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.

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

TABLE I

Compound	No. of birds	Clotting times, min. 5	5–10	10–20	20–30	>30	Per cent. 10-min. birds	Remarks
Controls	20	1	3	2	..	14	20	
2,3-Dimethyl-1,4-naphthoquinone	10	10	..	..	..	..	100	Very effective
Lomatiol	9	5	2	1	..	1	77	Effective
Hydroxyhydrolapachol	9	5	1	1	..	2	67	Effective
Lapachol	9	1	3	4	1	..	45	Borderline
Diallyl-1,4-hydroquinone[1]	10	3	2	2	..	3	50	Borderline
Lomatiol methyl ether[2]	9	1	3	..	..	5	45	Doubtful
Hydrolapachol	9	2	2	2	..	3	45	Borderline
Diallyl-1,4-hydroquinone diacetate[3]	8	..	3	3	2	..	37	Probably not effective
Lapachol methyl ether[4]	9	..	3	..	1	6	35	Not effective
Diallyl-1,4-benzoquinone[5]	10	3	..	..	..	7	30	Not effective

[1] M. p. 130–131°, found: C, 75.80; H, 7.40. By rearrangement of hydroquinone diallyl ether (H. B. Dunkle, Dissertation, Harvard University) along with an isomer, m. p. 87–90°, found: C, 76.17; H, 7.60 (E. M. F.).

[2] M. p. 61.5–62°, found: C, 70.96; H, 6.05; prepared with diazomethane (W. P. C.).

[3] M. p. 111–112°, found: C, 70.25; H, 6.70; from the higher melting isomer (E. M. F.).

[4] M. p. 51.5–52°, found: C, 74.76; H, 6.34 (W. P. C.).

[5] M. p. 16°, found: C, 76.71; H, 6.75; by oxidation of the 130–131° isomer with silver oxide (E. M. F.).

resistant to alkaline hydrolysis (compare Doisy's dihydro vitamin diacetates); on cleavage with a Grignard reagent and air oxidation in ether it gave a quinone, m. p. 129–130° (absorption maxima at 245, 267, and 330 mμ in ethanol). 2,3-Dimethyl-1,4-naphthoquinone shows maxima at 246 and 265 mμ (log ε between 4.2 and 4.3) and 330 mμ (log ε = 3.4). The spectra (R. N. J. and D. M. B.) resemble those reported for vitamins K_1 and K_2 (Doisy, *et al.*, Dam, Karrer, *et al.*) except for the absence of fine structure in the two intense bands.

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RECEIVED JUNE 12, 1939

SYNTHESIS OF ANTIHEMORRHAGIC COMPOUNDS

Sir:

The further bio-assays by Dr. W. L. Sampson given in Table I lend added support to our conception of the nature of vitamin K_1 and K_2. Of special significance is the contrast between the highly active 2,3-dimethyl-1,4-naphthoquinone and the much less potent 2,6- and 2,7-isomers, the contrast between quinones of the benzene and naphthalene series, and the high potency encountered in a 1,4-naphthoquinone having a β-unsaturated side-chain (allyl) in the quinonoid ring, as postulated for both vitamins. The spectrographic data of Table II also indicate a close correspondence between the natural vitamins and model substances of the postulated structure of 2,3-dialkyl-1,4-naphthoquinones. Dr. T. J. Webb of the Merck Research Laboratories independently examined 2,3-dimethyl-1,4-naphthoquinone and pointed out to us definite indications of fine structure in the two intense bands; this was subsequently discerned in a new reading of our plates. The resolution of the most intense band of the 6,7-dimethyl-2,3-diallyl compound is still more distinct, particularly in hexane. We are indebted to Dr. Webb for the other determinations indicated.

The following examples illustrate methods developed for the synthesis of quinones of the type considered favorable for vitamin K activity. (1) 2,6-Dimethyl-8-naphthol was allylated, the ether rearranged, and the once distilled allyldimethylnaphthol (b. p. 152–157° at 2 mm. Found: C, 84.20; H, 7.74) converted to the 5-amine and this oxidized in acetone suspension with ferric chloride to give 2,6-dimethyl-3-allyl-1,4-naphthoquinone, m. p. 42–42.5° (Found: C, 79.82; H, 6.36). (2) The lower melting diallylhydroquinone (87–90°) was oxidized with silver oxide to the quinone (oil); 2,3-dimethylbutadiene was added to this, the product was isomerized to a hydroquinone, and on oxidation with chromic acid this afforded 6,7-dimethyl-2,3-diallyl-1,4-naphthoquinone, m. p. 69.5–70.7° (Found: C, 81.46; H, 6.96). 6,7-Dimethyl-1,4-naphthoqui-

