laidic acids, respectively, with periodic acid, resulted in the same products, β-hydroxy-pelargonic aldehyde and aldehydo-azelaic acid.

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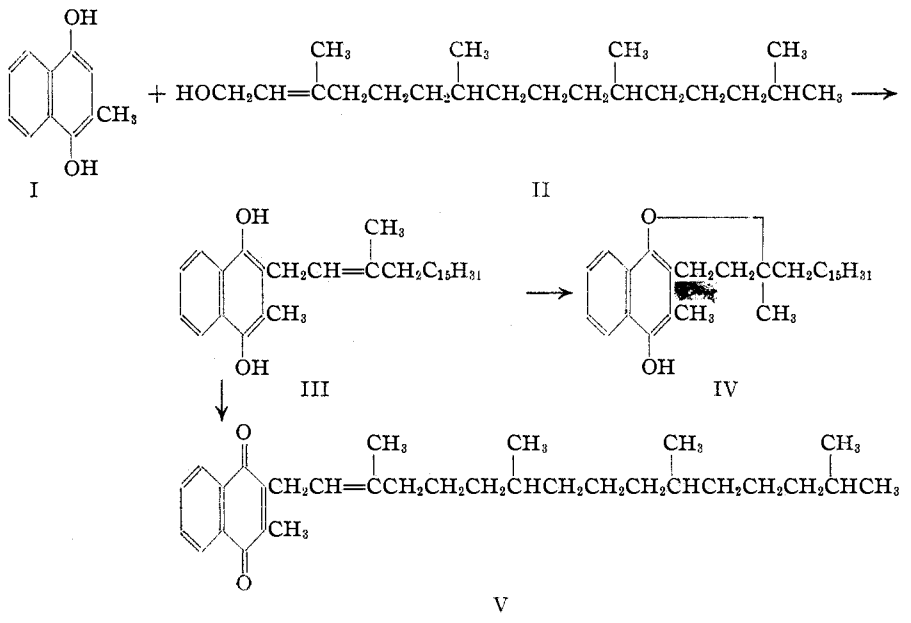
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Synthesis of Vitamin K_1[1]

BY LOUIS F. FIESER

In seeking to apply to the synthesis of vitamin K_1 the method of condensation employed successfully[2] for the synthesis of simple quinones of the structural type postulated[3] for the natural substance, trial was first made[2] of the condensation of 2-methyl-1,4-naphthohydroquinone with an equivalent amount of phytol in molten oxalic acid dihydrate at 140°. A smooth reaction ensued but the product had the properties of the naphthotocopherol IV, rather than of the desired substituted hydroquinone III. The result was much the same on conducting the reaction in the presence of anhydrous oxalic acid in dioxane solution at the reflux temperature.[2] On attempting to avoid the cyclization step by further moderating the conditions, it was found that reaction occurs at a reasonable rate at 75° and there were indications of the formation of 2-methyl-3-phytyl-1,4-naphthohydroquinone (III). A further improvement consisted in using a large excess of methylnaphthohydroquinone (up to 5.7 equivalents) in order to accelerate the bimolecular condensation reaction and thus give it precedence over the monomolecular cyclization. The tabulation of results given in the Experimental Part shows that this definitely increases the yield based upon phytol. The best results were obtained with a reaction time of thirty-six hours; the yield falls off on more prolonged heating, evidently because of cyclization to the naphthotocopherol. The proportion of oxalic acid can be varied considerably without change in the results and this reagent can be replaced by trichloroacetic acid.



The problem of separating the primary product III from the reaction mixture was solved very simply by working with the material in the reduced condition. The unchanged methylnaphthohydroquinone can be separated completely and recovered in a usable condition by extraction of an ethereal solution of the mixture with 1–2% alkali containing sodium hydrosulfite to prevent oxidation, the yellow vat color providing a convenient index of the course of the extraction. The more highly substituted naphthohydroquinone is not extracted from ether even with 25% aqueous alkali, and the separation of the two dihydroxy compounds is therefore very sharp. The impurities retained in the ether, including the naphthotocopherol, phytol, and possibly phytadiene, are all very soluble in petroleum ether whereas methylphytylnaphthohy-

(1) See preliminary communications, THIS JOURNAL, **61**, 2559, 2561 (1939).

(2) Fieser, Campbell, Fry and Gates, *ibid.*, **61**, 2559, 3216 (1939).

(3) Fieser, Bowen, Campbell, M. Fieser, Fry, Jones, Riegel, Schweitzer and Smith, *ibid.*, **61**, 1925 (1939).

droquinone is sparingly soluble in this solvent and separates as a waxy white solid. By repeated centrifugation and digestion with fresh solvent, the substance can be freed completely from contaminants, and no difficulty was experienced in isolating the material from an oil containing as little as 6% of product. Although the substance can be oxidized to the quinone by deliberately shaking an ethereal solution with air, as observed for vitamin K_1 hydroquinone by Doisy, *et al.*,[4,5] no difficulty was experienced in manipulating and evaporating ethereal solutions without protection from the air. When covered with petroleum ether the white solid shows little tendency to undergo air oxidation.

Conversion to the quinone was accomplished by shaking an ethereal solution of the well washed hydroquinone with silver oxide and magnesium sulfate. Evaporation of the filtered yellow solution left a light yellow oil consisting of 2-methyl-3-phytyl-1,4-naphthohydroquinone in a directly pure condition. The substance gives an intense Dam–Karrer color test with alcoholic alkali and the spectrum[1] corresponds closely to the pattern of simple 2,3-dialkyl-1,4-naphthoquinones.[2,3,6,7] That the structure is correctly represented by formula V was inferred from considerations already outlined.[1] The synthesis can be completed in a very short working period and provides a practical method for the preparation of the quinone in quantity. The yield amounts to 44% of the weight of phytol used, or 29% of the theoretical amount.

