VITAMIN K ACTIVITY AND STRUCTURE

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(Received for publication, September 24, 1940)

This paper presents the results of assays for antihemorrhagic activity carried out on an array of naphthoquinones and related compounds synthesized as described in a series of previous papers. Some of the compounds were prepared as synthetic models of the structural types postulated (1) for vitamins K₁ and K₂ (1-5); others were synthesized subsequent to the establishment of the structure of vitamin K₁ (6-9) in a study of the relationship of vitamin K activity and structure (10-15). Preliminary bioassay results reported at the outset of the investigations (1, 2) were subsequently recognized as uncertain (3), and judgment was reserved until the assay procedure could be more fully explored. Later reports have included purely preliminary indications of the degree of antihemorrhagic potency encountered in some of the new compounds examined, and the results given in the present paper supersede all previous data.

Assay Method

The assay procedure is a modification of the short curative method described by Ansbacher (16) and is similar to the 18 hour method of Thayer et al. (17). Day-old chicks were placed in electrically heated brooders and fed the vitamin K-free diet of Almquist (18-20). Clean water was supplied twice daily and the brooder trays were removed and cleaned daily. After 12 days a trial bleeding was made on ten or twelve birds selected at random from the group. Approximately 0.5 cc. of blood from the brachial vein was collected in a small glass vial which was placed immedi-
ately in the device for rocking at constant temperature described by Almquist and Klose (20). The blood samples were examined at half minute intervals and the time of coagulation determined. When 90 per cent of the birds taken for trial bleeding showed coagulation times of 60 minutes or more, the entire group was considered ready for assay purposes. The birds employed in the preliminary bleeding tests were not used in the assays, since birds so bled frequently have been observed to show a marked reduction in the clotting time within the next 18 hours without any treatment with antihemorrhagic substances. No chicks over 21 days old were employed in the assays.

Except in the case of certain water-soluble compounds, the material to be assayed was dissolved in peanut oil and the solution diluted with the same solvent to such an extent that the quantity of substance to be fed was always contained in exactly 0.1 cc. of the solution. This precaution was taken in order to avoid irregularities in response due to the use of varying volumes of solvent, as observed by Ansbacher (16). After administration of the dose into the crop the birds were held for 18 hours without access to food but with a supply of clean water and the coagulation time of whole blood was then determined. Our criterion for the effective dose is the minimum amount of material which, when administered in 0.1 cc. of peanut oil, will reduce the clotting times of 60 to 80 per cent of the vitamin K-deficient birds to less than 10 minutes in the 18 hour period. Ten or more chicks were used at each dosage level, and with every assay there was included a group of untreated control birds and three groups receiving, respectively, 0.1 \( \gamma \), 0.3 \( \gamma \), and 0.5 \( \gamma \) of standard 2-methyl-1,4-naphthoquinone. The latter three groups were included to control possible variation in the degree of deficiency among different lots of chicks. The substance is initially given at a level deemed likely to be ineffective and then at higher levels.

Various units for vitamin K activity have been proposed with dried spinach or alfalfa, extracts of alfalfa, or the more recently suggested 2-methyl-1,4-naphthoquinone (21) or 2-methyl-1,4-naphthohydroquinone diacetate (22, 23) as the reference standard. Pending general acceptance of a reference standard, we have chosen to report our findings in terms of the effective dose in micrograms, but, since 2-methyl-1,4-naphthoquinone has been employed throughout as a comparison control, our results can be
referred to this substance as a standard. Although some minor variations have been observed, we have found that with the large majority of the groups of deficient chicks investigated 0.3 $\gamma$ of 2-methyl-1,4-naphthoquinone meets our criterion for the effective dose.

Our most reliable estimate of the minimum dose of pure vitamin K$_1$ (synthetic) by the above procedure is 1 $\gamma$, and hence our results indicate that 2-methyl-1,4-naphthoquinone is 3.3 times as potent as the vitamin in the chick assay. Almquist and Klose (24) found the ratio 3.8:1, Emmett, Brown, and Kamm (25) found 2.2:1, and Dam, Glavind, and Karrer (23) found 2.1:1. Ansbacher, Fernholz, and MacPhillamy (26) report a ratio of 4:1 between the potencies of the two compounds when assayed by the 18 hour procedure, but state that the ratio was 30:1 in the 6 hour assay. We have compared the compounds repeatedly by the shorter method without observing any such disparity in the results; by the 6 hour method we find the effective doses of vitamin K$_1$ and 2-methyl-1,4-naphthoquinone to be 1.5 $\gamma$ and 0.5 $\gamma$, respectively. Thayer et al. (21) likewise found no significant difference in the ratio of potencies of the two compounds after assay periods of 6, 18, and 72 hours. To test the possible influence of the solvent medium, we assayed vitamin K$_1$ by the 6 and 18 hour methods in cod liver oil, sesame oil, and corn oil, and the results were substantially the same as described for peanut oil.

2,3-Disubstituted Naphthoquinones

The following discussion will be limited to a consideration of the features of structural specificity encountered among substances of pronounced antihemorrhagic activity. An action so feeble as to be manifested only at a dosage of 1000 $\gamma$ or more is considered to have little biological significance. The key point of interest is vitamin K$_1$, or 2-methyl-3-phytyl-1,4-naphthoquinone (I), and a
first group of compounds for comparison with this substance embraces those 2-methyl-1,4-naphthoquinones which carry at position 3 some hydrocarbon radical in place of the phytol group. Table I lists assay results for a series of such compounds and references to papers describing their synthesis and characterization as to purity.

Comparison with results from other laboratories can be made in two cases. Karrer and Epprecht (27) report an assay result by Dam for the $\beta,\gamma$-dihydride of vitamin K$_1$ (II) which diverges considerably from ours. Dam found the compound to be only $1/24$ as active as the vitamin (23), while we find it to possess $1/8$ the potency of the $\beta,\gamma$-unsaturated substance. Karrer and Epprecht prepared the quinone by oxidation of the hydrocarbon and isolated a "practically pure" sample from the reaction mixture by chromatographic adsorption. Our material was prepared by hydrogenation of synthetic vitamin K$_1$, and contamination with

