di-*t*-butylphenol, yield 36%, m.p. 105° (lit.[11] 103.5–104.5°); 1,2-bis(3,5-di-*t*-butyl-4-hydroxyphenyl)ethane, yield 6%, m.p. 171–172° (lit.[5] 169–170°).

E.s.r. Measurements.—The spectra of the phenoxy radicals were obtained in solution with a Varian V-4500 spectrometer utilizing 100 kc. modulation.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

The Chemistry of 9-Hydroxy-α-tocopherone, a Quinone Hemiacetal[1]

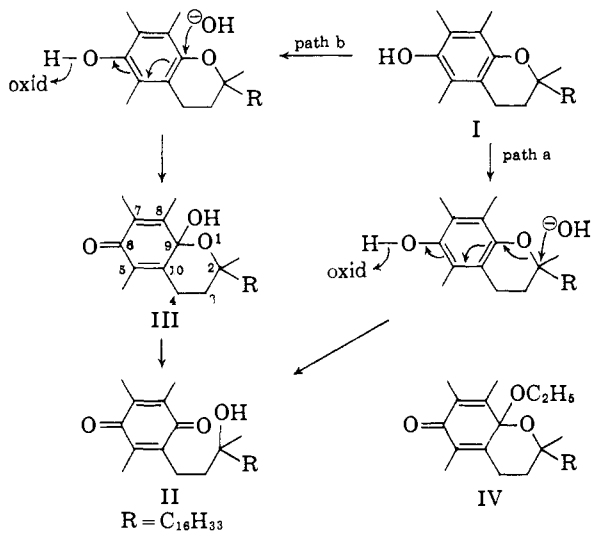
BY WALTER DÜRCKHEIMER[2] AND LOUIS A. COHEN

RECEIVED FEBRUARY 5, 1964

Oxidation of α-tocopherol with N-bromosuccinimide or with tetrachloro-*o*-quinone in aqueous acetonitrile leads to the formation of 9-hydroxy-α-tocopherone (III), the cyclic hemiacetal tautomer of tocopherylquinone. At pH 5.5, the dienone has a half-life time of 44 min.; in petroleum ether, the half-life time is extended to 3–4 hr. The compound is converted into the quinone by acid or alkali and is readily reduced to tocopherol by a variety of agents. The oxidation–reduction potential of α-tocopherol, measured for the first time under reversible conditions, was found to be +720 mv. Oxidation of α-tocopherol in the presence of acetate ion leads to an analogous, highly labile acetoxydienone. The energetics of chromanols and quinones in oxidative phosphorylation are discussed in light of the new data.

Recent years have witnessed a number of efforts to elucidate the mechanisms by which the energy generated in a biological oxidation process may be conserved through the formation of covalent bonds, particularly and ultimately by the conversion of ADP to ATP.[3] The demonstration that chromanols (such as α-tocopherol) and quinones (such as ubiquinone) occur extensively in mitochondrial aggregates,[4] a site of such oxidative phosphorylation, has prompted various proposals regarding the metabolic role of these materials and the manner in which they may serve in electron transport and energy conservation.[3]

The design of a chemical approach to the problem has been hampered, in part, by limitations in the present-day understanding of oxidation mechanisms, particularly with respect to complex phenolic systems. For example, the oxidation of a chromanol (I; or of a hydroquinone monoether, in general) to the corresponding quinone II requires the introduction of an additional oxygen atom; two pathways may be considered which differ in the final disposition of the chroman oxygen atom.[5] The identification of IV as an oxidation product of α-tocopherol in ethanol[6] supports path b and encourages consideration of III as an intermediate in the oxidation of I in the presence of water.[7] It is with the preparation, isolation, and properties of III that the present paper is principally concerned.



The reaction of α-tocopherol with a variety of oxidants (Ag^{+1},[8a] Fe^{+3},[8a] Au^{+3},[8b] Ce^{+4},[8c] Pb^{+4} [8d]) in aqueous media leads ultimately to the quinone, as may be observed by a rapid shift in the ultraviolet spectrum from 292 to 265 mμ. With *neutral, organic* oxidants, such as tetrachloro-*o*-quinone (TClQ) or N-bromosuccinimide (NBS), however, a labile intermediate with a spectral peak at 242 mμ may be detected. We conclude, from the evidence set forth below, that the intermediate is 9-hydroxy-α-tocopherone (III).[9,10] In the presence of stoichiometric quantities of oxidant, the peak at 242 mμ reaches a maximum value in 1–2 min. The rate at which the intermediate is converted

(1) Paper I of a series "Oxidation Mechanisms in Biochemical Processes." A preliminary account of this work has been published: *Biochem. Biophys. Res. Commun.*, **9**, 262 (1962).

(2) Associate in the Visiting Program of the USPHS, 1961–1962.

(3) For recent comprehensive and critical reviews, see (a) Ciba Foundation Symposium, "Quinones in Electron Transport," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1960; (b) E. Racker, "Advances in Enzymology," Vol. 23, F. F. Nord, Ed., Interscience Publishers, Inc., New York, N. Y., 1961, p. 323; (c) E. C. Slater, *Rev. Pure Appl. Chem.*, **8**, 221 (1958); (d) E. C. Slater, *Proc. Intern. Congr. Biochem., 4th, Vienna*, **9**, 316 (1958); (e) P. D. Boyer, "The Enzymes," Vol. III, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1960, p. 353.

