chemical evidence was inconclusive, nevertheless it appeared as though the solid form might be the *trans*-isomer and the liquid form the *cis*-isomer. The moments found for the two compounds, namely, 1.27 *D* for the solid and 1.47 *D* for the liquid, support the assumption of *cis–trans* isomerism.

Acknowledgment.—We wish to acknowledge the assistance of Dr. Peter Oesper in the experimental work on the $C_{13}H_{11}Cl$ compounds.

Summary

1. The precision of the calculated P_2 values and consequently of the dipole moment of a solute in a given solvent does not materially depend on the absolute values of the dielectric constants and densities.

2. Marked upswings and downswings in the polarization curves at high dilution are shown to be due to experimental error in measuring ϵ_0. It is suggested that an extrapolated ϵ_0 value obtained from an ϵ *vs.* N_2 plot be accepted as the dielectric constant of the pure solvent.

3. The dipole moments of friedelin, low and high-melting friedelinol, cerin and the solid and liquid forms of 3-α-naphthyl-1-chloro-1-propene have been determined.

4. The observed dipole moments of these compounds are compatible with their postulated structures.

COLLEGE PARK, MARYLAND RECEIVED OCTOBER 25, 1943

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, STATE UNIVERSITY OF IOWA]

The Kinetics of the Antioxygenic Synergism of Quinones with Ascorbic Acid in Fat Systems[1]

BY VINCENT P. CALKINS[2] AND H. A. MATTILL

In a study of the well-recognized capacity of ascorbic acid, under certain conditions, to act as an inhibitor of fat oxidation, Golumbic and Mattill[3] demonstrated that by itself it was a relatively ineffective antioxidant, but that in fats and oils containing tocopherols, it greatly enhanced the stabilizing action of these naturally occurring inhibitors. This reinforcing action of ascorbic acid was shown to extend to other fat stabilizers of phenolic character, including quinols and hydroxy chromans, and appeared to be accomplished by a delaying of the quinol ⇄ quinone oxidation that is fundamentally responsible for their stabilizing capacity. Isler[4] had earlier demonstrated that ascorbic acid markedly protected tocopherol from oxidation.

The oxidation potentials of the antioxygenic quinols[5] are appreciably higher than that of ascorbic acid. Since fat peroxides oxidize quinols, their oxidation potentials are still higher, but despite this considerable difference in potential between ascorbic acid and the fat peroxides, ascorbic acid added to fats is neither appreciably oxidized during their induction period nor does it sensibly prolong this period unless quinones, hydroquinones or other phenolic inhibitors are present.

This investigation is a kinetic study of the role of these intermediary agents in the antioxygenic action of ascorbic acid.

The systems used comprised the ethyl esters of lard fatty acids with and without ascorbic acid, with and without quinone; since quinone is antioxygenic because it is reduced to hydroquinone, the hydroquinone-lard ethyl esters system was also studied.

The ethyl esters of lard fatty acids were prepared according to the method of Olcott and Mattill[6] and were stored in the icebox under nitrogen. The induction period of each system was measured by the oxygen absorption method in oxygen at 75°; from time to time during its progress, determinations were made of hydroquinone, ascorbic acid, dehydroascorbic acid and glycolic acid, and these results were plotted against time.

Hydroquinone was determined by the α,α'-dipyridyl color reaction[7]; a calibration curve (Klett-Summerson photoelectric colorimeter, Filter 52) showed that the logarithmic reading was a linear function of the amount of hydroquinone present and obeyed Beer's law. Peroxides, except in a very low concentration, interfere with this determination.

Ascorbic acid and dehydroascorbic acid were determined by the method of Bessey,[8] slightly modified to fit the existing conditions. A calibration curve prepared from readings made on solutions of known concentration was linear and obeyed Beer's law. In the assay of dehydroascorbic acid, particular care was taken to remove

(1) The experimental data in this paper are taken from a dissertation submitted by Vincent P. Calkins to the Faculty of the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Doctor of Philosophy, April, 1943. A preliminary report of some of the results has appeared (*Fed. Proc.*, **1**, 104 (1942)).

