THE CHEMISTRY OF VITAMIN E[1]

LEE IRVIN SMITH

School of Chemistry, University of Minnesota, Minneapolis, Minnesota

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CONTENTS

I. INTRODUCTION

In 1922 Evans and his collaborators at the University of California (24, 25, 26, 27, 28, 29, 30, 31, 32) described the results of a long series of experiments which indicated that there was required, in animal nutrition, a dietary constituent necessary for normal reproduction. Young rats, fed for a sufficiently long time on a diet of purified foods with addition of the necessary salts and all of the known vitamins, lost the ability to reproduce. Upon the addition of certain vegetable products to the diet, the reproductive ability was regained. It followed that there existed, in the added vegetable products, an unknown factor which was necessary for the normal reproductive ability of rats. First designated as factor-X, the substance was later recognized as a vitamin and given the letter E (27, 130). It has also been called the antisterility factor or the reproductive vitamin. Although other vitamins, especially vitamin A, appear to exert an influence on the reproductive ability, this loss is most characteristic of a lack of vitamin E. The existence of vitamin E was at first disputed by several workers, but as the studies progressed, it was shown that these workers had used diets not quite free from vitamin E, and soon there was general

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agreement that the factor actually existed. Almost simultaneously with Evans' publications, Sure (129, 130, 131) and Mattill and his collaborators (95, 96, 97) published the results of their experiments, which also indicated the existence of the antisterility factor. These results have since been duplicated in many other laboratories. It is unfortunate, however, that the terms "antisterility vitamin" and "reproductive vitamin" were ever applied to vitamin E, for in no case has the vitamin been found to bring about reproductive ability where formerly this did not exist at all; neither does a dose larger than the minimum necessary for normal litters bring about any increase in the size of the litters. The function of the vitamin, so far as reproductive ability alone is concerned, is merely one of aiding or allowing a normal action to occur.

Further investigation of vitamin E was intensively undertaken by Evans and his associates, particularly by Evans and Burr (31, 32, 33). Extended series of experiments, involving many thousands of experimental animals, were carried out (31, 32, 33). The results showed that wheat-germ oil was the richest source of vitamin E, but the vitamin was also found in considerable amounts in cottonseed oil (100), lettuce oil, rice-germ oil, and other seed-germ oils (31, 103). The vitamin remains in the unsaponifiable part of the lipoid fraction (31, 32, 91). By processes of partition between different solvents, a sterol-free concentrate was obtained which was active in single doses of 10 mg. (31, 32).

The characteristic symptoms of lack of vitamin E differ in the sexes (24). In the female rat (31, 32) normal conception occurs, but this is followed by "resorption sterility." There is the usual pregnancy increase in weight for about 10 days, then the weight decreases and becomes normal at about the twentieth day. No litter is cast. The litter has been resorbed, but the resorption has no effect upon the next oestrus cycle. If, now, a female known to be in this state of resorption sterility is again mated, conception occurs as before. A day or so later, the animal is given in the food the substance to be tested. If this is active, the pregnancy will be terminated by the birth of a litter of living young. The vitamin E activity is often expressed as milligrams of the substance, fed in a single dose, necessary to cure the sterility and to produce litters in 50 per cent of the animals used (1, 2, 3, 4, 5, 31, 32, 105).

In male animals, the characteristic symptoms of lack of the vitamin are associated with the germinal epithelia and the spermatozoa. These degenerate until all sexual power is lost. These changes can be arrested by vitamin E only in the early stages; once the degeneration in the male animal has progressed very far, administration of the vitamin is of no use. At one time, it was suggested that there might be two vitamin E factors, one essential for male animals and the other essential for females (51), but later work has failed to substantiate this.

Along with these changes in the reproductive organs go other, more obscure, degenerative changes elsewhere. Recently Shimotori, Emerson, and Evans (106) have reported cases of muscular dystrophy caused by lack of vitamin E, and there are growth effects (22, 104) clearly discernible, as well as a characteristic paralysis of the hind quarters (33). Other effects, especially connected with the hypophysis and with the occurrence and growth of tumors, have been reported, but there is not complete agreement, as yet, about the connection between these effects and vitamin E.

At the close of the second stage in the study of vitamin E, it was possible to obtain a concentrate from wheat-germ oil which showed activity in doses of 10 to 20 mg. These were yellow to red oils which were extremely difficult to concentrate further. By high-vacuum distillation, Olcott and Mattill (100, 101, 103) were able to obtain a fraction boiling at 200–250°C. under 0.05–0.1 mm. pressure, which was active in doses of 5 mg., but the vitamin was damaged in this process by the high temperature necessary for the distillation. Evans, Emerson, and Emerson (34), as well as Todd, Bergel, and Work (133), subjected the concentrates to partition between petroleum ether and methanol and obtained highly active preparations; Drummond and collaborators (20, 21) used chromatographic adsorption to achieve the same end. But none of these procedures yielded the vitamin in crystalline form. At each stage in these separations the various fractions were assayed biologically, and, also at this time, measurement of the ultraviolet absorption spectra of these concentrates was begun. It was found that a parallel existed between the activity and the height of an absorption band at 2940 Å. (13, 14, 20, 21, 41, 98, 100, 101, 103, 137), and this proved to be a reliable guide in following the process of concentration. The curves are given in figure 1 (137): A is the curve for natural α-tocopherol, with circles and squares representing two different preparations; B is the curve for synthetic *dl*-α-tocopherol; and C is the curve for *m*-xylotocopherol. In figure 2 are given, for comparison, the curves of three model substances related in structure to the tocopherols; the similarities, as well as the differences, of the chroman and coumaran types are apparent from these curves.

These vitamin E concentrates are readily soluble in all lipoid solvents, and only slightly soluble in water. They withstand a temperature of about 200°C. and are fairly stable in the air when in mass, although when finely divided they are attacked by air, and lose their activity. Ultraviolet light quickly destroys all of the activity (21). The concentrates are quite stable toward acids, much less so toward alkalis (31). They are resistant to reduction but are quickly attacked by oxidizing agents, even by such mild oxidizing agents as ferric chloride. Potassium permanganate,

in pyridine solution, is rapidly reduced even in the cold (103). Ozone inactivates the vitamin (101). The presence of an active hydrogen atom was shown by the Zerewitinoff procedure (20). Acetyl chloride and benzoyl chloride react to produce esters (100, 101, 103), and these esters have practically the same activity as the original material. By comparing the

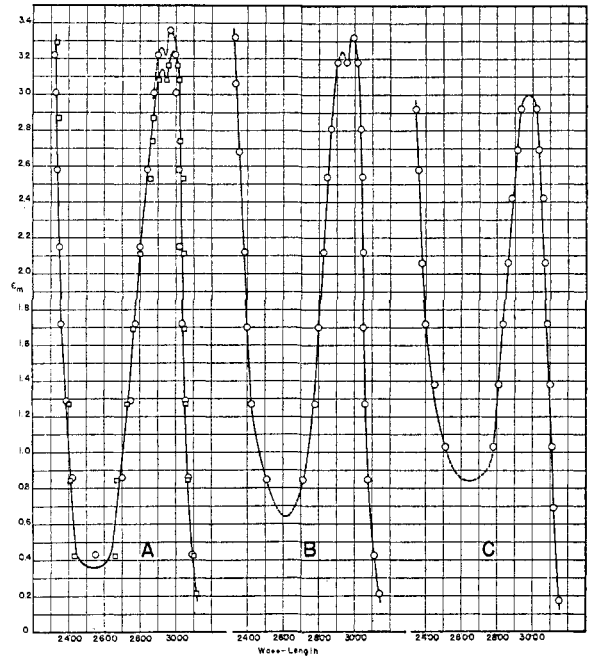
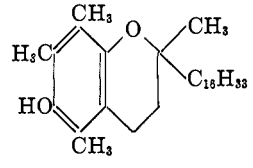
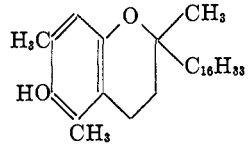


FIG. 1. Ultraviolet absorption spectra. A, curve for natural α-tocopherol; B, curve for synthetic *dl*-α-tocopherol; C, curve for *m*-xylotocopherol.



A and B



C

shift in the maximum of the absorption spectrum that takes place when phenol is acetylated, with that occurring when vitamin E concentrates are acetylated, John, Dietzel, and Günther (63) were able to deduce that the hydroxyl group in vitamin E was phenolic in nature.

However, esterification of these concentrates by various acids failed to

produce solid esters, and it was not until Evans, Emerson, and Emerson (34) treated the concentrates with cyanic acid that a solid derivative of

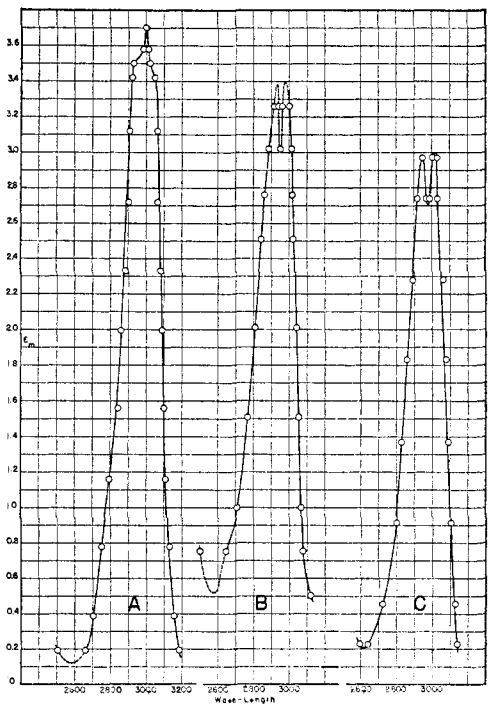
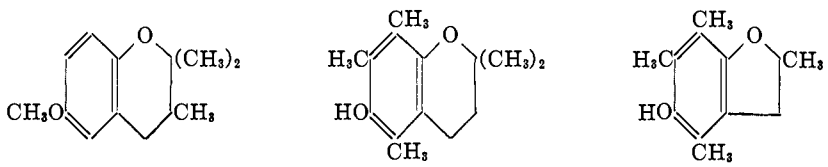


FIG. 2. Ultraviolet absorption spectra of three model substances related in structure to the tocopherols.



A B C

the vitamin was obtained. This reaction, characteristic of the hydroxyl group, leads to esters known as allophanates.

$$2HNCO + ROH \rightarrow H_2NCONHCOOR$$

By careful purification of the solid obtained in this way from wheat-germ oil concentrates there was obtained an allophanate melting at 159–160°C. and another melting at 138°C. These allophanates were soon isolated in other laboratories (20, 133); careful recrystallization of the allophanate

melting at 138°C. gave a product which melted at 144–146°C. (41, 133). These allophanates were hydrolyzed, and each yielded a pale yellow oil. These oils were both highly active, the first in 3-mg. doses, the second in 8-mg. doses. For these individual vitamin E factors Evans coined the name tocopherol; the tocopherols were then designated as α- and β-tocopherols. From 1 kg. of wheat-germ oil, about 1 g. of α-tocopherol allophanate may be obtained, although the yield is often much less than this. α-Tocopherol was converted into the *p*-nitrophenylurethan, m.p. 129–131°C.; it was then recovered from this derivative by hydrolysis, and reconverted into the allophanate, which again melted at 158–160°C.

α-Tocopherol possesses all of the properties of the highly active concentrates from wheat-germ oil. It shows the same solubility behavior, and the absorption band at 2940 Å. is the same. Analysis shows the composition to be $C_{29}H_{50}O_2$. The homogeneity of the preparation was shown by converting it, as stated above, into a solid *p*-nitrophenylurethan (and a solid *p*-nitrobenzoate) and transformation of these into allophanates with the same melting point as that possessed by the original allophanate from the concentrates.

β-Tocopherol, obtained in the same way from its allophanate, is likewise an oil. Its properties are almost identical with those of α-tocopherol, but its composition is $C_{28}H_{48}O_2$ and so it is a lower homolog of α-tocopherol. The yield of β-tocopherol from wheat-germ oil is usually much smaller than the yield of α-tocopherol, but often, from oils of different sources, normal amounts of β-tocopherol can be isolated, while almost no α-tocopherol can be found.

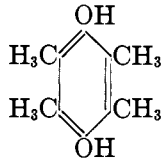
A third allophanate, melting at 138–140°C., has been isolated from cottonseed oil by Emerson, Evans, and their associates (41). This has been named γ-tocopherol allophanate. γ-Tocopherol is likewise an oil, active in 8-mg. doses, and it is an isomer of β-tocopherol, having the composition $C_{28}H_{48}O_2$.

We have, then, three antisterility factors which are responsible for vitamin E activity. These three tocopherols appear to be the only substances isolated from natural material which certainly possess vitamin E activity, for reports of still other active principles have not been substantiated (57, 80, 88, 89).

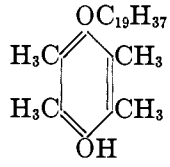
II. α-TOCOPHEROL

As mentioned above, α-tocopherol possesses the composition $C_{29}H_{50}O_2$. This composition is very close to that of some of the sterols,—sitosterol, for instance, having the composition $C_{29}H_{50}O$. As dehydrogenation with selenium had been of such great value in connection with studies of structure in the field of the sterols, it was natural that this method should be

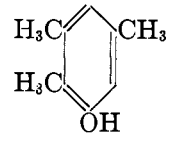
applied to α-tocopherol. McArthur and Watson (92) heated α-tocopherol with selenium; the result was a yellow sublimate, duroquinone, and a red oil. Somewhat later Fernholz (44) pyrolyzed α-tocopherol at 350°C. in the absence of any dehydrogenating agent. There was obtained a good yield of a white crystalline sublimate, identified as durohydroquinone (I), together with a red oil. Similarly, β-tocopherol gave trimethylhydroquinone (10, 55). The simplest assumption which would account for these decomposition products was that α-tocopherol was a monoether of



I



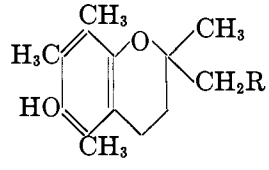
II



III

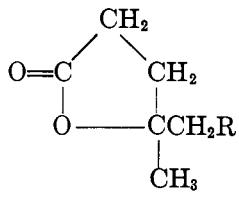
hydroduroquinone (44, 55), such as II (in which the group $C_{19}H_{37}$ contained one saturated ring), for it was known that many alkyl ethers of phenols were cleaved by pyrolysis into the phenol and an unsaturated hydrocarbon. Accordingly, in several laboratories monoethers of hydroduroquinone and of other hydroquinones were synthesized. Some of these showed activity when assayed biologically, but these ethers differed markedly from α-tocopherol in chemical properties and their ultraviolet absorption spectra were also quite different from that of the vitamin. As a result of these studies, it quickly became apparent that α-tocopherol could not be a simple monoether of hydroduroquinone (11, 58, 89, 99). John, Dietzel, and Günther (63) had also obtained pseudocumenol-6 (isopseudocumenol), III, by heating α-tocopherol with hydriodic acid; this result was also difficult to reconcile with the assumption that α-tocopherol was a simple monoether of hydroduroquinone, but it could be reconciled with the assumption that a second ring was condensed with the aromatic nucleus, probably involving an oxygen atom. Bergel, Todd, and Work (11) found that α-tocopherol, when energetically hydrogenated, absorbed 4 moles of hydrogen and they, too, supposed that an oxide ring was a part of the structure of the vitamin.

