temperature not exceeding 25°. The addition of chlorine is stopped only after the precipitate, at first yellow, and then violet-brown, becomes light-yellow again. The latter is filtered by suction, washed at first with water to eliminate $Cl^-$ and $SO_4^{--}$, and then with 250–300 ml. of acetone until complete removal of 2-methyl-1,4-naphthoquinone is accomplished; weight 14.5 g. Another portion of 1.4 g. is isolated from the washings by precipitating with an equal volume of saturated potassium chloride solution. The product without further purification was analyzed. *Anal.* Calcd. for $C_{11}H_7O_5SK$: K, 13.47. Found: K, 13.44.

Unchanged 2-methyl-1,4-naphthoquinone (1.1 g.) was obtained from the acetone extracts as in Experiment 1a. Another portion of 2-methyl-1,4-naphthoquinone (2.5 g.) is precipitated from the reaction filtrate by adding an excess of a saturated soda solution; yield 57%.

2. **Preparation of Potassium 2-Methyl-1,4-naphthoquinone-3-sulfonate after Moore.**[5]—Sulfonation and oxidation were carried out according to Moore's direction starting with 17.5 g. of 2-methyl-1,4-naphthoquinone; 6.5 g. of substance was isolated which was purified as in Experiment 1b: weight of potassium 2-methyl-1,4-naphthoquinone-3-sulfonate, 2.8 g.; weight of unreacted 2-methyl-1,4-naphthoquinone 1.5 g.; yield 10%.

## Summary

New conditions for the sulfonation of 2-methyl-1,4-naphthoquinone by potassium bisulfite are described and the mechanism of sulfonation of quinones by bisulfite is discussed. A preparative method for potassium 2-methyl-1,4-naphthoquinone-3-sulfonate is described with yields up to 60%.

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[CONTRIBUTION FROM ALL-UNION INSTITUTE OF EXPERIMENTAL MEDICINE. LABORATORY OF VITAMIN SYNTHESIS (TOMSK) AND CHEMICAL LABORATORY (MOSCOW)]

# Studies in the Vitamin K Group. II. The Mechanism of Biological Action of Vitamin K and of Its Synthetic Analogs

BY M. M. SHEMIAKIN, L. A. SCHUKINA AND J. B. SHVEZOV

The relationship between structure and biological activity of the group of compounds having vitamin K activity has been a subject of considerable discussion.[1]

It has been accepted generally that a prerequisite of anti-hemorrhagic activity is the 1,4-naphthoquinone grouping or one capable of being converted into this system. Not less significant is the presence of certain groups attached to the second carbon atom of the quinoid ring. Whereas the introduction of methyl group into 1,4-naphthoquinone increases its activity about 1000 times, all other alkyl groups, irrespective of size, are ineffective. With the methyl group in position 2, the introduction of alkenyl and particularly alkyl radicals in position 3 generally decreases biological activity. A positive, although slight, effect is exerted by the double bond in the $\beta,\gamma$-position of the alkenyl radicals, which is augmented by branching (particularly of the isoprenoid type) and by increasing the number of carbon atoms in the chain. The introduction of the sulfonic acid[2,3] or the dimethylamino[3] group in position 3 reduces the activity of 2-methyl-1,4-naphthoquinone only slightly whereas a great decrease in activity is noted in the 3-hydroxy compound. If the benzene ring is modified by the introduction of an alkyl group, the antihemorrhagic activity disappears almost completely.

All these empirical conclusions do not afford a general interpretation of the correlation between biological action and structure. Nor do they account for the fact that a slight change in structure of vitamin $K_1$ results in a striking change in biological activity whereas a radical change in structure, such as the elimination of the phytyl group, causes an actual increase in activity. It is also difficult to understand why 2-methyltetralone has only slightly less activity than 2-methyl-1,4-naphthoquinone whereas the removal of the methyl group in the latter compound or the replacement of methyl by ethyl results in an almost complete loss of activity. In addition to these inadequacies, the empirical conclusions contribute very little to our comprehension of the biological action of the antihemorrhagic compounds.

With respect to the relation of structure to antihemorrhagic activity, Fieser[4] suggested in 1939 and elaborated further[1] in 1941 the concept that the biological activity of the synthetic analogs of vitamin K is due to their transformation within the organism, through a biosynthesis with phytol or other natural isoprenoid alcohols, into quinones of the type of the naturally occurring vitamins $K_1$ and $K_2$.

