an isotope-dilution procedure. The necessary radioactive BLMB was prepared by benzoylating the Grignard derivative of leucomethylene blue with carbonyl-labeled benzoyl chloride. In the absence of oxygen, the hydrolysis over 3 half-lives showed good first-order kinetics, with a rate constant of $26.5 (\pm 3) \times 10^{-7} \text{ sec}^{-1}$ (99% confidence level). A series of runs was also performed with oxygen present and, therefore, with methylene blue color developing simultaneously. Some of the runs were simply the continuation of the

(1) The formation of benzoylmethylene blue (IV) from BLMB is analogous to the formation of reactive, electron-deficient intermediates from hydroquinone precursors in oxidative phosphorylation. Cf. D. M. Brown, *Advan. Org. Chem.*, **3**, 138 (1963).

anaerobic hydrolyses after admitting air; some were open from the start. Under such aerobic conditions, the rate of disappearance of BLMB again followed first-order kinetics, with a rate constant of $29.3 (\pm 0.7) \times 10^{-7} \text{ sec}^{-1}$ (99% confidence level). Since the two values were the same within experimental error, all the experimental points—covering 9 half-lives—were combined to give a rate constant of $29.3 (\pm 0.8) \times 10^{-7} \text{ sec}^{-1}$ (99% confidence level). Clearly, the rate of hydrolysis of BLMB is unaffected by air and proceeds at the same rate with or without concurrent oxidative development of methylene blue. This result rules out eq 4.

Figure 1 follows the development of methylene blue (measured spectrophotometrically) in mixtures that had been allowed to hydrolyze anaerobically until 17–99% of the initial BLMB disappeared before admitting air. The curves show how the longer the hydrolysis period and the greater the extent of hydrolysis, the faster the development of color. This behavior receives a straightforward interpretation if the disappearance of BLMB does, in fact, correspond to its hydrolysis, so that the readily autoxidizable leucomethylene blue accumulates during the preliminary anaerobic period and then forms methylene blue relatively rapidly as soon as air is admitted.

This interpretation is tantamount to admitting eq 3 and rejecting eq 2. The kinetics of color formation from BLMB point to the same conclusion.

Methylene Blue from BLMB.—Color development in the autoxidation of BLMB to methylene blue was followed spectrophotometrically. The bottom curve of Figure 1 is typical of most of the concentration-time plots. However, although this curve indicates how the rate of methylene blue formation first increases and then decreases, it does not show how the concentration of methylene blue tends to level off short of the theoretical yield and then to decrease. Figure 2 presents a first-order plot—again representative of most of the runs—for the production of methylene blue. Three sections of this curve may be recognized: (a) an induction period in which the initial near-horizontal curve gradually becomes steeper and approaches a limiting slope; (b) a linear portion where, over a considerable part of the reaction course, the plot fits first-order kinetics; and (c) a final section where the plot deviates from the straight line.

Initially, the autoxidative development of methylene blue from BLMB is very slow. However, over the first 20–40 hr of reaction, the rate gradually increases until the process takes on first-order characteristics. Generally, 10–20% of the BLMB originally present is converted to methylene blue during this induction phase. An induction period is to be expected if the autoxidation is correctly described by eq 3 and if the rate constants for the BLMB to leucomethylene blue step and the leucomethylene blue to methylene blue step are not too different.² However, the lengths of the induction periods are variable and the reproducibility is poor, so that our data offer little support to this uncomplicated picture of eq 3 as two consecutive first-order steps. We suggest, provisionally, that the slow color development at the early stages of the autoxi-

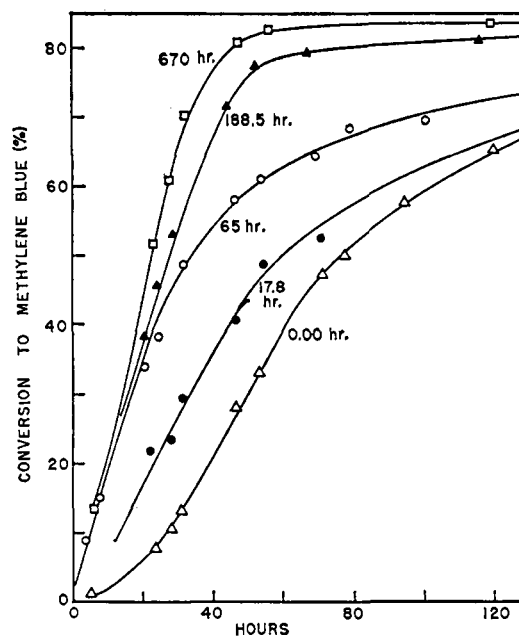


Figure 1.—Methylene blue development on exposing BLMB solutions to air after anaerobic hydrolysis at 50° . The solvent is $0.50 M$ sulfuric acid. The zero point on the abscissa corresponds to the point at which air was admitted after the indicated periods of hydrolysis. The extent of hydrolysis during each period is as follows: 0.0 hr, 0.0%; 17.8 hr, 22%; 65 hr, 51%; 188.5 hr, 84%; 670 hr, >99.9%.

dation involves not only the slow buildup of leucomethylene blue but also the oxidation of leucomethylene blue to methylene blue through a mechanism itself subject to an induction period. Clarification here must await the results of a separate study of the autoxidation of leucomethylene blue.³

Figure 2 shows that, after the induction period, the first-order plot becomes a straight line. At 50° , where most of the experiments were conducted, good linearity was observed during the 15–210-hr period after the start of the run and over the 10–20 to 55–75% range of conversion of BLMB to methylene blue—*i.e.*, over about half the reaction course. All first-order rate constants for color production were derived from this part of the curves.