The correct structure for α-tocopherol (IV) was proposed by Fernholz (45) as a result of oxidative degradation, using chromic acid as the oxidizing

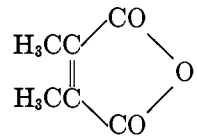


IV

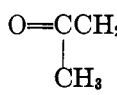
agent. The products were a C_{21} lactone (V), dimethylmaleic anhydride (VI), a C_{18} ketone (VII), a C_{16} acid (VIII), together with diacetyl and acetone.



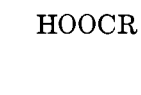
V



VI

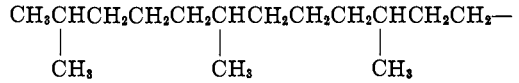


VII



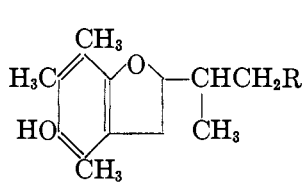
VIII

$CH_3COCOCH_3$ CH_3COCH_3

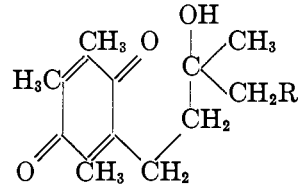
R = $C_{15}H_{31}$— or 

The hydroxy acid corresponding to the lactone V was transformed into the lactone with extreme ease, indicating that it was a γ-hydroxy acid; moreover, the hydroxyl group of the acid could not be oxidized to a carbonyl group, and was esterified only with difficulty. These facts indicated that the hydroxyl group was tertiary. The C_{16} acid (VIII), when analyzed for C—CH_3 groups, showed three such groups. The structure for the lactone (V) can only be written as shown in order to explain the formation from it of a C_{18} ketone and a C_{16} acid, and when these degradation products are assembled, they lead unequivocally to the structure IV, that of a chroman, for α-tocopherol. These results do not, of course, lead to the structure shown for the group R, $C_{15}H_{31}$. The structure for this group was written on the basis of the C-methyl determination and the experiences gained in other fields of natural products which frequently contain chains of "isoprene" units joined head to tail.

Karrer (80, 89), although considering both the chroman (IV) and the coumaran (IX) structures for α-tocopherol, at first preferred the latter. However, John and his associates (58) showed that α-tocopherol, when



IX



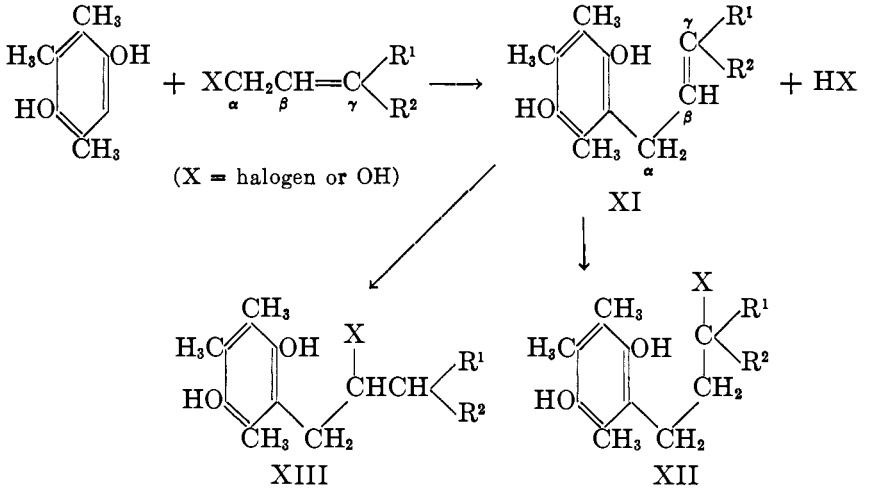
X

R = $C_{15}H_{31}$—, as in structure IV

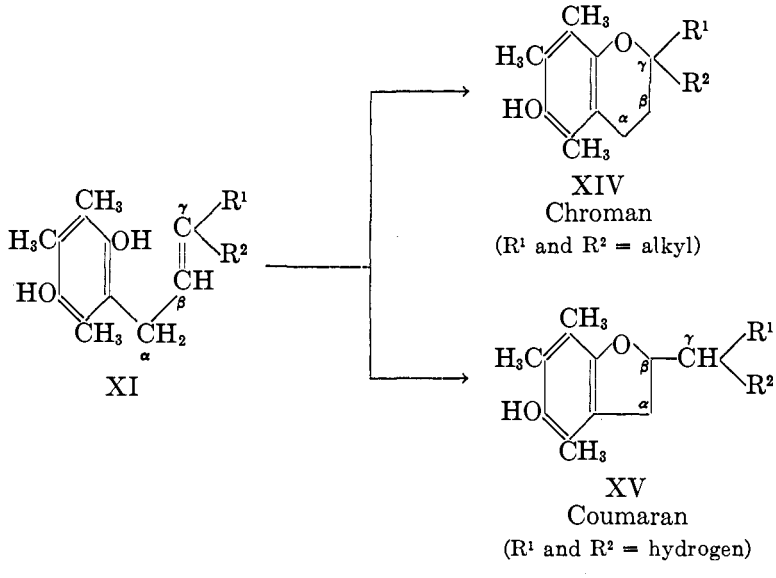
oxidized carefully with silver nitrate or ferric chloride, gave a yellow quinone (X). This quinone could be reduced to a hydroquinone, the di-*p*-bromobenzoate of which was quite stable toward chromic oxide, a fact which indicated that the hydroxyl group in X was tertiary. This could only be true if the oxygen ring in α-tocopherol were a chroman, for the coumaran IX would on oxidation give a hydroxyquinone, the hydroxyl group of which would be secondary and so susceptible to ready oxidation by chromic oxide. Karrer based his selection of the coumaran formula (IX) upon the fact that allyl bromide, when condensed with trimethylhydroquinone, does give a coumaran, and when he synthesized α-tocopherol from phytyl bromide, trimethylhydroquinone, and zinc chloride (79) he stated that structure IX was "highly probable."

The synthesis of chromans such as IV is often, but by no means always, rather simple and easy. The starting materials are hydroquinones (or phenols) having vacant one position in the ring ortho to the hydroxyl group (42, 81, 116). These are condensed with allylic halides or alcohols, or with conjugated dienes (7, 8, 79, 80, 119, 126). Frequently the reaction proceeds so smoothly that neither solvent nor catalyst is required, especially when allylic bromides or chlorides are used. When the alcohols or the dienes are used, it is customary to employ both a catalyst and a solvent. But in any event, because of the great reactivity of the allylic compounds, coupled with the enhanced activity of the aromatic nucleus in polyalkyl benzene derivatives, reactions between the two classes of compounds take place readily and the products are often obtained in good yields.

Using the halides or the alcohols, the first step in the reaction appears to be a direct introduction of the allyl group (121, 128) without rearrangement, to give XI.



Frequently, when X is halogen, the HX addition products (XII or XIII) of the allylic compounds (XI) can be isolated. The second step in the reaction, the ring closure, involves the addition of the hydroxyl group to the double bond in the side chain of XI, in accordance with Markownikoff's rule.

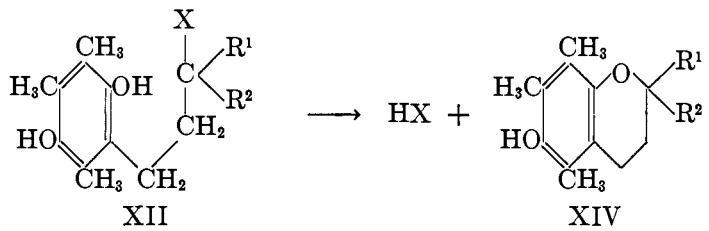


Hence, whether a chroman or a coumaran will be formed in this reaction will depend upon the nature of the groups or atoms attached to the γ-carbon atom in the allylic compound. If these groups are both alkyl, the oxygen of the hydroxyl group will add to the γ-carbon atom and the product will be a chroman (XIV); while if these groups are both hydrogen, addition will occur in the reverse manner and the product will be a coumaran (XV). When one of the groups is alkyl and the other is hydrogen, the product might be either the chroman or the coumaran, or a mixture of the two, although in most of these cases which have been studied so far it is largely the chroman. In a recent paper, Karrer, Escher, and Rentschler (74) have made similar generalizations about these ring closures; they have isolated, as condensation products of trimethylhydroquinone and crotyl bromide, *both* the chroman (XXXIII) and the coumaran (XXXIIIa). The structure of the latter was proved by an independent synthesis, using the sequence of reactions shown for the synthesis of XVI, substituting propionylacetic ester for acetoacetic ester.

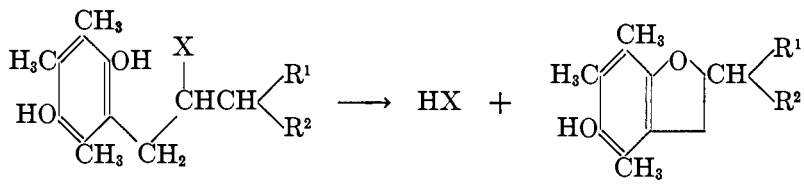
However, it is very curious in this connection that condensation of

trimethylhydroquinone with either crotyl *chloride* or butadiene (111) gave the same product, melting at 145°C. Mixtures of these two products did not show any depression in melting point, and according to previous work (74) this product was 2,5,7,8-tetramethyl-6-hydroxychroman (XXXIII; page 22). The isomeric 2-ethyl-4,6,7-trimethyl-5-hydroxycoumaran (XXXIIIa), isolated along with the chroman from the condensation product of crotyl *bromide* and trimethylhydroquinone (74), was reported to melt at 120°C. alone or when mixed with the chroman XXXIII, m.p. 145°C. Yet the series of reactions involving compounds LII, LV, LVI, LVII, and XXXIII has been reported (66). The last step, from LVII to XXXIII, involved the action of hydrobromic acid upon a solution of the ketone LVII in acetic acid, whereby the methoxyl groups were cleaved, and, simultaneously, reduction and ring closure occurred. The chroman XXXIII, made in this way, was reported to melt at 145°C. This experiment was repeated, *via* LII, LIII, LIV, and LVII (111), but the product, although it melted at 143°C., was *not* identical with XXXIII prepared from trimethylhydroquinone and crotyl chloride, crotyl bromide, or butadiene. A mixture of the two substances (both melting at 143–145°C.) melted at 115–130°C. Moreover, the product prepared from the ketone LVII gave a red color immediately with alcoholic silver nitrate, while the product prepared by condensation of trimethylhydroquinone and crotyl chloride or butadiene gave only a yellow oil with alcoholic silver nitrate. This yellow oil, when reductively acetylated, gave a monoacetate which melted at 79–80.5°C. and which was identical with the monoacetate (m.p. 84–85°C.) obtained from the condensation product itself (111). It then appears that the ketone LVII can be converted into the same product as that obtained by condensation of trimethylhydroquinone with crotyl bromide, but this product is certainly different from the substance obtained by condensation of the hydroquinone with either crotyl chloride or butadiene.

The halogen-containing products, XII and XIII, follow the same general rules. These can be readily cyclized to ring compounds, HX being eliminated between the halogen atom and the hydrogen atom of the hydroxyl group. It is to be noted that Markownikoff's rule also plays a part in these reactions, for although XII cyclizes to XIV (R^1 and R^2 are alkyl), the addition of HX to the double bond in XI could occur in two ways and the mode of addition will be governed by the rule. Thus when R^1 and R^2 in XI are alkyl groups, HX will add so as to produce XII; but when R^1 and R^2 are hydrogen atoms, HX will add so as to produce XIII. The ensuing ring closure by elimination of HX would then give the chroman XIV from XII and the coumaran XV from XIII.

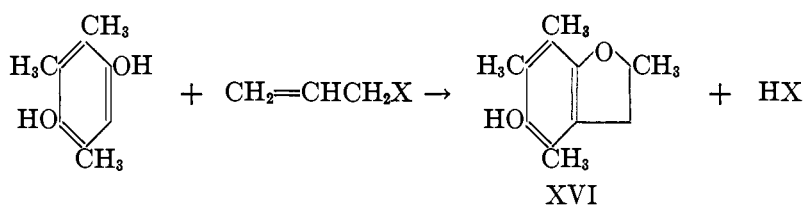


XII XIV

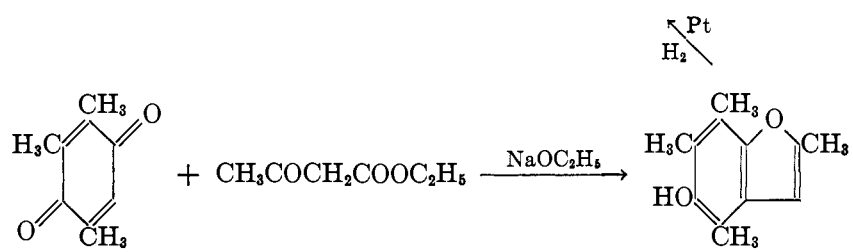


XIII XV

The generalities stated above regarding these reactions were carefully checked by means of model experiments upon simple compounds, the structures of which could be proved by independent syntheses. Thus, when allyl bromide or chloride is condensed with trimethylhydroquinone, the product is the coumaran XVI (121, 126), which is also produced by reduction of the coumarone XVII whose structure had previously been proved (115).



