PART IV

INTERMEDIATE METABOLISM

FREE RADICALS DERIVED FROM TOCOPHEROL AND RELATED SUBSTANCES

by

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Tocopherol is known to exhibit two properties: It serves as a vitamin, and also as an antioxidant with respect to the autoxidation of unsaturated fatty acids. The latter property is shared with many substances of phenolic character. Although the mechanism of the antioxidant effect is not fully understood, and the mechanism of its effect as vitamin E is not understood at all, the suggestion as to some relationship of those two effects is almost inescapable. The vitamin effect may be closely related to the antioxidant effect, except of course for the fact that the more specific effect of the vitamin requires a special structure in addition to the general feature of being a substituted hydroquinone. It may be left undecided whether the specific structure is just to make it more fat-soluble or to adapt it to any function as a coenzyme to some enzyme.

Hydroquinone is an efficient antioxidant[1]. Although the mechanism of its action is not known in every respect, it can scarcely be doubted that this effect is in some way connected with its reversible oxidizability. However, also phenols with only one (or at least one unsubstituted) hydroxyl group are antioxidants[2]. Here no reversible oxidation comparable with that of hydroquinone can take place. The reversible oxidation of hydroquinone leads to quinone, by a bivalent oxidation passing through the intermediate stage of a semiquinone. For monophenols, no such bivalent reversible oxidation is imaginable. However, a reversible univalent oxidation to a free radical is imaginable both for hydroquinone and for mono-phenols**, including tocopherol. Such a radical would be a rather unstable compound. Ordinary oxidizing agents may not be able to produce the semiquinone radicals to any readily recognizable extent; yet, if a free radical may be produced only to a slight extent, not recognizable directly, the high energy content of the radical would make it a powerful reactant; just as the free OH radical, although never existing to any directly recognizable extent in an aqueous solution, has been recognized as a powerful reagent in many chain reactions.

However, any speculation about such free radicals is all too vague unless there is more direct evidence for their existence. It is the purpose of this paper to produce such evidence. It is based on a method devised by G. N. LEWIS[3, 4] and consists of the following procedure. The substance to be oxidized is dissolved in an organic solvent such as, at the temperature of liquid air, will freeze to a homogeneous glass without crystallizing,

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[**] At the present time, it will not be discussed whether even one unsubstituted hydroxyl group is necessary at all for the establishment of a free radical of comparable structure.

and is irradiated with ultraviolet light through quartz windows in a Dewar vessel. Such an irradiation may have two effects: one is, to raise the energy of some electron to a higher level. The spontaneous return of this electron to its ground level will be manifested by some luminescence, either fluorescence or phosphorescence of longer duration, according to conditions discussed by LEWIS. In the second place, if there be an electron of sufficiently low ionization potential, the electron may be knocked out altogether, a process comparable to oxidation by a chemical oxidizing agent. At the temperature of liquid air and in the rigid medium molecular collisions are inhibited. Free radicals, once created, will accumulate to a concentration far above that permissible by thermodynamics, provided the electrons ejected are trapped in the molecules of the solvent and do not re-combine with the free radicals. In this case, no equilibrium in which the radical may be involved, can be established. Reactions such as dismutation, or dimerization of the radicals cannot occur. If the radical happens to be stable in so far as not to suffer a decay by a spontaneous unimolecular reaction (such as occurs in a radioactive atom), it will accumulate to a thermodynamically impermissible concentration. If the radical should be coloured, it could be seen in the frozen medium and remain as long as the temperature is kept low. On slight warming up the solution the colour should disappear. This may be taken as evidence for the that fact the colour belongs to a compound capable of existence to a noticeable extent only under conditions where the establishment of chemical equilibria is inhibited*.

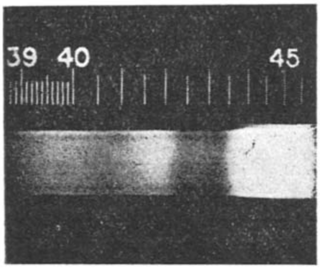

Fig. 1 shows the absorption spectrum of irradiated α-tocopherol at liquid air temperature, photographed with a spectrograph.