**Table I**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effective dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methyl-3-phytyl-1,4-naphthoquinone (8, 9)</td>
<td>7</td>
</tr>
<tr>
<td>2-Methyl-3-farnesyl-1,4-naphthoquinone (11, 14)</td>
<td>5</td>
</tr>
<tr>
<td>2-Methyl-3-((\beta,\gamma)-dihydrophytyl)-1,4-naphthoquinone (11, 15)</td>
<td>8</td>
</tr>
<tr>
<td>2-Methyl-3-geranyl-1,4-naphthoquinone (9)</td>
<td>25</td>
</tr>
<tr>
<td>2-Methyl-3-cinnamyl-1,4-naphthoquinone (4)</td>
<td>25</td>
</tr>
<tr>
<td>2-Methyl-3-((\beta,\gamma,\gamma)-trimethylallyl)-1,4-naphthoquinone (4)</td>
<td>50</td>
</tr>
<tr>
<td>2,3-Dimethyl-1,4-naphthoquinone</td>
<td>50</td>
</tr>
<tr>
<td>2-Methyl-3-benzyl-1,4-naphthoquinone (4)</td>
<td>200</td>
</tr>
<tr>
<td>2-Methyl-3-hydrocinnamyl-1,4-naphthoquinone (15)</td>
<td>300</td>
</tr>
</tbody>
</table>

1/24 as active as the vitamin (23), while we find it to possess 1/8 the potency of the $\beta,\gamma$-unsaturated substance.
unreduced starting material is possible but seems unlikely in view of the completely negative color test with alcoholic alkali (15). In the case of this and other liquid quinones we feel a certain confidence in the freedom of our samples from incidental impurities and products of cyclization because of the use of a highly effective method of purification through the solid hydroquinones (8, 9). The effect of saturation of a $\beta,\gamma$ double bond has been studied in two other instances and the loss in potency found is of the same order of magnitude as is indicated by our results for the above case. 2-Methyl-3-cinnamyl-1,4-naphthoquinone and its $\beta,\gamma$-dihydride (Table I) constitute a favorable pair for comparison, since they are both crystalline and easily purified, and the latter is found to be 1/12 as potent as the former. In the case of 2-phytyl-1,4-naphthoquinone (Table III), saturation of the double bond of the side chain is attended also with a 12-fold decrease in activity.

Whether the true ratio of the potency of the hydride to that of the vitamin is closer to 1:8 or 1:24, it is clear that the presence of the $\beta,\gamma$ double bond is important for maintenance of high potency. The first six quinones in Table I all possess this significant structural feature, and the 2-methyl-3-benzyl compound has the weakly unsaturated phenyl group in the corresponding position of the side chain.

Fernholz, Ansbacher, and MacPhillamy (28) assayed 2,3-dimethyl-1,4-naphthoquinone by their 6 hour method and their results place the compound in much the same position with respect to vitamin $K_1$ and to methylnaphthoquinone as do ours. Among the compounds listed in Table I the position of 2,3-dimethyl-1,4-naphthoquinone appears rather anomalous, for this simplest member of the series of 2,3-dialkyl compounds possesses neither a long side chain nor a $\beta$-unsaturated center but nevertheless exhibits activity of a fairly high degree.

In the series of 3-$\beta$-alkenyl compounds the size of the group appears to be a factor of definite significance. The activity bears no proportionality to molecular weight, but decreases in the order of the size of the group, as shown in Table II. The additional double bonds present in the farnesyl and geranyl derivatives evidently are of little influence. It is of interest that the quinone containing the phytol group (vitamin $K_1$) would show a still greater superiority in potency over the other compounds if com-
Vitamin K Activity and Structure

Comparison were made on a molar rather than on a weight basis. Vitamin K\textsubscript{2} (29), which according to the findings of Doisy and coworkers (30) is probably 2-methyl-3-difarnesyl-1,4-naphthoquinone, is reported to possess from 60 (29) to 67 per cent (23) of the activity of vitamin K\textsubscript{1}. Since the molecular weight is 1.3 times that of vitamin K\textsubscript{1}, the activity per mole is within about 82 per cent of that of vitamin K\textsubscript{1}. In the series of isoprenoid derivat-

### Table II

<table>
<thead>
<tr>
<th>3-Substituent</th>
<th>Molecular weight of group</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{CH}_3)</td>
<td>279</td>
<td>1</td>
</tr>
<tr>
<td>(-\text{CH}_2\text{CH}=\text{CCH}_2\text{CH}_2\text{CH}=\text{CCH}_3) (farnesyl)</td>
<td>205</td>
<td>1/5</td>
</tr>
<tr>
<td>(-\text{CH}_2\text{CH}=\text{CCH}_2\text{CH}=\text{CCH}_2\text{CH}=\text{CCH}_3) (geranyl)</td>
<td>137</td>
<td>1/25</td>
</tr>
<tr>
<td>(-\text{CH}_2\text{CH}=\text{CCH}_3\text{H}_5) (cinnamyl)</td>
<td>127</td>
<td>1/25</td>
</tr>
<tr>
<td>(-\text{CH}_3\text{C}=\text{CCH}_3) (trimethylallyl)</td>
<td>83</td>
<td>1/50</td>
</tr>
</tbody>
</table>

The branched chain isoprenoid structure of the 3-substituent appears to be a factor favorable to the development of antihemorrhagic activity. Thus the straight chain 2-methyl-3-octadecyl-1,4-naphthoquinone (III) has been synthesized by Karrer and Epprecht (27) and by Fernholz, Ansbacher, and MacPhillamy (28) and found by the latter workers and by Dam to be but weakly
active. The quinone is appropriately compared with the \( \beta,\gamma \)-dihydride of vitamin \( \text{K}_1 \) (II), and Dam's direct comparison (27) indicates that (III) is less than 1/3 as potent as (II). The assays by Fernholz, Ansbacher, and MacPhillamy of (III) and our assays of (II) would both indicate a still greater disparity between the straight and branched chain compounds. The octadecyl derivative is a solid melting at about 100°, whereas (II) is a liquid at room temperature, as are the corresponding phytol, farnesyl, and geranyl derivatives. Vitamin \( \text{K}_2 \) is a solid, if a low melting one. It is interesting that biological potency reaches a high point in the liquid, low melting compounds having a branched chain of a certain optimum carbon content, for the situation is reminiscent of that existing among fatty acids having a leprocidal action and those isolated from the tubercle bacillus, as pointed out by Robinson (31). Another point of interest is that the 2-methyl-3-geranyl and 2-methyl-3-farnesyl compounds exhibit considerable vitamin K potency. These compounds are easily produced by the condensation reaction applicable to the synthesis of vitamin \( \text{K}_1 \) (9), and since geraniol and farnesol are widely distributed in nature these lower isoprenologues of vitamins \( \text{K}_1 \) and \( \text{K}_2 \) may conceivably occur as additional vitamin K factors.

