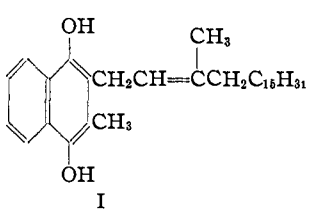
[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MERCK AND CO., INC., AND THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

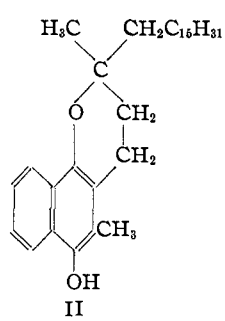
Nature of the By-product in the Synthesis of Vitamin K_1

BY MAX TISHLER, LOUIS F. FIESER AND NORMAN L. WENDLER

In previous work on the synthesis of vitamin K_1,[1] it was observed that the condensation of 2-methyl-1,4-naphthohydroquinone with phytol using oxalic acid in dioxane at 75° affords the desired 2-methyl-3-phytyl-1,4-naphthohydroquinone (I) in only moderate yield and that a considerable amount of a liquid by-product is formed. A preliminary characterization[2] indicated that this is a rather inert substance, probably isomeric with I, and in view of the established formation of tocopherols in analogous condensations, the substance was provisionally regarded[1] as a naphthotocopherol resulting from the cyclization of I and having the chroman structure II, or the less likely coumaran structure. This idea of the nature of



I

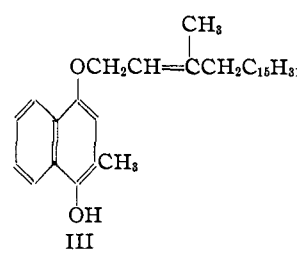


II

the side reaction seemed to be supported by the observation that the yield of I fell off when the reaction time was extended much beyond an optimum period of about thirty-six hours.

Further investigation has shown this hypothesis to be untenable. When the pure hydroquinone I was heated with oxalic acid in dioxane under the conditions of the synthesis over 90% was recovered unchanged, showing that the by-product can hardly arise from the cyclization of this substance in the course of the reaction. The observed decrease in yield on prolonging the reaction period may have been due to air oxidation of the product and to the failure of the quinone formed to be reduced completely in the extraction step employing ether and aqueous hydrosulfite solution. The reaction is extremely slow in this two-phase system and it has been found expedient to introduce a more efficient reduction step consisting in treatment with hydrosulfite solution in alcoholic suspension.

The by-product can be isolated easily in a pure condition by extraction of a petroleum ether solution of the crude material with Claisen's alkali and hydrosulfite to remove traces of the hydroquinone I, followed by distillation of the neutral residue in high vacuum. After collecting a fore-run of phytadiene amounting to about 17%, the by-product is obtained as a pale yellow oil in 20–22% yield. Further doubt was cast on a naphthotocopherol structure such as II by the observation that the substance differs from the known tocopherols in not reducing alcoholic silver nitrate and in giving a negative Furter–Meyer test.[3] An alternate formulation considered is that of the monophytyl ether III.



III

The lack of solubility of the by-product in Claisen's alkali might be taken as an argument against both the ether and the naphthotocopherol formulas were it not for a contradictory observation concerning a similar by-product, m. p. 73°, resulting from the condensation of methylnaphthohydroquinone and 2,3-dimethylbutadiene.[4] In contrast to the behavior of the K_1 by-product, which has a much higher molecular weight, this colorless, crystalline compound dissolves in alcoholic alkali with a yellow color and can be recovered unchanged. There is evidence against the presence of a phenolic hydroxyl group, however, in the observation that the crystalline substance was recovered unchanged after being refluxed for four hours with acetic anhydride in dimethylaniline. An ether of the allylic type should rearrange under these conditions and either a mono ether or a tocopherol should undergo acetylation. The vitamin K_1 by-product likewise resisted various attempted acylations.

(1) Fieser, THIS JOURNAL, **61**, 3467 (1939).

(2) Fieser, Campbell, Fry and Gates, *ibid.*, **61**, 2559 (1939).

(3) Furter and Meyer, *Helv. Chim. Acta*, **22**, 240 (1939).

(4) Fieser, Campbell, Fry and Gates, THIS JOURNAL, **61**, 3216 (1939).

The two by-products resemble each other closely in absorption spectra,[5] as shown in Fig. 1

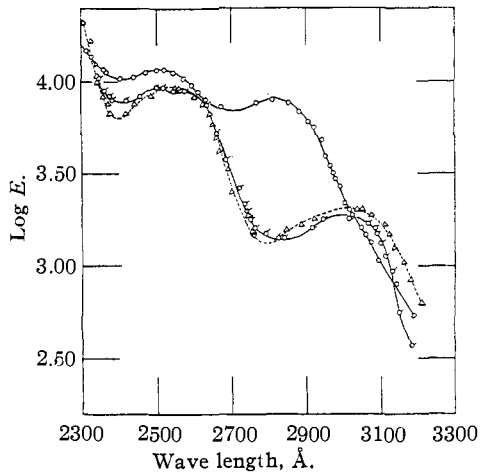


Fig. 1.—Curve shown by ♂: by-product $C_{31}H_{48}O_2$ of the vitamin K_1 synthesis. Curve shown by △: by-product $C_{19}H_{20}O_2$, m. p. 73°, from the condensation of 2-methyl-1,4-naphthohydroquinone with 2,3-dimethyl-butadiene. Curve shown by △ (determination by R. N. Jones): 1-oxy-4-oxo-2,3-dimethyl-1-phenyldihydronaphthalene (VIII).

and Table I, and must therefore be of the same structural type. Although the absorption curves

TABLE I

ABSORPTION MAXIMA IN mμ, WITH LOG E_{Molar} VALUES IN PARENTHESES

Vitamin K_1 by-product $C_{31}H_{48}O_2$ (VII)	253 (3.97), 300 (3.27)
By-product $C_{19}H_{20}O_2$, m. p. 73°	253 (3.98), 298 (3.31)
2,3-Dimethyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone (X)	253 (3.96), about 300 (3.2)
Methylnaphthohydroquinone monoethyl ether	243 (4.26), about 320 (3.7)
The naphthotocopherol $C_{31}H_{48}O_2$	246 (4.54), about 320 (3.6)
Compound VIII	251 (4.07), 281 (3.91)

do not appear consistent with a naphthalenoid formulation, it seemed desirable to provide adequate data for comparison. 2-Methyl-1,4-naphthohydroquinone was converted by treatment with ethanol and hydrogen chloride into a monoethyl ether probably analogous to III. It is evident from Fig. 2 that the spectrum of this substance diverges considerably from that characteristic of the by-products. The sharp band at the left is displaced to a region of shorter wave length

(5) We are indebted to Dr. T. J. Webb and W. A. Bastedo, Jr., for the spectrographic determinations reported except that indicated in the legend of Fig. 1, kindly made by Dr. R. N. Jones. The solvent used throughout was 95% alcohol.