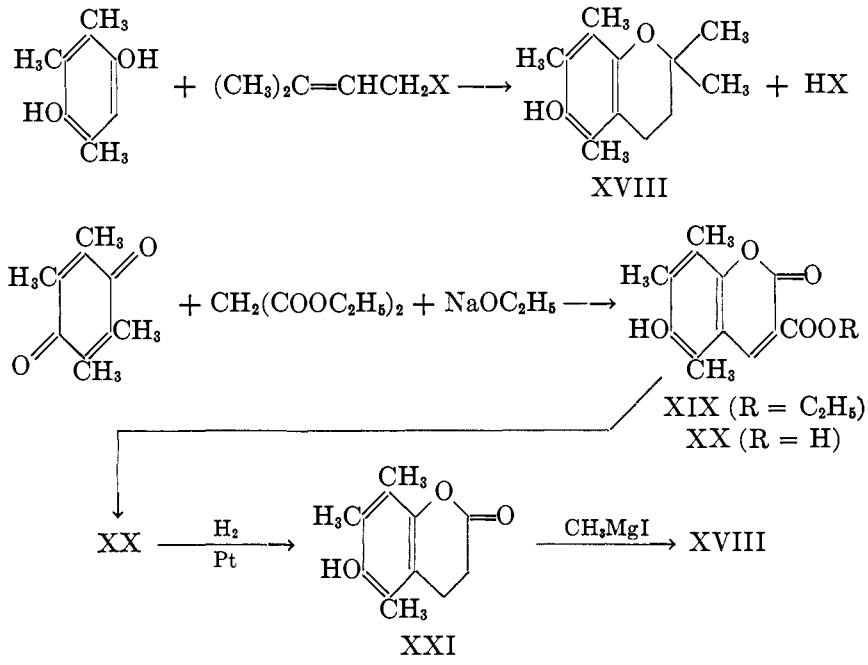
XVI



XVII

When γ,γ-dimethylallyl bromide is used, the ring closure occurs in the reverse direction and a chroman (XVIII) is produced (121, 126, 127). The structure of this chroman also was proved by an independent synthesis

from coumarin derivatives (XIX, XX, and XXI) of known structure (109, 110).

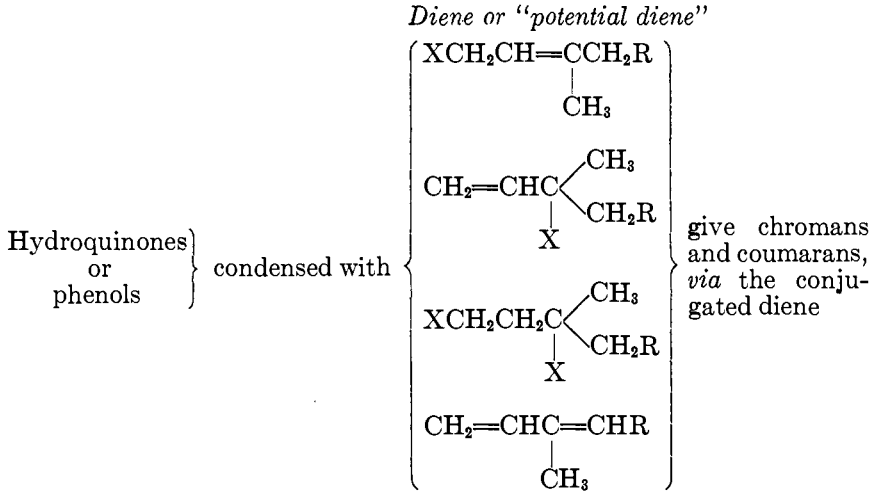


This same chroman (XVIII) has also been synthesized in other ways (66, 68, 125), so that there can be no doubt as to its structure; it is also the product of the reaction between trimethylhydroquinone and isoprene (122, 126, 128), a fact which has an important bearing upon the mechanism of these condensations (128).

In all this work involving condensations of hydroquinones with allylic compounds and with dienes, Markownikoff's rule has been assumed to apply fully, since the nature of one of the reactants (hydroquinones) insured that the reaction occurred under antioxidant conditions. Any "peroxide effect" therefore was considered to be negligible. Recently, however, it has been shown that the antioxidant effect of allylic phenols could be avoided by conversion of the phenols into acetates, and under these conditions there was a definite "peroxide effect" when the allylic phenol acetates were cyclized (52). Thus, when *o*-allylphenol was cyclized, the product was 2-methylcoumaran under all conditions. However, the *acetate* of this phenol, subjected to the action of hydrobromic acid, gave *2-methylcoumaran* when hydroquinone was present, and *chroman* when ascaridole (or other peroxides) was present. In a similar fashion, the ace-

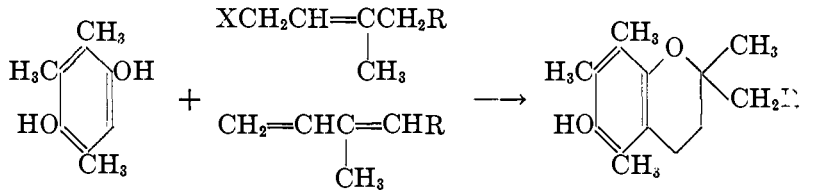
tate of *o*-allyl-*p*-cresol was converted into 2,5-dimethylcoumaran when hydroquinone was present, and into 6-methylchroman when peroxides were present. Other allylic phenols were found to be susceptible to this directed ring closure: these included *o*-allyl-*o*-cresol, *o*-allyl-*p*-bromophenol, and *o*-crotylphenol. But in the case of the one γ,γ-disubstituted allylic phenol studied, namely *o*-(γ,γ-dimethylallyl)phenol, the tendency toward chroman formation was so great that the product from the acetate was 2,2-dimethylchroman regardless of whether hydroquinone or benzoyl peroxide was present (52).

As stated above, conjugated dienes can be condensed with phenols and hydroquinones to give chromans and coumarans, and it is a fact that allylic carbinols, halides, and conjugated dienes, as well as appropriate diols and dihalides which give conjugated dienes when treated with catalysts, all condense with phenols to give chromans and coumarans (16, 128). These reactions have recently been extended to methylated hydroquinones (122, 126, 128) and in particular to the synthesis of tocopherols (7, 8, 79, 119, 126, 128). Claisen (16), who carried out the early work upon the phenols, implied that in all of these condensations the conjugated diene was an intermediate regardless of the halogen or hydroxyl compound used.



X = OH or halogen; R = alkyl

When it was found that phytadiene could be condensed with trimethylhydroquinone to produce α-tocopherol (122, 126), Claisen's mechanism appeared to offer a common explanation of the formation of tocopherols from hydroquinones and phytyl halides (7, 8, 75, 79, 119, 126), phytol (126, 128), and phytadiene (122, 126, 128).

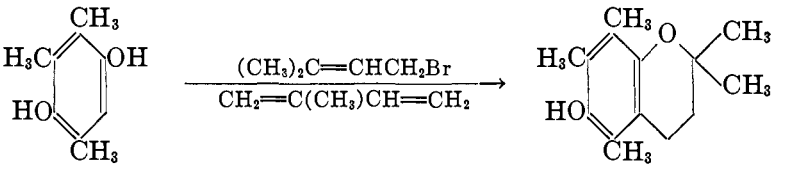


IV

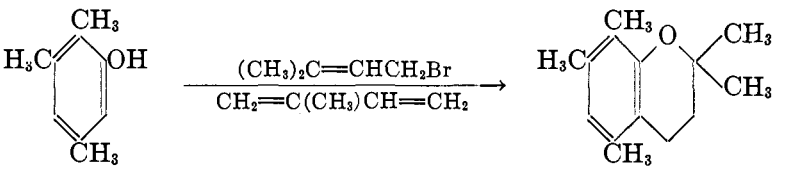
X = OH or halogen α-Tocopherol

R = $C_{15}H_{31}$ = $CH_3CHCH_2CH_2CH_2CHCH_2CH_2CH_2CHCH_2CH_2$—
CH_3 CH_3 CH_3

In harmony with this conception is the formation of the chromans XVIII and XXII when either γ,γ-dimethylallyl bromide or isoprene is used (121, 122). But this mechanism

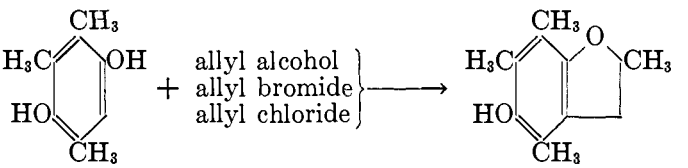


XVIII



XXII

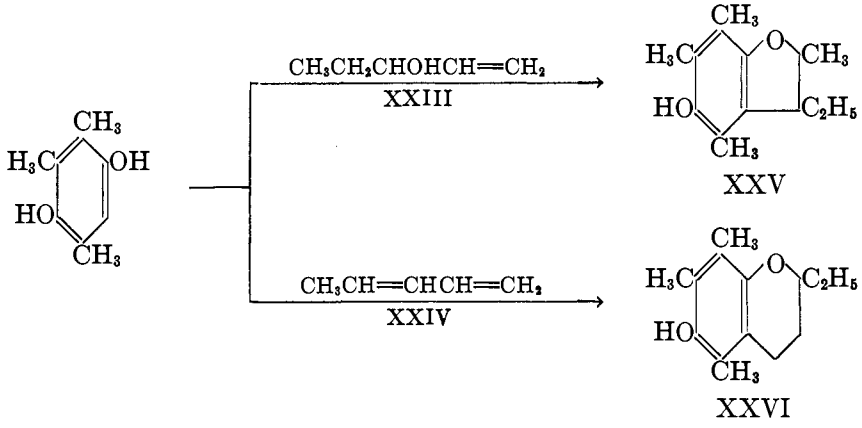
offers some difficulties when considered in the light of known facts regarding the addition of phenols to double bonds. In the first place, allyl alcohol, allyl bromide, and allyl chloride all condense with 2,3,5-trimethylhydroquinone to give the coumaran XVI and it is obvious that the corresponding diene (allene, $CH_2{=}C{=}CH_2$) cannot be an intermediate in this case unless an unusual mode of addition of the hydroquinone is postulated. Secondly, in some of the reactions between conjugated dienes and phenols,



XVI

hydroquinones, and their derivatives, allylic phenols or their HX addition products (XI, XII, XIII) are formed (122), and these may be cyclized in a separate operation, giving rise to the same cyclic compound whether the reaction is carried out in one step or in two. With a substituted diene such as isoprene, therefore, it is again difficult to interpret the reaction as a direct addition of the phenol or hydroquinone to the diene without assuming an unusual mode of addition, since the intermediate is known to contain a γ,γ-dimethylallyl group.

In order to reach a decision as to whether or not the diene is an intermediate, ethylvinylcarbinol (XXIII) and 1,3-pentadiene (XXIV) were condensed with trimethylhydroquinone under identical conditions (128). Since the diene to be expected from XXIII is XXIV, these two substances should give the same products when condensed with the hydroquinone, providing that the diene is an intermediate. Actually, however, the two products are different; the carbinol gave the coumaran XXV, while the diene gave the chroman XXVI.



Thus the diene cannot be an intermediate in the formation of XXV, and some other mechanism must be devised to account for the known products obtained in these condensations. A tentative hypothesis, which accounts for all of the known facts, is as follows: the *alcohols* and *halides*, as stated above, react with the phenol or hydroquinone by direct nuclear "allylation" without rearrangement; ring closure then follows in accordance with Markownikoff's rule. The *dienes* react first by 1,4-addition of the acidic catalyst (proton); this intermediate then "allylates" the aromatic nucleus without rearrangement, and ring closure follows as before. Hence 1,3-pentadiene (XXIV), isoprene, 2,3-dimethylbutadiene, phytadiene (XXIX), and other similarly constituted dienes with terminal CH_2 groups (XXVII; R^1, R^2, R^3 = hydrogen or alkyl) all give the same products as would be obtained using the halide or alcohol XXVIII (R^1, R^2, R^3 =

hydrogen or alkyl; X = hydroxyl or halogen). On the other hand, the secondary and probably also the tertiary allylic alcohols and halides give products different from those obtained from the diene, and this regardless of whether the alcohols and halides are single substances or mixtures of allylic isomers, so long as one of the allylic isomers is not related to the diene as XXVIII is to XXVII. Thus methylvinylcarbinol and hydroquinone give the chroman XXXII *via* the intermediate XXX, while crotyl bromide gives the chroman XXXIII (74, 128) and the coumaran XXXIIIa (74) *via* the intermediate XXXI.

$R^1CH{=}C(R^2)CR^3{=}CH_2$ (XXVII)

$CH_3CH{=}CHCH{=}CH_2$ (XXIV)

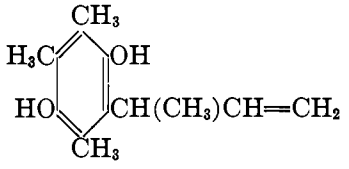
$CH_2{=}C(CH_3)CH{=}CH_2$

$CH_2{=}C(CH_3)C(CH_3){=}CH_2$

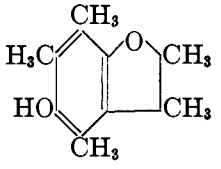
$C_{15}H_{31}CH{=}C(CH_3)CH{=}CH_2$ (XXIX)

All dienes with terminal CH_2 group

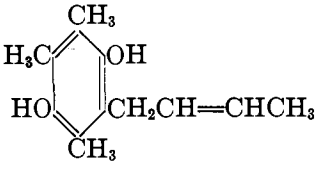
$R^1CH_2C(R^2){=}C(R^3)CH_2X$ (XXVIII)



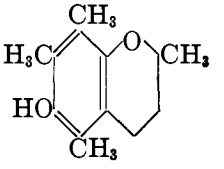
XXX
(Intermediate using methylvinylcarbinol)



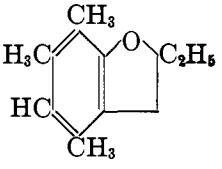
XXXII



XXXI
(Intermediate using crotyl bromide)



XXXIII
(largely)

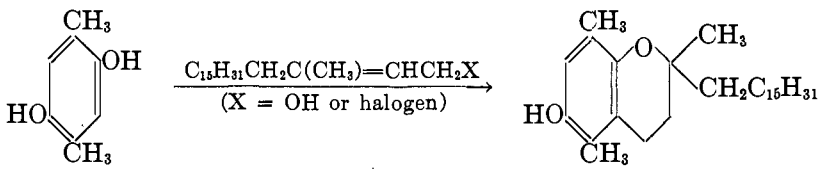


XXXIIIa
(by-product)

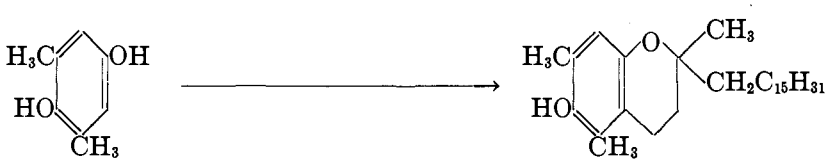
Using this reaction scheme, it can be readily understood that phytol, phytyl halides, and phytadiene should all condense with trimethylhydroquinone to give α-tocopherol, a prediction in complete accord with the facts. Thus it is possible to use a variety of starting materials and so to synthesize in several ways compounds containing the chroman or coumaran ring. These reactions all take place readily, and the syntheses of α-tocopherol described here involve no laborious syntheses of complicated intermediates, as is so often the case in vitamin syntheses.