(4) D. M. Ziegler, *Am. J. Clin. Nutr.*, **9** (Part II), 43 (1961); F. L. Crane, *Biochemistry*, **1**, 510 (1962).

(5) The duality of pathways has been studied by O^{18} tracer techniques: (a) A. Lapidot and D. Samuel, *Biochem. Biophys. Acta*, **65**, 164 (1962); (b) E. Adler, I. Falkehag, and B. Smith, *Acta Chem. Scand.*, **16**, 529 (1962); (c) P. Schudel, H. Mayer, J. Metzger, R. Rüegg, and O. Isler, *Helv. Chim. Acta*, **46**, 333 (1963). For studies based on product identification, see paper II of this series (W. Dürckheimer and L. A. Cohen, *J. Am. Chem. Soc.*, in press.

(6) (a) C. Martius and H. Eilingsfeld, *Ann.*, **607**, 159 (1957); (b) P. D. Boyer, *J. Am. Chem. Soc.*, **73**, 733 (1951).

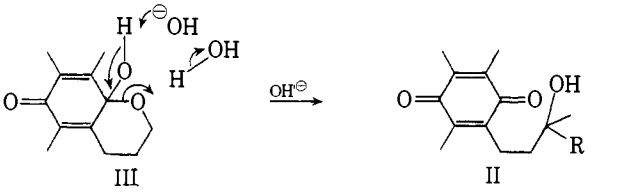
(7) The existence of III as an oxidation intermediate has been considered previously. See W. H. Harrison, J. E. Gander, E. R. Blakley, and P. D. Boyer, *Biochim. Biophys. Acta*, **21**, 150 (1956).

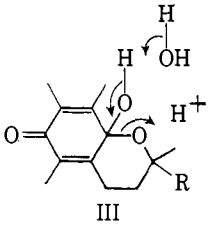
(8) (a) W. John, E. Dietzel, and W. Emte, *Z. physiol. Chem.*, **257**, 173 (1939); (b) P. Karrer and A. Geiger, *Helv. Chim. Acta*, **23**, 455 (1940); (c) M. Kofler, *Verhandl. Schweiz. Naturforsch. Ges.*, 239 (1941); (d) A. Issidorides, *J. Am. Chem. Soc.*, **73**, 5146 (1951).

(9) The parent compound, α-tocopherone, would then be the dienone tautomer of α-tocopherol. Although the 9-hydroxyl function may be *cis* or *trans* to the phytyl side chain, no information has been obtained on the geometrical homogeneity or lack of it in preparations of III.

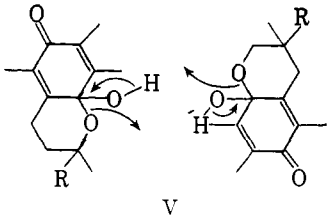
(10) Although III is almost certainly an intermediate in oxidation reactions effected by inorganic cations, its presence is difficult to demonstrate since such cations generally mask the 242 mμ peak of the dienone and become either unreactive or insoluble in the pH range in which III has optimal stability.

into the quinone (242 → 265 mμ) is markedly dependent on pH, as shown in Fig. 1. At all pH values examined, the decomposition follows approximately first-order kinetics. When prepared using NBS (acetate buffer–acetonitrile, 2:5, apparent pH 5.5), the compound exhibits a half-life time of 44 min.; with TClQ (water–acetonitrile, 2:5), a half-life time of 53 min. may be realized. It is evident from Fig. 1 that even a small deviation in the pH′ of the medium from the optimal value of 5.5 results in a rapid conversion of the intermediate into the more stable quinone II. Both the acid- and base-catalyzed rearrangements may be mechanistically analogous to the opening of simple hemiacetals.[11]





From the aqueous oxidation mixtures, III may be extracted into petroleum ether (30–40°), in which solvent the half-life time is extended to 3–4 hr. Despite careful purification and drying of the petroleum ether, the conversion of III into the quinone II could not be prevented or delayed (except by lowering the temperature). It was observed, however, that the stability of III in petroleum ether increases with dilution. Taking advantage of marked differences in the infrared spectral characteristics of III and II,[12] the rate of the conversion in petroleum ether was determined. In this solvent, approximate second-order kinetics were observed, suggesting a bimolecular process (V being the transition state) which leads to the quinone as the principal product.[13]



V

The ultraviolet spectral bands for III, λ_{max} 238 mμ (ε 12,000, petroleum ether), λ_{max} 242 mμ (ε 12,000, aqueous