(2) A part-time grant to V. P. C. from Merck and Co., Inc., is gratefully acknowledged.

(3) Calvin Golumbic and H. A. Mattill, THIS JOURNAL, **63**, 1279 (1941).

(4) O. Isler, *Helv. Chim. Acta*, **21**, 1756 (1938).

(5) J. B. Conant and L. F. Fieser, THIS JOURNAL, **46**, 1868 (1924).

(6) H. S. Olcott and H. A. Mattill, *ibid.*, **58**, 2204 (1936).

(7) A. Emmerie and C. Engel, *Rec. trav. chim.*, **57**, 1351 (1938).

(8) O. A. Bessey, *J. Biol. Chem.*, **126**, 771 (1938).

the last traces of hydrogen sulfide because of the interference of sulfhydryl compounds.[9]

To determine total ascorbic and dehydroascorbic acids, both dissolved and undissolved, in the ester mixture, the entire contents of the test flask was extracted with a mixture of benzene and 3% metaphosphoric acid in a separatory funnel. The acid layer was analyzed for ascorbic and dehydroascorbic acids. For the occasional determination of dissolved ascorbic and dehydroascorbic acids in the ester mixture, a portion of this was weighed out, made up to a definite volume with benzene and centrifuged to remove any undissolved acids. The supernatant liquid was then extracted with 3% metaphosphoric acid solution, the benzene fraction retaining any hydroquinone or quinone present, the metaphosphoric acid solution containing the ascorbic acid and its oxidation products. This solution was buffered with citrate to *p*H 3.5–3.6 and was centrifuged again to remove remaining traces of benzene. Absence of any emulsion was absolutely necessary for the successful use of the modified method for determining ascorbic acid; the second centrifugation aided the process of separation without danger of emulsification.

Glycolic acid was estimated by a new method[10] based on a color reaction with 2,7-dihydroxynaphthalene in concentrated sulfuric acid.

The average induction period of the esters was one to two hours, but since this varied with the freshness of the sample, a blank was always run simultaneously with the protected fat, thus permitting a comparison between the various runs on the basis of an antioxygenic index. Figure 1 shows the rate of disappearance of hydroquinone (0.02% in 20 cc. of esters) during the induction period, which in this case was nineteen hours as against one hour for the unstabilized fat. When plotted logarithmically, this curve suggests an order of reaction lying between 0 and 1. Half of the hydroquinone was oxidized in the first five hours; oxidation of the remainder required fourteen hours. As has appeared in other connections,[11,12] the length of the induction period is a function of the concentration of quinol; at its close, all the hydroquinone was in the form of quinone for, on reduction, all that had originally been introduced could be recovered. When very pure quinone was added to the ethyl esters, the original content of quinol, 1.2 μg per gram of quinone, was increased to 6.5 μg during fifteen minutes warming in air or under nitrogen. Quinone apparently acts as a hydrogen acceptor, and the hydrogen may come from the fat as a result of the formation of further double bonds (oxidation).[13]

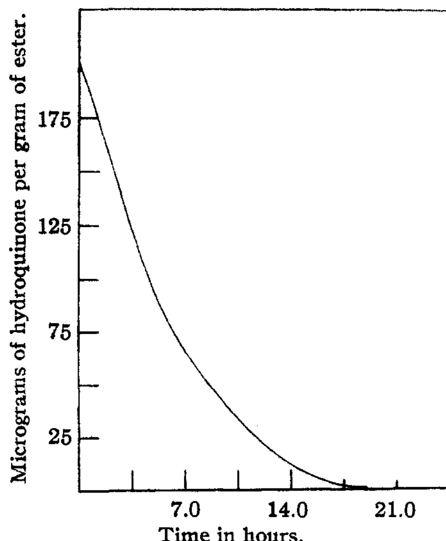

Fig. 1.—Disappearance of hydroquinone during the induction period of ethyl esters of lard fatty acids.