The synthetic quinone corresponded closely in properties with vitamin K_1 from alfalfa as described by Doisy, *et al.*,[8] and by the Dam–Karrer group.[9] In addition to the properties noted, the substance was found to have high antihemorrhagic activity[1] and to yield a crystalline hydroquinone diacetate. The fully purified derivative melted at 60–61.5° and remelted at 60–60.5°, whereas Doisy's dihydro vitamin K_1 diacetate was reported[4] to melt at 59° (purified sample,[5] m. p. 62–63°). At the time the synthetic diacetate was first isolated in a crystalline condition (Aug. 10) it was known from Doisy's degradative work[10] that the vitamin very probably is either 2-methyl or 2-ethyl-3-phytyl-1,4-naphthoquinone. While Doisy had tentatively favored the latter structure, the close correspondence to the synthetic methyl compound strongly suggested that we had the vitamin in hand. This view was strengthened by the consideration that in this series ethyl compounds melt considerably lower than the methyl homologs, as shown in the following comparison of data previously published.[2] Difference in m. p. between 2-methyl and 2-ethyl compounds:

2-Alkyl-1,4-naphthohydroquinone diacetates	+ 8°
2-Alkyl-1,4-naphthohydroquinone dibenzoates	+16°
2-Alkyl-3-cinnamyl-1,4-naphthohydroquinone diacetates	+44°
2-Alkyl-3-cinnamyl-1,4-naphthoquinones	+ 9°

A direct comparison was of course essential, and it seemed possible that the isolation of the natural vitamin might be accomplished far more easily than heretofore by applying the information and experience gained in the synthesis. While previously reported methods of isolation involve processing the material in the quinone form, it was found that considerable advantage can be taken of the distinctive chemical and physical properties of the vitamin hydroquinone. The starting material was very kindly supplied by Dr. Byron Riegel and consisted of a purified alfalfa concentrate containing 3–5% of vitamin prepared as described by Riegel, Schweitzer and Smith.[11] The vitamin present was reduced by shaking an alcoholic suspension of the concentrate with aqueous sodium hydrosulfite and the material was then extracted with petroleum ether. Unlike the experience of the synthesis, the vitamin hydroquinone did not separate at this point but remained in solution, apparently because of the high proportion and nature of the companion substances. Another method of separation was sought and in trials with pure synthetic 2-methyl-3-phytyl-1,4-naphthohydroquinone it was soon found that this very weakly acidic substance can

(4) Binkley, MacCorquodale, Cheney, Thayer, McKee and Doisy, THIS JOURNAL, **61**, 1612 (1939).

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

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

(7) In this connection belated reference may be made to the extensive study of the spectra of naphthoquinones by Macbeth, Price and Winzor, *J. Chem. Soc.*, 325 (1935). This paper was of considerable value to us in arriving at the conception of vitamins K_1 and K_2 as α-naphthoquinones; the marked similarity of the spectra of the vitamins to that reported by the British investigators for 2,3-diacetoxy-1,4-naphthoquinone was noted by Dr. R. N. Jones on May 19.

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

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

(10) MacCorquodale, Binkley, Thayer and Doisy, THIS JOURNAL, **61**, 1928 (1939).

(11) Riegel, Schweitzer and Smith, *J. Biol. Chem.*, **129**, 495 (1939).

be extracted from petroleum ether with Claisen's alkali (aqueous potassium hydroxide–methanol). When hydrosulfite is added to keep the material in the reduced condition the alkaline liquor acquires a bright yellow vat color which clearly indicates the course of the extraction. The yellow alkaline layer is then separated and merely diluted with water in the presence of ether, when the hydroquinone is liberated from its salt and passes into the ether layer. On processing the reduced concentrate in this way the vitamin hydroquinone was extracted easily and completely, and on evaporation of the ether layer and treatment of the residue with petroleum ether it separated as a white solid. The well washed hydroquinone was then oxidized and afforded a light yellow oil having the characteristics of very pure vitamin K_1. The entire process appears very simple and efficient and avoids difficulties arising from the sensitivity of the vitamin in its oxidized form to heat, light, alkali, and adsorbents.

The sample of natural vitamin K_1 was found by Dr. W. L. Sampson to have the same degree of antihemorrhagic activity as synthetic 2-methyl-3-phytyl-1,4-naphthoquinone. Assayed with chicks by the eighteen-hour procedure,[1] the minimum dose for both substances was approximately 2 γ.[12] The absorption curve determined by D. M. Bowen for the synthetic quinone has already been reported,[1,13] and this corresponds well in form and in the position of the maxima with the curve for vitamin K_1 published by Dam, Karrer, *et al.*[9] A direct comparison of the present samples was kindly made by Dr. T. J. Webb. For the synthetic quinone he found the following maxima (mμ) and log ϵ values (in alcohol): 241(4.18), 248(4.24), 263(4.22), 271(4.23), 330(3.45); the only significant difference between this and Bowen's determination is in placing one of the subsidiary maxima in the region of intense absorption at 263 instead of 260.5 mμ. The maxima for the natural sample occurred at the same wave lengths as above but were slightly lower, the log ϵ values at the several maxima differing by the constant amount of 0.04 unit. This indicates that the natural sample at the time of examination was not quite as pure as the synthetic.

The natural vitamin K_1 gave a crystalline diacetate corresponding in melting point with the synthetic sample and giving no depression when mixed with this substance. While it is perhaps questionable if, among closely related compounds of this series, the absence of a depression in the mixed melting point determination constitutes a rigid proof of identity, it seems significant that the mixture was observed to exhibit the behavior characteristic of both individual samples of solidifying in a few hours and remelting at a sharper and slightly lower temperature. That the synthetic and natural quinone samples give diacetates which thus appear to be identical does not exclude the possibility of the presence in the samples of a certain amount of the geometrical isomer differing from that affording the crystalline diacetate, for the yields of crystalline product in the few reductive acetylations conducted were not quantitative. Although this point merits further investigation, the present indication from the behavior and assay of various samples is that stereoisomerism probably plays a minor role.

