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Natural α-, β- and γ-Tocopherols and Certain Esters of Physiological Interest[1]

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Only two crystalline or solid esters of the natural tocopherols[2] appear to have been reported which have a vitamin E activity equal to their tocopherol content and are non-toxic to human beings. These are α-tocopherol acetate[3] and calcium α-tocopherol succinate.[4] The present investigation had the primary objective of preparing such esters of the three tocopherols for a projected research on the physiological action of the tocopherols in human beings and in animals. The esters promised to be useful in this work because they are more stable to oxidation *in vitro* than the free tocopherols. It, therefore, seemed probable that the tocopherols would be better protected from oxidation in the animal body if fed in ester form rather than in the free condition.

As a result of the research these crystalline esters were prepared: α-tocopherol acid succinate,[5] α-tocopherol palmitate, β-tocopherol azobenzene-4-carboxylate and γ-tocopherol palmitate (Figs. 1, 2, 3, 4). For comparison, crystalline synthetic *d,l*-α-tocopherol palmitate was prepared and an unsuccessful attempt was made to secure crystalline synthetic α-tocopherol acid succinate. With the exception of the β-tocopherol compound these esters were found to be non-toxic and on a molecular basis to have the same vitamin E activity as the free tocopherols.

The purified tocopherols were prepared from the esters by saponification, followed by distillation, and were identified by conversion to the known allophanates.

Properties of Esters

Extinction Coefficient.—With the exception of β-tocopherol azobenzene carboxylate (maximum at 328 mμ) all of the esters prepared had an absorption band with a maximum at 286 mμ, in ethyl alcohol.[6] The extinction coefficients ($E^{1\%}_{1\,cm.}$) at the maximum are given in Table IB. On an equivalent basis the esters possessed only about 60% of the extinction coefficients of the free tocopherols.

TABLE I

EXTINCTION COEFFICIENTS OF (A) TOCOPHEROLS AND (B) TOCOPHEROL ESTERS IN ETHYL ALCOHOL

Tocopherol		Absorption maximum in (mμ)	$E^{1\%}_{1\,cm.}$	Persistence ratio: E Max. (292–300 mμ) / E Min. (253–259 mμ)
(A) Natural	α-	292	73.7	17.5
	β-	297	87.6	20.6
	γ-	298	92.8	18.0
Synthetic[a]	α-	292	74.7	6.8
	β-	297	85.1	7.3
	γ-	300	89.2	9.2
(B) Tocopherol Esters:				
Natural:				
α-acid succinate		286	38.5	5.7
α-palmitate		286	26.8	5.7
β-azobenzene carboxylate		328	414.0	3.6
γ-palmitate		286	39.0	4.4
α-allophanate		286	33.6	5.6
β-allophanate		286	44.5	5.0
γ-allophanate		286	46.0	5.9
Synthetic:				
α-palmitate		286	30.9	7.8

[a] These were specimens supplied by the Merck Company and were not further purified.

Crystallization.—The following observations were made on the crystallizing properties of the esters:

(1) The esters separated from solution in better yield than the allophanates. This is indicated by the data in Table II in which the yields of tocoph-

TABLE II

RELATIVE YIELDS OF PURE TOCOPHEROLS CRYSTALLIZED AS NEW ESTERS AND AS ALLOPHANATES

Ester	% tocopherol in conc. before esterification	Crystallizations to attain constant m. p.	% yield of tocopherol as pure ester
α-Tocopherol acid succinate	51	2	81
α-Tocopherol allophanate	50	5	35
β-Tocopherol azobenzene-4-carboxylate	83	2	59
β-Tocopherol allophanate	100	2	18
γ-Tocopherol palmitate	75	2	55
γ-Tocopherol allophanate	100	4	21

(1) Presented before the Division of Organic Chemistry of the American Chemical Society, Buffalo meeting, September, 1942.

(2) Hereafter the term "tocopherol" means "natural tocopherol."

(3) Robeson, THIS JOURNAL, **64**, 1487 (1942).

(4) Smith, Renfrow and Opie, *ibid.*, **64**, 1084 (1942).

(5) Synthetic α-tocopherol acid succinate [Demole, Isler, Ringier, Salomon and Karrer, *Helv. Chem. Acta*, **22**, 65 (1939)] and natural α-tocopherol acid succinate [McArthur and Watson, *Can. Chem. Process Inds.*, **23**, 350 (1939)] have previously been prepared in non crystalline form.

(6) The spectrographic measurements were made by Mr. G. Wait of this Laboratory using a Hilger quartz spectrograph, Model E-498, and a Spekker ultraviolet photometer. The light source was a tungsten-steel spark.

