trimethylhydroquinone to give the chromane III (R is perhydrofarnesyl) and not the coumarane I.

We have synthesized α-tocopherol from trimethylhydroquinone and phytylbromide without a catalyst, and also from the hydroquinone and phytadiene. The products were distilled under high vacuum (10^{-6} mm) and they are thick, viscous, nearly colorless oils. The analyses of the samples of α-tocopherol prepared and purified as outlined (the products were not chromatographed), agree fairly well with the theoretical values. The sample of α-tocopherol prepared from phytylbromide gave an allophanate which melted at 168–170°; when mixed with the allophanate (mp. 157–160°) derived from natural α-tocopherol, shows a melting point between the two.[6]

Compounds II and IV have been tested biologically and found to be completely inactive in doses of 50 mg.[7] Several of our synthetic compounds, both coumaranes and chromanes, which have been examined in the laboratories of Merck and Company, Inc., show absorption curves strikingly similar to that of α-tocopherol. John[8] has also shown that α-tocopherol is either a coumarane or a chromane, and he has succeeded in opening the hetero ring without the loss of any carbon atoms. He states, however, that his work does not enable a decision to be made between the 5- and 6-membered hetero ring, nor as to the exact location of the aliphatic side chain attached to the ring.

Our syntheses of the pentamethyl-6-hydroxy chromane (II) and the proof of the structure of this compound, together with the two analogous syntheses of α-tocopherol, indicate to us that the structure proposed by Fernholz[9] for α-tocopherol is the correct one; that is, the vitamin is a chromane with two substituents in the α-position.[10]

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THE CHEMISTRY OF VITAMIN E. II. BIOLOGICAL ASSAYS OF VARIOUS SYNTHETIC COMPOUNDS[1]

THE fact that more than a single chemical substance can function as Vitamin E was demonstrated with the isolation of alpha, beta and gamma tocopherol. This need not occasion surprise, since it is already well established that several chemical entities function as Vitamins A and D, respectively, and the same phenomena have been discovered as regards the hormones—the so-called endocrine principles, for example, estrogenic activity.

Since the detection by Fernholz[2] of durohydroquinone amongst the products of the pyrolysis of alpha tocopherol, biological study of this substance and its related compounds for the detection of Vitamin E activity was urgently indicated.

PARENT SUBSTANCES

Hydroquinone itself was fed at the 100 mg level and though sufficiently toxic to be lethal at this level for three of the animals, did not confer fertility upon four animals in which the phenomena of insemination, implantation and resorption were all typically established. As regards durohydroquinone, one is at some disadvantage because of the difficulty of its solution. After various preliminary expedients, we at last hit on the plan of giving 4 cc of water by stomach tube, followed by a solution of durohydroquinone in 95 per cent. ethyl alcohol. Durohydroquinone was thus fed at 50, 100 and 500 mg levels. While no litters were secured at the 50 mg level, a single excellent litter resulted at the 100 mg level, though there were four clear resorptions at the same level. Both the animals to whom we succeeded in feeding a total of 0.5 g of durohydroquinone as ten 50 mg doses cast litters.

ETHERS OF DUROHYDROQUINONE[3]

The following ethers of durohydroquinone were fed: mono-cetyl, mono-octadecyl, mono-do-decyl and mono-(n-nonadecyl). At the lowest level fed (50 mg) two of four of the animals fed the mono-cetyl ether of durohydroquinone cast small, somewhat undersized, but living, litters. Our experience with the same substance at the 100 mg level demonstrated that this partial result at 50 mg was exceptional, since only two of eight littered at 100 mg. Two other ethers, namely, the mono-do-decyl ether and the mono-nona-decyl ether, showed some potency at the 100 mg level. Four

[6] We are greatly indebted to Dr. O. H. Emerson and to the Research Laboratory of Merck and Company, Inc., for the preparation and comparison of these allophanates.

[7] This work as well as the bioassay of our synthetic tocopherol has been done by Dr. H. M. Evans, of the University of California. See Dr. Evans' paper in this issue of SCIENCE.

[8] *Zeits. für Physiol. Chem.*, 252: 222, 1938.

[9] *Jour. Am. Chem. Soc.*, 60: 700, 1938.

[10] Note added to proof: Since this paper was written, Dr. John has very kindly sent us a reprint of his note in *Naturwiss.* 26: 366, 1938, to which we had not previously had access. Based upon his very elegant degradative work on α-tocopherol, John has reached the same conclusion with regard to the structure of the vitamin as we have, namely, the substance is a chromane with two substituents in the α-position (III).

[1] Aided by grants from the Board of Research and from the Department of Agriculture of the University of California, from Merck and Company, Inc., Rahway, N. J., and the Rockefeller Foundation, New York. Assistance was rendered by the Works Progress Administration, Project No. 8823 A-5. The following materials were generously contributed: cod liver oil by E. R. Squibb and Sons, and brewers' yeast by The Vitamin Food Company of New York.

