After ten hours of refluxing 250 cc. of water was added, the alcohol distilled off, and the residual solution treated with 275 cc. of concentrated hydrochloric acid with stirring and external cooling. The reaction mixture, now acid to congo red, was extracted with five 200-cc. portions of ether. The combined ethereal extracts were washed with saturated salt solution, dried over sodium sulfate and the ether distilled. The resulting crude dicarboxylic acid weighed 157 g. which was recrystallized from benzene–petroleum ether, giving 136 g. of a white crystalline product melting at 105–106.5° in a yield of 85%. This was decarboxylated by heating at 190–200° for five hours and then fractionated *in vacuo*, giving 61.2 g. of α-allylcyclobutanepropionic acid, b. p. 142–150° at 17 mm., in a yield of 85.8%.

*Anal.* Calcd. for $C_{10}H_{16}O_2$: C, 71.39; H, 9.58. Found: C, 71.09; H, 9.33.

This acid was converted to the acid chloride by means of phosphorus trichloride, as above, and fractionated *in vacuo*, giving a 70.5% yield of α-allylcyclobutanepropionyl chloride, b. p. 90–95° at 13 mm. Subsequently, improved yields were obtained by chlorinating with thionyl chloride: 31 g. of the acid and 33 g. of thionyl chloride were mixed and heated on a water-bath maintained at 40–50° for thirty minutes and then refluxed for three hours. The thionyl chloride was removed and the residue fractionated yielding 28.7 g. of the desired acid chloride, b. p. 95–99° at 14 mm. in a yield of 83.5%.

*Anal.* Calcd. for $C_{10}H_{15}ClO$: Cl, 19.03. Found: Cl, 18.59.

To prepare the ureide, the acid chloride and urea in molar proportions of 1:4 were mixed in the absence of water and allowed to stand in a Petri dish in an oven maintained at 110–130° for five hours. The mixture was then heated on a steam-bath for five hours, after which it was cooled, triturated with 5% sodium carbonate solution and the insoluble material extracted with ether. The combined ether extracts were washed with 5% sodium carbonate solution and then dried over sodium sulfate. On distilling the ether there was obtained an 81.2% yield of crude product which was recrystallized from benzene–petroleum ether giving a product, m. p. 124.5–125.5°, in a yield of 58%.

*Anal.* Calcd. for $C_{11}H_{18}N_2O_2$: N, 13.33. Found: N, 13.52.

The thioureide was similarly prepared, m. p. 108–109°, in a yield of 29%.

*Anal.* Calcd. for $C_{11}H_{18}N_2OS$: S, 14.15. Found: S, 14.41.

## Summary

A number of alicyclicalkyl alkyl malonic esters have been prepared and their properties described.

The corresponding barbituric acids as well as some thiobarbituric acids and 1-methyl derivatives have been prepared.

Several carbamates and ureides containing the alicyclicalkyl group have been prepared and their properties described.

NEW BRUNSWICK, N. J.      RECEIVED AUGUST 14, 1946

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[COMMUNICATION 93 FROM THE LABORATORIES OF DISTILLATION PRODUCTS, INC.]

# δ-Tocopherol. I. Isolation from Soybean Oil and Properties[1]

BY MAX H. STERN, CHARLES D. ROBESON, LEONARD WEISLER AND JAMES G. BAXTER

During the assay of tocopherol concentrates prepared from soybean oil by molecular distillation, evidence was obtained of the presence of a previously unidentified member of the vitamin E complex. α-, β- and γ-tocopherols give substantially complete color formation in a modified Emmerie and Engel assay method,[2] using a reaction time of two minutes; and there is only a small percentage increase in color intensity or "rise" as the time is increased to ten minutes. The mixture of tocopherols in the distillate from soybean oil, however, exhibited a rise of about 10%. The substance responsible for this has been separated in pure form and found to be a tocopherol, which we have named δ-tocopherol following the nomenclature introduced by H. M. Evans.

The evidence to be described indicates that δ-tocopherol is 8-methyltocol with the formula
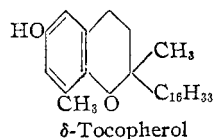


δ-Tocopherol

It differs from α-, β- and γ-tocopherols in having only one methyl group in the aromatic ring of the chroman nucleus and is the first monomethyl tocol isolated from natural sources.

This paper is concerned with the occurrence of δ-tocopherol, with the method used for its isolation, with certain of its physical, chemical, and biological properties and with the methods used to investigate its structure.