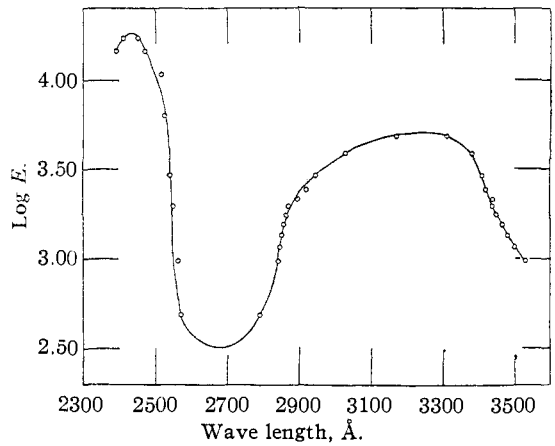


Fig. 2.—2-Methyl-1,4-naphthohydroquinone monoethyl ether.

(Table I) and the second band is shifted in the opposite direction and is much broader and more intense.

To obtain conclusive evidence regarding the naphthotocopherol formulation, we cyclized the hydroquinone I by refluxing vitamin K_1 in acetic acid with stannous chloride and hydrochloric acid. This procedure gave a nearly colorless liquid sensitive to air oxidation, reducing silver nitrate and giving the Furter–Meyer test. The substance has the expected composition, gives a crystalline *p*-nitrobenzoate, and appears to be the true naphthotocopherol. The absorption spectrum (Fig. 3) corresponds closely in the nature and position of the two bands with the analogous methylnaphthohydroquinone monoethyl ether and does not resemble that of the isomeric by-product.

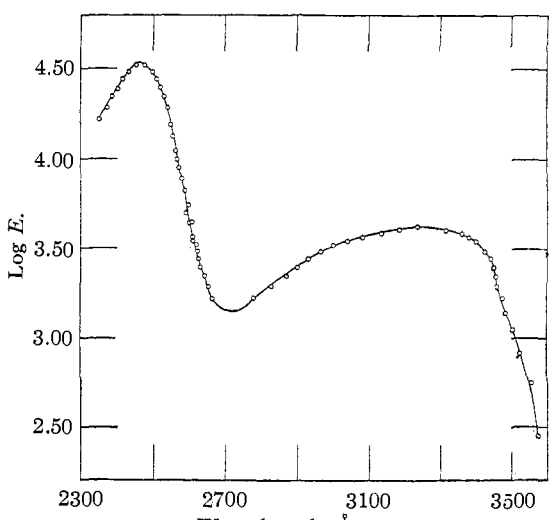
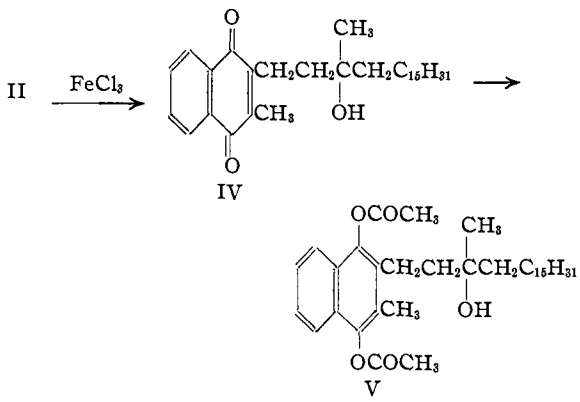


Fig. 3.—The naphthotocopherol $C_{31}H_{48}O_2$ (Formula II, or the corresponding coumaran).

The naphthotocopherol, which in analogy with known cases may be formulated provisionally as the chroman II, was oxidized to a hydroxyquinone



most satisfactorily with ferric chloride in a methanol–water–ether mixture. The crude product, a red oil, was effectively purified by reduction, separation of the hydroquinone from petroleum ether as a white solid, and oxidation with silver oxide, as in the vitamin K_1 synthesis. This afforded the quinone IV as a bright yellow liquid. On reductive acetylation the substance formed a crystalline diacetate of low melting point, the alcoholic hydroxyl group in the side chain being unattacked. This result, which would appear consistent with the formulation of the compound as a tertiary carbinol, is of special interest in connection with the reported acylation of similar carbinol groups in the α-tocopherol and related series.[6,6a,6b]

(6) John, Dietzel and Emte, *Z. physiol. Chem.*, **257**, 173 (1939); Smith, Ungnade and Irwin, THIS JOURNAL, **62**, 142 (1940).

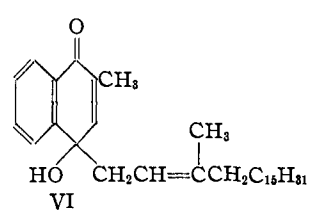
(6a) In assays kindly conducted by Dr. H. M. Evans, the naphthotocopherol showed a high degree of vitamin E activity at a dosage of 25 mg. The previously reported moderate vitamin K activity of the compound [Ref. 9] has been confirmed in assays on several samples, the effective dose in the eighteen-hour test being between 300 and 600 γ. This apparently is the first instance of a compound combining the biological actions of two vitamins. Several samples of the quinone IV when assayed as above showed no antihemorrhagic activity at 500 or 1000 γ. The samples were purified through the solid hydroquinone, in one case by extraction with Claisen's alkali before precipitation with petroleum ether. The crystalline diacetate V was likewise inactive at 1000 γ.

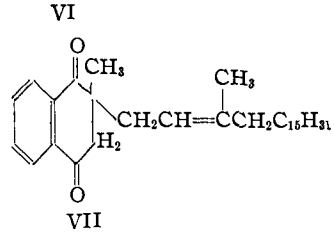
Jacob, Sutcliffe and Todd, *J. Chem. Soc.*, 327 (1940), made a preliminary attempt to prepare the naphthotocopherol by condensing methylnaphthohydroquinone with phytol in the presence of zinc chloride and obtained a mixture having no vitamin E activity at 250 mg. By an apparently similar method, Fernholz, MacPhillamy and Ansbacher, THIS JOURNAL, **62**, 1619 (1940), obtained a substance regarded as the pure naphthotocopherol and stated to be inactive at 1000 γ. This result, as well as that on the "dark orange oil" obtained as an oxidation product (indicating activity at 1000 γ), presumably was obtained by an assay procedure (six-hour) differing significantly from ours.

(6b) By refluxing the diacetate V with acetic anhydride and sodium acetate this has been converted into the triacetate, obtained as small needles from absolute alcohol; m. p. 65° (Calcd. for $C_{37}H_{56}O_6$: C, 74.50; H, 9.45. Found: C, 74.71; H, 9.47).