TABLE I

BIO-ASSAYS BY THE ANSBACHER PROCEDURE

Substance (In 1 cc. peanut oil)	No. of birds	Clotting times, min. 5	5–10	10–20	20–30	> 30	% 10-min. birds	Remarks
Controls (1st series)	20	2	4	6	..	8	30	
Peanut oil (1 cc.)	10	..	3	..	..	7	30	
Alfalfa (100 mg.)	10	5	3	1	1	..	80	
Alfalfa (50 mg.)	9	2	5	1	1	..	77	
Alfalfa (25 mg.)	10	1	4	2	1	2	50	
Alfalfa (10 mg.)	10	1	3	2	2	2	40	
2-α-Heptenyl-3-hydroxy-1,4-naphthoquinone (0.2 mg.)	10	3	4	2	..	1	70	Some activity
2-n-Heptyl-3-hydroxy-1,4-naphthoquinone (0.2 mg.)	10	2	6	2	..	..	80	Some activity
2-Allyl-1,4-naphthoquinone (0.2 mg.)	10	10	..	..	..	..	100	Very active
Controls (2nd series)	11	..	..	..	..	11	0	All over 60 min.
Alfalfa (150 mg.)	10	7	3	..	..	..	100	
2,3-Diallyl-1,4-naphthohydroquinone diacetate (0.2 mg.)	10	..	1	1	5	3	10	Inactive
2,6-Dimethyl-1,4-naphthoquinone (0.2 mg.)	10	..	4	..	..	6	40	Very sl. act.
2,7-Dimethyl-1,4-naphthoquinone (0.2 mg.)	10	3	3	2	..	2	60	Active

TABLE II

ABSORPTION MAXIMA

In ethanol, except as noted; log ϵ values given in parentheses

	Maxima (in mμ)		
Vitamin K_1 (Doisy, *et al.*)	243, 248	261, 270	323
(Dam, Karrer, *et al.*)	248	261, 270	328
Vitamin K_2 (Doisy, *et al.*)	249	261, 269	320
2,3-Dimethyl-1,4-naphthoquinone (T. J. Webb)	244, 249 (4.29)	264, 270 (4.27)	332 (3.39)
(D. M. B., new reading)	243, 249 (4.26)	262, 267 (4.24)	330 (3.38)
6,7-Dimethyl-2,3-diallyl-1,4-naphthoquinone	253, 260 (4.40)	273, 278 (4.12)	343 (3.45
(In hexane)	253, 260 (4.41)	271, 276 (4.14)	338 (3.46)
2-Allyl-1,4-naphthoquinone	246, 251 (4.31)	Shoulder	332 (3.43)
(In hexane)	243, 251 (4.1)	260 (3.9)	Not measured
2-Methyl-1,4-naphthoquinone (T. J. Webb)	250 (4.29)	263 (4.24)	334 (3.38)
2,6-Dimethyl-1,4-naphthoquinone (T. J. Webb)	256 (4.33)		340 (3.47)
2,6-Dimethyl-3-allyl-1,4-naphthoquinone[a]	249 (4.30) 256 (4.35)	266, 272 (4.17)	335 (3.39)

none, m. p. 118–119° (Found: C, 77.43; H, 5.61), was similarly prepared from the known diene addition product. (3) Monobutadiene-1,4-benzoquinone with allyl bromide and potassium carbonate in acetone gave 5,8-dihydro-1,4-naphthohydroquinone diallyl ether, m. p. 64–65° (Found: C, 79.51; H, 7.55), and when heated in kerosene this rearranged smoothly to 5,8-dihydro-2,3-diallyl-1,4-naphthohydroquinone, m. p. 108–109° (Found: C, 79.36; H, 7.78). Chromic acid oxidation in acetic acid gave 2,3-diallyl-1,4-naphthoquinone, m. p. 29–30° (Found: C, 80.53; H, 6.02). The substance (m. p. 130°) obtained previously by the action of ethyl or n-butylmagnesium bromide on 2,3-diallyl-1,4-naphthohydroquinone diacetate is a naphthoquinone (spectrum) having two hydrogen atoms more than the expected diallyl compound (Calcd. for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 80.06, 79.93; H, 6.78, 6.76). More gentle cleavage with methylmagnesium bromide and silver oxide oxidation gave 2,3-diallyl-1,4-naphthoquinone, m. p. 28.5–29.5°, identical with the above sample.

