THE CHEMISTRY OF VITAMIN E
TOCOPHEROLS FROM VARIOUS SOURCES*

BY OLIVER H. EMERSON, GLADYS A. EMERSON, ALI MOHAMMAD,
AND HERBERT M. EVANS

(From the Institute of Experimental Biology, University of California,
Berkeley)

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In a previous communication (1) some of us described the isolation from wheat germ oil of an alcohol, C_{29}H_{50}O_{2}, having marked vitamin E activity, for which we proposed the name \( \alpha \)-tocopherol. The substance itself was an oil, and was isolated in the form of the allophanate melting at 158–160\(^\circ\) from which it could be easily regenerated by alkaline hydrolysis. From it was also obtained a \( p \)-nitrophenylurethane, crystallizing in fine needles melting at 129–131\(^\circ\). The substance was believed to be homogeneous because adsorption of the allophanate on a column of calcium carbonate from benzene solution gave end-fractions of the same melting point, which on hydrolysis yielded alcohols of the same biological activity. Furthermore, \( \alpha \)-tocopherol from the allophanate was converted to the \( p \)-nitrophenylurethane, which was recrystallized to constant melting point, and then reconverted to the allophanate which, after a few recrystallizations, melted at 158–160\(^\circ\). The extra step in purification effected no change in the biological activity or in the absorption spectrum of the \( \alpha \)-tocopherol.

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We wish to acknowledge the assistance rendered by the Federal Works Progress Administration (Project No. 5778).
Dr. T. R. Hogness and Dr. F. P. Zscheile, Jr., of the Chemistry Department, University of Chicago, very kindly measured the absorption spectrum of α-tocopherol in isooctane. They found that although the shape of our curve was correct, the absolute

![Graph showing absorption spectrum with peaks and labels](image)

values were too high, at the 2880 Å. maximum $E_{1\text{cm.}}^{1\text{per cent}} = 73$ instead of 90. Drummond and Hoover (2) reported 75. Since Hogness and Zscheile were able to extend their measurements into the much further ultraviolet, we are reproducing their curve (Fig. 1).
As mentioned in the previous communication, there was also isolated from wheat germ oil a beautifully crystalline allophanate melting at 136–138°, the analysis of which indicated that it was derived from an isomer of α-tocopherol. In spite of its homogeneous appearance and the fact that this allophanate could be recrystallized a number of times from alcohol without change in melting point, it was possible by careful recrystallization from acetone to bring the melting point up to 144–146°. This allophanate appeared to have a slight optical rotation. The alcohol from this allophanate was very similar in its chemical properties and absorption spectrum to α-tocopherol, but had distinctly less biological activity. For it the name β-tocopherol is proposed.

A short report (3) referred to the isolation of two tocopherols from cottonseed oil, one of which appeared to be identical with α-tocopherol from wheat germ oil.

We now wish to report in more detail our work with cottonseed oil, and, in addition, the isolation of tocopherols from lettuce leaves and palm oil. Olcott and Mattill (4) prepared a potent concentrate of vitamin E from lettuce leaves, which was of particular interest in that it represented a source quite different from the seed oils previously investigated. Moreover, they reported that their concentrate showed merely general absorption of increasing intensity toward the shorter ultraviolet, without any definite maximum between 2900 and 3000 Å. (5). Palm oil possessed interest in that Olcott (6) reported a preparation of its non-saponifiable matter to possess strong absorption at 2940 Å, without corresponding biological activity.

From each of the three sources an allophanate has been secured which appeared to be identical with that of α-tocopherol from wheat germ oil in that the preparations showed the same melting point, showed no depression on mixed melting, agreed reasonably well in analysis, and on hydrolysis yielded alcohols having, within the limits of accuracy of the test, the same degree of biological activity (Table I). Further, the preparations from cottonseed oil and lettuce were converted to the p-nitrophenylurethane, which gave no melting point depression when mixed with that of α-tocopherol from wheat germ oil, or with each other.

From lettuce, only α-tocopherol was obtained, but from cottonseed oil a third allophanate was isolated, melting at 138–140°.
This allophanate separated as round aggregates which occasionally appeared to be made up of extremely fine thread-like needles. In this respect it differed from the \( \beta \)-allophanate, which often crystallized beautifully, even from impure solutions. A mixture of the \( \beta \) and the new allophanate gave a distinct depression of the melting point (132-136\(^\circ\)). Like the \( \beta \)-allophanate it appeared to have a slight optical rotation, but this might have been due to an impurity. For this third member of the series the name \( \gamma \)-tocopherol is proposed. The free tocopherol was not potent at a level of 3 mg., but did show activity when administered in a single dose of 8 mg.

