

[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

## The Kinetics of the Thermal Decomposition of Peresters. III. The Effect of *p*-Substituents on the Unimolecular Decomposition of *t*-Butyl Perbenzoates<sup>1</sup>

BY A. T. BLOMQUIST AND IRVING A. BERSTEIN

A kinetic study of the thermal decomposition in phenyl ether of *t*-butyl perbenzoate and four *p*-substituted peresters has been accomplished. It was observed that, in this solvent, the rates of decomposition were all first order and completely independent of the initial peroxide concentration. The effects of a series of potent radical inhibitors were investigated and it was determined that none of them lowered the over-all rate of decomposition of the perester most prone to radical attack, *viz.*, the *p*-nitro compound. From consideration of the above evidence and the unlikelihood of the existence of any significant amount of heterolytic decomposition in the medium employed, it is believed that the effects of substituents on the rate of the unimolecular, homolytic decomposition have been measured. The rates of the decomposition of all of the peresters have been closely correlated by means of the Hammett relationship. The energy and entropy of activation for the decomposition of the peroxide bond in each of the peresters have been determined and the results clearly show that substituents affect both factors, a definite correlation existing between increasing energy of activation and increasing entropy of activation.

The purpose of the present investigation was to ascertain the effects of various substituents on the energy and entropy of activation of the unimolecular decomposition of the peroxide bond in *p*-substituted *t*-butyl perbenzoates and to determine whether the Hammett equation<sup>2</sup> would satisfactorily correlate these rates of decomposition with the electronic characteristics of the substituents, this relationship having been applied with considerable success to other free radical reactions.<sup>3,4,5</sup>

It has recently been found that the thermal decomposition of *t*-butyl perbenzoate proceeded at about the same rate in all the aromatic solvents investigated and at much faster and widely different rates in aliphatic solvents.<sup>6,7</sup> Furthermore, since the over-all rate of decomposition was dependent on the initial perester concentration and since free radical inhibitors lowered the over-all rate of decomposition, it was concluded that the perester decomposition was essentially of the same complex nature as that of benzoyl peroxide,<sup>8</sup> namely, a radical-induced decomposition superimposed on the homolytic, unimolecular cleavage of the perester.

Therefore, for true comparisons of substituent effects on rate of decomposition and the energy and entropy of activation of the peroxy bond in the substituted perbenzoates it was necessary to obtain reaction conditions in which no significant amount of induced decomposition occurred.

In the present study, all the peresters were decomposed in phenyl ether, and, it is believed, in this solvent no radical-induced decomposition occurred. As part of the evidence for this view are the strictly first order plots observed for all the compounds even to a high degree of decomposition (Fig. 1) and the independence of the rate constant even over a tenfold concentration range (Table I).

(1) Taken from the thesis of I. A. Berstein presented to the Graduate School of Cornell University in February, 1951, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) L. P. Hammett, *Chem. Revs.*, **17**, 125 (1935); "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

(3) C. Walling, E. R. Briggs, K. B. Wolfstirn and F. R. Mayo, *THIS JOURNAL*, **70**, 1537 (1948); C. Walling, D. Seymour and K. B. Wolfstirn, *ibid.*, **70**, 1544 (1948).

(4) C. G. Swain, W. H. Stockmayer and J. T. Clarke, *ibid.*, **72**, 5426 (1950).

(5) A. T. Blomquist and A. J. Buselli, *ibid.*, **73**, 3883 (1951).

(6) A. T. Blomquist and A. F. Ferris, *ibid.*, **73**, 3408 (1951).

(7) A. T. Blomquist and A. F. Ferris, *ibid.*, **73**, 3412 (1951).

(8) (a) D. J. Brown, *ibid.*, **62**, 2657 (1940); (b) K. Nozaki and P. D. Bartlett, *ibid.*, **68**, 1686 (1946); (c) B. Barnett and W. E. Vaughan, *J. Phys. Colloid Chem.*, **51**, 926, 942 (1947).