To obtain a point of reference, rate constants from eleven standard autoxidation runs were averaged. The standard run was considered to be one in which the temperature was 50° , air was the source of oxygen, and only BLMB and $0.5 M$ sulfuric acid were introduced into the reaction mixture. At the 95% confidence level, this average rate constant for color development is $30.0 (\pm 1.8) \times 10^{-7} \text{ sec}^{-1}$. Therefore, within experimental error, the rate constants for methylene blue formation and for BLMB hydrolysis ($29.3 \times 10^{-7} \text{ sec}^{-1}$) are identical. Equation 2, accordingly, is not acceptable since it has the rate-determining step for color formation residing in the oxidation of leucomethylene blue, a process which is distinct and differ-

(3) The autoxidation of leucomethylene blue has been studied, although under conditions considerably different from those used here.^{4–6} Ferrous as well as cupric ions in trace amounts can accelerate the process.

(4) T. F. Macrae, *Ber.*, **64**, 133 (1931); A. Schöberl, *ibid.*, **64**, 546 (1931).

(5) H. Wieland and A. Bertho, *Ann.*, **467**, 95 (1928); A. Reid, *Ber.*, **63**, 1920 (1930).

(6) W. M. Clark, B. Cohen, and H. D. Gibbs, *Chem. Abstr.*, **19**, 2770 (1924); *Chem. Zentr.*, **100 II**, 3153 (1929) [*Public Health Rept.*, **40**, 1311 (1925)].

(2) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1961, p 167.

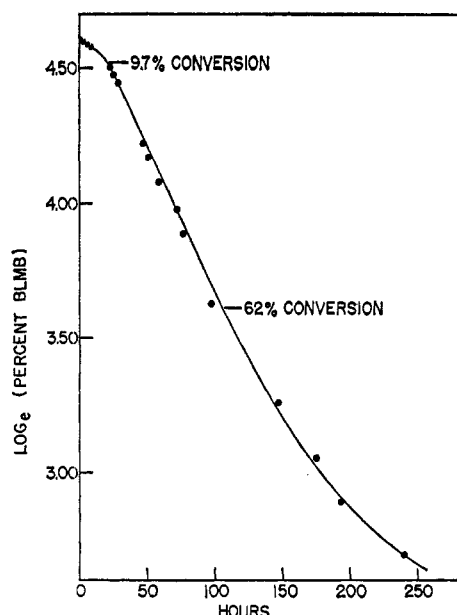


Figure 2.—First-order plot for methylene blue formation in the autoxidation of $1.02 \times 10^{-3} M$ BLMB in $0.50 M$ sulfuric acid at 50° . The ordinates in this representative plot refer to the per cent of BLMB remaining in the reaction mixture and are calculated from the expression $\log_e[100([\text{BLMB}]_0 - [\text{MB}])/[\text{BLMB}]_0]$. The curve is linear over the 9.7–62% range of conversion; $k_{av} = 30.0 \times 10^{-7} \text{ sec}^{-1}$.

ent from the hydrolysis of BLMB. Equation 3, with hydrolysis of BLMB as the rate-determining step, is consistent with the facts.

In the late stages of BLMB autoxidation runs, the measured rate of color production deviates from first-order kinetics (Figure 2). Also, the experimental points tend to scatter more here than before. We believe that this behavior reflects degradative changes in the methylene blue product. The shift in the absorption spectrum,^{7,8} the tendency for oxygen consumption to become excessive at longer reaction times, and the development of a colored body showing ether-solubility^{7,9,10} suggest that oxidative demethylation of methylene blue is taking place.

Whatever the details of the deterioration, the methylene blue product survives long enough in the 50° reaction mixtures to enable useful kinetic data to be derived from spectrophotometric measurements. At 39° , where the stoichiometry was determined, this kind of deterioration fortunately was small enough to be disregarded.

Effect of Reaction Parameters on the Rate of Methylene Blue Development.—Varying the BLMB concentration 40-fold (*i.e.*, from 0.0001 to 0.004 M) did not change the first-order rate constant; the maximum value was 30.3×10^{-7} , the minimum, $27.4 \times 10^{-7} \text{ sec}^{-1}$. Since all these experiments were performed at 50° , under air, and in $0.5 M$ sulfuric acid, the derived rate constants were included in the standard set of 11 experiments on which the average of $30 \times 10^{-7} \text{ sec}^{-1}$ is based (see above).

Although most of the kinetic runs were conducted in $0.5 M$ sulfuric acid, some exploratory work was done

with $1.5 M$ acid. The development of color was observed to follow the same general pattern. The first-order rate constant is approximately $80 \times 10^{-7} \text{ sec}^{-1}$. Thus, increasing acidity increases the rate of color production, a result consistent with BLMB hydrolysis as the slow step in the autoxidation.¹¹

With oxygen at a partial pressure of 150 mm (air), the first-order rate constant for methylene blue production is not dependent on oxygen pressure. Even in runs with concentrations of BLMB larger than usual, and so with heavier demands on the oxidant the rate constant was unchanged. When air was replaced by a mixture of two parts of nitrogen to three parts of oxygen, the rate constant did not change ($k_{50} = 27.4 \times 10^{-7} \text{ sec}^{-1}$). The same was true when pure oxygen was bubbled into the reaction mixtures at the start of the reaction and thereafter several times daily ($k_{50} = 28.6, 30.0, 29.6, 26.3,$ and $28.8 \times 10^{-7} \text{ sec}^{-1}$). Thus under the conditions chosen for the autoxidations, neither transfer of oxygen from the gas phase nor combination of oxygen with the organic substrate(s) is rate determining.