Before drawing a final conclusion concerning the identity of the vitamin it seemed desirable to investigate the alternate possibility, and 2-ethyl-naphthohydroquinone was consequently condensed as above with phytol. The reaction proceeded less readily than in the methyl series and the phytyl-substituted hydroquinone proved to be much more soluble in petroleum ether and failed to separate. The method of extraction with Claisen's alkali was successfully applied, but even after removal of all but traces of impurities in this way the substance, in sharp contrast to the behavior noted with both the synthetic methyl compound and the vitamin hydroquinone, did not solidify on treatment with petroleum ether. Oxidation of the unpurified material gave a sample of 2-ethyl-3-phytyl-1,4-naphthoquinone having the characteristic vitamin K type of spectrum but a lower extinction coefficient and estimated from the value found and from the extinction coefficient of the pure synthetic methyl compound to be 95 $\pm$ 3% pure (T. J. Webb). Dr. Sampson found this material inactive at a dosage of 160 γ. The lack of activity and the marked contrast in the solubility relationships of the hydroquinone eliminate any possibility of identity with vitamin K_1.

The evidence is therefore conclusive that vitamin K_1 is identical with synthetic 2-methyl-3-phytyl-1,4-naphthoquinone. The conclusion based upon the present synthesis, which was completed before the structure of the vitamin had

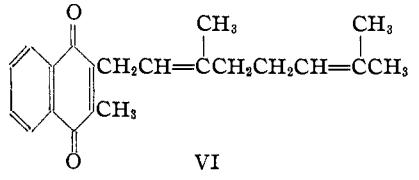
(12) A full account of the assays mentioned in this series of papers will be reported elsewhere by Dr. Sampson.

(13) The log ϵ value for the broad band at 328 mμ was inadvertently printed as 2.42 in place of 3.42.

been established by other evidence, agrees with that reached by Doisy, *et al.*, from degradative experiments[14] and from a further synthesis.[14,15]

In confirmation of a prediction[16] based on the behavior of simpler synthetic models, it has been established that vitamin K_1 (synthetic) yields phthiocol as one product of the Dam–Karrer color reaction with alcoholic alkali.

2,6 - Dimethyl- 3- phytyl - 1,4 - naphthoquinone was synthesized by M. D. Gates, Jr., by the procedure given above and found to be devoid of antihemorrhagic activity at a dosage of 50 γ (W. L. Sampson). D. M. Bowen observed the following absorption maxima (mμ) and log ϵ values in alcohol): 247(4.24), 256.5(4.33), 264.5(4.22), 271-(4.22), 331(3.47). A comparison with Bowen's results[1] for the 2-methyl compound shows that the nuclear methyl group at the 6-position displaces the absorption bands 4.4 mμ (av.) in the direction of longer wave lengths while the logarithm of the extinction coefficient is 0.05 unit higher. A similar shift attending substitution in the benzenoid ring has been observed with simpler models.[16] 2-Methyl-1,4-naphthohydroquinone was condensed under comparable conditions with geraniol and with the diene myrcene, which presumably bears the relationship to geraniol which phytadiene bears to phytol. The reaction proceeded considerably better with the alcohol than with the diene. In each case the substituted hydroquinone separated from petroleum ether in such a gelatinous condition that full purification was not achieved. The quinone obtained from geraniol is referred to as 2-methyl-3-geranyl-1,4-naphthoquinone and assigned the structure VI; it is not yet known whether the product from myrcene is identical with this substance. 2-Methyl-3-geranyl-1,4-naphthoquinone is active at a dosage of 25 γ but not at 5 γ (W. L. Sampson).



VI

The assay results now available for the series of 2,3-dialkyl-1,4-naphthoquinones for the most part point to a certain specificity in the structures associated with significant antihemorrhagic activity, while the 2,3-dimethyl compound constitutes a rather anomalous example which seems to indicate a lack of such specificity. Since the natural vitamins may be described as 2,3-dialkyl derivatives having at least one long side chain, it is odd that activity[16] should be encountered in the 2,3-dimethyl compound and not in substances more closely related to the vitamins in structure such as the 2,3-diallyl, 2-methyl-3-trimethylallyl, and 2-methyl-3-benzyl compounds. Excepting this anomalous case the results form a consistent picture. Activity begins to appear as the alkyl groups are increased in size beyond the limits just noted (total of 6–8 carbons) and is encountered in the 2-methyl-3-cinnamyl (10 carbons) and 2-methyl-3-geranyl (11 carbons) compounds. Activity reaches a high point in vitamins K_1 and K_2 with a total of 21 and 30 carbon atoms, respectively, in the side chains. The structure 2-methyl-3-phytyl-1,4-naphthoquinone seems highly specific, for the activity is lost on introducing a methyl group into the benzenoid nucleus or enlarging the 2-methyl to an ethyl group.

In view of these indications of specificity it seems very remarkable that the simple and rather remotely related 2-methyl-1,4-naphthoquinone should exhibit striking potency, as first reported by Ansbacher and Fernholz.[17] It is to be noted that early assays in three different laboratories[18,19,20] indicated the compound to be but very weakly active and that the more recent results, while all pointing to a high order of potency, still differ in the exact relative activity assigned to the compound with respect to vitamin K_1. Two groups of investigators[20,21] find the 2-methyl compound about four times as potent as the vitamin, another regards the two compounds as equally active,[22] while according to a third group methylnaphthoquinone "is by no means as active as vitamin K."[23] A possible explanation both for

(14) Binkley, Cheney, Holcomb, McKee, Thayer, MacCorquodale and Doisy, THIS JOURNAL, **61**, 2558 (1939).