TABLE I

DECOMPOSITION OF THE PERESTERS IN PHENYL ETHER AT 120.2°. VARIATION IN FIRST ORDER CONSTANT WITH CONCENTRATION

Substituent	Approx. initial concn., <i>M</i>	First order constant, hr <sup>-1</sup> Range	Average
<i>p</i> -Methoxy	0.03	0.456-0.461	0.459
	.2	.460-.461	.461
<i>p</i> -Methyl	.03	.371-.377	.374
	.2	.383-.386	.385
Unsubstituted	.03	.319-.327	.323
	.2	.324-.327	.326
<i>p</i> -Chloro	.03	.227-.229	.228
	.2	.228-.232	.230
<i>p</i> -Nitro	.03	.114-.115	.115
	.2	.114-.116	.115

As pointed out by Leffler,<sup>9</sup> the sole fact that first order rate constants are independent of variations in initial peroxide concentration is not in itself sufficient to signify a unimolecular decomposition since the same results are obtained if the induced decomposition is caused primarily by attack of solvent radicals and if chain termination is between solvent radicals and peroxide radicals. Therefore, further experimental evidence had to be obtained to prove whether any induced decomposition was actually occurring.

Such evidence was obtained from investigation of the effect of additives on the decomposition of the perester most prone to radical attack, the *p*-nitro compound (Table II).

TABLE II

DECOMPOSITION OF *t*-BUTYL PER-*p*-NITROBENZOATE IN PHENYL ETHER WITH INHIBITORS ADDED AT 120.2°

Initial perester concn., <i>M</i>	Inhibitor and concn., <i>M</i>	First order rate constant, hr <sup>-1</sup>
0.2	None	0.115
.2	Ethyl urethan	.173
.2	Acetanilide	.140
.2	Styrene	.130
.2	Dimethyl fumarate	.127

It was determined from kinetic experiments with *p*-xylene as solvent that the greatest amount of induced decomposition occurred in the most negatively substituted perester, the *p*-nitro compound. Swain, *et al.*,<sup>4</sup> in their studies on the substituted benzoyl peroxides have also indicated that

(9) J. E. Leffler, *THIS JOURNAL*, **72**, 67 (1950).

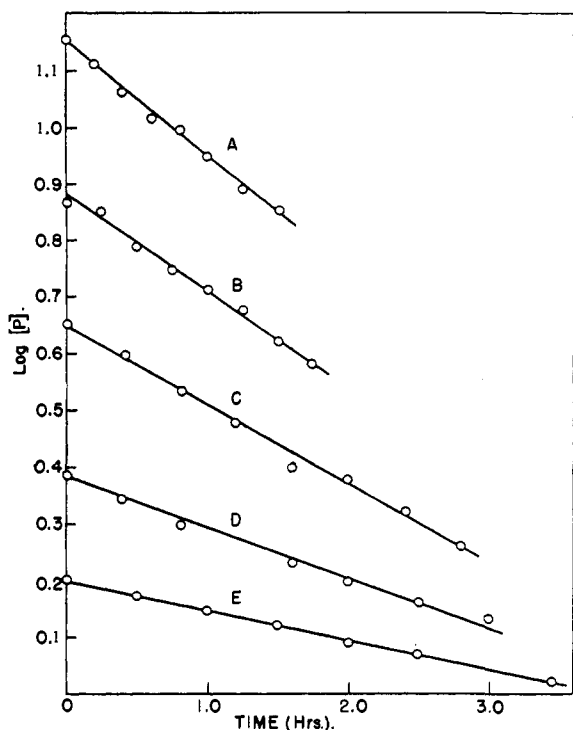


Fig. 1.—Typical first order plots for the thermal decomposition of the unsubstituted and *p*-substituted *t*-butyl perbenzoates in phenyl ether at 120.2°: A, *p*-methoxy; B, *p*-methyl; C, unsubstituted; D, *p*-chloro; E, *p*-nitro. The position of the scale of the ordinate is arbitrary.

the nitro-substituted peroxides are the most susceptible to radical attack. The additives employed were substances which have all shown themselves to be among the most efficient radical inhibitors.<sup>4</sup>