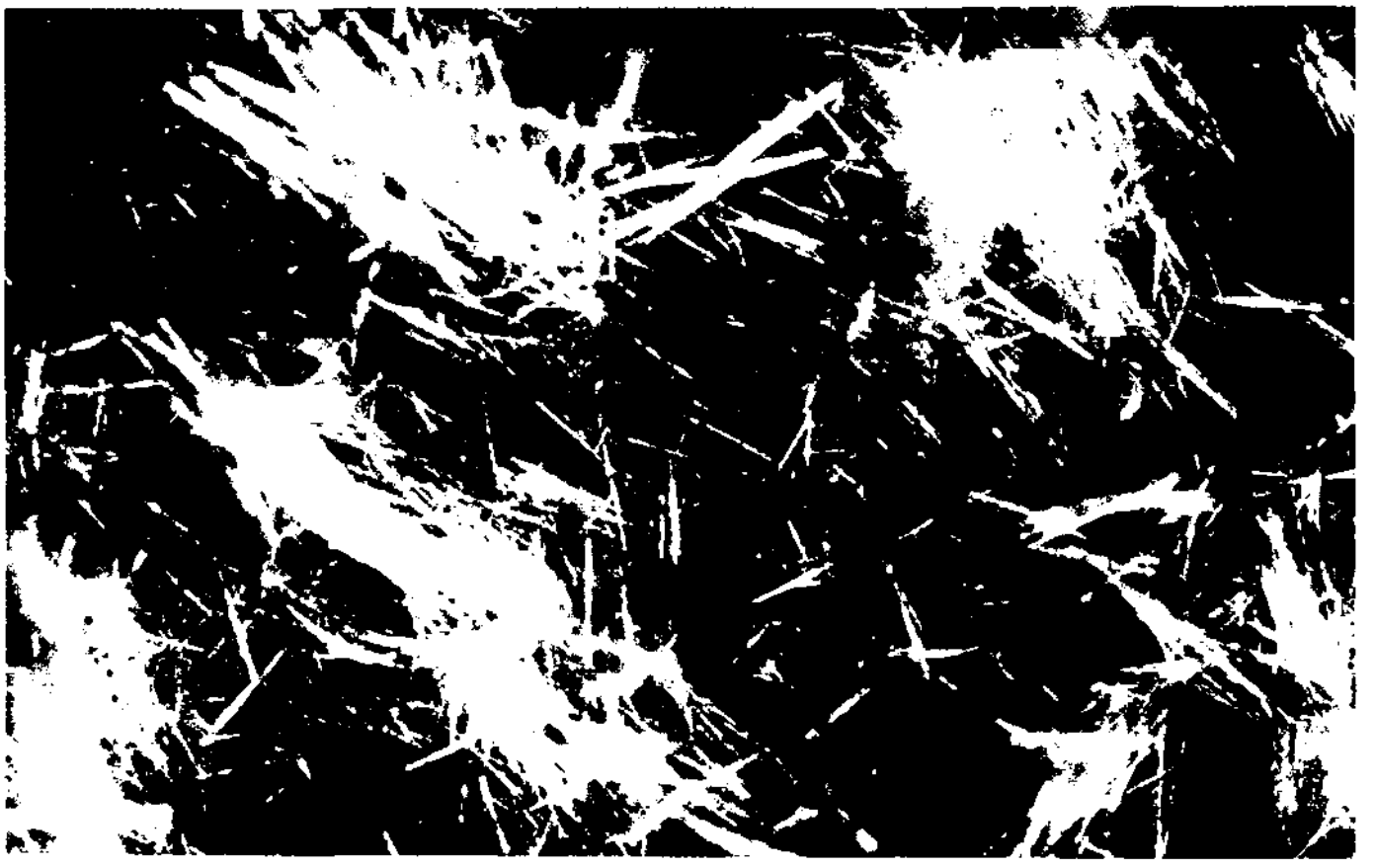

Fig. 1.—Natural α-tocopherol acid succinate × 25 (photomicrographs, Figs. 1, 2, 3, 4, courtesy of Mr. R. P. Loveland, Eastman Kodak Company, Research Laboratories).

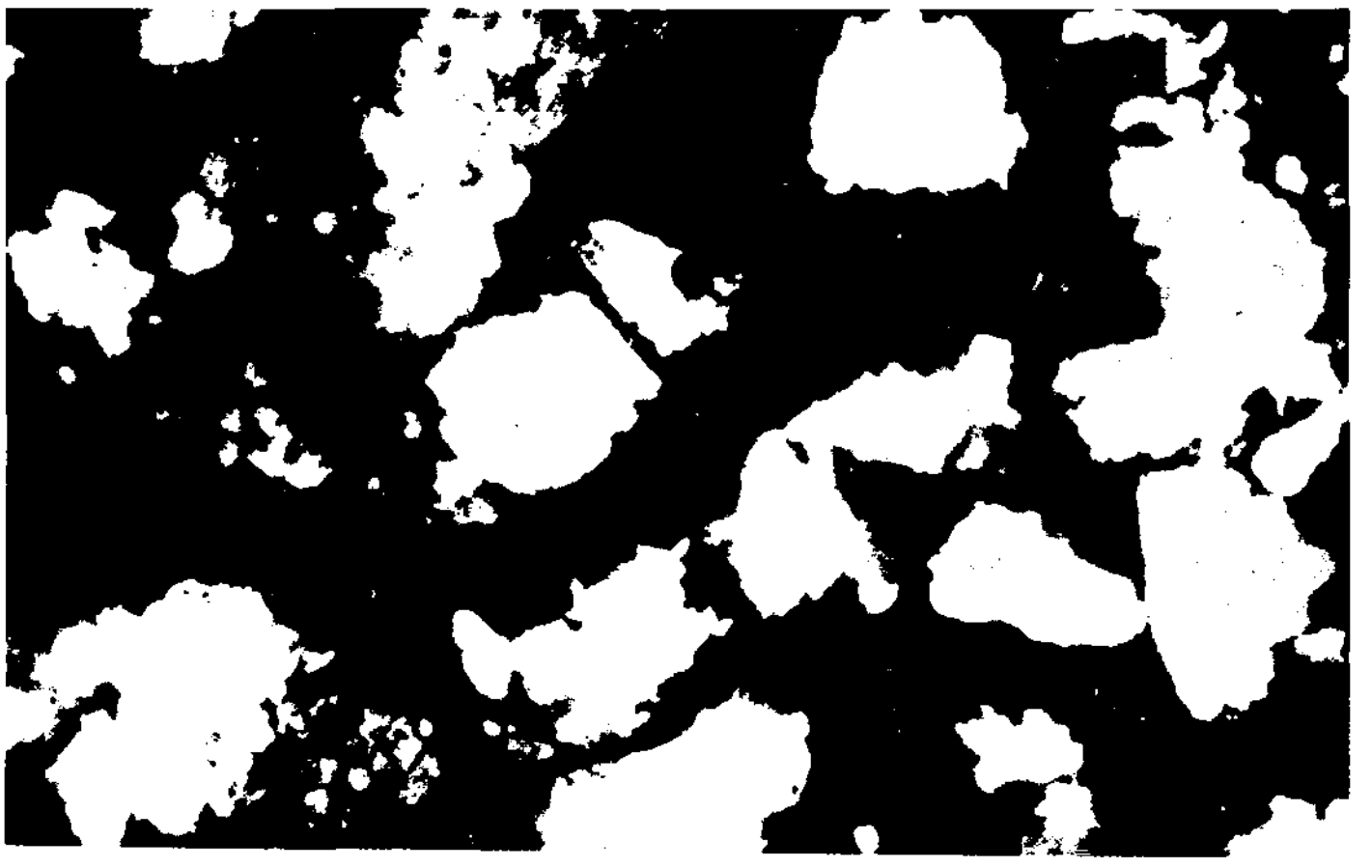

Fig. 2.—α-Tocopherol palmitate × 25.