(15) MacCorquodale, McKee, Binkley, Cheney, Holcomb, Thayer and Doisy, *J. Biol. Chem.*, **130**, 433 (1939).

(16) Fieser, Campbell and Fry, THIS JOURNAL, **61**, 2206 (1939). Essentially the same interpretation of the initial phase of the color reaction has been advanced more recently by Karrer, *Helv. Chim. Acta*, **22**, 1146 (1939).

(17) Ansbacher and Fernholz, THIS JOURNAL, **61**, 1924 (1939).

(18) Thayer, Cheney, Binkley, MacCorquodale and Doisy, *ibid.*, **61**, 1932 (1939).

(19) Almquist and Klose, *ibid.*, **61**, 1923 (1939).

(20) Tishler and Sampson, *ibid.*, **61**, 2563 (1939).

(21) Fernholz and Ansbacher, *Science*, **90**, 215 (1939).

(22) Thayer, Binkley, MacCorquodale, Doisy, Emmett, Brown and Bird, THIS JOURNAL, **61**, 2563 (1939).

(23) Almquist and Klose, *ibid.*, **61**, 2557 (1939). Since this paper was written Almquist and Klose, *J. Biol. Chem.*, **130**, 787 (1939), have observed high potency in the compound when administered orally.

the high potency shown in certain of the chick-assays and for the evident variability of the results is suggested by the present observation that methylnaphthoquinone in the reduced form is a component in a simple synthesis of vitamin K_1 which can be conducted in the laboratory at a moderate temperature and with a mildly active catalyst. It is conceivable that the material administered orally in the assays merely provides one component for a similar synthesis in the organism. The substance in the reduced form may combine with phytol or similar component, available to a greater or less extent according to the nature of the diet and the technique of the assay, giving vitamin K_1 or a related compound. A given amount of the simple quinone could then give rise to as much as 2.6 times its weight of active vitamin, which might account for an apparently greater activity on the weight basis.

The author is indebted to various workers in the Merck Laboratories for their coöperation in conducting the bio-assays and for spectrographic determinations and analyses kindly carried out after the summer closing of the Harvard Laboratory.

Experimental Part[24]

Synthesis of 2-Methyl-3-phytyl-1,4-naphthoquinone.—The procedure found most satisfactory is illustrated by the following experiment. A mixture of 5 g. of 2-methylnaphthohydroquinone, 1 g. of powdered anhydrous oxalic acid, 1.48 g. of phytol, and 15 cc. of dioxane (purified with sodium) was warmed to 75° and shaken to bring the solid into solution. The flask was closed with a stopper carrying a thermometer and heated on a hot-plate for thirty-six hours at 75° (±3–4°). During this time the initially light orange or orange-red solution becomes deep red. The solution was cooled, washed into a separatory with ether, diluted with water, and the mixture extracted with ether. The red ethereal solution was separated, washed with a fresh portion of water, and then extracted with successive 50-cc. portions of 2% potassium hydroxide in which sodium hydrosulfite was dissolved just before use (1–2 g. per 100 cc.). Shaking with the first portion of alkali-hydrosulfite should result in the discharge of the red color and the appearance of a bright yellow alkaline layer; more hydrosulfite is added if the red color persists, while more alkali is used in case the yellow color does not appear. The mixture often forms a rather persistent emulsion and in this case the funnel is kept stoppered until the layers separate in order to avoid oxidation by the air. In the present experiment the unchanged methylnaphthohydroquinone responsible for the yellow vat was nearly all removed in three extractions, and two more were made to eliminate traces of the substance, the fourth being weakly yellow and the fifth colorless. The alkaline extracts were run into a large separatory funnel containing about 50 cc. of ether to protect the solution from oxidation, and after collecting the total yellow liquor in this way it was shaken with the ether and the yellow layer run into another separatory funnel containing 75–100 cc. of ether. Sufficient acetic acid was then added to discharge the yellow color and the liberated methylnaphthohydroquinone was extracted into the ether. The aqueous layer was extracted with a fresh portion of ether and the total extract was dried with magnesium sulfate and evaporated nearly to dryness. The residual cake of hydroquinone was covered with petroleum ether, broken up, collected, and washed with petroleum ether. The recovered material was obtained as a colorless or slightly gray crystalline powder suitable for further use and weighing 3.55 g.

The weakly yellow ethereal solution remaining after extraction with alkali was washed with water and with a small volume of saturated sodium chloride solution, dried with magnesium sulfate and the solvent evaporated, eventually in vacuum, leaving 2.00 g. of a light orange-brown oil. When cooled and rubbed, this had the consistency of a soft wax, and on stirring with 30–50 cc. of petroleum ether (b. p. 20–40°) a fine white solid separated. Lumps of brown wax were disintegrated by rubbing and by digesting the mixture at the boiling point, and the pasty, white suspension was then transferred to centrifuge tubes and cooled in ice. On centrifugation the 2-methyl-3-phytyl-1,4-naphthohydroquinone separated as a white, somewhat waxy, and fairly voluminous sludge. The brownish supernatant liquor was decanted and the solid stirred with a rather liberal quantity of fresh solvent, cooled, and centrifuged, when the liquor was definitely but faintly colored. Evaporation of the combined petroleum ether liquors and washings gave 1.29 g. of residual, dark brown oil.

When the petroleum ether paste of the hydroquinone is covered with a little fresh solvent it may be manipulated or kept for several hours without protection from the air. Two digestions as above seem to give a very pure product, but the substance is so sparingly soluble in cold petroleum ether that the process can be repeated with little loss. The hydroquinone can be dissolved in hot ligroin but separates on cooling as a fine powder which is not easily retained on a filter paper.

