After careful filtration, the saturated solutions *containing* NaLS were diluted with the original solvent (0.01 *M* NaLS, *p*H 10.2) until the optical density at the maximum of the visible absorption band was accurately measurable. In equation 10, this optical density will be denoted by *OD'*, and the dilution factor by *f*. To the filtered saturated solutions *not* containing NaLS, enough solid NaLS was added to produce a medium 0.01 *M* in detergent, in which a measurable fraction of the carbinol was converted to the dye. The optical density, *OD''*, was measured at the maximum of the visible absorption band of R^+ after equilibrium between ROH and R^+ had been established. The solubility ratio, $S_{ROH}^{\ominus}/S_{ROH}$, may then be computed from equation 10.

$$\frac{S_{ROH}^{\ominus}}{S_{ROH}} = \frac{(OD')/f}{(OD'')} \times \frac{K^{\ominus}[OH^-]}{1 - K^{\ominus}[OH^-]} \qquad (10)$$

Values of $K^{\ominus}$ were taken from Table I. The final results reported in Table II are average values based on 11 experiments. The accuracy is believed to be within a factor of two.

Acknowledgment.—It is a pleasure to acknowledge helpful discussions with Professor H. M. Walborsky.

TALLAHASSEE, FLA.

[CONTRIBUTION FROM THE GENERAL ELECTRIC RESEARCH LABORATORY]

A Study of the Mechanism of Cumene Autoxidation. Mechanism of the Interaction of *t*-Peroxy Radicals[1]

BY HARRY S. BLANCHARD

RECEIVED FEBRUARY 13, 1959

The liquid phase autoxidation of cumene has been investigated at 60° in the presence of α,α'-azodiisobutyronitrile. The rates of oxidation and yields of hydroperoxide were determined as functions of the kinetic chain lengths. Moreover, acetophenone was identified as a primary oxidation product and its yield was also determined as a function of the kinetic chain lengths. The results of the study support the idea that cumylperoxy radicals undergo a *non-terminating* interaction resulting in acetophenone and methyl radicals. The major termination product of cumene autoxidation is di-α-cumyl peroxide. The formation of acetophenone as well as the formation of di-α-cumyl peroxide support the idea that cumyloxy radicals result from the non-terminating interaction of cumylperoxy radicals. However, the fact that little α,α-dimethylbenzyl alcohol is formed indicates that if cumyloxy radicals are present, they behave differently from cumyloxy radicals resulting from the decomposition of di-α-cumyl peroxide at higher temperatures. Alternatively, both the terminating interaction resulting in di-α-cumyl peroxide as well as the non-terminating interaction resulting in acetophenone may come about *via* a common intermediate or transition state involving cumylperoxy radicals directly.

The mechanism of autoxidation of hydrocarbons has received considerable attention in recent years[2]; yet, certain aspects are still incompletely understood. In general, much of the work has been devoted to kinetic investigations, resulting in an understanding of the kinetically important propagation reaction but leading to little understanding of some other aspects of the mechanism. In particular, such kinetic studies yield little information regarding the termination reaction of autoxidation. The purpose of this paper is to present results which have a bearing on the interaction of cumylperoxy radicals. From these results, along with the over-all kinetics of autoxidation, it is now possible to write a fairly complete mechanism for the autoxidation of cumene.

Results

Autoxidations of cumene were performed in the liquid phase at 60° in the presence of α,α'-azodiisobutyronitrile (AIBN). Under the conditions of the experiments the rates of oxygen absorption were independent of the oxygen pressure (200–760 mm.) thus indicating that only oxygenated radicals were involved in the termination reaction.[2]