III. β-TOCOPHEROL

As stated before, β-tocopherol possesses the composition $C_{28}H_{48}O_2$ and is a lower homolog of α-tocopherol. On thermal decomposition, it gives a sublimate of trimethylhydroquinone (11, 55); cleavage with hydriodic acid leads to *p*-xylenol (63). These reactions indicate that β-tocopherol possesses one less methyl group in the aromatic nucleus than does α-tocopherol, and the proof that this is the only difference between β- and α-tocopherol was supplied by Emerson (39) who, using the procedure of Fernholz, obtained from β-tocopherol practically the same degradation products as had been obtained from α-tocopherol. All of these facts indicated that the two methyl groups in the aromatic ring of β-tocopherol were para to each other and that this tocopherol was a derivative of *p*-xylohydroquinone with the structure XXXIV. This structure was proved by the synthesis of β-tocopherol; in fact, all three of the "xylotocopherols" have been synthesized (7, 8, 54, 75, 76, 78, 79, 80, 86, 119).



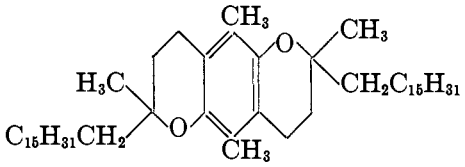
XXXIV
"*p*-Xylotocopherol"
(β-Tocopherol)



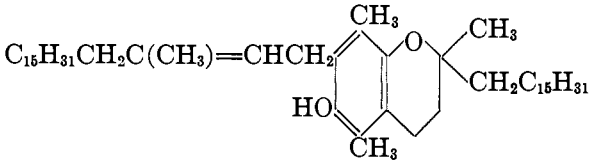
XXXV
"*m*-Xylotocopherol"

XXXVI
"*o*-Xylotocopherol"
(γ-Tocopherol)

These syntheses, however, proved to be much more difficult than the synthesis of α-tocopherol. The great reactivity of the allylic alcohol or halide (phytol or phytyl bromide) used, coupled with the enhanced reactivity of the aromatic nucleus due to the presence of the methyl groups, led to the easy introduction of more than one phytyl group, resulting in the formation of complicated mixtures from which the separation of the tocopherols was extremely tedious and difficult. These by-products were substances of the type of XXXVII, XXXVIII, XXXIX, XL, and XLI.

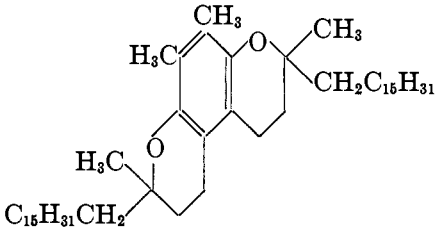


XXXVII

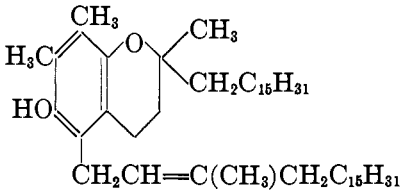


XXXVIII

By-products in the synthesis of *p*-xylo(β)tocopherol

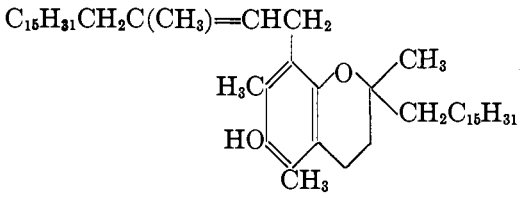


XXXIX



XL

By-products in the synthesis of *o*-xylo(γ)tocopherol



XLI

By-product in the synthesis of *m*-xylotocopherol

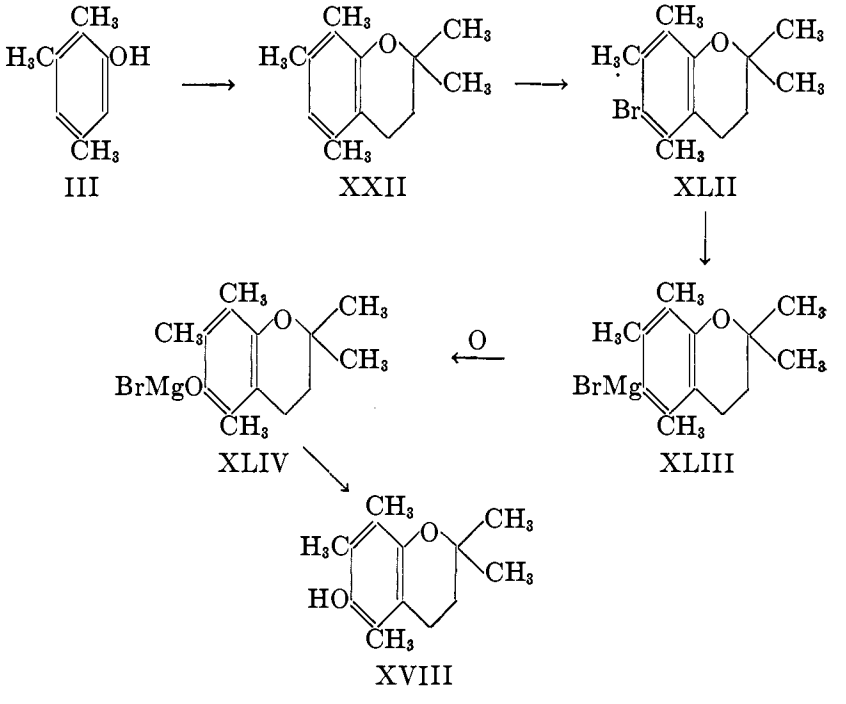
The allophanates of the xylotocopherols do not crystallize well from these mixtures, but by repeated chromatographic adsorption combined with crystallization of the allophanates, Karrer (76) was able to isolate the allophanate and the *p*-nitrophenylurethan of *m*-xylotocopherol (XXXV), which melted at 150° and 90°C., respectively, as well as the allophanate and the *p*-nitrophenylurethan of *p*-xylotocopherol (XXXIV), which melted at 154–155° and 91°C., respectively. The allophanate of *o*-xylotocopherol (XXXVI) was also isolated (78); it melted at 146°C. These derivatives of *p*-xylotocopherol (XXXIV) showed no depression in melting point when mixed with the corresponding derivatives of natural β-tocopherol (m.p. 146° and 90°C., respectively). This synthesis therefore completed the proof that β-tocopherol is *p*-xylotocopherol (XXXIV). The English group, however, report the melting point of the *p*-nitrophenylurethan of *p*-xylotocopherol as 111–112°C. (Karrer (86) later checked this and also reported 111–112°C.), and that of *o*-xylotocopherol as 89°C. (54). All three of the xylotocopherols are biologically active when fed at 5- to 10-mg. levels (54, 75, 76, 119). Recent work (86) indicates that the xylotocopherols are somewhat more active biologically than was at first supposed. All three are 100 per cent active in 10-mg. doses; *o*- and *m*-xylotocopherols are about 50 per cent active in 5-mg. doses, although *p*-xylo(β)tocopherol, curiously enough, is inactive at this level. The para- and ortho-compounds are inactive in 2.5-mg. doses; data on the meta-compound were not reported at levels below 5 mg.

IV. γ-TOCOPHEROL

This tocopherol is also a lower homolog of α-tocopherol (39, 54). It is isomeric with β-tocopherol, and it has the composition $C_{28}H_{48}O_2$. On oxidation, γ-tocopherol gives many of the same products as were obtained from α- and β-tocopherols (39), particularly dimethylmaleic anhydride (38) (8 per cent yield as compared with 24 per cent yield from α-tocopherol (19)); it also yields trimethylhydroquinone on pyrolysis (39). These data indicate strongly that γ-tocopherol is *o*-xylotocopherol. The allophanate of γ-tocopherol melts at 137–140°C. γ-Tocopherol is extremely difficult

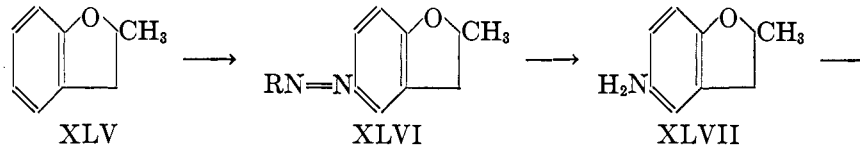
to isolate and purify, because the allophanate of α-tocopherol is very similar to that of γ-tocopherol and mixtures of the two show depressions of only a few degrees in melting point. It has recently been found, however, that corn-embryo oil is relatively rich in γ-tocopherol and contains very little α-tocopherol, so that the isolation of the allophanate of γ-tocopherol from this source is much simplified (40). With larger amounts of γ-tocopherol available, the structure of this substance will doubtless be definitely settled soon.

Because the synthesis of simpler tocopherols is accompanied by so many side reactions and by-products, attempts have been made to devise syntheses which avoid these complications (77, 112, 113, 117, 124). Since phenols are much less troublesome than hydroquinones as far as these by-products are concerned, the heterocyclic ring was built onto the phenol and the *p*-hydroxyl group then introduced as the final step in the synthesis (113). Thus 2,3,5-trimethylphenol (III) was condensed with isoprene to give the chroman XXII.

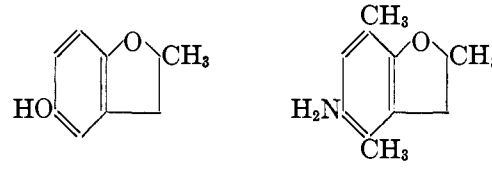


This was converted to the monobromo derivative (XLII), which reacted with metallic magnesium to produce the Grignard reagent XLIII. Oxidation of the magnesium derivative, followed by hydrolysis, produced the

hydroxychroman XVIII, identical with that synthesized by other methods. No by-products were produced in these syntheses, but the over-all yield of XVIII left much to be desired. Experiments were also carried out in which chromans and coumarans were coupled with aromatic diazonium compounds (77, 113). Thus Karrer (77), by coupling the coumaran XLV with diazotized 2,4-dinitroaniline, prepared the azo compound XLVI, which was reductively cleaved to the aminocoumaran XLVII. The amino group of this coumaran was then diazotized and replaced by the hydroxyl



XLV XLVI XLVII

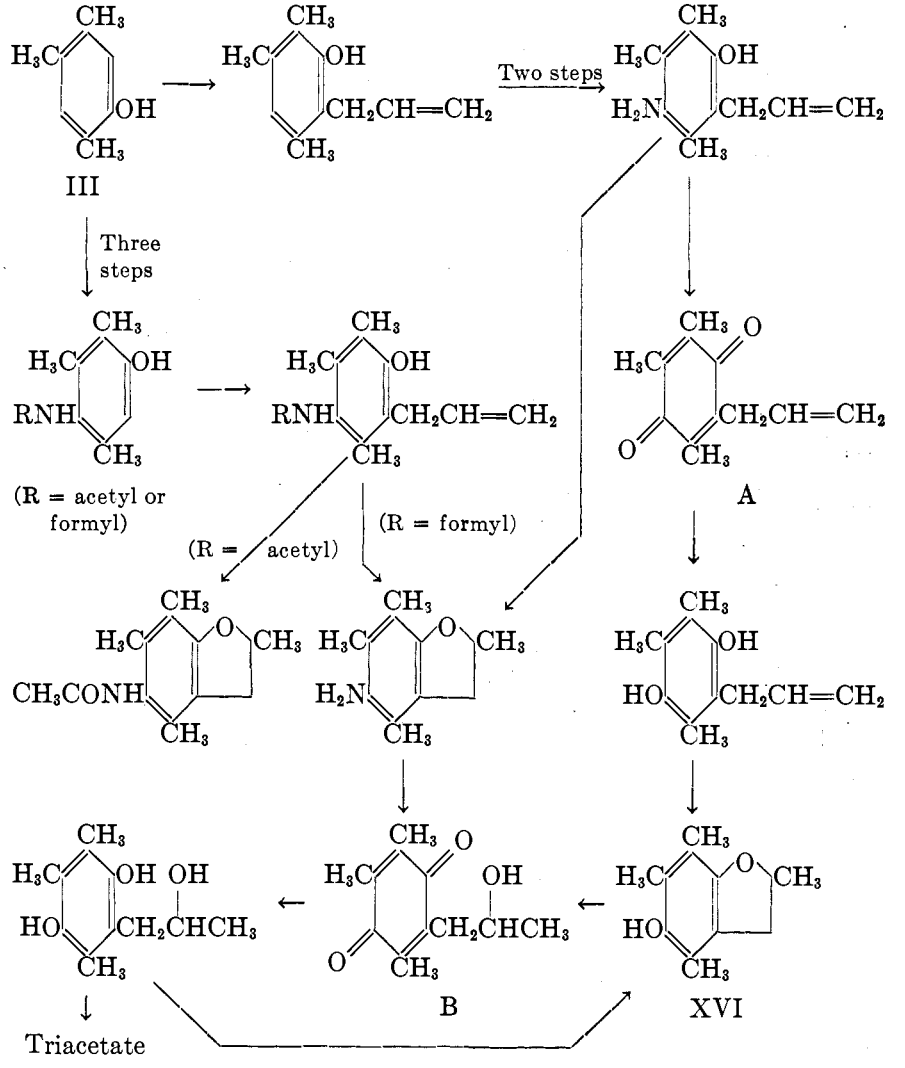


XLVIII XLIX

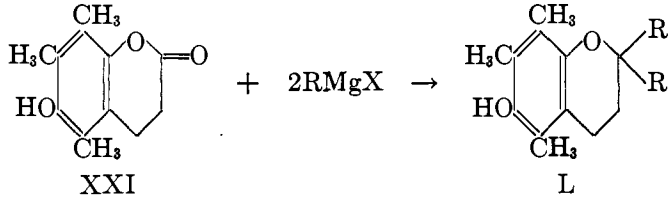
group in the usual way, yielding the hydroxycoumaran XLVIII. The aminocoumaran XLIX was prepared in a similar manner.