2-Alkynaphthoquinones

The observation that 1,4-naphthohydroquinone condenses very smoothly with phytol, farnesol, or geraniol (11, 14) has provided ready access to a series of monosubstituted naphthoquinones of unambiguous structure differing from the compounds of the foregoing section only in the absence of the methyl group. A substance designated 2-phytyl-1,4-naphthoquinone has been prepared by Karrer and coworkers (32, 33) by another synthesis
and found by Dam (23) to have only 1/240 the activity of vitamin K₁, whereas our material shows 1/50 the potency of the vitamin (Table III). The synthesis of Karrer and coworkers, however, involved establishment of the side chain double bond by a reaction which can proceed in two ways, and these investigators state that

![Diagram]

the material obtained probably was not completely homogeneous but contained the γ,δ-unsaturated isomer. Evidence cited above shows that any such isomerism should detract from the activity of the sample. A mixture of bond isomers would be expected to show a potency somewhere between that of pure (IV) and its β,γ-dihydride, or from 1/600 to 1/50 the activity of vitamin K₁. As a check on our results for (IV) three independent samples were prepared, one at Harvard and two at the Merck laboratories, and assayed at different times; they all evoked essentially the same biological response.

The monosubstituted naphthoquinones having isoprenoid side
chains exhibit the same relationships encountered in the 2-methyl-3-alkyl series. The activity again decreases in the order phytyl > farnesyl > dihydrophytyle > geranyl, and these compounds are 1/50, 1/100, 1/75, and 1/40 as active as the corresponding methylated quinones. The liquid dihydrophytyl compound appears to be somewhat more active than the crystalline 2-n-hexadecyl- and 2-n-octadecyl-1,4-naphthoquinones synthesized and assayed by Fernholz, Ansbacher, and MacPhillamy (28).

Our early report that 2-ethyl- and 2-n-propyl-1,4-naphthoquinones are decidedly less effective than the methyl homologue (34) has been confirmed by others (28, 35), and our present assays indicate that neither compound possesses activity of any biological significance. The 2-allyl compound appears from present indications to be effective at 800 y.

2-Methyl-1,4-naphthoquinone clearly occupies a unique position, for it is some 170 times as potent as the next most active of the monosubstituted naphthoquinones (phytyl) and about 3.1 times (average) as active as vitamin K₁. It has been suggested by one of us (9) that the remarkable potency of methylnaphthoquinone may be due not to the functioning of the compound as such but to its conversion in the organism into a quinone of the true vitamin K type. The simple quinone in the reduced form condenses readily under mild conditions with the naturally occurring phytol, farnesol, and geraniol, and presumably would react with other β-unsaturated isoprenoid alcohols to give antihemorrhagic products. A rapid biosynthesis is thus entirely conceivable. Material given by mouth may combine in the gut with one of the reactive alcohols derived from foodstuffs, while that administered parenterally may be supplied with an isoprenoid side chain by a process of synthesis in the liver. In the latter case the alcoholic component may be vitamin A.

If some such transformation of administered methylnaphthoquinone does occur, a given weight of the simple quinone could give rise to much more than its own weight of a vitamin K type of compound. The ratios of molecular weights are such that 1 part by weight of methylnaphthoquinone is equivalent to 2.6 parts of vitamin K₁ or 3.4 parts of vitamin K₂. The assays of Dam et al. (23), which provide a direct comparison of all three compounds, indicate that a given weight of methylnaphthoquinone
possesses antihemorrhagic activity equivalent to that of 2.1 parts of vitamin \( K_1 \) or of 3.1 parts of vitamin \( K_2 \). A quantitative biological conversion to either vitamin thus would more than account for all of the activity observed. While some of the other estimates of the relative potencies of methylnaphthoquinone and vitamin \( K_1 \) give a picture which is not quite so favorable to the hypothesis, the average ratio of 3.1:1 is not widely different from the inverse ratio of the molecular weights. It may be argued that a condensation reaction linking phytol to methylnaphtho-hydroquinone would have to proceed more efficiently in the organism than has been realized in the laboratory in order to account for the relationship, for the maximum yield in the vitamin \( K_1 \) synthesis is only about 30 per cent \( (9) \). A superior efficiency of the biological process seems entirely possible, and furthermore it must be noted that the actual alcoholic component may well be a substance more reactive than phytol and may possibly give a vitamin \( K \) principle more potent than the quinones isolated from alfalfa or fish meal. The substance also might differ somewhat from these quinones in therapeutic qualities and persistence.

The approximate equivalence of vitamin \( K_1 \) and methylnaphtho-quinone on a molecular basis would also be consistent with the hypothesis of a transformation of the former substance into the latter in the body, but an experimental basis for such a postulate is lacking. There are no indications that the synthesis can be reversed, and degradative cleavage of vitamin \( K_1 \) under mild conditions affords phthiocol and not methylnaphthoquinone \( (8, 9) \). Furthermore, the 3-phytyl, 3-farnesyl, 3-geranyl, and 3-cinnamyl derivatives of 2-methyl-1,4-naphthoquinone differ in potency much more than would be expected if they function as precursors of a common product of biodegradation. The alternate hypothesis of a biosynthesis is thus more plausible. The case is in no wise weakened by the fact that 1,4-naphthoquinone is very feebly active and falls far short of being equivalent in potency to 2-phytyl-1,4-naphthoquinone. 1,4-Naphthoquinone differs significantly from its 2-methyl derivative in being highly susceptible to addition reactions, and the bulk of the quinone administered may well be diverted from the condensation with an isoprenoid alcohol by interaction with an amino acid or a protein. The 2-methyl group should protect the material, while in the oxidized form, from such dissipating side reactions.
In view of the many indications of a specificity associated with the 2-methyl-3-(β-alkenyl)-1,4-naphthoquinone structure, the apparent glaring exception of the 2-methyl compound is in itself an argument in favor of the above hypothesis. In the monoalkyl series antihemorrhagic activity is observed over a considerable range of chain length, and yet the high potency of the 2-methyl compound is completely abolished on lengthening the chain by a single carbon atom. In terms of the hypothesis of a conversion in the body, the lack of activity of ethylnaphthoquinone finds a simple explanation on the basis of the observation that 2-ethyl-3-phytyl-1,4-naphthoquinone is practically devoid of activity (Table IV).