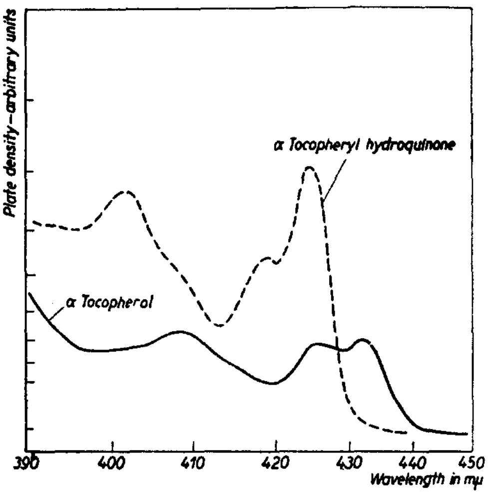

Fig. 2 shows tracings, obtained with a recording micophotometer, of the spectrum of irradiated α-tocopherol, and of irradiated α-tocopherylhydroquinone**.

The colour produced in this way can, in suitable cases, be compared with the colour of free radicals produced by chemical oxidation. In fact, the absorption spectrum of the compound generated by either method was found to be identical[3] on working with such substances as asymmetrical dimethyl-p-phenylene diamine, or tritolylamine[5, 6].

In this paper we shall describe the absorption spectra of several coloured substances considered as free semiquinone radicals prepared in this way from substances related

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* According to LEWIS and his associates, there may be still another effect: dissociation of a large molecule (such as tetraphenylhydrazin) either into two free radicals, or into a positive and a negative ion. Considering the structure of the compounds investigated, the possibility of such effects may be disregarded here. The fact that all the spectra obtained from the various compounds are similar, is further evidence as to the absence of any essential photodecomposition.

** The authors are indebted to the SUN CHEMICAL COMPANY, New York, for their permission to use their recording microphotometer.

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to tocopherol. They are all produced by irradiation of a solution in a mixture of ether, ethanol and pentane*, in the volume proportions 5:2:5, respectively, with an ultraviolet lamp for the duration of a few minutes to about twenty minutes. Although the method is not suitable in its present form to tell anything about the yield, it may be stated, that the radical of tocopherol is produced with ease to a readily recognizable extent.

Among the substances irradiated during this experimental study there is, first of all, hydroquinone. It is irradiated, then the decay of the phosphorescence is awaited (usually several seconds), without lifting the vessel out of the liquid air environment. Now the colour in transmitted light is observed. It is yellow, its absorption spectrum consists of several bands in the visible, the maxima of which are reproduced in Fig. 3. The yellow substance is not quinone. Firstly, its absorption spectrum is different from that of quinone; secondly, this colour vanishes on slightly warming up the frozen mixture. In addition, a spectrum of the same character is produced in this way from hydroquinone-monomethyl ether. This, of course, cannot be oxidized to the level of a regular quinone but there is no reason why it should not be oxidized to the level of a semiquinone.

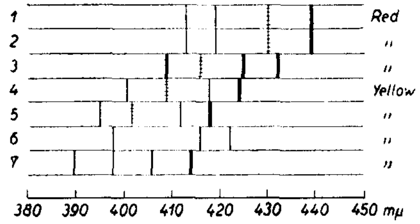
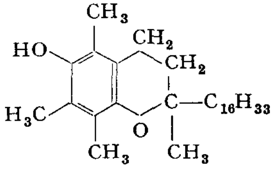


Fig. 3 shows the location of the absorption bands after irradiation as obtained according both to photographs such as Fig. 1 and to tracings such as Fig. 2.

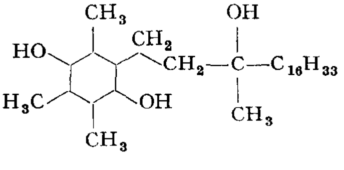
1. δ-tocopherol
2. γ-tocopherol
3. α-tocopherol
4. α-tocopheryl hydroquinone
5. hydroquinone monomethyl ether
6. durohydroquinone
7. hydroquinone

Of the various tocopherols, samples of pure α, δ, and γ tocopherol** and several samples of commercially available α-tocopherol were compared. The latter showed the same behaviour as the pure α-tocopherol, whereas the δ and γ compound showed, after irradiation, absorption bands slightly different from the α-compound. Whereas the colour of the radicals from hydroquinone and its methyl-ether are yellow, that of all the tocopherols is red, of slightly orange tint. This difference corresponds to the location of the absorption bands in Fig. 3.