For conversion to the quinone the petroleum ether sludge was dissolved in a little dry ether (very soluble) and the colorless solution shaken for twenty minutes with excess silver oxide (1 g.) and magnesium sulfate. The clear yellow solution was evaporated on the hot-plate and the last traces of ether removed in vacuum. (A brownish color observed during the filtration or on evaporation is due to the presence of quinhydrone and indicates incomplete oxidation.) 2-Methyl-3-phytyl-1,4-naphthoquinone was left as a residue in the form of a pure yellow, heavy, but rather mobile oil; yield 0.65 g. (29%, based on phytol). The sample for analysis was dried at 78° and 2 mm. pressure.

Anal. Calcd. for $C_{31}H_{46}O_2$: C, 82.61; H, 10.29. Found: C, 82.76; H, 10.53.

The results of a study of the effect of various changes in the proportions and conditions are given in Table I.

(24) Microanalyses by Lyon Southworth and Herbert S. Wight.

TABLE I

SUMMARY OF YIELDS

Condensation of 1.48 g. (0.005 *m.*) of phytol, or indicated multiple, in dioxane: 10 cc. for 0.86 g. = 0.005 *m.* of the hydroquinone, 15 cc. for larger amounts.

Methylnaphtho-hydroquinone, g.	Condens. agent	Temp., °C.	Time, hrs.	Recov. starting material, g.	Total oil, g.	Residual oil, g.	Yield of quinone, g.
0.86	1 g. $(CO_2H)_2$	Refl.	22	..	1.65	..	0.0
.86	1 g. $(CO_2H)_2$	75	18	0.21	..	1.47	.20
.86	1 g. $(CO_2H)_2$	75	41	.1	1.67	1.49	.11
3	1 g. $(CO_2H)_2$	75	18	1.8	1.97	1.54	.36
3	3 g. $(CO_2H)_2$	75	18	1.92	1.97	1.62	.32
5	1 g. CCl_3CO_2H	75	24	..	1.98	1.40	.53
5[a]	1 g. $(CO_2H)_2$	75	25	3.51	1.96	1.33	.62
5	1 g. $(CO_2H)_2$	75	27	3.56	1.96	1.29	.60
5	1 g. $(CO_2H)_2$	50	27	4.01	1.48	..	.00
5-Fivefold[b]	1 g. $(CO_2H)_2$	75	31	3.35	2.08	1.45	.58
5	1 g. $(CO_2H)_2$	75	36	3.55	2.00	1.29	.65
5-Fourfold	1 g. $(CO_2H)_2$	75	36	3.42	1.98	1.30	.64
5	1 g. $(CO_2H)_2$	75	69	3.72	1.94	1.52	.40

[a] With the addition of 4 g. of anhydrous magnesium sulfate. [b] Exposed to bright sunlight in the daylight hours.

At 50° there was no evidence of reaction and the mobile oil left on extraction with alkali seemed to be unchanged phytol. In the experiment conducted at the reflux temperature the product was a viscous brown oil which gave only a trace of solid when cooled in petroleum ether solution and which evidently consisted very largely of the naphthotocopherol. At the intermediate temperature of 75° the reaction proceeds satisfactorily and the optimum time seems to be about thirty-six hours. The results show that a too prolonged reaction period results in a marked drop in the yield (formation of the naphthotocopherol) and that a large excess of methylnaphthohydroquinone is definitely advantageous. The addition of a drying agent or a threefold increase in the amount of condensing agent does not materially influence the results, and trichloroacetic acid seems to be about as effective as anhydrous oxalic acid. No differences were observed on conducting the condensation on a four- or five-fold scale, or on exposing the solution to bright August sunlight in a Pyrex Erlenmeyer flask. In the latter experiment the solution was flushed with nitrogen at 75° before stoppering the flask.

Properties of the Quinone.—Synthetic 2-methyl-3-phytyl-1,4-naphthoquinone is very soluble in petroleum ether, moderately soluble in acetone, and sparingly soluble in methyl or ethyl alcohol; the solutions are pure yellow. The substance can be caused to separate from acetone in fluffy yellow crystals by cooling in a bath of solid carbon dioxide and rubbing. There was no evidence of decomposition on evaporation of ethereal solutions of the quinone to dryness on the hot-plate, even at atmospheric pressure, or in manipulating the substance in the diffused light of the laboratory, although undue exposure to light was always avoided. On overnight distillation of a 0.7-g. sample with a mercury vapor pump at 150–180° some decomposition occurred, for the distillate was dark reddish-yellow. As initially prepared the quinone has a pure, light yellow color, and a useful test of purity consists in shaking a yellow ethereal solution of the substance with aqueous sodium hydrosulfite. With vigorous shaking the yellow color is slowly discharged and both layers become colorless.

On shaking the quinone with equal volumes of petroleum ether and 90% aqueous methanol, the yellow color seemed to be retained completely in the hydrocarbon layer. Such a distribution was tried as a possible method of isolating the substance from the reaction mixture in the above synthesis. After removal of phenolic starting material the solution was shaken with silver oxide and the total oil (1.91 g.) taken into petroleum ether (40 cc.) and extracted with eight 40-cc. portions of 90% methanol. The extracts were all weakly yellow and when tested by warming with an equal volume of 10% potassium hydroxide the first and second were negative, the third intermediate, and the remainder gave an identical positive response consisting in the appearance of a clear light purple color persisting for one or two minutes. The yellow petroleum ether layer retained 1.41 g. of material which gave a strong but rather dull color test; when shaken with ether–hydrosulfite the color faded slightly to a straw-yellow, and on separation of the hydroquinone from petroleum ether and oxidation there was obtained 0.16 g. of the pure quinone (under the conditions used the total yield should be 0.65 g.). The material collected from the fourth to eighth methanol extracts by back extraction with petroleum ether was a light yellow oil (0.19 g.), but it gave only a weak and dull color with alcoholic alkali and the yellow color was not completely discharged on shaking with ether–hydrosulfite. It therefore appears that while a certain amount of contaminant (phytol?) is removed in one or two distributions between petroleum ether and 90% methanol, the latter solvent then extracts some other (yellow) impurity (naphthotocopherol?) along with small amounts of the quinone.

