# An Addition Compound of Oxidized Tocopherol and Linoleic Acid

**W.L. PORTER, L.A. LEVASSEUR** and **A.S. HENICK**,
U.S. Army Natick Laboratories, Food Laboratory, Natick, Massachusetts 07160

## ABSTRACT

There is evidence that an addition compound of oxidized *dl*-alpha-tocopherol and linoleic acid is formed when the components are adsorbed in mixed monolayer on silica gel at a molecular ratio of 1:20, and subjected to heating in air at 80 C. A relatively nonpolar tocopheryl quinone is also formed in smaller amounts. These are the major tocopherol oxidation products isolated in this system and do not correspond to any known to the authors. The addition compound has about the same mobility as linoleic acid in most thin layer chromatography (TLC) and chromatographic systems, but can be isolated by successive chromatography on silica and gel filtration on Sephadex LH-20. It yields a single spot in TLC in several systems. The elemental analysis is reproducible and consistent with a simple addition compound of linoleic acid and bivalently oxidized tocopherol. The compound has a carboxyl group which can be esterified. The ester has about the same TLC mobility as methyl linoleate. The molecular weight of the ester is 722.6. The UV spectrum shows a single peak, $\lambda_{max}^{ETOH}$ = 3000 Å, E = 4.74. The IR spectrum shows a very strong chroman ether band at 9.12 $\mu$, a strong methyl band at 7.24 $\mu$ and carboxyl but no hydroxyl absorption. The NMR spectrum shows, in contrast to that of tocopherol, a reduction in aromatic methyl protons, a carboxyl proton exchangeable with deuterium oxide, but no hydroxyl proton. The compound does not reduce Emmerie-Engel reagent prior to treatment with concentrated hydriodic acid, nor do the ether-extractable products after such treatment. The present data are consistent with an addition product whose bridging group is a new chroman ring.

## INTRODUCTION

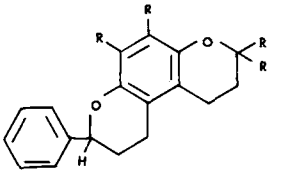
The oxidation of statistical monolayers of fatty acids formed by adsorption on silica gel, both with and without added *dl*-alpha-tocopherol, has been reported from this laboratory (1,2). The products of these oxidations were studied to determine the relative translational mobility of the molecules in the monolayer. Since tocopherol and its oxidation products have characteristic UV absorptions indicative of monomeric and polymeric forms, and since we have shown that the silica gel system, under suitable experimental conditions, permits UV study of the products while in monolayer (2), it was decided to study first the alterations of this molecule. Although quinones and self-polymeric forms have been frequently reported (3-8), compounds of oxidized *dl*-alpha-tocopherol with molecules other than tocopherol under conditions presumably productive of free radicals, have not been frequently demonstrated. Only the 9-hydroxy-alpha-tocopherone (9), the 9-ethoxy-alpha-tocopherone (10), and the 5-benzoyloxymethyl-*dl*-alpha tocopherol (11) appear to have been reported from in vitro reactions. From the alpha-tocopherol model compound, 6-hydroxy-2,2,5,7,8-pentamethyl chroman, Nilsson (12) prepared a styrene Diels-Alder type adduct (Fig. 1) during alkali ferricyanide oxidation in styrene solution. Also from the same model compound, Skinner and Parkhurst (13) have prepared Diels-Alder type adducts with dihydropyran and tetracyanoethylene in aprotic solvents.

About 40% of the tocopherol adsorbed from petroleum ether at a 1:20 molar ratio with linoleic acid in monolayer on silica gel and oxidized at 80 C in air was recovered as a 1:1 adduct of oxidized tocopherol with linoleic acid. The adduct is quite stable, has a free carboxyl group and about the same chromatographic mobility as the free fatty acid in all systems tested, except gel permeation chromatography.

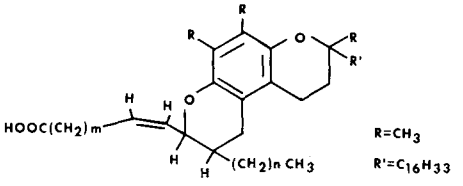
About 10% of the adsorbed tocopherol was recovered as a relatively nonpolar monomeric quinone of tocopherol without a hydroxyl group. Neither of the products corresponds to compounds previously reported (6).

## EXPERIMENTAL PROCEDURES

Silica Gel G was supplied by Warner-Chilcott Laboratories Instruments Division, Richmond, California. It was acid-washed four times by the procedure of Stahl (14). Iron content of the final acid washings was 0.75 ppm, as deter-



CHROMANOL-STYRENE ADDUCT




TOCOPHEROL-LINOLEIC ACID ADDUCT

FIG. 1. Structure of chromanol-styrene adduct and of tocopherol-linoleic acid adduct; n + m = 12. Relative location of methyl, carboxyl and double bond of linoleic acid moiety not determined.

mined by atomic adsorption spectrophotometry.