Although this hypothesis is in accord with the published data it is difficult to reconcile it with the high activity of derivatives of 2-methyl-1,4-naphthoquinone containing the sulfonic acid or dimethylamino group in position 3. No adequate explanation is offered by this hypothesis for the exceptional role of the methyl group in position 2. Finally, the observation made in this Laboratory[5] that phthalic acid also possesses a perceptible antihemorrhagic activity is at variance with

(1) For a detailed bibliography *cf.* Fieser, Tishler and Sampson, *J. Biol. Chem.*, **137**, 659 (1941).

(2) Moore, THIS JOURNAL, **63**, 2049 (1941).

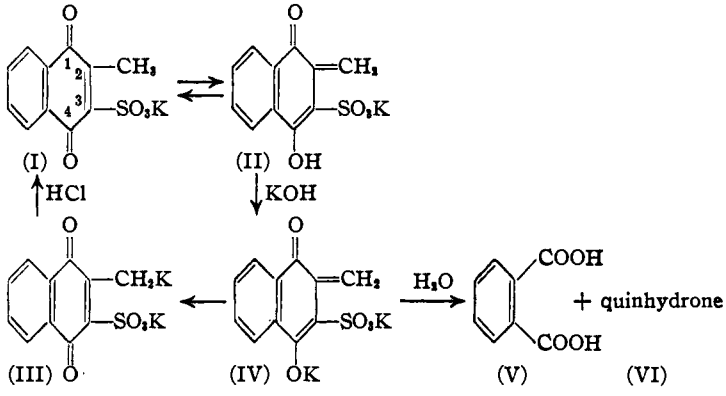
(3) Kudriashev, *Bull. Exptl. Biol. Med.* (Russ.), 510 (1941).

(4) L. F. Fieser, THIS JOURNAL, **61**, 3467 (1939).

(5) K. G. Packendorf, B. A. Kudriashev and E. N. Lazareva, *Doklady Akad. Nauk U. S. S. R.*, **XXXI**, 484 (1941) (Russ.).

Fieser's concept. This finding suggests a contrary hypothesis that the biological activity of natural vitamins $K_1$ and $K_2$, as well as the synthetic analogs is due to their oxidative bio-degradation to phthalic acid or to other oxidative products of the benzene series. In order to test this concept, we have studied the properties and reactions of 2-methyl-1,4-naphthoquinone and potassium 2-methyl-1,4-naphthoquinone-3-sulfonate. We have found that the two compounds are transformed into phthalic acid under mild conditions and with extreme ease. Indeed, mere heating with water is sufficient to accomplish this change, although it is greatly accelerated by hydrogen and hydroxyl ions.

A study of the effect of alkali on potassium 2-methyl-1,4-naphthoquinone-3-sulfonate, I, has revealed why this profound change, obviously connected with oxidation and reduction, occurs under such mild conditions and also the mechanism involved. When the sulfonate is dissolved in concentrated potassium hydroxide solution, a precipitate is soon formed in quantitative amounts which is the enolate IV. On storage in potassium hydroxide solution the latter is gradually converted into the more stable isomeric compound, III. The striking difference in the properties of III and IV suggests the following structures of these compounds and their places in the conversion of sulfonate to phthalic acid.




Compound III is an orange-red solid, soluble in alcohol and rather stable in water solutions, from which the quinone, I, is regenerated upon acidification. The enolate, IV, is a white substance, insoluble in alcohol and its aqueous solutions are very unstable. In water, precipitation of an orange quinhydrone occurs with the simultaneous formation of phthalic acid. No such potassium compounds have been isolated in the case of 2-methyl-1,4-naphthoquinone since the reaction proceeds too rapidly.

We believe that this peculiar behavior, the transformation of 2-methyl-1,4-naphthoquinone and related quinones to phthalic acid, is due to the character of the methyl group in position 2. The great ionization capacity of the hydrogen atoms of the methyl group, the possibility of the existence of such quinones in two tautomeric forms I and II, and the ease with which the enol form, II, can be oxidized by the quinoid form, I, may account for this remarkable reaction. This particular case of quinone tautomerism belongs to the keto–enol type of cationotropic changes which are known to be catalyzed by hydrogen and hydroxyl ions. The tautomeric change may also be caused by mere heating of aqueous solutions of quinones and may even proceed at lower temperatures. It is our opinion that in the tautomeric system I $\rightleftarrows$ II, which at the same time is an oxidation–reduction system, the quinoid form oxidizes the enol form, II, to phthalic acid whereas the quinone is reduced to a hydroquinone which either forms a quinhydrone or undergoes secondary reactions of condensation and polymerization as is apparently the case with 2-methyl-1,4-naphthoquinone.