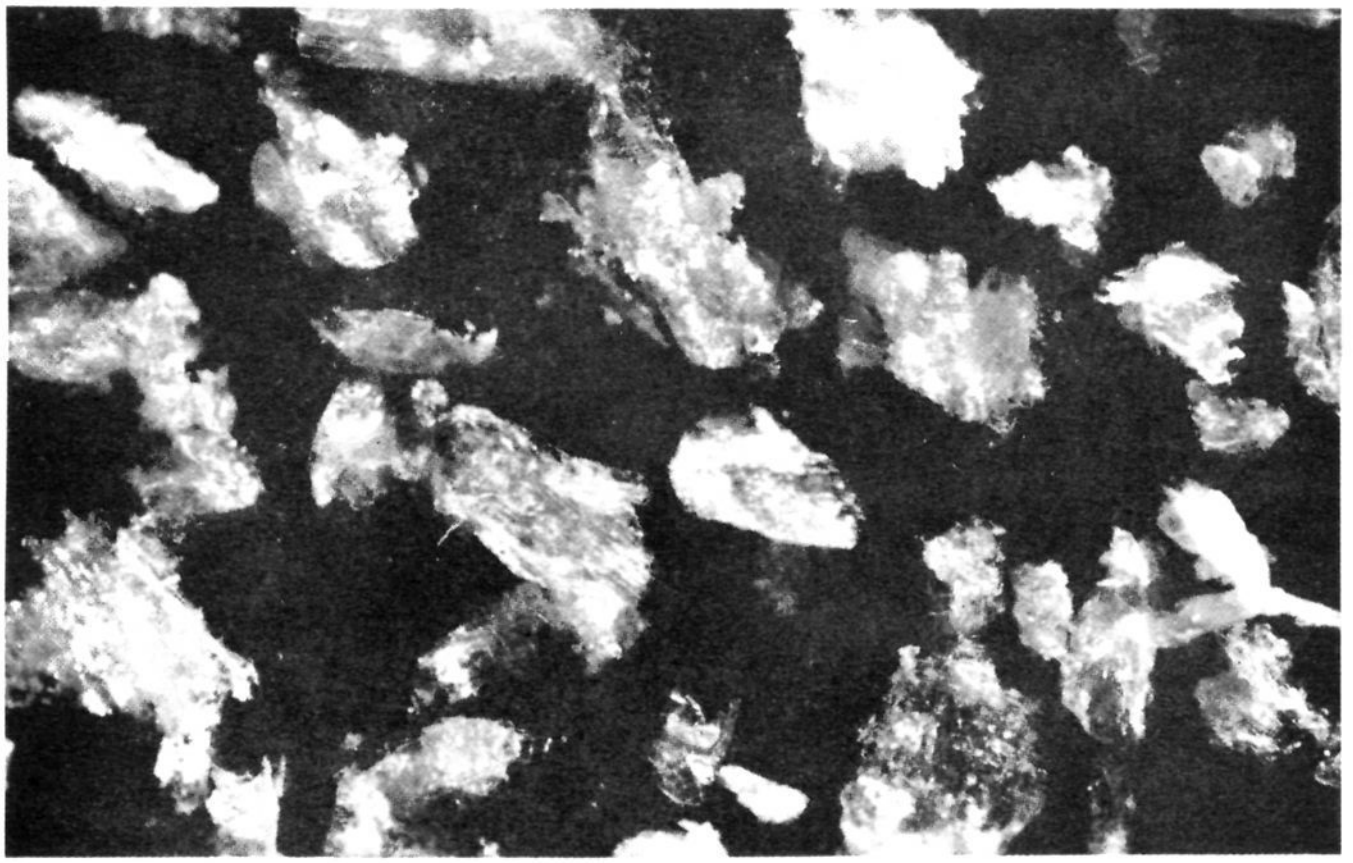

Fig. 3.—β-Tocopherol azobenzene-4-carboxylate × 25.

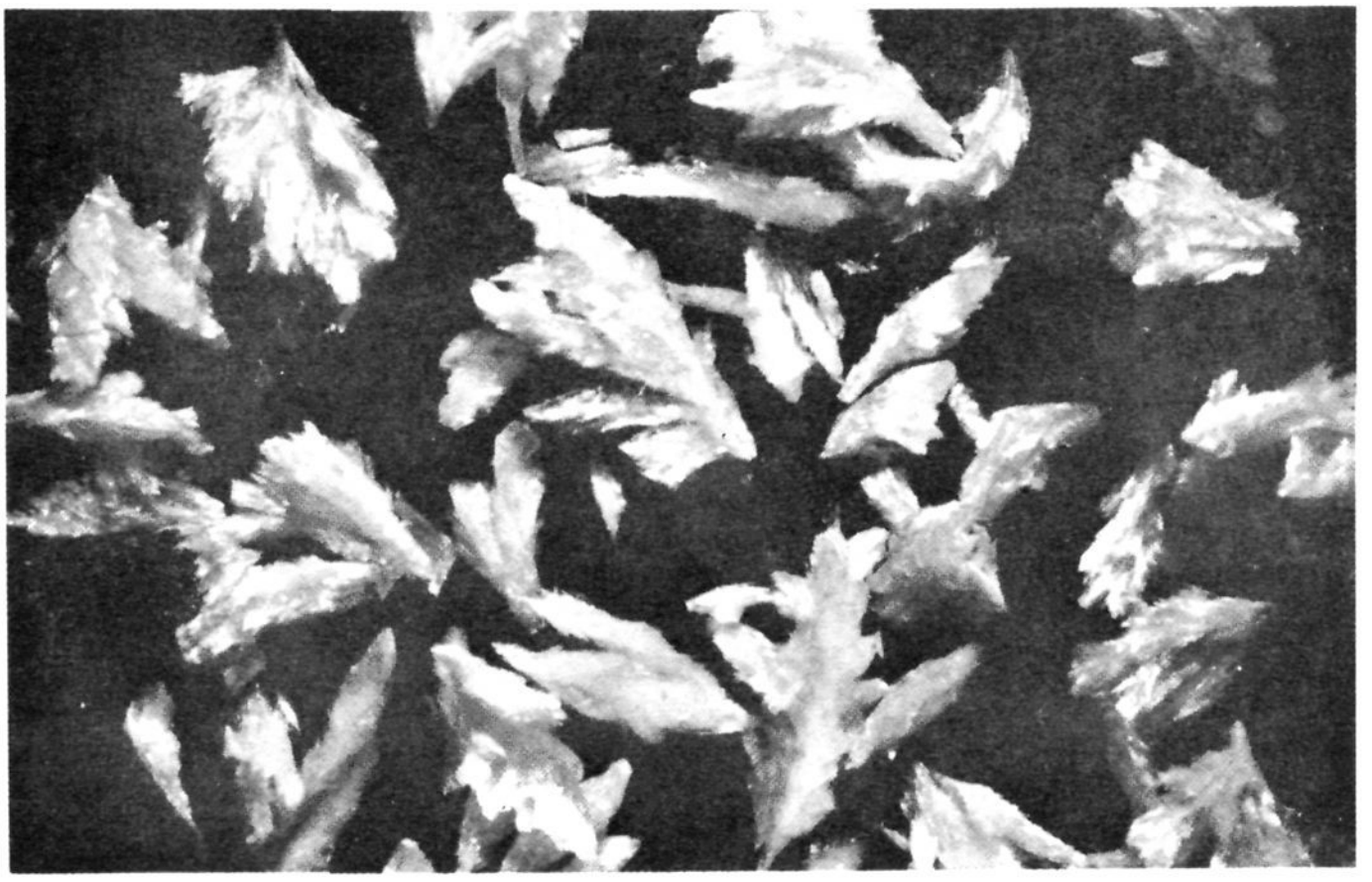

Fig. 4.—γ-Tocopherol palmitate × 10.

erols crystallized as the new esters and as allophanates are compared.