From palm oil an allophanate fraction melting at 135\(^\circ\) was obtained, which gave no melting point depression when mixed with the slightly impure \( \gamma \)-tocopheryl allophanate of the same
melting point from cottonseed oil. Unfortunately the amount of this fraction obtained was insufficient for its complete purification, but it seemed that the tocopherol make-up of palm oil was qualitatively similar to that of cottonseed oil.¹

**EXPERIMENTAL**

**Wheat Germ Oil**

3.5 kilos of wheat germ oil were saponified and the non-saponifiable fraction was handled as previously described (1) except that the last traces of sterols were not precipitated with digitonin, a step which, in the case of wheat germ and cottonseed oils at least, appeared to be unnecessary. The allophanates were also treated as described before, except that it was found that the crude α-tocopheryl allophanate was more conveniently freed from oily impurities by recrystallization from small amounts of acetone than by recrystallization from alcohol and washing with petroleum ether.

From 3.5 kilos of wheat germ oil, 3.2 gm. of α-tocopheryl allophanate, melting at 158–160°, and 1.5 gm. of β-allophanate, melting at 136–138°, were obtained. Since further recrystallization from alcohol effected no change in the melting point of the latter allophanate, acetone was tried. Fractions melting over a broad range, e.g. 138–144°, were soon obtained, which on further recrystallization melted sharply at 144–146° (uncorrected). The allophanate was much more soluble in acetone than in alcohol, so that much material remained in the mother liquors, making it necessary to work up the intermediate fractions, and rendering this further purification very laborious and time-consuming.

**Optical Rotation of β-Tocopheryl Allophanate**—52.6 mg. in 3 cc. of benzene, 1 dm. tube, gave a rotation of +0.10°.

\[ [\alpha]_D^\circ = \frac{+0.10 \times 3 \times 1000}{52.6} = +5.7^\circ \]

**Analysis**—Found, C 71.71, 71.97; H 10.00, 10.06; N 5.53, 5.76
C\(_{32}\)H\(_{46}\)N\(_2\)O\(_4\). Calculated. C 72.04, H 10.15, N 5.42
C\(_{32}\)H\(_{46}\)N\(_2\)O\(_4\). “ 71.65, “ 10.03, “ 5.57

¹ We have since learned that palm oil concentrates possessing both biological activity and the absorption band at 2980 Å have been prepared at the Iowa laboratory.
Cottonseed Oil

3.5 kilos of cottonseed oil were treated as described above for wheat germ oil. By recrystallization from alcohol, 300 mg. of an allophanate melting at 158–160° were obtained without difficulty. This preparation gave no melting point depression when mixed with \( \alpha \)-tocopheryl allophanate from wheat germ oil. However, on analysis, carbon values were observed about 0.5 per cent higher than those for the wheat germ oil product, but by recrystallization from acetone a preparation was obtained whose analytical values were in better agreement with those previously observed.

Analysis of \( \alpha \)-Tocopheryl Allophanate from Cottonseed Oil—Found, C 72.23, 72.32; H 10.09, 10.18; N 5.54
C\(_{35}\)H\(_{53}\)N\(_2\)O\(_4\). Calculated. C 72.04, H 10.15, N 5.42

Conversion to \( p \)-Nitrophenylurethane—This was carried out as described before for \( \alpha \)-tocopherol from wheat germ oil (1). With the original sample of \( p \)-nitrophenyl isocyanate, a \( p \)-nitrophenylurethane was obtained, melting at 129–131°, which gave no depression on mixed melting with a sample of \( \alpha \)-tocopheryl \( p \)-nitrophenylurethane from wheat germ oil. However, with a different preparation of the reagent, it was possible to secure with ease a \( p \)-nitrophenylurethane melting sharply at 133°.

Analysis of \( p \)-Nitrophenylurethane—Found, C 72.77, 72.67; H 9.12, 9.27; N 4.54, 4.61
C\(_{36}\)H\(_{44}\)N\(_2\)O\(_4\). Calculated. C 72.67, H 9.15, N 4.71
C\(_{37}\)H\(_{46}\)N\(_2\)O\(_4\). “ “ 72.98, “ 9.27, “ 4.60

\( \gamma \)-Tocopheryl Allophanate—From the mother liquors of the \( \alpha \)-allophanate a much more soluble allophanate fraction, melting at 135°, was obtained. This was a mixture of \( \alpha \)-tocopheryl allophanate and the \( \gamma \)-allophanate. With small amounts of material, a separation of the two by fractional crystallization did not prove easy. At least a partial separation may be obtained by absorption on a column of calcium carbonate from benzene solution.

100 gm. of calcium carbonate, which had been dried by heating at 150° for 3 hours, were packed into a 45 mm. glass tube, and 107 mg. of the allophanate mixture in 100 cc. of benzene were poured into the top of the column, and the chromatogram developed with
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benzene. The first 850 cc. of filtrate contained no allophanates; the next 900 cc. washed through 30 mg. of an allophanate which, after one recrystallization from a small amount of alcohol, melted at 150–153° and was, no doubt, slightly impure α-allophanate. The next 750 cc. of solvent contained 28 mg. of material which melted at 135–137°. The material remaining in the column was eluted with a mixture of alcohol-benzene, and it also melted at 135–137°. By several recrystallizations it was possible to bring the melting point up to 136–138°.