It should be noted that none of the "inhibitors" lowered the over-all rate but some actually increased it. The acceleration in rate in the presence of these additives can be interpreted as follows. It is well established that even though a substance may behave as an effective radical inhibitor in one system, it may be quite ineffective in other systems. Thus, although phenyl ether alone may be a superior inhibitor for radicals derived from the perester (*i.e.*, the *t*-butoxy and the substituted benzoxy radicals), the addition of substances, which are known to behave as inhibitors in other systems, might conceivably give rise to radicals sufficiently reactive to cause some induced decomposition and, therefore, to effect an acceleration in rate of decomposition over that obtained in phenyl ether alone. Complete proof for the view that phenyl ether is acting as the radical trap would require isolation of the reaction products.

It was concluded from the above evidence on concentration effect and additive effect that no significant amount of induced decomposition occurred and, thus, it was deemed permissible to use a single convenient perester concentration in the determination of the rates of decomposition at three other temperatures. From a plot of  $\log k$  against  $1/T$  at four temperatures, Fig. 2, the energies of activation of the unimolecular, homolytic cleavage of the peresters were estimated. Using these values of the activation energy, the entropy

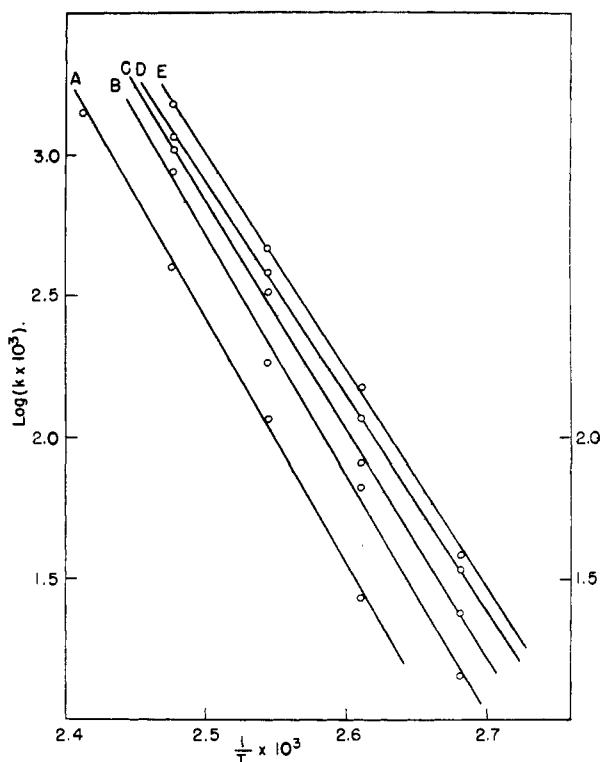


Fig. 2.—Activation energy plots for the decomposition of the unsubstituted and *p*-substituted *t*-butyl perbenzoates: A, *p*-nitro; B, *p*-chloro; C, unsubstituted; D, *p*-methyl; E, *p*-methoxy.

of activation for the thermal decomposition of each of the peresters in phenyl ether at 120.2° was calculated according to the formula from transition state theory for reactions occurring in ideal solution.<sup>10</sup> The energies and entropies of activation and the data from which they were obtained are listed in Table III.

Assuming an error of 3% in  $k$ , the values of the energies of activation are significant to  $\pm 0.7$  kcal. mole<sup>-1</sup> and the entropies of activation to  $\pm 1.5$  cal. mole<sup>-1</sup> degree<sup>-1</sup>. The substituent effects on the rates of decomposition seem to correlate well with their effects on the energy of activation, the methoxy perester decomposing fastest and having the lowest value for the activation energy and the nitro derivative decomposing slowest and having the largest activation energy. However, it would be expected that such a large difference in activation energies of 5.5 kcal. between the two compounds would cause a greater difference in magnitude between the unimolecular rates than that observed. The reason this is not the case is readily seen from inspection of the  $\Delta S^*$  values for each of the peresters. The trend of increasing  $\Delta S^*$  with increasing energy of activation is a real one and considerably above the limits of experimental error. Large values for the activation energy, of course, cause a lowered rate of reaction but increasing entropy values tend to balance this effect by making reaction more probable.