**Occurrence.**—δ-Tocopherol appears to be one of the more common members of the vitamin E complex. It was found to constitute approximately 30% of the mixed tocopherols in soybean oil,[3] 5% of those in wheat germ oil,[4] and there is evidence of its occurrence in cottonseed and peanut oils.[3]

**Isolation.**—The mixture of δ-, γ- and α-tocopherols in soybean oil was concentrated by molecular distillation, separated from glycerides by saponification and freed of α-tocopherol by selective adsorption. The resulting concentrate was then esterified with palmitoyl chloride to give an ester concentrate which was crystallized from acetone at 5°.

γ-Tocopherol palmitate was found to crystallize readily while δ-tocopherol palmitate stayed in solution. After saponification of the soluble fraction, a concentrate was obtained which was separated from the last traces of γ-tocopherol by

selective adsorption. Esterification of the concentrate with $p$-phenylazobenzoyl chloride gave a crystalline ester which, upon saponification and distillation, gave δ-tocopherol as a yellow oil.

## Experimental

### Isolation of δ-Tocopherol from Soybean Oil

**Concentration.**—Alkali-refined soybean oil (0.19% mixed α-, γ- and δ-tocopherols) was distilled in a centrifugal-type molecular still and the tocopherol fraction, which distilled below 240° at a pressure of 0.004 mm. of mercury, was collected. After removal of as much of the sterols and other crystallizable substances as possible by crystallization from acetone at −10° and removal of glycerides by saponification, the tocopherols in the unsaponifiable matter were further concentrated by molecular distillation to give a fraction of 557 g. containing 61% mixed tocopherols. An aliquot (250 g.) was freed of α-tocopherol by selective adsorption on zinc carbonate using the liquid chromatogram method.[3] After again removing as many solids as possible by low temperature crystallization a concentrate was obtained (145 g., 87.5% tocopherols) which was esterified with palmitoyl chloride,[5] giving a crude ester (210 g.). This was crystallized from acetone (1500 ml.) at 5° for five days. γ-Tocopherol palmitate was removed by filtration and the solvent was evaporated from a portion of the filtrate (475 ml.), leaving a residue (44 g.) consisting of δ-tocopherol palmitate mixed with a small amount of the γ-ester. The mixed esters were saponified as previously described,[5] giving a reddish-yellow oil (34 g., 66% mixed tocopherols) which was freed of the remaining γ-tocopherol, palmitic acid, and certain other impurities by selective adsorption on zinc carbonate.[3] By readsorption of the less concentrated fractions a δ-tocopherol concentrate (18.5 g., 96% tocopherol) was finally obtained.

**δ-Tocopherol Phenylazobenzoate.**—This concentrate was esterified with $p$-phenylazobenzoyl chloride as described.[5] The resulting orange oil was crystallized from isopropyl alcohol at 0°, giving δ-tocopherol phenylazobenzoate (m. p. 41–42°, 18.7 g.) as orange-yellow leaflets.

*Anal.* Calcd. for $C_{40}H_{54}O_3N_2$: C, 78.64; H, 8.91; N, 4.59. Found: C, 78.96; H, 8.95; N, 4.67.

**δ-Tocopherol.**—By saponification of the ester and molecular distillation from castor oil residue as described,[5] δ-tocopherol was obtained as a yellow oil.

*Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.53; H, 11.52; mol. wt., 402. Found: C, 80.74; H, 11.11; mol. wt. (in benzene), 377. The molecular weight determination was

done by Mr. D. Ketchum, Kodak Research Laboratories, using the Menzies–Wright method.

## Properties of δ-Tocopherol

**Behavior with Ferric Chloride–Dipyridyl Reagent.**—δ-Tocopherol has proved to be unique among the known natural tocopherols in giving with the ferric chloride–dipyridyl reagent a slow, steady increase in color intensity after the initial, rapid oxidation is completed. The intensity is most nearly the same on a molecular basis for the four tocopherols when a reaction time of two and one-half minutes is chosen for the procedure described in ref. 2. If time is extended to ten minutes δ-tocopherol gives an intensity which is approximately 22% greater. This leads to errors in the assay of mixed tocopherol preparations containing δ-tocopherol if the shorter reaction time is not chosen or if a suitable correction is not applied. A method for assaying total tocopherols in mixtures which may contain δ-tocopherol is described in ref. 2.

**Antioxidant Activity.**—δ-Tocopherol proved to be the most active antioxidant for vitamin A and β-carotene, *in vitro*, under our experimental conditions, of the tocopherols so far isolated.



Fig. 2.—Relative activities of tocopherols (0.1%) as antioxidants for vitamin A acetate in olive oil (50,000 units/g.) at 55°.



