color, if sufficient vitamin K is present and interfering pigments are practically absent. Soon the color changes to a reddish-purple and finally to a reddish-brown. At this point, carotenoid pigments may be removed by partition with a hydrocarbon solvent. The color due to reaction of the vitamin with sodium methylate remains in the methanol phase. To test the agreement of color reaction with activity, we have applied this reaction to a variety of sources of the vitamin assayed by a procedure already described [*Biochem. J.*, **32,** 1897 (1938)]. Results are given in Table I.

Fractions obtained by chromatographic adsorption showed a consistent relation of color test to activity. This was also true of fractions obtained by incomplete molecular distillation and of a preparation (concentrate 1270) obtained by repeated precipitation from methanol by chilling with solid carbon dioxide [*J. Biol. Chem.*, **120,** 635 (1937)] but not purified from sterols. A preparation of the molecular compound of the vitamin with deoxycholic acid [THIS JOURNAL, **61,** 745 (1939)] showed a color reaction consistent with its activity, which was also true of the residue of this preparation remaining after partial extraction of the vitamin with xylene. A strong color reaction was also produced on testing an active concentrate prepared by repeated molecular distillation of soybean oil, followed by removal of sterols, free fatty acids and waxes. In addition to the data in the table, we may report that the color reaction has been obtained in extracts of several kinds of bacteria known to be good sources of the vitamin [*Proc. Soc. Exp. Biol. Med.*, **38,** 336 (1938)].

TABLE I

ANTIHEMORRHAGIC ACTIVITY AND COLOR REACTION INTENSITY OF VITAMIN K CONCENTRATES

	Concentrate	Level fed per kilo of diet, mg.	Av. blood clotting time, min.	Rel. intensity of color test
	Chromatographic adsorption fractions			
1	Orange zone	10	2.8	4
2	Light yellow zone	10	1.8	8
3	Yellow zone	10	6.3	2
4	Colorless zone	10	>30	0
	Incomplete distillation fractions			
1	Low temp. distillate, P11	80	>30	0
2	Vitamin distillate, P11	20	4.2	2
3	Residue, P11	20	7.3	1
4	Vitamin distillate, P8	10	3.5	4
5	Residue, P8	10	3.7	4
6	Vitamin distillate, P12	20	3.7	2
	Other preparations			
1	Concentrate 1270	10	4.7	4
2	K-choleic acid, 8P	30	2.7	4
3	K-choleic acid, 8P xylene extracted	30	14.1	1
4	Soybean oil concentrate	400	3.0	strong

The results strongly indicate that the color reaction is due to the vitamin itself. The character of the pigment is being studied further.

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RECEIVED MAY 19, 1939

THE ANTI-HEMORRHAGIC ACTIVITY OF PURE SYNTHETIC PHTHIOCOL

Sir:

We wish to announce the discovery of the anti-hemorrhagic activity of pure synthetic phthiocol, 2-methyl-3-hydroxy-1,4-naphthoquinone. The physical and chemical properties of this compound are in general similar to those known for vitamin K. When fed to chicks at a level of 20 mg. per kg. of vitamin K-free diet, phthiocol maintained the average blood-clotting time at 2.1 minutes in one test and 1.6 minutes in a second test. At a level of 10 mg. the blood-clotting time was maintained at 1.8 minutes. Chicks fed only the basal ration had prolonged blood-clotting times. The minimum required level is being determined. It is probable that phthiocol is the simplest member of an homologous series of anti-hemorrhagic substances.

We are indebted to Professor R. J. Anderson for the phthiocol used in these experiments.

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RECEIVED MAY 31, 1939

CHROMATOGRAPHIC ADSORPTION AND DIPOLES

Sir:

The use of the method of chromatographic adsorption has become extremely important for the separation of complex mixtures of organic molecules.

A careful survey of numerous experimental investigations has revealed the importance of dipoles in determining the order of adsorption of a mixture on a polar medium (*i. e.*, aluminum oxide). Thus Karrer [Karrer and Nielsen, *Ber.*

Ges. Physiol. Exptl. Pharmakol., **86**, 529–530 (1935)] has shown that on aluminum oxide the order of occurrence in the tube of mono-nitrophenols is para, meta, and ortho. These authors obtained similar results with the corresponding nitroanilines. This proves that basicity and acidity are not important factors. The above order, however, is exactly that of the decreasing permanent dipoles of these substances. Cook [*J. Chem. Soc.*, 876 (1938)] has shown recently that *cis*-azobenzene is more strongly adsorbed on alumina than the *trans* modification. This is in accord with this author's idea concerning the dipole interaction between the solute dipoles and the fixed dipoles in the polar adsorbing media.