Such a dependence of entropy of activation on

(10) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 199.

TABLE III

ENERGY AND ENTROPY OF ACTIVATION OF THE THERMAL DECOMPOSITION OF THE UNSUBSTITUTED AND *p*-SUBSTITUTED *t*-BUTYL PERBENZOATES AS DETERMINED BY DECOMPOSITION IN PHENYL ETHER

Substituents	<i>t</i> , °C.	<i>k</i> , hr. <sup>-1</sup>	$\Delta E^*$ , kcal. mole <sup>-1</sup>	$\Delta S^*$ , cal. mole <sup>-1</sup> degree <sup>-1</sup>
<i>p</i> -Methoxy	100.0	0.0386		
	110.1	0.150	35.8 ± 0.7	12.2 ± 1.5
	120.2	0.461		
	130.9	1.54		
<i>p</i> -Methyl	100.0	0.0339		
	110.1	0.115	36.1 ± .7	12.6 ± 1.5
	120.2	0.380		
	130.9	1.17		
Unsubstituted	100.0	0.0241		
	110.1	0.0820	37.5 ± .7	15.8 ± 1.5
	120.2	0.324		
	130.9	1.05		
<i>p</i> -Chloro	100.0	0.0140		
	110.1	0.0665		
	120.2	0.230	39.3 ± .7	19.7 ± 1.5
	130.9	0.871		
<i>p</i> -Nitro	110.1	0.0272		
	120.2	0.115	41.3 ± .7	23.7 ± 1.5
	130.9	0.401		
	141.5	1.41		

\* Computed from data at 120.2°.

the energy of activation,  $\Delta E^*$ , has been observed in a number of cases, among which are the unimolecular decarboxylations of a series of related compounds such as substituted malonic acids<sup>11</sup> and other carboxylic acids.<sup>12</sup>

The correlation of substituent effects on the bond strengths of the substituted peresters can be interpreted in the following way. The *t*-butoxy part of the perester is strongly electron-repelling and substitution of increasingly negative groups (increasing  $\sigma$  value) on the benzene ring causes the benzyloxy portion of the molecule to have increasing electron-attracting power. Thus, these mesomeric and inductive effects cause a relatively greater electron density on one oxygen atom of the peroxy bond attached to the butoxy group and a relative deficiency of electron density on the oxygen atom

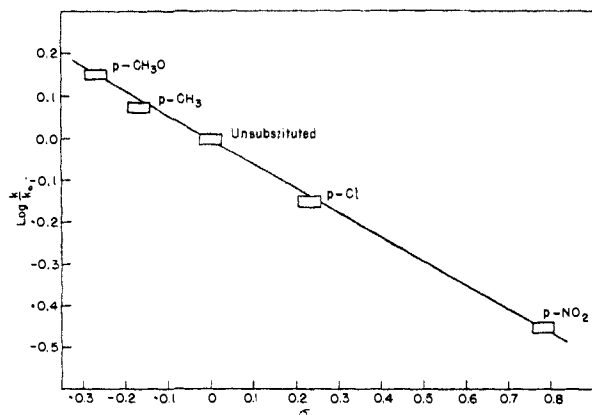


Fig. 3.—Correlation of first order rate constants with Hammett's sigma values.

(11) A. L. Banoulli and W. Wege, *Helv. Chim. Acta*, **2**, 511 (1919).

(12) R. A. Fairclough, *J. Chem. Soc.*, 1186 (1938).

connected to the substituted benzoyl group, the magnitude of which depends on the electron-attracting power of the substituent. The result of the above effects is to induce more ionic character in the —O—O— bond in the negatively substituted compounds than in the unsubstituted or positively substituted molecules. The increase in ionic nature of the peroxide bond would result in an increase in bond strength.

The rates of decomposition have been very closely correlated by means of the Hammett relationship<sup>3</sup>

$$\log k/k_0 = \rho\sigma$$

where *k* and *k*<sub>0</sub> are rate constants for the side chain reaction of the substituted and unsubstituted benzenes,  $\sigma$  is a parameter for each substituent, independent of the reaction, and  $\rho$ , a parameter dependent only upon the reaction and independent of the substituent.

