yield of satisfactory material was 0.35 g. (35%). In the test with alcoholic potassium hydroxide in the cold, the quinone gives an intense blue changing slowly to yellow.

Anal. Calcd. for $C_{20}H_{22}O_2$: C, 81.59; H, 7.53. Found: C, 81.60; H, 7.62.

2 - (δ - Methyl - γ - pentenyl) - 1,4 - dihydroanthraquinone (V).—The myrcene employed was obtained from a commercial terpene fraction of bay oil. About half of this distilled at 64–69° (20 mm., 1-m. column), and on redistillation of 250 cc. of this material the myrcene collected chiefly in a fraction boiling at 64.5–66° (20 mm.) and weighing 120 g. Semmler[13] reports the b. p. 67–68° at 20 mm. For identification a sample was converted into the maleic anhydride addition product, which corresponded in b. p. (188° at 8 mm.) and m. p. (34–35°) with the material described in the literature.[9,14]

The reaction between myrcene (30 cc.) and α-naphthoquinone (10 g.) was conducted by refluxing in dioxane (30 cc.) for twelve hours. After evaporation in vacuum the oily product was taken up in petroleum ether (b. p. 30–60°) and the solution was chilled and seeded with crystals obtained by cooling a dilute solution in petroleum ether to −70°. There was obtained 15.4 g. (83%) of cream colored crystalline product, m. p. 60–61°. Two crystallizations gave colorless crystals, m. p. 61–61.3°; yield 12 g. (64%). Arbusow and Abramow[9] found the m. p. 58–58.5°.

Anal. Calcd. for $C_{20}H_{22}O_2$: C, 81.59; H, 7.53. Found: C, 81.70; H, 7.66.

The **hydroquinone diacetate IV** was prepared by heating the addition product (12 g.) in acetic anhydride (20 cc.) and pyridine (4 cc.) for one hour on the steam-bath. The solution was cooled and stirred with dilute hydrochloric acid; with the hydrolysis of the excess anhydride the diacetate separated as a tan colored solid. This was taken into ether and the solution was washed free of acid, dried, and the solvent evaporated in vacuum. Three crystallizations from alcohol gave 13 g. (84%) of colorless needles having a fluorescent greenish tinge and melting at 121–122°.

Anal. Calcd. for $C_{24}H_{26}O_4$: C, 76.16; H, 6.93. Found: C, 76.34; H, 6.98.

For conversion to the quinone, 13 g. of the diacetate was added in ether solution to the Grignard reagent prepared from 12 g. of magnesium, 400 cc. of ether, and the required amount of methyl chloride. After refluxing for forty-five minutes the ether was largely displaced with benzene and refluxing was continued for one-half hour longer. The mixture was decomposed with ammonium chloride and hydrochloric acid and the yellow organic layer was washed, dried, and shaken for one-half hour with 3 g. of silver oxide and 20 g. of sodium sulfate. The filtered solution was evaporated eventually under vacuum, leaving a residue of 7 g. (70%) of the quinone, m. p. 88–89°. Crystallized from alcohol, the substance formed glistening yellow plates, m. p. 89.8–90.8°. With alcoholic alkali the substance gives a red color changing rapidly on shaking with air to pale yellow.

Anal. Calcd. for $C_{20}H_{20}O_2$: C, 82.20; H, 6.89. Found: C, 82.25; H, 7.00.

Summary

As a route to 2,3-dialkyl-1,4-naphthoquinones of high molecular weight which might show vitamin K activity, a study has been made of the addition of suitable dienes to α-naphthoquinone and the partial dehydrogenation of the products. Two satisfactory syntheses were developed and applied to the preparation of C_{20}-quinones of the type desired. Neither of these substances shows activity.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASS. RECEIVED NOVEMBER 29, 1939

(13) Semmler, *Ber.*, **34**, 3126 (1901).
(14) Diels and Alder, *Ann.*, **470**, 65 (1929).

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

Vitamin K-Active Derivatives of 2-Methyl-1,4-naphthohydroquinone

BY S. ANSBACHER, ERHARD FERNHOLZ AND M. A. DOLLIVER[1]

About six months ago we reported[2] that 2-methyl-1,4-naphthoquinone has a vitamin K potency of 1 unit[3] in about 0.5 γ. Simultaneously other investigators[4,5,6] published their work with this substance, and it is apparent that they then failed to observe the outstanding biological activity of this compound. However, the same authors,[7,8,9] and others[10,11] fully confirmed our results in more recent communications. Meanwhile we prepared and investigated several derivatives which are the object of the present report.