**Table IV**

*Highly Alkylated Naphthoquinones*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effective dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Ethyl-3-phytyl-1,4-naphthoquinone (6, 9)</td>
<td>1000</td>
</tr>
<tr>
<td>2,3-Diallyl-1,4-naphthoquinone (2, 3)</td>
<td>1000</td>
</tr>
<tr>
<td>1,1-Dimethyl-3-tert.-butyl-1,4-dihydroanthraquinone (5)</td>
<td>Inactive at 1000</td>
</tr>
<tr>
<td>2-(β-Methyl-γ-pentenyl)-1,4-dihydroanthraquinone (5)</td>
<td>“ “ 1000</td>
</tr>
<tr>
<td>2,6-Dimethyl-3-phytyl-1,4-naphthoquinone (9)</td>
<td>“ “ 1000</td>
</tr>
<tr>
<td>2,5-Dimethyl-1,4-naphthoquinone (15)</td>
<td>500 (Slight)</td>
</tr>
<tr>
<td>2,6-Dimethyl-1,4-naphthoquinone</td>
<td>Inactive at 1000</td>
</tr>
<tr>
<td>2,7-Dimethyl-1,4-naphthoquinone</td>
<td>1000</td>
</tr>
<tr>
<td>2,8-Dimethyl-1,4-naphthoquinone (15)</td>
<td>500 (Slight)</td>
</tr>
<tr>
<td>6,7-Dimethyl-1,4-naphthoquinone (2, 3)</td>
<td>Inactive at 1000</td>
</tr>
</tbody>
</table>

**Extension of the 2-Substituent and Alkylation in Benzenoid Ring**

The quinones included in Table IV are for the most part mono-methyl homologues of vitamin K₁ or 2-methyl-1,4-naphthoquinone, and the lack of any very significant activity in the series as a whole shows that this slight modification in structure results in the almost complete loss of the potencies characteristic of the parent substances. Replacement of the 2-methyl group of vitamin K₁ by a 2-ethyl group gives a compound (V) which possesses only 1/1000 the activity of the vitamin. Inasmuch as the chain of the 3-substituent group can be lengthened or shortened over a considerable range with relatively minor variation in activity, it is remarkable that the least possible extension of the 2-substituent
group, which involves increasing the molecular weight by only 3 per cent, should lead to almost complete abolishment of vitamin K activity. Another comparison of interest can be made between 2-methyl-3-geranyl-1,4-naphthoquinone ((VI), see Table I) and 2-(δ-methyl-γ-pentenyl)-1,4-dihydroanthraquinone (VII). The latter compound is closely related to (VI) and differs only in

![Diagram of compound VI](image)

(VI)

having a methylene bridge extending between the 2 position and the γ-carbon atom of the side chain in place of methyl groups at both points. In contrast to (VI), which is effective at a level of 25 γ, (VII) is completely inactive at 1000 γ. For highest potency, the 2-methyl group evidently must be intact, and a lengthening of the group or its incorporation in a side ring interferes very seriously with effective physiological functioning of the quinone.
Karrer and Epprecht (27) recognized this structural relationship and its implications regarding the structure of vitamin K₂.

The specificity with regard to the 2 position is striking, but so is that with respect to substitution in the benzenoid ring. Thus 2,6-dimethyl-3-phytyl-1,4-naphthoquinone (VIII) is almost wholly devoid of activity, showing that a methyl group at the 6 position has the same inhibiting influence as one substituted into the 2-methyl group. Of the five possible methyl derivatives of the potent 2-methyl-1,4-naphthoquinone, only 2,3-dimethyl-1,4-naphthoquinone (Table I) shows appreciable activity (about 1/170 that of the 2-methyl compound). Substitution into the structure (IX) of a methyl group at any one of the positions 5 to 8 results in almost complete deactivation. Since the specificity with regard to the absence of a methyl group from one part of the molecule is comparable with that concerning its presence at another position, there seems to be little foundation for supposing that the favorably located 2-methyl group is involved in the functioning of the antihemorrhagic agent.

**Naphthoquinones with Oxygen-Containing Substituents**

Phthiocol (X) was the first simple compound reported to have antihemorrhagic activity (36), and both this substance and lawsone have been assayed in several laboratories. The results are in substantial agreement in indicating that the former substance is rather weakly active and the latter either inactive (Table V) or feebly active (23). Phthiocol is an end-product of the color reaction of vitamin K₁ with alcoholic alkali (8, 9) and can be converted in low yield into vitamin K₁ by condensation in the reduced form with phytol (13). While the latter observation suggests the possibility that administered phthiocol may owe its
activity to a biological conversion to a vitamin K type principle, the fact that the isomeric plumbagin (XI) exhibits activity of

### Table V

*Hydroxy- and Carbethoxynaphthoquinones*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effective dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plumbagin, or 2-methyl-5-hydroxy-1,4-naphthoquinone (37)</td>
<td>400</td>
</tr>
<tr>
<td>Phthiocol,* or 2-methyl-3-hydroxy-1,4-naphthoquinone</td>
<td>500</td>
</tr>
<tr>
<td>Juglone, or 5-hydroxy-1,4-naphthoquinone</td>
<td>Inactive at 1000</td>
</tr>
<tr>
<td>Lawsone, or 2-hydroxy-1,4-naphthoquinone</td>
<td>&quot; &quot; 1000</td>
</tr>
<tr>
<td>Lapachol*</td>
<td>&quot; &quot; 1000</td>
</tr>
<tr>
<td>2-9-Heptenyl-3-hydroxy-1,4-naphthoquinone*</td>
<td>&quot; &quot; 1000</td>
</tr>
<tr>
<td>Crude 2-farnesyl-3-hydroxy-1,4-naphthoquinone (15)</td>
<td>&quot; &quot; 1000</td>
</tr>
<tr>
<td>2-Methyl-3-(γ-hydroxydihydrophytyl)-1,4-naphthoquinone (13)</td>
<td>&quot; &quot; 1000</td>
</tr>
<tr>
<td>Hydroquinone diacetate (13)</td>
<td>&quot; &quot; 1000</td>
</tr>
<tr>
<td>2-Methyl-3-carbethoxy-1,4-naphthoquinone† (38)</td>
<td>25</td>
</tr>
</tbody>
</table>

*From the collection of Samuel C. Hooker.
† We are indebted to Dr. C. F. Koelsch for this sample.

about the same order points more in the direction of a weak effectiveness of both compounds acting as such. Thus the introduction of a hydroxyl group into 2-methyl-1,4-naphthoquinone at either the 3 or 5 position results in a profound reduction of antihemorrhagic activity without its complete abolishment. A deactivating influence attending hydroxyl substitution is clearly evident in the results for farnesylhydroxynaphthoquinone and for the 2-methyl-3-γ-hydroxydihydrophytyl compound (XII). The lack of activity of (XII) is striking, for this is the γ-hydroxy
derivative of $\beta,\gamma$-dihydromvitamin K$_1$, which is fully effective at a dosage of 8 $\gamma$. The hydroxyl group in this case is alcoholic, whereas in the other instances cited it is phenolic. It is interesting to note that in the field of the similarly lipid-soluble carcinogenic hydrocarbons the introduction of both alcoholic and phenolic hydroxyl groups at various positions results in a complete or considerable loss of biological activity (39).