[2] E. Fernholz, *Jour. Am. Chem. Soc.*, 59: 1154, 1937.

[3] We wish to thank Dr. E. Fernholz and Mr. J. Finkelstein for these preparations.

TABLE 1

VITAMIN E ACTIVITY OF SYNTHETIC COMPOUNDS OF KNOWN CHEMICAL STRUCTURE

Preparation	Level fed mg	Number rats fed	Number resorptions	Number litters	Per cent. littering	Average number living young per litter	Average weight g	Dead young	Remarks
Hydroquinone	100	7	4	0	0	..	..	..	3 died from toxicity of compound
Durohydroquinone	50	4	4	0	0	..	..	..	
Same	100	5	4	1	20	10	5.9	..	
Same (10 feedings of 50 mg.)	500	2	0	2	100	7	5.4	6	1 good, 1 very poor litter
Mono-cetyl ether of durohydroquinone	50	4	2	2	50	3	4.8	1	
Same	100	8	6	2	25	4	4.8	..	
Mono-octadecyl ether of durohydroquinone	50	4	4	0	0	..	..	..	
Same	250	2	1	1	50	2	5.0	..	litter on autopsy
Mono-do-decyl ether of durohydroquinone	100	6	5	1	16.5	3	3.8	4	1 late resorption
Same	250	2	1	1	50	10	5.4	..	
Mono-(n-nonadecyl) ether of durohydroquinone	100	4	2	2	50	6	4.7	2	1 litter consisted of 2 living young recovered on autopsy on 23rd day of gestation
Same	250	2	1	1	50	4	6.0	..	2 of young found at autopsy
[illegible] ether of durohydroquinone	250	2	2	0	0	..	..	..	
2, 2, 5, 7, 8 pentamethyl 6-hydroxy chromane	50	2	2	0	0	..	..	..	
2, 4, 6, 7 tetramethyl 5-hydroxy coumarane	50	4	4	0	0	..	..	..	
2, 2, 3 trimethyl 6-methoxy-chromane	100	4	4	0	0	..	..	..	
5, 7, 8 trimethyl 6-hydroxy 3–4 dihydrocoumarine	50	4	4	0	0	..	..	..	1 late resorption
5-hydroxy 2, 4, 6, 7 tetramethyl coumarone	25	4	4	0	0	..	..	..	
Synthetic alpha tocopherol	7.5	4*	0	4	100	7.3	5.5	..	
Same	3	4†	0	4	100	9	5.1	4	

* In another case a precipitous drop in the mother's weight indicated a litter was cast and eaten. Autopsy revealed 4 placental sites.

† In another case a precipitous drop in the mother's weight indicated a litter was cast and eaten. Autopsy revealed 10 placental sites.

ethers were tested with two animals each at the high level of 250 mg (divided dosage). In the case of one of these ethers (mono-2-methyl-n-octadecyl ether of durohydroquinone) neither of the two pregnancies resulted in living young. In the case of the other three ethers at the 250 mg level, in each instance one animal resorbed and one gave birth to living young.

CHROMANES, COUMARANES, COUMARINES AND COUMARONES

Fernholz'[4] degradation studies showed that alpha tocopherol was not a simple mono-ethyl ether of durohydroquinone, but a cyclic ether, probably a chromane.

Of the five simple cyclic substances shown in Table 1 all gave negative results.[5]

SYNTHETIC ALPHA TOCOPHEROL

Karrer and co-workers[6] have now accomplished the synthesis of alpha tocopherol, but no bioassays were reported. Two similar syntheses here separately published have just been accomplished by L. I. Smith *et al.*[7,8] One of these, that accomplished by the employment of trimethyl hydroquinone and phytyl bromide, was fed to 5 animals each at the 3 and 7.5 mg levels, practically all animals littering with satisfactory healthy young. This represents, therefore, the only biological test of the efficacy of a synthetic tocopherol at a level comparable with that exhibited by alpha tocopherol from natural sources.

SUMMARY

(1) The results of the feeding of durohydroquinone and of various of its ethers and of certain cyclic ethers indicate that Vitamin E activity is displayed by some of these substances when fed at high levels; more critical levels are now being fed.

(2) Synthetic alpha tocopherol was demonstrably active at the same level (a single dose of three milligrams) as that characterizing the same substance when secured from natural sources.

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[4] E. Fernholz, *Jour. Am. Chem. Soc.*, 60: 700, 1938.

[5] These substances were prepared by L. I. Smith, H. E. Ungnade and W. W. Prichard, of the University of Minnesota.

[6] P. Karrer, H. Fritzsche, B. H. Ringier and H. Salomon, *Helv. Chim. Acta.*, 21: 520, 1938.

[7] L. I. Smith, H. E. Ungnade and W. W. Prichard, this Journal, this issue.

[8] We wish to thank Dr. L. I. Smith and his collaborators, Messrs. H. E. Ungnade and W. W. Prichard, for the synthetic alpha tocopherol.