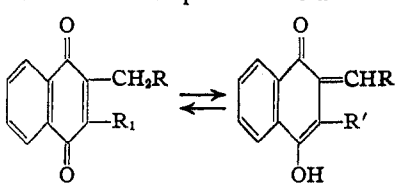
The fact that an aqueous solution of the enolate of 2-methyl-1,4-naphthoquinone-3-sulfonate, II, is readily converted to phthalic acid even at ordinary temperature and that phthalic acid has antihemorrhagic activity, strongly supports the hypothesis of the *biological oxidative degradation* of vitamin K and its active analogs within the organism. They also indicate that it is phthalic acid itself that is the true carrier of the biological function of vitamin K. Vitamins $K_1$ and $K_2$ and the active analogs of these vitamins related to naphthalene should, therefore, be regarded as *pro-vitamins*.

At first it would appear that the very short and slight antihemorrhagic activity of phthalic acid would weaken our hypothesis.

We have felt for some time that the apparent slight activity of phthalic acid is due to its rapid elimination from the organism. It seemed, therefore, worth while to test a number of less soluble derivatives such as esters, diamides, etc., which are readily hydrolyzed by water. It was presumed that smaller solubility in water and different resorption by the tissues of such a derivative may slow up its elimination, but yet its ability to hydrolyze to phthalic acid would assure a more pronounced and lasting biological effect. Preliminary data obtained by B. A. Kudriashev on rats with thrombinemia caused by ligating the bile duct have actually revealed the high activity of some of these derivatives, in particular, diethyl phthalate. The latter is also of practical interest; the experimental data of this study will be reported in a forthcoming publication.

The properties and transformations of 2-methyl-1,4-naphthoquinone-3-sulfonate thus far studied by us not only may account for the mechanism of biological action of vitamin K and its analogs but

also elucidate the role of the structural peculiarities responsible for the biological action of the molecules. In our view, the antihemorrhagic activity of a compound is largely a function of its ability to be transformed into phthalic acid and the degree of biological activity is essentially due to the effect of one or another structural factor on the processes leading to the formation of this acid. Transformation into phthalic acid of vitamins $K_1$ and $K_2$ and their analogs (and hence their biological activity) becomes only possible if a tautomeric system VII ⇄ VIII is possible. The quinone, VII, should also be capable of oxidizing the enol VIII to phthalic acid.[6]




VII VIII

where R is hydrogen or a hydrocarbon residue, and $R_1$ is hydrogen, a hydrocarbon radical or some other group.

Inasmuch as the tautomeric system VII ⇄ VIII is cationotropic of the keto–enol type, the effect of structure in this step is obvious. In addition, structural changes should influence the oxidation-reduction capacity of the same system, affecting to a certain degree the oxidation capacity of the quinone, VII, and the reduction ability of the enol, VIII. Of course, certain secondary effects which are connected with biological action also depend on structural factors. The properties associated with resorption by the tissues, penetration to the respective points of the organisms, etc., are to a large extent dependent on structure.

It is not intended to discuss in this paper the relationships between structure and biological activity of the vitamin K group in detail. It is sufficient to point out that they are not only in accord with our concept, but offer a better explanation of the facts. On our basis, it becomes quite comprehensible why the methyl group in position 2 plays such an exceptional role, and it is clear why its elimination or replacement by any other alkyl radical practically results in complete loss of activity, whereas substitution by alkenyl radicals although producing a negative action is not so effective. It is also clear why it is possible to introduce in position 3 most diverse atom groupings while the methyl group is in position 2.

## Experimental

**Cleavage of 2-Methyl-1,4-naphthoquinone.**—Twenty grams of the quinone in 2 liters of water is boiled for thirty hours. The unchanged quinone is removed by steam distillation and the resulting solution is acidified with hydrochloric acid. The black precipitate is removed and the filtrate, after treatment with charcoal, is evaporated to dryness. The dried residue is either sublimed or subjected to a prolonged ether extraction in a Soxhlet extractor. Phthalic anhydride (0.9 g.) melting at 130–131° is obtained from the ether extract after treatment with charcoal. The black precipitate (*cf.* above) is crystallized from acetic acid. From this solvent it is obtained as ill-defined dark violet crystals which are stable at temperatures up to 350°.

