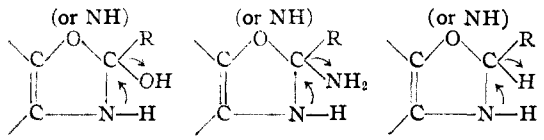
water, ammonia, some derivative of ammonia, or hydrogen. This may be shown as



Such cleavages would be facilitated by the presence of acids or bases and the reactions noted 1 to 12 are actually carried out under the influence of acids or bases.

The splitting out of hydrogen presents a more complicated situation. In the examples given above, where hydrogen is eliminated, it is believed that a so-called hydrogen acceptor must be present to aid in the elimination of hydrogen. In one case (reaction 1) the hydrogen acceptor has been shown to be a quinone and a mechanism has been proposed to account for the reaction.[2] In the other cases mentioned, suitable hydrogen acceptors were present and so similar mechanisms may be assumed for them.

Summary

1. It has been shown that *ortho* condensations leading to oxazole or imidazole formation may be represented by a common intermediate. The ring closure of the intermediate involves the addition of OH, NH_2 or RNH across a Schiff base linkage, —N=C—, with subsequent aromatization of the resulting dihydroöxazole or dihydroimidazole to the corresponding oxazole or imidazole.

PHILADELPHIA, PA. RECEIVED MAY 27, 1943

[CONTRIBUTION FROM THE MOSCOW TEXTILE INSTITUTE, THE ALL-UNION INSTITUTE OF EXPERIMENTAL MEDICINE]

Studies in the Vitamin K Group. I. Synthesis of Potassium 2-Methyl-1,4-naphthoquinone-3-sulfonate[1]

BY D. A. BOCHVAR, L. A. SCHUKINA, A. S. CHERNYSHEV, N. G. SEMENOV AND M. M. SHEMIAKIN

Since the discovery of the high antihemorrhagic activity of 2-methyl-1,4-naphthoquinone (I), exceeding that of the naturally occurring vitamins K_1 and K_2, considerable interest has been drawn to the compounds of the 1,4-naphthoquinone series and to kindred substances. The relationship between structure and antihemorrhagic activity of these compounds has been studied in many laboratories, and the results have established that certain groupings are essential for antihemorrhagic action.[2]

One of the most serious disadvantages of both natural K_1 and K_2 vitamins and of most of their biologically active synthetic analogs (including 2-methyl-1,4-naphthoquinone) is their insolubility in water, which not only limits their application in medicine, but greatly prolongs the onset of the maximum antihemorrhagic effect within the organism (eventually up to one and one-half to two days in the case of 2-methyl-1,4-naphthoquinone). Hence, numerous attempts have been made to obtain highly active water-soluble analogs of vitamins K_1 and K_2.[2]

In connection with a study of water soluble antihemorrhagic agents in the Laboratory headed by one of the authors (M. M. S.), the potassium salt of 2-methyl-1,4-naphthoquinone-3-sulfonate, V, was prepared at the beginning of 1941 by K. G. Packendorf and E. N. Lazareva. As the antihemorrhagic activity of this compound was found to be only slightly less than 2-methyl-1,4-naphthoquinone,[3] its preparation and properties seemed worthy of greater study.

The compound was first synthesized by a method resembling that developed by Fieser and Fieser[4] for converting 1,4-naphthoquinone to potassium 1,4-naphthoquinone-2-sulfonate in 80% yield which consists of treatment of the quinone with sodium bisulfite followed by oxidation with potassium dichromate. This procedure, however, was not suited to 2-methyl-1,4-naphthoquinone; our yields under similar conditions of sulfonation and oxidation did not exceed 9%. It was found that the yield depends not on the conditions of oxidation but rather on the manner in which the sulfonation of the quinone is carried out.

