49. *Studies on Vitamin E. Part III. Observations on the Structure of α- and β-Tocopherol.*

By F. Bergel, A. R. Todd, and T. S. Work.

β-Tocopherol, isolated as its allophanate from wheat germ oil (Part II, *Biochem. J.*, 1937, **31**, 2257) and showing full vitamin E activity in a dosage of 5 mg., is probably identical with the cumotocopherol of John (*Z. physiol. Chem.*, 1937, **250**, 11) and the neotocopherol of Karrer, Salomon, and Fritzsche (*Helv. Chim. Acta*, 1937, **20**, 1422); the name "β-tocopherol" is retained. α-Tocopheryl allophanate is obtained in varying amounts along with β-tocopheryl allophanate from the same sample of wheat germ oil. Pyrolysis of vitamin E concentrates from wheat germ oil gives mixtures of duroquinol and ψ-cumoquinol. Pure β-tocopherol yields on pyrolysis ψ-cumoquinol, accompanied by a small amount of a quinol of higher m. p. Synthetic *cetyl* and *allyl* ethers of duroquinol differ markedly from the tocopherols in absorption spectrum and reducing properties. The possibility that the tocopherols may be chroman or coumaran derivatives is under investigation.

In Part II (*loc. cit.*) we described the isolation of a crystalline optically active allophanate, m. p. 143·5—144·5°, from the unsaponifiable matter of wheat germ oil. This substance yielded on hydrolysis an oil, β-tocopherol, of approximate formula $C_{29}H_{50}O_2$, which showed full vitamin E activity in rats in a dosage of 5 mg. John (*loc. cit.*) and Karrer, Salomon, and Fritzsche (*loc. cit.*) have independently isolated in similar fashion allophanates, m. p. 146° and 143—144°, respectively, which from their physical and chemical properties are almost certainly identical with our compound.

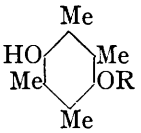
John describes the parent alcohol of his substance as cumotocopherol, and the Swiss workers use the name neotocopherol. We prefer to retain the term β-tocopherol which Evans, Emerson, and Emerson (*J. Biol. Chem.*, 1936, **113**, 319) introduced, since the β-tocopheryl allophanate they described had m. p. 138°, this being the normal m. p. of our crude β-tocopheryl allophanate. The main impurity in this material, m. p. 138°, appears to be a little β-amyrin allophanate, m. p. 273—275°; its removal is a matter of considerable difficulty and is accompanied by a loss of material. The introduction of entirely new names to describe a substance which is almost certainly only a purer specimen of one already in the literature seems to us undesirable. John (*loc. cit.*) states that his tocopherol was active in a minimum dosage of 8 mg., whereas we have had partial activity in dosages as low as 3 mg.; Karrer and his collaborators (*loc. cit.*) give no values for biological activity. This disagreement with regard to biological activity is probably of little significance,

however, as such differences are almost certain to arise where no standardised biological test method is available.

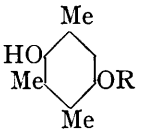
In Part II (*loc. cit.*) we reported that only traces of an allophanate corresponding to the α-tocopheryl allophanate of Evans, Emerson, and Emerson (*loc. cit.*) were obtained. This result we were at first inclined to attribute to a difference between our starting material and that of the American workers, but we have since isolated varying amounts of this compound as well as β-tocopheryl allophanate in different isolations from the same batch of wheat germ oil concentrate. This is the more remarkable as our isolation method has been apparently identical in all cases.

This unaccountable variation in the yield of α-tocopheryl allophanate suggests the possibility that it may be an artefact. The properties and analytical values of our α-tocopheryl allophanate are in agreement with those given by Evans, Emerson, and Emerson (*loc. cit.*) and we confirm their observation of its optical inactivity. The suggestion of Drummond and Hoover (*Biochem. J.*, 1937, **31**, 1852) that β-tocopherol is formed from the α-compound during the isolation process seems unlikely in view of the optical activity of β-tocopheryl allophanate. The total amount of α-tocopheryl allophanate which has been available to us is small, so we have been unable to examine it very closely.

