THE ISOLATION FROM WHEAT GERM OIL OF AN ALCOHOL, α-TOCOPHEROL, HAVING THE PROPERTIES OF VITAMIN E*

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(Received for publication, October 28, 1935)

The first attempt to concentrate vitamin E was made by Evans and Burr in 1927 (1). The non-saponifiable matter from wheat germ oil was treated successively with pentane and methanol, from which the bulk of the sterols and some oily material separated. By distribution between pentane and 92 per cent methanol the xanthophyll pigments and some other inactive material were removed. There was thus obtained a red oil which in a single dose of 10 mg. enabled the test rats to bear litters. Further concentration was secured by high vacuum distillation, but this step was accompanied by considerable loss.

Olcott (2) and Olcott and Mattill (3, 4) also prepared concentrates from wheat germ oil, cottonseed oil, and lettuce by procedures analogous to those of Evans and Burr, except that in their hands vacuum distillation was a much more effective tool. Concentrates potent in a single dose of 3 mg. were secured. A very

* Aided by grants from the Research Board of the University of California and from the Rockefeller Foundation. A significant part of the research herein reported was carried out during the occupancy of an Eli Lilly fellowship by two of the authors (O. H. E. and G. A. E.). We desire to express particular thanks to Professor Adolf Butenandt, with whom part of the work herein reported was actually done, and to Professors Butenandt and A. Windaus for the delightful and profitable year which the two authors mentioned enjoyed at the University of Göttingen. Dr. Adolf Pabst of the Geology Department and Dr. Kenneth R. More and Mr. Louis A. Strait of the Physics Department of the University of California have all contributed highly important data to this study, as hereinafter specifically mentioned, and we desire to tender them also our sincerest thanks.

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potent concentrate was obtained by Drummond, Singer, and MacWalter (5) by fractionally adsorbing the sterol-free non-saponifiable fraction of wheat germ oil on a column of Brockmann's alumina.

Spectroscopic studies by Martin, Moore, Schmidt, and Bowden (6), Olcott (2, 7), and Drummond, Singer, and MacWalter showed that vitamin concentrates from cottonseed and wheat germ oils possessed a strong absorption band with a maximum at 2940 Å. Martin, Moore, Schmidt, and Bowden and Drummond, Singer, and MacWalter expressed the belief that this band is probably due to the vitamin. Drummond's concentrates showed considerable parallelism between the intensity of the absorption and the vitamin potency; the most potent preparations showed maximal absorption at 2940 Å, $E_{1}^{\text{percent}} = 54$. However, Olcott (8) has concluded that the substance showing this absorption is not the vitamin. He based this conclusion upon four lines of evidence; *viz.*, (1) he obtained a fraction from palm oil showing intense absorption here but with little or no vitamin activity; (2) acetylation caused a shift in the absorption maximum of both the wheat germ oil and palm oil concentrates to 2810 Å, but it did not interfere with the vitamin E potency of the wheat germ oil concentrate; (3) the concentrate from lettuce, although quite potent, showed no band at 2940 Å.; (4) by treating a cottonseed oil concentrate with methyl alcoholic silver nitrate, it was possible to destroy completely the absorption at 2940 Å. and still retain a considerable proportion of the vitamin activity.

Olcott (8) has shown that the vitamin is an alcohol, since it is inactivated by phenyl isocyanate, and the activity can subsequently be restored by hydrolysis with dilute alkali. By conversion to the methyl ether the vitamin activity was completely destroyed.