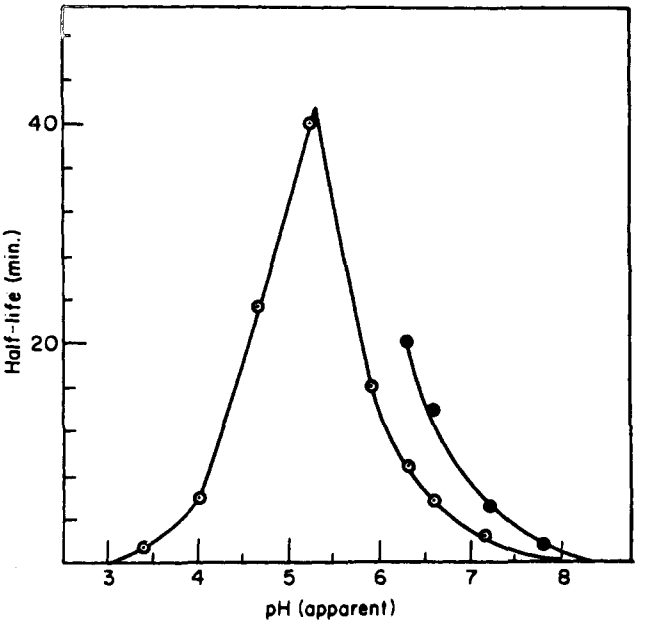
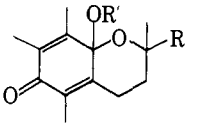


Fig. 1.—Effect of pH on stability of 9-hydroxy-α-tocopherone (I) in acetonitrile buffer (5:2); —⊙— $M/5$ acetate; —●— $M/15$ phosphate.

acetonitrile), are very similar to those reported for 9-ethoxy-α-tocopherone (IV) (see Experimental)[6] and are quite reasonable for a fully alkylated 2,5-dienone system.[14] The infrared spectrum of III (petroleum ether) shows hydroxyl absorption at 3485 (2.87 μ), a carbonyl band at 1678 (5.96 μ), a conjugated olefinic band at 1628 (6.14 μ), and the chroman band at 1250 cm.$^{-1}$ (8.0 μ). The purity of fresh preparations of III is estimated to be at least 90%, α-tocopherol and its quinone being the principal contaminants. Such estimates are based on ultraviolet spectral ratios and on quantitative thin layer chromatography of samples of III both before and after conversion to II[15] or reduction to I.

That III is not a dimeric substance of structure VI is clearly shown by several facts: decomposition leads almost exclusively to the quinone II, without the accompanying liberation of an equivalent of α-tocopherol; spectral titration indicates a consumption of 1 mole of NBS per mole of substrate; the infrared spectrum shows a strong hydroxyl band which disappears in the course of the decomposition of III to the quinone; the ultraviolet spectrum shows no significant phenolic absorption.



VI, R=$C_{16}H_{33}$
R′=α-tocopheryl

The dienone is converted to α-tocopherol by reducing agents such as aqueous ascorbic acid, pyrogallol, potassium iodide, or toluhydroquinone, but is unaf-

(11) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p. 542.

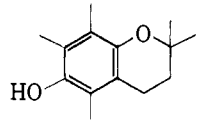
(12) The stretching band for the side-chain hydroxyl of II appears in the infrared at 3550 cm.$^{-1}$ and has a low intensity; on the other hand, the hemiacetal hydroxyl of III shows a sharp band of high intensity at 3485 cm.$^{-1}$. This difference is ample to permit the following of rates of conversion of III to II.

(13) *Cf.* C. G. Swain and J. F. Brown, Jr., *J. Am. Chem. Soc.*, **74**, 2534, 2538 (1952).

(14) (a) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 19; (b) J. Derkosch and W. Kaltenegger; *Monatsh.*, **88**, 778 (1957); (c) E. Müller and K. Ley, *Chem. Ber.*, **88**, 601 (1955).

(15) Decomposition of III in acidic media leads to traces of dimeric substances in addition to II. For a discussion of dimer formation, see paper III of this series, W. Dürckheimer and L. A. Cohen, in preparation.

fected by hydroquinone; on the other hand, α-tocopherol is oxidized, in inert solvents, to dimers by benzoquinone but much more slowly by tolu-*p*-quinone. In earlier efforts to determine the oxidation–reduction potential of α-tocopherol,[16] the limitations introduced by the irreversibility of the tocopherol–tocopheryl-quinone transformation, as well as by the instability of a postulated intermediate, have been noted. With the demonstration of a moderate stability for III at pH′ 5.5, it became possible to reinvestigate the problem under conditions of reversibility. Preliminary experiments indicated that the rate of oxidation of α-tocopherol by various quinones or indophenol dyes was lower than, or comparable to, the rate of decomposition of the intermediate. By following the increase in optical density at 242 mμ, it could be shown that partial oxidation of α-tocopherol by half the stoichiometric amount of NBS is complete in 6 min.; subsequently, the optical density of the dienone peak decreases as III is transformed slowly into II. A similar pattern was observed when the electrode potential was measured with respect to time. Within 6 min. of addition of the NBS, the potential had decreased approximately 150 mv., further changes being relatively small as III decomposed. Indeed, the rate of potential changeover 2 hr. after addition of NBS could be correlated with the rate of decomposition of III determined previously by ultraviolet spectroscopy. In this way E_0 for α-tocopherol was found to be +710 mv. in 80% acetonitrile or +720 mv. following correction for the medium effect. Basing their calculations on polarographic measurements, Smith, *et al.*, found for α-tocopherol E_0 = +770 mv. (75% ethanol, pH 4) but for the simpler chroman VII, E_0 = +691 mv. (50% methanol, pH 3.6).[17]