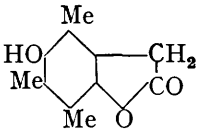
In early experiments (Todd, Bergel, Waldmann, and Work, *Nature*, 1937, **140**, 361) we found, in accordance with Fernholz's results (*J. Amer. Chem. Soc.*, 1937, **59**, 1154), that highly active vitamin E concentrates from rice and wheat germ oils gave duroquinol on heating to about 360°; moreover, concentrates yielding no duroquinol had no biological activity. It was observed, however, that the crude crystalline distillate obtained on pyrolysis generally had a m. p. considerably below 200° and that reasonably pure duroquinol could only be obtained from it after several recrystallisations. Further work on wheat oils showed that this low m. p. was due to the presence in the distillate of varying amounts of a second quinol, m. p. 165—166°, giving on oxidation a steam-volatile quinone, m. p. 28—30°. When slightly impure β-tocopherol was used, this lower-melting quinol was the main product, although some duroquinol was also formed. While these experiments were in progress John (*loc. cit.*) announced that "cumotocopherol" gave ψ-cumoquinol (II; R = H), m. p. 165—170°, on pyrolysis and that it must be an ether of this quinol of possible formula (II; R = $C_{19}H_{37}$) and α-tocopherol correspondingly (I; R = $C_{19}H_{37}$), *i.e.*, that the two compounds were homologues. Our quinol, m. p. 165—166°, we identified by analysis and direct comparison with synthetic ψ-cumoquinol.



(I.)



(II.)



(III.)

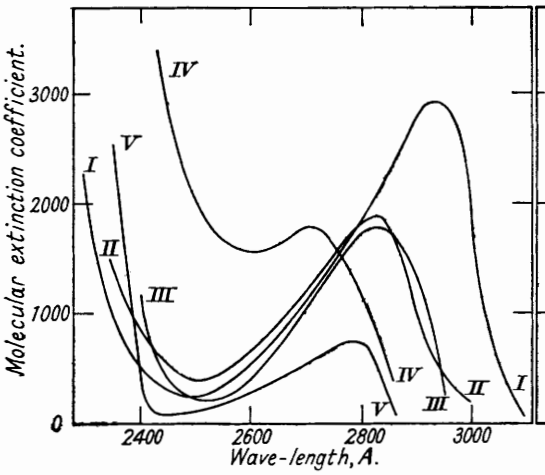
Since the appearance of Fernholz's publication we have synthesised several duroquinol ethers in order to compare their properties with those of the tocopherols. Examination of the absorption spectra of these compounds revealed at once a remarkable difference from the tocopherols. The collected data for the substances examined are shown in Figs. 1 and 2.

Duroquinol and ψ-cumoquinol show single absorption maxima at about 2950 A. In this connection it is worthy of mention that the spectrum given for duroquinol by Karrer, Salomon, and Fritzsche (*loc. cit.*) is incorrect. An absorption curve of the type they describe is only shown by partly oxidised solutions of duroquinol (observation of Dr. H. Waldmann; cf. also John, *loc. cit.*). This oxidation occurs with extreme ease and an accurate spectrum can only be obtained if a freshly prepared duroquinol solution is examined. The mono-ethers of duroquinol also show a maximum, but it occurs at about 2830 A. and is of smaller intensity (mono-acylation of duroquinol causes a similar shift of the maximum); introduction of a second ether grouping as in the dicetyl ether causes a slight shift in the position of this maximum. Esterification of a mono-ether as in *O-benzoylduroquinol allyl ether* causes a further shift of the maximum to about 2710 A.