(1) Chemist at the Development Laboratory of E. R. Squibb & Sons, Brooklyn, N. Y.
(2) Ansbacher and Fernholz, THIS JOURNAL, **61**, 1924 (1939).
(3) Ansbacher, *J. Nutrition*, **17**, 303 (1939).
(4) Almquist and Klose, THIS JOURNAL, **61**, 1923 (1939).
(5) Fieser, Bowen, Campbell, Fry and Gates, Jr., *ibid.*, **61**, 1926 (1939).
(6) Thayer, Cheney, Binkley, MacCorquodale and Doisy, *ibid.*, **61**, 1932 (1939).
(7) Fieser, *ibid.*, **61**, 2559 (1939).
(8) Thayer, Binkley, MacCorquodale, Doisy, Emmett, Brown and Bird, *ibid.*, **61**, 2563 (1939).
(9) Almquist and Klose, *J. Biol. Chem.*, **130**, 787 (1939).
(10) Tishler and Sampson, THIS JOURNAL, **61**, 2563 (1939).
(11) Personal communication of September 25, 1939, from Dr. H. Dam of the University of Copenhagen, Denmark.

Experimental Part[12]

2 - Methyl - 1,4 - naphthohydroquinone - dipropionate.—A mixture of 10 g. of 2-methyl-1,4-naphthoquinone, 30 ml. of propionic acid, 100 ml. of propionic anhydride, 25 g. of zinc dust and 5 g. of fused sodium propionate was refluxed for thirty minutes in an oil-bath at 160–170°, then poured into water. An oily product separated and solidified on standing. It was removed by filtration and recrystallized from dilute methanol to a constant m. p. of 74–75°; yield 12 g. (72%).

Anal. Calcd. for $C_{17}H_{18}O_4$: C, 71.29; H, 6.34. Found: C, 71.48; H, 6.36.

The procedure followed for preparing the dipropionate is similar to the one used by Anderson and Newman[13] for the synthesis of the diacetate.

2 - Methyl - 1,4 - naphthohydroquinone - dibenzoate.—12.5 g. of 2-methyl-1,4-naphthohydroquinone was treated with 30 g. of benzoyl chloride in 60 g. of pyridine while cooling. After standing for one hour, the mixture was heated for thirty minutes at a bath temperature of 110–120° and poured into water. The resulting precipitate was collected by filtration and recrystallized from dilute acetone to a constant m. p. of 179°; yield 16.2 g. (59%).

Anal. Calcd. for $C_{25}H_{18}O_4$: C, 78.51; H, 4.97. Found: C, 78.71; H, 5.00.

A second crop of 9 g. (m. p. 174–176°) was obtained from the mother liquors, bringing the total yield to 25.2 g. (84%).

2 - Methyl - 1,4 - naphthohydroquinone - di - *n* - butyrate. Method A.—A mixture of 10 g. of 2-methyl-1,4-naphthoquinone, 30 ml. of butyric acid, 100 ml. of butyric anhydride, 5 g. of fused sodium butyrate, and 25 g. of zinc dust was refluxed for one hour in an oil-bath at 170–180°, then poured into water. As crystallization could not be induced, the oily product was extracted with ether, dried over anhydrous sodium sulfate and fractionated to give 12.5 g. of an oil distilling at 175–185° (1 mm.). The distillate solidified on cooling and stirring, and was recrystallized from dilute methanol to a constant m. p. of 52–53°; yield 10 g. (55%).

Anal. Calcd. for $C_{19}H_{22}O_4$: C, 72.57; H, 7.06. Found: C, 72.66; H, 7.31.

Method B. A mixture of 15 g. of *n*-butyryl chloride and 100 ml. of pyridine was added to 8.5 g. of 2-methyl-1,4-naphthohydroquinone and gently refluxed for one hour. After standing for one hour the mixture was poured into 800 ml. of water and the resulting oil extracted with ether. The ethereal solution was washed with 10% hydrochloric acid to remove pyridine, then freed from acid with water, and finally dried over anhydrous sodium sulfate. Fractionation gave 7.9 g. of an oil distilling at 190–195° (1.5 mm.). The distillate solidified on cooling and stirring. Recrystallization from dilute methanol yielded 3.5 g. of a compound melting at 52–53° and with no depression of melting point when mixed with the product resulting from method A.