It is hardly necessary to state that if much progress is to be made in the elucidation of the structure of vitamin E, it must be isolated in pure form. Our plan of attack was to find a solid derivative of the vitamin which could be purified, and from which the vitamin could readily be regenerated. We first tried ketone reagents, but could find no indication of reaction, an observation which has been confirmed by Girard (quoted by Drummond, Singer, and MacWalter (5)). Next we tried a number of alcohol
reagents, but although most of these obviously reacted, they yielded only oily products. However, cyanic acid gave us three allophanates. One of these yielded an alcohol devoid of physiological activity. The other two were obviously related to the vitamin. The first of these melted at 158°-160° after repeated recrystallization from ethyl or methyl alcohol or acetone. The analysis was in good agreement with the monoallophanate of an alcohol, C\textsubscript{29}H\textsubscript{50}O\textsubscript{2}.\textsuperscript{1} It separated as fine balls rather than definite crystals. On hydrolysis it yielded an oily alcohol; when this was fed in a single dose of 3 mg. litters were produced quite regularly, but only sporadically at the 1 mg. level. For this alcohol we propose the name "\(\alpha\)-tocopherol."\textsuperscript{2} The optical rotation of the allo-

\textsuperscript{1} Analytical data secured by Drummond, Singer, and MacWalter (5) on their most potent preparation and its acetate are in excellent agreement with the value required by an alcohol, C\textsubscript{29}H\textsubscript{50}O\textsubscript{2}, and its monoacetate.

\textsuperscript{2} Tokos = childbirth; phero = to bear; -01, indicating an alcohol. We wish to thank our colleague, Professor George M. Calhoun of the University of California, for the suggestion of this designation.
The 

\( \alpha \)-tocopherol shows strong absorption, with a maximum at 2980 Å, \( E_{1cm}^{1\%} = 90 \pm 10 \), and a secondary slightly less intense maximum at 2920 Å. (Fig. 1, Curve 2).

The second allophanate forms beautiful needles melting at 138°, and appears to be isomeric with \( \alpha \)-tocopheryl allophanate, whose

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>Level fed</th>
<th>Littering</th>
<th>Average No. per litter</th>
<th>Average weight of young per rat</th>
<th>Source of ( \alpha )-tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>per cent</td>
<td>gm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>66</td>
<td>10</td>
<td>4.6</td>
<td>Allophanate as isolated</td>
</tr>
<tr>
<td>87-A</td>
<td>1</td>
<td>20</td>
<td>1</td>
<td>4.0</td>
<td>&quot; treated with bromine</td>
</tr>
<tr>
<td>87-A</td>
<td>3</td>
<td>100</td>
<td>3.6</td>
<td>5.0</td>
<td>&quot; mixed with oil from</td>
</tr>
<tr>
<td>71-A</td>
<td>3</td>
<td>75</td>
<td>7.6</td>
<td>5.6</td>
<td>mother liquor of Preparation</td>
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<tr>
<td>87-B</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>5.2</td>
<td>87-A</td>
</tr>
<tr>
<td>87-B</td>
<td>3</td>
<td>25</td>
<td>6</td>
<td>5.2</td>
<td>Allophanate converted to p-nitrophenylurethane</td>
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<tr>
<td>90-B</td>
<td>2.5</td>
<td>100</td>
<td>10</td>
<td>5.2</td>
<td>Allophanate fractionally adsorbed on CaCO₃; weakest adsorbed fraction</td>
</tr>
<tr>
<td>110-D</td>
<td>3</td>
<td>75</td>
<td>8.5</td>
<td>4.8</td>
<td>Allophanate adsorbed on CaCO₃; strongest adsorbed fraction</td>
</tr>
<tr>
<td>112-A</td>
<td>3</td>
<td>66</td>
<td>5.5</td>
<td>5.5</td>
<td>Original allophanate recrystallized to melting point 158-160°, converted to p-nitrophenylurethane, then reconverted to allophanate</td>
</tr>
<tr>
<td>146-A</td>
<td>1</td>
<td>0</td>
<td>5.2</td>
<td>5.0</td>
<td>Silver nitrate reaction product of ( \alpha )-tocopherol</td>
</tr>
<tr>
<td>146-A</td>
<td>3</td>
<td>100</td>
<td>5.2</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>71-B</td>
<td>4</td>
<td>50</td>
<td>5.5</td>
<td>4.4</td>
<td></td>
</tr>
</tbody>
</table>