The mechanism of autoxidation is now generally formulated as

$$(CH_3)_2C(CN)-N=N-C(CN)(CH_3)_2 \xrightarrow{k_i} 2eR'\cdot + N_2$$

$$R'\cdot + O_2 \longrightarrow R'OO\cdot$$

$$R'OO\cdot + RH \longrightarrow R'OOH + R\cdot$$

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$ROO\cdot + RH \xrightarrow{k_p} ROOH + R\cdot$$

$$2\ ROO\cdot \xrightarrow{k_t} \text{non-radical products} + O_2$$

Application of the usual steady-state treatment to this scheme results in the expression

$$-d[O_2]/dt = k_p[RH](R_i)^{1/2}/(2k_t)^{1/2} + R_i/2 \qquad (1)$$

in which, R_i, the rate of initiation, $= 2ek_i[AIBN]$. In the present work, R_i has been calculated to be 1.32×10^{-6} mole $l.^{-1}$ $sec.^{-1}$ for 0.097 *M* AIBN based on a value of 1.15×10^{-5} $sec.^{-1}$ for k_i at 60° and a value of 0.60 for *e*. The value for k_i was selected from a compilation of various literature values[3] while the value of *e* appears established by the work of Hammond, Sen and Boozer.[4] The constancy of the data in column 4 of Table I show that (1) is obeyed rigorously in the autoxidation of cumene in chlorobenzene solution as well as in pure cumene itself.

Such a kinetic treatment provides assurance that the broad general features of the above mechanism are correct for cumene autoxidation. However, this treatment gives no insight into the termination reaction other than the fact that termination takes place in a bimolecular process involving two oxygenated radicals. Such insight can be gained from a more detailed study of the products of the reaction.

(1) Presented before the Division of Organic Chemistry at the 132nd Meeting of the American Chemical Society, New York, N. Y., September, 1957.

(2) J. L. Bolland, *Quart. Revs.*, **3**, 1 (1949); L. Bateman, *ibid.*, **8**, 147 (1954).

(3) See footnote 3 in ref. 9 for the data used.

(4) G. S. Hammond, J. N. Sen and C. E. Boozer, THIS JOURNAL, **77**, 3244 (1955).

TABLE I

OXIDATION OF CUMENE AT 60° IN CHLOROBENZENE SOLUTION IN THE PRESENCE OF α,α'-AZODIISOBUTYRONITRILE (0.097 M)

[Cumene], mole l.$^{-1}$	Kinetic chain length[a]	Rate,[b] mole l.$^{-1}$ sec.$^{-1}$ × 10^5	Rate = $R_i/2/(RH)[AIBN]^{1/2}$ × 10^5	Yield of products[c] Hydroperoxide	Dialkyl peroxide	Acetophenone
0.50	2.3	0.25	1.25	60	18.5	11.3
0.85	3.0	.39	1.22	70	13.3	10.0
1.05	3.6	.48	1.26	75	12.5	9.4
1.75	5.7	.76	1.25	85	..	7.1
2.10	6.7	.88	1.22	87	..	6.0
2.80	8.9	1.18	1.25	90	..	4.4
3.50	10.9	1.44	1.23	91	..	3.5
4.20	12.9	1.70	1.23	92	..	2.7
5.25	15.6	2.06	1.21	94	..	2.2
6.90[d]	20.0	2.68	1.20	96	..	..
6.90[d,e]	29.0	1.90	1.21	97	..	..
6.90[d,f]	66.0	0.86	1.24	97	..	..

[a] See text for definition. [b] Corrected for evolution of nitrogen from the AIBN. [c] Calculated as per cent. of oxygen absorbed. [d] Pure cumene. [e] 0.05 M AIBN. [f] 0.01 M AIBN.

It has long been known that the major oxidation product from cumene is cumene hydroperoxide.[5] However, the reported oxidations were usually carried out in pure cumene, precisely the conditions favoring a high yield of hydroperoxide since the oxidation chains are long. In the present study, the yields of hydroperoxides were determined as a function of the kinetic chain length, which is defined as the number of molecules of oxygen absorbed per initiating radical, *i.e.*

$$\text{kinetic chain length} = -\mathrm{d}[O_2]/\mathrm{d}t/R_i$$

Since the yields of hydroperoxide reported in Table I were surprisingly low at short kinetic chain lengths, and since independent experiments showed that cumene hydroperoxide was completely stable under the experimental conditions, a search was made for other oxidation products.