2 - Methyl - 1,4 - naphthohydroquinone - di - isobutyrate.—Thirty grams of isobutyryl chloride in 75 ml. of pyridine was added to 19.5 g. of 2-methyl-1,4-naphthohydroquinone, the mixture allowed to stand for ninety minutes, then heated in an oil-bath at 120–135° for one hour, and finally poured into water. The solids thus obtained were removed by filtration, washed with water, and recrystallized from dilute methanol to a constant melting point of 73–74°; yield 24.6 g. (69%).

Anal. Calcd. for $C_{19}H_{22}O_4$: C, 72.57; H, 7.06. Found: C, 72.64; H, 7.02.

2 - Methyl - 1,4 - naphthohydroquinone - di - *n* - valerate.—Sixteen grams of 2-methyl-1,4-naphthohydroquinone was dissolved in 80 ml. of pyridine and treated dropwise while cooling and shaking with 30 g. of *n*-valeryl chloride. After standing for one hour the mixture was refluxed for one hour at a bath temperature of 120–130°, allowed to stand overnight and finally poured into water. The oily product was extracted with ether, the extract washed with water, 10% hydrochloric acid, and again with water. After being dried over anhydrous sodium sulfate, the ether was removed by distillation and the residual red oil was distilled three times under reduced pressure from a modified Claisen flask. The final product was a yellow oil distilling at 210° (1 mm.); yield 22 g. (70%).

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.64; H, 7.66. Found: C, 73.43; H, 7.77.

After standing for some time, it crystallized and was recrystallized from dilute methanol to a constant melting point of 40–41°.

2 - Methyl - 1,4 - naphthohydroquinone - di - isovalerate.—Fifteen grams of 2-methyl-1,4-naphthohydroquinone was treated with 30 g. of isovaleryl chloride in 50 ml. of pyridine, then heated at a bath temperature of 130° for one hour. The mixture was poured into water and the resulting oil extracted with ether. The ethereal solution was washed with water, 10% hydrochloric acid, and again with water. After being dried over anhydrous sodium sulfate the ether was removed by evaporation and the residue was fractionated by three vacuum distillations from a modified Claisen flask. The final product was a yellow oil distilling at 185° (1 mm.); yield 14.2 g. (48%).

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.64; H, 7.66. Found: C, 73.02; H, 7.44.

2-Methyl-1,4-dimethoxynaphthalene.—18.4 g. of 2-methyl-1,4-naphthohydroquinone was added to 53.6 g. of dimethyl sulfate in a flask equipped with a reflux condenser, mercury-seal stirrer, and a gas inlet tube. Nitrogen was passed through the apparatus while adding 1-ml. portions of a solution of 47.6 g. of potassium hydroxide in 100 ml. of water by way of the condenser. The mixture was then heated on the steam-bath for forty-five minutes, allowed to stand overnight, finally diluted to 500 ml. with water and extracted with ether. The extract was washed free from alkali with water, dried over anhydrous sodium sulfate and fractionated to yield 16.5 g. of an oily product distilling at 174–178° (18 mm.). The oil solidified on treatment with 50 ml. of 1 *N* sodium hydroxide and cooling. Recrystallization from petroleum ether gave a white crystalline product melting at 48–49°; yield 11.8 g. (55%).

Anal. Calcd. for $C_{13}H_{14}O_2$: C, 77.19; H, 6.98. Found: C, 77.09; H, 6.87.

The procedure followed for preparing the dimethyl ether

(12) Analyses by Dr. F. A. Smith, Chemist at the Development Laboratory of E. R. Squibb & Sons, Brooklyn, N. Y.

(13) Anderson and Newman, *J. Biol. Chem.*, **103**, 405 (1933).

is essentially the one outlined by Giral.[14] However, the product obtained by this investigator had a b. p. of 175–178° (18 mm.) and a m. p. of 23–24°.

Bio-assays.—The rapid assay procedure, carried out to date on more than 15,000 chicks, was employed for determining the vitamin K unit of the various naphthohydroquinone derivatives summarized in Table I. The method has been described in detail[3] and found applicable to the testing of concentrates,[3,15,16] synthetic vitamins[15] and synthetic substitutes.[2,15,17,18] Certain minor, but nevertheless important, points seem to warrant emphasis, since they were overlooked by other investigators when attempting to make use of a rapid assay procedure.