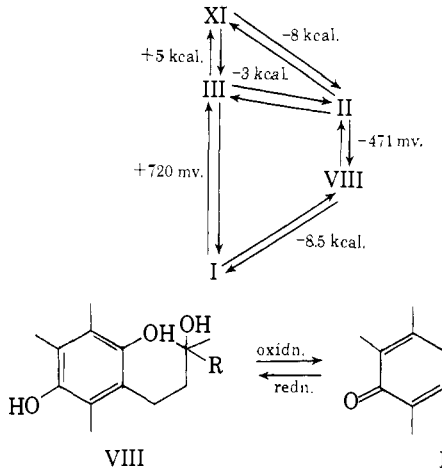


VII

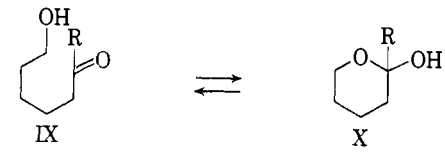
Since these results may be pertinent to biological systems, it is desirable to obtain quantitative data for the various transformations shown in Chart I. For the system II ⇄ VIII, E_0 is taken as +471 mv., based on polarographic data.[18] By use of the relationship

$$\Delta F^\circ = -n\mathfrak{F}\,\Delta E^\circ$$
$$= (-2)(23{,}063)(0.720 - 0.471) = -11.5 \text{ kcal./mol.}$$

it follows that the total free-energy change for the combined steps III → II and VIII → I is −11.5 kcal./mol.[19] Separation of this value into its components requires a knowledge of the equilibrium constant for at least one of these two transformations. Experimental determination of K for either equilibrium could not be realized; however, several considerations lead to an estimate of the extent to which III may exist in equilibrium with II: (a) δ-hydroxyaldehydes and ketones (IX) exist predominantly in the cyclic form X[20]; (b) the relatively facile hydration of a quinone carbonyl group has been demonstrated by the fact



VIII II



that complete O^{18} exchange in H_2O occurs in 10 days at 25°[21]; (c) the loss in resonance energy in going from the quinone to the dienone system may be estimated at 1–2 kcal.[22]; (d) earlier studies[16d] suggest that reduction of the quinone II to α-tocopherol in acidic media may proceed *via* III or the corresponding carbonium ion XII rather than through VIII. Using various spectral techniques, we have been unable to measure the equilibrium concentration of III in the presence of a very large excess of the quinone II; nevertheless, we feel that the preceeding arguments support the contention that II may cyclize back to III, if only to a small extent (see also footnote 30a). With respect to the system VIII ⇄ I, available evidence indicates that the equilibrium is overwhelmingly on the side of the chroman: (a) in various syntheses of α-tocopherol by Friedel–Crafts alkylation of trimethylhydroquinone, VIII has not been detected as a by-product[23]; (b) in the present investigation, efforts to effect a detectable cleavage of the chroman ring of I have been unsuccessful, using conditions comparable to those under which the hydroquinone system is readily converted to the chroman.[24] If we assume that 1% of III exists in equilibrium with II, the free-energy change of the transformation would be about 3 kcal. per mole.

(16) (a) L. I. Smith, L. J. Spillane, and I. M. Kolthoff, *J. Am. Chem. Soc.*, **64**, 447 (1942); (b) C. Golumbic and H. A. Matill, *J. Biol. Chem.*, **134**, 535 (1940); (c) W. Wachs, *Biochem. Z.*, **319**, 561 (1949); (d) W. H. Harrison, J. E. Gander, E. R. Blakley, and P. D. Boyer, *Biochim. Biophys. Acta*, **21**, 150 (1956); (e) E. Knobloch, F. Machà, and K. Mňouček, *Chem. Listy*, **46**, 718 (1952).

(17) L. I. Smith, I. M. Kolthoff, S. Wawzonek, and P. M. Ruoff, *J. Am. Chem. Soc.*, **63**, 1018 (1941).

(18) L. I. Smith, L. J. Spillane, and I. M. Kolthoff, *ibid.*, **64**, 644 (1942).

(19) J. S. Fruton and S. Simmonds, "General Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1959, p. 300.

(20) C. D. Hurd and W. H. Saunders, Jr., *J. Am. Chem. Soc.*, **74**, 5324 (1952), and references cited therein; G. S. Hammond, "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 460.

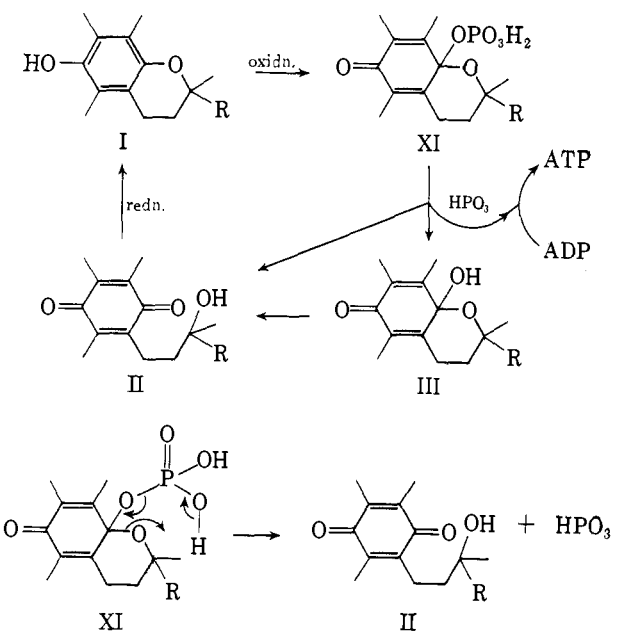
(21) V. V. Fesenko and I. P. Gragerov, *Dokl. Akad. Nauk SSSR*, **101**, 695 (1955); see also ref. 5b.

