THE BIOLOGICAL ACTIVITY OF DL-UBICHROMENOL AND AN ANALOGOUS DL-UBICHROMANOL IN VITAMIN E DEFICIENCIES

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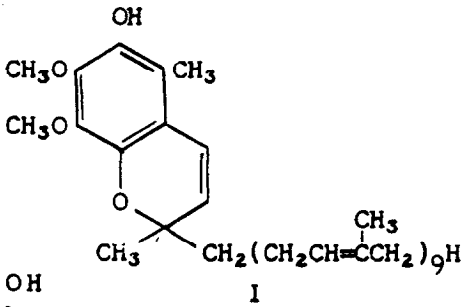
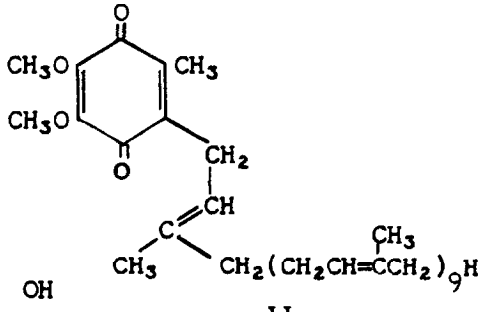
DL-Ubichromenol (I), having the chemical properties as herein described and prepared by a modified procedure, was found to have biological activity in preventing the resorption-gestation syndrome in the rat.

Ubichromenol (I) is isomeric with and is formed from coenzyme Q_{10} (ubiquinone, II), but differs from α-tocopherol (III) in that the latter is a chromanol with additional differences in substituents which are significant in structure-activity relationships for vitamins.



I



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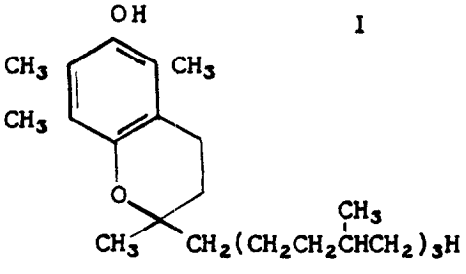

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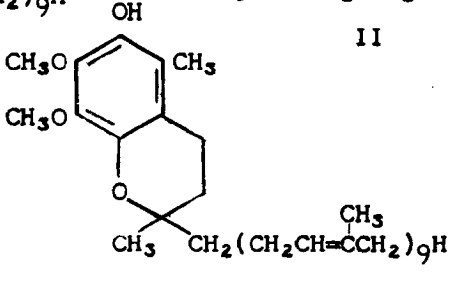

IV

* Paper XXVI in the Coenzyme Q series.

It has been previously demonstrated that certain antioxidants will prevent and cure the resorption-gestation syndrome brought about in rats by vitamin E deficiency (Draper et al., 1958; Crider et al., 1961). On the basis of the discovery of the coenzyme group of quinones and their importance in mitochondrial enzymic reactions, it was considered that vitamin E might function by the transfer of electrons to coenzyme Q_{10}, perhaps, with concomitant biochemical transformations involving either the corresponding ubichromenol (I) or ubichromanol (IV) (Hoffman, et al., 1960). One of these latter compounds might then be the biochemically active compound in the prevention of the syndrome brought about by vitamin E deficiency in the animal body. This idea might also explain the activity of certain other chemically unrelated antioxidants which replace vitamin E, and the formation of compounds O and F as mild oxidation products of vitamin E metabolism (Alaupovic et al., 1961).

To explore this idea, DL-ubichromenol (from CoQ_{10}) and DL-ubichromanol (from hexahydro CoQ_{10}) (Shunk et al., 1960b) were tested as replacements for vitamin E in the cure of the resorption-gestation syndrome in the rat. The methods used in the rat experiments are the same as those reported (Crider et al., 1961). The results are summarized in Table I.

It is apparent that DL-ubichromenol prevented the resorptio gestation syndrome and permitted the birth of live young, when administered to proven vitamin E-deficient female rats at the time of mating. While it is clear, as has been reported previously (Crider et al., 1961), that certain antioxidants are also active, coenzyme Q_{10} was not found to be active in this test. The limited testing of ubichromanols is being extended. There is no evidence for the existence of any ubichromanol in nature.

Table I

Reproduction of female rats given ubichromenol and ubichromanol

Treatment	No. of females	No. positive matings	No. of litters	No. of pups born
Vitamin E, 10 mg.[1]	8	6	6	49
Ubichromenol,* 20 mg.[2]	20	13	8	47
Ubichromenol,* 3 mg.[3]	6	2	2	22
Ubichromanol,** 10 mg.[2]	10	3	1	6
None	25	13	0	0

[1]Two oral doses 5 and 7 days following onset of mating.
[2]Two subcutaneous doses 5 and 7 days following onset of mating.
[3]One intraperitoneal dose 6 days following onset of mating.
*From CoQ_{10}.
**From hexahydro CoQ_4.