Linoleic acid was purchased from the Hormel Institute, and was used as received, since further purification in our hands gave a product not noticeably more free of UV absorption at 233 mμ than the original, which shows less than 0.1% conjugated diene. There is about 0.1 moles percent of an impurity with the UV spectrum and chromatographic behavior of conjugated trienoic acid.

Both synthetic, racemic *dl*-alpha-tocopherol and natural *d*-alpha-tocopherol were used. The synthetic tocopherol was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, and was of N.F. grade (96% tocopherol). It was purified by eight repetitions of the acid and alkali wash procedure of Parker and McFarlane (15). The product gave a single spot in several systems of thin layer chromatography (TLC) and UV absorbance in agreement with literature values.

Natural *d*-alpha-tocopherol was purchased both from Fisher Scientific Company and from Distillation Products Industries. These preparations, which are initially of high purity, were used without further purification from an unopened ampoule and were pure to TLC and UV analysis.

Redistilled, low-boiling (35-52 C) petroleum ether of A.C.S. Reagent Grade was used as adsorbing solvent. It was deoxygenated by 1 hr of flushing with dry nitrogen. Benzene and chloroform used as eluting solvents in chromatography were also of A.C.S. Reagent Grade and were used as received.

Silicic acid used for the initial chromatography was Mallinckrodt A.R. Grade, "Suitable for Chromatographic Analysis by the Method of Ramsey and Patterson." It was activated at 110 C for at least 10 hr, and column beds were washed with about three bed volumes of benzene.

For the final gel filtration of the compounds, Sephadex LH-20 was purchased from Pharmacia Fine Chemicals, Inc. The gel was allowed to swell overnight in a mixture of chloroform and cyclohexane (8:2 v/v). After column packing, it was washed with about three bed volumes of chloroform.

The methods of adsorption on silica gel of a monolayer of lipid containing linoleic acid and tocopherol at a molar ratio of 20:1, subsequent controlled air oxidation at 80 C, and monitoring of the oxidation by oxygen uptake and UV measurements of the monolayer while on silica, have been reported elsewhere (1,2).

Briefly summarized, the method entails adsorption on 2 g of acid-washed, activated Silica G at room temperature of approximately 370 mg of linoleic acid and 25 mg of tocopherol from solution in 45 ml redistilled petroleum ether in an acid-washed, 100 ml round bottom flask. After 40 min of shaking and 3 min of settling, the supernatant solvent is decanted and the adhering residual solvent removed during 1 hr under dry nitrogen flow at slightly above atmospheric pressure. The reaction vessel is then flushed with filtered laboratory air for 5 min, capped with a rubber serum bottle stopper, placed in an 80 C oil bath, and sampled for headspace gas at appropriate intervals, using a Fisher Gas Partitioner (16). After this procedure, no petroleum ether peak could be detected by the Gas Partitioner.

It has been previously shown (1) that adsorption conducted under the above conditions conforms to the Langmuir adsorption isotherm. The amount of linoleic acid adsorbed at the solution concentration used was equivalent to about 75% of theoretical monolayer coverage and was approximately the molecular equivalent of the available oxygen in the flask.

Flasks were removed at appropriate intervals and samples of lipid-coated silica were subjected to successive elution and two-stage chromatography, first in a silica-benzene and later in a gel filtration system. Final purification involved repeated gel filtration, which accomplished the difficult separation of the tocopherol-linoleic acid adduct from linoleic acid.

For the silicic acid column chromatography, since the compounds had been calculated to be in monolayer form on the silica, they were eluted directly from the oxidized lipid-coated