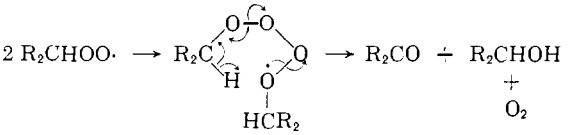
The infrared spectra of the oxidates showed a carbonyl absorption at 5.9 μ which is the position for the carbonyl absorption in acetophenone. That the carbonyl component was indeed acetophenone was shown by isolation and identification as its DNP derivative. Moreover, the yield of acetophenone (Table I) also was found to be a function of the kinetic chain length thus indicating that it too is a primary oxidation product. The acetophenone was detectable even in the oxidations conducted in pure cumene, although the intensity of its absorption was too small for accurate measurement.

Considerable effort was expended in searching for other oxidation products *via* vapor phase chromatography. By this technique, it was learned that the oxidates contained at least one other component in addition to hydroperoxide and acetophenone. Comparison of the chromatograms of the oxidates with those containing known compounds showed that both α-methylstyrene and α,α-dimethylbenzyl methyl ether were absent. It was not possible to positively identify the other component but some evidence was obtained that this component was, in fact, α,α-dimethylbenzyl alcohol. This was shown by adding hexamethyldisilazane to the oxidates[6] and comparing the resulting chromatograms to known mixtures of the disilazane and the carbinol. The important point which was established unequivocally was that no more than 7–8% of the absorbed oxygen could be accounted for as α,α-dimethylbenzyl alcohol.

Finally, some of the oxidates were subjected to total peroxide assays utilizing the technique of Vaughan[7] and co-workers as modified by Hercules Powder Co., Inc.[8] These analyses were complicated by the fact that the oxidates still contained undecomposed AIBN which would react with the iodine formed under the conditions of the experiment. By utilizing known mixtures of di-α-cumyl peroxide and cumene hydroperoxide, it was found that this complication could be circumvented by heating the oxidates to 110° for approximately 3–5 minutes prior to the analysis, and thus completely decomposing the residual AIBN. In this manner, it was unequivocally established that the oxidates contained dialkyl peroxide as well as hydroperoxide. Thus, in a typical analysis of an oxidate resulting from the absorption of 4.86×10^{-4} mole of oxygen under such conditions that the kinetic chain length was 3, it was found that 3.4×10^{-4} mole (70%) of the oxygen was accounted for as hydroperoxide, 0.65×10^{-4} mole (13.3%) of the oxygen was accounted for as dialkyl peroxide and 0.49×10^{-4} mole (10%) of the oxygen was accounted for as acetophenone. Thus, 93.2% of the total oxygen absorbed was accounted for by direct analysis. The remainder, 6.8%, was presumably present as α,α-dimethylbenzyl alcohol.

Discussion

Recently, Russell[9] demonstrated convincingly that *sec*-peroxy radicals undergo the termination reaction



As pointed out by Russell, it seems likely that this termination process is also general for *prim*-peroxy radicals. Moreover, Russell[10] previously had shown that many *prim*- and *sec*-peroxy radicals terminate much more readily than do *t*-peroxy radicals. Obviously, *t*-peroxy radicals cannot undergo the above termination reaction since they have no α-hydrogens. Furthermore, the fact that α,α-dimethylbenzyl methyl ether is not found among the oxidation products eliminates the somewhat unlikely possibility that a methyl group is transferred in a manner analogous to the hydrogen transfer in the above reaction.

In the past, the most common proposal regarding the termination reaction of *t*-peroxy radicals has

(5) H. Hock and S. Lang, *Ber.*, **77**, 257 (1944).

(6) S. H. Lange, S. Connell and I. Wender, *J. Org. Chem.*, **23**, 50 (1958).

