substituted naphthoquinones. A positive color reaction for 2-hydroxy-1,4-naphthoquinones [*ibid.*, **58,** 1174 (1936)] was obtained with highly purified concentrates of vitamin K from alfalfa.

The positions of ultraviolet absorption maxima reported in vitamin K concentrates, especially at 328 and 248 mμ [*Helv. Chim. Acta*, **22,** 310 (1939)], are close to the 334 and 250 maxima of phthiocol [*J. Biol. Chem.*, **115,** 479 (1936)], and the general shapes of the absorption curves are similar.

We have made quantitative assays by our improved method [*Biochem. J.*, in press] of phthiocol and several related compounds. In addition to the substances listed in Table I, we have tested lapachol and lomatiol. Both of these proved inactive, first at a 20-mg. level and later at a 100-mg. level. A preliminary test of a preparation of phthiocol monoacetate indicated a greater degree of activity than that of phthiocol.

An aqueous solution of phthiocol was made by dissolving 2 mg. in each cc. of a 0.05 molal phosphate buffer, *p*H 7.4. The *p*H of the final solution was 7.0. Sufficient of this solution was injected daily into the breast muscle of chicks to equal the amount consumed by chicks on the 20-mg. level. A control group received the same amount of solution orally. The average prothrombin time for 6 injected chicks was 31.9 seconds, while that for 6 orally fed chicks was 32.4 seconds. An 0.05 molal phosphate buffer, *p*H 7.8, dissolved 4 mg. of phthiocol per cc. to a final *p*H of 7.1. Chicks receiving no vitamin K in the diet were given intravenously 2 mg. each of phthiocol in aqueous solution. A comparable group of chicks was given the same dosage orally. After an interval of two days, the prothrombin time of 5 injected chicks was 29.6 seconds, and that of 5 orally fed chicks 30.3 seconds. Phthiocol appeared to exhibit approximately the same activity whether given in the diet or as a solution orally, intramuscularly or intravenously.

The antihemorrhagic activity of phthiocol lies between that of the methyl naphthoquinone and the hydroxy naphthoquinone (Table I). Consideration of the activities of various compounds indicates that the methyl group is functionally important, while the hydroxyl group seems to reduce activity. The latter effect may be largely physical. Phthiocol is obviously lower in activity than the more complex form of vitamin K existing in alfalfa. This lower activity is more than compensated for by the low cost of preparation and great convenience of administration of the compound.

TABLE I[a]

ANTIHEMORRHAGIC ACTIVITY OF SEVERAL NAPHTHOQUINONES

Substance	Level fed per kg. of diet	Av. prothrombin time, seconds	Act. in terms of cc. of ref. std. per g.
Ref. std.[b]	2 cc.	54.5	
Ref. std.	4 cc.	37.4	
Ref. std.	8 cc.	26.7	
Phthiocol	5 mg.	80.6	268
Phthiocol	20 mg.	32.0	263
Phthiocol	20 mg.	31.6	270
2-Methyl-1,4-naphthoquinone	20 mg.	26.1	435
	50 mg.	23.2	
2-Hydroxy-1,4-naphthoquinone	100 mg.	26.4	84
Alfalfa concentrate	2 mg.	26.1	4350

[a] Received June 22, 1939.

[b] Standard hexane extract of dried alfalfa representing 1 g. per cc.

We are indebted to Professor R. J. Anderson for the phthiocol and a sample of lapachol, and to Professor L. F. Fieser for samples of lapachol and lomatiol. Our work has been aided by a grant from Merck and Co., Inc.

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RECEIVED JUNE 21, 1939

SIMPLE COMPOUNDS WITH VITAMIN K ACTIVITY

Sir:

The announcement of Almquist and Klose [THIS JOURNAL, **61,** 1611 (1939)] that pure synthetic phthiocol has anti-hemorrhagic activity prompts us to publish certain observations on related compounds. We have found that 2-methyl-1,4-naphthoquinone is practically as active as vitamin K, and that the diacetate of the corresponding hydroquinone appears to be somewhat inferior in potency. The chicks survived doses of several thousand units of these compounds, the cure was as dramatically rapid as with natural vitamin K and the animals developed normally thereafter. The activity of phthiocol reported by Almquist and Klose was confirmed with a preparation made from the above compounds, but the potency of the phthiocol thus prepared was several hundred times less than that of vitamin K. Duroquinone was found to be inactive in doses of as high as 1 mg.