α-Tocopherol (John, *loc. cit.*) and β-tocopherol show a single absorption maximum at 2950 A. and allophanation of the hydroxyl group (cf. John, *loc. cit.*) causes a shift of the maximum to about 2860 A. accompanied by a marked fall in intensity. On this evidence alone the view that either α- or β-tocopherol is a simple duroquinol ether is highly improbable.

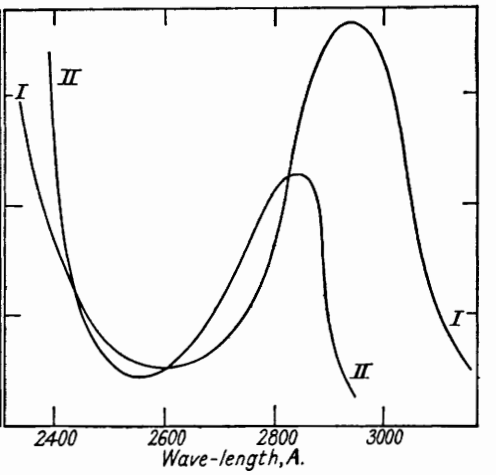
Further evidence pointing in the same direction was obtained by comparing the behaviour of the various compounds towards silver nitrate. Duroquinol, ψ-cumoquinol, and the tocopherols all reduce methyl-alcoholic silver nitrate on warming. This property is not shown by the duroquinol ethers we have prepared, although, of course, the mono-ethers will reduce ammoniacal silver nitrate. Finally, we were unable to isolate either duroquinol or ψ-cumoquinol from the products obtained on heating β-tocopheryl allophanate with hydriodic acid or with hydrochloric acid in acetic acid.

FIG. 1.



I. *Duroquinol in ether.*
II. *Monocetylduroquinol in alcohol.*
III. *Monoallylduroquinol in alcohol.*
IV. *O-Benzoylallylduroquinol in ether.*
V. *Dicetylduroquinol in ether.*

FIG. 2.



I. *β-Tocopherol in alcohol.*
II. *β-Tocopheryl allophanate in alcohol.*

Duroquinol monocetyl ether undergoes pyrolysis much more rapidly than α- or β-tocopherol and at a slightly lower temperature (*ca.* 325°); the crude crystalline distillate of duroquinol has a m. p. above 220° and is already practically pure. The corresponding *dicetyl* ether decomposes at about the same rate as the tocopherols and again yields a very pure product. As mentioned above, we observed the production of both ψ-cumoquinol and duroquinol on pyrolysis of our active materials. We have re-investigated this, using for pyrolysis a specimen of β-tocopherol prepared from a five-times recrystallised sample of the allophanate. Here again we obtained ψ-cumoquinol as the main product, but by washing the crude crystalline distillate, m. p. 160—170°, with ether a small, less soluble fraction of quinol was obtained, m. p. 195—200° with considerable sublimation. The total yield of crystalline material being only about 3% of the weight of tocopherol used, it was impossible for us, with the quantities at our disposal, to identify this material properly. The possibility admittedly exists that our β-tocopheryl allophanate was contaminated with the α-compound, but we consider this highly unlikely. Pyrolysis of a very small amount of α-tocopherol yielded a crude crystalline material of m. p. between 180° and 190°, again suggestive of a mixture of duroquinol with a lower-melting quinol, when contrasted with the behaviour of duroquinol cetyl ether. The evidence is admittedly not conclusive, but we consider it at least possible that both α- and β-tocopherol yield mixtures of ψ-cumoquinol and duroquinol on pyrolysis.

As has been pointed out by Evans, Emerson, and Emerson (*loc. cit.*) α-tocopheryl

allophanate does not behave like an unsaturated compound towards bromine or potassium permanganate; the same is true of β-tocopheryl allophanate. Work on the oxidative degradation of β-tocopherol has been hampered by scarcity of material, but oxidation of the allophanate with alkaline potassium permanganate gave an acid yielding a *p-phenylphenacyl* ester, m. p. 84°. The small amount available precluded complete identification, but it appeared from analysis to be an aliphatic acid containing about 18 carbon atoms. Catalytic hydrogenation of β-tocopherol is difficult to effect, but 4 mols. of hydrogen were taken up slowly on heating. The allophanate also absorbed 4 mols. of hydrogen, but here, curiously enough, 1 mol. was rather easily taken up.