The value of  $\sigma$  used for the *p*-nitro substituent is the value given for all benzene derivatives except those of aniline and phenols in which the effects of resonance modify the extent of electron-release. In Fig. 3 are plotted the logarithms of the ratio of rate of decomposition of the substituted perester to the unsubstituted one against the value for the substituent on the benzene ring. A standard error in *k* of 3% was assumed and the median error given for Hammett's  $\sigma$  values has been included in the graph.

From a consideration of the plot of Fig. 3, the linear relationship between the log of the rate constants and Hammett's sigma function that is obtained would appear to provide further confirmation of the applicability of the Hammett relationship to substituent effects in free radical decomposition as previously reported for the relative rates of addition of the styryl radical to *m*- and *p*-substituted styrenes<sup>3</sup> and for the decomposition of the substituted benzoyl peroxides.<sup>4,5</sup> However, it should be recognized that such a satisfactory correlation certainly would not be expected in this case in which variations in entropy of activation occur since the Hammett relationship is generally effective only for those series of reactions in which solely potential energy terms are involved.

The possibility that there is any significant amount of heterolytic decomposition occurring when the peresters were decomposed in phenyl ether is held unlikely in view of the low dielectric constant of the solvent, the comparatively large variations in the rate constants with changes in temperature, and the fact that previous work by Blomquist and Ferris<sup>7</sup> showed the decomposition of *t*-butyl perbenzoate responded to acid catalysis only with the very strong acid, trichloroacetic. Further evidence can be obtained from consideration of the magnitudes of the energies of activation. The activation energies for the nitro and chloro peresters were found to be 41.3 kcal. mole<sup>-1</sup> and 39.3 kcal. mole<sup>-1</sup>, significantly larger than for the unsubstituted or positively substituted compounds. Since the greatest amount of ionic decomposition should occur in the most electrically unsymmetrical molecules, *i.e.*, those with negative substituents, lower rather than higher values for the activation energy of the negatively substituted molecules

TABLE IV  
 SUBSTITUTED *t*-BUTYL PERBENZOATES

Substituent	M.p. perester °C.	$n_D^{20}$	$d_4^{20}$	Purity, %	Yield, %	Analyses, %			
						Calcd. Carbon	Found Carbon	Calcd. Hydrogen	Found Hydrogen
<i>p</i> -Methoxy <sup>a</sup>	18.5-19	1.5157	1.103	97.8	85	64.3	64.1	7.20	7.11
<i>p</i> -Methyl <sup>b</sup>	...	1.5022	1.027	97.6	78	69.3	69.3	7.76	7.64
Unsubstituted	8	1.4992	1.035	98.0	..	..	..	..	..
<i>p</i> -Chloro <sup>c</sup>	49 <sup>e</sup>	....	...	98.5	77	57.8	57.8	5.74	5.58
<i>p</i> -Nitro <sup>d</sup>	79 <sup>e</sup>	....	...	100.0	83	55.3	55.4	5.49	5.32

<sup>a</sup> B.p. of acid chloride, 90° (1 mm.); cf. J. Marsh and H. Stephen, *J. Chem. Soc.*, 127, 1635 (1925). <sup>b</sup> B.p. of acid chloride, 70° (4 mm.); cf. G. Morgan and E. Carlson, *ibid.*, 2208 (1929). <sup>c</sup> B.p. of acid chloride, 79-80° (2 mm.), supplied by Heyden Chemical Co. <sup>d</sup> B.p. of acid chloride, 140° (10 mm.); cf. R. Adams and R. Jenkins, *Org. Syntheses*, 3, 75 (1923). <sup>e</sup> Both peresters recrystallized from hexane. All temperatures determined are uncorrected.

would be expected if there were appreciable heterolytic cleavage.