(22) G. W. Whelaud, "Resonance in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1955, pp. 85, 99.

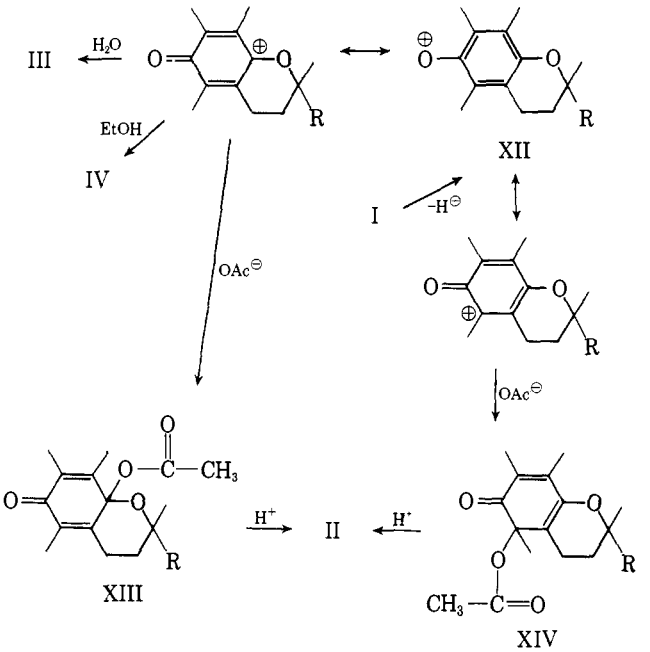
(23) L. I. Smith, *Chem. Rev.*, **27**, 287 (1940).

(24) (a) W. John, E. Dietzel, and W. Emte, *Z. physiol. Chem.*, **257**, 173 (1939); (b) L. I. Smith and H. C. Miller, *J. Am. Chem. Soc.*, **64**, 440 (1942); (c) M. Tishler and N. L. Wendler, *ibid.*, **63**, 1532 (1941).

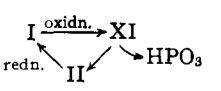
In various discussions of the possible role of chromanols and quinones in oxidative phosphorylation,[3] a phosphorylated dienone such as XI has been considered as a donor of the metaphosphate which converts ADP to ATP. It may be estimated that the difference in free-energy content between XI and III is comparable to that between glucose 1-phosphate and glucose, *i.e.* 5 kcal.,[25] a value at least 3 kcal. too low to permit the conversion of ADP to ATP.[26] If species such as XI are to have significance in oxidative phosphorylation,[27] energy considerations require the omission of III as a member of the oxidation–reduction cycle, with the direct conversion of XI to II being a more reasonable alternative.



Just as the formation of III may be considered to occur *via* the reaction of a carbonium ion (XII) with water, the formation of derivatives of III should be possible in the presence of other anionic species. If α-tocopherol is oxidized in water-containing media saturated with acetate ion, the only species extractable into petroleum ether is the hydroxydienone III. The same result is observed in aprotic media containing a large excess of acetate ion and only a trace of water. Whether the carbonium ion XII has a greater preference for water than for acetate or whether an initially formed acetoxydienone is solvolyzed by water very rapidly has yet to be determined. Under rigorously anhydrous conditions, however, oxidation of α-tocopherol in aprotic media containing acetate ion leads to the formation of a mixture of 9-acetoxy-α-tocopherone (XIII) and the *o*-acetoxydienone (XIV). Both species show an acid lability comparable to that of III.[28] The structures assigned to XIII and XIV are based on spectral data, acetyl group determination, and their rapid conversion to α-tocopherylquinone by aqueous acid (see Experimental).[29] The stringent requirements for the formation of XIII reinforce the argument for an energy difference between XIII and III at least comparable to that between glucose and glucose 1-acetate (*cf.* glucose and glucose 1-phosphate[25]). The mixture of XIII and XIV was found to be incapable of acetylating aniline or benzylamine. In view of the preceding considerations, it is, therefore, not surprising to find that the free energy to be derived from the conversion of XIII to III is insufficient to permit the former to be an acetylating agent. In this respect, the dienone phosphate XI is *not* analogous to the acetate since, in principle, it is capable of providing some 8 kcal. of free energy by its conversion to the quinone II and metaphosphate in a *single* step.[30]



We have shown that, under aprotic conditions, 9-acetoxy-α-tocopherone (XIII) may be formed in the course of oxidation of α-tocopherol, thus storing potentially useful energy in a labile species; furthermore, the stabilities of both III and XIII are enhanced in nonpolar media (compare the lipoid region in mitochondrial aggregates). Encouraged by these results, we are presently concerned with an experimental demonstration of a three-step oxidative cycle resulting in the generation of metaphosphate from inorganic phosphate.[31]



Experimental[32]

9-Hydroxy-α-tocopherone (III). Method A.—A solution of 0.043 g. (0.1 mmole) of DL-α-tocopherol in 20 ml. of acetonitrile (purified by distillation from phosphorus pentoxide) was cooled to 0° and added rapidly to a stirred, chilled solution of 0.027 g. (0.11

(25) P. Oesper, "Phosphorus Metabolism," Vol. I, W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1954, p. 523.