(2) Although the tocopherols are similar in structure no one ester was best suited to crystallize the three. Thus, α-tocopherol acid succinate crystallized but not the acid succinates of β- and γ-tocopherols. α- and γ-tocopherol palmitates crystallized but not β-tocopherol palmitate. β-Tocopherol azobenzene carboxylate was the only ester of β-tocopherol, with the exception of the allophanate, which we could crystallize. Unsuccessful attempts were made to crystallize the acetate, 3,5-dinitrobenzoate, β-naphthoate, α-naphthyl urethan and phenyl urethan.

(3) Certain esters co-crystallized. Thus, γ-tocopherol acid succinate, which would not crystallize when pure, co-crystallized with α-tocopherol acid succinate. The formation of mixed crystals of the α- and γ-allophanates has previously been reported by Emerson and Smith.[7] The melting point of a mixture of esters containing 75% α- and 25% γ- acid succinates was only 4° lower than that of α-tocopherol acid succinate. This made it difficult to detect the presence of the γ- in the α-ester by the melting point depression.

α- and β-tocopherol palmitates did not co-crystallize. This property was utilized (Experimental Part) to prepare a β-tocopherol concentrate (II), substantially free of α-tocopherol.

(4) Synthetic α-tocopherol acid succinate could not be crystallized, but separated from solvents as a gel. This was surprising in view of the excellent crystallizing properties of the natural ester.

Sodium α-Tocopherol Succinate.—α-Tocopherol acid succinate forms a solid calcium salt.[4] We prepared the sodium salt. The latter formed a dilute solution (0.2%) in water or in propylene glycol which may be useful for the injection of α-tocopherol. The *p*H of a 0.5% solution of the sodium salt in 75:25 ethyl alcohol–water mixtures was approximately 3.5. Upon neutralization with dilute hydrochloric acid, the propylene glycol solution remained clear but after several hours the water solution became turbid.

Properties of Tocopherols

The free tocopherols were prepared by the saponification of α-tocopherol acid succinate, β-tocopherol azobenzene carboxylate and γ-tocopherol palmitate, respectively, and were purified by distillation. They were light yellow or colorless oils which were unchanged in appearance after months of storage at 5°, in darkness, in an atmosphere of nitrogen, but turned red after exposure to sunlight for one day.

Extinction Coefficient.—The tocopherols were distinguished from one another by the position of their absorption maxima, in ethyl alcohol (Table IA and Fig. 5) and by their extinction coefficients.

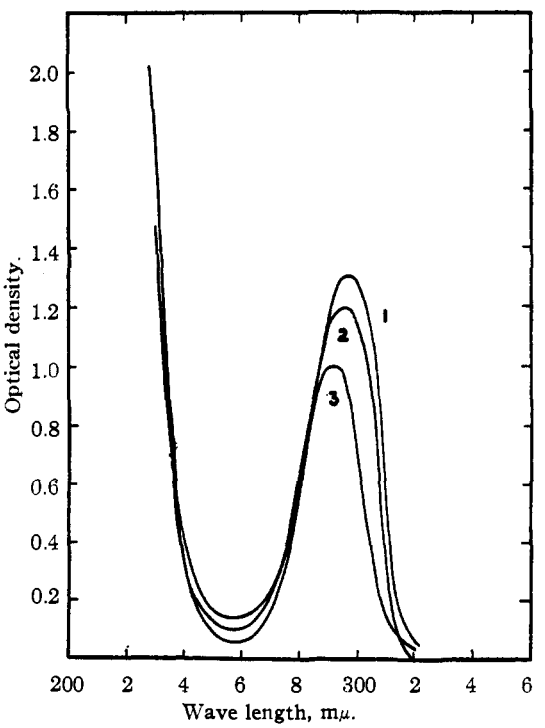

Fig. 5.—Spectrophotometric curves of natural tocopherols in ethyl alcohol: α-(3), β-(2), γ-(1); concns. (g./100 cc.), 0.0137, 0.0138, and 0.0142, respectively.

The extinction coefficient found for α-tocopherol agreed closely with the value $E^{1\%}_{1\,cm.}$ (292 mμ) = 70 reported by Moss and Drummond[8] and was substantially the same as that found for a specimen of synthetic α-tocopherol (Merck).

The extinction coefficient of β-tocopherol agreed with the value reported by Todd, Bergel and Work [$E^{1\%}_{1\,cm.}$ (295 mμ) = 87][9] but the absorption maximum was at 297 mμ with our instrument. An intermediate value for the position of the maximum was determined by Dr. F. P. Zscheile and L. F. Green of Purdue University who kindly assayed our preparation of β-tocopherol and found $E^{1\%}_{1\,cm.}$ (295.8 mμ) = 86.0.[10] A specimen of synthetic β-tocopherol (Merck) had substantially the same

(7) Emerson and Smith, THIS JOURNAL, **62**, 1870 (1940).

(8) Moss and Drummond, *Biochem. J.*, **32**, 1953 (1938).

(9) Todd, Bergel and Work, *ibid.*, **31**, 2257 (1937).

(10) For a description of the instrument used, see Zscheile and Henry, *Ind. Eng. Chem., Anal. Ed.*, **14**, 422 (1942).

extinction coefficient and absorption maximum as natural β-tocopherol.

The extinction coefficient of γ-tocopherol does not appear to have been previously reported. The value we found [$E^{1\%}_{1\,cm.}$ (298 mμ) = 92.8] agreed closely with the value found by Zscheile [$E^{1\%}_{1\,cm.}$ (298.0 mμ) = 90.0]. A specimen of synthetic γ-tocopherol (Merck) had a lower extinction coefficient than that of natural γ-tocopherol and the maximum was at 300 mμ.

Optical Rotation.—Determination of the specific rotation of the purified tocopherols and two of their esters gave the values in Table III.[11] The three tocopherols were dextrorotatory in ethyl alcohol and α- and γ-tocopherols were levorotatory in benzene. Thus the sign of rotation varied with the solvent. This result was unexpected but it was confirmed by repeated determinations. Neither the ethyl alcohol nor the benzene used gave a detectable rotation by itself.