The properties of α- and β-tocopherol so far as they are known indicate that they are in some way related to duroquinol and ψ-cumoquinol. The absorption of 4 mols. of hydrogen by β-tocopherol, coupled with the apparently saturated nature of its allophanate, suggests the possibility that it may be a cyclic ether, *i.e.*, a coumaran or chroman derivative bearing a long side chain in the heterocyclic nucleus. A similar structure would be expected for α-tocopherol. Such a structure for these compounds might also explain the production of a mixture of quinols on pyrolysis. This possibility is at present being investigated by degradative and synthetic methods, and our results will be communicated later. As a preliminary model we have examined 5-hydroxy-4 : 6 : 7-trimethyl*iso*coumaranone (III) (Smith and MacMullen, *J. Amer. Chem. Soc.*, 1936, **58**, 630). This compound we find shows a striking resemblance to the tocopherols in both its absorption spectrum (max. *ca.* 2930 A., min. 2650 A.) and reducing properties.

As regards the molecular size of β-tocopheryl allophanate our analysis figures do not permit of a differentiation between $C_{30}H_{50}O_4N_2$ and $C_{31}H_{52}O_4N_2$. Miss D. M. Crowfoot, to whom we are greatly indebted, has made a crystallographic examination of β-tocopheryl allophanate. Her results, reproduced in the experimental section, are indicative of a C_{30} formula for this compound, corresponding to a C_{28} formula for β-tocopherol itself.

Experimental.

α-Tocopheryl Allophanate.—The purified substance had m. p. 158—159° and its properties were in agreement with those given by Evans, Emerson, and Emerson (*loc. cit.*) (Found : C, 72·3; H, 10·0. Calc. for $C_{31}H_{52}O_4N_2$: C, 72·0; H, 10·2%). In chloroform solution it was optically inactive. α-Tocopherol was obtained from it by hydrolysis as a colourless oil.

β-Amyrin Allophanate from Crude β-Tocopheryl Allophanate.—The crude β-tocopheryl allophanate, m. p. *ca.* 138° (cf. Part II, *loc. cit.*), shows a reddish-violet colour in the Liebermann reaction, whereas the pure allophanate, m. p. 143·5—144·5°, does not. Purification is best effected by boiling methyl-alcoholic solutions with charcoal; the charcoal, then eluted with acetone, yields small amounts of a beautifully crystalline allophanate, m. p. 273—275°, showing the reddish-violet Liebermann reaction characteristic of β-amyrin derivatives. Mixed with a specimen of β-amyrin allophanate (m. p. 272—273°), it produced no depression of the m. p.

Micro-hydrogenations.—β-Tocopherol (1·02 mg.), $C_{29}H_{50}O_2$, absorbed 0·2055 c.c. of hydrogen at N.T.P. (= 3·87 mols. H_2). The solvent was decalin–acetic acid, and the catalyst platinum oxide. No absorption occurred in the cold, but slow regular absorption occurred at 90° and was complete after 8 hours.

β-Tocopheryl allophanate (1·905 mg.), $C_{31}H_{52}O_4N_2$, absorbed 0·3274 c.c. of hydrogen at N.T.P. (= 3·96 mols. H_2), the solvent being acetic acid, and the catalyst platinum oxide. One mol. of hydrogen was absorbed in the cold, and the remainder by heating the mixture to 90°, shaking it until it was cold, and keeping it for 12 hours.