Most of the kinetic work was done at 50° . Higher temperatures were avoided so as to keep deterioration of methylene blue within bounds; lower temperatures gave inconveniently slow reactions. However, enough information was collected at 39° to indicate that the first-order rate constant at this lower temperature is approximately one-third as large as the value at 50° .

Further modifications in the reaction mixture were tried in an effort to uncover factors effecting the rate of methylene blue production. All the following runs were conducted in $0.5 M$ sulfuric acid at 50° with BLMB at an initial concentration close to $10^{-3} M$.

Using conductivity water in place of ordinary distilled water did not change the first-order rate constant ($30.1 \times 10^{-7} \text{ sec}^{-1}$). The presence of methylene blue ($6 \times 10^{-5} M$ under oxygen; $10 \times 10^{-5} M$ under air) at the start of autoxidation runs had little effect on the rate constant (27.2×10^{-7} and $28.9 \times 10^{-7} \text{ sec}^{-1}$) but did shorten the induction period somewhat. Benzoic acid in low or high concentrations had no effect on the rate constants ($27.2\text{--}31.7 \times 10^{-7} \text{ sec}^{-1}$) but did lengthen the induction period (*e.g.*, to 40 hr). Also, with benzoic acid present in $1.5 \times 10^{-3} M$ concentration, the first-order plot was linear over 18–81% of the reaction course. In further experimentation, an autoxidation mixture corresponding to 20% conversion to methylene blue was simulated by allowing $0.2 \times 10^{-3} M$ methylene blue chloride and $0.2 \times 10^{-3} M$ benzoic acid in $0.5 M$ sulfuric acid to stand at 50° for 10 days. To this mixture enough solid BLMB was added to furnish an $0.8 \times 10^{-3} M$ solution, and the autoxidation was allowed to run as usual. No noteworthy change occurred either in the induction period or in the linear portion ($k = 29.2 \times 10^{-7} \text{ sec}^{-1}$).

Sodium chloride in concentrations two to eight times that of BLMB gave unexceptional results ($k = 30.7, 30.8,$ and $33.1 \times 10^{-7} \text{ sec}^{-1}$). Mannitol as well as ethanol in $0.1 \times 10^{-3} M$ concentrations stretched out the induction period (to *ca.* 45 hr; 15% conversion) and also lengthened the linear portion of the log plots

(7) F. Kehrman, E. Havas, and E. Grandmougin, *Ber.*, **46**, 2131 (1913).

(8) N. Wotherspoon and G. Oster, *J. Am. Chem. Soc.*, **79**, 3992 (1957).

(9) Y. Usui, H. Obata, and M. Koizumi, *Bull. Chem. Soc. Japan*, **34**, 1049 (1961).

(10) H. Obata, *ibid.*, **34**, 1057 (1961).

(11) A decrease in the rate of acid-catalyzed amide hydrolyses has been observed with increasing acidity, but only when the sulfuric acid concentration becomes greater than $2.5\text{--}5 M$ [See M. L. Bender, *Chem. Rev.*, **60**, 53 (1960)].

(up to 77% conversion). The rate constants, however, were unchanged (28.6 and $29.1 \times 10^{-7} \text{ sec}^{-1}$).

Accordingly, whatever the effects of these modifications on the autoxidation reaction, they do not influence the rate-determining stage.

Behavior of Peroxides and Other Oxidants on Color Formation.—The possible involvement of hydrogen peroxide was probed by following an autoxidation run in which hydrogen peroxide was present in one-tenth the initial concentration of BLMB (10^{-4} vs. $10^{-3} M$). Figure 3, giving the first-order plot for methylene blue production, shows how the points taken in the first 4 hr fall on a straight line whose slope corresponds to a rate constant of $30.6 \times 10^{-7} \text{ sec}^{-1}$. During this time—when, with no added peroxide, approximately 0.6% conversion to methylene blue is realized and the induction period is very much in evidence—5% of the theoretical amount of methylene blue develops and no sign of induction is noted.¹² After this initial period, the rate drops off, but then increases until the curve again becomes linear. From this part of the curve (19–83% conversion), the rate constant was determined to be $26 \times 10^{-7} \text{ sec}^{-1}$, a value not significantly different from that of the standard runs. Actually, Figure 3 shows that, after the initial period, the plot resembles that of a standard run with no peroxide added.

The same kinds of results were obtained when $10^{-4} M$ potassium peroxydisulfate was used in place of hydrogen peroxide. For the first 6 hr, the first-order plot follows an induction-free straight line ($k = 32.0 \times 10^{-7} \text{ sec}^{-1}$); after the first 30 hr, the plot is again linear ($k = 28.6 \times 10^{-7} \text{ sec}^{-1}$).

These experiments offer no support to the possibility that either hydrogen peroxide or peroxydisulfuric acid participates in the autoxidation of BLMB.¹³ Although both reagents in low concentration develop methylene blue with no induction period, neither eliminates the induction period for autoxidation. Evidently, the set of intermediates operating in the air oxidation of leucomethylene blue to methylene blue does not coincide with the set of intermediates operating in the peroxide oxidation. The initial rates in the peroxide and peroxydisulfuric acid processes are the same as the rate of hydrolysis of BLMB, a feature suggesting that the peroxides, at least in low concentrations, react rapidly with leucomethylene blue but only very slowly with BLMB.

At higher concentrations, the peroxides behave quite differently. Thus, when $10^{-2} M$ hydrogen peroxide was taken with $10^{-3} M$ BLMB, methylene blue was formed with little if any induction period and with a rate two to three times greater than the hydrolysis of BLMB. The methylene blue concentration reached a maximum in the unusually short time of 47 hr and then just as rapidly fell off. A mixture of $10^{-2} M$

(12) In a duplicate experiment, the first six experimental points, well spaced over the first 7 hr, fell on a straight line corresponding to a rate constant of $30.0 \times 10^{-7} \text{ sec}^{-1}$. The conversion here after 7 hr was 7% compared with 1% (or less) in the runs without peroxide.