In cases where no permanent dipole exists, it is to be expected that those substances with highest polarizability (*i. e.*, ease to form an induced dipole) would be more strongly adsorbed. This is actually the case with Kuhn's polyenes.

Adsorption of this type is competitive between the solvent and adsorbing media.

The number of isolated dipoles in a molecule is important, however. It has been shown here that picric acid with three nitro groups is more strongly adsorbed on aluminum oxide (from benzene–petroleum ether solutions) than is *o*-nitrophenol, although the latter has a larger permanent dipole. The same was found for 4-methyl-2-nitrophenol.

It appears that in isomeric molecules containing the same number and kind of functional groups, those with the larger dipole moments are more strongly adsorbed on polar media.

A detailed study is being made in this Laboratory and will be reported later.

DEPARTMENT OF CHEMISTRY RICHARD T. ARNOLD
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RECEIVED MAY 5, 1939

DERIVATIVES OF VITAMINS K_1 AND K_2

Sir:

As further evidence that we have actually isolated vitamins K_1 and K_2 and that the two vitamins contain the suggested quinone structure [THIS JOURNAL, **61**, 1295 (1939)], we have now prepared the diacetates of dihydro vitamin K_1 and dihydro vitamin K_2. These diacetates are colorless, crystalline derivatives which possess the activity of vitamin K. When vitamin K_1 is reductively acetylated the diacetate of dihydro vitamin K_1 is obtained. This derivative may be crystallized readily from low boiling petroleum ether (30–60°) or methyl alcohol (solvents in which vitamin K_1 is soluble and insoluble, respectively) in fine white needles melting at 59°. *Anal.* Found: C, 78.21, 78.01; H, 10.07, 10.03; mol. wt., 531. Calcd. for $C_{36}H_{56}O_4$: C, 78.21; H, 10.21; mol. wt., 552; for $C_{36}H_{54}O_4$: C, 78.50; H, 9.88; mol. wt., 550. Microhydrogenation: uptake of H_2, 3.04 moles (of vitamin K_1 4.08 moles). Assay: approximately 500 units per mg. There is general absorption in the region from 220 mμ to beyond 300 mμ with intense absorption at 230 mμ where the extinction coefficient of $E^{1\%}_{1\,cm.} = 1250$.

The compound is not readily hydrolyzed by alkali or acid in an aqueous or alcoholic medium. In alcoholic solution its activity is unstable to 1% potassium hydroxide and thirty-six hours of exposure to sunlight but is stable to one hundred hours of exposure to light from a 100-watt bulb at a distance of 4 feet (1.2 meters).

Diacetyl dihydro vitamin K_1 was converted to vitamin K_1 by treating it with an excess of methylmagnesium iodide followed by shaking an ether solution of the hydrolyzed product with air. After fractionation by distillation at 2×10^{-4} mm. pressure, 85–90% of the theoretical yield of the vitamin was obtained. *Anal.* Found: C, 82.34; H, 10.13. Calcd. for $C_{32}H_{48}O_2$: C, 82.70; H, 10.41; for $C_{32}H_{50}O_2$: C, 82.33; H, 10.80. Assay: 1000 units per mg.

Repetition of the reductive acetylation of this vitamin K_1 preparation gave a compound which according to melting point, mixed melting point and bio-assay was identical with the original diacetate of vitamin K_1.

Vitamin K_2 was converted to the diacetate of dihydro vitamin K_2 by the same method as used for K_1. The derivative after purification by several recrystallizations from low boiling petroleum ether (30–60°) and methyl alcohol melted at 57–58°. *Anal.* Found: C, 80.89, 81.03; H, 9.94, 9.79; mol. wt., 628. Calcd. for $C_{44}H_{60}O_4$: C, 80.93; H, 9.26; mol. wt., 652; for $C_{44}H_{62}O_4$: C, 80.68; H, 9.54; mol. wt., 654. Microhydrogenation: uptake of H_2, 7.99 moles; (of vitamin K_2 8.73 moles). Assay: approximately 300 units per mg. The ultraviolet absorption is very similar to that of the diacetate of dihydro vitamin K_1. The extinction coefficient of $E^{1\%}_{1\,cm.} = 1250$ at 232 mμ.