The last compound listed in Table V is a hydroquinone ester of the structure (XIII), and the high degree of activity (25 $\gamma$) is noteworthy. The substance may function in the form of the corresponding quinone, which would then be in a class with 2,3-dimethyl-1,4-naphthoquinone, a compound effective at a not greatly different dosage level (50 $\gamma$). Another possibility is suggested by the observation of Koelsch and Byers (38) that esters of this type can be converted very readily into the 2-alkyl-1,4-naphthoquinones by saponification and oxidation. A partial conversion of (XIII) in the organism to methylnaphthoquinone would account for the observed activity.
Naphthroquinone Oxides

The colorless oxides listed in Table VI are easily prepared from the yellow quinones in almost quantitative yield (4), and they have been found capable of reduction under very mild conditions (11, 15) with elimination of the oxidic bridge and formation of the corresponding hydroquinone. This generally applicable reduction reaction affords a basis for the hypothesis that the antihemorrhagic activity observed with certain members of the series may be due to a similar reduction of the administered material in the organism, with formation of either the quinone or the biologically equivalent hydroquinone component of the oxidation-reduction system. This would account for the very high potency of vitamin K₁ oxide (XIV), which is hardly distinguishable in the bioassays from the vitamin itself. That 2-methyl-1,4-naphthoquinone oxide (XV) is considerably less potent than (XIV) and exhibits only 1/17 the activity of methylnaphthoquinone may be an indication that the reversion of this oxide to the quinone proceeds less efficiently than in the case of the vitamin oxide. Such an inference is supported by the observation that the oxides of monoalkylated quinones can enter into a second type of chemical transformation.
to which the dialkyl compounds are not amenable. The oxide of the 2-methyl compound can be converted smoothly with sulfuric acid at a low temperature into phthiocol (40). Under the influence of alkali it is in part isomerized to phthiocol and in part degraded to lawsone, with loss of the methyl group (15). A partial transformation of administered oxide to either compound would involve a distinct loss in total potency, and the former change, since it requires merely the migration of the hydrogen atoms at position 3, may well occur to some extent in the course of the test. A 2,3-dialkynaphthoquinone oxide such as that of vitamin K₁ (XIV) has no hydrogen available for isomerization. Of the other two oxides of the same type which were examined (Table VI), the 2-methyl-3-cinnamyl derivative retains about 1/3 of the activity of the parent quinone, while the oxide of 2,3-dimethyl-1,4-naphthoquinone was found to possess about twice the potency of this compound. The latter result appears anomalous and inconsistent with the hypothesis that the oxides function merely as precursors of the quinones from which they are derived. Perhaps also inconsistent is the fact that the oxides of 2-phytyl- and 2-farnesyl-1,4-naphthoquinones show 1/4 to 1/2 the potencies of the quinones, although the difference from the behavior noted in the 2-methyl series may well be due to a hindering influence of the large isoprenoid groups manifested in protecting the oxide linkage from hydrolytic cleavage. It may also be that differences in absorbability of the oxide and the quinone account for variations in relative potency of the order of from 1:4 to 2:1, noted with all oxides studied except that of methylnaphthoquinone. The pronounced deviation of this compound from the general rule seems attributable to a greater chemical reactivity, with consequent greater opportunity for a diversion of a part of
the material to less potent by-products, and this interpretation holds good whether the oxides function as such or by virtue of a reduction to the quinones.

The highly potent vitamin K\textsubscript{1} oxide may find practical application by virtue of its stability, the substance being much less sensitive to light than the vitamin. Thus solutions of the two substances in petroleum ether (20 mg. in 25 cc.) were exposed together to direct sunlight for 2 hours and then assayed. The vitamin sample had lost about 90 per cent of its activity in this period, while the oxide showed a diminution in potency of less than 30 per cent. This comparatively light-stable derivative, which is easily produced from and converted into vitamin K\textsubscript{1}, may possibly have a bearing on the question of the form in which the light-sensitive vitamin exists in green plants. Indeed, Fernholz, Ans- bacher, and coworkers (26, 41) report the isolation from alfalfa concentrates of a colorless fraction having properties reminiscent of those of our oxide. Thus their most potent concentrates, like the oxide, gave no purple-blue color with alcoholic alkali and showed about the same activity as vitamin K\textsubscript{1} in the 18 hour assay.

Compounds of Other Than the Naphthoquinone Type

A number of quinones other than those of the \(\alpha\)-naphthoquinone series have been investigated in various laboratories, but where antihemorrhagic activity has been encountered it has been of a low order. The only such exploratory assays included in the present report are those for the first few compounds listed in Table VII. The first is analogous in structure to 2-methyl-1,4-naphthoquinone, the second is a benzoquinonoid model of the active 2,3-dimethyl compound, and the third, of formula (XVI),

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{CH}_3\text{CH} = \text{CCH}_3\text{C}_6\text{H}_4 \\
\text{H}_3\text{C} & \quad \text{CH}_3
\end{align*}
\]

(XVI)
differs from vitamin K\textsubscript{1} only in having two methyl groups in place of the 4-carbon benzenoid side ring. The absence of activity in all three compounds is a further indication of the specificity associated with the structures of the corresponding naphthoquinones. 9-Methylperinaphthenone-7 (XVII) resembles 2-methyl-1,4-naphthoquinone in having an \(\alpha,\beta\)-unsaturated ketonic system with a methyl group at the \(\beta\) position. The lack of activity suggests that the character of the unsaturated system is of importance only if this is present in a compound of the true quinone type.

The naphthotocopherol (XVIII) assayed in the present investigation was prepared by a method which precludes its contamination with the isomeric ketonic substance (XIX), which is formed in considerable amounts as a by-product in the synthesis of vitamin K\textsubscript{1} and which, like (XVIII), is not extracted by Claisen's

\begin{table}
\centering
\caption{Miscellaneous Compounds}
\begin{tabular}{lc}
\hline
Compound & Effective dose \\
\hline
2,3,5-Trimethyl-1,4-benzoquinone & Inactive at 1000 \\
Duroquinone & “ “ 1000 \\
2,3,5-Trimethyl-6-phytyl-1,4-benzoquinone (11, 14) & “ “ 1000 \\
\(\alpha\)-Tocopherylquinone (14) & “ “ 1000 \\
9-Methylperinaphthenone-7 (15) & “ “ 1000 \\
Naphthotocopherol (11, 13) & 500 \\
2-Methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone (11, 13) & 50 \\
\hline
\end{tabular}
\end{table}
alkali. The naphthotocopherol occupies a unique position in that it evokes the biological responses characteristic of both vitamins E and K (13). The manifestation of antihemorrhagic activity of even a rather low order (500 γ) raises an interesting point. The substance conceivably is convertible into a quinone by either oxidation or reduction. Chemical oxidation gives the naphthotocopherylquinone, or γ-hydroxy-β,γ-dihydrovitamin K₁, but this substance is inactive (Table V). A partial, inefficient reductive cleavage of the chroman ring would explain the observed activity, for the β,γ-dihydride of vitamin K₁ which would result is effective at 8 γ.