(13) This does not necessarily hold for autoxidations at different conditions. Thus, hydrogen peroxide appears to be involved either as product or intermediate in the autoxidation of leucomethylene blue at pH values close to neutrality.⁴ In this connection, leucomethylene blue as well as BLMB in benzene solution containing trifluoroacetic acid can be oxidized to methylene blue with a variety of organic peroxides [S. Sorge and K. Ueberreiter, *Angew. Chem.*, **68**, 486 (1956); M. I. Eiss and P. Giesscke, *Anal. Chem.*, **31**, 1558 (1959)].

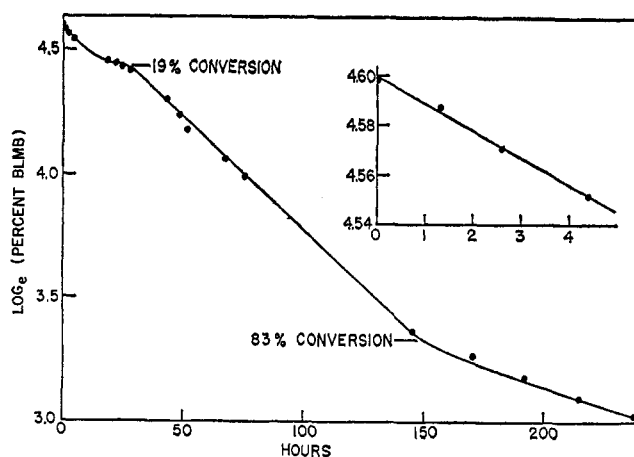


Figure 3.—First-order plot for BLMB air oxidation to methylene blue in the presence of small amounts of hydrogen peroxide. The reaction mixture consisted of $1.18 \times 10^{-3} M$ BLMB and $1.0 \times 10^{-4} M$ hydrogen peroxide in $0.50 M$ sulfuric acid at 50° . The insert expands the plot for the first 4 hr, where $k = 30.6 \times 10^{-7} \text{ sec}^{-1}$. The second linear portion corresponds to $k = 26 \times 10^{-7} \text{ sec}^{-1}$.

potassium peroxydisulfate with $10^{-3} M$ BLMB followed the same pattern, but in a more exaggerated manner. Approximately 66% of the theoretical amount of methylene blue appeared in 0.5 hr. Afterwards, the change was almost as rapid in the opposite direction and, after 4 hr, less than 4% of the theoretical amount of methylene blue remained.

Since the initial rates of color production are greater than the rate of BLMB hydrolysis, hydrolysis cannot be rate limiting. The peroxidic reagents at the higher concentrations must attack BLMB directly, possibly according to the sequence described in eq 4. Control experiments demonstrated that the loss of MB color in these runs can be accounted for by the instability of methylene blue to $10^{-2} M$ peroxides.

Both ceric ion and lead dioxide in $0.5 M$ sulfuric acid oxidize BLMB rapidly to methylene blue. Both reagents, however, attack the developed methylene blue, and, if the reagents are present in excess, the reaction mixtures can be completely bleached.

Bromine water develops color instantaneously, but the product is probably a brominated methylene blue instead of methylene blue itself.¹⁴

Treatment of $10^{-3} M$ BLMB in $0.05 M$ sulfuric acid with a few drops of 5% hydrogen peroxide produces very little methylene blue. However, addition of a few milligrams of ferrous chloride produces an immediate coloration.¹⁵

Judging from the rapid production of color, we conclude that ceric ion, lead dioxide, bromine, and ferrous ion–hydrogen peroxide—like peroxydisulfuric acid or hydrogen peroxide in excess—can attack BLMB directly and do not require preliminary hydrolysis to leucomethylene blue.

Autoxidation of *p*-Nitro- and *p*-Methoxybenzoyl-leucomethylene Blue.—The development of methylene blue by autoxidation of *p*-nitro- and *p*-methoxy-BLMB was studied. The reaction with the two derivatives followed the familiar pattern of an induc-

(14) Private communication from C. Adams, National Cash Register Co.

(15) Note that ferric chloride converts BLMB in alcohol solution to methylene blue [G. Cohen, *Ber.*, **33**, 1567 (1900)].

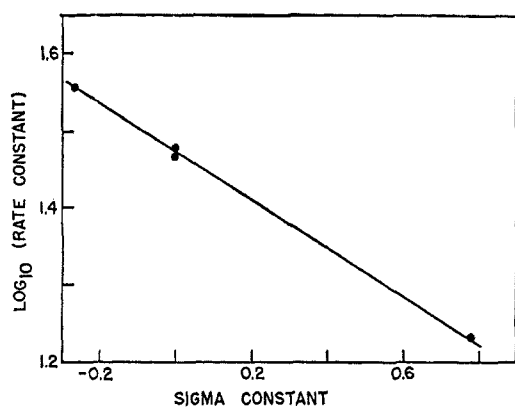


Figure 4.— σ - ρ plot for autoxidation of substituted BLMB compounds in 0.50 *M* sulfuric acid at 50°. The rate constants used here are as follows: *p*-methoxybenzoyl, 36×10^{-7} ; unsubstituted benzoyl, 30×10^{-7} ; and *p*-nitrobenzoyl, 17×10^{-7} sec^{-1} . The σ values for *p*-methoxy (-0.268) and *p*-nitro (0.778) are taken from Jaffé's compilation.¹⁷ The lower of the two points for unsubstituted BLMB refers to its directly measured rate of hydrolysis ($k_{50} = 29.3 \times 10^{-7}$ sec^{-1}).

tion period, a first-order region, and a terminal stage. The nitro compound showed a first-order rate constant of 17 ± 1 (standard derivation) $\times 10^{-7}$ sec^{-1} , and thus autoxidized more slowly than unsubstituted BLMB ($k = 30 \times 10^{-8}$ sec^{-1}). The methoxy compound showed a rate constant of 36 ± 3 (standard deviation) $\times 10^{-7}$ sec^{-1} , and thus autoxidized more rapidly than unsubstituted BLMB.