Biological Activity of \( \alpha \)-Tocopherol and Its Silver Nitrate Reaction Product

Attempts to fractionate \( \alpha \)-tocopheryl allophanate by crystalliza-
tion from methyl or ethyl alcohol or acetone failed. To test its homogeneity, we adsorbed 106 mg. on a column of 100 gm. of calcium carbonate, developing the "chromatogram" with 1150 cc. of benzene. This washed 26 mg. of material through the column, which had the same melting point as the most strongly adsorbed fraction. The alcohols from the two end-fractions showed the same biological activity (Preparations 110-D and 112-A, Table I).

It was found that \( \alpha \)-tocopherol reacted with \( p \)-nitrophenyl isocyanate to yield a nitrophenylurethane crystallizing in fine needles, which melted at 129–131°. The analysis of this substance was also in good agreement with the values required by a mono derivative of an alcohol, \( C_{29}H_{50}O_2 \), although this formula must be accepted with some reservation, since the difference in percentage composition between adjacent homologues is not great. The alcohol regenerated from the nitrophenylurethane, fed to four rats at a level of 2.5 mg., enabled all of them to bear good litters.

The nitrophenylurethane was reconverted to the allophanate, and the product so obtained appeared microscopically identical with the original allophanate, and after a few recrystallizations, melted at 158–160°. \( \alpha \)-Tocopherol from this reconverted allophanate showed the same physiological activity and absorption spectrum as the original allophanate (Table I, Preparation 146-A; Fig. 1, Curve 1). Hence we feel justified in believing that we have obtained a homogeneous product which plays the biological rôle of vitamin E.

Olcott's finding that a concentrate from palm oil shows strong absorption at 2940 Å., with weak or no vitamin activity, is analogous to our experience with the alcohol from the 138° allophanate. His observation that treatment with methyl alcoholic silver nitrate caused the destruction of the absorption band at 2940 Å. with simultaneous persistence of vitamin activity was of great interest to us, although, as the reader will see, our interpretation of this phenomenon is different. We repeated the experiment with our pure substance—\( \alpha \)-tocopherol. Blackening occurred as soon as the solution became warm\(^3\) and after 5 minutes on the steam bath the

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\(^3\) There is a strange difference in the behavior of \( \alpha \)-tocopherol and \( \alpha \)-toco-phenyl allophanate. When the allophanate is warmed with methyl alcoholic silver nitrate, no blackening takes place. The free alcohol readily decolorizes bromine, but the allophanate reacts extremely slowly. A sample of the allophanate was allowed to stand 1 hour in an excess of bromine in chloroform solution, and the alcohol regenerated from it showed both the
heating was discontinued. The absorption spectrum showed no sign of the band at 2920 to 2980 Å., but there were two new bands with maxima at 2710 and 2620 Å., having an intensity $E_{1}^{1}$ per cent of 480 (Fig. 2). The reaction product was fed in a dose of 4 mg., and vitamin activity, although reduced, was not lost (Table I). If we assume that our $\alpha$-tocopherol is a homogeneous (uncontaminated) substance, which seems justifiable, we are forced to the conclusion that the silver nitrate experiments have demonstrated that

![Fig. 2. Absorption spectrum of the reaction product of $\alpha$-tocopherol with methyl alcoholic silver nitrate.](image)

characteristic spectrum and normal vitamin activity (Table I, Preparation 71-A). A sample of the allophanate was allowed to stand in a weighed flask for 50 hours in an excess of bromine in chloroform. The chloroform and bromine were carefully removed, and there was no significant increase in weight. On standing 4 days with an excess of perbenzoic acid in chloroform solution, a sample of the allophanate produced no significant reduction of the perbenzoic acid. Were it not for the fact that the allophanate gives an intense color with tetranitromethane, one would imagine it to be a saturated body. Olcott and Mattill (4) noted that acetylated vitamin E concentrates are not destroyed by rancidity to which the free vitamin is extraordinarily sensitive.
vitamin E activity is not the property of a single molecular species; the situation in this respect thus resembles that in regard to vitamin D, to the precursors of vitamin A, and to the male and female sex hormones.