The above evidence definitely excludes both the tocopherol and the ether formulations for the vitamin K_1 by-product. One clue to the nature of the substance was found in the results of Zerewitinoff determinations pointing to the presence of one carbonyl group and one active hydrogen; analogous results were obtained with the crystalline by-product, m. p. 73°.[7] The ketonic nature of the K_1 by-product was further substantiated by the isolation of a crystalline mono-2,4-dinitrophenylhydrazone. On oxidation of the by-product with chromic acid two fragments were isolated accounting for all of the carbon atoms. One was an acid, $C_{13}H_{12}O_4$, and the other was identified as 2,6,10-trimethylpentadecanone-14 by the preparation of a semicarbazone which did not depress the m. p. of that from the oxidation product of phytol. The reaction thus involves cleavage at the external double bond, $C_{31}H_{48}O_3 \longrightarrow C_{12}H_{11}O_2COOH + CH_3COCH_2C_{15}H_{31}$, and demonstrates the presence of the phytyl group.

The requirements of a substance isomeric with vitamin K_1 hydroquinone and having an intact primary phytyl group, a carbonyl group, and an active hydrogen atom would be met by either of the formulas VI or VII (in the former case the methyl group could alternately be placed at position 3). Formula VI, which is in agreement with the results



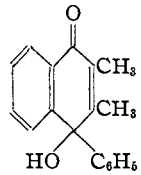


of the Zerewitinoff determination and with the resistance of the substance to acylation, finds a close parallel in the structure VIII, which was established by Crawford[8] for a compound resulting from the 1,4-addition of phenylmagnesium bromide to 2,3-dimethyl-1,4-naphthoquinone. The absorp-

(7) In a determination by R. D. Cramer conducted in isoamyl ether the substance of m. p. 73° liberated 0.76 equivalent of methane and added 0.84 equivalent of Grignard reagent. The complex dissolved better using xylene as the solvent and the results were 0.78 and 1.14 equiv., respectively.

(8) Crawford, THIS JOURNAL, **57**, 2000 (1935); **61**, 3310 (1939).

tion spectrum (Fig. 1), determined with a sample of VIII kindly supplied by Dr. Crawford, shows



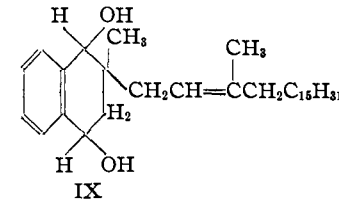
VIII

an intense maximum corresponding well with that observed for the by-products, but the secondary band is entirely different. The presence of an absorbing phenyl substituent in VIII of course introduces an element of uncertainty, and in the absence of adequate data for comparison the spectrographic evidence does not distinguish between an α,β-unsaturated ketone (VI) and a 1,4-diketone (VII) or its enolic modification.

The formulation of the K_1 by-product as the keto alcohol VI would require some special explanation of the solubility of the analogous compound m. p. 73° in alcoholic alkali. A further objection to VI is that neither the by-products nor the C_{13}-acid obtained from the phytyl compound show any tendency to undergo dehydration. Whereas a substance with a doubly activated tertiary hydroxyl group as in VI should lose water readily, the K_1 by-product was recovered unchanged after being heated with acetic and hydrochloric acids and distilled in high vacuum.

The alternate formula VII accords with the stability to dehydrating agents and with the alkali solubility of the 73° compound but requires the assumption that the K_1 by-product retains the diketonic structure under acylating conditions although it is enolized by the Grignard reagent. There is a certain analogy for this situation in Crawford's observations concerning two ketonic substances somewhat similar to VII, for one compound (m. p. 208°) enolized under the conditions of the Zerewitinoff determination while the other (m. p. 123°) did not.

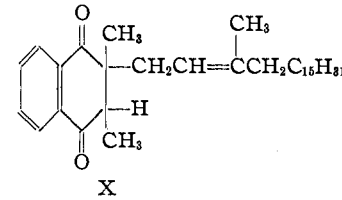
Fortunately an unambiguous means for distinguishing between formulas VI and VII was found in the reaction with aluminum isopropoxide. The keto alcohol VI should give a dihydride having only one secondary alcoholic group, and the diketone VII should yield a tetrahydride with two such groups. Actually the reaction product, obtained in good yield as a waxy solid, proved to be a tetrahydro derivative (IX) which gave an oily diacetate and two crystalline *bis*-3,5-dinitrobenzoates, m. p. 75 and 120°, which evidently are steroisomers. As expected for such a secondary diol, the substance lost water readily when warmed with acetic–hydrochloric acid. A mixture of products resulted and the isolation of a small amount of β-methylnaphthalene suggests that a rearrangement is involved.



IX

A further observation, which will be reported in a later paper, is that the condensation of phytol with α-naphthohydroquinone[9] affords phytylnaphthohydroquinone in comparatively high yield and is not attended with the formation of a ketonic by-product. Clearly, no by-product would be expected if the side reaction consists in the introduction of the phytyl group at a β-position as postulated. On the other hand, the yield of an α-substituted product of the type VI should not be influenced greatly by the presence or absence of a β-methyl group.

The diketone formulation VII was further substantiated by observations concerning an analogous compound obtained by condensing 2,3-dimethyl-1,4-naphthohydroquinone with phytol. This compound (X) was found to correspond to



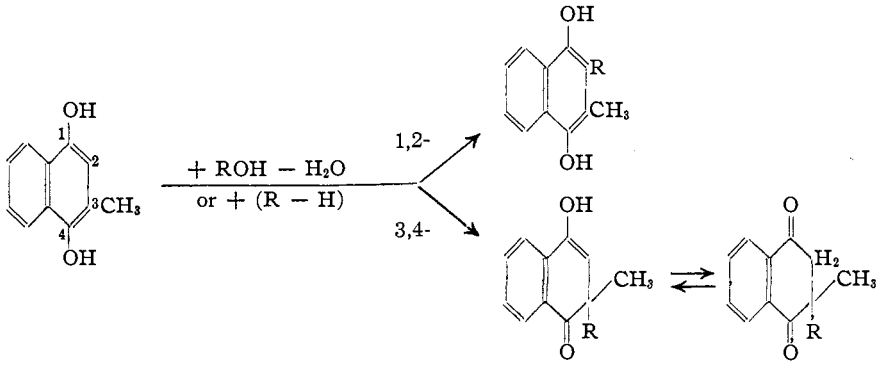
X

VII in spectrum (Table I) and it took up four atoms of hydrogen on reduction with aluminum isopropoxide. In the Zerewitinoff determination the substance liberated only a negligible amount of gas but consumed two moles of reagent. This result clearly disposes of the alternate keto alcohol formulation. The contrasting behavior in the Zerewitinoff test noted by Crawford thus finds a complete parallel in compounds VII and X of the present series, since one of these enolizes in the presence of RMgX and the other does not.

