reaction of these compounds with the corresponding acid chlorides in pyridine. Catalytic hydrogenation of the estrone derivatives in neutral medium yielded the mono esters of α-estrodiol. Estrone *t*-butylacetate was also prepared by the Schotten–Baumann procedure. The esters prepared appear to be somewhat more soluble than are many of the other well known esters of these substances.

We wish to thank Parke, Davis and Company for their generous help and assistance in the various phases of this work.

Experimental Part

Estrone Trimethylacetate.—To a solution of 300 mg. of estrone in 12 cc. of dry pyridine was added 1 cc. of trimethylacetyl chloride. The resulting mixture, after standing at room temperature for thirty-six hours, was diluted with water and the precipitated solid taken up in ether. The ethereal extract was washed with dilute hydrochloric acid and dilute sodium carbonate solution. Evaporation of the ether gave white needles which was recrystallized from acetone–methanol as thick white needles, m. p. 164–166°.

Anal. Calcd. for $C_{23}H_{30}O_3$: C, 77.9; H, 8.5. Found: C, 77.6; H, 8.3.

α-Estrodiol-3-trimethylacetate.—A mixture of 200 mg. of estrone trimethylacetate, 300 mg. of Adams catalyst, 50 cc. of ether and 50 cc. of ethanol was shaken with hydrogen at 1 atmosphere pressure at room temperature for eighteen hours. The mixture was filtered and the filtrate evaporated *in vacuo*. The residual sirup was treated with Norite and crystallized from aqueous methanol as white needles, m. p. 178–180°.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.5; H, 9.0. Found: C, 77.7; H, 9.0.

α-Estrodiol-3,17-*bis*-trimethylacetate.—A mixture of 100 mg. of α-estrodiol, 10 cc. of pyridine and 0.5 cc. of trimethylacetyl chloride was treated as described for the preparation of estrone trimethylacetate. The product was crystallized from acetone–methanol as white needles, m. p. 174–176°.

Anal. Calcd. for $C_{28}H_{40}O_4$: C, 76.3; H, 9.15. Found: C, 76.0; H, 9.2.

Estrone *t*-Butylacetate.—Estrone *t*-butylacetate was prepared by the pyridine method as described for estrone trimethylacetate. The product was crystallized from methanol as white plates, m. p. 148–150°.

Anal. Calcd. for $C_{24}H_{32}O_3$: C, 78.2; H, 8.7. Found: C, 78.1; H, 8.6.

To a solution of 50 mg. of estrone in 150 cc. of 10% aqueous potassium hydroxide was added 1 cc. of *t*-butylacetyl chloride. The mixture was shaken vigorously for five minutes, and the solid collected, washed and dried. The product crystallized from methanol as white plates, m. p. 147.5–149.5°. This gave no depression with that prepared above.

α-Estrodiol-3-*t*-butylacetate.—This was prepared as described for α-estrodiol-3-trimethylacetate. The product was crystallized from aqueous methanol as white needles, m. p. 127–129°.

Anal. Calcd. for $C_{24}H_{34}O_3$: C, 77.8; H, 9.2. Found: C, 78.1; H, 9.4.

α-Estrodiol-3,17-di-*t*-butylacetate.—This was prepared from α-estrodiol as described for α-estrodiol-3,17-*bis*-trimethylacetate. The product was crystallized from methanol as white plates, m. p. 98–100°.

Anal. Calcd. for $C_{30}H_{44}O_4$: C, 76.9; H, 9.5. Found: C, 76.9; H, 9.5.

SCHOOL OF CHEMISTRY AND PHYSICS
THE PENNSYLVANIA STATE COLLEGE
STATE COLLEGE, PENNA. RECEIVED APRIL 28, 1939

COMMUNICATIONS TO THE EDITOR

THE ANTIHEMORRHAGIC ACTIVITY OF CERTAIN NAPHTHOQUINONES

Sir:

We have briefly reported on the antihemorrhagic activity of phthiocol, 2-methyl-3-hydroxy-1,4-naphthoquinone, the first completely identified form of vitamin K [THIS JOURNAL, **61** 1611 (1939)]. Phthiocol has been isolated as the pigment of *Mycobacterium tuberculosis* (human) and synthesized by Anderson and co-workers [*J. Biol. Chem.*, **101,** 773 (1933); **103,** 197 (1933); **105,** 279 (1934)]. This organism is known to contain vitamin K [*Proc. Soc. Exp. Biol. Med.*, **38,** 336 (1938)].

Treatment of vitamin K concentrates with sodium methylate produces a reddish pigment the quantity of which is proportional to the activity of the concentrate [THIS JOURNAL, **61,** 1610 (1939)]. The pigment has a strong red color in alkaline media, from which it can be extracted by adding hexane or ethyl ether and acidifying. It then assumes a yellow color. These color changes of the derived pigment are very similar to those exhibited by phthiocol and similarly

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.