A test that has been applied by some investigators to the extent that ionic character can be introduced into the decomposition of peroxides is to carry out decomposition in solvents of greatly varying dielectric constants, but this approach may be misleading unless, for each solvent, it is carefully determined that no radical-induced decomposition is occurring. Perhaps a better method is to determine the susceptibility to acid catalysis of the peroxide in a solvent in which no significant amount of induced decomposition can be occurring. Investigations of acid catalysis on the series of peresters should be particularly interesting because of their remarkable gradation in electrical dissymmetry. The *t*-butyl per-*p*-nitrobenzoate should probably respond even more to acid catalysis than the unsymmetrical benzoyl peroxide investigated by Leffler.<sup>9</sup>

It is hoped that such studies on acid catalysis and product investigations will provide further insight on the decompositions of this series of interesting and theoretically significant molecules.

### Experimental

**Materials. Phenyl Ether.**—The phenyl ether, obtainable from the Paragon Chemical Company, was first recrystallized and then distilled at reduced pressure (b.p. 135.5° at 23 mm.). No peroxide could be detected in this solvent even on standing for several months.

**Ethyl Urethan.**—The ethyl urethan was synthesized using a modification of a method of preparation of methyl urethan.<sup>13</sup> Before being used, the product was distilled twice *in vacuo*. A main cut boiling at 88-89° (30 mm.) was collected.

**Acetanilide.**—Acetanilide available in the laboratory having a m.p. of 112-114° was utilized.

**Dimethyl Fumarate.**—Eastman Kodak White Label grade dimethyl fumarate having a m.p. 119-120° was used.

**Styrene.**—The styrene was Eastman Kodak Yellow Label grade which was distilled under reduced pressure directly before being used.

**Acetic Acid.**—Reagent Grade glacial acetic acid, sold by General Chemical Company was used directly without special treatment. This material was peroxide-free, showing consistently negligible blanks.

**Phosphoric Acid.**—Reagent Grade 85% orthophosphoric obtained from the Baker and Adamson Company was used in the analytical determinations.

**Dioxane.**—Paragon dioxane when distilled over sodium wire at atmospheric pressure yielded a completely peroxide-free product. Even after several months standing, no peroxide formation could be detected in the dioxane when stored over sodium wire.

**Hydriodic Acid.**—The 57% hydriodic acid (sp. gr., 1.7) employed in the analytical procedure was prepared from

commercial hydriodic acid (J. T. Baker Co.—sp. gr., 1.5) by distillation under an atmosphere of carbon dioxide in an all-glass apparatus and was stored in 35-ml. black bottles sealed with paraffin.

**Preparation of Peresters.**—The unsubstituted *t*-butyl perbenzoate was supplied by Dr. A. F. Ferris.<sup>6,7</sup>

All the substituted peresters investigated were synthesized utilizing a modification of the method for the preparation of the unsubstituted *t*-butyl perbenzoate.<sup>14</sup>

The *t*-butyl hydroperoxide used in the syntheses of the peresters was prepared from commercial *t*-butyl hydroperoxide obtained from the Lucidol Division of Novadel-Agene Corporation by fractional distillation under reduced pressure (b.p. 34-35° at 11 mm.).

The preparation of the substituted peresters was carried out by simultaneously adding approximately 0.63 mole of 30% aqueous potassium hydroxide and 0.50 mole of the substituted benzoyl chloride dropwise to 0.51 mole of 60% aqueous *t*-butyl hydroperoxide while stirring vigorously. The temperature of the reaction medium was kept between 0-6°. Stirring was continued for about 10 hours after the addition of the acid chloride and the potassium hydroxide.

Of the two distinct layers in the reaction mixture, the lower aqueous layer was drawn off in a separatory funnel and discarded. Methylene chloride (sp. gr., 1.34) was then added to the upper organic layer to prevent any emulsion formation when it was washed. To remove any acidic impurities, the methylene chloride was washed twice with 50 ml. of 5% sodium carbonate and then three times with 50 ml. of distilled water. The organic extracts were then dried by allowing them to stand for several hours over anhydrous magnesium sulfate. After removal of the magnesium sulfate by filtration through a sintered glass funnel, the perester was freed of most of the methylene chloride by putting it under vacuum for several hours.