TABLE III

SPECIFIC ROTATION OF TOCOPHEROLS AND ESTERS IN ETHYL ALCOHOL AND BENZENE

Sample		Ethyl alcohol $[\alpha]^{25}_{546.1}$	l[a]	c[b]	Benzene $[\alpha]^{25}_{546.1}$	l	c
Tocopherols	α-	+0.32	1	14.28	−3.0	1	13.50
	β-	+2.9	1	7.15	...	..	...
	γ	+2.2	1	9.32	−2.4	1	8.59
Tocopherol esters	α-acid succinate	+4.4	2	8.35	+2.6	2	15.10
	γ-palmitate	...	..	...	+3.4	1	11.20

[a] l = length of tube in dm. [b] c = concentration of solution in g./100 cc.

α-Tocopherol acid succinate and γ-tocopherol palmitate were dextrorotatory in benzene. Thus the esters can have a different sign of rotation in a given solvent from that of the corresponding free tocopherols. It is evident that the magnitude of the specific rotations of the esters was greater on an equivalent basis than that of the tocopherols. The specific rotations found for α-tocopherol acid succinate in alcohol and benzene indicate that the zero rotation observed for α-tocopherol allophanate in benzene[12] does not hold for all esters of natural α-tocopherol.

Iodine Value.—It was shown by Olcott that hydrogenated vitamin E concentrates had an appreciable iodine value[13] but it was not established whether this was due to the tocopherols or to impurities. Therefore, iodine values (Wijs) were determined on purified α-, β- and γ-tocopherols giving 143, 138, and 152, respectively. The iodine values, therefore, determined by Olcott were due to the tocopherols and not to impurities. The reaction responsible for the iodine value is being studied.

Oxidation: (a) By Ferric Chloride and αα′-Dipyridyl.—The tocopherols are oxidized by ferric chloride to yellow *p*-quinones. In the presence of αα′-dipyridyl, α-tocopherol is said to be quantitatively oxidized to α-tocopheryl quinone,[14] the simultaneously formed ferrous chloride giving with αα′-dipyridyl a red color. This reaction is utilized in the well-known Emmerie–Engel method for the assay of tocopherols.

We have compared the rate of oxidation of the three purified tocopherols to *p*-quinones by ferric chloride and αα′-dipyridyl using a procedure designed to retard the rate of oxidation sufficiently so that measurements of the speed could be made.[15]

Solutions of α-, β-, and γ-tocopherols (23 cc., approximately 0.0007% in absolute ethyl alcohol) were added to a series of amber bottles. To each solution in turn 1 cc. of dipyridyl solution (0.25% in ethyl alcohol) and 1 cc. of ferric chloride solution (0.1% in ethyl alcohol) were added at room temperature (23°). After intervals of from twenty seconds to twenty minutes the "L values" ($L^{1\%}_{1\,cm.}$ 520 mμ) of the solutions were measured in an Evelyn photoelectric colorimeter using a 520 mμ filter. The "L value," otherwise written $L^{1\%}_{1\,cm.}$, is a quantity measured by the Evelyn instrument which is proportional to $E^{1\%}_{1\,cm.}$, the extinction coefficient. The maximum of the ferrous chloride–

TABLE IV

VARIATION OF "L VALUE" ($L^{1\%}_{1\,cm.}$ 520 mμ) OF α-, β-, γ-TOCOPHEROLS WITH TIME DURING OXIDATION BY FERRIC CHLORIDE AND αα′-DIPYRIDYL

Time in seconds	L Value α-	β-	γ-
20	340	243	231
40	354	343	338
80	354	370	372
Minutes			
2.6	352	372	372
5	350	365	374
10	352	372	372
20	359	372	382

(11) We wish to thank Mr. T. F. Murray, Eastman Kodak Company, Research Laboratories, for measuring the optical rotations. A Hilger instrument, Lippich type, was used with a 546.1 mμ filter. The light source was a mercury arc.

(12) Emerson, Emerson, Mohammad and Evans, *J. Biol. Chem.*, **122**, 103 (1937).

(13) Olcott, *ibid.*, **105**, lxv (1934).

(14) Emmerie and Engel, *Rec. trav. chim.*, **57**, 1351 (1938).

(15) This procedure is based on a modification of the original Emmerie Engel method which was devised by Mr. H. Rawlings of this Laboratory and is routinely employed here.

dipyridyl complex is at 520 mμ. The relative rates of oxidation of the tocopherols were determined by comparing the rate of increase of the L values (Table IV).

It appears that under the experimental conditions α-tocopherol was oxidized more rapidly than β- or γ-tocopherol. β-Tocopherol was oxidized slightly more rapidly than the γ-isomer but the difference was too small to be significant. Within the experimental error, the L values for the three tocopherols were constant after eighty seconds and were inversely proportional to the molecular weights.

Since the final L value for a mixture of pure tocopherols may vary by as much as 4% depending on which are present and in what relative amounts, there is an inherent error of this magnitude in assaying mixtures of tocopherols whose composition is not known by the usual Emmerie–Engel procedure. This error can be largely eliminated by modifying the procedure, such as in the way described, to retard the reaction rate and choosing a reaction time so that the three tocopherols give the same L value. In the procedure outlined a reaction period of one minute was necessary. This method of determining the true total tocopherol content in mixtures of tocopherols is now in use in this Laboratory.

Oxidation: (b) By Silver Nitrate.—On prolonged oxidation by either ferric chloride or silver nitrate in alcohol solution, the tocopherols are oxidized to red *o*-quinones.[16] The red quinones of α- and γ-tocopherols have an absorption maximum at 480 mμ while that of β-tocopherol has a maximum at 465 mμ.

We have studied the oxidation of the purified tocopherols by silver nitrate. It was found that under experimental conditions which caused γ-tocopherol to be substantially completely oxidized to the *o*-quinone, the α- and β-, compounds were only partially oxidized. Thus, when α-, β- and γ-tocopherols (0.3% concentration in a 1.15% solution of silver nitrate in methyl alcohol) were separately oxidized for fifteen minutes at 65°, red-colored solutions were formed with extinction coefficients at the maxima of 5.1, 6.4 and 19.2, respectively, corresponding approximately to 24, 30 and 91% conversion to *o*-quinone. The completeness of the oxidation of γ-tocopherol was determined by oxidizing a separate sample to the *o*-quinone with nitric acid and comparing the intensities of the red colors produced by the two methods of oxidation.

It appears that the order of relative ease of oxidation of the tocopherols to *o*-quinones is the reverse of the order in which they are oxidized to *p*-quinones. The ready oxidation of γ-tocopherol to an *o*-quinone appears to be due to the lack of a methyl group in position 5. This methyl group, present in the α- and β- compounds, must be oxidized or displaced before *o*-quinones can be formed.

The difference in the rate with which the three tocopherols are oxidized to *o*-quinone has been made the basis of an assay method for mixtures of tocopherols which was used to determine the efficiency with which mixed tocopherols were separated by adsorption (Experimental Part).