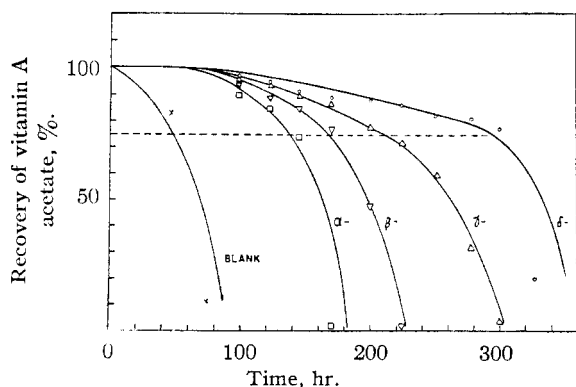Fig. 3.—Relative activities of tocopherols (0.1%) as antioxidants for β-carotene in olive oil (900 micrograms/g.) at 39°.



Fig. 1.—Relative activities of tocopherol (concn. = 0.1%) as antioxidants for vitamin A acetate in olive oil (50,000 U. S. P. units/g.) at 39°.

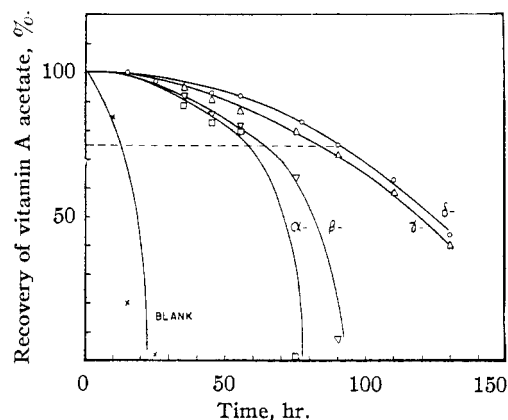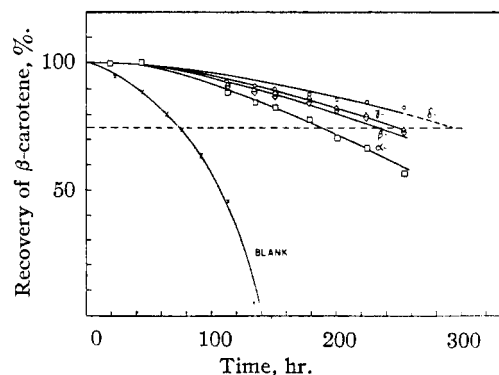(5) Baxter, Robeson, Taylor and Lehman, THIS JOURNAL, **65**, 923 (1943).

This was determined by an accelerated test in which olive oil solutions of vitamin A acetate and β-carotene, containing 0.1% of each of the natural tocopherols, were uniformly exposed to air at 39°. The vitamin A acetate experiment was also repeated at 55°. Blank solutions were provided containing the test substance but no tocopherol. The relative effectiveness of the tocopherols as antioxidants was determined by comparing the percentage recovery of vitamin A and carotene at suitable time intervals. A plot of the data is given in Figs. 1, 2 and 3.

## Experimental

**Test Solutions.**—Crystalline vitamin A acetate was dissolved in olive oil (peroxide value less than 1) and diluted with olive oil containing the various tocopherols so as to give solutions containing approximately 1.7% vitamin A acetate (approximately 50,000 U. S. P. units/gram) and 0.1% tocopherol. The percentage recovery of vitamin A during the stability test was determined by the antimony trichloride method.

Crystalline β-carotene (90% β-, 10% α-) was dissolved in olive oil to give a solution containing approximately 900 micrograms/g. and 0.1% tocopherol. The percentage recovery of carotene during the stability tests was determined colorimetrically using the Evelyn photoelectric colorimeter.

**Oxidation.**—The accelerated tests at 39° were performed by bubbling air through the oil samples in the usual Swift apparatus, using a bath of refluxing methylene chloride to control the temperature.

The tests with vitamin A acetate at 55° were carried out by uniformly exposing samples of the oil (2 g.) to air in an oven whose temperature was controlled within a degree. V-shaped tubes were used to contain the oil which was caused to move periodically from one limb to the other by a mechanical rocking device. This avoided the danger inherent in static accelerated tests that oxidation tends to occur more rapidly at the surface of the oil than in the interior.