Attempted Acid Fission of β-Tocopherol.—β-Tocopheryl allophanate (100 mg.) was heated in a sealed tube at 200° with hydrochloric–acetic acid (5 c.c. of a mixture of 10 c.c. of glacial acetic acid and 30 c.c. of concentrated hydrochloric acid saturated with hydrogen chloride at 0°) during 18 hours. After a further 2 hours at 220° the tube was cooled, and the dark gummy product extracted with ether. The ethereal solution, on drying and evaporating, gave a dark brown gum which did not crystallise. Oxidation with ferric chloride gave a mixture which had a slight quinone-like odour, but no appreciable steam-volatile product could be detected. The combined material from this experiment was refluxed for 3 hours with hydriodic acid (*d* 1·7), and the product submitted to chromatographic analysis on aluminium oxide (Merck). A small amount of a yellowish oil was washed through the column. The rest of the material, which was

very strongly absorbed, was a brown gum; this did not crystallise, nor did it appear to give a volatile quinone on oxidation.

Oxidation of β-Tocopheryl Allophanate.—The allophanate (145 mg.), dissolved in pure hexane (50 c.c.), was shaken for 12 hours with a solution of potassium permanganate (570 mg. ≡ 19 mols.) in sodium hydroxide solution (75 c.c. of 3%). Much of the permanganate was then unchanged and the concentration of alkali was increased to 6% and shaking continued for a further 24 hours. The hexane layer was separated and gave on evaporation a small amount of a substance, m. p. 140°, possibly unchanged allophanate. The aqueous layer was freed from manganese dioxide and unchanged permanganate by means of sulphur dioxide, and the resulting solution, after acidification with sulphuric acid, was extracted continuously with ether during 48 hours. The extract was shaken with alkali. A small amount of an unidentified neutral oil remained in the ether, and the acidic material was recovered by acidifying the alkaline layer and again extracting it with ether. Evaporation of this ethereal extract gave a thick oil (26 mg.). This oil had no detectable rotation in chloroform solution ($c = 0{\cdot}65\%$; $l = 1$ dm.) and titration against N/20-sodium hydroxide indicated a mol. wt. of about 260, assuming it to be a monobasic acid. The material was esterified with *p*-phenylphenacyl bromide, and the *ester* crystallised from methyl alcohol. After several recrystallisations a product, m. p. 84°, was obtained (Found: C, 80·7; H, 9·4. $C_{33}H_{46}O_3$ requires C, 80·3; H, 9·6%. $C_{33}H_{44}O_3$ requires C, 80·7; H, 9·3%). A portion of the crude acidic material remained unesterified in this experiment.

Pyrolysis of Active Concentrates from Wheat Germ Oil.—The oil (600 mg.), prepared by the method described in Part II (*loc. cit.*) and showing vitamin E activity in a dosage of 15 mg., was heated in a nitrogen atmosphere to about 360° in a bulb tube with a long side arm. From about 300° onwards a yellowish oil distilled, followed at 350—360° by a product which crystallised in colourless needles in the side tube. After about 20 minutes the distillation was complete. The crystalline distillate was freed from oily impurities by washing with a little light petroleum (b. p. 40—60°); it had a rather indefinite m. p. 170—180°. The material was recrystallised from petroleum (b. p. 100—120°) and the crystals (A) separating were washed with low-boiling petroleum, the washings being combined with the crystallisation mother-liquors (B).

The crystals (A) were treated with a little sodium hyposulphite solution, filtered off, and sublimed in a vacuum. The sublimate was again washed with light petroleum, then with a very small amount of ether, and again sublimed. The product (*ca.* 6 mg.), which had the crystalline form of duroquinol, had m. p. 223—224° (Found: C, 72·5; H, 8·6. Calc. for $C_{10}H_{14}O_2$: C, 72·3; H, 8·4%). A mixed m. p. with synthetic duroquinol showed no depression, and oxidation with ferric chloride gave a yellow quinone, m. p. 107—108°, undepressed by duroquinone (m. p. 108—109°).