Dam–Karrer Color Reaction.—The following tests were made with a 10% solution of potassium hydroxide in 95% ethanol. A solution of 1–2 mg. of the quinone in 1 cc. of this solvent when treated with 1–2 drops of the alkali in the cold slowly developed a light purple color (three minutes) which became stronger on warming and then faded in one or two minutes to dull red. On treating a similar solution of the quinone with an equal volume of the alcoholic alkali, a clear intense indigo-blue color appeared

almost at once in the cold. This changed to purple in about one minute and in about three minutes more the dull red end stage had been reached.

In a typical exploratory cleavage experiment 0.62 g. of the quinone was dissolved in 30 cc. of alcohol by warming to about 40° and the solution was treated with 12 cc. of alcoholic potassium hydroxide solution. The solution became indigo blue at once, cloudy (one minute), dull purple (two to three minutes), and then dull red (five minutes). It was diluted, giving a cloudy precipitate, extracted with ether, and the orange-red aqueous layer separated from the brown ethereal solution and acidified. The brown-yellow solution was extracted with ether and the latter shaken with soda solution; the light red extract was acidified and the liberated material taken into ether. Evaporation gave 30–40 mg. of an orange-yellow solid, which was extracted with successive portions of boiling ligroin (b. p. 60–90°). On clarifying and concentrating the solution there was obtained a small amount (2–3 mg.) of a yellow crystallizate melting at 166–168°. The results were not as good when less alkali was used. On conducting the reaction at 0° the indigo-purple phase persisted for about ten minutes. When the alkali was added to the quinone solution at the boiling point the color changes were soon over; the yield of yellow pigment was lower and the bulk of the quinone seemed to be converted into a neutral, bright red oil (isomerization product?).

The combined crude cleavage product from several experiments was further purified by solution in soda and precipitation from the clarified deep cherry-red solution. The crystalline product was then sublimed at 90–105° (2 mm.) and obtained as pure yellow microcrystals, m. p. 170–171°. A mixture of this with a sample of **phthiocol**, m. p. 171–172°, from the collection of Samuel C. Hooker[25] melted at 171.5–172.5°.

Anal.[26] Calcd. for $C_{11}H_8O_3$: C, 70.21; H, 4.26. Found: C, 70.33; H, 4.29.

2-Methyl-3-phytyl-1,4-naphthohydroquinone Diacetate.—For reductive acetylation 0.50 g. of the synthetic quinone was suspended in 5 cc. of acetic anhydride and treated with 0.5 g. of zinc dust and then, while cooling in ice, with 5 drops of pyridine. A part of the quinone was reduced at once but some adhered to the walls and formed a mat with the zinc. On working this with a spatula under ice cooling the oil soon dissolved to an almost colorless solution leaving the zinc as a clean powder. After ten minutes in the ice-bath and one-half hour at room temperature, acetic acid was added and the mixture boiled and filtered and the residue extracted with acetic acid and with water. After further dilution with water the filtrate was warmed for ten minutes, cooled, and the oily product extracted with ether. After washing with acid, alkali, and water, the solution was dried and evaporated and gave 0.55 g. of the diacetate as a very faintly yellow, viscous oil. An earlier sample prepared in this way and dried at 80° and 2 mm. was analyzed with the following results.

Anal. Calcd. for $C_{35}H_{52}O_4$: C, 78.31; H, 9.77. Found: C, 78.43; H, 10.01.

Even when in a good and analytically pure condition the diacetate often is slow to crystallize. The most successful expedient found was to dissolve the oil in a rather liberal quantity of methanol, cause some of the material to separate as an oil by cooling (well below 0° if necessary), and rub this thoroughly with a stirring rod. Once the oil is obtained in a somewhat waxy condition, solidification usually occurs after standing for a time at room temperature and is further promoted by rubbing and cooling. The first crystallizate was obtained on exposure of an alcoholic solution containing suspended oil to bright sunlight for a few hours in a Pyrex flask, but it now seems likely that the prior rubbing was of more significance than the irradiation. The sample in question melted at 57–59° and the m. p. was the same on forced crystallization from methanol (Found: C, 78.34; H, 9.87). In the experiment referred to above the material crystallized within a few minutes without irradiation and on merely rubbing with methanol and cooling under the tap. The first crop (0.23 g.) softened at 56° and melted at 59.5° to a clear liquid, the behavior resembling that of camphor. Recrystallization from methanol was not very satisfactory, giving a white powder, m. p. 59–60°, and 95% ethanol was used in the further purification with better success. On seeding a not too concentrated alcoholic solution at room temperature and allowing the solution to stand without external cooling, the diacetate slowly formed cottony clusters of fine needles, m. p. (camphor-like) 60.5–63°. The melt solidified within a few hours and remelted sharply at 60–60.5°. After a further crystallization the material had the same appearance and m. p. characteristics. When heated extremely slowly, the sample melted at 60–61.5°; it remelted at 60–60.5°. While the temperature of first remelting seems significant, the m. p. becomes slightly lower on repeating the process, and if the sample is heated to 160° it fails to solidify in the tube.

In duplicate determinations with the best sample of the diacetate, Dr. R. N. Jones found for the extinction coefficient at 230 mμ (absolute ethanol, per cent. by volume) the values $E^{1\%}_{1\,cm.}$ = 1770, 1780. Doisy reports a much lower coefficient (1250,[4] 1300, or 1600[5]). It may be noted that the extinction coefficient for the quinone found both in this Laboratory[1] and by Dr. Webb ($E^{1\%}_{1\,cm.,\,248\,m\mu}$ = 386) agrees with Doisy's first value (385[3]) but not with that more recently reported (540[4]).