It is apparent that all the tocopherols are effective antioxidants for vitamin A acetate and β-carotene and that the order of decreasing activity is δ-, γ-, β-, and α-. This conclusion, so far as α-, β-, and γ-tocopherols are concerned, is qualitatively the same as that reached by Olcott and Emerson,[6] and Hove and Hove[7] from experiments on markedly different systems. We did not observe, as did Hove and Hove, that the activity of α-tocopherol increased relative to that of γ-tocopherol as the temperature was reduced from 55 to 39°. Considering the difference in the two experimental methods used, and in the concentrations of tocopherol tested, this discrepancy is not surprising. It appears that the relative activity of the tocopherols as antioxidants may be expected to vary on a quantitative basis, depending on the test procedure.

It is convenient to define the induction period of vitamin A acetate or β-carotene in an oil as the number of hours of exposure to air required for the initial concentration to be reduced by 25%. We may also, following reference 7, measure the antioxidant activity by the percentage increase in the induction period of the protected sample over that

(6) Olcott and Emerson, THIS JOURNAL, **59**, 1008 (1937).

(7) Hove and Hove, J. Biol. Chem., **156**, 623 (1944).

of the blank. Measured in this way, the relative activities of α-, β-, γ- and δ-tocopherols as antioxidants for vitamin A acetate in olive oil at 39° were as 1:1.3:1.8:2.7, respectively. The corresponding ratios at 55° were as 1:1.1:1.6:1.7. Thus, the activity of γ- and δ-tocopherols increased, relative to that of α-tocopherol, as the temperature was decreased. For β-carotene at 39° the ratios were as 1:1.4:1.5:(1.9?).

The work of Swift, Rose, and Jamieson[7a] and also unpublished work from this laboratory have indicated that the length of the induction period is not a linear function of the tocopherol concentration. The function appears to be logarithmic. One cannot, therefore, use the data given, except in an approximate way, to estimate the concentration of one tocopherol needed to achieve the protection afforded by a given concentration of another.

**Resistance to Atmospheric Oxidation.**—Since active formation of peroxides in fats has been shown to follow destruction of the tocopherols,[8] one might expect to find a parallelism between the antioxidant activity of the tocopherols in vitro and their resistance to atmospheric oxidation. This point was tested by uniformly exposing olive oil solutions of each of the tocopherols to air at 55° in an oven, and determining the percentage recovery at various time intervals by the ferric chloride–dipyridyl method. Since fat peroxides inhibit the formation of the red color, measurements were made only during the time when the tocopherol concentration remained high and the peroxide concentration was, therefore, low. A portion of the data is given in Table I.

### TABLE I

RELATIVE RESISTANCE OF TOCOPHEROLS TO OXIDATION IN OIL

Test solution: Olive Oil + 0.1% Tocopherol (by wt.)

| Added tocopherol | % Recovery of tocopherol at 55° in: | |
|---|---|---|
| | 88 hr. | 108 hr. |
| δ- | 94.2 | 93.5 |
| γ- | 93.5 | 92.5 |
| β- | 83.8 | 76.8 |
| α- | 81.2 | 75.5 |

It is apparent that the resistance of the tocopherols to atmospheric oxidation parallels their activity as antioxidants, δ-tocopherol being the most resistant and α-tocopherol the least resistant to oxidation. Evidently while protecting vitamin A the tocopherol itself is attacked and its ability to withstand this destruction is related to its effectiveness as an antioxidant. This is not the entire explanation, however, since it has been shown that the oxidation products of certain of the tocopherols (such as γ-tocopherol o-quinone) have antioxidant activity in their own right.[8a]

Hove and Hove[7] have given data on the oxida-

(7a) Swift, Rose and Jamieson, Oil and Soap, **19**, 176 (1942).

(8) Golumbic, Oil & Soap, **20**, 105 (1943).

(8a) Golumbic, THIS JOURNAL, **63**, 1163 (1941).

tion of $\alpha$-, $\beta$-, and $\gamma$-tocopherols by peroxidized peanut oil which indicated the same order of resistance to oxidation which we have found.

**Activity in Resorption Sterility Test.**—The activity of $\delta$-tocopherol in the Evans resorption sterility test for vitamin E was determined by Miss M. I. Ludwig and Dr. P. L. Harris of the Biological Department of this Laboratory. The experimental details will be described elsewhere. It was found that $\delta$-tocopherol has an activity which is less than 1/100 that of $d$-$\alpha$-tocopherol. Thus, it is the least active of the natural tocopherols in the antisterility test. This result on the natural compound agreed with that found by Karrer and coworker, and Jacob and co-workers, for synthetic 8-methyltocol.[9]

**Ultraviolet Absorption.**—The absorption curve for $\delta$-tocopherol is similar to that of $\alpha$-, $\beta$- and $\gamma$-tocopherols.[5] The purest preparation so far obtained had $E_{1\,cm}^{1\%}$ (298 m$\mu$) = 91.2.