The combined liquors and washings (B) were evaporated, and the residue washed with sodium hyposulphite solution and taken up in ether. The dried ethereal solution was evaporated, and the residue purified by crystallisation from light petroleum (b. p. 60—80°) and sublimation in a vacuum. The product (2·5 mg.) had m. p. 165—166° (Found: C, 70·7; H, 8·1. Calc. for $C_9H_{12}O_2$: C, 71·1; H, 7·9%). A mixed m. p. with ψ-cumoquinol (m. p. 168—170°) showed no depression and oxidation with ferric chloride gave a yellow quinone, m. p. 28—30° (ψ-cumoquinone has m. p. 30°).

The relative proportions of the two quinols varied in different experiments even with the same sample of oil.

Duroquinol Cetyl Ethers.—A mixture of duroquinol (3 g.), methyl ethyl ketone (80 c.c.), cetyl iodide (6·3 g.), and anhydrous potassium carbonate (2·55 g.) was refluxed in a nitrogen atmosphere during 10 hours. The mixture was now filtered, and the filtrate kept at 0° for a few hours. The crystalline material which separated was dissolved in light petroleum (b. p. 40—60°), and the filtered solution evaporated. The residue, recrystallised from acetone, gave *duroquinol dicetyl ether* in colourless waxy crystals, m. p. 81—84° to a cloudy liquid which became clear at 86—87° [Found: C, 82·0; H, 12·5; *M* (Rast), 550. $C_{42}H_{78}O_2$ requires C, 82·2; H, 12·5%; *M*, 614].

The methyl ethyl ketone filtrate from the dicetyl ether was evaporated to dryness, and the residue freed from remaining traces of the latter compound by dissolution in cold acetone. The acetone solution was now steam-distilled to remove duroquinone, and the distillation residue shaken with light petroleum. A yellow solid (0·85 g.) remained undissolved; it had m. p. 204—205° after recrystallisation from methyl alcohol and was probably diduroquinone (v. Pechmann, *Ber.*, 1889, **22**, 2115). The light petroleum solution was washed with aqueous caustic potash, then with water, dried over sodium sulphate, and evaporated. The residue was taken up in a small amount of acetone, in which *duroquinol monocetyl ether* is readily soluble, filtered, and cooled to − 5°. The crystalline material which separated had m. p. 91—92°, but

still contained traces of iodine (contamination with cetyl iodide). By repeated crystallisation from methyl alcohol and finally from light petroleum (b. p. 60—80°) the ether was obtained in colourless waxy crystals, m. p. 98° (Found: C, 79·4; H, 11·4. $C_{26}H_{40}O_2$ requires C, 80·0; H, 11·7%). On oxidation with chromic acid by the Kuhn–Roth method the substance gave 3·2 mols. of acetic acid. It showed no vitamin E activity when tested biologically in doses up to 50 mg.

At about 325° the monocetyl ether decomposes within a few minutes, giving duroquinol (m. p. crude 221°). The dicetyl ether behaves similarly, but the decomposition occupies about 20 minutes (m. p. of crude duroquinol 225—227°). The yield in each case is *ca.* 20% of the theoretical.

O-*Monobenzoylduroquinol.*—To a solution of duroquinol (5·2 g.) in dry pyridine (26 c.c.) at 0°, benzoyl chloride (3·8 c.c.) was added dropwise with shaking, the whole operation being carried out in an atmosphere of nitrogen. After standing overnight, the mixture was heated on the water-bath for 1 hour, cooled, and poured on a mixture of ice and dilute sulphuric acid. The solid which separated was collected, washed thoroughly first with sodium hydroxide solution, then with water, dried, and boiled with methyl alcohol (80 c.c.); this treatment left much of the product undissolved but removed duroquinone and unchanged duroquinol. After cooling, the suspension was filtered; the filter residue (5·2 g.) had m. p. 204—215°. The product was extracted three times with a mixture of benzene (4 parts) and petroleum (7 parts; b. p. 100—120°), which removed the more soluble dibenzoyl derivative. The residue (4 g.), m. p. 220—221°, gave after two recrystallisations from methyl alcohol colourless prismatic needles of O-*monobenzoylduroquinol*, m. p. 221—223° (Found: C, 75·6; H, 6·4. $C_{17}H_{18}O_3$ requires C, 75·6; H, 6·6%).