This investigation was interrupted in the autumn of 1941 and was resumed in the beginning of 1942. In the meantime, Moore[5] reported the

(1) The original versions of the manuscript of this article and of the following article, "Studies in the Vitamin K Group. II. The Mechanism of Biological Action of Vitamin K and of its Synthetic Analogs," by Shemiakin, Schukina and Shvezov, were received from the Soviet Embassy in Washington for publication in the JOURNAL. While their subject matter for the most part was acceptable for publication, a revision of the presentation to meet the requirements of the JOURNAL was necessary, and, in view of the inaccessibility of the authors, this revision was made at our request by an expert familiar with this special field, for whose assistance we are very grateful. With minor exceptions the revision involved only alterations in form and deletions. The revised manuscripts, therefore, with the approval of the Soviet Embassy, are being published without submission to the authors.—*The Editor.*

(2) For a complete bibliography, *cf.* L. F. Fieser, M. Tishler and W. L. Sampson, *J. Biol. Chem.*, **137**, 659 (1941).

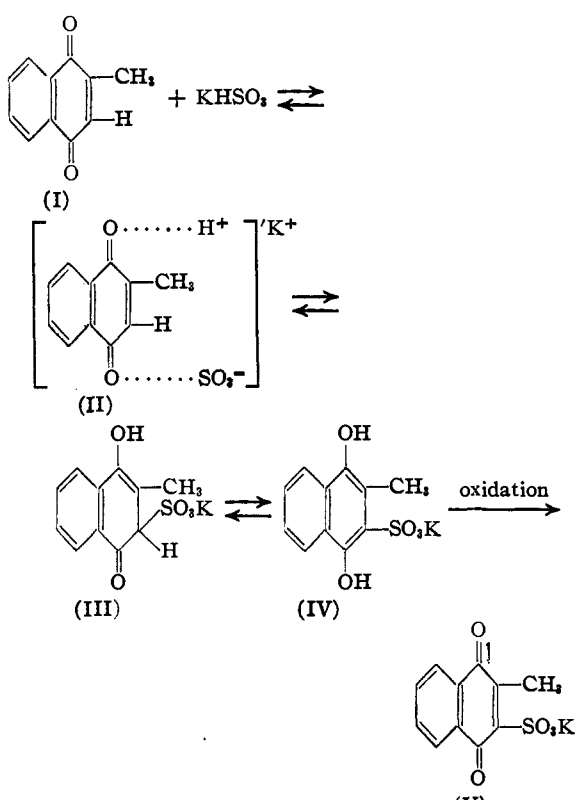
(3) B. A. Kudriashev, *Bull. Exptl. Biol. Med.*, 510 (1941) (Russ.) Sov. Zdravookhranenie Turkmenii No. 1 (1942) (Russ.). It is of interest to note that the biological action of this derivative sets in more promptly than 2-methyl-1,4-naphthoquinone although its duration is shorter.

(4) L. F. Fieser and M. Fieser, THIS JOURNAL, **57**, 491 (1935).

(5) M. B. Moore, *ibid.*, **63**, 2049 (1941).

synthesis and a study of the antihemorrhagic activity of potassium 2-methyl-1,4-naphthoquinone-3-sulfonate which was prepared by a method closely resembling that for 1,4-naphthoquinone-2-sulfonate.[4] Our biological results[3] are in disagreement with those of Moore which place the antihemorrhagic activity of this compound appreciably less than that of the parent quinone. We have examined Moore's preparative procedure and find that it not only gives a poorer yield (9 to 10%) than stated (30%) but that it is also inadequate in the isolation of pure product. To evaluate Moore's method, it was necessary for us to modify the purification procedure.[6]

Further study by us of the preparation of the sulfonate has indicated that the conditions of the first step, the sulfonation of the quinone, are the most important. In the procedure for 1,4-naphthoquinone,[4] the quinone was dissolved in potassium bisulfite containing 5% sulfuric acid and the mixture was then boiled for one hour. In the case of 2-methyl-1,4-naphthoquinone this procedure is detrimental; leaving out the sulfuric acid increases the yield to about 60%. Sulfonation of 2-methyl-1,4-naphthoquinone apparently consists of three stages, II, III and IV.