**Infrared Spectrum.**—The infrared spectrum of $\delta$-tocopherol is compared with that of the other tocopherols in Fig. 4. The curves were determined by Dr. S. F. Kapff of our Laboratory, using a Perkin–Elmer instrument. The maximum at 2.9 microns due to the hydroxyl group is common to all four tocopherols and is not shown in the figure. The infrared spectrum has proved valuable in determining the percentage of $\gamma$-tocopherol in $\delta$-tocopherol concentrates at various stages in the purification process.
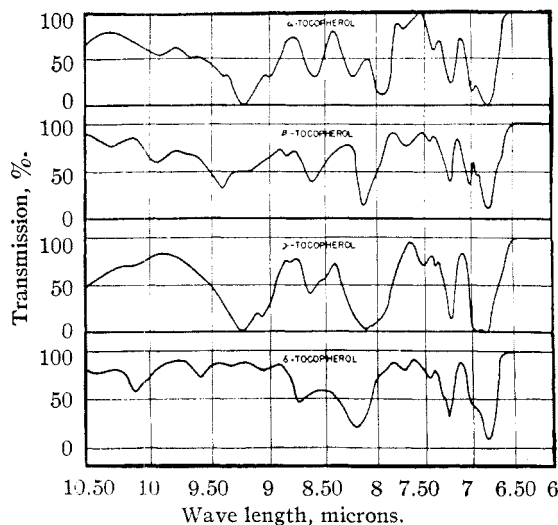


Fig. 4.—Infrared transmission spectra of natural tocopherols.

**Optical Rotation.**—The optical rotation of $\delta$-tocopherol is compared with that of the other tocopherols in Table II. Some of the data have been previously reported.[5]

$\delta$-Tocopherol is dextrorotatory, as are all the natural tocopherols, in ethyl alcohol and the mag-

(9) Jacob, Sutcliffe and Todd, *J. Chem. Soc.*, 327 (1940); Karrer and Fritzsche, *Helv. Chim. Acta*, **22**, 260 (1939).

TABLE II

SPECIFIC ROTATION OF TOCOPHEROLS AND ESTERS IN ETHYL ALCOHOL AND BENZENE

| Toco-pherols | Ethyl alcohol | | | Benzene | | |
|---|---|---|---|---|---|---|
| | $(\alpha\text{-})^{25}_{546.1}$ | L$^a$ | $c^b$ | $(\alpha\text{-})^{25}_{546.1}$ | L$^a$ | $c^b$ |
| $\delta$- | 3.4 | 1 | 15.48 | 1.1 | 1 | 10.86 |
| $\beta$- | 2.9 | 1 | 7.15 | 0.9 | 1 | 8.00 |
| $\gamma$- | 2.2 | 1 | 9.32 | −2.4 | 1 | 8.59 |
| $\alpha$- | 0.32 | 1 | 14.28 | −3.0 | 1 | 13.50 |

$^a$ L = length of tube in dm.   $^b$ $c$ = concentration of solution in g./100 cc.

nitude of the rotation is greater than for the $\alpha$-,$\beta$- or $\gamma$-compounds. $\delta$-Tocopherol is also dextrorotatory in benzene, as might be expected from the fact that the degree of negative rotation in this solvent decreases as the molecular weight is decreased.

**Diazo Reaction.**—$\delta$-Tocopherol reacts with diazo reagents, such as diazotized $o$-dianisidine, to give pigments whose color intensity varies with the degree of alkalinity of the solution. This has provided a method for the estimation of $\delta$- and also $\gamma$-tocopherols in mixtures. The procedure is described elsewhere.[3]

**Oxidation.**—Oxidation of $\delta$-tocopherol with gold chloride gave a compound which had the properties of a $p$-quinone. One preparation had an absorption maximum at 257 m$\mu$ with an extinction coefficient of 222 (Fig. 5). The position of the maximum and the order of value of the extinction coefficient are similar to those of the other tocopherol $p$-quinones, all of which absorb at about 260 m$\mu$.
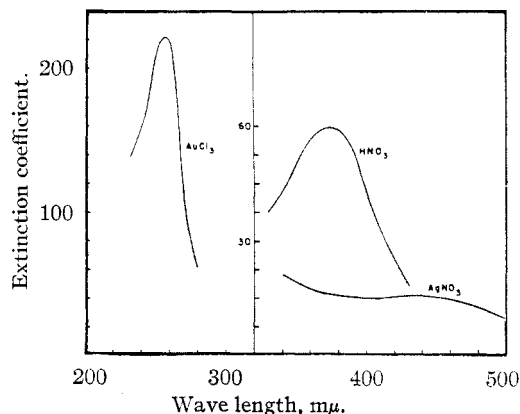


Fig. 5.—Absorption curves of the oxidation products of $\delta$-tocopherol.