In another such series (112), 2,3,5-trimethylphenol (III) was converted into 2,4,6,7-tetramethyl-5-hydroxycoumaran (XVI) by two routes, as shown below. All of the reactions leading to the coumaran proceeded smoothly and gave good yields, and whether the allyl group was introduced before or after the coupling made no difference. The use of diazotized sulfanilic acid in the coupling reactions was advantageous, because after cleavage the by-product (sulfanilic acid) was water-soluble and non-volatile. This series of reactions, in which ring closure occurs *after* the coupling, is of especial value, since chromans and coumarans, being essentially phenol ethers, often do not couple well even with very active diazonium salts. The only step in the whole series of reactions which gave rise to any difficulty was the cleavage of the acetaminocoumaran to the aminocoumaran; this cleavage could not be achieved by any of the methods tried. But the formylamino compounds offered no difficulty at any step; treatment of trimethylallylformaminophenol with hydrobromic acid gave the aminocoumaran hydrobromide in good yield. The hydroxycoumaran XVI was prepared equally well *via* the two quinones A and B, one of which (A) contained the unsaturated allylic side chain, while the other (B) contained the saturated, but hydroxylated, side chain. Many alternative paths from III to XVI involving the compounds in the chart naturally

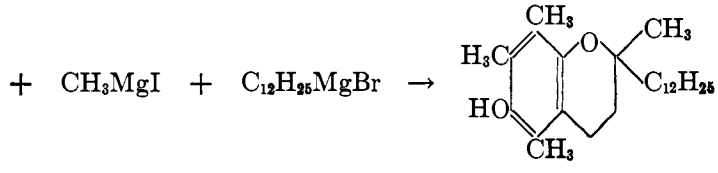
suggest themselves; while all of these were not tried, there was no evidence that the introduction of the various groups, as well as the ring closure, could not have been done in any desired order. An analogous series of reactions was also used for preparing 2-methyl-5-hydroxycoumaran. *o*-Allylphenol was converted, *via* the azo compound, into *o*-allyl-*p*-aminophenol, which was then oxidized to allylquinone. The quinone was reduced to the hydroquinone and the latter cyclized to the coumaran. The over-all yield of 2-methyl-5-hydroxycoumaran was excellent.



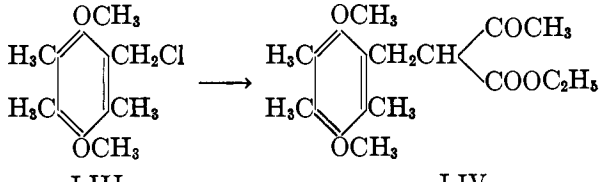
While such syntheses as these are interesting and some of them appear, from the results of model experiments, to be promising as general methods for the synthesis of *p*-hydroxychromans and *p*-hydroxycoumarans, no tocopherols have as yet been synthesized by any of these methods. Likewise unsuccessful as yet, are experiments leading to the synthesis of tocopherols without the use of phytol. The action of Grignard reagents upon dihydrocoumarins leads to α,α-disubstituted chromans (L) in which



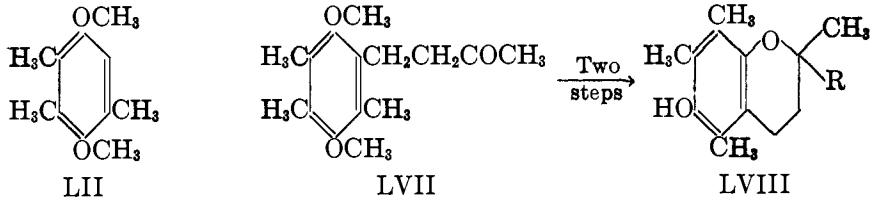
XXI L



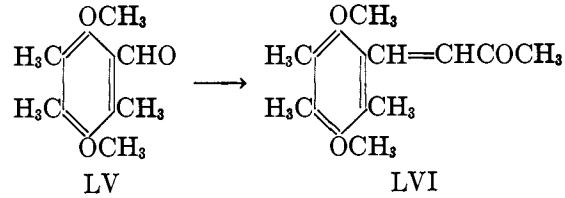
LI



LIII LIV



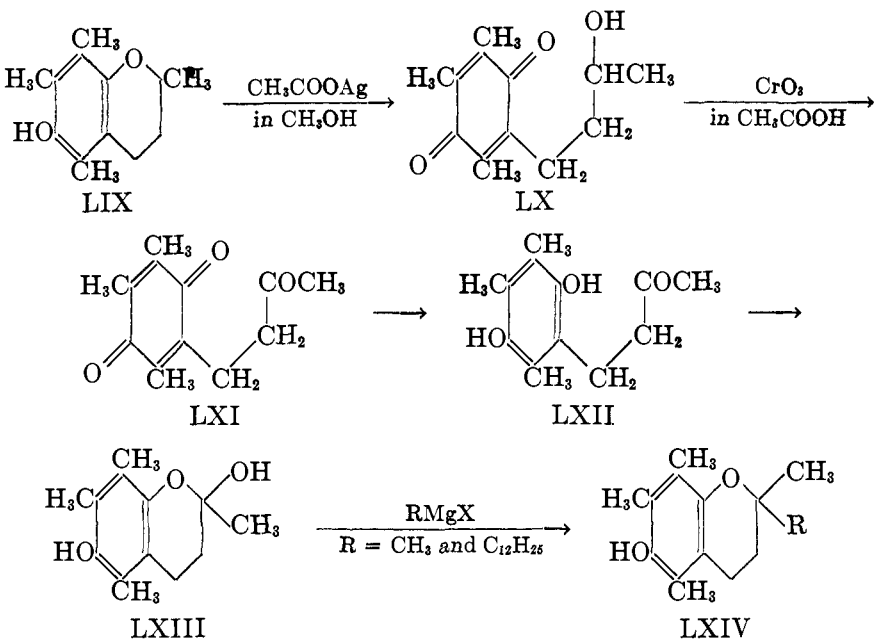
LII LVII LVIII



LV LVI

the two substituents are the same (9, 15, 67, 107, 118, 126, 127), although by subjecting the dihydrocoumarin to the action of a mixture of two different Grignard reagents, the chroman LI can be obtained (67).

The ketone LVII has been synthesized in two different ways (66, 125), and addition of RMgX to this ketone (R = CH_3 and $C_{12}H_{25}$), followed by cleavage of the ether linkages by hydrobromic acid leads directly to the chroman LVIII (66). Finally, the introduction of the second substituent into the α-position of a *p*-hydroxychroman has been achieved, starting with the monosubstituted chroman (68), through the series of compounds LIX to LXIV. The yields in this synthesis were fair, but the synthesis cannot compare in efficiency with the direct synthesis of tocopherols from



LIX LX LXI LXII LXIII LXIV

hydroquinone and phytol or the phytyl halides. Indeed, it is difficult at present to see how any other synthesis can possibly compare favorably with the direct synthesis, for the intermediates necessary, if phytol is not used, are themselves extremely complicated and difficult to prepare (117, 120, 124). The synthesis of relatively large amounts of alkylated quinones and hydroquinones was at first a formidable task, but these preparations have been studied in two laboratories (42, 81, 116) with the result that this phase of the tocopherol syntheses may now be regarded as solved. Good yields of alkylated quinones and hydroquinones can be obtained from readily accessible methylated phenols by means of efficient processes which do not involve very many steps.

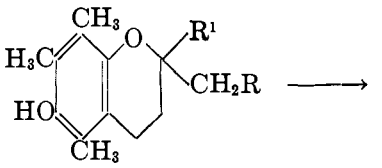
V. CHEMICAL PROPERTIES OF THE TOCOPHEROLS

The chemical properties of these substances depend upon the presence of the phenolic hydroxyl group and upon the fact that they are essentially monoethers of hydroquinones. By acylation, esters are produced and a series of these esters has been prepared (19). Among the acids used are the simple fatty acids, such as acetic and propionic acids; dibasic acids, such as succinic acid; and one of the aromatic series, benzoic acid. Some of these esters are fully as active biologically as α-tocopherol,—indeed, the acetate appears to surpass α-tocopherol in biological activity. Although many esters have been prepared, all except the allophanate, the *p*-nitrophenylurethan, and the nitrobenzoates are oils.

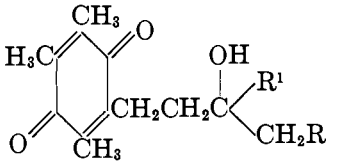
Aside from the reactions of the hydroxyl group, the chemical properties of the tocopherols center about the easy oxidation of these compounds. They show the properties of oxidation inhibitors (102), and their activity in this respect increases in the order α-, β-, and γ-, that is, the antioxidant activity is the reverse of the biological activity. The tocopherols are slowly attacked by oxygen, gradually darkening and acquiring a reddish color. They are also very sensitive to ultraviolet light (84). Not all specimens behave alike in this respect, however, and traces of impurities apparently affect the rate of oxidation very much. As the oxidation proceeds, the biological activity diminishes. The oxidation of the tocopherols is greatly retarded by the action of inhibitors such as hydroquinone or ascorbic acid, and is greatly *accelerated* when the exposed surface is increased,—i.e., when the tocopherols are chromatographed, or incorporated into finely divided powders of any sort (53). The esters, however, are stable in air over long periods of time, even when the exposed surface is greatly increased.

The oxidative degradation of the tocopherols, using permanganate or chromic acid, has already been discussed. When subjected to mild oxidation, however, no carbon is lost in the initial stages, and there results a series of compounds closely related to the tocopherols (36, 62, 64, 74, 78, 90, 114). The end product of this type of oxidation,—which is shown by all 6-hydroxychromans and 5-hydroxycoumarans so far investigated (114),—depends upon the oxidizing agent used.

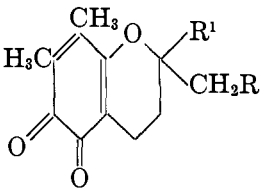
When the *p*-hydroxychroman IV (R = hydrogen, alkyl, and, for α-tocopherol, $C_{15}H_{31}$—) is oxidized by ferric chloride (62, 64, 72), gold chloride (78), silver acetate (64, 68), silver sulfate (64) or, under certain circumstances by silver nitrate, the product is the yellow *p*-quinone X. This quinone can be reduced to the hydroquinone LXV and this, in turn, can be converted either to the quinone X or the original chroman IV. When, however, the action of silver nitrate is prolonged (36, 62, 64, 78), or when nitric acid in ethanol is used as the oxidizing agent (49, 78, 114),



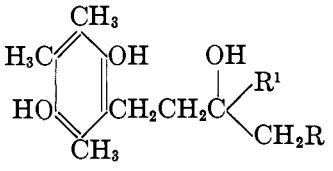
IV



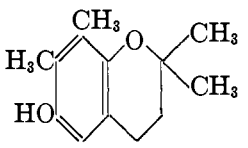
X



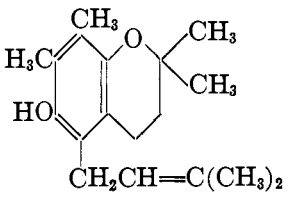
LXVI



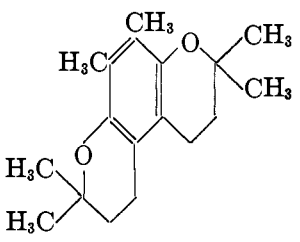
LXV



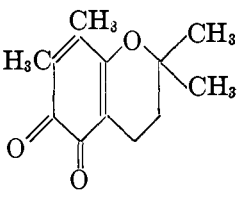
LXVII



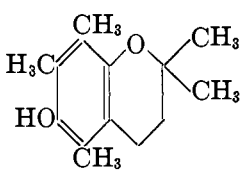
LXVIII



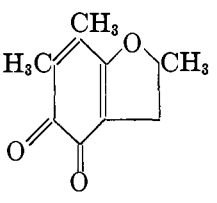
LXIX



LXX

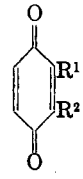


XVIII



LXXa

the reaction proceeds beyond the yellow *p*-quinone (X) stage and brilliant red solutions are obtained. From these solutions, red crystalline substances can be obtained. These substances are *o*-quinone derivatives (LXVI), formed as the result of a curious reaction in which the substituent in the 5-position of the original chroman IV has been eliminated (114). The red solids form phenazines and show other typical reactions of *o*-quinones. That it is the group in the 5-position that is eliminated in this reaction was shown by oxidation of the three substances LXVII, LXVIII, and LXIX, prepared by condensation of *o*-xylohydroquinone with isoprene. All three of these substances gave the same red crystalline oxidation product LXX; this is also the product when the chroman XVIII is oxidized in the same manner. The red oxidation product of 2,4,6,7-tetramethyl-5-hydroxycoumaran (XVI) has also been isolated (112). This substance is likewise an *o*-quinone (LXXa), but it is a much more sensitive substance than the chroman LXX. This reaction appears to be given by all chromans and coumarans which have an hydroxyl group para to the bridge oxygen, and it can be concluded that if the heterocyclic compound can be oxidized readily to a quinone of the type illustrated by LXXI (vacant positions may be occupied by alkyl or substituted alkyl groups), a red *o*-quinone of the type LXX can be obtained, with elimination of the group R^2, when the group R^1 is any of those listed in the first column below:

	GROUPS R^1 WHICH GIVE RED *o*-QUINONES	GROUPS R^1 WHICH DO NOT GIVE RED *o*-QUINONES
 LXXI	$-CH_2CH_2CHOHCH_3$	$-CH_2CH_2COOH$
	$-CH_2CHOHCH_3$	$-CH_2COCH_3$
	$-CH(C_2H_5)CHOHCH_3$	$-COCH_2COCH_3$
	$-CH_2CH_2C(OH)(CH_3)_2$	$-CH_2Cl$
	$-CH(CH_3)CHOHCH_3$	$-CH{=}C(COOH)COOC_2H_5$
	$-CH_2CH_2C(OH)(CH_3)R$	$-CH_3$
	$-CH_2CH{=}CH_2$	$-C_2H_5$

When the group R^1 is any of those listed in the second column, no red *o*-quinone can be obtained (114). It was at first supposed that these red compounds had double the molecular weight required by the simple formulas (62, 64, 78), and this is true when camphor is used as the solvent. However, the molecular weight in benzene is normal (74).