O-*Benzoylduroquinol Allyl Ether.*—*O*-Monobenzoylduroquinol (0·6 g.) was added to potassium powder (0·1 g.) in toluene (20 c.c.) and heated, a thick gelatinous mass being formed. When most of the potassium had disappeared, allyl bromide (0·24 c.c.) was added together with more dry toluene (10 c.c.), and the whole refluxed for *ca.* 4 hours; the gelatinous material then disappeared and potassium bromide separated. The liquid was now filtered and evaporated to dryness, yielding a thick oil which crystallised on trituration with light petroleum. Recrystallised from methyl alcohol, O-*benzoylduroquinol allyl ether* formed colourless needles (0·4 g.), m. p. 111—112° (Found: C, 76·9; H, 7·0. $C_{20}H_{22}O_3$ requires C, 77·4; H, 7·1%).

Duroquinol Monoallyl Ether.—The above benzoate was refluxed in a nitrogen atmosphere with methyl-alcoholic potassium hydroxide (5%) during 2 hours. The resulting solution was diluted with water and extracted with ether, and the extract dried and evaporated. The oily residue crystallised from light petroleum (b. p. 40—60°) in colourless needles, m. p. 108° (Found: C, 75·8; H, 8·8. $C_{13}H_{18}O_2$ requires C, 75·7; H, 8·7%). The substance reduced ammoniacal methyl-alcoholic silver nitrate solution but had no action on methyl-alcoholic silver nitrate alone, even on heating. Duroquinol monocetyl ether also may be prepared in an analogous manner *via* the benzoyl derivative; a sample thus prepared had m. p. 102°.

Migration Experiments.—When a solution of *O*-benzoylduroquinol allyl ether in dimethylaniline was refluxed for 6 hours, the allyl group was removed and *O*-monobenzoylduroquinol, m. p. 221—223°, was isolated in nearly quantitative yield. On similar treatment duroquinol monoallyl ether yielded duroquinone (oxidation of initially formed quinol), but duroquinol monocetyl ether was recovered unchanged.

Crystallographic Data.—β-Tocopheryl allophanate crystallises in the monoclinic space group P_2; $a = 13{\cdot}52$ A. (limits, 13·57, 13·45); $b = 5{\cdot}26$ A. (limits 5·27, 5·245); $c \sin\beta = 21{\cdot}35$ (limits 21·40, 21·05); $p = 1{\cdot}092$ (limits 1·097, 1·087). The number of molecules in the unit cell being taken as 2, the minimum molecular weight required by the crystallography is 502 (limits 509, 490). This indicates definitely a C_{30} formula ($C_{30}H_{50}O_4N_2$ requires *M*, 502).

We are indebted to Mr. F. Boston, Manchester University, for the micro-hydrogenation figures. Our thanks are also due to Messrs. Glaxo Laboratories Ltd. and Messrs. Hoffmann La Roche & Co. for their generous assistance and gifts of material and to Dr. E. L. Smith of the former firm for measuring many absorption spectra. We have to thank Miss. A. M. Copping, who has undertaken the biological testing necessary in these investigations, and the Medical Research Council, whose grant to her has made this possible.

(Note added, February 11th) Emerson, Emerson, Mohammad, and Evans (*J. Biol. Chem.*, 1937, **122**, 99) have now reported that their purified β-tocopheryl allophanate has m. p. 144—146°.

THE LISTER INSTITUTE, LONDON, S.W. 1. [*Received, December 20th,* 1937.]