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.

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RICHARD T. ARNOLD

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 $E^{1\%}_{1\,\text{cm.}}$ = 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 $E^{1\%}_{1\,\text{cm.}}$ = 1250 at 232 mμ.

In our previous communication (*loc. cit.*) we gave an extinction coefficient of $E^{1\%}_{1\,cm.}$ = 385 at 248 mμ for vitamin K_1 but since that time a value of 540 has been obtained. The analyses of both preparations indicated that each was analytically pure. Our first value was probably due to instability of the vitamin on storage and toward light. For this reason we are not at present certain that 540 is the maximum value attainable. In this same communication line 38 column 1 should read "all have a potency of about *1000* units per mg." instead of, "all have potency of about 100 units per mg."

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RECEIVED MAY 22, 1939

DENATURATION OF MYOSIN

Sir:

Myosin is a protein particularly susceptible to "denaturation" by very mild chemical agents. We have employed four criteria of alteration in the molecule: alteration in –SH groups,[1] loss of double refraction of flow,[2] changes in viscosity and in solubility. Seven preparations of myosin from rabbit muscle, and one from lobster, have been studied with very consistent results.

Some of our observations are briefly summarized in Table I, from which several conclusions may be drawn. (1) The extreme asymmetry of the molecule, on which its double refraction depends, is diminished rapidly by all commonly employed denaturing agents, and by many other substances as well. (2) The content of titratable –SH groups is greatly increased in concentrated solutions of urea and even more in guanidine hydrochloride; in the presence of the latter at 16 *m*, nearly all the non-methionine sulfur of myosin[3] can be accounted for as –SH. The concentrations of urea and guanidine needed to produce marked increase in –SH content are much greater than those needed to destroy double refraction. (3) Other substances, such as lithium, calcium and magnesium chlorides, destroy double refraction but do not affect –SH groups. Substances containing an ammonium group abolish titratable –SH completely (but the full content of titratable –SH is immediately restored by adding concentrated guanidine hydrochloride). Such substances may or may not destroy double refraction (see table). There appears to be no systematic correlation between the effect of reagents on –SH groups and their effect on double refraction.

(1) Greenstein, *J. Biol. Chem.*, **125**, 501 (1938); **128**, 233 (1939).
(2) Von Muralt and Edsall, *ibid.*, **89**, 351 (1930).
(3) Bailey, *Biochem. J.*, **31**, 1406 (1937).

TABLE I

EFFECT OF REAGENTS ON DOUBLE REFRACTION OF FLOW AND ON SULFHYDRYL CONTENT OF RABBIT MYOSIN

All substances tested were added to myosin dissolved in KCl, 0.4–0.5 *n*, at *p*H 6.2–7.4. *T* denotes the time required for disappearance of double refraction of flow. –SH content is expressed as percentage cysteine.

Substance added	Molality in solution	*T*	–SH content, %
KCl	0.5	>2 weeks	0.42 ± 0.03
KBr	0.80	15 min.	..
KI	0.27	15 min.	0.46
Guanidine HCl	0.30	5 min.	0.42
Guanidine HCl	16.6	At once	1.14 ± 0.03
Guanidine HI	0.075	>5 days	..
Guanidine HI	0.14	1 hour	..
Guanidine HI	0.20	5 min.	..
Guanidine HI	0.28	<30 sec.	..
Urea	1.4	15 min.	0.42
Urea	16.6	At once	0.66 ± 0.03
LiCl	1.0	10 min.	0.46
$MgCl_2$	0.35	2 min.	0.46
$CaCl_2$	0.25	5–10 min.	..
NH_4Cl	1.4	10 min.	0
CH_3NH_3Cl	1.4	10 min.	0
Arginine mono-HCl	0.35	10 min.	0
Glycine	1.7	>1 week	0

The viscosity of myosin solutions decreases markedly on addition of reagents which destroy double refraction of flow. This is explicable on the assumption that the very long molecules of native myosin are broken up into smaller and less asymmetrical chains by the action of such reagents. This decrease in viscosity is in marked contrast to the increase produced by denaturation in solutions of "globular" proteins.[4]

The solubility of myosin is not fundamentally altered by any of the reagents studied; it retains the characteristics of a typical globulin. This is in marked contrast with the loss of solubility produced by heating myosin[5] and with the effect of all denaturing agents in decreasing the solubility of such proteins as egg albumin or hemoglobin.

(4) Anson and Mirsky, *J. Gen. Physiol.*, **15**, 341 (1932).
(5) Mirsky, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 150 (1938).

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ON THE COLOR REACTION FOR VITAMIN K

Sir:

Recently Dam, Karrer and co-workers [*Helv. Chim. Acta*, **22**, 310 (1939)] described a vita-