VI. METHODS OF ASSAY AND ANALYSIS FOR TOCOPHEROLS

Besides the biological assay, already discussed, and the careful measurement of absorption spectra (137), there exist a number of chemical methods for assay of materials containing tocopherols.

Potentiometric titration with gold trichloride (73, 83, 84, 85) has proved to be of great value. Since carotenoids also react with gold chloride, these must either be absent or determined independently, although the amount of carotenoids is so small in most cases that the error introduced is very slight. The method does not distinguish between α- and β-(or γ-) tocopherols, but gives the sum of all the tocopherols. Since the biological activities of the tocopherols differ, this chemical method cannot be used to replace the bioassay, although there usually is a good agreement between this method and the bioassay. A number of natural oils have been analyzed by this method; the results are shown in table 1 (83).

TABLE 1

Tocopherol content ($\alpha + \beta$) of various materials

MATERIALS	CONTENT
	per cent
Unsaponifiable fraction from wheat-germ oil*	13.4
Wheat-germ oil	0.52
Wheat germs	0.0259
Unsaponifiable fraction from corn-germ oil†	10.2
Corn germs	0.0164
Unsaponifiable fraction from lettuce‡	4.3
Lettuce (dry)	0.055
Unsaponifiable fraction from linseed oil	2.34
Linseed oil	0.023
Unsaponifiable fraction from olive oil	0.935
Olive oil	0.008
Unsaponifiable fraction from sesame oil	0.63
Sesame oil	0.005
Unsaponifiable fraction from coconut oil	0.55
Coconut oil	0.0027

* Sterols partly removed; carotenoids less than 0.1 per cent.

† Part of the sterols removed; no carotenoids present.

‡ Most of the sterols removed; corrected for 0.3 per cent carotenoids.

A second method of analysis involves the oxidation of the tocopherols with ferric chloride in ethanol; the ferrous ions so formed are converted into a red complex by addition of α,α'-dipyridyl, and the intensity of the color is measured (43). Besides α,α'-dipyridyl, α,α'-dipiperidyl or *o*-phenanthroline may be used, but the first of these gives the best straight-line relationship between color and concentration. Only 10 to 15 min. are required to determine 0.1–0.4 mg. samples using the Zeiss Pulfrich photometer with 1-cm. cell. In this method also, carotenoids interfere and a correction factor must be applied if these are present, or they must be removed by filtration of the benzene solution of the concentrate through a

layer of floridin. The method is more sensitive than the potentiometric titration for very small amounts of material, although the two methods check very well and both agree well with the figures obtained by biological assay.

A third method, also colorimetric, is based upon the red color which is formed when solutions of the tocopherols in ethanol are oxidized with nitric acid under specified conditions (49). This method has the advantage that carotenoids do not interfere, but the values obtained are frequently slightly high because of the red *o*-quinoid oxidation products

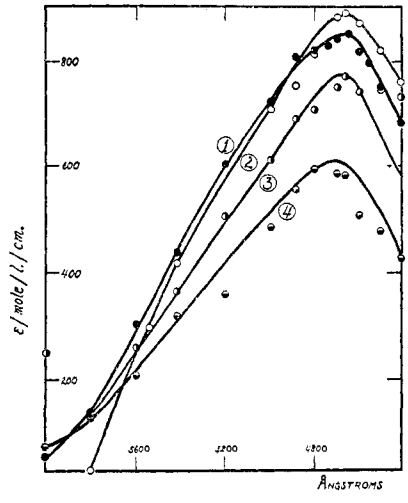


FIG. 3

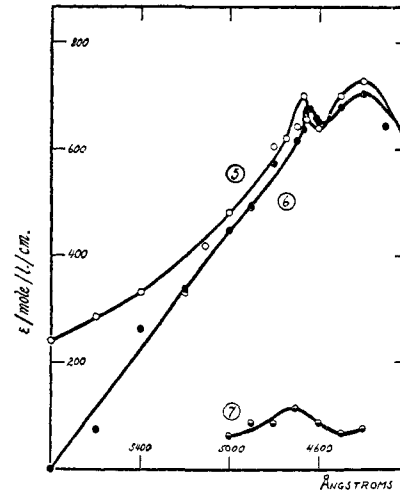


FIG. 4

FIG. 3. Absorption spectra. Curve 1, 2,2,5,7,8-pentamethyl-6-hydroxychroman; curve 2, α-tocopherol (Furter); curve 3, α-tocopherol, once distilled (impure); curve 4, γ-tocopherol (impure?).

FIG. 4. Absorption spectra. Curve 5, 2,3,4,6,7-pentamethyl-5-hydroxycoumaran; curve 6, 2,4,6,7-tetramethyl-5-hydroxycoumaran; curve 7, 2,4,6,7-tetramethylcoumaran.

often already present in the oils. The procedure is very convenient and rapid, and the limits of the method have recently been explored (134). The structure of the compound responsible for the red color has also been determined (114). In figures 3 and 4 are shown some curves obtained by this method; it is surprising how closely the curve for the simple chroman (curve 1) follows that of α-tocopherol (curve 2). It is apparent from the differences between curves 1 to 4 (chromans) and curves 5 to 6 (coumarans) that this method provides a rapid means of distinguishing between the two classes of ring structures, provided that the compounds are hydroxylated para to the oxygen atom of the heterocyclic ring (curve 7).

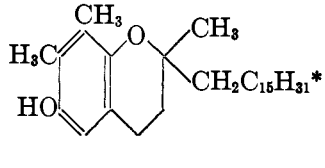
VII. SPECIFICITY OF VITAMIN E

Vitamin activity is usually very specific, and even slight changes in the structure of the vitamin molecule are sufficient to reduce the biological activity greatly or to remove it completely. Contrary to the usual experience in this field, vitamin E activity is shown by a great number of compounds widely different in nature, and only slightly related in structure to the tocopherols. Over one hundred and thirty individual compounds have been assayed biologically, and a complete list of these has recently been published (37). The list of substances examined includes chromans chromenes, coumarans, coumarins, coumarones, phenols, quinones, hydroquinones, and their esters and ethers. Of the one hundred and thirty or more compounds tested, over forty show vitamin E activity. It is true that, with very few exceptions, none of these synthetic substances compares with the tocopherols in activity and many of them have to be fed at levels (50 to 100 mg.) approaching the toxic in order to obtain positive results in the bioassays, yet these results do show quite definitely that vitamin E activity is quite widespread and is by no means confined to a single class of compounds.

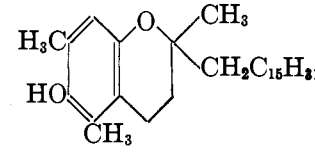
α-Tocopherol has been synthesized from trimethylhydroquinone and synthetic phytol (87); this product showed approximately the same activity as natural α-tocopherol. In all the tocopherol syntheses using phytyl derivatives, the product is racemic about the α-carbon atom of the heterocyclic ring; it is customary to refer to such a tocopherol as *dl*-α-tocopherol. This substance has been resolved *via* the bromocamphorsulfonates (79, 80), but the activity is unchanged. Hence symmetry or asymmetry about the α-carbon atom of the heterocyclic ring does not influence the activity of the tocopherol, nor does the optical state of any of the asymmetric carbon atoms in the phytol side chain exert any effect, since the product from synthetic phytol was as active as the natural tocopherol (87; especially 86). In fact, it appears that natural tocopherol is racemic about all three asymmetric centers (86). Synthetic α-tocopherol and its acetate are nontoxic, and very large doses (50 g. per kilogram of body weight to mice) have no ill effects (17). Nor do these substances have any carcinogenic properties (18).

The homologs of α-tocopherol in which the homology is due to changes in the benzene ring show a decreasing activity as methyl groups are removed, or as methyl groups are replaced by ethyl groups. Thus *o*(or *dl*-γ)-, *m*-, and *p*(or *dl*-β)-xylotocopherols (LXXII, LXXIII, LXXIV) are active when fed at 5- to 20-mg. levels (37, 54a, 76, 86); the ethyl homolog LXXV is active when fed at 10- to 16-mg. levels (81, 86). The tolutocopherols (LXXVI) (isolated as a mixture; position of the methyl group undetermined) are inactive at levels of 40 to 50 mg. (54a, 76); the

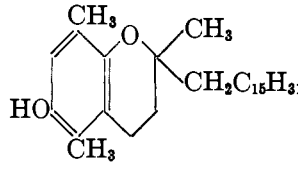
tocopherol with no methyl groups in the benzene ring is inactive in 50-mg. doses (54a), while 6-desoxy-*dl*-α-tocopherol (LXXVII) is inactive in 100-mg. doses (140). When the phytol side chain in α-tocopherol is



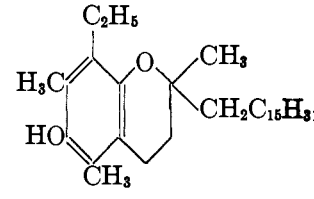
LXII



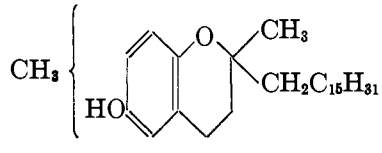
LXXIII



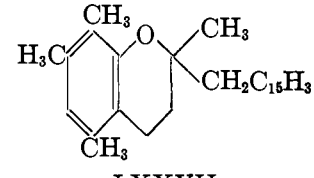
LXXIV



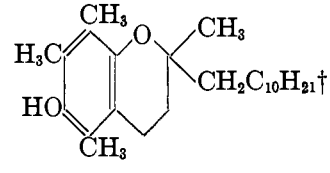
LXXV



LXXVI



LXXVII



LXXVIII

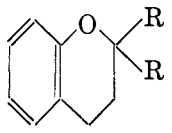
* $C_{15}H_{31}$— = $CH_3CHCH_2CH_2CH_2CHCH_2CH_2CH_2CHCH_2CH_2$—
CH_3 CH_3 CH_3

† $C_{10}H_{21}$— = $CH_3CHCH_2CH_2CH_2CHCH_2CH_2$—
CH_3 CH_3

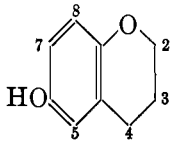
shortened by one isoprene unit, the compound (LXXVIII) is inactive at the 20-mg. level (82). From these results it follows that, starting with α-tocopherol (activity 3 mg.), any change in the groups in the benzene ring, or in the nature of the long aliphatic side chain, reduces very much

the activity of the compound. Further, the hydroxyl group para to the bridge oxygen is necessary for any activity, although it can be masked as any one of several *carboxylic* esters without reducing the activity appreciably (19). This hydroxyl group *cannot* be masked as the allophanate, or as an ether, without complete loss of activity.

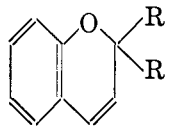
Turning to the simpler compounds, chromans represented by LXXIX



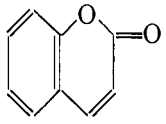
LXXIX



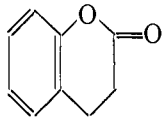
LXXX



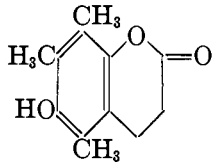
LXXXI



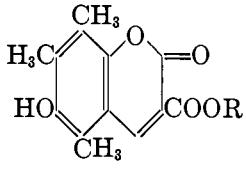
LXXXII



LXXXIII



LXXXIV

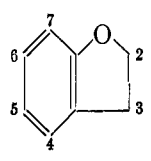


LXXXV

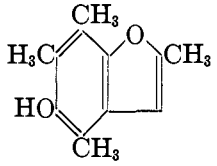
are active when the groups R are hydrogen (140), ethyl (37), or *n*-butyl (37), but inactive when the groups R are methyl or *n*-propyl (37). This alternation in activity with groups containing even and odd numbers of carbon atoms is very curious and it would be interesting to extend the series further. Of the other chromans with the 6-position vacant, 2,2,3-trimethyl- (37), 2-methyl-4-ethyl- (37), and 2,2,5,7-tetramethyl- (37) chromans are inactive, while 2,5,7,8-tetramethylchroman (140) is active. A number of chromans with the hydroxyl group in position 6 (LXXX) have been examined. These include 2,5,7,8-tetramethyl- (67, 82, 140), 2,2,5,7,8-pentamethyl- (37), 2,3,5,7,8-pentamethyl- (67), 2,5,7,8-tetramethyl-2-dodecyl- (60), and 2,5,7,8-tetramethyl-2-isohexyl- (37) -6-hydroxychromans, all inactive except the 2,2,5,7,8-pentamethyl compound, which in one test out of three showed a faint activity at the level of 100 mg. (37). The other 6-hydroxychromans studied are all closely related to the tocopherols, and the bioassays of these compounds are discussed in the preceding paragraph. Three chromenes (LXXXI) have been ex-

amined (37),—those in which the groups R are methyl, ethyl, and *n*-butyl. All are inactive. Of the six coumarin derivatives examined (37), coumarin (LXXXII) and dihydrocoumarin (LXXXIII) are inactive; indeed, the former is toxic at the level fed (100 mg.). The dihydrocoumarin LXXXIV, which has the same substituents in the benzene ring as α-tocopherol, is inactive. Likewise inactive are the substituted coumarins LXXXV when R is hydrogen or isoamyl, but, astonishingly, when R is ethyl the coumarin LXXXV shows a very high activity,—being effective in doses of 20 mg. This compound LXXXV, R = C_2H_5, is the most active compound known outside of the tocopherols themselves; the activity exceeds that of tolutocopherol (LXXVI) and is comparable to that of the xylotocopherols (LXXII, LXXIII, and LXXIV). This high activity of a compound quite different in structure from the tocopherols is very mysterious, and it becomes all the more inexplicable in view of the inactivity of the two closely related substances obtained when the ethyl group (R) in LXXXV is replaced by hydrogen or isoamyl.