(7) F. H. Dickey, J. H. Raley, F. F. Rust, R. S. Tresedor and W. E. Vaughan, *Ind. Eng. Chem.*, **41**, 1673 (1949).

(8) Hercules Powder Co., Inc., Wilmington, Del., Bulletin D 31.5-1.

(9) G. A. Russell, THIS JOURNAL, **79**, 3781 (1957).

(10) G. A. Russell, *ibid.*, **77**, 4583 (1955); **78**, 1047 (1956).

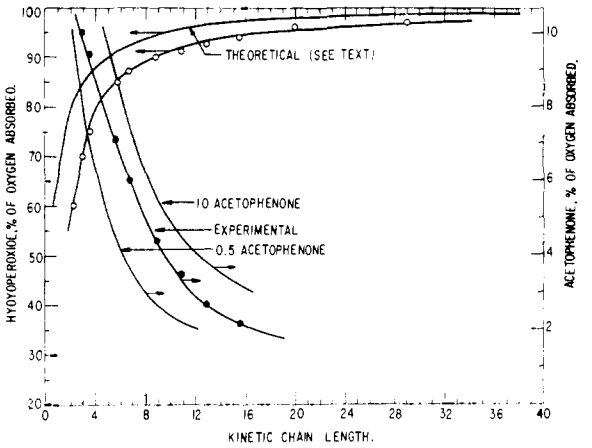


Fig. 1.—Percentage of oxygen absorbed found as hydroperoxide or acetophenone as a function of the kinetic chain length.

been the dimerization reaction

$$2C_6H_5C(CH_3)_2OO\cdot \longrightarrow C_6H_5C(CH_3)_2OO(CH_3)_2CC_6H_5 + O_2$$

Although this reaction has been proposed a number of times in the literature, experimental evidence for its occurrence never has been brought forth.

More recently, Boozer, *et al.*,[11] studied the autoxidation of cumene and of two β-deuteriocumenes. From the results they concluded that the termination reaction for cumylperoxy radicals involves the breaking of a β-carbon–hydrogen bond and, therefore, should be formulated

$$2C_6H_5C(CH_3)_2OO\cdot \longrightarrow C_6H_5C(CH_3)_2OOH + C_6H_5(CH_3)=CH_2 + O_2$$

If cumylperoxy radicals undergo this termination reaction, all of the oxygen absorbed will be fixed as hydroperoxide, resulting in a quantitative yield, regardless of the kinetic chain length. As shown in Fig. 1, such is not the case. Rather, it is seen that the yield of hydroperoxide is a function of the number of molecules of oxygen absorbed per termination reaction (*i.e.*, per two kinetic chains). Thus, at a kinetic chain length of 3, the yield of hydroperoxide is only 70%. Moreover, the experimental curve consistently lies below the theoretical curve calculated on the basis that hydroperoxide is formed in the initiation and propagation reactions and that one molecule of oxygen entered into non-hydroperoxidic products in the termination reaction. Furthermore, as shown in Fig. 1, the yield of acetophenone is also a function of the kinetic chain length, thus identifying it as a primary oxidation product. It might be argued that the acetophenone could arise by autoxidation of the α-methylstyrene formed in the above proposed termination reaction. Indeed, from the work of Mayo and Miller,[12] it is known that acetophenone

$$C_6H_5C(CH_3)=CH_2 + O_2 \longrightarrow C_6H_5COCH_3 + CH_2O$$

is an autoxidation product of α-methylstyrene. This possibility, however, can be eliminated on two grounds. First, if the acetophenone arises from autoxidation of α-methylstyrene, then the yields

(11) C. E. Boozer, B. W. Ponder, J. C. Trisler and C. E. Wrightman, THIS JOURNAL, **78**, 1506 (1956).

(12) F. R. Mayo and A. A. Miller, *ibid.*, **80**, 2480 (1958).