By reduction with sodium hydrosulfite a hydroquinone was formed ($E_{1\,cm}^{1\%}$ 294 m$\mu$ = 81.6; 59% apparent tocopherol by ferric chloride–dipyridyl assay). This, on cyclization with sulfuric acid in ethanol, gave impure $\delta$-tocopherol ($E_{1\,cm}^{1\%}$ 299 m$\mu$ = 90; 60% tocopherol).

Oxidation of $\delta$-tocopherol with silver nitrate gave a red pigment which had the ultraviolet light absorption properties of an $o$-quinone. The identification of the red pigments formed from

the tocopherols with silver nitrate and nitric acid as *o*-quinones was accomplished by Smith and co-workers.[10]

The position of the absorption maximum for the pigment from δ-tocopherol is at approximately 435 mμ with an extinction coefficient of 15 (Fig. 5). The maxima for the pigments from α-, β- and γ-tocopherols are at 460–480 mμ, with extinction coefficients of 11–15 (Fig. 6). The broad absorption band in the case of the pigment from δ-tocopherol suggests that the reaction gives a mixture of products.

Nitric acid oxidation proceeded differently with δ- than with the other tocopherols. An orange-yellow pigment was formed having an extinction coefficient at 373 mμ of 59 (Fig. 5). A preparation of synthetic 8-methyl tocol gave with nitric acid a pigment absorbing at the same wave length ($E_{1\,cm.}^{1\%}$ (373 mμ) = 41) (Fig. 6). Another synthetic preparation gave an oxidation product with $E_{1\,cm.}^{1\%}$ (373 mμ) = 48.

α-, β- and γ-tocopherols give *o*-quinones with nitric acid just as they do with silver nitrate (Fig. 6). This observation may be of importance in developing an analytical method for δ-tocopherol in mixtures with other tocopherols. Conceivably one might oxidize the mixture with nitric acid and determine the δ-tocopherol spectrophotometrically by the intensity of absorption at 373 mμ.

### Experimental

#### Oxidations

**Gold Chloride.**—δ-Tocopherol was oxidized with gold chloride (1.7 mole prop.) in ethanol for forty-five minutes, according to Karrer and Geiger.[11] A reddish oil was obtained ($E_{1\,cm.}^{1\%}$ (257 mμ) = 222) which contained 12% unchanged δ-tocopherol.

**Silver Nitrate.**—δ-Tocopherol (0.055 g.) and silver nitrate (0.15 g.) in ethanol (1 ml.) were heated to 50° for one hour. The solution was diluted with water, extracted with ether, the latter washed with water, dried and the solvent removed. A reddish oil was obtained ($E_{1\,cm.}^{1\%}$ (435 mμ) = 15.2) which contained 6.5% unchanged tocopherol.

**Nitric Acid.**—To δ-tocopherol (0.03 g.) in ethanol (10 ml.) was added slowly, concentrated nitric acid (2 ml.). After shaking the solution was allowed to stand for two and one-half hours at room temperature. The extinction coefficient at 373 mμ was then 59.7. Oxidation at 100° caused bleaching of the orange-yellow color in one minute.

### Structure

δ-Tocopherol was suspected of having a chroman structure because of its empirical formula and the similarity of its properties to those of the known tocopherols in soybean oil. Its elimination maximum, determined by analytical distillation from constant yield oil[12] (170°) was ten degrees lower than that of γ-tocopherol which suggested that the compound was either unmethylated or monomethylated in the aromatic ring of the chroman nucleus.

(10) Smith, Irwin and Ungnade, THIS JOURNAL, **61**, 2424 (1939).

(11) Karrer and Geiger, *Helv. Chim. Acta*, **23**, 455 (1940).
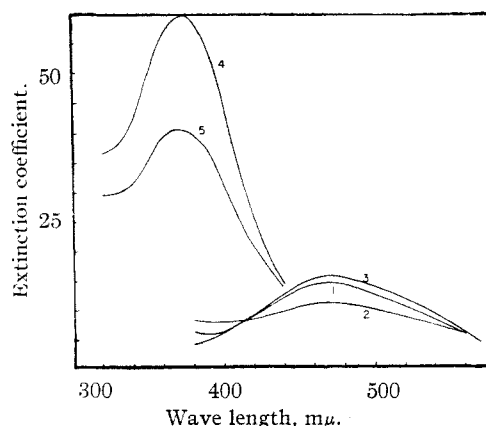
(12) Hickman, *Ind. Eng. Chem.*, **29**, 968 (1937).