TABLE I

VITAMIN K UNIT OF METHYLNAPHTHOHYDROQUINONE AND ITS DERIVATIVES

Compound, 2-Methyl-1,4-	1 unit equals, γ
Naphthohydroquinone	0.5
Naphthohydroquinonediacetate	1
Naphthohydroquinonedipropionate	1
Naphthohydroquinonedibenzoate	1
Naphthohydroquinone-di-*n*-butyrate	1.25
Naphthohydroquinone-di-isobutyrate	5
Naphthohydroquinone-di-*n*-valerate	1.25
Naphthohydroquinone-di-isovalerate	3
Dimethoxynaphthalene	5

The medium employed for administering the test substances was cod liver oil in most of our experiments. Other oils, such as peanut or corn oil, are likewise satisfactory vehicles. However, the amount of the menstruum plays a decisive role. In accordance with the definition of our unit,[3] we administer fat-soluble test substances always in 0.10 ml. of oil. If a product is soluble in water, its potency can be determined with aqueous solutions. The 2-methyl-1,4-naphthoquinone is soluble in water to the extent of about 100 γ/ml.; it shows a biological response indicative of 1 unit in about 0.5 γ, irrespective of whether it is dissolved in 0.10 ml. of cod liver oil or 1 ml. of water. Incidentally, such aqueous solutions can be administered intravenously.

If a vitamin K experiment is carried out properly, the chicks become deficient within two weeks, no spontaneous cures occur, the blood clotting time of all the animals remains prolonged, and hemorrhages develop as a result of which death occurs. If a bird shows hemorrhages but has a normal or only slightly prolonged coagulation time after the depletion period, it undoubtedly has had access to vitamin K. There are chicks which will be more resistant to vitamin K deficiency than others, as already pointed out for birds with enlarged thyroid glands.[3] Furthermore, many investigators have commented on the apparent resistance of animals hatched during the summer months, and we have found that exposure of day-old chicks to freezing temperatures for about six hours, as a preliminary step to the depletion period, is a contributing factor to a high incidence of vitamin K deficiency. A biological assay properly conducted will permit the determination not only of the minimum effective dose, but also of the duration of the curative effect, summarized for the naphthohydroquinone derivatives in Table I and Table II, respectively.*

TABLE II

DURATION OF THE CURATIVE EFFECT OF THE VARIOUS COMPOUNDS

Compd.	Amount, γ	Hours
2-Methyl-1,4-naphthoquinone	0.75	72
	.50 (1 unit)	48
	.25	24
2-Methyl-1,4-naphthohydroquinonediacetate	1.5	72
	1.0 (1 unit)	48
	0.75	24
2-Methyl-1,4-naphthohydroquinonedipropionate	1.5	72
	1.0 (1 unit)	48
	0.75	24
2-Methyl-1,4-naphthohydroquinone-di-*n*-butyrate	1.5	72
	1.25 (1 unit)	48
	1.0	24

Discussion

Since 2-methyl-1,4-naphthohydroquinone may be oxidized easily to methyl-1,4-naphthoquinone, the equal potency of the two compounds was to be expected and has been mentioned already by Thayer, *et al.*[8] The mechanism of the biological effect of vitamin K active naphthohydroquinone derivatives has not been explained; it appears possible that an oxidation–reduction phenomenon is involved and certain data apparently substantiate such a hypothesis. The diacetate, dipropionate and dibenzoate esters are identical as far as potency, speed of action and period of efficacy within the animal body are concerned. This fact could be interpreted to mean that esters are

(14) Giral, *Anales soc. espan. fis quim.*, **31**, 861 (1933).

(15) Ansbacher, Fernholz and MacPhillamy, *Proc. Soc. Exp. Biol. Med.*, **42**, 655 (1939).

(16) Fernholz, Ansbacher and Moore, THIS JOURNAL, **61**, 1613 (1939).

(17) Fernholz and Ansbacher, *Science*, **90**, 315 (1939).

(18) Ansbacher and Fernholz, *J. Biol. Chem.*, **131**, 399 (1939).