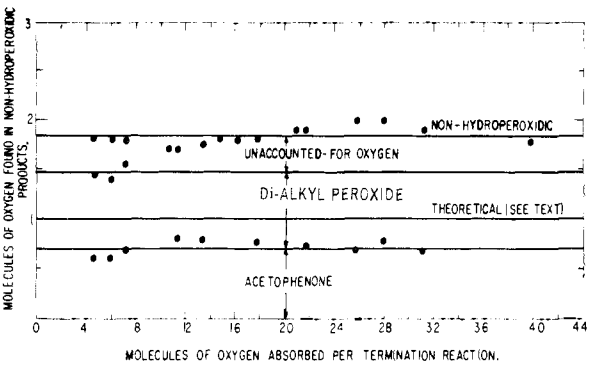


Fig. 2.—Molecules of oxygen present in non-hydroperoxide products per termination reaction in the autoxidation of cumene.

of hydroperoxide should be even higher than the theoretical curve of Fig. 1. For example, at a kinetic chain length of three, seven molecules of oxygen would be absorbed per termination reaction, of which six, or 85.8%, would be present as hydroperoxide. Secondly, only one molecule of acetophenone per termination reaction would be expected, since only one molecule of α-methylstyrene is formed per termination reaction. Actually, 1.4 molecules (Fig. 1) of acetophenone per termination reaction result in the autoxidation of cumene. Thus it is clear that autoxidation of α-methylstyrene cannot account for all of the acetophenone and in view of the yields of hydroperoxide seems unlikely as a source of any of it.

In Fig. 2, the total amount of oxygen fixed in non-hydroperoxidic products, as calculated from the yields of hydroperoxide, are seen to group around the average value of 1.85 molecules per termination reaction regardless of the kinetic chain lengths. Such a situation can arise only under steady-state conditions and suggests that all of the non-hydroperoxidic material arises from a common intermediate. If cumylperoxy radicals interacted cleanly to give non-radical, non-hydroperoxidic products in the termination reaction, then 1 molecule of oxygen per termination reaction would be fixed in non-hydroperoxidic products. The fact that a constant value of 0.73 molecule of oxygen per termination reaction is accounted for as acetophenone regardless of the kinetic chain lengths suggests that the common intermediate is cumyloxy radicals resulting from a *non-terminating* interaction of cumylperoxy radicals.

$$2C_6H_5C(CH_3)_2OO\cdot \longrightarrow 2C_6H_5C(CH_3)_2O\cdot + O_2$$

From the work of Kharasch[13] and co-workers as well as that of Vaughan[14] and co-workers, it would be expected that the cumyloxy radicals would undergo the reactions shown, both of

$$C_6H_5C(CH_3)_2O\cdot \xrightarrow{RH} \begin{cases} C_6H_5C(CH_3)_2OH + R\cdot \\ C_6H_5COCH_3 + CH_3\cdot \end{cases}$$

which will serve as propagation reactions since

(13) M. S. Kharasch, A. Fono and W. Nudenberg, *J. Org. Chem.*, **16**, 105 (1951).

(14) E. R. Bell, J. H. Raley, F. F. Rust, F. H. Seubold and W. E. Vaughan, *Disc. Faraday Soc.*, **10**, 242 (1951); F. H. Seubold, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **73**, 18 (1951).

both produce radicals. However, under the conditions of the oxidations, a third reaction path, namely, dimerization, is also available to the cumyloxy radicals since di-α-cumyl peroxide is completely stable at 60°. This reaction results in ter-

$$2C_6H_5C(CH_3)_2O\cdot \longrightarrow C_6H_5C(CH_3)_2OO(CH_3)_2CC_6H_5$$

mination and apparently represents the major termination process in the autoxidation of cumene. As shown in Fig. 2, based on the analyses at short kinetic chain lengths, 0.8 molecule of oxygen per termination reaction is accounted for as dialkyl peroxide. Considering the difficulties inherent in the analyses, this must be considered excellent agreement with the expected value of one molecule of oxygen per termination reaction based on the assumption that *all* chain termination results in di-alkyl peroxide.