That the ketonic by-product (XIX) shows activity at 50 γ seems remarkable. To be sure, the substance is isomeric with vitamin K₁ hydroquinone and it has been found convertible into the vitamin in low yield by a process of pyrolysis and oxidation (11, 13). A biological conversion proceeding to the extent of 1 to 2 per cent would thus appear at least possible.

**Naphthohydroquinone Esters and Ethers**

**Inorganic Esters**—Among water-soluble antihemorrhagic compounds of various types which have been suggested for use in parenteral therapy, a series of phosphate and sulfate esters of types (XX) and (XXI) were prepared and examined in our laboratories (10). Subsequently Foster, Lee, and Solmsen (42) published the results of a careful pharmacological study of the diphosphate ester of methyl-naphthohydroquinone, and Ansbacher, Fernholz, and Dolliver (43) reported assays for this compound and for the corresponding sulfate ester. Our completed assays
(Table VIII) agree very closely with those of Foster, Lee, and Solmssen and are at variance with those of the second group of workers. We find the diphosphate ester to be effective at a dosage of 0.5 \( \gamma \), an amount which is equivalent to only 0.2 \( \gamma \) of methyl-naphthoquinone, and, since our value for the minimum dose of the latter compound is 0.3 \( \gamma \), it is evident that on a molecular basis the ester is at least as potent as the quinone. We agree with Foster, Lee, and Solmssen on this point, but discount their suggestion that the action of methylnaphthoquinone or a similar compound is mediated through a phosphate ester, for the corresponding ester derived from vitamin \( K_1 \) should in this case show much higher potency than is actually observed (Table VIII). This derivative of the vitamin is about 5 times as potent when given intravenously as when administered by mouth, whereas no appreciable differences were observed when the diphosphate and disulfate esters of the 2-methyl compound were assayed by these two methods. Another point of difference is that the hydroquinone esters of vitamin \( K_1 \) are much less active than the parent quinone, whereas the esters of methylnaphthoquinone exhibit molecular potency of the same order of magnitude as this substance.

We consider the most likely interpretation of the results to be that the inorganic esters undergo more or less complete hydrolysis.

<table>
<thead>
<tr>
<th>Table VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-Soluble Inorganic Esters</td>
</tr>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Sodium 2-methyl-1,4-naphthohydroquinone diphosphate (10)</td>
</tr>
<tr>
<td>Sodium 2-methyl-1,4-naphthohydroquinone disulfate (10)</td>
</tr>
<tr>
<td>Vitamin ( K_1 ) hydroquinone diphosphoric acid (10)</td>
</tr>
<tr>
<td>Sodium 2,3-dimethyl-1,4-naphthohydroquinone disulfate (10)</td>
</tr>
<tr>
<td>Potassium vitamin ( K_1 ) hydroquinone disulfate (10)</td>
</tr>
</tbody>
</table>
in the body with liberation of quinones in the reduced form and that the latter are the agents which actually function. The differences noted would then signify that the phosphates are more easily hydrolyzed than the sulfates, which is compatible with biochemical information, and that a given ester of 2-methyl-1,4-naphthohydroquinone is more readily hydrolyzed than the ester of 2-methyl-3-phytyl-1,4-naphthohydroquinone. The latter proposition is reasonable, for in the second case both ester groups are subject to the hindering influence of an adjacent alkyl group, and one of these is a bulky isoprenoid side chain.

**Organic Esters and Ethers**—Interest in acylated hydroquinone derivatives of quinones possessing antihemorrhagic activity originated in the observation by Doisy and coworkers (44) that the

<table>
<thead>
<tr>
<th>Organic Esters and Ethers</th>
<th>Effective dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methyl-1,4-naphthohydroquinone</td>
<td>7</td>
</tr>
<tr>
<td>Diacetate (4)</td>
<td>1</td>
</tr>
<tr>
<td>Dibenzoate (4)</td>
<td>1</td>
</tr>
<tr>
<td>Dimesitoate (12, 15)</td>
<td>300</td>
</tr>
<tr>
<td>Monoethyl ether (13)</td>
<td>1</td>
</tr>
<tr>
<td>Dimethyl ether</td>
<td>5</td>
</tr>
<tr>
<td>Dibenzyl &quot; (4)</td>
<td>7</td>
</tr>
</tbody>
</table>

crystalline hydroquinone diacetates of vitamins K$_1$ and K$_2$ are about 1/2 as active as the quinones. The diacetate of 2-methyl-1,4-naphthohydroquinone has been assayed in several laboratories and the corresponding dibenzoate and dimethyl ether have been studied by Ansbacher, Fernholz, and Dolliver (45). Our results for these esters and ethers (Table IX) are in substantial agreement with those of other investigators.

As a test of the obvious hypothesis that the acylated naphthohydroquinones undergo hydrolysis in the organism, and exert an antihemorrhagic action merely by virtue of the functioning of the liberated hydroquinone, methylnaphthohydroquinone dibenzoate was compared with the highly hindered dimesitoate (XXII). The latter ester is very resistant to hydrolysis, and the results indicate that it possesses no more than about 1/300 the potency of the
dibenzoate. This observation lends plausibility to the hypothesis mentioned.

The ethers of methylnaphthohydroquinone would be expected to be much more resistant to hydrolysis than the acetates and, on observing that the dimethyl ether is nevertheless about 1/10 as active as the quinone, Ansbacher, Fernholz, and Dolliver (45) were inclined to favor the idea that the hydroquinone derivatives in general function as such and not in the form of the hydroquinone-quinone system. Our assays place the dimethyl ether in the same relative position with regard to activity. The dibenzyl ether, which probably is subject to more ready hydrolytic fission, appears somewhat less active on a weight basis but actually is slightly more potent than the dimethyl ether on a molecular basis. The monoethyl ether of the probable structure (XXIII) is significantly more active than either of the diethers, and it occurred to us that this ether, being of a type susceptible to oxidation, might be convertible to the quinone in the organism by an oxidative mechanism. The observation of Doisy and coworkers (46) that
1,4-diacetoxy-2-methylnaphthalene-3-acetic acid is very resistant to hydrolytic fission but can be converted to the corresponding quinone by oxidation suggested that a diether might also be susceptible to oxidation. In a trial experiment it was found that the methylnaphthohydroquinone dimethyl ether (XXIV) can indeed be converted smoothly into the corresponding quinone (XXV) by oxidation with chromic acid (12). It therefore seems possible that hydroquinone esters and ethers function as anti-hemorrhagic agents by virtue of a biological conversion to the hydroquinones or quinones by either the hydrolytic or the oxidative route.