Experimental

Preparation of Tocopherol Esters

The tocopherol esters were prepared by the following procedures: (1) concentrates containing mixed α-, γ- and α-, β-, tocopherols were prepared by the molecular distillation of cottonseed and wheat germ[17] oils, respectively; (2) the tocopherols were further concentrated and separated from one another by chromatographic adsorption; (3) the individual tocopherol concentrates thus prepared were esterified with the appropriate acylating agents and the esters crystallized.

Molecular Distillation of Vegetable Oils.—Alkali-refined cottonseed oil (0.1% mixed α-, γ-tocopherols) and wheat germ oil (0.3% mixed α-, β-tocopherols) were distilled in a cyclic molecular still.[18] The tocopherols and sterols distilled from 150–170° at 0.003 mm. pressure. The distillates were freed of sterols by crystallization from acetone at low temperatures and the desterolated material was redistilled. The distillates were reddish-orange oils containing 25–40% mixed α-, γ- and α-, β-tocopherols. The yield of mixed tocopherol concentrates was from 40–60% of the tocopherols present in the undistilled oils. The impurities consisted of sterols, glycerides, and unknown compounds of a hydrocarbon-like nature.

Molecular distillation was particularly useful for this preliminary concentration because it separated the tocopherols from the bulk of the glycerides without the destruction of vitamin E which frequently accompanies concentration by saponification.

Adsorptive Separation and Concentration of Tocopherols.—The distillates were further concentrated and the tocopherols separated from one another by adsorption on Special Filtrol and Doucil,[19] using the liquid chromatogram method.[20] The efficiency of the separation of the

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

(17) We wish to thank General Mills, Inc., and Dr. A. E. Taylor for the gift of this oil.

(18) Hickman, *Ind. Eng. Chem.*, **29**, 968 (1937).

(19) Special Filtrol (Filtrol Corp., 315 West 5th Street, Los Angeles, California) is an activated clay. Doucil (American Doucil Company, 121 South 3d Street, Philadelphia, Pennsylvania) is a sodium aluminum silicate.

(20) Zechmeister and Cholnoky, "Principles and Practice of Chromatography," John Wiley and Sons, Inc., New York, N. Y. 1941, p. 76.

tocopherols was determined by an assay method which, as was previously mentioned, involved oxidation by silver nitrate in methyl alcohol solution. The adsorption procedure and the silver nitrate method for the assay of mixed tocopherols are closely related and will be described separately.

By adsorption, concentrates containing 51% α-tocopherol (I) and 75% γ-tocopherol (III) were prepared. The impurities were chiefly hydrocarbons and glycerides. The adsorptive separation of α- and β-tocopherols was not complete, necessitating the removal of α-tocopherol from the β tocopherol concentrate (87% mixed tocopherols; β:α ratio approximately 60:40) by esterifying it with palmitoyl chloride and crystallizing α-tocopherol palmitate (see next section). The β-palmitate did not crystallize. Saponification of the soluble fraction gave an 83% β-tocopherol concentrate (II) whose freedom from α-tocopherol could not be accurately determined by the mixed tocopherol assay method. For the removal of any remaining α-tocopherol, reliance was placed on the subsequent repeated crystallization of β-tocopherol azobenzene carboxylate. This reliance appeared justified because the β-tocopherol prepared by saponifying the ester had similar spectrographic properties to that prepared by Todd and Bergel[9] from wheat germ oil containing little or no α-tocopherol. Also, the crystalline allophanate prepared from the purified β-tocopherol was homogeneous under the microscope and had the characteristic lath-like structure of β-tocopherol allophanate.[9]

Concentrates I, II and III containing 25–30% of the tocopherol present in the original cottonseed and wheat germ oils, were sufficiently potent for the preparation of α-tocopherol acid succinate, β-tocopherol azobenzene-4-carboxylate and γ-tocopherol palmitate, by esterification with succinic anhydride, azobenzene carboxyl chloride and palmitoyl chloride, respectively. The esters crystallized without difficulty in good yield. The synthetic esters were made from *d,l*-α-tocopherol (Merck).

Separation of α-Tocopherol from the β-Tocopherol Concentrate.—An impure β-tocopherol concentrate (53.5 g., 87% mixed tocopherols) was esterified with palmitoyl chloride by the procedure used for γ-tocopherol palmitate. The mixed tocopherol palmitate (86 g.), was crystallized from isopropyl alcohol (800 cc.) at 3°. After three days α-tocopherol palmitate was filtered (37.5 g.) and the filtrate was allowed to evaporate slowly at 3° in a flask stoppered with a plug of glass wool. After two weeks a trace of α-tocopherol palmitate was filtered, the filtrate was evaporated and the residue was saponified in an atmosphere of nitrogen, as described under "Preparation of Free Tocopherols." An 83% β-tocopherol concentrate (II) was obtained (29 g.).

Preparation of (A) α-Tocopherol Acid Succinate.—An α-tocopherol concentrate, (I), (131 g., 51.7% α-tocopherol, 0.157 mole) was heated at 90° with succinic anhydride (74 g., 0.74 mole) in pyridine (304 cc.) for four hours. The mixture was diluted with ethyl ether (220 cc.) and allowed to stand fifteen hours at 3°. Succinic anhydride was filtered and the crystals were well washed with ethyl ether. The filtrate, further diluted with ether, was washed with dilute hydrochloric acid, water and dried. After distillation of ether a dark oily residue (150 g.) was obtained. This was dissolved in petroleum ether (650 cc., b. p. 30–65°) and adsorbed on 854 g. of a 70:30 mixture of Special Filtrol and Hyflo Super Cel in an adsorbent column 4″ × 7″. The solution was forced through the column under nitrogen pressure. The column was washed with petroleum ether to remove unadsorbed impurities, then the strongly adsorbed α-tocopherol succinate was eluted with ethyl ether (3000 cc.). After distillation of ether the residue (85 g.) was crystallized from petroleum ether (850 cc.) at 5° and recrystallized from a 10% solution in petroleum ether at 20°. α-Tocopherol acid succinate crystallized in needles, m. p. 76–77°. The yield was 65 g., 80% of the theoretical.

Anal. Calcd. for $C_{33}H_{54}O_5$: C, 74.66; H, 10.26. Found: C, 74.59; H, 10.07.

Sodium α-Tocopherol Succinate.—α-Tocopherol acid succinate (0.3 g.) in absolute ethyl alcohol (1 cc.) was neutralized with a 2% solution of sodium hydroxide in ethyl alcohol. The neutralized solution was evaporated to dryness and extracted with a hot 10% solution of methyl alcohol in acetone (3 cc.). The solvent was decanted leaving the salt as a white powder.