It is clear from this evidence that the side reaction occurring to a significant extent during the

(9) Fieser, Tishler and Sampson, THIS JOURNAL, **62**, 996 (1940).

formation of 2-methyl-3-phytyl-1,4-naphthohydroquinone results in the production of a diketone having the methyl and phytyl groups both attached at the 2-position. One conceivable mechanism is that the reaction proceeds through a tautomerization of 2-methyl-1,4-naphthohydroquinone to the 1-keto or 1,4-diketo form, and a corollary hypothesis would be that vitamin K_1 hydroquinone is formed in a like manner. Against this supposition is the fact that the hydroquinone shows little tendency to ketonize and that the completely or almost completely ketonic substances VII, X, and $C_{19}H_{20}O_2$ do not enter into further condensation under the conditions of the synthesis. The only alternate interpretation seems to be in terms of an addition mechanism involving an enolic double bond, and indeed products evidently arising from some form of primary addition to an enolic group have been encountered frequently in the naphthalene series.[10] In the case of phytol or other β,γ-unsaturated alcohol, or of a substance of the benzyl alcohol type, this may consist in an actual addition of the alcohol as R·OH to the enolic double bond or in the formation of an equivalent complex, with subsequent elimination of water, but it is perhaps well to refrain from any specific speculation regarding the hypothetical intermediates and postulate merely the involvement of either the 1,2- or the 3,4-double bond in the formation of compounds of the type of vitamin K_1 hydroquinone and the by-product, respectively. Condensations in both directions have been observed with dienes (R-H) corresponding to certain of the reactive alcohols, and in this case, particularly as applied to the reaction leading to a by-product, it is not clear whether an alternate mechanism is involved or the diene initially combines with a trace of moisture, the acid catalyst or the phenol to give an alcohol or its derivative. Some such circuitous route may be indicated by the fact that the dienes react less readily than the corresponding alcohols.



The observation that 3,4-addition to the enol system —C(CH$_3$)=C(OH)— takes place about as readily as 1,2-addition to —CH=C(OH)— indicates that the side-reaction which detracts from the yield of vitamin K_1 cannot be suppressed by adjustment of the concentrations or the reaction time. The finding that a group can be introduced at an already substituted β-position has led to the development by M. D. Gates, Jr., and one of us (L. F. F.) of a new synthesis of vitamin K_1 which consists in condensing phytol with the hydroquinone of phthiocol.

It has been found in the present work that vitamin K_1 can be obtained in small amounts by the pyrolysis of the by-product VII. When the material was refluxed in decalin for fifteen hours and the product worked up as in the synthesis, there was isolated about 10% by weight of methylnaphthohydroquinone and 5% of a substance having the composition of vitamin K_1 and giving a positive Dam–Karrer color test.[11] It cannot be argued that a small amount of the phytyl ether III, present in the starting material as an impurity, may have rearranged to give a substituted hydroquinone, for the phytyl group would then be linked through the quaternary γ-carbon atom and should not give a color reaction which is dependent upon the presence of an enolizable hydrogen at the point of attachment of the side chain.[12] Proof of the normal phytyl structure was obtained by conversion to crystalline vitamin K_1 hydroquinone diacetate.[13] Evidence that the pyrolysis products do not arise from other impurities is at hand in the observation that the

(10) Fieser, in Gilman, "Organic Chemistry," John Wiley and Sons, New York, N. Y., Vol. I, 81–97 (1937).

(11) Dam, Geiger, Glavind, P. Karrer, W. Karrer, Rothschild and Solomon, *Helv. Chim. Acta*, **22**, 310 (1939).

(12) Fieser, Campbell and Fry, THIS JOURNAL, **61**, 2206 (1939).

(13) In reporting experiments in our laboratory with the use of the pyridine procedure for the reductive acetylation of quinones,[4] we overlooked the prior description of this convenient method by Rosenhauer, Braun, Pummerer and Riegelbauer, *Ber.*, **70**, 2281 (1937) [see also Kuhn and Wallenfels, *ibid.*, **72**, 1407 (1939); Price and Robinson, *J. Chem. Soc.*, 1522 (1939)].—L. F. F.

material not extracted by aqueous or alcoholic alkali had all the properties of the unchanged starting material and gave further small amounts of the above products when pyrolyzed a second and a third time. The most likely interpretation of the reaction is that rupture occurs at the bond linking the phytyl group to the nucleus, for this is so located as to be activated by one of the carbonyl groups and by the β,γ-double bond. The methylnaphthohydroquinone and phytadiene thus produced could partially recombine to give methylphytylnaphthohydroquinone. It is noteworthy that the by-product VII shows marked antihemorrhagic activity, giving a positive response in the chick assay at dosages as low as 50 γ. A biological transformation of the substance into vitamin K_1 or methylnaphthoquinone would seem a remarkable process, but this has some support in the analogous laboratory conversion described.

In the course of this work a number of carefully purified preparations of synthetic vitamin K_1 have been submitted to spectrographic determination, and in view of the divergent reports concerning the extinction coefficient of the intense band at 248 mμ these newer experiences may be summarized. Dam, Karrer, *et al.*,[11] on isolating the natural vitamin, reported the coefficient $E^{1\%}_{1\text{ cm.}} = 280$, corresponding to log $E_M = 4.10$, while Doisy and associates subsequently published the log E_M values 4.24[14], 4.39[15,16] and 4.28.[16] In an initial determination[17] at Harvard, D. M. Bowen found log $E = 4.24$, and twelve subsequent spectrographic analyses in our two laboratories have given values within the limits 4.24–4.27 (alcoholic solution). Dr. R. N. Jones investigated the possible photodecomposition of the vitamin during the determination by comparing on the same plate the maxima at 248 mμ observed with separate fresh portions of a solution after their exposure to the hydrogen discharge tube for two and one-half and twenty-two minutes, respectively. The spectra appeared identical, and on traversing the plate at the 252-mμ level with a recording photoelectric photometer no differences were detectable in the tracings. Dr. T. J. Webb has ascertained that the probable error due to exposure to the spark discharge in the normal course of measurement would not exceed 5%. Both workers agree that the most reliable value is log $E_M = 4.26$ (alcohol) and that this figure is not appreciably in error due to alteration of the vitamin during exposure. It is significant that this value agrees well with the molar extinction coefficients found for the intense absorption bands of three crystalline 2,3-dialkyl-1,4-naphthoquinones, as shown in Table II.[18]

TABLE II

WAVE LENGTH AND INTENSITY OF THE PRINCIPAL ABSORPTION BAND (ALCOHOL SOLUTION)

-1,4-naphthoquinone	λ, mμ	Log E_{Molar}
2-Methyl-3-phytyl	248	4.26
2,3-Dimethyl	249	4.24[12] 4.26[12,19]
2,3-Diallyl	249	4.24
2-Methyl-3-(β,γ,γ-trimethylallyl)	249	4.27[4]
2,6-Dimethyl-3-phytyl	256.5	4.33[1]
2,6-Dimethyl-3-allyl	256	4.35[12,19]
6,7-Dimethyl-2,3-diallyl	260	4.41[12,19]

It may be noted that the extinction coefficient found by the Doisy group[15,20] for the 249-mμ band of vitamin K_2 corresponds to a log E_M value of 4.25 on the basis of the molecular weight 581. The introduction of an additional methyl group at the 6-position of the benzenoid ring displaces the characteristic band to a region of longer wave length and significantly increases the extinction coefficient (fifth and sixth entries), and another methyl group at the 7 position (last entry) produces further changes in the same direction.