*Hydrogenated Naphthoquinones*

An interesting field for the further consideration of the possible biological transformation of the administered material is provided by a series of hydrides (Table X) obtained in part by catalytic hydrogenation of vitamin K₁ or methylnaphthoquinone, and in part by an independent method of synthesis. The 5,8-dihydride of vitamin K₁ (XXVI) is 1/4 as active as the vitamin and twice as potent as the isomeric β,γ-dihydride (Table I). Partially hydrogenated quinones of this type are easily converted into the fully
aromatized naphthoquinones by chemical oxidation, the hydrogen atoms at positions 5 and 8 being highly activated and subject to either direct attack or to oxidative removal subsequent to an enolic shift to oxygen. Thus the observed activity of (XXVI) may well be the result of a partial dehydrogenation to the vitamin. In sharp contrast to the behavior of the 5,8-dihydride, hexahydrovitamin K₁ (XXVII) gave only a feeble response at the high level of 1000 γ. Fernholz, MacPhillamy, and Ansbacher (47) observed no activity at twice this dosage by the 6 hour method. The substance should be compared with the β,γ-dihydride, for which the effective dose is 8 γ, and it is evident that complete saturation of the non-quinonoid ring produces a profound change. Chemically,

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effective dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,8-Dihydrovitamin K₁ (11, 14)</td>
<td>4</td>
</tr>
<tr>
<td>β,γ,5,6,7,8-Hexahydrovitamin K₁ (11, 15)</td>
<td>1000 (Very slight)</td>
</tr>
<tr>
<td>2-Methyl-5,8-dihydro-1,4-naphthoquinone (14)</td>
<td>6</td>
</tr>
<tr>
<td>2-Methyl-5,8,9,10-tetrahydro-1,4-naphthoquinone (14)</td>
<td>8</td>
</tr>
<tr>
<td>2-Methyl-5,6,7,8-tetrahydro-1,4-naphthoquinone (15)</td>
<td>500</td>
</tr>
</tbody>
</table>

the compound is to be regarded as a benzoquinone with four alkyl substituents, and from this point of view it is most nearly comparable with the inactive 2,3,5-trimethyl-6-phytyl-1,4-benzoquinone ((XVI) above). When the side ring contains no activating double bond, it does not share the susceptibility to dehydrogenation characteristic of compounds of the type (XXVI). The lack of significant activity thus appears to be attributable to the inability of the substance to undergo biological transformation into a naphthoquinone.

Similar relationships were observed among various hydrides of 2-methyl-1,4-naphthoquinone. The condensation of butadiene with toluquinone affords the 5,8,9,10-tetrahydride (XXVIII), which in the presence of a trace of an acid or a base is smoothly
isomerized to 2-methyl-5,8-dihydronaphthohydroquinone (XXIX). The latter compound was assayed as such rather than in

\[
\begin{align*}
\text{(XXVIII)} & \quad \text{H}^+ \\
\text{(XXIX)}
\end{align*}
\]

the form of the quinone, of which it is doubtless the biological equivalent, because the quinone is a highly sensitive compound subject to rapid deterioration. The substance does not show as high a ratio of potency to methylnaphthoquinone as does the 5,8-dihydrider of vitamin K1 to the vitamin, possibly because of the destruction of a part of the sensitive quinone formed as an intermediate in the process of aromatization of the end ring. As in the vitamin series, the 5,6,7,8-tetrahydride of methylnaphthoquinone shows activity of only a low order (compare Fernholz, MacPhillamy, and Ansbacher (47)). The isomeric 5,8,9,10-tetrahydride (XXVIII), however, possesses considerable potency, being about 1/8 as active as vitamin K1. This diene addition product does not possess the structural characteristics and properties of a quinone and is quite far removed from a naphthoquinone type. An explanation of the vitamin K activity on the basis of the structure as such is far to seek, and the biological action of the compound seems, rather, to be a manifestation of the stepwise conversion of the substance into methylnaphthoquinone, and possibly to a true vitamin K type principle.

**Naphthalene and Tetralin Derivatives**

In a further study of possible biochemical changes 2-methyl-1-naphthol (XXX) and 3-methyl-1-naphthol (XXXI) were examined for comparison with 2-methyl-1,4-naphthohydroquinone (XXXII), which is effective at practically the same level as the quinone. The two methylnaphthols were found to possess striking antihemorrhagic potency, giving a full response at 1 \( \gamma \) and at 0.6 \( \gamma \), respectively (Table XI). It would seem remarkable
that these simple naphthalene derivatives should be capable of exerting in their own right an action characteristic of complex

TABLE XI
Methylnaphthols, Methyltetralones, and Related Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effective dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methyl-1,4-naphthohydroquinone</td>
<td>0.5</td>
</tr>
<tr>
<td>2-Methyl-1-naphthol (12, 15)</td>
<td>1</td>
</tr>
<tr>
<td>3-Methyl-1-naphthol (12, 15)</td>
<td>0.6</td>
</tr>
<tr>
<td>4-Methyl-1-naphthol (12, 15)</td>
<td>Inactive at 1000</td>
</tr>
<tr>
<td>1-Methyl-2-naphthol (12, 15)</td>
<td>Inactive at 1000</td>
</tr>
<tr>
<td>3-Methyl-2-naphthol (12, 15)</td>
<td>Inactive at 1000</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>1000 (Slight)</td>
</tr>
<tr>
<td>2-Methyl-1-tetralone (12, 15)</td>
<td>0.6</td>
</tr>
<tr>
<td>3-Methyl-1-tetralone (12, 15)</td>
<td>1</td>
</tr>
<tr>
<td>2-Methyl-1-naphthylamine (12, 15)</td>
<td>5</td>
</tr>
<tr>
<td>3-Methylnaphthalene</td>
<td>1000 (Slight)</td>
</tr>
<tr>
<td>α-Methyl-γ-phenylbutyric acid (15)</td>
<td>Inactive at 1000</td>
</tr>
</tbody>
</table>

vitamin principles of highly specific structures. 1-Naphthol evokes no such response. In order to determine whether this is a general property associated with methylnaphthols we investigated the three other possible isomers having the two groups in the same ring, (XXXIII) to (XXXV). These are all completely inactive, and yet they do not depart greatly in structure from the highly potent isomers, 4-methyl-1-naphthol having the reactive α-hydroxyl group characteristic of these compounds, and 3-methyl-2-naphthol the methyl group at the β position. There is, however, a striking differentiation in this series of five isomers in that two of them are convertible by direct processes into 2-methyl-1,4-naphthoquinone and the other three are not. It can hardly
be a coincidence that those two which can yield the quinone share its biological action and that the non-convertible isomers are inactive. The results thus provide a strong indication that the active methylnaphthols when administered to animals undergo efficient hydroxylation at the position para to the hydroxyl group.