The fact that α,α-dimethylbenzyl alcohol is apparently formed in very small amounts is puzzling since the work of Kharasch[13] and co-workers as well as that of Bailey and Godin[15] has shown that when di-α-cumyl peroxide is decomposed in the presence of a good hydrogen donor such as cumene, appreciable quantities of the alcohol are formed along with the acetophenone. Moreover, the formation of alcohol is favored at lower temperatures. Thus, at 140° the ratio of acetophenone to α,α-dimethylbenzyl alcohol is 1.40 decreasing to 0.68 at 112°. In view of this, it would be expected that alcohol formation would be highly favored at 60° whereas ketone formation is actually predominant. The explanation of this apparent discrepancy may be that it is unfair to compare cumyloxy radicals derived from cumylperoxy radicals with those derived from di-α-cumyl peroxide since Kharasch[13] has further shown that the ketone–alcohol ratio is also dependent on the source of the cumyloxy radicals as well as on the temperature. For example, the ratio of acetophenone to α,α-dimethylbenzyl alcohol at 140° is 3.00 in the decomposition of *t*-butyl cumyl peroxide in cumene while the same ratio is only 1.40 from the decomposition of di-α-cumyl peroxide under the same conditions.

A more plausible explanation might be that cumylperoxy radicals interact to form a transition state or unstable intermediate similar to that postulated by Russell[9] for *sec*-peroxy radicals.

$$2C_6H_5C(CH_3)_2OO\cdot \longrightarrow C_6H_5C(CH_3)_2OO\text{–}OO(CH_3)_2CC_6H_5$$

Since cumylperoxy radicals have no α-hydrogens, they cannot undergo a hydrogen transfer reaction as can *prim*- and *sec*-peroxy radicals. Conceivably this intermediate could decompose directly by either of two alternative paths. On the one hand, it could decompose to di-α-cumyl peroxide, resulting in termination. On the other hand, it could decompose to acetophenone, a methylperoxy radical and a methyl radical, resulting in a non-terminating interaction.

$$C_6H_5C(CH_3)_2OO\text{–}OO(CH_3)_2CC_6H_5 \longrightarrow \begin{cases} C_6H_5C(CH_3)_2OO(CH_3)_2CC_6H_5 + O_2 \\ 2C_6H_5COCH_3 + CH_3OO\cdot + CH_3\cdot \end{cases}$$

Another possible interpretation is that this intermediate decomposes to cumyloxy radicals and oxygen which are capable of undergoing either a non-terminating interaction or a terminating interaction before they diffuse apart and escape from the solvent cage. Thus the acetophenone as well as the

$$[C_6H_5C(CH_3)O\cdot O_2\cdot O(CH_3)_2CC_6H_5] \longrightarrow \begin{cases} C_6H_5C(CH_3)_2OO(CH_3)_2CC_6H_5 + O_2 \\ 2C_6H_5COCH_3 + CH_3OO\cdot + CH_3\cdot \end{cases}$$

di-α-cumyl peroxide can be accounted for by a reaction not involving "free" cumyloxy radicals. The small amount of α,α-dimethylbenzyl alcohol apparently formed can then be accounted for on the basis of the relatively few free cumyloxy radicals formed by diffusion out of the solvent cage.

From the data in Fig. 2, it is possible to calculate the relative amounts of chain termination as opposed to chain propagation, based on the assumption that all of the termination results in dialkyl peroxide. Since 1.85 molecules of oxygen, on the average, are present in non-hydroperoxidic products and since, under steady state conditions, the rate of initiation must equal the rate of termination, 1 molecule of oxygen must be present as dialkyl peroxide. Thus, to account for this amount of peroxide as well as the acetophenone, assuming the difference between the two, namely, 0.12 molecule, is present as α-cumyl alcohol, requires 3.70 cumyloxy radicals. Therefore, 39.5% of the time these radicals interact with decomposition to acetophenone, 54% of the time they interact with dimerization to dialkyl peroxide and 6.5% of the time they abstract hydrogen atoms.