(26) E. A. Robbins and P. D. Boyer, *J. Biol. Chem.*, **224**, 121 (1957); R. A. Alberty, R. M. Smith, and R. M. Bock, *ibid.*, **193**, 425 (1951).

(27) Although quantitative data for the ubiquinone system would be more pertinent to the argument, we assume the replacement of ring-methyl by methoxyl to have a fairly constant effect upon the oxidation–reduction potentials and free energies of the various members of the cycle.

(28) See also ref. 3a, p. 201.

(29) A detailed report on the chemistry of acyloxydienones derived from tocopherol is in preparation.

(30) It should be noted that an analogous one-step conversion to quinone of the phosphate corresponding to XIV is not feasible.

(31) The direct reduction of tocopherylquinone to tocopherol with sulfhydryl reagents such as dihydrolipoic acid in nonpolar media has recently been achieved (M. A. Oxman and L. A. Cohen, in preparation).

(32) Ultraviolet spectra were measured using a Cary recording spectrophotometer, Model 14, and infrared spectra with a Perkin–Elmer Infracord spectrophotometer. Microanalyses were performed by Mr. H. G. McCann and his associates of this Institute.

mmole) of tetrachloro-*o*-quinone in 40 ml. of 50% aqueous acetonitrile. When the reaction mixture had discharged its initial yellow color (20–30 sec.), it was shaken vigorously with 40 ml. of purified petroleum ether (30–40°); the petroleum ether layer was washed four times with ice-cold water and dried rapidly with sodium sulfate.[33] On the basis of ultraviolet spectra, the petroleum ether solution contained, in addition to the dienone (238 mμ), small amounts of α-tocopherol and α-tocopherylquinone (less than 10%). The solvent was removed by concentration *in vacuo* at 25° and the residual oil used without further attempts at purification.

Method B.—The oxidation was performed as in method A except that the solution of α-tocopherol (0.1 mmole) was added to a solution of 0.071 g. (0.4 mmole) of N-bromosuccinimide in a mixture of 20 ml. of 0.1 *M* phosphate (KH_2PO_4) and 20 ml. of acetonitrile. Since petroleum ether extracts containing III gave no precipitate with silver nitrate, either before or after treatment with formic acid,[34] contamination by bromodienones or by benzyl bromides may be excluded.

Variation in the Stability of III with pH.—To a mixture of 1 ml. of acetate buffer (0.2 *M*) and 2 ml. of NBS (0.4 m*M*) in acetonitrile, contained in a quartz cuvette, was added 0.5 ml. of α-tocopherol (0.4 m*M*) in acetonitrile. The mixture was shaken for several seconds and the ultraviolet spectrum determined at various time intervals, with particular attention to intensity changes at 242 and at 265 mμ. Since the extinction coefficient of the quinone (18,700) is greater than that of the dienone (12,000), calculations were based on optical density changes at 265 mμ. To a reasonable approximation, the collapse of III to α-tocopherylquinone followed first-order kinetics. The half-life times at various pH′ values were determined graphically and the results are shown in Fig. 1.[35] For pH′ values beyond the range of the buffer, 0.2 *M* acetic acid and 0.2 *M* sodium acetate were used. Maximum stability (half-life 44 min.) was observed at pH′ 5.5 (pH 3.9).

Analogous studies were carried out with phosphate buffer, a slightly greater stability being observed in the limited pH range examined (Fig. 1).[35] When α-tocopherol was oxidized with tetrachloro-*o*-quinone in water–acetonitrile (2:5), the hydroxydienone showed a half-life of 53 min. at pH′ 5.8. Thus, any specific effect of the buffer in catalyzing the decomposition of II may reasonably be excluded.

Spectral Properties of III.—The dienone exhibits an absorption band at 238 (ε 12,000 ± 500, petroleum ether) and at 242 mμ (ε 12,000 ± 500, water–acetonitrile, 2:5). The molar extinction coefficient was calculated by determining the ratios of the optical density of III to that of α-tocopherylquinone at various stages of decomposition and extrapolating back to zero time. By comparison, 9-ethoxy-α-tocopherone (IV) shows a maximum at 237 mμ (ε 12,000, isooctane).

Infrared spectra of III (in petroleum ether) show a single, sharp hydroxyl band at 3485, a carbonyl band at 1678, a conjugated double bond absorption at 1628, and a chroman band at 1250 cm.$^{-1}$. Fresh preparations of the dienone exhibit a weak band caused by α-tocopherylquinone at 1648 cm.$^{-1}$ (6.07 μ). Absorption bands in the carbonyl region agree well with those of 9-ethoxy-α-tocopherone[6b] and of similarly constituted dienone systems.[14]

By performing the operations of oxidation, extraction with petroleum ether, washing, drying, and concentrating as rapidly as possible, the infrared spectrum of III could be obtained 12–15 min. after addition of oxidant. At that time the extent of conversion to the quinone was still relatively low; even in dry petroleum ether, the spectrum of III changes slowly and, after 18 hr., is almost identical with that of α-tocopherylquinone. Since the hydroxyl stretching band of III at 3485 cm.$^{-1}$ is of considerably greater intensity than that of the quinone II at 3550 cm.$^{-1}$, the rate of conversion of III to II could be followed by the decrease in intensity of the hydroxyl band. The reaction was found to follow second-order kinetics up to at least 70% conversion. Initial and final concentrations of both dienone and quinone were determined from ultraviolet spectra of the same solutions following 500-fold dilution. The kinetics were followed by infrared rather than by ultraviolet spectroscopy since the logarithmic record obtained by the former method permitted analysis over a much wider concentration range. It could be demonstrated qualitatively that the stability of III in petroleum ether was increased by increasing dilution as well as by reduction in temperature. At concentrations of 0.01–0.05 *M* in petroleum ether, the dienone was found to have a half-life of 3–4 hr.