**EXPERIMENTAL**

*Biological Assay*—The procedure of Evans, Murphy, Archibald, and Cornish (9) was slightly modified. The diet of commercial casein 27.0, cooked corn-starch 35.0, fresh lard 22.0, dried brewers' yeast 10.0, Salt Mixture 185 (10) 4.0 was mixed and allowed to stand for 2 weeks at room temperature to allow the incipient rancidity of the lard to destroy any vitamin E present. Cod liver oil, 2 parts, was added just before feeding.

Rats 21 days old were placed on the diet, and were bred for their trial gestation at 60 days of age, or as soon thereafter as their cycles permitted. The percentage of initial fertility under these conditions was very low. The test substances, dissolved in ethyl laurate, were administered by stomach tube to the rat under light ether anesthesia. Four animals were used for the test, and successful implantation was noted by finding the erythrocyte sign on the 13th day.

*Measurement of the Absorption Spectra*—The absorption spectra were obtained with a small quartz spectrograph which photographed the region between 2000 and 5000 Å. on a 6 inch Eastman No. 33 plate, and had an average dispersion of 24 Å. per mm. between 2500 and 3000 Å. A hydrogen discharge tube was used as a continuous source of ultra-violet light. On each plate were recorded the absorption spectrum of the pure solvent (hexane, purified according to Twyman and Allsopp (11)) and that of the solution, several concentrations of the latter being used. With a cell 1.93 cm. long, a concentration of α-tocopherol of 0.005 per cent gave the most satisfactory range of blackening density. The blackening on the plate was determined with a Zeiss recording microphotometer.

The calibration of the plates was made with screens of known transmission, as described by Harrison (12). Six calibration curves were made for the region between 2500 and 3000 Å., but as

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4 By Kenneth R. More and Louis A. Strait, Physics Department, University of California.
these were found not to differ materially, an average curve was used for the interval.

By means of the calibration curves the intensity of the light was determined from the degree of blackening measured by the microphotometer, as described by Langstroth (13). Thus were measured the intensity of light passing through the cell filled with pure solvent, $I_1$, and the intensity of the light passing through the cell filled with solution, $I_2$.

The extinction coefficient, $E_{1\text{cm.}}^1 \text{per cent}$, was calculated from the formula

$$E_{1\text{cm.}}^1 \text{per cent} = \frac{1}{cL} \log_{10} \frac{I_1}{I_2}$$

where $c$ equals the concentration of the solution in per cent and $L$ the length of the cell in cm. For the curves of $\alpha$-tocopherol, $E$ was evaluated at intervals of every 10 Å over the region of maximum absorption.

**Preparation of Sterol-Free Non-Saponifiable Matter**—The saponification of the wheat germ oil was conducted as described by Evans et al. (9) and the non-saponifiable fraction was extracted with ether rendered peroxide-free by distillation over stannous chloride. The non-saponifiable matter was taken up in petroleum ether, b.p. 85–110°, and allowed to stand overnight at 0° to permit the separation of the bulk of the sterols, which were filtered off and washed free of pigment. The petroleum ether solution was concentrated to contain about 10 per cent of solute and was washed several times with small volumes of 92 per cent methanol, which removed the xanthophylls and other inert material. The hydrocarbon solution, again concentrated to contain about 10 per cent solute, was extracted six times with an equal volume of dry methanol, saturated with petroleum ether, at 0°. This extracted the vitamin fairly completely, and was an effective means of removing certain oily substances difficultly soluble in methanol. The methanol solution was concentrated to small volume and allowed to stand at $-18^\circ$ to allow as much as possible of the remaining sterols to separate. The final trace of sterols was removed with digitonin from 90 per cent ethyl alcohol solution.