A non-terminating interaction of *t*-peroxy radicals has been postulated by Vaughan[14] and co-workers based on their studies involving the decomposition of di-*t*-butyl peroxide in the presence of oxygen as well as in the induced chain decomposition of *t*-butyl hydroperoxide. Although their decompositions were carried out at much higher temperatures than the oxidations in the present paper, the products found indicated quite clearly that such a non-terminating reaction was occurring. Under the conditions of their experiments, however, the dimerization reaction resulting in peroxide would not be important since the peroxides are unstable at the temperatures utilized. However, more recently Dean and Skirrow[16] studied the cobaltous ion catalyzed decomposition of *t*-butyl hydroperoxide and concluded that *t*-butylperoxy radicals must undergo a non-terminating interaction resulting in alkoxy radicals. Moreover, in this case also, di-*t*-butyl peroxide is a product of the reaction.

Acknowledgment.—It is a pleasure to acknowledge several helpful discussions with Dr. Glen A. Russell.

Experimental

Materials.—Cumene (Phillips 99 mole % minimum) was rectified in a large Podbielniak column (>50 plates) and the center fraction collected; b.p. 69–70° at 41 mm., $n^{20}D$ 1.4910. The fraction was chromatographically filtered through activated silica gel or alumina and stored under nitrogen at 0°.

Chlorobenzene was extracted with concentrated sulfuric acid, washed with water, dried and rectified in a Podbielniak

(15) H. C. Bailey and G. W. Godin, *Trans. Faraday Soc.*, **52**, 68 (1956).

(16) M. H. Dean and G. Skirrow, *ibid.*, **54**, 899 (1956).

column (>50 plates); b.p. 130–132°. It was stored at 0° under nitrogen.

Azodiisobutyronitrile (Eastman Kodak Co.) was recrystallized several times from methanol; m.p. 102° dec.

Oxidation Procedure.—The initiator was added to the oxidation flask as an 0.1 *M* solution in benzene. The benzene was removed by evacuating at 15–20 mm. for approximately two hours. The cumene and chlorobenzene were added by pipet and the oxidation flask, which consisted of a heavy-walled erlenmeyer flask attached to a 30-cm. length of 16-mm. tubing, was connected to a gas manometer, evacuated and filled with oxygen several times. The flask then was attached to a reciprocating rack contained in a thermostated oil-bath and the rate of oxygen absorption measured by manual control of the mercury level in the gas buret.

Analytical Procedure. Hydroperoxides.—The hydroperoxide yields were determined by the stannous chloride procedure of Barnard and Hargrave[17] and carried out as follows: a 1–5-ml. aliquot of the oxidate was added to 10 ml. of 0.1 *N* stannous chloride solution containing 10 ml. of glacial acetic acid. The resulting mixture, contained in an erlenmeyer flask, was flushed with nitrogen, stoppered, and allowed to stand 1–2 hours in a nitrogen atmosphere with occasional shaking. Finally, the solutions were titrated with standard potassium triiodide solution. In each determination, a blank was carried through the entire procedure and the amount of hydroperoxide was thus determined by the difference in the titer of the blank and the solution containing the oxidate.

Acetophenone.—The yields of acetophenone were determined by infrared absorption utilizing a differential technique. In this procedure standards are prepared and their absorption at 5.9 μ measured in a 1-mm. cell utilizing a Perkin–Elmer model 21 spectrometer. Following this, these absorptions are balanced against a second set of standards placed in a matched cell in the reference beam of the spectrometer. In this manner, a calibration curve can be prepared from which it is possible to determine the amount of acetophenone present in the oxidates. The acetophenone was isolated and identified as its DNP derivative, m.p. 248–249°, mixed m.p. 249°; reported[18] 250°.

(17) D. Barnard and K. R. Hargrave, *Anal. Chim. Acta*, **5**, 476 (1951).