The problem arises whether this "oxidation" by irradiation is a reversible one. Only in this case the substance could serve in metabolism as something analogous to a coenzyme of an oxidative enzyme. When tocopherol is chemically oxidized (say by ferric chloride), the first oxidation product obtainable is a quinone, tocopherylquinone[7],



α-tocopherol, parent substance of radical No. 3 in Fig. 3



α-tocopherylhydroquinone, parent substance of radical No. 4 in Fig. 3

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* G. N. Lewis recommends isopentane. We had no trouble with ordinary commercial pentane. If the mixed solvent shows any inclination to crystallize at liquid air temperature, it can be corrected by adding slightly more ether.

** We owe these to the courtesy of Distillation Products Corporation, Rochester, N.Y.

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which cannot be re-reduced directly to the original tocopherol because the phytol side-ring is opened to make the quinone. When this quinone is reduced to its corresponding hydroquinone, and this "tocopherylhydroquinone" is irradiated under proper conditions, the absorption spectrum of the free radical is different from that produced by the irradiated tocopherol itself. It resembles, with its yellow colour, more that of the hydroquinone-methyl-ether. Hereby it is shown that the red radical produced from tocopherol does not involve the opening of the phytol side-ring. The preservation of the free radical will also be aided by the fact that the opening of the phytol ring represents a hydrolysis which cannot occur in the absence of water. There is, then, no reason, why the univalent oxidation of tocopherol, especially in a non-aqueous solvent, should not be reversible.

SUMMARY

Tocopherol, dissolved in a suitable mixture of organic solvents such as will, at the temperature of liquid air, form a homogeneous glass, is irradiated with ultraviolet light. A red colour is developed which disappears at slightly higher temperature. Similar observations are made with some other substances related to hydroquinones. The coloured substance is interpreted as a free semiquinone radical. Its possible function for the vitamine and the antioxidant effect of tocopherol is discussed.

RÉSUMÉ

Le tocophérol, dissous dans un mélange approprié de solvants organiques, mélange qui, à la température de l'air liquide, forme un verre homogène, est irradié au moyen de lumière ultraviolette. Une coloration rouge apparaît, qui redisparaît lorsqu'on élève quelque peu la température. Des observations similaires ont été faites avec quelques autres substances de nature hydroquinonique. La substance colorée est considérée comme étant un radical semiquinonique libre. Son rôle possible dans l'action vitaminique et antioxydante du tocophérol est discuté.

ZUSAMMENFASSUNG

Tocopherol, gelöst in einer geeigneten Mischung von organischen Lösungsmitteln, welche bei der Temperatur der flüssigen Luft zu einem homogenen Glas erstarren, wird mit ultraviolettem Licht bestrahlt. Es entsteht eine rote Färbung, welche bei höherer Temperatur wieder verschwindet. Ähnliches wird mit anderen Hydrochinon-ähnlichen Verbindungen beobachtet. Die gefärbte Substanz wird als ein Semichinon gedeutet und ihre mögliche Funktion bei der Rolle des Tocopherols als Vitamin und als Antioxidant erörtert.

REFERENCES

[1] J. L. Bolland and P. ten Have, *Trans. Faraday Soc.*, 43 (1947) 201.

[2] J. L. Bolland and P. ten Have, in *The Labile Molecule, "Discussions of the Faraday Soc.,"* London 1947.

[3] G. N. Lewis and D. Lipkin, *J. Am. Chem. Soc.*, 64 (1942) 2801–8.

[4] G. N. Lewis and Biegeleisen, *J. Am. Chem. Soc.*, 65 (1944) 2424–6; 65 (1944) 2419.

[5] L. Michaelis, M. P. Schubert, and S. Granick, *J. Am. Chem. Soc.*, 61 (1939) 1981–92.

[6] S. Granick and L. Michaelis, *J. Am. Chem. Soc.*, 62 (1940) 2241.

[7] Walter John, *Z. physiol. Chem.*, 250 (1937) 11; 257 (1939) 173.

[8] L. T. Smith, *Chem. Revs*, 27 (1940) 287–320 (Review of the chemistry of vitamin E).

[9] R. A. Morton, *The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes*, 2dn Edition, Adam Hilger, Ltd., London 1942.

[10] Biological Antioxidants, *Transactions of the first conference*, Josiah Macy, Jr. Foundation, N.Y., 1946. *Second Conference*, 1947; *third Conference* (in press) 1948.

Received February 14th, 1949