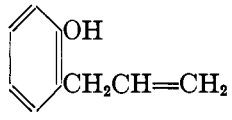
Several compounds with the heterocyclic ring consisting of five instead of six atoms have been examined. These are for the most part coumarans (LXXXVI), although one coumarone (LXXXVII) has been studied and it is inactive (37). Of the coumarans, the unsubstituted molecule (LXXXVI) is inactive (140). 2-Methylcoumaran showed great activity



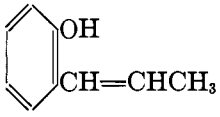
LXXXVI



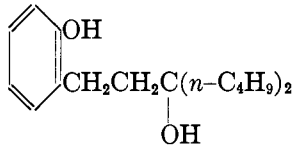
LXXXVII



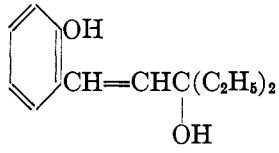
LXXXVIII



LXXXIX



XC



XCI

at a level of 50 mg. in one of four assays; the other three assays (25, 50, and 100 mg.) were negative (37). 2,2,7-Trimethylcoumaran was also active (37), as was 2,3,4,6,7-pentamethyl-5-hydroxycoumaran (37), while 3-methyl- (37), 2,4,6,7-tetramethyl- (37), 2,4,6,7-tetramethyl-5-hydroxy (37, 140), and 4,6,7-trimethyl-2-*n*-heptadecyl-5-hydroxy- (9) coumarans were all inactive.

Some phenols—mostly containing allylic groups—have been studied

(37), since these are possible intermediates in the syntheses of chromans and coumarans from allylic compounds. *o*-Allylphenol (LXXXVIII) is inactive in 25-mg. doses, but active when the dose is 50 mg. *o*-Propenylphenol (LXXXIX) is inactive. A di-*o*-hexenylphenol (mixture of isomers) is active, as is *p*-amino-*o*-allylphenol. All the other phenols tested are inactive; these included *o*-α-methylallyl-, *o*-hexenyl-, 2,3,5-trimethyl-6-allyl-, *p*-capryl-, *p*-*tert*-octyl-, *o*-allyl-*p*-carboxy-, *o*-allyl-*p*-carbethoxy-, and two more complicated phenols (XC and XCI).

By far the most extensively investigated compounds, however, are the quinones and hydroquinones, together with esters and ethers of the latter. These compounds were examined in some detail, because early in the work on the structure of vitamin E there was some evidence which indicated that possibly the vitamin might be a monoether of a methylated hydroquinone. With one exception, the *p*-quinones studied are all inactive. These include duroquinone (36, 65), tetraethylquinone (toxic) (37), thymoquinone (toxic) (37), trimethylethylquinone (37), 1,4-naphthoquinone (140), 1,2-naphthoquinone (toxic) (37), 2,3-dimethyl-1,4-naphthoquinone (37, 140), 2-methyl-1,4-naphthoquinone (37), 2-hydroxy-1,4-naphthoquinone (37), 2-methoxy-1,4-naphthoquinone (37), anthraquinone (37), and β-methylanthraquinone (37). The one exception to the inactive *p*-quinones is α-tocopherylquinone (X), obtained by mild oxidation of α-tocopherol, and the results of different workers who have tested this substance do not agree. It has been reported active once (35), but three other assays were negative (62, 140). The red *o*-quinone oxidation product of α-tocopherol (LXVI; $R^1 = CH_3$, $R = C_{15}H_{31}$) is inactive in doses of 3 and 6 mg., but active in doses of 12 mg. (37).

Of the hydroquinones examined, the unsubstituted hydroquinone is inactive (36), as is *m*-xylohydroquinone (37, 140), but *o*-xylohydroquinone is active (139), while *p*-xylohydroquinone is inactive in 50-mg. doses (37) but active in 100-mg. doses (140). Trimethylhydroquinone has been reported as inactive (100 mg.) (37) and also active at this same level (140). Durohydroquinone is active (65, 68), while trimethylethylhydroquinone and trimethyl-5-acetohydroquinone are inactive (138, 140). The only naphthohydroquinone tested is 2,3-dimethyl-5,6,7,8-tetrahydro-1,4-naphthohydroquinone; this compound exhibits good activity (139), as does its mono-*n*-dodecyl ether (65).

Table 2 shows the results obtained with a series of esters and ethers of trimethylhydroquinone.

Table 3 shows the results obtained with a series of esters and ethers of tetramethylhydroquinone (durohydroquinone).

Three simple phenol ethers,—phenylhexenyl ether, phenylcinnamyl ether, and *p*-carboxyphenylallyl ether,—have been examined (37); all are

inactive. A ketone, 4-(2,5-dimethoxy-3,4,6-trimethylphenyl)-2-butanone, is likewise inactive (37). Finally, phytol, alone or in combination with trimethylhydroquinone, shows no activity (37); hence, even though

TABLE 2

Ethers and esters of trimethylhydroquinone

DERIVATIVE	ACTIVITY	REFERENCE
Monobenzoate	+	(140)
Bis-β-iodopropionate	−	(140)
Mono-*n*-hexyl ether	+	(139)
Mono-*n*-dodecyl ether	+	(139)
Mono-*n*-dodecyl ether acetate	+	(139)
Monodihydrochaulmoogryl ether	+	(139)
Di-*n*-dodecyl ether	−	(139)

TABLE 3

Ethers and esters of tetramethylhydroquinone

DERIVATIVE	ACTIVITY	REFERENCE
Mono-*n*-butyl ether	+	(139)
Di-*n*-butyl ether	+	(139)
Mono-*n*-hexyl ether	+	(139)
Di-*n*-hexyl ether	+	(139)
Mono-*n*-heptyl ether	−	(139)
Di-*n*-heptyl ether	+	(139)
Mono-*n*-octyl ether	+	(139)
Di-*n*-octyl ether	+	(139)
Monocetyl ether	+ (twice); − (once)	(36, 46)
Monododecyl ether propionate	−	(139)
Monododecyl ether palmitate	+	(139)
Monododecyl ether	+	(36, 46, 139)
Didodecyl ether	−	(139)
Monohydrophytyl ether	+	(139)
Mono-*n*-octadecyl ether	−	(36, 46)
Mono-*n*-nonadecyl-2 ether	+	(36, 46)
Mono-2-methyloctadecyl ether	−	(36, 46)
Mono-*n*-nonadecyl ether	+	(140)
Di-*n*-nonadecyl ether	−	(140)
Mono-3-methyl-5-(1′, 1′, 3′-trimethyl-2′-cyclohexyl)pentyl-1 ether	−	(140)
Monodihydrochaulmoogryl ether	+	(139)
Monobenzyl ether	+	(139)
Dibenzyl ether	+	(139)

the synthesis of α-tocopherol from these compounds in the laboratory is surprisingly easy, the synthesis does not occur *in vivo*, at least when the substances are fed.

From the results of the bioassays presented here, it is clear that many compounds exhibit some vitamin E activity, and there are one or two fairly simple compounds which show considerable activity. These results, until recently at variance with all other results in the vitamin field, appear now to be paralleled in the field of the K vitamins, where a number of substances aside from the vitamins themselves possess great potency. Notable among these are certain 1,4-naphthoquinones, especially 2-methyl-1,4-naphthoquinone. None of these naphthoquinones possesses any vitamin E activity, but the methylnaphthoquinone has about as much antihemorrhagic activity as vitamin K_1 itself.

There is no adequate theory at present to account for vitamin E activity in terms of organic structure. One theory has been advanced (37, 62, 67) independently from two laboratories, but there remain objections to the theory which will have to be overcome before it can be accepted (37, 62, 67, 71).

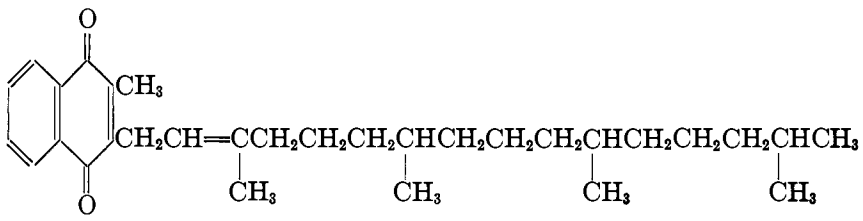
VIII. USES AND IMPORTANCE OF VITAMIN E

Vitamin E appears to be a most promising substance to be used in the treatment of habitual abortion in women, and for similar use in the veterinary field. Some rather startling successes have been reported when the vitamin has been used in these cases. There is good evidence also that the young of both sexes need vitamin E for normal growth, and that there is some connection between the amount of vitamin E and the functioning of the thyroid gland as well as that of the hypophysis. That muscular dystrophy can result from a lack of vitamin E appears to be well established (106). The writer is not competent to discuss this field, and since reviews covering the use of vitamin E in medicine have recently been published (6, 23, 50, 56, 59, 61, 69, 70, 93, 94, 135; especially 50, 61) only the most general statements have been made here. Most of the studies so far have been carried out using wheat-germ oil concentrates, but now that the synthetic vitamin is available in pure form, a standard preparation of known potency is available and it is to be hoped that the clinical work will proceed rapidly so that the usefulness, as well as the limits, of vitamin E therapy may soon be known.

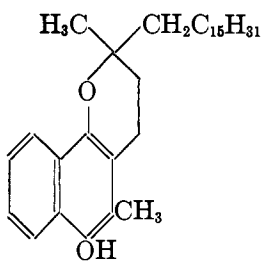
IX. VITAMIN K

Before closing this review, a word about vitamin K may not be out of place, for this vitamin is unique in that it is the first of all the vitamins whose chemistry has been aided and simplified by the chemical knowledge gained in the study of any other vitamin. Until the recent work on the structure and synthesis of vitamins E and K, no vitamin had ever been found which was in any way related chemically to any other vitamin,—

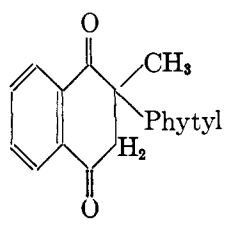
each vitamin belonged to an entirely different class of chemical compounds; indeed, vitamins A and D might almost be said to have represented, at the time they were isolated, new classes of organic compounds. Vitamins E and K, however, are closely related in their chemistry, and the knowledge and experience gained in studying one of these vitamins has been of great value in the study of the other. There are two K vitamins, K_1 (XCII) and K_2, both of which show a powerful antihemorrhagic activity, although K_1 surpasses K_2 in this respect (16a).



XCII
Vitamin K_1



XCIV



XCIII

Vitamin K_1 has been synthesized in two laboratories (12, 47, 48). However, the methods used for the synthesis of vitamin E had to be modified considerably in order to avoid ring closures which would lead to compounds of the tocopherol type. The yield of vitamin K_1 from phytol and 2-methyl-naphthohydroquinone was good (132); very little of the naphthotocopherol (XCIV) was produced, but relatively large amounts of a by-product, probably XCIII, resulted (132). Unfortunately it has not been found possible to convert compounds of the tocopherol types into compounds of the vitamin K types, although the reverse transformation has been realized in the transformation of vitamin K_1 (XCII) into the naphthotocopherol XCIV (48a). Vitamins E and K also have in common the fact that the specificity is not limited to the vitamins themselves,—indeed, 2-methyl-1,4-naphthoquinone appears to be much more active than vitamin K (16a). Curiously enough, no compounds have been found so far which

show both kinds of activity: if a substance exhibits vitamin E activity, it does not show any vitamin K activity, and *vice versa* (but in this connection, see references 16a, 54a, 91a).

REFERENCES[2]

(1) BACHARACH: Biochem. J. **31**, 2287 (1937).
(2) BACHARACH: Biochem. J. **32**, 1298 (1938).
(3) BACHARACH: Nature **142**, 35 (1938).
(4) BACHARACH AND ALLCHORNE: J. Soc. Chem. Ind. **57**, 600 (1938).
(5) BACHARACH AND VAN ROSSUM: J. Soc. Chem. Ind. **57**, 600 (1938).
(6) BERGEL: Chem. Products and Chem. News **2**, 39 (1939).
(7) BERGEL, COPPING, JACOB, TODD, AND WORK: J. Chem. Soc. **1938**, 1382.
(8) BERGEL, COPPING, JACOB, TODD, AND WORK: Nature **142**, 36 (1938).
(9) BERGEL, JACOB, TODD, AND WORK: J. Chem. Soc. **1938**, 1375.
(10) BERGEL, TODD, AND WORK: Chemistry & Industry **1937**, 1054.
(11) BERGEL, TODD, AND WORK: J. Chem. Soc. **1938**, 253.
(12) BINKLEY, CHENEY, HOLCOMB, MCKEE, THAYER, MACCORQUODALE, AND DOISY: J. Am. Chem. Soc. **61**, 2558 (1939).
(13) BOWDEN AND MOORE: Nature **131**, 512 (1933).
(14) BOWDEN AND MOORE: Nature **132**, 204 (1933).
(15) BRIDGE, CROCKER, CUBIN, AND ROBERTSON: J. Chem. Soc. **1937**, 1530.
(16) CLAISEN *et al.*: (a) Ann. **401**, 26 (1913); (b) Ann. **442**, 210 (1935); (c) Ber. **58**, 279 (1925); (d) German patent 394797 (1924).
(16a) DAM, GLAVIND, AND KARRER: Helv. Chim. Acta **23**, 224 (1940).
(17) DEMOLE: Z. Vitaminforsch. **8**, 338 (1939).
(18) DEMOLE: Z. Vitaminforsch. **8**, 341 (1939).
(19) DEMOLE, ISLER, RINGIER, SALOMON, AND KARRER: Helv. Chim. Acta. **22**, 65 (1939).
(20) DRUMMOND AND HOOVER: Biochem. J. **31**, 1852 (1937).
(21) DRUMMOND, SINGER, AND MACWALTER: Biochem. J. **29**, 456, 2510 (1935).
(22) EVANS: J. Nutrition **1**, 23 (1928).
(23) EVANS: J. Am. Med. Assoc. **99**, 469 (1932).
(24) EVANS: J. Am. Med. Assoc. **99**, 470 (1932).
(25) EVANS AND BISHOP: Am. J. Physiol. **63**, 396 (1922).
(26) EVANS AND BISHOP: J. Am. Med. Assoc. **81**, 889 (1922).
(27) EVANS AND BISHOP: Science **56**, 650 (1922).
(28) EVANS AND BISHOP: J. Metabolic Research **1922**, I, 319, 335; **1923**, III, 202, 233.
(29) EVANS AND BISHOP: Am. J. Physiol. **63**, 396 (1923).

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[2] References 6, 23, 50, 56, 59, 61, 69, 70, 93, and 94 are review articles; the whole issue of *Angewandte Chemie* **52**, 413 32 (July 17, 1939) was devoted to the review articles of John (59) and Grandel (50), and to a verbatim report of the Vitamin E Conference held in London on April 22, 1939 (135). The papers given at this Conference, together with the discussions that followed, have been printed in book form (136). Those interested in the physiological aspects of vitamin E will find the position of such matters as of April, 1939, well defined in this book. For a more detailed review of this book, see reference 108. Of the above references to review articles, those of fairly recent date, which deal particularly with the physiological aspects of vitamin E, are 50, 61, and 69.