(B) Synthetic α-Tocopherol Acid Succinate.—Synthetic α-tocopherol (Merck) was succinated by the procedure used for natural α-tocopherol. The acid succinate (2.2 g.) was adsorbed on Special Filtrol. The column was washed with petroleum ether (100 cc.), benzene (100 cc.), and ethyl ether (100 cc.), the filtrate being collected in fractions. The synthetic ester appeared to be adsorbed less strongly than the natural ester and was collected mainly in the petroleum ether and benzene fractions. These were combined and the solvent distilled, leaving the acid succinate as a brown oil (1.73 g.). This separated from petroleum ether only as a gel, even when the solution was seeded with natural α-tocopherol succinate. Attempts to crystallize the ester from other solvents were also unsuccessful.

(C) α-Tocopherol Palmitate.—An α-tocopherol concentrate (I), (4 g.) was esterified with palmitoyl chloride as in the preparation of γ-tocopherol palmitate. The residue, after adsorption, was a pale yellow oil which was crystallized successively from a 20% solution in isopropyl alcohol at 5°; from a 5% solution in acetone at 5°; and from a 15% solution in acetone at room temperature. α-Tocopherol palmitate crystallized in glossy laths, m. p. 42–43°. The yield was 1.0 g., 33% of the theoretical.

Anal. Calcd. for $C_{45}H_{80}O_3$: C, 80.76; H, 12.06. Found: C, 80.96; H, 11.89.

(D) Synthetic α-Tocopherol Palmitate.—Synthetic α-tocopherol (Merck) (2.0 g.) was esterified with palmitoyl chloride by the method used for γ-tocopherol palmitate. After adsorption a light yellow oil was obtained (2.9 g.) which was crystallized successively from a 10% solution in isopropyl alcohol at 5°; from a 5% solution in acetone at 5°, with slow cooling to prevent oiling out; and from a 10% solution in acetone at 5°. The ester consisted of white granules, m. p. 36–38°. The yield (0.9 g.) was 29% of the theoretical.

Anal. Calcd. for $C_{45}H_{80}O_3$: C, 80.76; H, 12.06. Found: C, 81.00; H, 12.01.

(E) **β-Tocopherol Azobenzene-4-carboxylate: (1) Azobenzene Carboxylic Acid.**[21]—A solution of *p*-aminobenzoic acid (25.5 g.) in glacial acetic acid (106 cc.) was added to a solution of nitrosobenzene (27.7 g.) in glacial acetic acid (82 cc.). After standing two hours at room temperature the flask was heated to 40° for five minutes and allowed to stand at room temperature for one day. The orange plates of the acid were filtered, washed three times with glacial acetic acid, and dried at 100° under reduced pressure. The yield was 29.2 g., 69% of the theoretical, m. p. 240–241°.

(2) **Azobenzene-4-carboxyl Chloride.**—This was prepared by the method of Ladenburg, *et al.*, from the free acid (29.2 g.).[22] The acid chloride formed orange-red plates, m. p. 92–93°. The yield was 15.7 g., 50% of the theoretical, based on the acid.

(3) **Esterification.**—A β-tocopherol concentrate (II), (8.6 g., 83% β-tocopherol, 0.017 mole) was dissolved in pyridine (12 cc.) and ethylene chloride (40 cc.) and azobenzene-4-carboxyl chloride (6.35 g., 0.026 mole) in ethylene chloride (40 cc.) was slowly added. The mixture was allowed to stand at room temperature for one hour and then was heated to 80° for forty-five minutes. Water (2 cc.) was added to hydrolyze the excess acid chloride, the mixture was allowed to stand thirty minutes and then was taken up in ether and washed with dilute hydrochloric acid, then with 0.5 *N* potassium hydroxide and water. After removal of solvent, a red solid was obtained (12.6 g.).

This solid was dissolved in hot isopropyl alcohol (218 cc.) and the well-lagged flask containing the solution was allowed to crystallize at room temperature for eighteen hours. The ester was filtered and dried. The yield was 7.2 g. of orange plates, m. p. 69.5–70.5°. One recrystallization from a 10% solution in isopropyl alcohol, at room temperature, gave 6.3 g. of ester, m. p. 70–71°, 59% of the theoretical. Repeated recrystallization of the ester did not change its melting point.

Anal. Calcd. $C_{41}H_{56}O_3N_2$: C, 78.79; H, 9.04; N, 4.49. Found: C, 78.74; H, 8.87; N, 4.54.

(F) **γ-Tocopherol Palmitate.**—A γ-tocopherol concentrate (III), (20.9 g., 75% γ-tocopherol, 0.038 mole) was dissolved in ethylene chloride (75 cc.) and pyridine (20 cc.). To this solution was slowly added palmitoyl chloride (10.9 g., 0.041 mole, 1.1 mol. prop.) in ethylene chloride (75 cc.). After standing twenty hours at room temperature, the mixture was poured into ether and the extract was washed with dilute hydrochloric acid, 10% aqueous potassium carbonate, 0.5 *N* potassium hydroxide and water. After distillation of ether the residue (30.9 g.) was adsorbed from petroleum ether (200 cc.) on Doucil[19] (140 g.). This was to remove color and some palmitic acid. The adsorbent was washed with petroleum ether (1500 cc.) and the filtrate was evaporated. The residue (28.5 g.) was crystallized from isopropyl alcohol (140 cc.) at 5°. The crystals (18 g.) were recrystallized from acetone (340 cc.) at 5°. γ-Tocopherol palmitate formed glossy laths, m. p. 44–45°. The yield was 13.6 g., 55% of the theoretical. Repeated recrystallization of the ester did not change its melting point.

Anal. Calcd. for $C_{44}H_{78}O_3$: C, 80.66; H, 12.01. Found: C, 80.89; H, 11.96.

(G) **Allophanates.**—These esters were made for characterization purposes from the purified tocopherols by a modification of the method used by Evans, *et al.*[23]

α-Tocopherol Allophanate.—α-Tocopherol (2.84 g.), purified through the acid succinate, in dry toluene (20 cc.) was cooled to −50°. Then cyanic acid (2.5 cc., 10 mol. prop.) was added. The acid was distilled from cyanuric acid (20 g.) at 360° and was condensed in a vessel packed with dry-ice. The reaction mixture was allowed to stand for three days at 5° during which time it set to a gel. This gel was repeatedly extracted with benzene in the usual way and the solvent was removed. The ester was considered to be substantially free of cyanuric acid and cyamelide when a 10% solution in benzene deposited no solids overnight at room temperature. The ester was crystallized three times from a 10% solution in acetone at 5° before the melting point was constant. It consisted of granules, m. p. 157–158°. The yield was 1.5 g. or 45% of the theoretical.