In the Dam–Karrer color test with sodium ethylate in ethanol, regarded by some as characteristic of vitamin K_1 (Almquist and Klose, THIS JOURNAL, **61**, 1610 (1939); see, however, Fernholz, *et al.*, *ibid.*, **61**, 1613 (1939)), our synthetic naphthoquinones having at least one allyl group in the quinonoid ring all give intense and transient blue or purple colors and contrast sharply with the 2,3-dimethyl compound (weak, purplish color).[a]

[a] Received June 26, 1939.

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RECEIVED JUNE 23, 1939

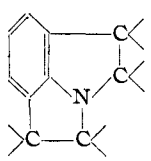
THE STEREOCHEMISTRY OF TERVALENT NITROGEN

Sir:

Jackson and Kenner [*J. Chem. Soc.*, 573 (1928)] briefly reviewed the existing evidence bearing

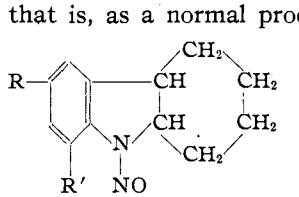
on the spatial configuration of tervalent nitrogen compounds in which three separate groups are attached to the nitrogen atom. They pointed out that "the search for isomerides demanded by a non-planar configuration has been (almost completely) unsuccessful, and it would therefore appear that, in general, the non-planar readily passes into a planar form, from which the original or its enantiomorph may be regenerated; or else the normal configuration is plane [compare Meisenheimer, *Ber.*, **57**, 1747 (1924)].

"Before, however, such conclusions can be accepted, it is desirable that the negative results on which they rest should be supplemented by positive evidence. This would be supplied by the preparation of a compound in the molecule of which a nitrogen atom is common to two ring structures which are at the same time plane and co-planar. Since . . . there is no evidence available which renders doubtful the plane configuration of five-membered ring structures, it would appear that these conditions would be fulfilled by a structure of type I, if Kekulé's formula for benzene and its derivatives be accepted."

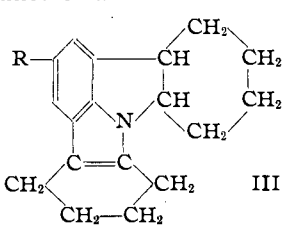


I

Jackson and Kenner were unable to report the preparation of a compound of this type. However, there had been recorded just previously some experiments by Manjunath [*J. Indian Chem. Soc.*, **4**, 271 (1927)] in which it was shown that treatment of a glacial acetic acid solution of 9-nitroso-hexahydro-carbazole (II, R,R = H) containing cyclohexanone with zinc dust, and then warming, led to formation of a crystalline substance $C_{18}H_{21}N$, which he described as "8,9-(1,2-cyclohexyl)-tetrahydro-carbazole." His structural formula for this substance is obviously incorrect as it represents a substance of formula $C_{19}H_{23}N$. Every analogy suggests that Manjunath's compound should be formulated as III (R = H), that is, as a normal product of a Fischer indole ring closure, and as a substance fulfilling the requirements of Jackson and Kenner's test.



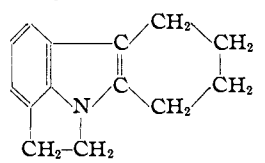
II



III

We have now confirmed Manjunath's result, and his further observation that a similar substance (III, R = CH_3) can be obtained in a similar manner from 9-nitroso-6-methyl-hexahydro-carbazole (II, R = CH_3, R′ = H). On the other hand, we have been unable to prepare a substance of similar structure from 9-nitroso-8-methyl-hexahydro-carbazole (II, R = H, R′ = CH_3) because the ortho position essential for indole ring closure has been "blocked" by the methyl group.

That the substances III have the structures assigned to them is strongly supported by the fact that addition of zinc dust to an acetic acid solution of 1-nitroso-indoline containing cyclohexanone, followed by warming, leads to formation of a colorless crystalline neutral substance, $C_{14}H_{15}N$, melting at 154°. There can be no doubt that this must be formulated as IV, so that it, also, must be regarded as fulfilling the requirements of Jackson and Kenner's test.