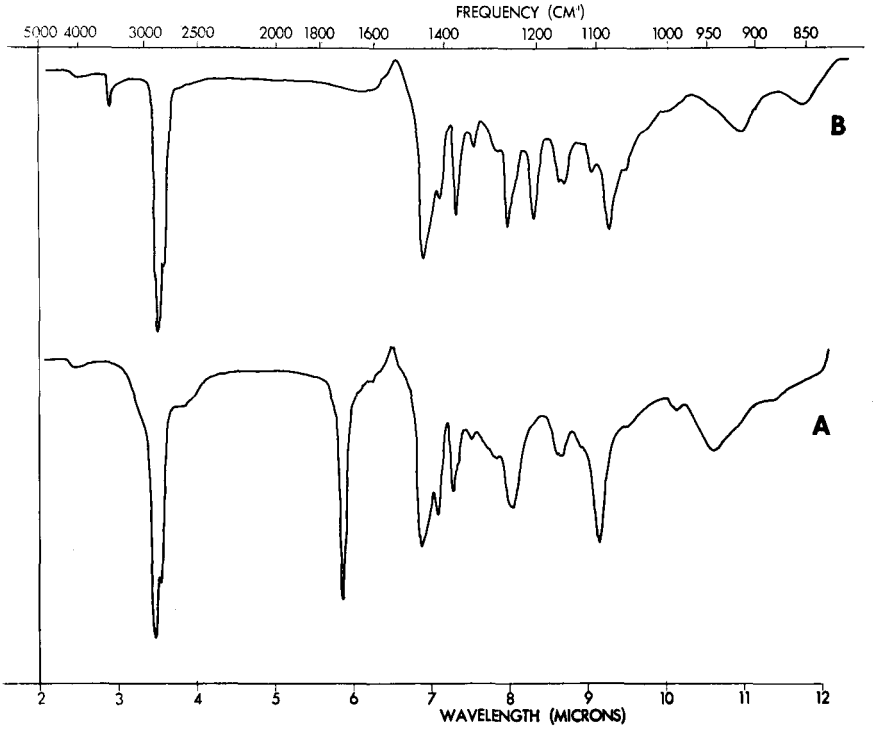



FIG. 2. IR spectrum of tocopherol adduct (A) and of tocopherol (B).

silica onto the top surface of the silicic acid column bed. The 2 g sample of lipid-coated silica, representing the charge of one flask, was dusted through a long-stemmed funnel onto a filter paper disk covering the top of the silicic acid bed surface, which was submerged in ca. 1 ml of the slurrying solvent, benzene. A second disk was floated into place above the charge, solvent was added cautiously, and elution commenced. Among other minor fractions, about 2 mg of a bright yellow, relatively nonpolar tocopheryl quinone was usually eluted at about 1.2 bed volumes. The nearly colorless tocopherol-linoleic acid adduct was eluted at two to three bed volumes, depending on the silica activation. Linoleic acid (and its conjugated trienoic acid impurity) was eluted shortly after the emergence of the adduct, and cochromatographed with it during much of the elution. Of the initial charge of 375 mg, there were retained for gel filtration the middle fractions containing about 165 mg of linoleic acid and 20 mg of tocopherol-derived products.

Since the adduct was highly contaminated with fatty acid after silica chromatography, it was twice rechromatographed by gel filtration on Sephadex LH-20, using chloroform as eluent. In this system, using newly swelled Sephadex, or Sephadex regenerated by chloroform and methanol washing and overnight vacuum drying before swelling, the adduct separates as a pure fraction just preceding the fatty acids, at about two bed volumes.

UV spectrophotometry was performed on a Cary Recording Spectrophotometer, Model 14, using 95% ethanol, unless otherwise specified. IR spectrophotometry was done on a Perkin-Elmer IR Spectrophotometer, Model 21, using $CCl_4$ as solvent. NMR spectrometry was carried out on a Varian HA-100 NMR Spectrometer. Spectra were recorded at 100 MHz, operating in the frequency-swept mode, using deuterated chloroform as solvent and tetramethylsilane as

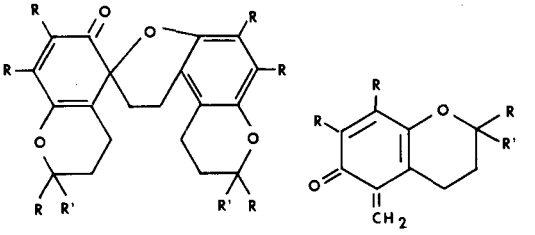



FIG. 3. Structure of tocopherol dimer and of hypothetical quinone methide of tocopherol.

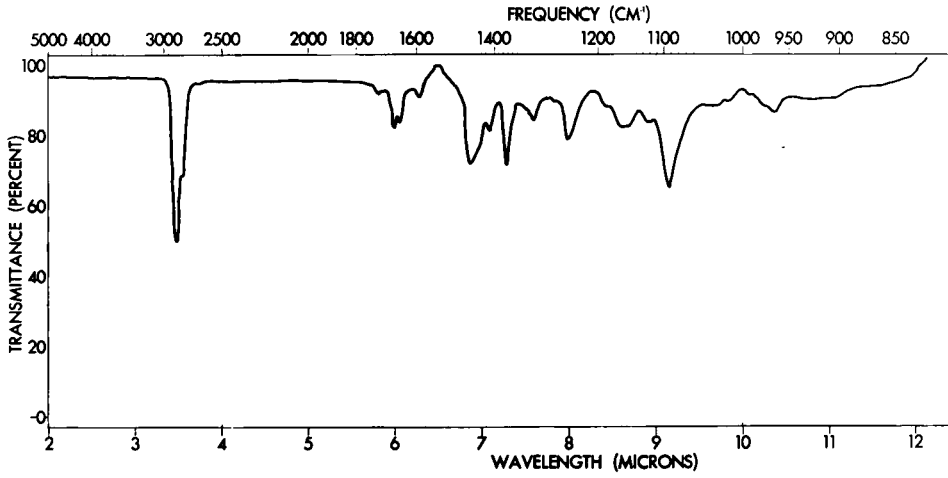



FIG. 4. IR spectrum of tocopherol dimer.

internal standard. Peak values are reported in the tau convention, as parts per million. Mass spectrometry was performed on the methyl ester of the adduct using a CEC Mass Spectrometer, Model 21-110B, with perfluorokerosene as an internal standard and a source temperature of 240 C at time of recording the parent molecular ion. Molecular weight and a confirmation of the elemental analysis were determined by the peak-matching method of Nier (17).