2-Methyl-1-naphthylamine (XXXVI) shows considerable activity but is only 1/5 as potent as the corresponding methylnaphthol (XXX). The amine may conceivably undergo deamination to (XXX) or para-hydroxylation and oxidation, and in either case the over-all process must involve some losses. The p-hydroxy compound (XXXVII) has been assayed by Emmett, Kamm, and Sharp (48) and found nearly as potent as methylnaphthoquinone, whereas the methylnaphthylamine is only about 1/17 as effective as this reference substance. A p-aminonaphthol probably requires no special process of deamination, since this can occur spontaneously following oxidation of the substance to a quinoneimine.

The results for the tetralones (XXXVIII) and (XXXIX) in Table XI show that these substances give every indication of functioning as precursors of the corresponding methylnaphthols, for they are effective at practically the same level. There is a
slight difference in potency between the two tetralones and the order is the reverse of that of the methylnaphthols. Differences in the relative efficiencies of the various steps in the conversion and in the susceptibility to side reactions would account for such minor variations.

There is no inconsistency in the view that the alicyclic ring of a tetralone undergoes aromatization in the body, whereas that present in 2-methyl-5,6,7,8-tetrahydro-1,4-naphthoquinone does not, for the tetralone can function in the enolic form in which a double bond is supplied to the ring in question and only 2 hydrogen atoms have to be removed in order to produce a phenol. The energy required for aromatization of a tetrahydrobenzenoid ring is far greater than for a dihydride with the bond structure of the enol (XL). The aromatization of a tetralone is thus comparable with that indicated as occurring biologically with the 5,8-dihydroronaphthoquinones mentioned.

As far as our observations go, the limit of biological conversion, as indicated by high antihemorrhagic activity, is reached with the tetralones and methylnaphthols. An acid precursor which yields one of the tetralones on cyclodehydration was tested with negative results, and β-methylnaphthalene showed little indication of undergoing biological oxidation.
Vitamin K Activity and Structure

SUMMARY

Bioassays for antihemorrhagic activity in chicks by the 18 hour method are reported for 79 compounds synthesized or selected with the view of obtaining information on the relationship between vitamin K activity and structure. A number of interesting relationships are suggested by the data, and, although the nature of the phenomenon is such that any interpretation must be tempered with reservation, the following main conclusions seem indicated.

Antihemorrhagic activity of any biologically significant magnitude is found only in the series of the 1,4-naphthoquinones, or among compounds convertible into such quinones. Considerable specificity is evident in the series which embraces vitamins K₁ and K₂ and all other 1,4-naphthoquinones having a methyl group at the 2 position and some more extended group at position 3. A double bond in the β,γ position of the 3-substituted side chain contributes to the potency of the compound, while unsaturation at points more remote from the quinonoid nucleus is without influence. A branched chain isoprenoid structure of the 3-substituent is more favorable for the development of antihemorrhagic activity than a straight chain arrangement, and the carbon content is also a factor of importance. In the series of quinones having a branched side chain the activity tends to increase as the chain is lengthened and reaches a maximum when the chain includes some 20 or 30 carbon atoms.

Although the methyl group at position 2 is important for the development of highest potency, β-alkenynaphthoquinones lacking this group retain an average of about 1/65 the activity of the methylated quinones and exhibit all the above features of specificity. Any extension of the 2-methyl group, however, or any alkyl substitution in the benzenoid ring, results in complete obliteration of the activity. The high potency of 2-methyl-1,4-naphthoquinone is similarly almost completely wiped out by replacing the methyl group by ethyl or propyl, or by introducing a methyl group at any of the four positions in the benzenoid ring. Hydroxyl substitution in nuclear positions of methylnaphthoquinone or into the side chain of β,γ-dihydrovitamin K₁ results in loss in potency.

The antihemorrhagic activity observed with a considerable number of the compounds tested seems to be attributable not to
the functioning of the actual substance administered but to its conversion in the animal body to a vitamin K type principle in the course of the assay. It appears possible that methylnaphthoquinone exhibits high potency by virtue of its functioning as a component in the biosynthesis of a quinone of the general type exemplified by vitamins K₁ and K₂. Inorganic and organic esters of methylnaphthohydroquinone and vitamin K₁ hydroquinone most likely owe such activity as they exhibit to a more or less efficient hydrolysis in the organism, whereas ether derivatives may be convertible into the corresponding quinones by a process of metabolic oxidation. Still more extensive changes which appear from the assay data to be of likely occurrence are illustrated in the accompanying chart. A figure is included for each compound giving the effective dose in micrograms as found in our assays, and the arrows indicate postulated metabolic changes. Thus the remarkably potent methylnaphthalones are considered to suffer dehydrogenation to the methylnaphthols, which in turn are converted by hydroxylation into methylnaphthohydroquinone; the latter substance, or the biologically equivalent quinone, may then combine with an isoprenoid alcohol to give a vitamin K
factor. The potencies observed for all of the compounds in this complex series do not depart very widely from molecular equivalence. Other biological processes indicated as likely by the assay results apparently proceed with varying efficiency. Thus the oxide of vitamin K₁ is almost equivalent to this substance in activity and therefore may be subject to nearly quantitative bio-reduction, whereas that of methylphthiocolquinone probably is in part dissipated by conversion into the less active phthiocol. Compounds having a dihydrobenzenoid ring, as exemplified by 5,8-dihydrovitamin K₁, appear capable of moderately efficient dehydrogenation, but the corresponding tetrahydrides are resistant to such a change.

Addendum (January 15, 1941)—Since this paper was submitted for publication Lee, Solmsen, Steyermark, and Foster (49) have published additional assay data for sodium 2-methyl-1,4-naphthohydroquinone diphosphate which are in close agreement with the earlier results from the same laboratory (42) and with the present results (Table VIII). Lee et al. are in error in stating that Fieser and Fry (10) reported assays indicating that sodium 2-methyl-1,4-naphthohydroquinone diphosphate is only moderately active. The statement which they quote refers to the phosphate ester of vitamin K₁ hydroquinone and not to that of 2-methyl-1,4-naphthohydroquinone; the exact dose level of the latter ester had not at the time been determined.

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Louis F. Fieser, Max Tishler, W. L. Sampson and With the technical assistance of Saramae Woodford


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