Dialkyl Peroxide.—This analysis was carried out essentially by the technique of Vaughan[7] and co-workers as modified by Hercules Powder Co., Inc.[8] Prior to analysis, an appropriately sized aliquot of the oxidate was heated in a nitrogen atmosphere to 110° for 3–5 minutes to decompose the residual AIBN. Then 50 ml. of glacial acetic acid, 3 g. of sodium iodide and 3 ml. of water were added and the whole heated in a nitrogen atmosphere at 110–115° for 1 hour. Finally, the resulting iodine was titrated in an atmosphere of carbon dioxide with standard thiosulfate solution. The difference between this determination and the stannous chloride determinations for hydroperoxide thus gave the amount of dialkyl peroxide present. The complete analytical procedure, including the pre-heating in the presence of AIBN, was checked with known quantities of pure di-α-cumyl peroxide and cumene hydroperoxide. It was found that the method was reproducible within $\pm 12\%$.

Vapor Phase Chromatographic Analyses.—Several of the oxidates were subjected to vapor phase chromatography utilizing an A-1 column at 120°. By this method, utilizing known solutions, both α-methylstyrene and α,α-dimethylbenzyl methyl ether were positively eliminated as oxidation products. Moreover, all of the peaks, with one exception, could be accounted for by comparison with the chromatograms obtained from known mixtures of acetophenone and cumene hydroperoxide. The one exception was a broad peak of very low intensity due to a compound with a relatively long retention time. Comparison with the chromatogram of a solution of α,α-dimethylbenzyl alcohol indicated that it could be due to this compound. Moreover, the intensity of this peak was slightly diminished upon addition of hexamethyldisilazane[6] as was the intensity of the corresponding peak from the chromatogram of the known solution containing the disilazane. The chromatograms of the known solutions containing enough α,α-dimethylbenzyl alcohol to correspond to an amount of oxygen absorbed in the oxidations of greater than 8% established this as an approximate upper limit for the amount of α,α-dimethylbenzyl alcohol which could be present in the oxidates.

(18) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948.

SCHENECTADY, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY AND THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, THE JOHNS HOPKINS SCHOOL OF MEDICINE]

The Influence of Mechanism on the Apparent pK_a' of Participating Groups in Enzymic Reactions

BY THOMAS C. BRUICE[1] AND GASTON L. SCHMIR[2]

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It has become an accepted practice to employ the *p*H dependence curves of enzymic activity to determine the dissociation constants of groups involved in the catalytic processes and thereby establish their structural identity. However, kinetically determined acid dissociation constants may or may not reflect the nature of the participating groups and the values of the apparent dissociation constants will depend, among other things, on the intricacies of mechanism. Limiting our considerations to the mechanism of the bond making and breaking processes within the enzyme–substrate complex, the way in which *the constant of any equilibrium* (in addition to the dissociation constant) *occurring prior to the rate-determining step becomes a part of the kinetically determined pKa' value* is illustrated by considering six plausible mechanisms for an esteratic enzyme. The possible application of the concepts developed here to certain enzymic reactions is pointed out.

Introduction

To determine the nature of the ionizable groups which participate in enzymic reactions it is conventional to plot V_{max} at constant total enzyme concentration (or an equivalent rate expression) *vs.* *p*H and then to determine what pK_a' values fit best to the resultant *p*H dependency curve. Thus, in the reaction of trypsin with benzoyl-L-arginine ethyl ester the *p*H dependence curve has the shape of a dissociation curve for a single group of pK_{app} 6.25,[3] while for the reaction of α-chymotrypsin with acetyl-L-tryptophan ethyl ester and acetyl-L-tyrosine ethyl ester the kinetic data fitted well the theoretical curves for the dissociation of a single group of $pK_{app'}$ 6.7–6.74.[4] For the reaction of α-chymotrypsin with methyl hydrocinnamate the variation of rate with *p*H implicates groups of $pK_{app'}$ 7.2 and 8.0.[5] In the instance of the choline

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(2) The National Institutes of Health, Bethesda, Md.

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