The rate of each of these successive transformations depends mostly on the concentration and temperature; higher temperatures favor a shifting of the equilibrium toward (IV). Since isomerization of the compound (III) into (IV) is a cationotropic process of the keto–enol type it should be greatly accelerated by H^+ and OH^-. Hence, the positive effect of subsequent heating with sulfuric acid for the particular case of 1,4-naphthoquinone. The introduction of a methyl group in position 2 of 1,4-naphthoquinone affects the ratios of the rates of transformation of II into III and III into IV. The sulfuric acid, therefore, causes the regeneration of II and possibly III to the original quinone I at a greater rate than the isomerization of III to IV. Such an interpretation would account for the poor yields encountered in the preparation of 2-methyl-1,4-naphthoquinone-3-sulfonate IV when sulfuric acid is employed in the sulfonation step. A detailed study of the conditions of the sulfonation step enables us to approach the solution of the mechanism of this process. This problem will be discussed in a forthcoming publication.

When the sulfonation is carried out by our method, the choice of a suitable oxidizing agent for the last step depends on the ease of subsequent manipulations. Chlorine is recommended as the product then can be purified readily.

Experimental

1. Sulfonation of 2-Methyl-1,4-naphthoquinone with Potassium Bisulfite.—Twenty grams of dry 2-methyl-1,4-naphthoquinone (the latter may also be used in the form of a 30–60% aqueous paste) is added to the solution of 40 g. of 90% potassium bisulfite in 100 cc. of water and heated in a porcelain vessel with agitation until the temperature of the thickening mass reaches 115–120°. The quinone is completely dissolved at 60–70°. Heating should last about two hours. After cooling, the mass is triturated in a mortar with 20 cc. of water and then oxidized with potassium dichromate.

1a. Oxidation with Potassium Dichromate.—To the agitated mixture at 30–35° is added over the course of an hour 230 cc. of a solution prepared by dissolving 76 g. of potassium dichromate and 40 cc. of concd. sulfuric acid in 300 cc. of water. Agitation is continued for another hour. The mixture is filtered by suction and the yellow product is washed with 80–100 ml. of water and then with 250–300 ml. of acetone. The product is recrystallized from water by quickly dissolving it in hot water (10 g. in 60–70 cc. water), treating with charcoal and filtering. Recrystallization should be carried out as promptly as possible as potassium 2-methyl-1,4-naphthoquinone-3-sulfonate gradually decomposes in a hot aqueous solution. In this way, 13.6 g. is obtained. On adding an equal volume of saturated potassium chloride solution to the mother liquors and washings, a second crop is obtained which is recrystallized (0.8 g.). Some unchanged 2-methyl-1,4-naphthoquinone is extracted from the acetone solution either through distillation or by precipitating with a large amount of water; weight 1.1 g. The yield of potassium 2-methyl-1,4-naphthoquinone-3-sulfonate, based on reacted quinone, is 45%.

1b. Oxidation with Chlorine.—The mixture obtained in Experiment 1 is diluted with an additional 280 cc. of water and subjected to a stream of chlorine (with stirring) at a

(6) Two recent papers: Baker, Davies, McElroy and Carlson, THIS JOURNAL, **64**, 1096 (1942), and Menotti, *ibid.*, **65**, 1209 (1943), which undoubtedly have not reached the authors, are concerned with the preparation of 2-methyl-1,4-naphthoquinone-3-sulfonate. The former publication indicates relatively low antihemorrhagic potency for the sulfonate in contrast to the view of this paper. It should be pointed out that the usual method of assaying antihemorrhagic activity in this country is quite different from that employed by the authors. The difference may account for the discrepancy discussed in this paper.—*The Editor.*

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%.

THE MOSCOW TEXTILE INSTITUTE
THE ALL-UNION INSTITUTE OF EXPERIMENTAL MEDICINE
RECEIVED MAY 7, 1943

[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 β,γ-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.).