The adduct was characterized by using TLC on Silica G with benzene, esterification with boron trichloride and methanol reagent (18), reaction with the Emmerie-Engel spray reagent and with 58% hydriodic acid (15,19). Analytical data for the compound are shown below.

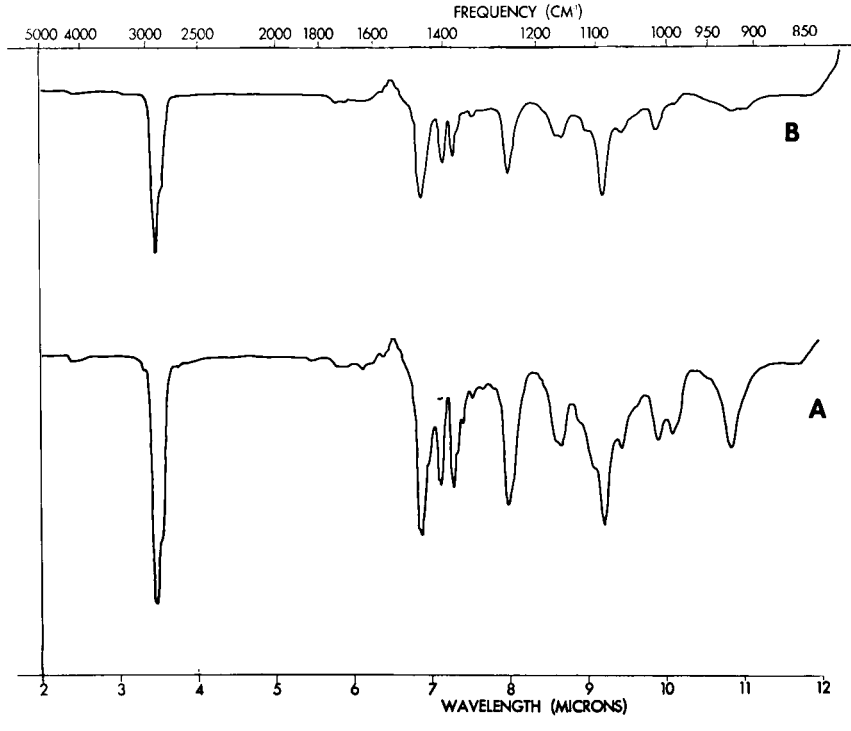



FIG. 5. IR spectrum of tocopheryl allyl ether (A) and of tocopheryl methyl ether (B).

All analytical data on the adduct except that from mass spectrometry were obtained from at least two separately purified samples and were reproducible: pale yellow oil; molecular weight (mass spectrometry) 708.6; $R_f$ linoleic acid 0.07, tocopherol-linoleic acid adduct 0.08, methyl linoleate 0.40, tocopherol-methyl linoleate adduct 0.42; $\lambda_{max}^{ETOH}$ 300 mμ ($E_{cm}^{g/l}$ 4.74) with blue shift on silica and with polar solvents; IR (μ) carboxyl at 5.84, strong bands at 9.12 (tocopherol chroman) and 7.24 (methyl); NMR, 7.88 (2 aromatic methyl groups), 4.56 (2 olefin protons), 5.93 (1 ether proton), carboxyl but no hydroxyl protons; adduct gives no Emmerie-Engel reaction; with $BCl_3$-MeOH is converted to an ester giving no Emmerie-Engel reaction; HI treatment does not produce tocopherol or ether-extractable products that give an Emmerie-Engel reaction.

Analysis. Calculated for $C_{47}H_{80}O_4$: C, 79.60; H, 11.37. Found: Sample 1, C, 79.45; H, 12.17. Sample 2, C, 79.32; H, 12.10.

The methyl and allyl ethers of *d*-alpha-tocopherol were prepared, using either methyl iodide or allyl bromide in acetone solution shaken with 36% sodium hydroxide solution at room temperature (20). After two repetitions of silica-benzene chromatography, the middle fractions in both cases were a clear oil for which UV, IR and NMR spectrophotometry were consistent with the relevant ether structure, as was elemental analysis. The ethers were readily cleaved to tocopherol using hydriodic acid (19).

Analysis of allyl ether. Calculated for $C_{32}H_{54}O_2$: C, 81.64; H, 11.56. Found: C, 81.93; H, 11.14. $N_D^{20}$: 1.4956.