Fig. 6.—Absorption curves for the nitric acid oxidation products of (1) α-tocopherol, (2) β-tocopherol, (3) γ-tocopherol, (4) natural δ-tocopherol, and (5) synthetic 8-methyltocol.

Heat degradation of δ-tocopherol, according to the method of Fernholz,[13] gave a hydroquinone (m. p. 144–147.5°) which was converted to a diacetate (m. p. 89–91°). 8-Methyltocol should give, on heating, 2,6-dimethylhydroquinone (m. p. 147–149°)[13,14] which, by esterification, gives a diacetate (m. p. 91–92°). Mixed melting point determinations on the two hydroquinones and diacetates showed no depression (mixture melting points, 147–150°, 90–92°, respectively). The infrared curves for the two hydroquinones were identical. The data thus indicated that the heat degradation product of δ-tocopherol is 2,6-dimethylhydroquinone and that δ-tocopherol is 8-methyltocol.

On the other hand, toluhydroquinone, the hydroquinone to be expected from a tocol unmethylated in the aromatic ring, has a melting point (124–125°) which is lower than that of the hydroquinone from δ-tocopherol. The mixed melting point of the two showed a marked depression (m. p. 105–110°). The melting points of the hydroquinones which would be expected to be derived from 7-methyl, 5-methyl, and 5,7-dimethyl tocols are so much higher than that found for the hydroquinone from δ-tocopherol (2,5-dimethyl-, 213°; 2,3-dimethyl-, 221°; 2,3,5-trimethyl-, 170°, respectively) that these possible formulas were excluded.

The identification was checked by synthesizing 8-methyl tocol from toluhydroquinone monobenzoate and natural phytol by a modification of the method of Jacob, *et al.*[9] The tocol was purified by chromatography on zinc carbonate and had a purity of 94% by ferric chloride–dipyridyl assay. The properties of the natural (*d*-) and the synthetic (racemic) tocopherol were found to be similar. This was true of their infrared and ultraviolet absorption spectral. On heat degradation the synthetic compound gave apparently the same hydro-

(13) Fernholz, THIS JOURNAL, **60**, 700 (1938).

(14) Emerson and Smith, *ibid.*, **62**, 1869 (1940).

quinone (m. p. 143–144°) as the natural compound since the melting points and infrared spectra were substantially the same. Oxidation of the synthetic compound with gold chloride, silver nitrate, and nitric acid gave pigments having absorption maxima at the same position as the natural compounds $E_{1\,cm.}^{1\%}$ (257 m$\mu$) = 227, $E_{1\,cm.}^{1\%}$ (435 m$\mu$) = 11 and $E_{1\,cm.}^{1\%}$ (373 m$\mu$) = 48, respectively). The slight divergencies observed were probably due to the fact that the oxidation products were not purified.

## Experimental

### Structure

**Heat Degradation of δ-Tocopherol.**—A preparation of δ-tocopherol (1 g.) was heated for two to three hours in a retort (50 ml.) provided with an inlet-tube through which a slow stream of carbon dioxide was passed. A bath temperature of 375–385° was maintained. A water-cooled receiver was provided to condense volatiles. A sublimate consisting of crystals and some oil collected in the neck of the retort and was dissolved in ether. After evaporation the residue was extracted twice with warm petroleum ether (Skellysolve F, 25 ml.). A white solid remained (0.11 g.) which was dissolved in ether (5 ml.) and Skellysolve F (25 ml.) added. The solution was evaporated to incipient precipitation, filtered into Skellysolve F (25 ml.) and allowed to stand for two hours. The crystals (I, 0.05 g.) were filtered and had a melting point of 129–134°. From the mother liquor, after concentration, more crystals (0.027 g.) were obtained. The crude hydroquinone was purified by the method of Martin and Synge[15] on silica gel saturated with water. A 50% yield of purified hydroquinone was obtained (m. p. 144–147.5°).

*Anal.* Calcd. for 2,6-dimethylhydroquinone $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.70; H, 7.30.

The hydroquinone diacetate was prepared with acetic anhydride and fused sodium acetate according to the procedure of Shriner and Fuson.[16] The mixture was worked up in the usual way and crystallized from a small amount of hot ethanol to which water was added to incipient turbidity. The crystals melted at 89–91°.

*Anal.* Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.30; H, 6.30.