Figure 4 presents a Hammett plot for the rates and shows how the three values fall on a straight line, for which the slope, ρ , is -0.32. If hydrolysis of the BLMB compounds is accepted as the rate-determining step in the development of methylene blue, these rate constants are, in fact, rate constants for hydrolysis. Accordingly, the derived ρ value should also refer to hydrolysis. The closest analogy we could find for comparison deals with the acid-catalyzed hydrolysis of simple, ring-substituted benzamides in 60% ethanol,¹⁶ a process for which $\rho_{52.4}$ is -0.483.¹⁷ Significantly, the ρ constant for autoxidative color production agrees in sign and compares closely in magnitude with this ρ constant.

Conclusion

Equation 3 for color production can accommodate the data for BLMB as well as for the *p*-nitro and *p*-methoxy derivatives. Hydrolysis to leucomethylene blue (III) is a preliminary and rate-limiting step. The oxidation to methylene blue is distinct from, occurs after, and is faster than the hydrolysis.

Experimental Section¹⁸

Description of Compounds.—A sample of Harleco methylene blue chloride was crystallized twice from distilled water. The

(16) I. Meloche and K. J. Laidler, *J. Am. Chem. Soc.*, **73**, 1712 (1951). These authors point out that the earlier results [E. Reid, *Am. Chem. J.*, **21**, 284 (1899); **24**, 397 (1900)] with the same reaction in which water alone is solvent and for which $\rho_{100} = +0.119$ are questionable, since the reaction mixtures were heterogeneous.

(17) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).

(18) Elementary analyses were obtained from Massachusetts Institute of Technology Microchemical Laboratory, Cambridge, Mass., and from Schwarzkopf Microanalytical Laboratories, Woodside, N. Y. The radioactivity of BLMB samples was determined by New England Nuclear Corp., Boston, Mass.

blue crystals were first dried *in vacuo* over phosphorus pentoxide at room temperature and then allowed to equilibrate in air for several days.

Anal. Calcd for $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S} \cdot 5\text{H}_2\text{O}$: C, 46.88; H, 6.88; Cl, 8.65; N, 10.25; O, 19.51; S, 7.82; H_2O , 22.0. Found: C, 46.91, 46.96; H, 7.01, 6.98; Cl, 8.47, 8.51; N, 10.40, 10.30; O, 19.37, 19.34; S, 7.98, 8.13.

Methylene blue perchlorate was prepared from 5 g of methylene blue chloride dissolved in 100 ml of distilled water by warming and stirring. After filtration, the solution was treated with a small volume of aqueous 8 *M* sodium perchlorate. The precipitate was collected, washed thoroughly with cold water, and recrystallized twice from aqueous 0.01 *M* sodium perchlorate. The deep blue crystals of methylene blue perchlorate, washed thoroughly with cold water, were dried *in vacuo* at 80° over phosphorus pentoxide to constant weight.

Anal. Calcd for $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{O}_5\text{S}$: C, 50.06; H, 4.73; Cl, 9.23; N, 10.95; O, 16.77; S, 8.35. Found: C, 50.28, 50.22; H, 4.90, 4.98; Cl, 8.98, 9.12; N, 10.94, 10.74; O, 16.36, 16.37; S, 8.52, 8.45.

N-Benzoylleucomethylene blue was obtained by purifying material furnished by The National Cash Register Co. The pale green BLMB (10 g) was warmed with *ca.* 100 ml of pure acetonitrile. Care was taken to blanket the materials here and subsequently with nitrogen. Before filtration, the mixture was decolorized with a small portion of attapulgite clay. The cooled filtrate deposited faintly yellow crystals, which were collected and washed with cold acetonitrile. This procedure was repeated, and the pale yellow benzoylleucomethylene blue was dried at 80° *in vacuo* over phosphorus pentoxide. This material in a sealed capillary showed mp 194°. Benzoylleucomethylene blue has been reported before with mp 196–198° (cor)¹⁹ and 190.5–192°.¹⁰

Anal. Calcd for $\text{C}_{23}\text{H}_{23}\text{N}_3\text{O}_5\text{S}$: C, 70.91; H, 5.91; N, 10.79; S, 8.22. Found: C, 71.25; H, 5.99; N, 10.74; S, 8.24.

Long storage under nitrogen and away from light did not change the properties of material prepared in this way. According to spectroscopic assay, methylene blue was present in amounts corresponding to less than 0.02%.

p-Nitrobenzoylleucomethylene blue and *p*-methoxybenzoylleucomethylene blue were supplied by The National Cash Register Co. The *p*-nitro derivative was crystallized from acetonitrile as above and dried at 80°. The material was also dried directly at 100° without purification. The recrystallized material showed mp 243–243.5° in a sealed capillary.

The *p*-methoxy derivative was crystallized from acetonitrile and dried at 80°. The small, diamond-shaped, light yellow crystals, mp 173–177.5°, were stored under nitrogen. The discrepancy between the melting point observed here and that reported before (106–107°)¹⁶ was not resolved.