Analysis of methyl ether. Calculated for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 80.92; H, 11.77. $N_D^{20}$: 1.4925.

The spiro-keto ether dimer of tocopherol (3) was prepared in over 90% yield using silver oxide. Twenty-two milligrams of *d*-alpha-tocopherol were dissolved in 7 ml of methyl iodide contained in a 50 ml round bottom flask with reflux condenser. One gram of silver oxide was added, whereupon the solution became bright yellow. The suspension was refluxed at 60 C for 3 1/2 hr and filtered. The solvent was removed at reduced pressure to leave 21.5 mg of a bright, orange-yellow oil, soluble with difficulty in ethanol, but very soluble in petroleum ether. The UV absorption spectrum in hexane showed an absorption maximum at 301 mμ and a secondary maximum at 338 mμ ($E_1^{1\%}{}_{cm}$ 5.3, 2.3). This spectrum was very little changed on further purification and closely corresponds to reported values (3-5,7) for the keto-ether dimer of tocopherol. The oil was further purified by column chromatography on silica, using benzene as eluent, the bright yellow oil emerging at 0.79 bed volumes. After two repetitions, the middle fractions of the yellow zone were collected for an analytical sample and were pure to TLC (Silica G-benzene). UV and IR spectra were in agreement with reported values, as was the elemental analysis.

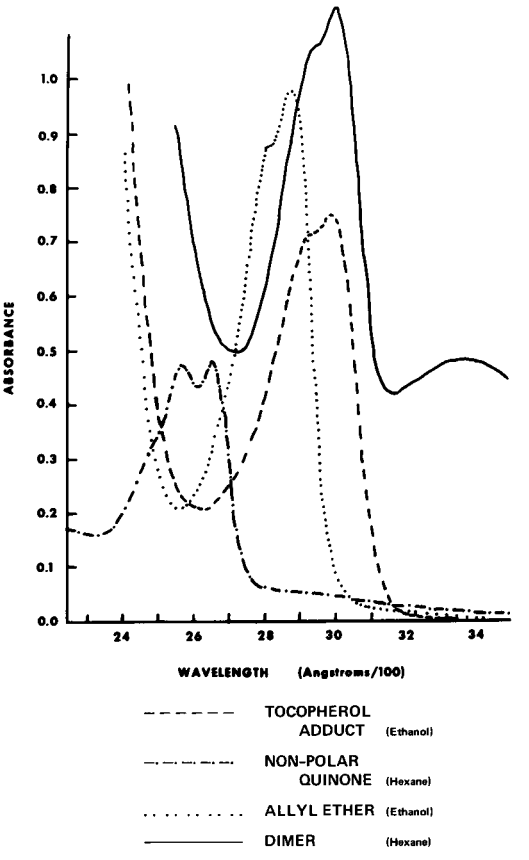



FIG. 6. UV spectra of tocopheryl derivatives. Tocopherol adduct, 0.16 mg/ml. Nonpolar quinone, 0.016 mg/ml. Allyl ether, 0.20 mg/ml. Dimer, 0.21 mg/ml.

Analysis. Calculated for $C_{58}H_{96}O_4$: C, 81.25; H, 11.29. Found: C, 80.62; H, 11.26.

## RESULTS AND DISCUSSION

### Course of Oxidation in the Linoleic Acid-Tocopherol Monolayer

It was found, using silica-UV and gravimetric measurement methods previously described (1,2), that about 4.3 moles per cent tocopherol were co-adsorbed with 185 mg of linoleic acid in a monolayer on 1 g of silica from 25 ml of petroleum ether solution initially containing 10 mg/ml of linoleic acid and 0.8 mg/ml of toco-

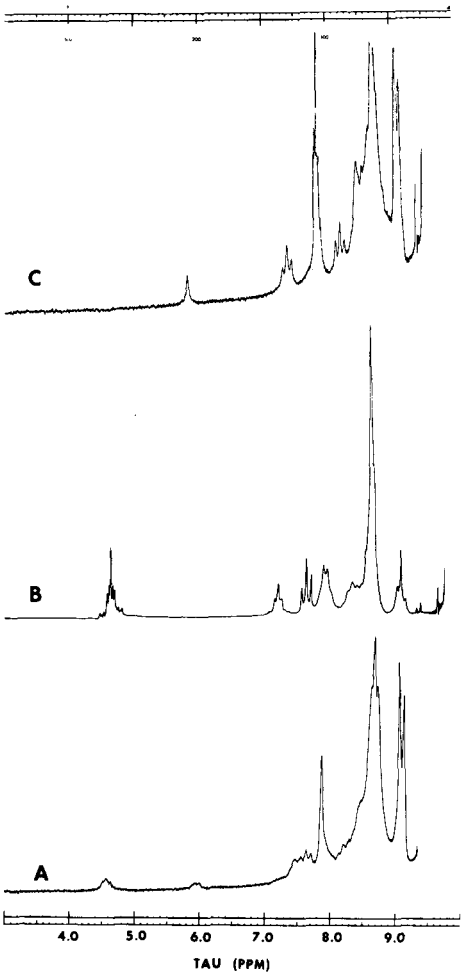



FIG. 7. NMR spectrum of tocopherol adduct (A), linoleic acid (B) and tocopherol (C).

pherol. In the typical experiment at this concentration and at a temperature of 80 ± 2 C, oxygen uptake was followed for five to seven days, or until headspace oxygen had reached less than 1%. This oxygen consumption was approximately equivalent to 1 mole of oxygen per mole of linoleic acid.