(30) EVANS AND BISHOP: Anat. Record **27**, 203 (1924).
(31) EVANS AND BURR: Proc. Natl. Acad. Sci. (U. S.) **11**, 334 (1925).
(32) EVANS AND BURR: Memoirs of the University of California, No. 8 (1927).
(33) EVANS AND BURR: J. Biol. Chem. **76**, 273 (1928).
(34) EVANS, EMERSON, AND EMERSON: J. Biol. Chem. **113**, 319 (1936).
(35) EVANS, EMERSON, AND EMERSON: J. Biol. Chem. **113**, 322 (1936); **131**, 409 (1939).
(36) EVANS, EMERSON, AND EMERSON: Science **88**, 38 (1938).
(37) EVANS, EMERSON, EMERSON, SMITH, UNGNADE, PRICHARD, AUSTIN, HOEHN, OPIE, AND WAWZONEK: J. Org. Chem. **4**, 376 (1939).
(38) EMERSON AND SMITH: J. Am. Chem. Soc. **62**, 1869 (1940).
(39) EMERSON: J. Am. Chem. Soc. **60**, 1741 (1938).
(40) EMERSON, EMERSON, AND EVANS: Science **89**, 193 (1939).
(41) EMERSON, EMERSON, MOHAMMAD, AND EVANS: J. Biol. Chem. **122**, 99 (1937).
(42) EMERSON AND SMITH: J. Am. Chem. Soc. **62**, 141 (1940).
(43) EMMERIE AND ENGEL: Rec. trav. chim. **57**, 1351 (1938); **58**, 283 (1939).
(44) FERNHOLZ: J. Am. Chem. Soc. **59**, 1154 (1937).
(45) FERNHOLZ: J. Am. Chem. Soc. **60**, 700 (1938).
(46) FERNHOLZ AND FINKELSTEIN: J. Am. Chem. Soc. **60**, 2402 (1938).
(47) FIESER: J. Am. Chem. Soc. **61**, 2559, 2561 (1939).
(48) FIESER, CAMPBELL, FRY, AND GATES: J. Am. Chem. Soc. **61**, 2559 (1939).
(48a) FIESER, TISHLER, AND SAMPSON: J. Am. Chem. Soc. **62**, 996 (1940).
(49) FURTER AND MEYER: Helv. Chim. Acta **22**, 240 (1939).
(50) GRANDEL: Angew. Chem. **52**, 420 (1939).
(51) GRIJNS AND DINGEMANSE: Proc. Acad. Sci. Amsterdam **36**, 342 (1933).
(52) HURD AND HOFFMAN: J. Org. Chem. **5**, 212 (1940).
(53) ISLER: Helv. Chim. Acta **21**, 1756 (1938).
(54) JACOB, STEIGER, TODD, AND WORK: J. Chem. Soc. **1939**, 542.
(54a) JACOB, SUTCLIFFE, AND TODD: J. Chem. Soc., **1940**, 327.
(55) JOHN: Z. physiol. Chem. **250**, 11 (1937).
(56) JOHN: Naturwissenschaften **26**, 449 (1938).
(57) JOHN: Z. physiol. Chem. **252**, 201 (1938).
(58) JOHN: Z. physiol. Chem. **252**, 222 (1938).
(59) JOHN: Angew. Chem. **52**, 413 (1939).
(60) JOHN: Angew. Chem. **52**, 418 (1939).
(61) JOHN: Ergeb. Physiol., biol. Chem., exptl. Pharmakol. **42**, 1 (1939).
(62) JOHN, DIETZEL, AND EMTE: Z. physiol. Chem. **257**, 173 (1939).
(63) JOHN, DIETZEL, AND GÜNTHER: Z. physiol. Chem. **252**, 208 (1938).
(64) JOHN AND EMTE: Z. physiol. Chem. **261**, 24 (1939).
(65) JOHN AND GÜNTHER: Z. physiol. Chem. **254**, 51 (1938).
(66) JOHN AND GÜNTHER: Ber. **72**, 1649 (1939).
(67) JOHN, GÜNTHER, AND SCHMEIL: Ber. **71**, 2637 (1938).
(68) JOHN AND SCHMEIL: Ber. **72**, 1653 (1939).
(69) KARRER: Schweiz. med. Wochschr. **68**, 954 (1938).
(70) KARRER: Helv. Chim. Acta **22**, 334 (1939).
(71) KARRER: Helv. Chim. Acta **22**, 349 (1939).
(72) KARRER AND ESCHER: Helv. Chim. Acta **22**, 264 (1939).
(73) KARRER, ESCHER, FRITZSCHE, KELLER, RINGIER, AND SALOMON: Helv. Chim. Acta **21**, 939 (1938).
(74) KARRER, ESCHER, AND RENTSCHLER: Helv. Chim. Acta **22**, 1287 (1939).
(75) KARRER AND FRITZSCHE: Helv. Chim. Acta **21**, 1234 (1938).
(76) KARRER AND FRITZSCHE: Helv. Chim. Acta **22**, 260 (1939).

(77) KARRER AND FRITZSCHE: Helv. Chim. Acta **22**, 657 (1939).
(78) KARRER, FRITZSCHE, AND ESCHER: Helv. Chim. Acta **22**, 661 (1939).
(79) KARRER, FRITZSCHE, RINGIER, AND SALOMON: Helv. Chim. Acta **21**, 520 (1938).
(80) KARRER, FRITZSCHE, RINGIER, AND SALOMON: Helv. Chim. Acta **21**, 820 (1938).
(81) KARRER AND HOFFMAN: Helv. Chim. Acta **22**, 654 (1939).
(82) KARRER AND JENSEN: Helv. Chim. Acta **21**, 1622 (1938).
(83) KARRER AND KELLER: Helv. Chim. Acta **21**, 1161 (1938).
(84) KARRER AND KELLER: Helv. Chim. Acta **22**, 253 (1939).
(85) KARRER AND KELLER: Helv. Chim. Acta **22**, 617 (1939).
(86) KARRER, KOENIG, RINGIER, AND SALOMON: Helv. Chim. Acta **22**, 1139 (1939).
(87) KARRER AND RINGIER: Helv. Chim. Acta **22**, 610 (1939).
(88) KARRER, SALOMON, AND FRITZSCHE: Helv. Chim. Acta **20**, 1422 (1937).
(89) KARRER, SALOMON, AND FRITZSCHE: Helv. Chim. Acta **21**, 309 (1938).
(90) KARRER, SALOMON, AND FRITZSCHE: Angew. Chem. **51**, 309 (1938).
(91) KARRER, SALOMON, RINGIER, AND FRITZSCHE: Helv. Chim. Acta **21**, 514 (1938).
(91a) KUHN, WALLENFELS, WEYGAND, MOLL, AND HEPDING: Naturwissenschaften **57**, 518 (1939).
(92) MCARTHUR AND WATSON: Science **86**, 35 (1937).
(93) MATTILL: J. Am. Med. Assoc. **110**, 1830 (1938).
(94) MATTILL: *The Vitamins*, A Symposium held under the auspices of the American Medical Association, Chapter 30. Published in book form by the Association (1939).
(95) MATTILL, CARMEN, AND CLAYTON: J. Biol. Chem. **61**, 729 (1924).
(96) MATTILL AND CONKLIN: J. Biol. Chem. **44**, 137 (1920).
(97) MATTILL AND STONE: J. Biol. Chem. **55**, 443 (1923).
(98) MARTIN, MOORE, AND SCHMIDT: Nature **134**, 214 (1934).
(99) MOSS, CUTHBERTSON, DANIELLI, AND DRUMMOND: Chemistry & Industry **1938**, 275.
(100) OLCOTT: J. Biol. Chem. **107**, 471 (1934).
(101) OLCOTT: J. Biol. Chem. **110**, 695 (1935).
(102) OLCOTT AND EMERSON: J. Am. Chem. Soc. **59**, 1008 (1937).
(103) OLCOTT AND MATTILL: J. Biol. Chem. **104**, 423 (1934).
(104) OLCOTT AND MATTILL: J. Nutrition **14**, 169 (1937).
(105) PALMER: Ind. Eng. Chem., Anal. Ed. **9**, 427 (1937).
(106) SHIMOTORI, EMERSON, AND EVANS: Science **90**, 89 (1939).
(107) SHRINER AND SHARP: J. Org. Chem. **4**, 575 (1939).
(108) SMITH: J. Am. Chem. Soc. **62**, 458 (1940).
(109) SMITH AND DENYES: J. Am. Chem. Soc. **58**, 304 (1936).
(110) SMITH AND DOBROVOLNY: J. Am. Chem. Soc. **48**, 1693 (1926).
(111) SMITH AND GUSS: Unpublished work.
(112) SMITH, HOEHN, AND WHITNEY: J. Am. Chem. Soc. **62**, 1863 (1940).
(113) SMITH, HOEHN, AND UNGNADE: J. Org. Chem. **4**, 351 (1939).
(114) SMITH, IRWIN, AND UNGNADE: J. Am. Chem. Soc. **61**, 2424 (1939); Science **90**, 334 (1939).
(115) SMITH AND MACMULLEN: J. Am. Chem. Soc. **58**, 629 (1936).
(116) SMITH, OPIE, WAWZONEK, AND PRICHARD: J. Org. Chem. **4**, 318 (1939).
(117) SMITH AND PRICHARD: J. Org. Chem. **4**, 342 (1939).
(118) SMITH AND RUOFF: J. Am. Chem. Soc. **62**, 145 (1940).
(119) SMITH AND UNGNADE: J. Org. Chem. **4**, 298 (1939).
(120) SMITH, UNGNADE, AUSTIN, PRICHARD, AND OPIE: J. Org. Chem. **4**, 334 (1939).
(121) SMITH, UNGNADE, HOEHN, AND WAWZONEK: J. Org. Chem. **4**, 305 (1939).

(122) SMITH, UNGNADE, HOEHN, AND WAWZONEK: J. Org. Chem. **4**, 311 (1939).
(123) SMITH, UNGNADE, AND IRWIN: J. Am. Chem. Soc. **62**, 142 (1940).
(124) SMITH, UNGNADE, LAUER, AND LEEKLEY: J. Am. Chem. Soc. **61**, 3079 (1939).
(125) SMITH, UNGNADE, OPIE, PRICHARD, CARLIN, AND KAISER: J. Org. Chem. **4**, 323 (1939).
(126) SMITH, UNGNADE, AND PRICHARD: Science **88**, 37 (1938).
(127) SMITH, UNGNADE, AND PRICHARD: J. Org. Chem. **4**, 358 (1939).
(128) SMITH, UNGNADE, STEVENS, AND CHRISTMAN: J. Am. Chem. Soc. **61**, 2615 (1939).
(129) SURE: Science **59**, 19 (1924).
(130) SURE: J. Biol. Chem. **58**, 693 (1924).
(131) SURE: J. Biol. Chem. **62**, 371 (1925).
(132) TISHLER, FIESER, AND WENDLER: J. Am. Chem. Soc. **62**, 1982 (1940).
(133) TODD, BERGEL, AND WORK: Biochem. J. **31**, 2257 (1937).
(134) UNGNADE AND SMITH: J. Org. Chem. **4**, 397 (1939).
(135) Vitamin E Conference in London, April 22, 1939. Reported in full in Angew. Chem. **52**, 427 (1939).
(136) Vitamin E, A Symposium held under the Auspices of the Food Group (Nutrition Panel) of the Society of Chemical Industry on Saturday, April 22, 1939. Published by the Society, Clifton House, Euston Road, London, N.W. 1. 88 pp.; 13.5 x 21 cm. Price: 5 shillings.
(137) WEBB, SMITH, BASTEDO, UNGNADE, PRICHARD, HOEHN, WAWZONEK, OPIE, AND AUSTIN: J. Org. Chem. **4**, 389 (1939).
(138) v. WERDER AND JUNG: Ber. **71**, 2650 (1938).
(139) v. WERDER AND MOLL: Z. physiol. Chem. **254**, 39 (1938).
(140) v. WERDER, MOLL, AND JUNG: Z. physiol. Chem. **257**, 129 (1939).

Notes added to proof, September 24, 1940

1. Karrer and Geiger (Helv. Chim. Acta **23**, 455 (1940)) have published a very careful study of the yellow α-tocopherylquinone (X). The quinone was prepared by oxidation of tocopherol using three different reagents: (*a*) gold trichloride, (*b*) ferric chloride, and (*c*) silver nitrate. The quinone prepared by method a contained no reducing substances. It was a golden-yellow oil which gave a very good absorption spectrum curve, and the authors believe it to be "the only homogeneous preparation of this quinone described in the literature." The quinone prepared by method b contained considerable α-tocopherol or other reducible substance. After 6 hr. contact with the reagent, 11.6 per cent of α-tocopherol was still present, and after 36 hr., 7 per cent. (Analytical values were obtained by potentiometric titration, as well as by the method of Emmerie and Engel). This product was again subjected to the action of ferric chloride, and the second treatment led to a product showing no reducing properties. It is interesting that the reducing substance cannot be completely removed by one treatment with ferric chloride, no matter how prolonged. The product obtained by method c contained α-tocopherol, the yellow quinone X, and the red *o*-quinone. The products obtained by methods a and b were

tested biologically at levels of 10 and 25 mg. Absolutely no activity was shown; hence it is unlikely that α-tocopherol owes its activity directly to any oxidation–reduction system in the body.

2. Karrer, Jaeger, and Keller (Helv. Chim. Acta **23,** 464 (1940)) have determined the tocopherol content of certain animal organs. The liver (horse, cattle) shows the highest content of tocopherol, but the vitamin is present also in the muscle, heart, and kidney.

3. Karrer and Yap (Helv. Chim. Acta **23,** 581 (1940)) have prepared the chroman IV, in which R = isoamyl,—that is, the substance which contains two "isoprene units" less than α-tocopherol in the side chain. This substance shows no bio-activity at a level of 40 mg.

4. Tishler, Fieser, and Wandler (reference 132, page 1984, footnote 6a) report that the naphthotocopherol XCIV shows vitamin E activity at the 25-mg. level and that this compound also possesses moderate vitamin K activity (between 300 and 600 γ). This is the first instance of any compound combining the biological actions of two vitamins.