TABLE I

BIOLOGICAL DATA OF TEST SUBSTANCES

Amount administered γ	No. of chicks	B. C. T.,[a] min. before treatment	after
2-Methyl-1,4-naphthoquinone			
2500	10	>90	<1[b]
1000	20	>90	<1
500	10	>90	<1
333	10	>90	<1
100	10	>90	<1
10	10	>90	<2
5	10	>90	<3
1/2	10	>90	<6
2-Methyl-1,4-acetoxynaphthalene			
1000	10	>90	<1[b]
100	10	>90	<1
10	10	>90	<4
5	10	>90	<8
2-Methyl-3-hydroxy-1,4-naphthoquinone			
1000	10	>90	<2[c]
100	20	>90	>30[d]
10	5	>90	>30
Duroquinone			
1000	10	>90	>30

[a]B. C. T. = blood clotting time.

[b]All vitamin K-deficiency symptoms disappeared within twenty-four hours and the chicks doubled their weight within ten days.

[c]Three chicks died during the six-hour test period.

[d]Four chicks died during the six-hour test period.

We are preparing and investigating a large number of quinones and hydroquinones, particularly those with a long aliphatic side chain to which class vitamins K_1 and K_2 [Binkley, *et al.*, THIS JOURNAL, **61**, 1612 (1939)] appear to belong.

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RECEIVED JUNE 20, 1939

QUINONES HAVING VITAMIN K ACTIVITY

Sir:

Certain indications from recent publications of others and from preliminary oxido-reduction potential measurements conducted at Northwestern University have led us to postulate that the antihemorrhagic vitamin K_1 of alfalfa is a 2,3-dialkyl-1,4-naphthoquinone. From the similarity of vitamin K_2 [Doisy, *et al.*, THIS JOURNAL, **61**, 1295, 1612 (1939)] in absorption spectrum, ease of oxidation of the hydroquinone and other properties, it is probable that this substance is of the same type. As a specific hypothesis it is suggested (L. F. F.) that vitamin K_1 may be 2,6(?)-dimethyl-3-phytyl-1,4-naphthoquinone (or the 2-monomethyl compound) and vitamin K_2 2,3-difarnesyl-1,4-naphthoquinone. These structures seem consistent with the spectra [Doisy, *et al.*; Dam, Karrer, *et al.*, *Helv. Chim. Acta.* **22**, 310 (1939)], the analyses and hydrogen absorption [Doisy, *et al.* (assuming the saturation of one ring of the naphthalene nucleus)], the sensitivity to heat and light, and the hindered character of the functional groups of the quinone (K_1) [Almquist, *et al.*, *J. Biol. Chem.*, **125**, 681 (1938); Riegel, Schweitzer and Smith, in press] and hydroquinone diacetates (K_1 and K_2) [Doisy, *et al.*]. They also accord with recognized processes of biogenesis: K_1 (alfalfa) from dimethylnaphthoquinone (or toluquinone plus isoprene) and phytol [occurrence in green leaves: Dam, *Z. Vitaminforsch.*, **8**, 248 (1938–39); relationship between vitamins E and K_1]; K_2 (putrefied sardine meal) from naphthoquinone and farnesol (relationship of the alcohol to squalene).

These considerations suggested (B. R. and L. F. F.) the testing of various quinones available from previous researches or from the collection of the late Samuel C. Hooker. The synthesis of compounds of the type indicated has been undertaken at Harvard. Exploratory assays of ten compounds were kindly carried out by Dr. W. L. Sampson of the Merck Institute by a procedure based on that of Ansbacher [*J. Nutrition*, **17**, 303 (1939)]. Day old chicks were placed on the Almquist vitamin K deficient diet for twelve days and given a dose of 250 γ of substance in 1 cc. of peanut oil, administered by a tube into the crop, and the blood clotting time determined (Almquist method) the following morning.

The preliminary results suggest that some of the 2-hydroxy-3-alkyl-1,4-naphthoquinones possess positive vitamin K activity at the dose level fed [compare phthiocol, Almquist and Klose, THIS JOURNAL, **61**, 1611 (1939)] and that the 2,3-dimethyl derivative is at least 1/250 as active as Doisy's vitamin K_1.

2-Allyl-1,4-naphthoquinone (m. p. 36–36.5°, found: C, 78.82; H, 5.14) was prepared from 2-allyl-1-naphthol through the azo compound and amine (W. P. C.). 2,3-Diallyl-1,4-naphthohydroquinone diacetate (m. p. 92.5–93°, found: C, 74.18; H, 6.33) was obtained by heating 1,4-naphthohydroquinone diallyl ether (m. p. 49.5–50°, found: C, 80.09; H, 6.74) with diethylaniline and acetic anhydride (M. F.). The diacetate is