**Other Compounds Synthesized.** (a) **2,6-Dimethyl-hydroquinone.**—This was prepared from 3,5-dimethylphenol (Eastman Kodak Company) by nitrosation[17] to form the quinone oxime. After crystallization from 50:50 ethanol–benzene the oxime had m. p. 168–173°. It was hydrolyzed to the quinone according to the method of Sumerford and Dalton[18]; needles, m. p. 71–72°. The quinone was dissolved in warm benzene and reduced with aqueous sodium hydrosulfite solution by shaking until the color disappeared (ten minutes). A white precipitate separated which was extracted with ether. The extract was dried over sodium hydrosulfite and sodium sulfate, the ether partly removed and the hydroquinone allowed to crystallize; white leaflets, m. p. 147–149°. The melting point was previously reported to be 149–151°.[19]

(b) **The Diacetate.**—The diacetate of 2,6-dimethylhydroquinone was made by the procedure previously described[16] and had m. p. 91–92°.

*Anal.* Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.70; H, 6.30.

(c) **Toluhydroquinone.**—This was made from the quinone (Eastman Kodak Co.) by reduction with sodium hydrosulfite (m. p. 124–125°). The melting point was previously reported as 124–125°.[20]

(d) **Toluhydroquinone Monobenzoate.**—This was prepared according to the method of Jacob, *et al.*[9]

**Synthesis of 8-Methyltocol.**—Toluhydroquinone monobenzoate (3.3 g.), natural phytol (6.0 ml.) and zinc dust (0.6 g.) were added to glacial acetic acid (25 ml.) in a flask provided with a condenser and a tube through which a gentle stream of nitrogen was passed over the surface of the solution. A solution of freshly-fused zinc chloride (1.5 g.) in glacial acetic acid (18 ml.) was added with agitation. The mixture was refluxed in an atmosphere of nitrogen for seven hours, adding acetic acid from time to time to maintain the volume. The solution was poured on ice, extracted with ether, and the extract was washed with 0.5 $N$ potassium hydroxide and with water. After drying the solvent was distilled and the residue (5.3 g.) was saponified in ethanol–*i*-propanol, 80:20, as in (5). The unsaponifiable matter (3.87 g.) assayed 23% tocopherol and showed a 17% rise between two and ten minutes.

This fraction was adsorbed from petroleum ether solution on zinc carbonate–Celite, 70:30 (150 g.) in a column approximately 2 in. × 12 in. The column was developed with petroleum ether. 8-Methyltocol was concentrated in a zone near the top of the column which fluoresced light-purple with ultraviolet light. This zone was eluted with ether–acetone, 50:50 (0.33 g., assay 83.5%, 19% rise). From the next lower zone in the column a fraction was eluted which, upon rechromatographing, gave a further yield of 8-methyltocol (I, 0.249 g., assay 94%, 20% rise).

*Anal.* of I. Calcd. for $C_{27}H_{46}O_2$: C, 80.52; H, 11.52. Found: C, 80.40; H, 11.50.

## Summary

1. A newly recognized tocopherol, called δ-tocopherol, has been isolated from soybean oil. The available evidence indicates that δ-tocopherol is 8-methyltocol which makes it the first tocopherol yet isolated from natural sources which is monomethylated in the aromatic ring of the chroman nucleus.

2. δ-Tocopherol produces more color with the ferric chloride–dipyridyl reagent on a molecular basis than α-, β- and γ-tocopherols, which causes errors in assaying mixtures of tocopherols containing δ-tocopherol unless a proper correction is made.

3. δ-Tocopherol constitutes approximately 30% of the mixed tocopherols in soybean oil, 5% of those in wheat germ oil, and there is evidence of its presence in cottonseed and peanut oils.

4. Certain properties of δ-tocopherol are described. It was found to be the most active of the tocopherols as an antioxidant for vitamin A acetate and β-carotene. It was the most resistant to atmospheric oxidation. It had only one-one hundredth of the activity of natural α-tocopherol in the Evans resorption sterility test for vitamin E. Its ultraviolet absorption, its infrared spectrum, its optical rotation, its reaction with diazo reagents, and the nature of its oxidation products formed with silver nitrate, nitric acid, and gold chloride are described.

(15) Martin and Synge, *Biochem. J.*, **35**, 1364 (1941).

(16) Shriner and Fuson, "Identification of Organic Compounds," 2nd ed., 1940, p. 137, John Wiley and Sons, New York, N. Y.

(17) "Organic Syntheses," Coll. Vol. I, 511 (1941).

(18) Sumerford and Dalton, THIS JOURNAL, **66**, 1331 (1944).

(19) Noelting and Baumann, *Ber.*, **18**, 1151 (1885).

(20) Nietzke, *Ber.*, **10**, 1935 (1877).