IV

There is thus no doubt that substances can be prepared containing a tervalent nitrogen atom with three separate atoms attached to the nitrogen, in which the three nitrogen valences must be regarded as definitely co-planar.

DEPARTMENT OF ORGANIC CHEMISTRY FRANCIS LIONS
THE UNIVERSITY OF SYDNEY ERNEST RITCHIE
SYDNEY, AUSTRALIA

RECEIVED MAY 22, 1939

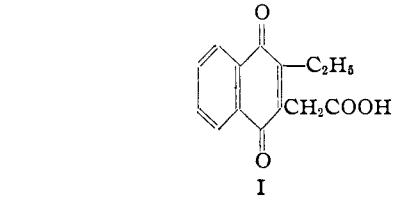
ON THE CONSTITUTION OF VITAMIN K_1

Sir:

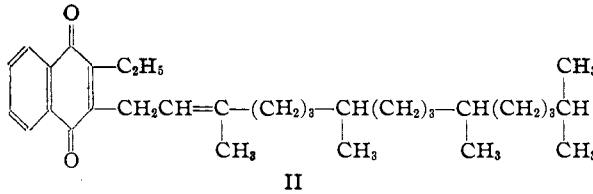
In a previous communication [THIS JOURNAL, **61**, 1295 (1939)] we suggested that vitamins K_1 and K_2 contain the quinone structure, and subsequently [*ibid.*, **61**, 1612 (1939)] we confirmed this by preparation of the diacetates by reductive acetylation. The pure yellow color of the vitamins suggests that they belong to the 1,4 series of quinones and this conclusion is supported by the discovery that 1,4-naphthoquinone has vitamin K activity whereas 1,2-naphthoquinone does not. Investigation of a considerable number of quinones has revealed only the derivatives of 1,4-naphthoquinone as having vitamin K activity.

On catalytic hydrogenation vitamin K_1 absorbs four moles of hydrogen with the formation of a hydroquinone. A naphthoquinone nucleus would account for three moles of hydrogen, the fourth mole being used in the saturation of an ethylenic linkage.

Ozonolysis of the diacetate of vitamin K_1 hydroquinone resulted in the formation of a ketone which gave a semicarbazone melting at 66–67°. *Anal.* Found: C, 70.04; H, 12.13; N, 12.83, 12.88. Calcd. for $C_{19}H_{39}ON_3$: C, 70.10, H, 12.08; N, 12.91. The semicarbazone of 2,6,10-trimethylpentadecanone-14 melts at 66–67° [G. F. Fischer and K. Lowenberg, *Ann.*, **464**, 69 (1928)]. The identity of our semicarbazone with that of Fischer and Lowenberg will be tested as soon as a specimen becomes available for determination of the mixed melting point. The formation of this ketone probably indicates the presence of a phytyl side chain in the vitamin molecule.



I



II

Vitamin K_1 was oxidized with chromic acid and the oxidation products separated into neutral and acidic fractions. Two crystalline acids were obtained from the latter fractions. One of these acids crystallized from water and had a melting point of 191° in a sealed tube. It was identified as phthalic acid by conversion to the anhydride. This melted at 127–128°. When mixed with authentic phthalic anhydride (m. p. 128–129°) the melting point was 128–129°. *Anal.* Calcd. for $C_8H_4O_3$: C, 64.87; H, 2.72. Found: C, 64.78; H, 3.05.

The second solid acid which was obtained in small amounts crystallized in well-formed yellow needles and melted with decomposition at 210°. If the phytyl radical is directly united to a 1,4-quinone ring, this would presumably be a substituted naphthoquinone acetic acid. This acid, like vitamin K_1, gives no color reaction with ethyl cyanoacetate [R. Craven, *J. Chem. Soc.*, 1605 (1931)] and so is presumably substituted in both the 3 and 4 positions. *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95. Found: C, 68.69; H, 4.85. The acid is probably 2-ethyl-1,4-naphthoquinone-3-acetic acid (I). The synthesis of this acid is at present in progress for purposes of comparison. On the basis of these degradation products we believe that the structure of the vitamin K_1 molecule is 2-ethyl-3-phytyl-1,4-naphthoquinone (II).