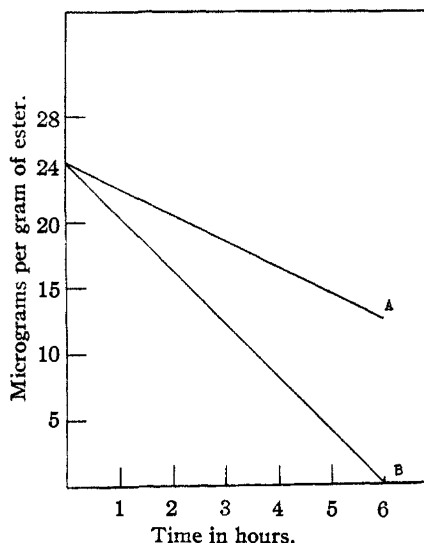

Fig. 2.—Disappearance of ascorbic acid (0.0024%) in ethyl esters: A, ascorbic acid alone; B, ascorbic acid and 0.02% quinone.

The disappearance of ascorbic acid (0.0024%), both dissolved and undissolved, during the oxidation of the ethyl esters is shown in Fig. 2. Both in the presence (Curve B) and in the absence (Curve A) of quinone, this follows a zero order reaction; the oxidation of ascorbic acid is independent of its concentration, as represented by the equation

$$-\mathrm{d}c/\mathrm{d}t = k$$

Upon integration this equation assumes the form

$$K = (C_0 - C_t)/(t_2 - t_0)$$

and from the slope of curve A, K_A is found to be 2.5×10^{-9} mole per liter per second. Similar

(9) C. G. King, personal communication.

(10) V. P. Calkins, *Ind. Eng. Chem*., *Anal. Ed*., **15**, 762 (1943).

(11) Calvin Golumbic, *Oil and Soap*, **19**, 181–182 (1942).

(12) Calvin Golumbic, *ibid*., **20**, 105 (1943).

(13) F. E. Deatherage and H. A. Mattill, *Ind. Eng. Chem*., **31**, 1425 (1939).

calculation for curve B gives a figure for K_B, 5.2×10^{-9} mole per liter per second. The rate of oxidation of ascorbic acid in the ethyl esters is thus approximately twice as great in the presence of quinone as in its absence. Traces of ascorbic acid were found in solution in the esters when it was present by itself (A), but never when quinone was also present (B). Apparently in the presence of quinone, ascorbic acid is oxidized as quickly as it goes into solution, but this does not necessarily imply that the interaction of quinone and ascorbic acid is solely a solution phenomenon. The significance of these two constants K_A and K_B will be discussed later.

The effect of the increased rate of oxidation of ascorbic acid in the presence of quinone is clearly demonstrated in Fig. 3. Curve E represents the peroxide formation in lard ethyl esters alone; curve D shows the slightly decreased rate of peroxide formation in esters containing 0.0024% of ascorbic acid. Curve C represents peroxide formation in the ester system containing 0.02% quinone in addition to 0.0024% ascorbic acid. Comparison of these curves shows the powerful synergistic action of ascorbic acid with quinone in the stabilization of the esters.

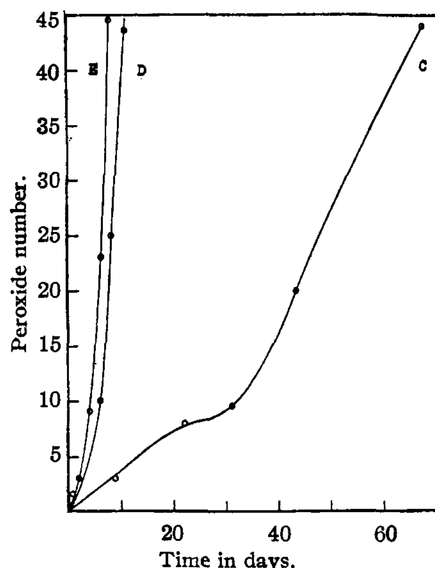

Fig. 3.—Peroxide formation in the ethyl esters containing: E, no additions; D, 0.0024% ascorbic acid; C, 0.0024% ascorbic acid and 0.02% quinone.