Using very much larger amounts of material, Dr. E. Fernholz of Merck and Company secured by fractional crystallization from alcohol a γ-tocopheryl allophanate melting at 138–140°.

Rotation—53.5 mg. dissolved in 3 cc. of benzene gave an observed rotation of +0.06°, 1 dm. tube.

\[
[a]_D = \frac{3 \times 0.06 \times 1000}{53.5} = +3.4°
\]

Analysis—Found, C 71.91, 71.60, 71.60; H 10.90, 9.90, 10.11; N 5.58, 5.49
C_{30}H_{48}N_4O_4. Calculated. C 72.04, H 10.15, N 5.42
C_{30}H_{48}N_4O_4. “ “ 71.65, “ 10.03, “ 5.57

Lettuce Leaves

In preparing our concentrate from lettuce leaves, we departed slightly from the procedure of Olcott and Mattill. High boiling petroleum ether was used in the extraction in place of ethyl alcohol, and saponification was effected by refluxing for 1 hour with methanol-KOH instead of overnight with cold ethyl alcoholic KOH. This concentrate was distilled in a molecular still in very high vacuum, instead of at 0.1 mm., which enabled the vitamin to be distilled at 110–140° instead of at 190–220°. Finally the concentrate was treated with digitonin in order to remove the last traces of sterols. Although our concentrate showed considerable general absorption, there was a definite band in the region shown by the tocopherols, namely 2900 to 3000Å.

250 pounds of fresh lettuce leaves were dried in a steam chamber, crushed, and extracted in the cold by standing overnight in high boiling petroleum ether. The solvent was filtered off and the leaves were extracted with a second portion of petroleum ether. The united filtrates were evaporated under reduced pressure,
leaving 500 gm. of a semisolid residue. This was saponified in an atmosphere of hydrogen by refluxing with 750 cc. of methanol, containing 72 gm. of KOH, and 1 gm. of hydroquinone as antioxidant. The cooled reaction mixture was diluted with 4 volumes of water and extracted with peroxide-free ether.

The fractionation of the non-saponifiable matter was carried out as described by Olcott and Mattill, and the concentrate so obtained was distilled in a molecular still at a pressure indicated by the MacLeod gage of 10⁻⁵ mm. The fraction boiling between 110–140° was taken up in methanol and chilled to −18°, which threw out additional waxy material. After the digitonin precipitation, 5 gm. of concentrate were obtained which were active as vitamin E in a single dose of 20 mg.

The conversion to the allophanate was carried out in the usual way. The isolation of α-tocopherol allophanate, melting at 158–160°, was rather easily accomplished, although the yield was small.

Analysis—C 72.10, 72.07; H 10.26, 0.70; N 5.73, 5.70
Calculated for C₁₁H₁₄N₂O₂; C 72.04, H 10.15, N 5.42

The allophanate was converted to the p-nitrophenylurethane as previously described. The p-nitrophenylurethane melted at 132–133° and gave no depression when mixed with α-tocopheryl p-nitrophenylurethane from cottonseed oil melting at 133°.

Palm Oil

3 kilos of palm oil were treated as described for the cottonseed oil and wheat germ oil, except that the final product was distilled in a molecular still, and treated with digitonin as described for the lettuce concentrate. There were thus obtained 2.1 gm. of a concentrate which showed clearly an absorption maximum at 2080 Å., as well as definite vitamin E activity, when administered in a single dose of 20 mg. From this concentrate, an allophanate was secured melting at 158–160°, which gave no depression when mixed with α-tocopheryl allophanate from wheat germ and cottonseed oils. The analysis gave high carbon values, as had been observed in samples of the corresponding cottonseed oil product. Lack of material prevented further purification.

* Analysis by Schoeller, Berlin,
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Analysis—C 72.65, 72.53; H 9.85, 9.77; N 5.19, 5.29
Calculated for C₃₆H₄₅N₂O₄; C 72.04, H 10.15, N 5.42

From the mother liquors of the α-allophanate there was obtained by crystallization from alcohol a fraction melting at 134–135° which gave no depression when mixed with the crude γ-tocopheryl allophanate of the same melting point from cottonseed oil. Lack of material prevented further purification.

SUMMARY

α-Tocopherol has been isolated by means of the allophanate from cottonseed oil, lettuce leaves, and palm oil; and from each of these sources has, within the limits of accuracy of the test, the same vitamin E activity as that originally isolated from wheat germ oil.

Two additional tocopherols have been isolated. β-Tocopherol from wheat germ oil was obtained as the allophanate crystallizing in well formed needles melting at 144–146°, and γ-tocopherol from cottonseed oil was obtained as the allophanate melting at 138–140°. These tocopherols appeared to be one-half to one-third as potent as α-tocopherol. The analysis of the allophanates of these tocopherols indicate that they may contain one CH₃ group less than α-tocopherol, though the possibility that all three are isomeric is by no means excluded.

A tocopherol apparently identical with γ-tocopherol was obtained from palm oil, but the amount was insufficient for its complete purification.

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