Experimental Part[21]

Variations in the Vitamin K_1 Synthesis.—The condensation of 2-methyl-1,4-naphthohydroquinone with phytol in dioxane in the presence of oxalic acid[1] proceeded satisfactorily on a 0.005-mole scale when conducted at or near the reflux temperature for short periods[22] (two to four hours), but with somewhat larger amounts the yield fluctuated. More regular results were obtained on conducting the reaction at 75° for thirty-four hours and also when a large excess of methylnaphthohydroquinone was used. With equal weights of the two reactants, the yield of vitamin K_1

(14) McKee, Binkley, MacCorquodale, Thayer and Doisy, THIS JOURNAL, **61**, 1295 (1939).

(15) Binkley, MacCorquodale, Thayer and Doisy, *J. Biol. Chem.*, **130**, 219 (1939).

(16) Ewing, Vandenbelt and Kamm, *ibid.*, **131**, 345 (1939).

(17) Fieser, THIS JOURNAL, **61**, 2559 (1939).

(18) Contrary to the statement of Ewing, Vandenbelt and Kamm,[16] the fine structure of the main absorption bands of 2,3-dimethyl-1,4-naphthoquinone in alcohol was observed by us and given specific and prominent consideration in the paper[12] which these authors cite. Our paper also reports an instance of the greater resolution obtained with hexane as solvent in place of alcohol.—L. F. F.

(19) Fieser, Bowen, Campbell, Fry and Gates, THIS JOURNAL, **61**, 1926 (1939).

(20) McKee, Binkley, Thayer, MacCorquodale and Doisy, *J. Biol. Chem.*, **131**, 327 (1939); Binkley, McKee, Thayer and Doisy, *ibid.*, **133**, 721 (1940).

(21) With the exceptions noted the experiments were by M. T. and N. L. W. and the microanalyses were conducted by D. Hayman, W. Reiss and H. C. Clark of the Merck Research Laboratories. The bio-assays were carried out by Dr. W. L. Sampson of the Merck Institute for Therapeutic Research.

(22) See procedure of Fieser, *J. Biol. Chem.*, **133**, 391 (1940).

was 15–24%. The amount of dioxane can be reduced to two-thirds that specified[1] and the commercial solvent is as satisfactory as that purified with sodium. For best recovery of the product it is advantageous to introduce a reduction operation prior to the precipitation of vitamin K_1 hydroquinone with petroleum ether, for some of the material invariably becomes oxidized and is not fully reduced by shaking in ether with hydrosulfite solution. The reduction is accomplished by stirring a methanol suspension of the brown total oil with aqueous hydrosulfite. After separation of the bulk of the vitamin K_1 hydroquinone, a small additional quantity can be obtained, after a further reduction operation, by extraction with Claisen's alkali.

Vitamin K_1 was also obtained (after processing the reaction mixture in the usual way) in 13–15% yield on refluxing methylnaphthohydroquinone and phytol in acetic acid in the presence of zinc dust for one to four hours (9% yield after sixteen hours). When the components were heated without solvent or catalyst under nitrogen at 150–160° for one hour and the residue processed as usual and extracted with Claisen's alkali, the yield of product, isolated as pure vitamin K_1, was 23%. The vitamin was also obtained in 11–19% yield by refluxing the components in dioxane in the absence of oxalic acid. The results were negative using pyridine containing a small amount of acetic acid. When phytadiene was used in place of phytol in an experiment conducted under the standard conditions of the synthesis the yield of K_1 was 3%.

In investigating the mechanism of the formation of the by-product it was found that after heating vitamin K_1 hydroquinone in dioxane in the presence of oxalic acid at 75° for thirty-four hours over 90% of the material can be recovered unchanged. However, after boiling 1 g. of the hydroquinone in 10 cc. of acetic acid with three drops of concentrated sulfuric acid for seven hours only about 10% of the substance was recoverable by extraction with Claisen's alkali. The neutral residue was shown to be the naphthotocopherol.

By-product of the Synthesis: 2-Methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone (VII).—After separation of the bulk of the vitamin K_1 hydroquinone from the reaction mixture, all traces of this material can be removed by extraction with Claisen's alkali and hydrosulfite, as shown by the complete absence of a blue or purple color on shaking a test portion of the residue with alcoholic alkali in the presence of air. On distillation of the residue from a pot still at 10^{-5} mm., a fraction consisting largely of phytadiene distilled at about 60°, and a second fraction came over at 140–150° (temperature of the liquid) as a pale yellow oil, leaving little residue. A small amount of methylnaphthoquinone sublimed in the course of the distillation. After two more distillations (inside temperature 145°) the by-product was obtained as a pale yellow viscous oil; $n^{29}D$ 1.5095.

Anal. Calcd. for $C_{31}H_{48}O_2$: C, 82.30; H, 10.66. Found: C, 82.53, 82.38, 82.50; H, 10.80, 10.65, 10.86.

Zerewitinoff determinations carried out by R. D. Cramer in isoamyl ether and xylene, respectively, gave the following results: methane liberated, 0.95, 0.74 equiv.; reagent added, 1.36, 1.22 equiv.

The substance gives a deep red color with concentrated sulfuric acid but shows no color changes when warmed with alcoholic alkali or when treated with cyanoacetic ester in the presence of alcohol and ammonia (Craven's test).[23] The compound fails to reduce alcoholic silver nitrate solution and does not respond to the Furter–Meyer test[3] for tocopherols. It reacts rapidly with bromine in carbon tetrachloride solution with evolution of hydrogen bromide, and in the presence of Adams catalyst in ethyl acetate or acetic acid absorbs hydrogen rapidly at first (1–2 moles) and then more slowly, but without a very sharp break in the rate. It does not react with diazomethane. Attempts to cleave an ether linkage with excess methylmagnesium bromide at 180°, with aluminum bromide, or sodium–potassium alloy were unsuccessful. No reaction was observed with *p*-nitrosodimethylaniline, *p*-nitrobenzaldehyde, *p*-nitrophenyl isocyanate, or 3,5-dinitrobenzoyl chloride, the reagents or their derivatives being recovered.