For the peresters whose parent-substituted benzoyl chlorides are liquids the procedure described above was followed exactly. Since *p*-nitrobenzoyl chloride is a solid, the method was modified by dissolving the acid chloride in methylene chloride and then adding the methylene chloride-acid chloride solution to the hydroperoxide-water mixture, stirring vigorously as in the addition of the liquid acid chlorides. The methylene chloride layer containing the nitro perester was then treated exactly as described above.

A complete list of the compounds synthesized and investigated is presented in Table IV.

All the peresters studied evidenced a remarkable ability to supercool, probably because of their quite unsymmetrical character. The compounds investigated were stored in a refrigerator, and no perceptible decomposition occurred on standing. At no time during preparation, purification, or general handling did a perester detonate.

**The Analytical Method.**—A modification of a method developed by Clarke<sup>15</sup> for the unsubstituted *t*-butyl perbenzoate was employed for the analyses of all the peresters.

In each of four 250-ml. erlenmeyer flasks were placed 5 ml. of peroxide-free dioxane, 5 ml. of chloroform and 15 ml. of a 3% solution of 85% orthophosphoric acid in glacial acetic acid. To three of the flasks were added by means of a pipet, a 2-ml. or a 5-ml. aliquot of the solutions to be

(14) N. A. Milas and D. M. Surgenor, *THIS JOURNAL*, 68, 642 (1946).

(15) J. T. Clarke, Ph.D. Thesis, Massachusetts Institute of Technology, 1949.

(13) *Org. Syntheses*, 12, 32 (1932).

analyzed, the size of the sample depending on the initial perester concentration in the kinetic run. In order to avoid crystallization of the phenyl ether on the inside of the pipets, the perester samples were warmed in water at a temperature of about 32° before pipetting. The fourth flask was used as a blank.

The flasks were placed in a black desiccator and after being flushed with carbon dioxide for about 25 minutes, 2 to 3 ml. of 57% hydriodic acid was quickly added to each flask. The desiccator was then closed and allowed to stand under continued flushing of carbon dioxide for a period of 30 minutes to one hour, the shorter reaction time for the methoxy, methyl and unsubstituted peresters and the longer one for the less reactive nitro and chloro compounds. At the end of the standing time, about 50 ml. of distilled water was added to each flask, and the liberated iodine was titrated with standard thiosulfate of 0.01 *N* while the flask was vigorously agitated to prevent retention of any iodine by the immiscible chloroform layer. The presence of chloroform was necessary to dissolve all colored organic substances, thus permitting the iodine to be the only colored substance in the aqueous layer and improving the ease of detection of the starch-iodide end-point considerably. From the titration value minus the blank titration, the concentration of the perester was calculated. The blank values were generally zero and never greater than 0.20 ml. The precision of the method is probably no better than ±3%.

To avoid difficulties in pipetting samples from kinetic experiments run on the 0.2 *M* solutions, in which considerable amounts of insoluble decomposition products resulted, a rapid, convenient method of filtration by centrifugation was developed. The bottom of a drying tube, cut off just above the bulb, was fitted into a 15-ml. centrifuge tube with a cork. After placing a small piece of filter paper and glass

wool in the drying tube bulb, the sample of liquid and solid was poured into the bulb. Separation of solids by their retention on the glass wool and filter paper was complete after centrifugation for about one minute.

When the peresters were analyzed for their purity, *i.e.*, the active oxygen content, the only variations in the method were the following: if liquids, the samples were weighed from a Lunge pipet and, if the peresters were solids, in a weighing bottle. The greater amount of iodine liberated in the purity determinations was titrated against 0.1 *N* thiosulfate solution.

**Apparatus and Procedure for the Kinetic Studies.**—The apparatus and procedure for the decompositions of the peresters were essentially the same as previously described by Blomquist and Buselli.<sup>5</sup>

The decomposition flasks were immersed in an oil-bath whose temperature was controlled by a bimetallic thermostat operating through an electronic time-delay relay. With this control apparatus, the temperature of the bath could be held to within ±0.1° of the desired temperature. The thermometer used in all the experiments was calibrated against a platinum resistance thermometer previously calibrated by the National Bureau of Standards and made available by Dr. E. R. VanArtsdalen. Decompositions were allowed to proceed to 50–70% of the initial concentration. After the perester samples for a given run had been taken, aliquot portions of the samples were analyzed for peroxide content according to the analytical method previously described. If the samples were not to be analyzed immediately or rechecks were to be made they were stored in a refrigerator, and on being stored for as long as several weeks, they evidenced no perceptible change in perester content.