The non-saponifiable matter from 3.7 kilos of wheat germ oil was allowed to stand overnight at $-18^\circ$ in 250 cc. of methanol. For
the removal of the last trace of sterols, 12 gm. of digitonin were sufficient. There were thus obtained 21 gm. of sterol-free oil, potent in a 10 mg. dose.

Preparation of Allophanates—This was done essentially as described by Windaus, Gaede, Koeser, and Stein (14) for the irradiation products of ergosterol. The sterol-free oil, in 10 gm. portions, dissolved in 250 cc. of benzene, was saturated with cyanic acid gas generated by heating 15 gm. of cyaniuric acid in a slow stream of CO₂. During the process the benzene solution was kept cool in an ice bath. The solution was allowed to stand a week at 5° for the reaction to go to completion. The cyamelide was filtered off and washed well with hot benzene, in which the desired allophanate was very soluble. The residue, on evaporation of the benzene, was dissolved in 10 volumes of methanol and allowed to stand overnight at 0°.

The oily precipitate was filtered off and the oil washed out with small amounts of petroleum ether, in which the desired allophanate was also, unfortunately, appreciably soluble. The allophanates were a mixture of a small amount of very sparingly soluble, high melting substance and a much larger amount of a much more soluble, lower melting product.

The cleanest separation was obtained by means of cold acetone, in which the low melting substance was moderately soluble and the high melting one very sparingly soluble.

The yield of high melting material from 3.7 kilos of wheat germ oil was about 100 mg., m.p. 250°.

Analysis—This analysis was made at Columbia University, through the kindness of Professor H. T. Clarke. The substance dried in vacuo at 80° without loss of weight.

C 74.77, 74.60; H 10.52, 10.30; N 5.39, 5.35
Calculated for C₃₂H₄₃N₂O₅. C 74.65, H 10.58, N 5.44

This may be the allophanate of β-amyrin, which Drummond, Singer, and MacWalter (5) found in their vitamin concentrate from wheat germ oil, and the formula of whose allophanate would be C₃₂H₃₂N₂O₅. The substance on hydrolysis yielded an alcohol having no indication of vitamin E potency, even in a dose of 10 mg. It was not further investigated.
Isolation of \( \alpha \)-Tocopheryl Allophanate—The acetone filtrate from the high melting allophanate was evaporated to dryness in vacuo and taken up again in hot methanol, from which, on cooling, the \( \alpha \)-tocopheryl allophanate separated, still contaminated with oil which was in part removed by careful washing with petroleum ether. Several recrystallizations and washing with petroleum ether were required to get the product completely free from oil, and several fractionations with acetone were required to remove completely the high melting allophanate. The pure \( \alpha \)-tocopheryl allophanate melts at 158–160° and consists of matted granules about 0.01 mm. in diameter which are isotropic; mean index of refraction about 1.51.

The mother liquors from this allophanate, on concentration, have yielded small amounts of the crystalline 138° substance, but we have been unable to detect other solid allophanates.

Optical Rotation—56.2 mg. of substance in 3 cc. of CHCl₃ in a 1 dm. tube, with sodium D light, gave no measurable rotation.

Analysis—The following analytical data have been secured.

The substance dried at 80° in vacuo gave no loss of weight.

\[
\begin{array}{ccccccc}
C & \text{Mean} & 72.05 & 72.20 & 72.14 & 72.33 & 72.14 & 72.16 \\
H & \text{Mean} & 10.16 & 10.17 & 10.11 & 10.42 & 10.31 & 10.23 \\
N & \text{Mean} & 5.46 & 5.30 & 5.23 & 5.33 \\
& \text{Calculated for } C_{27}H_{53}N_4O_4. & C 72.04, H 10.15, N 5.42 \\
& \text{For I the analyst was Dr. Ing. A. Schoeller, Berlin; for II, Herrler,} & \text{Leipsic; for III the analyses were made at Columbia University through} \\
& \text{the kindness of Professor H. T. Clarke.} & \\
\end{array}
\]

The allophanate was hydrolyzed by refluxing \( \frac{1}{2} \) hour with 4 per cent methyl alcoholic potassium hydroxide in an atmosphere of hydrogen. The reaction mixture was cooled, 4 volumes of water were added, and the alcohol was extracted with peroxide-free ether. The alcohol was a light colored, viscous oil. The biological assay of the alcohol obtained from various allophanate preparations is given in Table I.