The **dibenzoate** was prepared by adding pyridine to a petroleum ether sludge of the hydroquinone, removing the hydrocarbon solvent in vacuum, and adding benzoyl chloride. After standing overnight water was added and the product extracted with ether. The solution was washed well with dilute acid, 10% alkali and water and afforded a rather yellow, oily product on evaporation. After various trials the dibenzoate was obtained crystalline from alcohol, exposing the solution to direct sunlight, and melted at 85–86°. Recrystallization from methanol gave slightly waxy white nodules of the same m. p. and the small sample was not purified further.

Anal. Calcd. for $C_{46}H_{56}O_4$: C, 81.77; H, 8.54. Found: C, 81.63; H, 8.59.

2,6-Dimethyl-3-phytyl-1,4-naphthoquinone (M. D. Gates, Jr.).—The 2,6-dimethyl-1,4-naphthohydroquinone required as starting material was prepared by reduction of the quinone (11.5 g.) in suspension in alcohol (110 cc.)

(25) S. C. Hooker, THIS JOURNAL, **58**, 1174 (1936).

(26) Microanalysis conducted at the Merck Research Laboratory.

with stannous chloride (45 g.)–hydrochloric acid (50 cc.); water (100 cc.) was added, the solution was boiled with Norite, filtered (with the addition of 50 cc. of alcohol), and diluted with 100 cc. of water. The product separated as fine, slightly pink needles; yield 9.9 g. (85%). A solution of 4 g. of this material, 1.48 g. of phytol, and 1 g. of anhydrous oxalic acid in 15 cc. of dioxane was heated at 70–80° for twenty-four hours. The reaction mixture was worked up exactly as described in the above example, the recovered hydroquinone amounting to 2.37 g. and the yield of quinone being 0.54 g. In another experiment using 5 g. of starting material the yield was 0.59 g. The quinone was obtained as a viscous yellow oil having a slight reddish tinge. The Dam–Karrer color test is the same as with the lower homolog.

Anal. Calcd. for $C_{32}H_{48}O_2$: C, 82.70; H, 10.41. Found: C, 82.53; H, 10.44.

The **hydroquinone diacetate,** prepared as above, was obtained as a solid without difficulty from methanol, but the substance did not form good crystals from either methanol or ethanol. It separated as gelatinous masses which shrank to hard, solid granules on drying. When heated at the ordinary rate the substance melted at 55–56.5°.

Anal. Calcd. for $C_{36}H_{54}O_4$: C, 78.50; H, 9.88. Found: C, 78.84; H, 9.88.

2-Methyl-3-geranyl-1,4-naphthoquinone.—On heating 2 g. of methylnaphthohydroquinone, 1.13 g. of geraniol, 1 g. of anhydrous oxalic acid, and 10 cc. of dioxane at approximately 84° for twenty-four and one-half hours and working up the mixture by the above procedure, 1.0 g. of starting material was recovered and on adding petroleum ether to the alkali-insoluble oil the substituted hydroquinone separated as a voluminous white gel. The precipitated material was so gelatinous that removal of the impurities in the mother liquor was difficult. The substance was stirred and centrifuged with three additional portions of petroleum ether, but probably still retained impurities, as seen from the analysis below. Oxidation with silver oxide gave 0.26 g. of the quinone as a viscous yellow oil tinged slightly with red. A solution of the quinone in alcohol when treated with an equal volume of 10% alcoholic potassium hydroxide gave an intense indigo-blue solution changing to purple and then dull red. Yellow crystals resulted on cooling a methanol solution to −70°.

Anal. Calcd. for $C_{21}H_{24}O_2$: C, 81.78; H, 7.85. Found: C, 81.22; H, 8.01.

A parallel experiment was carried out with the quantities given above but replacing the geraniol by an equivalent quantity of myrcene (1.00 g.). The mixture was kept at 90–95° for twenty-four hours. Extraction with dilute alkali gave 1.31 g. of recovered starting material and left 1.0 g. of dark oil. The latter afforded a very small quantity of substituted hydroquinone as a petroleum ether sludge and on oxidation this afforded 79 mg. of the quinone as a yellow (slightly dark) oil (Found: C, 81.16, 81.24; H, 7.88, 7.99).

2-Ethyl-3-phytyl-1,4-naphthoquinone.—A mixture of 4.8 g. of 2-ethyl-1,4-naphthohydroquinone, 1.48 g. of phytol, 1 g. of anhydrous oxalic acid, and 15 cc. of dioxane was heated at 75° for twenty-four hours, diluted with water, extracted with ether, and the unchanged hydroquinone removed as usual. A brown oil (1.85 g.) of the usual appearance was obtained from the alkali-insoluble fraction, but this dissolved readily in petroleum ether and the substituted hydroquinone failed to separate even on cooling well below 0°. A partial purification was accomplished by shaking this in ether with silver oxide and magnesium sulfate and extracting a petroleum ether solution of the oxidized product with two portions of 90% methanol. Evaporation of the petroleum ether layer gave 1.37 g. of a rather reddish oil giving with alcoholic alkali a strong greenish-blue color changing to purple and then dull red. A fairly sharp separation finally was made by the extraction procedure described in detail below as applied to the isolation of vitamin K_1. Following the same procedure, 1.21 g. of the reddish oil was reduced in alcoholic suspension and a petroleum ether solution of the reduced material extracted with Claisen's alkali and hydrosulfite. On dilution of the yellow liquor with water, extraction with ether, and evaporation there was obtained 0.28 g. of crude substituted hydroquinone as a brownish oil which still failed to yield a solid from petroleum ether and which consequently was oxidized with silver oxide to the quinone (0.27 g.). This was obtained as a yellow (slightly dark) oil giving, when warmed with 5% alcoholic potassium hydroxide, an intense indigo-blue changing to purple and then dull red. On reductive acetylation it gave a clear, nearly colorless oil which failed to crystallize from methanol.