The course of oxygen uptake and the changes in the UV spectrum of such a monolayer on silica have been reported (1). Briefly summarized, during the first day of a four day "induction period," there is a pronounced uptake of oxygen (10% of available oxygen), coincident with a shift in the peak of maximum UV absorbance from 285 mμ (tocopherol adsorbed on silica) to 277 mμ (quinone adsorbed on silica). The oxygen consumption then declines slowly and the UV spectrum remains nearly constant for three more days. However, at the

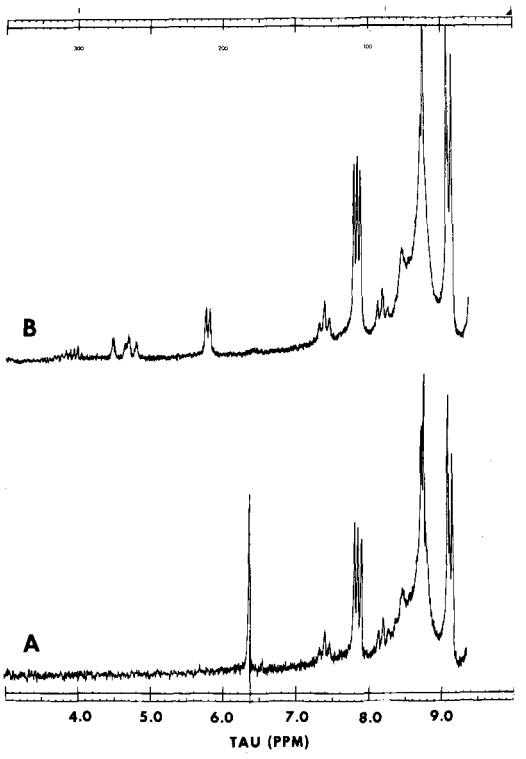



FIG. 8. NMR spectrum of tocopheryl methyl ether (A) and of tocopheryl allyl ether (B).

end of the fourth day about 25% of the available oxygen has been consumed, or about 5 mole/mole of tocopherol. After this four-day tocopherol-dependent "induction period," there is a sharp increase in oxygen consumption and an abrupt shift in the peak of the silica-UV spectrum back to 283 mμ (diene carbonyl on silica). Almost all of the oxygen is consumed within the next day or two.

#### Oxidation Products Derived From Tocopherol

Silicic acid chromatography of the products of oxidation direct from the monolayer on silica just prior to the end of the four-day "induction period" showed two predominant products traceable to tocopherol. A yellow, relatively non-polar, monomeric quinone without a hydroxyl group (Compound A) is eluted after about one bed volume of benzene as eluent. The UV absorption spectrum in ethanol showed maxima at 261, 267 mμ ($E_1^{1\%}{}_{cm}$ approx. 300). Compound B, a nearly colorless oil, appears after three or four bed volumes, just prior to linoleic acid and, in later fractions, merged with it. It is a tocopherol-linoleic acid adduct, the analytical characteristics of which have been described above.

These two compounds, at most, represent about 50% of the available tocopherol. Neither silica-UV spectrophotometry nor subsequent chromatography gave evidence for other monomers, dimers or trimers as elsewhere reported from tocopherol oxidation.

#### The Tocopherol-Linoleic Acid Adduct

Because it is present in larger amounts, and because of its possible importance to tocopherol function, the adduct was the more thoroughly characterized of the two products. Many of its characteristics have been stated above, but some should be discussed in more detail.

The elemental analysis most closely corresponds to that calculated for a simple or multiple adduct of the composition: linoleic acid + tocopherol, $C_{47}H_{82}O_4$. However, the parent ion molecular weight derived from mass spectral data for the ester, 722.617, corresponds more closely to that calculated for $C_{48}H_{82}O_4$, 722.621, consistent with methyl linoleate + tocopherol - 2H, the coupling of bivalently oxidized tocopherol with methyl linoleate (Fig. 1 for the acid).

The IR spectrum (Fig. 2) shows the presence of a carboxyl group at 5.83 $\mu$ and the methyl (7.24 $\mu$) and chroman (9.12 $\mu$) groups of tocopherol but no hydroxyl or unsaturated carbonyl groups. The chroman peak arises from the CO stretch vibration of the cyclic aromatic ether ring. The ratio of the absorbance of the methyl-methylene peak at 3.42 $\mu$ to that of the carboxyl group is 1.50 for the adduct, compared to 0.78 for linoleic acid alone, nearly a twofold increase, as expected for the hypothesized adduct.