Isolation of 138° Allophanate—The methyl alcoholic mother liquor from the first crude separation of the allophanates was allowed to stand 4 weeks at 0°. During this time the crystalline allophanate slowly separated, and was then filtered off and re-crystallized several times from ethyl and methyl alcohol. By
dissolving it in 50 volumes of hot 95 per cent ethyl alcohol and allowing the solution to cool slowly, about four-fifths of the allophanate separated as beautiful needles several mm. long. Although this allophanate separates very much more slowly than that of \( \alpha \)-tocopherol, it is, if anything, less soluble in ethyl alcohol and has little or no tendency to be accompanied by oil, so that its purification is much less bothersome.

**Analysis**—Heating at 80° *in vacuo* caused no loss of weight.

<table>
<thead>
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<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
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<tr>
<td>C</td>
<td>72.05</td>
<td>72.01</td>
<td>72.00</td>
</tr>
<tr>
<td>H</td>
<td>10.02</td>
<td>10.15</td>
<td>10.19</td>
</tr>
<tr>
<td>N</td>
<td>5.39</td>
<td>5.39</td>
<td></td>
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</table>

Calculated for \( C_{31}H_{62}N_2O_4 \). C 72.04, H 10.15, N 5.42

The analysis under I was made by Dr. Schoeller; under II at Columbia University.

**Fractional Adsorption of \( \alpha \)-Tocopheryl Allophanate**—100 gm. of Baker's Analyzed CaCO₃ powder, which had been heated for 3 hours at 150°, was packed tightly into a 35 mm. tube, as described by Strain (15), except that supercel was not necessary. 106 mg. of a sample of \( \alpha \)-tocopheryl allophanate, m.p. 156–157°, dissolved in a small volume of benzene were introduced, and the chromatogram developed with benzene. The first 400 cc. of filtrate were evaporated to dryness under reduced pressure, and left no residue. The following 750 cc. washed through 26 mg. of substance, which on recrystallization from a small amount of alcohol weighed 20.2 mg., m.p. 157–158°. This was hydrolyzed in the usual way, yielding Preparation 110-D; the results with feeding this are shown in Table I.

The filtration was then discontinued, and the column pushed out of the tube, divided into three approximately equal sections, and eluted with a mixture of alcohol and benzene. The yield and melting point of the eluates are as follows:

| Portion of column | Weight of crude material | Weight of recrystallized material | M.p.  
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td>63 mg.</td>
<td>49.2 mg.</td>
<td>158–159 C.</td>
</tr>
<tr>
<td>Middle</td>
<td>18 Ca.</td>
<td>12.0 mg.</td>
<td>157–158 C.</td>
</tr>
<tr>
<td>Top</td>
<td>1</td>
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<td></td>
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The allophanate from the middle section of the column was hydrolyzed in the usual way, yielding 9 mg. of \( \alpha \)-tocopherol (Table I, Preparation 112-A).

Conversion of \( \alpha \)-Tocopheryl Allophanate to the \( p \)-Nitrophenylurethane—500 mg. of the allophanate were hydrolyzed in the usual way, and the alcohol heated on the steam bath for an hour with 1 gm. of \( p \)-nitrophenyl isocyanate, no solvent being used. The reaction mixture was dissolved in benzene, and the excess reagent destroyed by adding acetone containing a small amount of water. After standing 24 hours, the di-\( p \)-nitrophenylurea was filtered off, and the residue taken up in petroleum ether. Some \( p \)-nitraniline separated. The filtrate was evaporated to dryness and taken up in methanol, from which, on cooling, the nitrophenylurethane separated. After several recrystallizations it melted at 129–131\(^\circ\), though not very sharply.