In a test of the stability of the by-product under dehydrating conditions (L. F. F.), 10 g. of the material was heated with 30 cc. of acetic acid and 1 cc. of concentrated hydrochloric acid for one hour on the steam-bath. Nearly all of the collected product distilled at 2×10^{-3} mm. at a bath temperature of 180–190° and was of unchanged composition (found[24]: C, 82.16; H, 10.77).

The **2,4-dinitrophenylhydrazone** was prepared by adding 7.5 cc. of an alcoholic solution containing 0.44 g. of 2,4-dinitrophenylhydrazine and 0.88 cc. of concentrated sulfuric acid to 1 g. of the by-product in 10 cc. of alcohol and refluxing for one-half hour, during which time a red oil separated. This slowly solidified and was obtained crystalline from 100 cc. of alcohol. After three recrystallizations the substance formed fine, orange-red needles, m. p. 107–108°; yield 1.2 g.

Anal. Calcd. for $C_{37}H_{52}O_2N_4$: C, 70.21; H, 8.28; N, 8.85. Found: C, 70.12; H, 8.27; N, 8.85.

Pyrolysis of the By-product.—In one preliminary experiment it was found that the by-product (1 g.) decomposes when heated at 250°, with sublimation of 2-methyl-1,4-naphthoquinone (0.1 g.) from the mixture. The same substance in the reduced form (0.25 g.) was isolated after heating the by-product (2 g.) in kerosene at about 220° for four hours and processing the mixture as in the vitamin K_1 synthesis. Extraction with Claisen's alkali and oxidation gave 60 mg. of an oil closely resembling vitamin K_1.

In the most satisfactory experiment a solution of 2 g. of the by-product in 5 cc. of dry decalin was refluxed vigorously for fifteen hours in a stream of nitrogen. The solvent was largely removed at reduced pressure and a suspension of the residue in 50 cc. of methanol was treated with an aqueous solution of 3 g. of sodium hydrosulfite, diluted and again shaken well. The product was extracted with ether and the solution extracted with 2% alkali containing 2% hydrosulfite until the alkaline liquor was colorless. The yellow liquor on acidification and extraction afforded 0.2 g. of 2-methyl-1,4-naphthohydroquinone, identified by

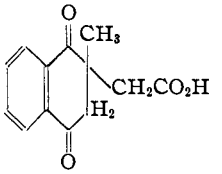
(23) Craven, *J. Chem. Soc.*, 1605 (1931). A deep blue or purple color is produced in the case of naphthoquinones (or the corresponding hydroquinones) having a free position in the quinone ring. Karrer and König, *Helv. Chim. Acta*, **23**, 272 (1940), state that crude 2,3-dimethyl-1,4-naphthoquinone obtained from the hydrocarbon gives a positive test. Samples which we have purified merely by crystallization have all failed to produce any color.

(24) Microanalysis by Lyon Southworth.

conversion to the quinone and the diacetate. The ethereal solution was evaporated under nitrogen and a solution of the residue in petroleum ether extracted with Claisen's alkali-hydrosulfite (100 cc.). The yellow alkaline liquor was diluted with 2% aqueous hydrosulfite and extracted with petroleum ether. On concentrating to a volume of about 8 cc. and chilling in ice and salt, a waxy white solid was obtained, and after washing three times by centrifugation this was oxidized in dry ether solution, giving 100 mg. of a yellow oil identified as vitamin K_1 by the absorption spectrum, Dam-Karrer color test, analysis (found: C, 82.38; H, 10.17), and conversion to the hydroquinone diacetate (m. p. 61°, no depression with an authentic sample).

The neutral material not extracted by Claisen's alkali was recovered and distilled at 150° and 10^{-5} mm., giving a pale yellow oil having the properties of the starting material: $n^{29}D$ 1.5090 (found: C, 82.29; H, 10.74). This recovered material on pyrolysis in decalin as above gave 2-methyl-1,4-naphthohydroquinone and vitamin K_1 in about the same yields as in the first treatment.

Oxidation of the By-product.—A solution of 2 g. of the material in 50 cc. of acetic acid was maintained at 60–70° while adding dropwise over a period of three hours a solution of 0.93 g. of chromic anhydride in 10 cc. of 80% acetic acid. After standing at 70° for one hour the solution was concentrated to dryness at reduced pressure and the residue was suspended in water and extracted three times with ether. After washing with water, the ethereal solution was extracted with sodium bicarbonate solution and the acidic product was taken into ether and obtained crystalline on concentrating and adding petroleum ether. The purified acid, which can be assigned the structure of 2-methyl-2,3-dihydro-1,4-naphthoquinone-2-acetic acid XI,



XI

was obtained as colorless platelets melting sharply at 126° (0.15 g.). A solution of the substance in aqueous sodium bicarbonate reduces permanganate rapidly in the cold.

Anal. Calcd. for $C_{13}H_{12}O_4$: C, 67.16; H, 5.17; mol. wt., 232. Found: C, 67.25; H, 5.40; mol. wt., 260 (Rast), 221 (titration).

The non-acidic fraction from the oxidation gave a positive Craven test and consequently the ethereal solution was shaken for ten minutes with hydrosulfite solution and extracted thoroughly with dilute alkali-hydrosulfite to remove any methylnaphthohydroquinone. The remaining ethereal solution was evaporated to dryness and steam distilled, giving a volatile fraction appearing as oily droplets and having an aromatic odor. Extraction with ether afforded 200 mg. of oil, and this was treated in methanol (1 cc.) with semicarbazide hydrochloride (150 mg.) and sodium acetate (125 mg.). After standing for several days at room temperature, the mixture was diluted with water and extracted with ether and the solution was washed, dried and evaporated. A solution of the residual oil in 1 cc. of methanol on standing for eighteen hours at −10° deposited a waxy solid. This was separated by centrifugation and recrystallized four times from small amounts of methanol, giving a crystalline product, m. p. 62–64°, which did not depress the m. p. of a sample of 2,6,10-trimethylpentadecanone-14, m. p. 64–66°, prepared by oxidation of phytol with chromic acid.[25]

In qualitative tests it was observed that the by-product is attacked also by lead tetraacetate and by selenium dioxide (in alcohol).