The three BLMB compounds were checked for homogeneity by thin layer chromatography, with silica gel plus 5% calcium sulfate as adsorbent and cyclohexane-ethyl acetate (3:2) as solvent. One or sometimes two very faint spots other than the main spot indicated the presence of persistent trace impurities.

Sulfuric acid reaction media were prepared from reagent-grade sulfuric acid (Baker Analyzed) and distilled water.

Benzoyl(carbonyl-¹⁴C)leucomethylene Blue.—A solution of methylene blue chloride (6.0 g; 0.019 mole) in 300 ml of warm water was shaken in a separatory funnel with a solution of sodium hydrosulfite dihydrate (14.0 g; 0.067 mole) in 300 ml of water. The dark blue color disappeared almost immediately, and a yellow flocculent solid formed on the surface. Addition of solid potassium carbonate made the mixture barely basic to litmus.

The leucomethylene blue was extracted into ether, and the solution was filtered through layers of diatomaceous earth (Celite), decolorizing carbon, and magnesium sulfate. The dry filtrate was concentrated and then cooled under a blanket of carbon dioxide while the leucomethylene blue crystallized as faintly yellow needles. The crystals were collected under a stream of carbon dioxide and stored in a vacuum desiccator.

A Grignard reagent was prepared under nitrogen from 1.06 g of methyl iodide (0.00743 mole), 0.18 g (0.00743 g-atom) of pure magnesium, and 35 ml of absolute ether. Leucomethylene blue (2.0 g or 0.0070 mole) was swirled with *ca.* 30 ml of absolute benzene in a dropping funnel, and the solution was added to the Grignard mixture over the course of 15 min. The dropping

(19) L. Chalkley, *J. Am. Chem. Soc.*, **47**, 2055 (1925).

funnel was rinsed with 25 ml of benzene, which was added to the reaction mixture. The dark blue-green solution was boiled under reflux for 1 hr.

A solution of benzoyl(carbonyl-¹⁴C) chloride (0.95 g; 0.00676 mole; 10 curie) in 10 ml of absolute ether was introduced. After a 4-hr period of boiling, the reaction mixture was allowed to stand overnight at room temperature.

The mixture was poured into a separatory funnel, with the solids adhering to the inside of the flask transferred with the help of 50–100 ml of 10% hydrochloric acid and a small volume of dimethylformamide. Ether (200 ml) was added, and then potassium carbonate until the mixture was basic to litmus. The mixture was extracted thoroughly with ether, which was dried with magnesium sulfate and then evaporated in a stream of nitrogen to give 2.12 g (80%) of BLMB. Recrystallizations from methanol plus benzene and from methanol alone gave 1.27 g of radioactive benzoylleucomethylene blue (48% based on benzoyl chloride).

Preliminary model runs according to these directions gave benzoylleucomethylene blue, mp 192–196°, in 77–86% yield.

A final purification of the radioactive material diluted with unlabeled material was effected by three crystallizations from acetonitrile. The almost colorless benzoylleucomethylene blue was radiochemically pure.

Radioactivity assay: after the second crystallization, 13.5×10^6 and 13.4×10^6 dps/g; after the third crystallization, 13.2×10^6 , 13.0×10^6 , 13.8×10^6 , and 13.1×10^6 average, 13.3×10^6 dps/g.

Spectrophotometric Measure of Methylene Blue.—Absorbance values were determined for 1-cm thicknesses with the help of a Bausch and Lomb Model 505 spectrophotometer (double beam; recording). Absorption was taken at the 668-m μ maximum with 0.50 M sulfuric acid as solvent. Experiments in which absorbance was determined at different concentrations of methylene blue (0.26×10^{-5} to 1.58×10^{-5} M) showed deviations from Beer's law.²⁰ An empirical correction was applied to the observed absorbances to obtain absorbance values directly proportional to the concentration of methylene blue. The molar extinction coefficient used in this work was determined with 7.49×10^{-6} M methylene blue perchlorate in 0.500 M sulfuric acid at 668 m μ (ϵ 6.69 $\times 10^4$) (0.9% maximum deviation).

Products from the Autoxidation of Benzoylleucomethylene Blue.—A solution of 0.780 g (2.00×10^{-3} mole) of benzoylleucomethylene blue in 2 l. of 0.45 M sulfuric acid was kept at 39° in the dark for 25 days. The concentration of methylene blue (0.82×10^{-3} M), determined spectrophotometrically, indicated a conversion of 82%.

Benzoic acid was isolated by extraction with ether. After removal of solvent, the residue was crystallized three times from water (including a treatment with decolorizing carbon) to give white crystals, melting alone or when mixed with authentic benzoic acid at 120–121°.

The acid solution after removal of benzoic acid was neutralized with solid sodium bicarbonate. The perchlorate of methylene blue was precipitated by adding 5 ml of concentrated perchloric acid and stirring for 20 min. The dark perchlorate salt was washed thoroughly on the funnel with water and then with alcohol-ether. The weight of perchlorate (0.54 g), dried at 110° for 1 hr, corresponded to a 70% conversion of benzoylleucomethylene blue to methylene blue. A spectral check of identity was made by comparing the absorption spectra of this autoxidation product in 0.035, 0.35, and 1.0 M sulfuric acid with those of authentic methylene blue perchlorate in the same solvents. At the same acidity, the absorption curves were practically superposable from 220 to 850 m μ .

After crystallization from 0.01 M aqueous sodium perchlorate, the perchlorate from the autoxidation was washed thoroughly with water and dried *in vacuo* over phosphorus pentoxide at room temperature.

Anal. Calcd for C₁₆H₁₈ClO₄S: C, 50.06; H, 4.73; Cl, 9.23; N, 10.95; S, 8.35. Found: C, 50.09, 50.11; H, 4.88, 4.80; Cl, 9.22; N, 10.79, 11.09; S, 8.05.