9-Ethoxy-α-tocopherone (IV).—Oxidation of α-tocopherol with ferric chloride in ethanol according to Martius and Eilingsfeld[6a] provided mostly starting material; the use of ferric chloride–α,α′-bipyridyl, according to Boyer,[6b] led to IV in high yield and good purity. The dienone acetal is also formed in excellent yield by oxidation of α-tocopherol with NBS in 95% ethanol containing 1 equiv. of sodium acetate. In our hands, the best oxidant, with respect to simplicity of procedure and purity of product, was found to be tetrachloro-*o*-quinone.

A solution of 0.215 g. of α-tocopherol in 80 ml. of ethanol was cooled to −5° and 0.13 g. of tetrachloro-*o*-quinone added over 15 min. to the stirred solution. A 25-ml. portion of cyclohexane was added, followed by 80 ml. of water. The layers were separated and the cyclohexane solution washed several times with water and dried over sodium sulfate. Thin-layer chromatography of the material on silicic acid resulted in decomposition; however, the compound was completely stable on alumina and purity could be estimated rapidly by chromatography on microslides. In the solvent system benzene–cyclohexane (1:1), the R_f of α-tocopherol is 0.30 and that of IV, 0.63. Samples of IV, prepared as described above, showed a trace of α-tocopherol as the only contaminant. By preparative thin-layer chromatography in the same solvent system, pure IV was obtained rapidly in quantities sufficient for a number of further studies.

Reactivity of III.—A solution of the hydroxydienone in petroleum ether was prepared by method A; aliquots were shaken for 3 min. with aqueous solutions of various reagents and the petroleum ether layer then examined by ultraviolet spectroscopy and by thin-layer chromatography. The results are summarized in Table I.

TABLE I

REACTION PRODUCTS OF 9-HYDROXY-α-TOCOPHERONE WITH VARIOUS REAGENTS

Reagent	Products
Ascorbic acid (10%)	α-Tocopherol (97%)[a]
Pyrogallol (5%)	α-Tocopherol (98%)[a]
Sodium borohydride (0.1 *M*)	α-Tocopherol (50%), α-tocopherylquinone (50%)
Potassium iodide, pH 3 (1 *M*)	α-Tocopherol (70%), α-tocopherylquinone (30%)
Hydroquinone (5%)	No change
Toluhydroquinone (5%), 3 min.	α-Tocopherol (27%)
Toluhydroquinone (5%), 12 min.	α-Tocopherol (91%)
Hydrochloric acid (6 *N*)	α-Tocopherylquinone (95%), dimers (5%)[b]

[a] Remainder of the material is quinone II. [b] The nature of the dimers will be discussed in a subsequent paper.

Reaction of α-Tocopherol with Quinones. A. Oxidation with Toluquinone.—To a solution of 0.43 g. of α-tocopherol in 10 ml. of benzene was added 0.61 g. of toluquinone (5 equiv.) and the mixture stored at 25° for 2 days. The solvent was removed *in vacuo* and the residue extracted with petroleum ether. The solution was washed with 10% ascorbic acid,[36] several times with water, and dried. Thin-layer chromatography of the solution on silica gel (developed with benzene) showed the major component to be starting material, together with a trace of dimeric material; no tocopherylquinone was observed.

B. Oxidation with Benzoquinone.—To a solution of 0.43 g. of α-tocopherol in 40 ml. of benzene was added 2.16 g. of benzoquinone. After several days at 25°, the black precipitate of quinhydrone was removed and the filtrate concentrated to dryness. The residue was extracted repeatedly with petroleumether (b.p. 30–40°) and the extract washed with aqueous ascorbic acid and water. The ultraviolet spectrum of the solution indicated both

(33) Judging from ultraviolet and infrared spectra, petroleum ether extracts neither tetrachloro-*o*-quinone, tetrachlorocatechol, nor acetonitrile from the aqueous layer.

(34) Formic acid is effective in reducing a bromodienone (positive halogen) to bromide ion.

(35) When acetate buffer (0.2 *M*) at various pH values (3.2–5.6) is diluted with acetonitrile (2:5), the apparent pH increases by a constant amount (1.60 ± 0.05 units). Phosphate buffer (0.66 *M*, pH 4.9–6.4), when diluted with acetonitrile in the same ratio, shows a constant increase in apparent pH of 1.43 ± 0.10 units.