The reported natural occurrence in human kidney of ubichromenol (Laidman, Morton, Paterson and Pennoch, 1960) has been re-appraised in terms of a possible artifact of isolation (Links, 1960; Draper and Csallany, 1960), but a statement (Morton, 1960) on positive rotatory dispersion for a sample from kidney supports the concept of natural occurrence. However, purity and optical rotatory data are not yet available for ubichromanol from tissue.

A direct chromatographic conversion of coenzyme Q_{10} into ubichromenol by alumina (Shunk *et al.*, 1960a) confirmed the finding of Links (1960) that DL-ubichromenol can be an artifact of isolation under certain experimental conditions. Further, coenzyme Q_{10} was chromatographically unstable over magnesium oxide (British Drug Houses, Ltd.). Although the reaction products were not identified, it was demonstrated that this adsorbent can induce structural changes during chromatography of coenzyme Q.

After stirring overnight at room temperature in an aqueous dioxane solution (two phase) which contained pyrogallol and sodium hydroxide, coenzyme Q_{10} was converted into several substances; one of these (low yield) had an R_f similar to that of DL-ubichromenol. This result gives some confirmation to the reported conversion of coenzyme Q_{10} into ubichromenol by alkali (Draper and Csallany, 1960).

The estimations of ubichromenol in tissue by procedures which include alkali treatment and/or chromatographic treatment over alumina or magnesium oxide should be based on quantitative control experiments which prove that artifactual formation of ubichromenol does not take place under the specific conditions used; otherwise, tissue data on ubichromenol do not necessarily exclude an artifact.

The artifactual formation of ubichromenol does not exclude its existence and function in tissue, perhaps as a transitory biochemical intermediate, but so far complete evidence for the existence of ubichromenol in nature is not available. It is now agreed (Hemming et al., 1961) that some of the ubichromenol isolated from tissue is an artifact of isolation. However, on the basis of recovery data, it is still believed that ubichromenol occurs in human kidney. Further ubichromenol from A. fumigatus is reported. (Packter and Glover, 1960).

It is as yet unknown whether the vitamin E-like activity of ubichromenol is biologically specific; if so, vitamin E or the anti-oxidants might be substituting for biologically active ubichromenol when the latter is present at inadequate levels. Perhaps DL-ubichromenol is merely another example of a nonspecific anti-oxidant which will replace vitamin E in nutrition. The vitamin E-like activity of DL-ubichromenol focuses new interest on the question of the natural occurrence of ubichromenol and

prompts further new biological comparisons of it with α-tocopherol in animals, and perhaps also in man.

The following modified conversion of coenzyme Q_{10} into DL-ubichromenol has yielded a crystalline product with the highest melting point (25-26°) yet described; a melting point of "about 18°" has been reported twice (Laidman et al., 1959, 1960). Whether this lower melting point (after seven crystallizations) signifies an optical isomer or tenacious optically active impurities in a DL-product is unknown.

DL-Ubichromenol

Coenzyme Q_{10} (1.0 g., m.p. 49-49.5°), dissolved in 10 ml. of isooctane, was adsorbed on a column of 100 g. of alumina (basic, No. 71707, Merck). Distilled isooctane, containing 10% ether, was passed through the column slowly for 3 hours. The brown band was then eluted with methanol-ether (1-1). The eluate containing the dark band was collected separately, and concentrated under reduced pressure. The residue was dissolved in isooctane and the solution was dried over sodium sulfate to remove the small amount of water present, filtered, and concentrated under reduced pressure, yielding 0.54 g. of a red-brown oil.

A portion of the above material (400 mg.) was dissolved in isooctane and adsorbed on a column of 40 g. of silica gel. The column was developed with isooctane (distilled) until the colored band had moved two-thirds of the way to the bottom of the column. The eluant was then changed to 2% ether in isooctane and ca. 15 ml. fractions were collected, using a fraction collector. Ultraviolet absorption measurements indicated the fractions which contained the desired product. Fractions were combined and concentration yielded the quantity given: Fraction 1, tubes 41-76, 12 mg.; fraction 2, tubes 77-106, 53 mg.; fraction 3, tubes 107-131,

118 mg.; fraction 4, tubes 132-171, 24 mg. Fraction 2 was dissolved in 4.5 ml. of warm ethanol. The solution was filtered and cooled in an ice bath. Crystals separated. These were collected, washed with cold ethanol and dried under reduced pressure; m.p. 25-26°, wt. 45 mg. In a similar manner, fraction 3 yielded 84 mg. of product, m.p. 25-26°, from 9 ml. of ethanol. Further recrystallizati did not change the melting point. The ultraviolet absorption spectrum in isooctane showed λmax. mu. (E%): 330 (39.5); 281 (91.6); 274 (96.8); 231 (233). The NMR spectrum is consistent with the structure. The IR spectrum is the same as that published (Laidman et al., 1960). Anal. Calcd. for $C_{59}H_{90}O_4$; C, 82.08; H, 10.51. Found: C, 81.94, 81.96; H, 10.57, 10.59.

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