The actual mechanism of this synergistic action is suggested by Fig. 4, where the curves represent the course of the oxidation of ascorbic acid in the quinone-ester system as studied by the tests available. Each curve is a composite of a series of determinations and shows the mean path followed by the various substances involved.

Curve C (ascorbic acid) shows that the ascorbic acid had completely disappeared in less than six hours although the induction period lasted for nearly seventy hours. Curve A shows the gradual appearance and subsidence of dehydroascorbic acid, that is, a typical curve for the primary resultant of a consecutive reaction. Part of the oxidized dehydroascorbic acid is found in the form of glycolic acid as shown by Curve B. The glycolic acid content seems to remain relatively constant throughout the course of the induction period.

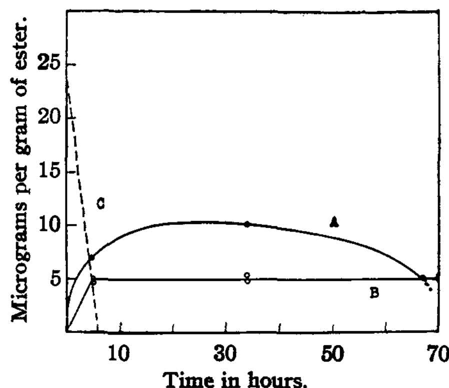

Fig. 4.—The course of oxidation of ascorbic acid in fat ethyl esters, in the presence of quinone: A, total dehydroascorbic acid; B, glycolic acid; C, total ascorbic acid.

Discussion

The significance of the two velocity constants, K_A and K_B, is best demonstrated by substituting their respective values in an equation developed by Eyring,[14] Gershinowitz[15] and others where K_1

$$K_1 = \frac{kTe^{-\frac{\Delta F^*}{RT}}}{h}$$

is the specific rate constant, ΔF^* is the free energy of formation of the activated state, T is the absolute temperature, R is the universal gas constant and k and h are the Boltzmann and Planck constants, respectively. This expression shows that the rate of a reaction is determined by a free energy change which usually is not the same as the free energy change for the total reaction. Use is made of an activated complex, and it is shown that the rate of a chemical reaction is determined by the concentration of the reacting systems in a certain fraction of the total phase space available, multiplied by the velocity with which the systems are passing through this space. ΔF^* cannot be the same as ΔF unless the energy of formation of the activated state is equal to the energy of the reaction.

By applying this equation to the velocity constants of both the ascorbic acid–lard ethyl esters and the ascorbic acid–quinone–lard ethyl esters systems, the respective ΔF^* of each system can be determined. By rearrangement of the equation and substitution of numerical values, ΔF^* values are obtained: $\Delta F_1^* = 34{,}404$ calories per mole per liter; $\Delta F_2^* = 33{,}894$ calories per mole per

(14) H. Eyring, *J. Chem. Phys.*, **3**, 107 (1935).
(15) H. Gershinowitz, *ibid.*, **4**, 363 (1936).

liter; where ΔF_1^* and ΔF_2^* are the free energies of formation of the activated states of the ascorbic acid–lard ethyl ester system and the ascorbic acid–quinone–lard ethyl ester system, respectively.

The difference between the two ΔF^* values, 510 calories per mole per liter, indicates that the addition of quinone to the ascorbic acid–lard ethyl ester system lowers the free energy level sufficiently for the ascorbic acid to be rapidly and completely oxidized. The quinone itself behaves as a catalyst, for at any time during the induction period the original amount introduced can be recovered.