(36) Control experiments showed that tocopherylquinone is not reduced by ascorbic acid under these conditions; see also ref. 7.

tocopherylquinone and tocopherol to be absent; a peak at 287 mμ was unaltered by addition of alkali and indicated the presence of nonphenolic material. That the tocopherol had been converted almost completely into dimers and trimers was demonstrated by thin layer chromatography.[15]

Determination of the Oxidation–Reduction Potential of α-Tocopherol.—All potential measurements were carried out at 25° using a Radiometer autotitrator, Model TTT-1a, equipped with platinum and calomel (satd. KCl) electrodes. In a 15-ml. beaker were placed 1 ml. of a solution of α-tocopherol (0.01 *M*) in purified acetonitrile, 2.5 ml. of acetonitrile, and 1 ml. of acetate buffer (0.2 *M*, pH 3.9). With the electrodes immersed, the solution was stirred magnetically until a constant reading of +70–75 mv. had been attained (*ca.* 5 min.); 0.5 ml. of a solution of NBS (0.01 *M*) in acetonitrile was then added rapidly. Immediately after addition of the oxidant, the potential rose to +275–290 mv. and, as NBS was consumed, decreased rapidly over 6 min. to a value of +135 mv. Examination of comparable reaction mixtures by ultraviolet spectroscopy indicated formation of the hydroxydienone III to be maximal at 6 min. Further decreases in the potential occurred much more slowly as dienone disappeared, reducing the ratio dienone/tocopherol from its value of 1.0 at 6 min. From the rate data which had been determined spectroscopically for the conversion of III to II at the same pH value,[37] it was possible to calculate the anticipated rate of change of potential, which agreed reasonably well with that observed over 1–2 hr. Additional runs were made, varying the pH (apparent) from 5.0 to 6.0 and the equivalents of NBS added from 0.25 to 0.75. A correction (+10 mv.) for the solvent system, which contained 80% acetonitrile, was obtained by measurement of the potential of a quinhydrone solution at pH′ 5.5 in 80% acetonitrile–buffer.[38] Based on the average of 15 runs, E_0 for α-tocopherol was calculated as +720 ± 5 mv.

Stability of α-Tocopherol to Acid Hydrolysis.—Taking advantage of the fact that tocopherylhydroquinone (VIII) is highly sensitive to air oxidation in neutral or alkaline media,[8a] the opening of the chroman ring of α-tocopherol was followed by the appearance of a quinone peak at 265 mμ. To solutions of tocopherol in ethanol, dioxane, and 1-butanol was added aqueous sulfuric acid so that final concentrations of acid ranged from *N* to 12 *N*. The solutions were refluxed for periods up to 8 hr. Aliquots were withdrawn, the acid neutralized with excess sodium acetate, and the mixtures were evaporated to dryness. The residues were extracted with cyclohexane and the extracts aerated for 30 min. following addition of 0.1 ml. of triethylamine to each sample. In no case was there evidence for quinone formation. By a similar procedure, the authentic hydroquinone VIII was converted rapidly to the quinone.

Acetoxy-α-tocopherones (XIII and XIV).—To a solution of 0.043 g. (0.1 mmole) of α-tocopherol in 5 ml. of absolute acetonitrile (purified by repeated distillation from phosphorus pentoxide) was added 0.051 g. of tetramethylammonium acetate (dried *in vacuo*) followed by 0.018 g. (0.1 mmole) of NBS. The mixture was vigorously shaken, a precipitate of tetramethylammonium bromide appearing almost immediately. Following removal of the precipitate, the solution was evaporated to dryness *in vacuo* at 25° and the residue extracted with cyclohexane. The cyclohexane solution showed an ultraviolet maximum at 330 mμ (shoulder at 360 mμ) and a strong shoulder at 240 mμ. Treatment of the cyclohexane solution with either strong acid or alkali resulted in the disappearance of the entire spectrum and the appearance of a new absorption band at 265 mμ (tocopherylquinone). The infrared spectrum in carbon tetrachloride of the mixture of acetoxydienones showed the absence of hydroxyl absorption and strong carbonyl bands at 1750 (5.72 μ), 1724 (5.80 μ), 1681 (5.95 μ), and 1665 cm.$^{-1}$ (6.01 μ). In one preparation, the cyclohexane solution was concentrated *in vacuo* at 25° and the residual oil pumped under high vacuum to remove traces of acetic acid.[39] The material was immediately analyzed for acetyl content.

Anal. Calcd. for 1 acetyl: 9.38. Found: acetyl, 7.18.

An excess of aniline was added to a solution of the acetoxydienones in cyclohexane. After storage overnight, the mixture was examined by thin-layer chromatography. The presence of acetanilide could not be demonstrated, dimers of tocopherol being the only transformation products found; similar results were obtained with benzylamine.

Thin-Layer Chromatography.—Compounds were adsorbed onto thin-layer plates (silica gel G, according to Stahl) in various nonpolar solvents. Spots were made visible by spraying the plates with 50% sulfuric acid followed by heating at 100° for 15 min. In the solvent system benzene–cyclohexane (1:1), R_f values are: α-tocopherol, 0.37; α-tocopherylquinone, 0.05; dimer, 0.80. With chloroform as solvent, R_f values are: α-tocopherol, 0.80; α-tocopherylquinone, 0.13.

(37) The apparent pH, 5.5, remained constant throughout the run.

(38) No additional correction was made for the change in potential owing to conversion of III to II during the first 6 min. of reaction; the correction, at pH′ 5.5, may be estimated at 1–2 mv.

(39) Infrared spectra prior to analysis failed to reveal acetic acid or other hydroxylic species.