Stoichiometry.—To minimize complications due to the deterioration of methylene blue, the stoichiometry experiments were conducted at 39° instead of at 50° (where the kinetic runs were made).

A. Relation between BLMB and Methylene Blue.—Solutions of BLMB in aqueous sulfuric acid were allowed to stand in a 39° water bath under an atmosphere containing excess oxygen. Generally 50 ml of solution was taken in a 100-ml volumetric flask, which was painted black or covered with aluminum foil. Aliquots were removed at intervals and assayed spectrophotometrically for methylene blue. Account was taken here of the effect of varying acidity on the spectrum of methylene blue. The concentration of methylene blue became constant after 340 hr when 1 M sulfuric acid was used and after 400–500 hr when 0.5 M sulfuric acid was used. The results of seven experiments were combined to give a BLMB:MB ratio of $1.00 \pm 2\%$ (standard deviation).

B. Relation between Oxygen Uptake and Methylene Blue Formation.—Oxygen absorption measurements were made manometrically in what is essentially a Warburg apparatus. The reaction mixture, 1.080 l. of 1.85×10^{-3} M BLMB dissolved in 1.15 M sulfuric acid, was maintained in the dark at 39° under an atmosphere of air enriched with oxygen. At each pressure measurement, a small aliquot of the reaction mixture was withdrawn for the spectrophotometric determination of methylene blue. The average value of the ratio of cumulative oxygen consumed to methylene blue formed was $0.48 \pm 5\%$ (standard deviation).

Hydrolysis and Autoxidation Experiments with Labeled Benzoylleucomethylene Blue.—Clean air was bubbled for 2 min through 250 ml of a solution of radioactive benzoylleucomethylene blue (0.001–0.006 M) in a 0.500 M sulfuric acid. The loosely stoppered flask, wrapped in aluminum foil, was then placed in a water bath at $50.0 \pm 0.1^\circ$. Three-milliliter samples were removed at intervals and analyzed either radiochemically for the benzoylleucomethylene blue remaining or spectrophotometrically for the methylene blue developed.

For the radiochemical analysis, the 3-ml sample was mixed with 0.500 M sulfuric acid into which a known amount (0.3–0.6 g) of pure benzoylleucomethylene blue had been dissolved just before use. Then solid sodium bicarbonate (*ca.* 1.3 g/10 ml of solution) was added with cooling and as rapidly as possible. The neutral mixture was extracted without delay with benzene. One rinse of the combined extracts with 20 ml of distilled water removed practically all the blue color. The benzene solution, after drying with magnesium sulfate, was evaporated, and the dry residual benzoylleucomethylene blue was recrystallized three times from acetonitrile. Samples from the second and third crystallizations were submitted for assay, which was done by liquid scintillation counting in toluene and reported as disintegrations per second per gram. The molar concentration of BLMB in the 3-ml sample was then calculated by the usual isotope dilution expressions.

For the experiments in which BLMB was hydrolyzed in the absence of air, a Kjeldahl flask (*ca.* 80 ml in volume) with a long neck fitted with a ground-glass joint served as the reaction vessel. The flask, containing radioactive BLMB dissolved in about 50 ml of 0.500 M sulfuric acid, was attached to the vacuum manifold and the contents were frozen at solid carbon dioxide-acetone temperatures. With the flask evacuated to 2–4 μ , the stopcock to the line was closed, and the reaction mixture stirred magnetically at room temperature. The degassing procedure was repeated, after which process the flask was cooled, evacuated, and sealed with a torch. The mixture was kept in the 50° water bath away from light for the desired period.

As soon as the flask was opened, samples were removed for methylene blue and benzoylleucomethylene blue determination. Then, after air had been bubbled into the mixture, it was treated as described above. In these experiments, the start of autoxidative development of methylene blue was taken as the time the flask was cut open. Figure 1 presents a plot of methylene blue developed in the BLMB solutions after preliminary periods of anaerobic hydrolysis.

Methylene Blue Development from BLMB.—Approximately 100 ml of a freshly prepared solution of BLMB in 0.500 M sulfuric acid was added to a volumetric flask. Air that had been passed successively through concentrated sulfuric acid, soda

(20) The experimental molar extinction coefficients at the 668-m μ maximum decreased with increasing concentration. This was true not only in 0.5 M but also in 0.1 M sulfuric acid. Others have reported the failure of methylene blue to follow Beer's law. Thus *cf.* G. N. Lewis, O. Goldschmidt, T. T. Magel, and J. Bigeleisen, *J. Am. Chem. Soc.*, **65**, 1150 (1943); D. Hayon, G. Scholes, and J. Weiss, *J. Chem. Soc.*, 301 (1957); L. Michaelis and S. Granick, *J. Am. Chem. Soc.*, **67**, 1212 (1945); E. Rabinowitch and L. F. Epstein, *ibid.*, **68**, 69 (1941); M. Schubert and A. Levine, *ibid.*, **77**, 4197 (1955); and G. Holst, *Z. Physik. Chem.*, **A175**, 99 (1935). Interestingly, G. N. Lewis and J. Bigeleisen found that both methylene blue and its monoprotonated form obey Beer's law [*J. Am. Chem. Soc.*, **65**, 1144 (1943)].

lime, concentrated sulfuric acid, and water was then bubbled through the BLMB reaction mixture for 2 min. The flask, carefully covered with aluminum foil, was mounted deep in the water bath at $50.0 \pm 0.1^\circ$. Aliquots were removed at intervals and diluted with 0.500 *M* sulfuric acid to bring the measured absorbance into the 0.3–0.8 range, where the absorbance-to-concentration dependence was known. The absorbance was determined without delay.