Dr. Adolf Pabst of the Geology Department very kindly examined the crystals of \( \alpha \)-tocopheryl \( p \)-nitrophenylurethane and described them thus:

"Very fine needle-like crystals. Parallel extinction, moderate to strong birefringence, biaxial positive, \( 2V \) small to moderate. Always shows interference figures normal to axial plane between optic axis and the obtuse bisectrix, with plane of the optic axes normal to the elongation of crystals. Elongation always negative. \( \beta = 1.514 \pm 0.002 \). The other indices could not be determined with certainty, but it is probable that \( \alpha \) is near 1.51 and \( \gamma \) near 1.53. It is highly probable that the crystals are orthorhombic, though they may be monoclinic or triclinic."

Analysis of \( \alpha \)-Tocopheryl-\( p \)-Nitrophenylurethane—The analysis was made by Dr. Ing. A. Schoeller, Berlin. The substance, dried at 80\(^\circ\) in vacuo, showed no loss of weight.

<table>
<thead>
<tr>
<th></th>
<th>73.02</th>
<th>73.09</th>
<th>72.98</th>
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<tr>
<td>C</td>
<td></td>
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<td></td>
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<tr>
<td>H</td>
<td>9.34</td>
<td>9.41</td>
<td>9.21</td>
</tr>
<tr>
<td>N</td>
<td>5.01</td>
<td>5.05</td>
<td>5.02</td>
</tr>
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</table>

Calculated for \( \text{C}_{22}\text{H}_{34}\text{N}_2\text{O}_5 \). C 72.67, H 9.15, N 4.71

Hydrolysis of the Nitrophenylurethane—The nitrophenylurethane was hydrolyzed with 4 per cent methyl alcoholic potassium hydroxide, as described for the allophanate. The ether residue was taken up in petroleum ether, from which a considerable amount of nitraniline separated and was filtered off. The remainder was
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removed by shaking a few times with 50 per cent methyl alcohol (Table I, Preparation 90-B).

Reconversion of Alcohol from p-Nitrophenylurethane to Allophanate—200 mg. of the p-nitrophenylurethane were hydrolyzed as described, and the alcohol, freed from p-nitraniline, was dissolved in benzene and saturated with cyanic acid gas from 2 gm. of cyano-nuric acid. After standing 4 days, the allophanate was worked up in the usual manner, and after a few recrystallizations from ethyl alcohol, melted at 158–160°.

50 mg. of the allophanate were hydrolyzed in the usual manner and used for biological assay and spectroscopic measurement (Table I, Preparation 146-A; Fig. 1, Curve 1).

SUMMARY

We have prepared from the non-saponifiable matter of wheat germ oil three allophanates:

1. M.p. 250°. This is possibly the allophanate of β-amyrin. The alcohol regenerated from the allophanate has no vitamin E potency.

2. M.p. 138°, readily crystallizing in long needles. The analysis agrees with values required by monoallophanates of an alcohol, C_{22}H_{50}O_{2}. The alcohol from this allophanate apparently has some vitamin E potency, but less than that from the third allophanate.

3. M.p. 158–160°. From this allophanate, the alcohol—for which we propose the name α-tocopherol—when given in a single dose of 3 mg. always enables vitamin E-deficient rats to bear young. α-Tocopherol shows a characteristic absorption band at 2980 Å, $E^{1}_{1\text{cm.}} = 90$ ca. Treatment with methyl alcoholic silver nitrate converts it to a substance which has absorption bands at 2710 and 2620 Å, respectively, $E^{1}_{1\text{cm.}} = 480$ ca., and possesses some vitamin E activity.

α-Tocopherol yields a crystalline p-nitrophenylurethane melting at 129–131°. Analyses of both the urethane and the allophanate indicate a provisional formula for α-tocopherol of C_{22}H_{50}O_{2}.

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332  \( \alpha \)-Tocopherol