Reduction of the By-product to 2-Methyl-2-phytyltetralindiol-1,4, IX (L. F. F.).—Aluminum isopropoxide was prepared from 3.5 g. of aluminum turnings (2-S) and 75 cc. of dry isopropyl alcohol using carbon tetrachloride and mercuric chloride as starters.[26] After distilling most of the solvent, 9 g. of the by-product was added in 75 cc. of toluene and the mixture was refluxed overnight. The solvent was then largely removed by slow distillation and the cooled residue was decomposed with dilute sulfuric acid and extracted with ether. After washing the nearly colorless solution well with dilute acid and with alkali the ether and toluene were completely removed by steam distillation and the product was collected and dried in ether. Evaporation of the ether left 8.50 g. of the diol as a very viscous, faintly yellow oil which solidified after a few hours to a wax of m. p. about 40–50°. The sample for analysis was dried in high vacuum at 80°.

Anal.[27] Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.67; H, 11.74.

In a Zerewitinoff determination (R. D. C.) the diol liberated 2.01 equiv. of methane (addition, 0.16 equiv.). On attempted distillation at 2×10^{-3} mm. some dehydration evidently occurred (found[24]: C, 83.81; H, 11.74). A trial dehydration was conducted by adding 1 cc. of concentrated hydrochloric acid to a solution of 8.08 g. of the diol in 40 cc. of acetic acid at room temperature. No change occurred on standing, but when the solution was heated on the steam-bath it very soon separated into two layers. After heating for four hours the mixture was treated with water and ether and the ether layer was washed with alkali and steam distilled. After removal of the ether, the distillate afforded on ether extraction 0.18 g. of β-methylnaphthalene, which yielded 0.32 g. of the picrate in the form of long yellow needles from alcohol, m. p. and mixed m. p. 116–117°. The non-volatile residue (7.32 g.) when distilled at 2×10^{-3} mm. gave 0.49 g. of a low boiling mobile fore-run (phytadiene?). The main portion distilled as a viscous oil at a bath temperature of about 150–155° and appeared to be a mixture (found[24,27]: C, 85.51, 85.43; H, 11.29, 11.52).

Diacetate of the Diol IX.—A solution of 1 g. of the diol and 1 cc. of acetic anhydride in 5 cc. of pyridine was heated on the steam-bath for four hours, and after treatment with water the product was extracted with ether. After washing with 5% hydrochloric acid and aqueous sodium bicarbonate solution the ethereal solution was dried, clarified with Norit and evaporated. The residue was a colorless liquid weighing 1 g.

(25) Fischer and Lowenberg, *Ann.*, **464**, 78 (1928).
(26) Bachmann and Struve, *J. Org. Chem.*, **4**, 456 (1939).
(27) Semi-microanalysis by D. M. Bowen.

Anal. Calcd. for $C_{35}H_{56}O_4$: C, 77.73; H, 10.43; CH_3CO, 15.90. Found: C, 77.57; H, 10.37; CH_3CO,[28] 16.36.

Bis-3,5-dinitrobenzoates of IX.—A mixture of 2 g. of the diol and 2 g. of 3,5-dinitrobenzoyl chloride in 15 cc. of pyridine was heated on the steam-bath for four hours and poured onto cracked ice. After decanting the supernatant liquor, the oily product adhering to the bottom of the flask was taken up in ether. The solution was washed with dilute acid and alkali, dried, treated with Norit and evaporated. On dissolving the oily residue in petroleum ether there soon separated a crop of fluffy needles having a faint green tinge and melting at 74–75°. Recrystallization from petroleum ether did not alter the m. p.

Anal. Calcd. for $C_{45}H_{56}O_{12}N_4$: C, 63.98; H, 6.64; N, 6.64. Found: C, 63.90; H, 6.54; N, 6.42.

The petroleum ether mother liquor after standing for a few weeks deposited a **stereoisomer**. This crystallized from ether in the form of fine, white, matted needles, m. p. 120°.

Anal. Found: C, 63.89; H, 6.66; N, 6.63.

Acylation of the diol as above with *p*-nitrobenzoyl chloride gave a product separating from petroleum ether as a waxy solid; this was not analyzed.

Naphthotocopherol Derived from Vitamin K_1 Hydroquinone (probably II).—A mixture of 5 g. of vitamin K_1, 50 cc. of acetic acid, 10 g. of stannous chloride and 5 cc. of concentrated hydrochloric acid was refluxed for four hours, during which time two layers had formed, poured onto ice, and extracted with ether. After washing with water and with 5% bicarbonate solution the ethereal layer was dried and concentrated and the product distilled at 10^{-5} mm. from a pot still. The material distilled without fore-run or residue at a liquid temperature of 155° as a pale straw colored, very viscous liquid. Unless the sample is protected from the air, it darkens rapidly on standing. A similar product was obtained by refluxing vitamin K_1 in acetic acid with zinc and a few drops of sulfuric acid.

Anal. Calcd. for $C_{31}H_{48}O_2$: C, 82.30; H, 10.66. Found: C, 82.30; H, 10.69.

The substance rapidly reduces alcoholic silver nitrate or ferric chloride in the cold. Unlike vitamin K_1 hydroquinone, it gives no purple-blue color when shaken with 5% alcoholic potassium hydroxide with access of air. The tocopherol is not extracted from petroleum ether solution by Claisen's alkali containing hydrosulfite, but this treatment gives a yellow color to the otherwise colorless petroleum ether solution.

The ***p*-nitrobenzoate** was prepared by heating 0.6 g. of the naphthotocopherol, 0.6 g. of *p*-nitrobenzoyl chloride in 10 cc. of pyridine on the steam-bath for two hours, diluting the pale orange solution with water and extracting with ether. The product, obtained as an oil after washing with acid, alkali and water, was taken up in 40 cc. of absolute alcohol and allowed to crystallize at room temperature and then at 4°. The crystallizate (0.71 g.) consisted of fine, pale yellow needles, m. p. 84–85°, which appeared waxy when rubbed on a clay plate. The melting point remained the same after several recrystallizations from alcohol, and the high carbon value noted in the first of the following analyses was found as well for a sample submitted to two further crystallizations.

Anal. Calcd. for $C_{38}H_{51}O_5N$: C, 75.84; H, 8.54; N, 2.32. Found: C, 76.55, 76.52; H, 8.48, 8.70; N, 2.35.

Naphthotocopherylquinone (γ-Hydroxy-β,γ-dihydrovitamin K_1, IV).—A solution of 4.5 g. of the naphthotocopherol in 100 cc. of ether was shaken with a solution of 6 g. of ferric chloride hexahydrate in 200 cc. of 50% aqueous methanol, added in portions. After each addition of oxidizing agent shaking was continued until the aqueous layer showed no immediate reaction with starch–iodide paper. When the test was positive the ethereal layer was washed well with water, dried, and concentrated at reduced pressure, giving an oily red residue. A suspension of the crude quinone in 200 cc. of methanol was shaken for ten hours with 10 g. of sodium hydrosulfite dissolved in 20 cc. of warm water. The mixture was then poured into a separatory funnel containing 400 cc. of water and a thin layer of petroleum ether. After shaking, the petroleum ether layer was separated and chilled to −5°, when the hydroquinone separated as a waxy solid. This was separated by centrifugation and washed several times with petroleum ether until the product was completely white and the washings colorless. The hydroquinone was then shaken with silver oxide and magnesium sulfate in absolute ether, and on concentrating the filtered solution under reduced pressure the quinone was obtained as a bright yellow oil which darkened rapidly on standing.