ITHACA, N. Y.

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[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY<sup>1</sup>]

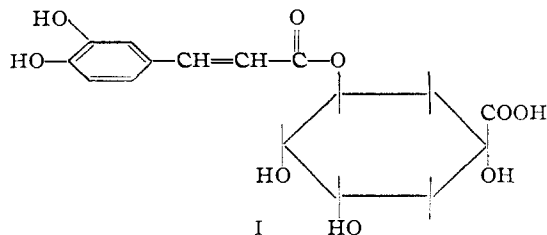
## Enzymatic Browning of Fruits. I. Autoxidation of Chlorogenic Acid

BY LLOYD L. INGRAHAM AND JOSEPH CORSE

The rate of absorption of oxygen by chlorogenic acid has been studied under various conditions. It has been found that the initial rates obey the expression:  $-\frac{dO_2}{dt} = \frac{k(\text{chlorogenic acid})P_{O_2}}{(H^+)}$  over the pH range of 7.49 to 8.74 and are little affected by light or cupric ions. The over-all activation energy for autoxidation was found to be 13.6 ± 1 kcal./mole.

### Introduction

It has long been recognized that rapid browning on the cut surfaces of fresh fruits is an enzymatic oxidation process and can be prevented by heat inactivation of the enzymes involved, but the kinetics and mechanism are not well understood and require further study. The part played by the non-enzymatic reaction (autoxidation) must also be known in such studies. There are indications that chlorogenic acid (I) which is a widely dis-



tributed compound in the plant kingdom,<sup>2</sup> occurs in

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) (a) K. Freudenberg, *Ber.*, **53**, 232 (1920); (b) J. Politis, *Compt. rend.*, **225**, 954 (1947); (c) G. O. Rudkin and J. M. Nelson, *This Journal*, **69**, 1470 (1947); (d) R. G. Moores, D. L. McDermott and T. R. Wood, *Anal. Chem.*, **20**, 620 (1948).

fruits,<sup>3</sup> and may be a substrate in the enzymatic darkening. The present paper describes autoxidation studies of chlorogenic acid.

### Experimental

The chlorogenic acid hemihydrate was isolated from coffee by the method of Moores, McDermott and Wood.<sup>3d</sup> It melted at 208–209° and appeared homogeneous when examined in a 25-plate Craig countercurrent extraction apparatus<sup>4</sup> with ethyl acetate and water as solvents. The solutions were made in water redistilled in an all-glass still. The rates were measured by the oxygen absorption in constant-volume manometers of the Summerson type<sup>5</sup> employing flasks of about 50-ml. capacity. One flask was kept as a control to compensate for temperature and pressure fluctuations. This flask contained buffer and oxygen but not the substrate. The center well of each flask was filled with paraffin; on this was placed a platinum cup containing a weighed sample of solid chlorogenic acid. Five milliliters of 1 *M* potassium phosphate buffer was placed in the bottom of the flask. The manometer was filled with Brodie's solution. The flask and manometer were evacuated with a water-pump and filled with oxygen. This was repeated

(3) K. Spitzer, *Biochem. Z.*, **231**, 309 (1931); W. V. Cruess and J. Sugihara, *Arch. Biochem.*, **16**, 39 (1948); G. Johnson, E. M. Foreman and M. M. Mayer, *Food Tech.*, **4**, 237 (1950); J. Corse and L. L. Ingraham, unpublished results.

(4) L. C. Craig and O. Post, *Anal. Chem.*, **21**, 500 (1949).

(5) W. W. Umbreit, R. H. Burris and J. F. Stauffer, "Manometric Techniques," Burgess Pub. Co., Minneapolis, Minn., 1945, pp. 119–120.