In general, then, the IR spectrum confirms the presence of both linoleic acid and tocopherol moieties. Only one oxidation product of tocopherol is known which has an esterifiable carboxyl group (21). The ring of this compound, alpha-tocopheronic acid, has a quinone structure and reactions, which Compound B, the adduct, does not. The chroman peak of the adduct is twice as intense as that in the tocopherol spectrum, and its wave length matches that of the chroman peak of the known tocopherol dimer (Fig. 3,4). The dimer contains a second chroman ring linking the monomers (4,8) and is probably derived from a quinone methide intermediate. The absorption by the adduct in the acyclic aromatic ether region around 8 $\mu$ is weak in contrast to the comparable absorption of the allyl and methyl tocopheryl ethers (Fig. 5).

Of the four oxygens in the 1:1 adduct suggested by the elemental analysis and mass spectrometry, the IR analysis permits assignment of two to the carboxyl and one to the chroman group. The assignment of the remaining oxygen is critical to the problem of structure. The IR analysis and the failure of the adduct to give an Emmerie-Engel reaction rule out hydroxyl and unsaturated carbonyl groups. Thus, for the group containing the remaining one atom of oxygen, there remain only simple or cyclic ethers as alternatives, either of which places a requirement that the aromatic structure be preserved, since no ether peak other than that of the intensified chroman is observed in the 9 $\mu$ cyclic and aliphatic ether region.

The UV spectrum in ethanol (Fig. 6) adds strong support to the aromatic ether assignment. The main 300 m$\mu$ band shows a slight bathochromic shift to 301 m$\mu$ in less polar solvents like chloroform, and a pronounced hypsochromic shift in more polar systems such as the silica-UV system (2), where the peak is at 295 m$\mu$. We have found such a hypsochromic shift in polar media to be associated with the aromatic ring of all tocopherol derivatives so far examined, whenever aromaticity is preserved. Thus, tocopherol and its allyl and methyl ethers, and the main 300 m$\mu$ band of the dimer, which contains one aromatic ring, shift in this manner. On the other hand, tocopherol quinones experience a bathochromic shift on silica of 7-10 m$\mu$, and the unsaturated ketones, in particular the ketone band of the dimer, also shift bathochromically, often as much as 30 m$\mu$. Pure conjugated polyene systems of up to three double bonds shift only slightly bathochromically.

Confirmation of aromaticity was provided by the NMR spectrum. In addition to the usual methyl peaks between 9.06-9.12 $\tau$ and methylene peaks between 8.63-8.73 $\tau$, representing the aliphatic chains of linoleic acid and tocopherol (Fig. 7), there occurred broad, asymmetric peaks at 4.56 $\tau$ and 5.93 $\tau$, which were equivalent to two and one protons, respectively. The former peak is in the region of nonconjugated olefinic protons, while the latter is not exchangeable with deuterium oxide, and is in the region of protons on carbon alpha to an aromatic ether oxygen. A carboxyl proton exchangeable with deuterium oxide was located below the zero of the scale. The most prominent peak below 8.0 $\tau$ was the strong singlet at 7.88 $\tau$, representing six protons of two tocopherol methyl groups. This low field position is indicative of methyl groups attached to aromatic or olefinic carbons (22).

The assignment most critical to structure is that of the 7.88 $\tau$ singlet, since it must arise from the ring methyl groups of tocopherol (Fig.

7), which have no adjacent alpha carbon with a proton in any known structures. Since it has been shown by the IR that there is no unsaturated carbonyl function in the ring, it appears confirmed that the ring remains aromatic, and that the single unassigned oxygen atom is part of a simple or cyclic aromatic ether link. Strong support for this assignment is found in the peak at 5.93 $\tau$, which is in the region of protons on carbon alpha to an aromatic ether oxygen, as is evident in the spectra of the allyl and methyl ethers (Fig. 8). This peak in the adduct is equivalent to a single proton, which suggests that the parent carbon atom is secondary, and thus, part of a chain.

It appears, however, from an inspection of the NMR spectra of the allyl and methyl ethers of tocopherol that the adduct does not have a simple aromatic ether link. Unlike the adduct, both of these ethers with widely differing oxygen substituents show a characteristic benzylic methylene triplet at 7.4 $\tau$, nearly the same as that in tocopherol, and a very distinct triple singlet structure of the aromatic methyls in the 7.85 $\tau$ region. In addition, both of these ethers have a UV absorption maximum at about 288 m$\mu$ in ethanol, in contrast to 300 m$\mu$ for the adduct and the main band of the dimer.