Anal. Calcd. for $C_{31}H_{48}O_3$: C, 79.43; H, 10.32 Found: C, 79.19; H, 10.17.

The hydroquinone diacetate (probably V) was obtained by treating 1.1 g. of the quinone with acetic anhydride, pyridine and zinc dust in the usual manner and obtained initially as a colorless oil. On standing in absolute ethanol solution at 6° the diacetate slowly separated as fine, fluffy needles, m. p. about 20°. Four recrystallizations from ethanol did not raise the m. p.

Anal. Calcd. for $C_{35}H_{54}O_5$: C, 75.77; H, 9.82; CH_3CO, 15.56. Found: C, 75.52, 75.77; H, 10.06, 10.05; CH_3CO, 17.20. The percentages calculated for a triacetate are: C, 74.35; H, 9.48; CH_3CO, 21.70.

2-Methyl-1,4-naphthohydroquinone Monoethyl Ether.—A solution of 5 g. of the hydroquinone in 50 cc. of absolute alcohol containing 2 g. of dissolved hydrogen chloride was allowed to stand at room temperature for twenty-four hours, when it had become reddish-purple. After dilution with ice water the material was extracted with ether and the solution washed and dried over magnesium sulfate, which adsorbed some of the pigment. The solution was clarified with Norit, concentrated to 15 cc. and treated with petroleum ether. On good scratching 3.1 g. of the ether separated, m. p. 115–116°, and 1 g. of less pure material was obtained from the mother liquor. On recrystallization from ether–petroleum ether, the substance formed colorless, silky needles of the same m. p. The ether dissolves readily in cold 1% aqueous sodium hydroxide and is precipitated on acidification.

(28) In conducting the acetyl determination on this and other substances containing the phytyl group, D. Hayman and H. S. Clark found it necessary to carry out the hydrolysis with sodium methoxide in boiling methanol; the usual procedure gave low and irregular results.

Anal. Calcd. for $C_{13}H_{14}O_2$: C, 77.17; H, 6.93; OC_2H_5, 22.25. Found: C, 76.98; H, 6.94; OC_2H_5, 21.64.

The monoethyl ether shows complete vitamin K activity at a dosage of 1γ.

2,3 - Dimethyl - 2 - phytyl - 2,3 - dihydro - 1,4 - naphthoquinone (X).—A solution of 7.5 g. of phytol, 15 g. of 2,3-dimethyl-1,4-naphthohydroquinone and 5 g. of oxalic acid in 75 cc. of dioxane was heated in a closed vessel at 75° for thirty-four hours. The mixture was diluted with water containing hydrosulfite, extracted with ether and unchanged hydroquinone was removed by extraction with 2% potassium hydroxide containing 2% sodium hydrosulfite. After a further washing with 10% alkali the product obtained from the dried ether was distilled at 10^{-4} mm. A fore-run of phytadiene and phytol distilled at an inside temperature of 90° and about 4 g. of X distilled at 140–150°, leaving about 0.5 g. of tarry residue. On redistillation the substance was obtained as a pale yellow oil. It reacted readily with bromine in carbon tetrachloride with the liberation of hydrogen bromide. No reaction was observed with 2,4-dinitrophenylhydrazine under the conditions employed with the K_1 by-product.

Anal. Calcd. for $C_{32}H_{50}O_2$: C, 82.36; H, 10.78. Found: C, 82.27; H, 10.67.

In the Zerewitinoff determination (R. D. C.) the substance liberated 0.28 equiv. of methane and added 2.07 equiv. of reagent.

Summary

The by-product isomeric with 2-methyl-3-phytyl-1,4-naphthohydroquinone in the vitamin K_1 synthesis is shown to be 2-methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone. The substance, which has been characterized by degradation, aluminum isopropoxide reduction, and other reactions affording crystalline derivatives, has marked antihemorrhagic activity and can be converted in small part into vitamin K_1 by pyrolysis.

The isomeric naphthotocopherol has been prepared by the action of stannous chloride and acid on vitamin K_1 and converted to γ-hydroxy-β,γ-dihydrovitamin K_1. This substance yields a hydroquinone diacetate on reductive acetylation.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF TORONTO]

The Methoxymercurials from *cis* and *trans* Styryl Cyanide[1]

BY WILLIAM H. BROWN AND GEORGE F. WRIGHT

Introduction

It has been shown[2] that the *cis* isomer of certain stereoisomeric pairs reacts more rapidly with a solution of mercuric acetate in methanol than does the *trans* isomer. Since the reaction is easy to follow analytically, this would seem to afford a method of ascertaining the geoisomeric configuration of such a pair. Furthermore, there is a possibility that the difference in rate of addition might be utilized as a method of evaluating the *cis–trans* content of mixtures. Accordingly several pairs of geoisomers whose configurations have been determined by other means have been tested by this procedure. One of these pairs was β-styryl cyanide.

Ghosez,[3] who separated *cis* and *trans* β-styryl cyanide by fractionation, considered the low-melting isomer to be the *cis* form. Kistiakowsky and Smith,[4] after a study of the thermal isomerization, agreed with this designation. It might then be expected that this isomer would react more rapidly with a methanol solution of mercuric acetate than the higher melting form. Actually a trial showed that neither reacted appreciably at room temperature. Since a small amount of nitric acid has been found to accelerate the addition,[2b] this modification was then used with success; both isomers added the elements of methoxymercuric acetate at a rate convenient to study. The analytical procedure, though modified to suit the present study was essentially that previously outlined[2b] involving chloroform extraction to separate organomercurial from mercuric acetate prior to titration with thiocyanate. This separation is necessary owing to the fact that the product, II, reacts with thiocyanate.

The low melting styryl cyanide reacted with an equivalent of mercuric acetate in excess of methanol at such a rate that the reaction half-life was one hundred eighty-five minutes at 25° and one hundred minutes at 35°. The corresponding half-life reaction periods for the high melting isomer

(1) Presented before the Division of Organic Chemistry, American Chemical Society at Boston, September, 1939.

(2) (a) Biilmann, *Ber.*, **35**, 2571 (1902). (b) Wright, THIS JOURNAL, **57**, 1993 (1935).

(3) Ghosez, *Bull. soc. chim. Belg.*, **41**, 477 (1932).

(4) Kistiakowsky and Smith, THIS JOURNAL, **58**, 2438 (1936).