It may seem surprising that such a small difference in energy can increase the rate of oxidation of ascorbic acid so significantly. Calculation of the number of molecules with an energy content above the free energy of each of the respective systems shows that the number of molecules with an energy content greater than 33,894 calories is double the number of molecules with an energy content greater than 34,404 calories.

Michaelis[16] has explored and reviewed the role of semiquinones as intermediate steps of reversible organic oxidation–reduction. The oxidation of organic compounds is frequently sluggish, even when an oxidant of thermodynamically sufficient oxidative power is applied, because it must, as a rule, go through two univalent steps; the intermediate step generally involves climbing over an energy hill; catalysts and enzymes concerned with oxidation–reduction processes convert the substance to be oxidized into some form or compound, in which the intermediate radical has a stronger resonance and so a greater stability than the original form.

These results with ascorbic acid and quinone can be interpreted on this basis, and the following hypothetical mechanism would explain them. The oxidation of ascorbic acid must go through two univalent steps, and to go through the intermediate step means climbing over a larger energy hill when quinone is absent than when it is present. In its presence, the oxidation of ascorbic acid can readily pass through its intermediate step since the free energy of formation of the activated complex, ascorbic acid + quinone, is less than that of the formation of the activated complex, ascorbic acid + peroxide radical. The compulsory univalent oxidation of ascorbic acid is favored more by the presence of quinone than by the presence of fat peroxide radical.

Quinone may act as a catalyst by being reduced to a semiquinone which latter is oxidized back to quinone by the activated peroxide radical, thus protecting the substrate. Necessarily, the free energy of formation of the activated complex of the semiquinone with the peroxide radical must be less than the free energy of formation of the activated complex of ascorbic acid with the peroxide radical. Otherwise, quinone could not act as an intermediary agent. Since only a trace of semiquinone, tested as quinol, could be found throughout the reaction, its rate of oxidation must be very high, significantly higher than that of ascorbic acid, and its free energy value correspondingly less.

In the ascorbic acid–quinone–ester system, the early and complete disappearance of ascorbic acid relative to the long induction period of the fat suggests that the real antioxidant involved is an oxidation product of ascorbic acid. This may be dehydroascorbic, 2,3-diketogulonic, threonic, glycolic, or oxalic acid, or a combination of all of them. The oxidation of ascorbic acid proceeded beyond dehydroascorbic acid and at least to glycolic acid.

The ascorbic acid–ester system differs from the quinone–ascorbic acid–ester system in two important aspects: (1) the presence of quinone permits the rapid oxidation of ascorbic acid, thus protecting the fat substrate until the oxidation products of ascorbic acid can exert their antioxygenic action; (2) the constant presence of quinone offers an intermediate and indirect mechanism of reaction between fat peroxides and ascorbic acid, or its oxidation products.

Summary

The absolute reaction rate of oxidation of ascorbic acid in ethyl esters of lard fatty acids has been measured in the presence and absence of quinone. The synergism of quinone with ascorbic acid in the stabilization of these esters has been shown to be due to the catalytic action of quinone. Quinone acts as a catalyst by being reduced to a semiquinone, which latter regenerates quinone by being oxidized by the activated peroxide radicals; this reduction of the peroxide radical prevents the accumulation of peroxides and thus protects the substrate. Quinone serves as an intermediary agent in the ascorbic acid–ester system by lowering the free energy of formation of the activated complex to such an extent that it doubles the number of particles of ascorbic acid possessing sufficient energy of reaction. The results follow closely the views of Michaelis on compulsory univalent oxidation, and on the basis of these data a mechanism for the synergistic action of quinone with ascorbic acid is proposed.

IOWA CITY, IOWA RECEIVED SEPTEMBER 14, 1943

(16) L. Michaelis, *Chem. Revs.*, **16**, 243 (1935).