The failure of hydriodic acid treatment to produce ether-extractable compounds reacting like tocopherol in the Emmerie-Engel reaction is further evidence that a simple aromatic ether bridge is not involved, since the simple allyl and methyl ethers readily produce tocopherol under these conditions.

All the above data, the similarity of the IR spectrum in the 8-9 $\mu$ region to that of the tocopherol dimer (Fig. 3,4), (to which has been assigned the structure of a second chroman ring as a bridge) and Nilsson's demonstration (12) of a styrene-chromanol adduct for which he postulates a second chroman ring bridge (Fig. 1), appear consistent with an adduct whose bridging group is a new chroman ring.

The present findings of a monomeric, non-hydroxylic quinone, and an adduct of oxidized tocopherol and linoleic acid, coupled with failure to find self-polymeric forms of oxidized tocopherol reported by other workers from one- and two-phase bulk liquid systems, suggest that tocopherol oxidation follows a different course in the silica monolayer system. Thus, tocopherol molecules appear to be prevented, either by separation or by steric restrictions, from mutual association and reaction. However, the existence of the adduct in proportionately large yield suggests that the chemical reactivity of the tocopherol molecule with other molecules is still considerable. The data are thus consistent with a relatively unrestricted reaction rate, but a greatly reduced migrational mobility. Since tocopherol is the more mobile of the two components in most silica chromatographic systems, it would seem plausible that both components are quite firmly anchored, that their initial distribution is quite random, with little tendency for segregation of the two components, and that the auto-oxidation reaction may be propagated in domino fashion, with little lateral translation.

If these findings are not unique to the system described, analogous compounds may be demonstrable using membrane constituents like unsaturated phospholipids on silica, or in lyophilized, or in vivo tissue systems.

ACKNOWLEDGMENTS

NMR spectrophotometry by R.C. Chalk and Maurice Halford, Pioneering Research Laboratory, U.S. Army Natick Laboratories, Mass spectrometry by W.G. Yeomans, Pioneering Research Laboratory, U.S. Army Natick Laboratories. Elemental analyses by Stephen Nagy. Technical assistance by Miss Anne McKinnon.

REFERENCES

1. AOCS Meeting, Chicago, October 1967, Abstract No. 89; also W.L. Porter, L.A. Levasseur, A.S. Henick and J.I. Jeffers, Lipids, in press.
2. AOCS Meeting, New York, October 1968, Abstract No. 117; also W.L. Porter, A.S. Henick, J.I. Jeffers and L.A. Levasseur, Lipids, in press.
3. Nelan, D.R., and C.D. Robeson, J. Amer. Chem. Soc. 84:2963-2965 (1962).
4. Skinner, W.A., and P. Alaupovic, Science 140:803-805 (1963).
5. Csallany, A.S., and H.H. Draper, Arch. Biochem. Biophys. 100:335-337 (1963).
6. Csallany, A.S., M. Chiu and H.H. Draper, Lipids 5:63-70 (1970).
7. Strauch, B.D., H.M. Fales, R.C. Pittman and J. Avigan, J. Nutri. 97:194-202 (1969).
8. Schudel, von P., H. Mayer, J. Metzger, R. Ruegg and O. Isler, Helv. Chim. Acta 46:636-649 (1963).
9. Dürckheimer, W., and L.A. Cohen, Biochem. Biophys. Res. Commun. 9:262-265 (1962).
10. Martius, C., and H. Eilingsfeld, Justius Liebigs Annalen 607:159-168 (1957).
11. Goodhue, C.T., and H.A. Risley, Biochem. Biophys. Res. Commun. 17:549-553 (1964).
12. Nilsson, J. Lars G., J.O. Branstad, H. Sievertsson, Acta. Pharm. Suecica 5:509-516 (1968).
13. Skinner, W.A., and R.M. Parkhurst, J. Org. Chem. 31:1248-1251 (1966).
14. Stahl, E., "Thin Layer Chromatography," Academic Press, New York, 1965, p. 475.
15. Parker, W.E., and W.D. McFarlane, Can. J. Res. 18B:405-409 (1940).
16. Bishov, S.J., and A.S. Henick, JAOCS 43:477 (1966).
17. Quisenberry, K.S., T.T. Scolman and A.O. Nier, Phys. Rev. 102:1071-1075 (1956).
18. Metcalfe, L.D., and A.A. Schmitz, Anal. Chem. 33:363-364 (1961).
19. Shriner, R.L., R.C. Fuson and D.Y. Curtin, "The Systematic Identification of Organic Compounds," Fourth Edition, John Wiley & Sons, Inc., New York, 1959, p. 116.
20. Green, J., S. Marcinkiewicz and D. McHale, J. Chem. Soc. C, Org. 1966 (6), 1422-1427.
21. Simon, E.J., A. Eisengart, L. Sundheim and A.T. Milhorat, J. Biol. Chem. 221:807-817 (1956).
22. Bovey, F.A., "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, 1969, p. 249.

[Received July 13, 1970]