When a mixture of 20 g. of the quinone and 200 cc. of aqueous potassium hydroxide solution is heated for forty-five minutes and worked up as above, 1.2 g. of phthalic anhydride and 17 g. of the high melting product are obtained. If a mixture of the quinone and concentrated hydrochloric acid is boiled for one hour, only a trace of phthalic acid can be isolated; the product in this case is the high melting solid mentioned above.

**Potassium 2-Methyl-1,4-naphthoquinone-3-sulfonate**

(a) **The Reaction with Water.**—A mixture of 20 g. of the sulfonate and 2 liters of water is boiled for five hours. The mixture is cooled and filtered from the yellow-brown quinhydrone precipitate. The filtrate is evaporated and acidified with hydrochloric acid (liberation of sulfur dioxide). The mixture is evaporated again and the residue extracted with boiling water. The extracts are concentrated to dryness leaving a residue from which phthalic anhydride is extracted by the methods indicated above. The weight of phthalic anhydride was 0.8 g.

The quinhydrone (3.3 g.) crystallizes from water, and separates as bright-orange platelets melting with decomposition at 243–244°. *Anal.* Found: S, 6.6; K, 6.2.

The quinhydrone is oxidized by chlorine to a light yellow quinone and is reduced by zinc dust in acetic acid to a white hydroquinone. The study of the quinhydrone structure is not yet complete.

(b) **Effect of Alkali; Preparation of Enolate IV.**—Five grams of finely ground sulfonate in 55 cc. of 25% potassium hydroxide solution (carbonate free) is shaken vigorously until solution is almost complete. The mixture is immediately filtered through a Schott No. 2A filter whereupon a white or slightly yellowish, crystalline precipitate (enolate IV) rapidly separates from the filtrate. The solid is promptly filtered off and washed with alcohol and acetone; weight 2.2 g. Using less alkali (27–30 cc.) the yield is quantitative. *Anal.* Calcd. for $C_{11}H_6O_5SK_2$: S, 9.76; K, 23.77. Found: S, 9.76; K, 23.77.

**Reactions of Enolate, IV.**—(a) On dissolving 5 g. of the enolate in 20 cc. of water a solution alkaline to litmus is obtained which darkens rapidly. After one-half to one minute the quinhydrone starts to precipitate. After ten to twenty-two hours it is filtered off and washed with water; weight 1.8 g. Part of the filtrate was used for isolating phthalic acid or its anhydride by the procedure described above whereas another part of the filtrate was used to determine by the iodometric method the amount of sulfite formed during the reaction. The sulfite content was 0.6 g.

**Conversion of Enolate IV to III.**—A mixture of 5 g. of the enolate in 100 cc. of 25% potassium hydroxide (carbonate free) was shaken vigorously for two hours. The mixture is filtered and from the dark red filtrate an orange-red crystalline solid slowly separates. After ten to twelve hours, it is collected and washed with alcohol and acetone; weight 0.5 g. *Anal.* Calcd. for $C_{11}H_6O_5SK_2$: S, 9.76; K, 23.77. Found: S, 9.47; K, 23.64.

Upon careful acidification of the filtrate from compound III, potassium 2-methyl-1,4-naphthoquinone-3-sulfonate precipitates rapidly and almost quantitatively.

## Summary

1. The antihemorrhagic effect of vitamin K and its synthetic analogs is due to biochemical degradation to phthalic acid and is largely a function of their capacity to be transformed into the latter. Phthalic acid is regarded as the true carrier of biological activity; natural vitamin K and its synthetic analogs belonging to the naph-

(6) It is quite probable that some other compounds within the organism may act as the oxidant.

thalene series should be regarded as provitamins.

2. A mechanism for the conversion of such compounds to phthalic acid has been proposed which was established by experimental data. Potassium 2-methyl-1,4-naphthoquinone-3-sulfonate and 2-methyl-1,4-naphthoquinone are readily converted into phthalic acid on heating with water or alkali. With the former, intermediate compounds have been isolated establishing the path of the conversion to phthalic acid.

3. The biochemical degradation hypothesis is consistent with all the known data and is in variance with the view of L. F. Fieser which holds that the biological activity of the simple synthetic analogs of vitamin K is not due to their action *per se* but to their transformation within the organism, by way of biosynthesis, into quinones of the type of vitamins $K_1$ and $K_2$.