After the first-order logarithmic plot was made, the set of points falling on the straight-line part of the curve was determined, and the slope was found by a least-squares calculation.

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Kinetics and Substituent Effects in Electrophilic Aromatic Substitution. II.¹ Tritylation of Catechol and its Monoethers^{2,3}

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The kinetics of the reaction of trityl perchlorate with catechol, guaiacol, 2-ethoxyphenol, and 2-isopropoxyphenol were followed dilatometrically in nitromethane solvent. Substitution took place by a bimolecular mechanism always at the *para* position relative to a hydroxy group. The monoethers showed an inductive order of activation: $\text{OCH}_3 < \text{OC}_2\text{H}_5 < \text{OCH}(\text{CH}_3)_2$. The rate of reaction of catechol was always larger than that of guaiacol but comparable with those of the other two ethers. The hydroxyl group has the ability of forming hydrogen bonds both with the solvent and the adjacent group; thus, in spite of not being the most activating when it is alone in the benzene ring (see part I), it can acquire the orientational control of the process. Catechol is faster than guaiacol probably because it can form two hydrogen bonds. Intramolecular hydrogen bonds are believed to be extensive in this series and to constitute the main factor in the orientational control. When no hydrogen bonding is possible, as in the case of 1-ethoxy-2-methoxybenzene, the reaction yields a mixture of the compounds tritylated at positions 4 and 5.

In part I¹ the behavior of phenol and some of its alkyl ethers was studied kinetically on tritylation with trityl perchlorate. In the present work catechol and some of its alkyl ethers were subject to the same reaction and under the same conditions, in order to estimate kinetically both the activation caused by the introduction of two "activating" groups toward electrophilic substitution, and the competition among these groups for orientational control of the process.

The tritylation reaction with trityl perchlorate in nitromethane presented several advantages and disadvantages for the kinetic study of aromatic substitution, which were mentioned and reviewed before.¹ Here it will be added that catechol,⁴ guaiacol,⁵ and veratrole⁵ were tritylated by other authors with triphenylmethanol in the presence of sulfuric acid, according to the Baeyer–Villiger method,⁶ and in all three substitution took place at position 4.^{7,8}

Results

The rate constants *k* in Table I represent the average values of second-order initial rate constants,

(1) Part I: G. Chuchani, H. Díaz, and J. Zabicky, *J. Org. Chem.*, **31**, 1573 (1966).

(2) Presented in part at the Third Caribbean Chemical Symposium, Caracas, Jan 1965.

(3) Abstracted in N. Barroeta, H. Díaz, G. Chuchani, and J. Zabicky, *Acta Cient. Venezolana*, **15**, 248 (1964).

(4) T. Zincke and E. Wugk, *Ann. Chem.*, **363**, 284 (1908).

(5) D. V. N. Hardy, *J. Chem. Soc.*, **1929**, 1000.

(6) A. Baeyer and V. Villiger, *Chem. Ber.*, **35**, 3013 (1902).

(7) Hardy⁵ reports a mixture of 4- and 5-tritylated guaiacols under similar conditions. Chuchani⁵ prepared the 4 isomer by hydrolysis of the diazonium salt of 4-amino-3-methoxytetraphenylmethane and found it to be identical with the Baeyer–Villiger derivative of guaiacol. This was also found in the present work.

(8) A chemical proof of the position of the trityl group can be deduced from ref 5 and the following papers: F. Sachs and R. Thonet, *Chem. Ber.*, **37**, 3327 (1904); M. Gomberg and G. C. Forrester, *J. Am. Chem. Soc.*, **47**, 2379 (1925); T. R. Lea and R. R. Robinson, *J. Chem. Soc.*, 2351 (1926); G. Chuchani, *ibid.*, 1753 (1959).

TABLE I

SECOND-ORDER INITIAL RATE CONSTANTS,^a *k*, OF THE REACTION OF TRITYL PERCHLORATE WITH AROMATIC SUBSTRATES OF THE TYPE *o*-C₆H₄(OH)X, IN NITROMETHANE SOLVENT

X	30°	40°	50°	60°
OH	0.1332	0.3439	0.5185	1.0080
OCH ₃	0.0901	0.2381	0.3992	0.8084
OC ₂ H ₅	0.1164	0.2772	0.5615	1.0801
OCH(CH ₃) ₂	0.1492	0.3199	0.5569	<i>b</i>

^a Units of *k*, liters mole⁻¹ hour⁻¹, estimated from *k*_{dilatometric} values in this table. ^b Could not be determined owing to decomposition.

of 9–16 runs, carried out on mixtures in which both reagents were present in concentrations ranging from 0.02 to 0.08 *M*. Being the experimental values "dilatometric rate constants," in units of milliliters liters mole⁻² hour⁻¹, these had to be divided by 18.69 ml mole⁻¹, which was the average contraction found for the system when 1 mole reacted. The estimates of *k* allow one to compare the results in this work with those in part I. The kinetic results were sufficiently accurate (about 6% standard deviation) as to enable the appreciation of substituent effects, but not of Arrhenius activation energies, which are shown in Table II. In this table the relative rates of phenol¹ and the substrates studied here compared with that of guaiacol are also shown.

1-Ethoxy-2-methoxybenzene was tritylated under Baeyer–Villiger conditions⁶ yielding a mixture of products tritylated at positions 4 and 5.

The tritylation product of 2-ethoxyphenol, with trityl perchlorate in nitromethane or under Baeyer–Villiger conditions, was shown to be 3-ethoxy-4-hydroxytetraphenylmethane, both from its infrared spectrum in the 800–900-cm⁻¹ region showing absorption bands typical