Isolation of Natural Vitamin K_1.—The starting material supplied by Dr. Riegel was described by him as an alfalfa concentrate containing 3–5% vitamin. In the Dam–Karrer test it gave a pale but distinct transient purple in dilute solution and a quite strong purple at higher concentrations. A 5.32 g. portion of the viscous yellow oil was suspended in 90 cc. of alcohol at room temperature, a fresh solution of 4.5 g. of sodium hydrosulfite in 30 cc. of water was added, the flask was stoppered, and the mixture containing oil droplets and precipitated salts was shaken vigorously for ten minutes. After adding 50 cc. of water to dissolve the salts the mixture was shaken again for ten minutes.[27] The suspension of light yellow oil was diluted further and extracted with about 100 cc. of petroleum ether and the weakly yellow solution was shaken thoroughly with 5% potassium hydroxide containing 1–2% of hydrosulfite, the colorless aqueous layer being separated and discarded (no precipitate with acetic acid). The petroleum ether solution was then extracted with 50 cc. of Claisen's alkali[28] to which 3 cc. of saturated aqueous hydrosulfite had been added, the alkaline liquor acquiring a bright yellow color characteristic of the salt of the vitamin hydroquinone. The solution is cloudy and apparently contains suspended salt, but a fairly clean separation is possible. The yellow alkaline layer was drawn off into a separatory funnel containing 50 cc. of ether, and two further extractions were made, the last being only pale yellow. The unextracted material recovered from the petroleum ether was a yellow oil weighing 4.61 g. The total alkaline liquor was shaken with the covering layer of

(27) When the pure synthetic vitamin is reduced by the above procedure the hydroquinone separates at this point as a white solid.

(28) Prepared from 35 g. of potassium hydroxide in 25 cc. of water, diluted to 100 cc. with methanol: Claisen, *Ann.*, **418**, 96 (1919).

ether, adding a little petroleum ether to facilitate the settling of the (colorless) ether layer. (If darkening occurs at any time in the ether layer due to oxidation, more hydrosulfite is added.) The washed yellow liquor was then run into another separatory funnel containing 50 cc. of ether and diluted with 2–3 volumes of 2–4% aqueous hydrosulfite solution (the hydroquinone is particularly sensitive to oxidation when in contact with aqueous alcohol). The dilution results in liberation of the free hydroquinone with disappearance of the yellow color, and on shaking the mixture the substance passes into the ether layer to give a pale yellowish solution. This was separated, washed with aqueous hydrosulfite solution and with water, dried and evaporated. The residual pale reddish-brown oil (0.17 g.) became waxy on cooling, and on adding 10 cc. of petroleum ether the vitamin hydroquinone separated at once as a fine white solid. The suspension was iced and centrifuged and the white sludge stirred with fresh solvent and the process repeated. The purified material was taken up in dry ether and shaken for one-half hour with silver oxide and magnesium sulfate. The filtered yellow solution on evaporation, eventually under vacuum, left a residue of 60 mg. of vitamin K_1 in the form of a pure yellow, rather mobile oil.

Anal.[26] Calcd. for $C_{31}H_{46}O_2$: C, 82.61; H, 10.29. Found: C, 82.64; H, 10.20.

The behavior in the Dam–Karrer test was exactly as described for the synthetic material.

Vitamin K_1 hydroquinone diacetate was obtained by reductive acetylation of 35 mg. of the quinone by the pyridine procedure and obtained initially as a colorless oil (40 mg.). After suitable rubbing in methanol (one hour) this formed waxy, semi-solid masses, and on standing overnight at room temperature with exposure to the morning sun solid particles appeared. On rubbing, the wax all went to a good white powder which was collected after cooling and afforded 6 mg. of material melting at 57–59.5° (camphor-like) and remelting at 57–58.5°. Recrystallized from 95% ethanol the substance formed compact clusters of microneedles, m. p. 58.5–60°.

Anal.[26] Calcd. for $C_{35}H_{52}O_4$: C, 78.31; H, 9.77. Found: C, 78.13; H, 10.11.

A mixture of this substance with the best sample of synthetic 2-methyl-3-phytyl-1,4-naphthohydroquinone diacetate melted at 59.5–61.5°. The melt solidified in about one hour and remelted sharply at 59.5–60°. Dr. E. A. Doisy kindly compared the synthetic diacetate with a sample of the natural vitamin K_1 hydroquinone diacetate prepared in his laboratory (m. p. 61.5–63°) and found that a mixture of equal parts melted at 59.5–61.5°.

Summary

2-Methyl-3-phytyl-1,4-naphthoquinone has been synthesized and found identical with natural vitamin K_1 from alfalfa by a direct comparison of samples with regard to analysis, spectrum, antihemorrhagic activity, color reaction, and the melting point and mixed melting point of a crystalline derivative.

The synthesis is an essentially one-step process utilizing 2-methyl-1,4-naphthohydroquinone and phytol and provides a practical method of preparing the vitamin in quantity. By separating and purifying the product in the reduced form prior to oxidation, the quinone is obtained in a pure condition without recourse to distillation or adsorption. Natural vitamin K_1 can be isolated very easily from alfalfa concentrates by a similar procedure.

Vitamin K_1 yields phthiocol on cleavage with alcoholic alkali.

The 2-ethyl-3-phytyl and 2,6-dimethyl-3-phytyl derivatives of 1,4-naphthoquinone were synthesized and found inactive in the chick-assay, while 2-methyl-3-geranyl-1,4-naphthoquinone shows moderate potency. Indications of a certain structural specificity are discussed and a tentative hypothesis is suggested concerning the action of 2-methyl-1,4-naphthoquinone.

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