Anal. Calcd. for $C_{31}H_{52}O_4N_2$: C, 72.03; H, 10.15; N, 5.43. Found: C, 72.22; H, 10.01; N, 5.32.

β-Tocopherol Allophanate.—β-Tocopherol (0.5 g.), purified through the azobenzene carboxylate, was dissolved in toluene (10 cc.) and cyanic acid (0.45 cc.) was added at −50°. After the solution had stood at 3° for two days, it was found that no esterification had occurred. Therefore, cyanic acid (0.45 cc.) was added. During three days esterification occurred. The ester was freed of cyamelide and crystallized twice from a 10% solution in acetone at 5°. It consisted of beautiful colorless laths, m. p. 138–139°. The yield was 0.088 g., 18% of the theoretical.

Anal. Calcd. for $C_{30}H_{50}O_4N_2$: C, 71.65; H, 10.03; N, 5.58. Found: C, 71.76; H, 9.85; N, 5.50.

γ-Tocopherol Allophanate.—γ-Tocopherol (0.47 g.), purified through the palmitate, in toluene (10 cc.) was treated with cyanic acid (0.85 cc.), at −50°. The esterification was complete in two days at 3°. After the usual purification the ester was crystallized four times from a 5% solution in acetone at 5°. It formed granules, m. p. 136–138°. Although the melting point agreed with that reported by Emerson, *et al.*,[12] the analysis indicated that the allophanate probably contained impurities from the allophanation procedure.

Anal. Calcd. for $C_{30}H_{50}O_4N_2$: C, 71.65; H, 10.03; N, 5.58. Found: C, 71.18; H, 9.78; N, 6.22.

Preparation of Free Tocopherols

The purified tocopherols were prepared by the saponification of α-tocopherol acid succinate, β-tocopherol azobenzene carboxylate and γ-tocopherol palmitate, respectively, in an atmosphere of nitrogen to prevent oxidation. The method used is illustrated by the preparation of *α-tocopherol.* A reaction flask was fitted with a three-way tube. The outlets were connected to a condenser, a dropping funnel and a tank of nitrogen. Gas from the

(21) This acid was previously prepared by Angeli (*Atti. Accad. Lincei*, **22**, I, 132 (1913), but as the journal was not available, this procedure was used.

(22) Ladenburg, Fernholz and Wallis, *J. Org. Chem.*, **3**, 277 (1938).

(23) Evans, Emerson and Emerson, *J. Biol. Chem.*, **113**, 328 (1936).

latter was led through a bubbler containing isopropyl alcohol before being admitted into the reaction flask in order to maintain a controlled stream of nitrogen.

α-Tocopherol acid succinate (21.3 g.) dissolved in absolute ethyl alcohol (173 cc.) was added to the reaction flask. The alcohol was refluxed for ten minutes while a gentle stream of nitrogen was passed through the apparatus. Then aqueous potassium hydroxide (17.3 cc., concn., 50 g./100 cc.) was added through the dropping funnel and refluxing was continued for fifteen minutes. The mixture, after cooling, was diluted with water and quickly extracted with ether. The extract was washed with 10% potassium carbonate solution and with water. After removal of solvent, under diminished pressure, α-tocopherol was obtained as an orange viscous oil (16.9 g.) which was dissolved in castor oil residue (45 cc.) for distillation.[24]

β-Tocopherol was prepared by saponifying the azobenzene carboxylate (6.8 g.) in 80:20 ethyl alcohol–isopropyl alcohol solution (50 cc.) with aqueous potassium hydroxide (5 cc., concn., 50 g./100 cc.). On adding the concentrated potassium hydroxide solution to the alcohol solution of the ester, a red solid was precipitated which appeared to be the potassium salt of azobenzene carboxylic acid. After refluxing for thirty minutes, the saponification mixture was cooled, extracted quickly with ether and the extract was filtered to remove the insoluble potassium salt. The ether solution was then washed with 0.5 *N* potassium hydroxide and water. After evaporation of ether under diminished pressure, β-tocopherol was obtained as an orange viscous oil [4.5 g. $E^{1\%}_{1\,cm.}$ (297 mμ) = 86.4] which was dissolved in castor oil residue (15 cc.) for distillation.

γ-Tocopherol was prepared by saponifying the palmitate (16.0 g.) in 60:40 ethyl alcohol–isopropyl alcohol (70 cc.) with potassium hydroxide solution (7.3 cc., concn., 50 g./100 cc.). After refluxing for thirty minutes the usual procedure was followed except that repeated washes with 0.5 *N* potassium hydroxide were necessary to remove palmitic acid from the ether solution. After evaporation of ether γ-tocopherol was obtained as a reddish orange oil (9.8 g.). It was dissolved in castor oil residue (30 cc.) for distillation.

Distillation.—The solutions of tocopherols were distilled in a cyclic molecular still at 140–170° (0.003 mm. pressure). Fractions were taken at 10°-intervals with five cycles at each temperature. The distillates were light yellow or colorless oils which were sealed in glass tubes under nitrogen for storage.

The tocopherols were also purified by distillation at 0.1 mm. in a "boiling point" still.[25] No carrier liquid was used and the distillation temperatures were 200–210° (bath temperatures 220–230°). When prepared in this way the tocopherols had similar spectrographic properties to those purified by molecular distillation but the α-tocopherol distillates were red rather than yellow in color. This seemed to indicate that slight decomposition might have occurred at the higher pressure and temperature. The β- and γ-tocopherol distillates were light yellow in color and showed no sign of decomposition.

(24) Castor oil residue, used as a carrier liquid, was prepared by stripping castor oil at 260° in a molecular still to remove the more volatile fraction.

Summary

1. The natural α-, β- and α-,γ- tocopherols in wheat germ and cottonseed oils, respectively, have been concentrated by molecular distillation and the individual tocopherols separated from one another by chromatographic adsorption.

2. From the concentrates four new crystalline esters of the tocopherols have been prepared: α-tocopherol acid succinate, α-tocopherol palmitate, β-tocopherol azobenzene carboxylate and γ-tocopherol palmitate. Crystalline synthetic α-tocopherol palmitate was also prepared. The new α- and γ-tocopherol esters appear to be non-toxic to human beings and have a vitamin E activity equivalent to their tocopherol content.

3. From the esters purified α-, β- and γ-tocopherols have been prepared.

4. The spectrophotometric curves of the tocopherols and their esters have been determined. The relative ease of oxidation of the tocopherols by ferric chloride and silver nitrate has been examined; and the significance of the results in the assay of tocopherols by the Emmerie–Engel method is discussed.

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(25) Hickman and Weyerts, THIS JOURNAL, **52**, 4714 (1930).