(*) The detailed data of Tables I and II are included in the authors' reprints of this paper.

first saponified and then act in the form of methylnaphthohydroquinone. However, the potencies of the di-*n*-butyrate and di-*n*-valerate were found to be significantly greater than those of the corresponding iso-compounds with their inherent branched chains. This interesting observation may be explained by a difference in the rate of absorption, but it is also possible that these derivatives act as a whole and not in the form of a methylnaphthohydroquinone–methylnaphthoquinone equilibrium. Furthermore, the activity of the dimethyl ether is remarkably great, although in all probability this compound cannot be transformed to methylnaphthohydroquinone, considering the difficulty with which methyl ethers of phenols are split *in vitro*.

Our data confirm the results of Binkley, *et al.*,[19] namely: "In general, the activities of the diacetates of the 1,4-diols were about one-half that of the corresponding quinones." Most recently Doisy and his co-workers[20] stated, furthermore: "Only 1,4-naphthoquinones and compounds which upon oxidation in the organism might yield 1,4-naphthoquinones showed activity." In view of our observations above, particularly in conjunction with the vitamin K potency of phlorone, already reported,[18] it would seem that vitamin K activity is not confined solely to 1,4-naphthoquinones. Incidentally, anthraquinone appears to have a potency of one unit in about 2 mg.

(19) Binkley, Cheney, Holcomb, MacCorquodale, Thayer and Doisy, 98th Meeting, Am. Chem. Soc., Boston, Mass., September 12, 1939.

Summary

The preparation of several esters and an ether of 2-methyl-1,4-naphthohydroquinone is described.

The various derivatives were found to have different vitamin K potencies.

A discussion of the relationship between structure and vitamin K activity is presented.

(20) Doisy, MacCorquodale, Thayer, Binkley and McKee, Nat. Acad. Sci., Brown U. Meeting, October 23–25, 1939 [*Science*, **90**, 407 (1939)].

NEW BRUNSWICK, N. J. RECEIVED NOVEMBER 13, 1939

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Sulfanilamide Compounds. II. Arylidine Derivatives of N^1-Substituted Sulfanilamides

BY H. G. KOLLOFF AND JAMES H. HUNTER

In 1936 Buttle, Gray and Stephenson[1] reported N^1-phenylsulfanilamide to be as active against streptococcal infections in mice as sulfanilamide and on the basis of our preliminary tests N^1-(4-nitro)-phenylsulfanilamide[2] shows considerable activity against both streptococcal and pneumococcal infections. Since 1938 N^1-(2-pyridyl)-sulfanilamide[3] (Sulfapyridine, M. and B. 693) has attained a foremost position in the ranks of antibacterial agents.

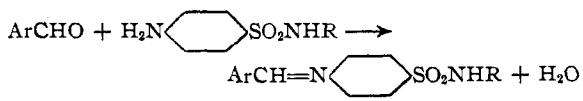
Goissedet, Despois, Galliot and Mayer[4] observed that some benzylidine sulfonamides were active and during the following year work along this line was continued by Gray, Buttle and Stephenson.[5]

The foregoing considerations suggested the preparation of a series of arylidine derivatives of the N^1-substituted sulfanilamides in order to ascertain in what respect their activity was generally affected by introducing an arylidine group.

For sake of comparison between structure and activity, the arylidine derivatives of the parent compound, sulfanilamide, have been included.

Sulfanilamide and sulfapyridine were obtained through commercial sources; N^1-phenylsulfanilamide was prepared according to Gelmo,[6] and for the preparation of N^1-(4-nitro)-phenylsulfanilamide the procedure of Webster and Powers[2] was successfully modified in that pyridine rather than dimethylaniline was used as a reaction medium.

The arylidine derivatives listed in Table I were prepared in 73% to practically quantitative yields by condensing the N^1-substituted sulfanilamides with the appropriate aldehyde in the absence of a solvent according to the equation



Purification of the new derivatives offered con-

(1) Buttle, Gray and Stephenson, *Lancet*, **1**, 1286 (1938).
(2) Webster and Powers, THIS JOURNAL, **60**, 1553 (1938).
(3) Whitby, *Lancet*, **1**, 1210 (1938).
(4) Goissedet, Despois, Galliot and Mayer, *Compt. rend. soc. biol.*, **121**, 1082 (1936).
(5) Gray, Buttle and Stephenson, *Biochem. J.*, **31**, 724 (1937).
(6) Gelmo, *J. prakt. Chem.*, [2], **77**, 374 (1908).