4. Less soluble derivatives of phthalic acid such as the diamide and the diethyl ester show a much higher and more protracted antihemorrhagic activity than phthalic acid.

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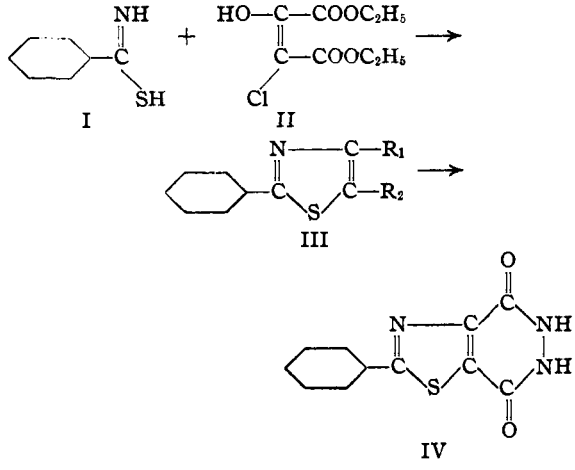
[CONTRIBUTION NO. 287 FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

# 2-Phenylthiazole-4,5-dicarboxylic Acid Derivatives

BY ERNEST H. HUNTRESS AND KARL PFISTER, 3RD[1]

In connection with a study of the chemiluminescence shown under certain circumstances by some cyclohydrazides, occasion arose to prepare 2-phenylthiazole-4,5-dicarboxylic acid cyclohydrazide (IV). This was successfully accomplished by the following reactions




Application of the Hantzsch thiazole synthesis to an equimolal mixture of thiobenzamide (I) and diethyl oxalo-$\alpha$-chloroacetate (II) gave excellent yields of 2-phenyl-4,5-dicarbethoxythiazole (III) (III, $R_1=R_2=COOC_2H_5$). Upon heating this ester in alcohol with an excess of strong hydrazine hydrate there resulted a mixture of the desired cyclohydrazide (IV) with the open chain 2-phenylthiazole-4,5-dicarboxylic acid dihydrazide. The relative amounts of these two products depended upon the duration of the reaction, the proportion of cyclohydrazide increasing with time. Since the open chain dihydrazide was insoluble in the excess hydrazine hydrate solution, it was readily filtered out and the soluble cyclohydrazide precipitated from the filtrate by acidification.

Hydrolysis of diethyl 2-phenylthiazole-4,5-dicarboxylate with methanolic potassium hydroxide gave according to the prevailing conditions either 2-phenylthiazole-4,5-dicarboxylic acid V (III, $R_1=R_2=COOH$) or its corresponding potassium acid salt VI (III, $R_1=COOK$, $R_2=COOH$). Pyrolysis of 2-phenylthiazole-4,5-dicarboxylic acid caused evolution of carbon dioxide and the formation of 2-phenylthiazole-4-carboxylic acid VII (III, $R_1=COOH$, $R_2=H$). The salt of this same monobasic acid also resulted from the pyrolysis of the potassium acid salt (VI) of the dibasic acid.

The structure of acid VII was demonstrated both positively and negatively. When 2-phenyl-4-chloromethylthiazole[2] is hydrolyzed with dilute alkali the corresponding 2-phenyl-4-hydroxymethylthiazole is readily obtained[3] and upon oxidation with aqueous chromic-sulfuric acid yields 2-phenylthiazole-4-carboxylic acid.[3] The product so obtained was in all respects identical with that from decarboxylation of the 2-phenylthiazole-4,5-dicarboxylic acid (either directly or through the potassium acid salt).

For comparison with 2-phenylthiazole-4-carboxylic acid (VII) the isomeric 2-phenylthiazole-5-carboxylic acid (VIII) (III, $R_1=R_2=COOH$) was also prepared by an independent synthesis. The condensation of thiobenzamide (I) with the sodium salt of ethyl $\alpha$-formyl-$\alpha$-chloroacetate gave 2-phenyl-5-carbethoxythiazole. Although the yield was not high (37%), use of the sodium salt gave better yields and purer product than did use of the conventional method with free aldehyde ester. Saponification of ethyl 2-phenylthiazole-5-carboxylate with methanolic potassium hydroxide gave a solution from which mineral acid

(1) This paper is constructed from part of a dissertation submitted in September, 1942, by Karl Pfister, 3rd, to the Faculty of the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Hooper and Johnson, THIS JOURNAL, **56**, 484 (1934).

(3) Huntress and Pfister, *ibid.*, **65**, 1669 (1943).