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RECEIVED JUNE 19, 1939

EVIDENCE FOR THE PRESENCE OF VITAMIN A AND CAROTENOIDS IN THE OLFACTORY AREA OF THE STEER

Sir:

In connection with our general project on the chemistry and sources of the fat-soluble vitamins, we became interested in examining the olfactory area of various animals for the presence of these vitamins, especially so for the presence of vitamin A's and their precursors. Since the absence of vitamin A from the diet causes the drying up of the mucous membranes of the body, it was suspected that the epithelia of the olfactory area together with the mucous membranes of the nasal passages in animals having normal diets might be rich in this vitamin. No work has been reported along these lines, and we decided to make a preliminary study of the olfactory area of the steer, but especially that area located at the upper end of the nasal cavity and known as the "yellow patch." This is composed of nerve filaments passing from the brain through the sieve-like cribriform plate into the nasal cavity and terminating at the upper third of this cavity. In the steer the epithelium of the olfactory area is dirty yellowish-brown in color while in the human being it is said to be yellow.

Forty heads of freshly killed steers were split open along the length of the nasal passages and by means of bone-cutters the olfactory area together with the bone and cartilage to which the epithelia were attached were removed. Most of the bone and cartilage were then removed from the yellowish-brown tissue, leaving a sample of about 470 g. This tissue was well ground and autolyzed with ethyl alcohol. Both the alcohol

extract and the autolyzed tissue were subjected to several extractions with pure ether in an atmosphere of nitrogen. The ether extracts were combined, washed with water and dried over anhydrous sodium sulfate.

An absorption spectrum of the deep yellow ethereal solution in the visible spectrum showed bands at 420, 442, 478 and 655 mμ, respectively. In petroleum ether the same extract showed bands at 420, 444, 472 and 656 mμ, respectively, while the antimony trichloride color in chloroform showed prominent bands with maxima at 420, 495 and 610 mμ, respectively.

The sample was then saponified in an atmosphere of nitrogen, the non-saponifiable fraction taken up in ether, the ethereal solution filtered at 0° and an absorption spectrum taken of the filtrate in the visible region of the spectrum. It showed bands at 446 and 474 mμ, respectively. The spectrum of the antimony trichloride color showed bands at 410, 440, 495, 620 and in one case at 690 mμ, respectively. An ultraviolet absorption spectrum of this sample in ethyl alcohol is shown plotted in Fig. 1. A maximum is readily

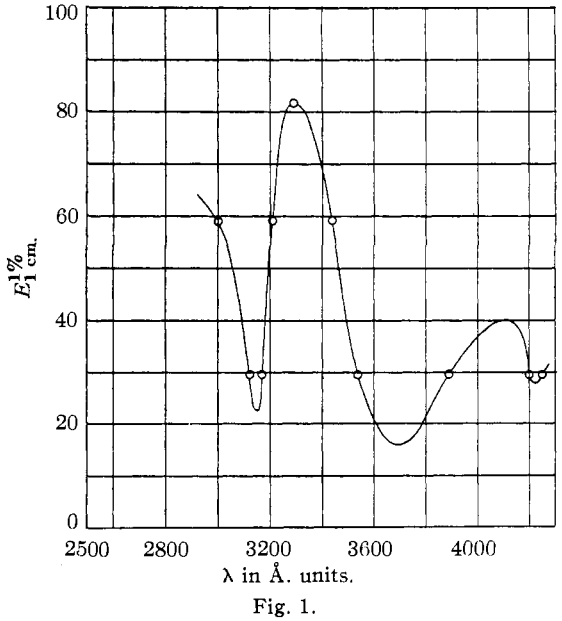

Fig. 1.

seen at 328 mμ which together with that of the antimony trichloride at 620 mμ is characteristic of vitamin A_1. The band of the antimony trichloride color at 690 mμ may be due to the presence of vitamin A_2, although the appearance of this band was not consistent. From the intensity of the maximum at 328 mμ and the concentration of the solution, we calculated $E^{1\%}_{1\ cm.}$ to be 82 for our sample which, on the basis of the purest vitamin A having a potency of 3,250,000 U. S. P. vitamin A units per gram [Milas and Heggie, unpublished results] would have a potency of 116,000 U. S. P. vitamin A units per gram. In another sample taken from a single steer head we found a potency of about 76,000 U. S. P. vitamin A units per gram.

The bands of the visible spectrum at 420, 442–446, 472–478 mμ, respectively, are due to carotenoids. We have not as yet identified the other bands reported in this paper and inasmuch as we are continuing with our work, we are hoping to report a more complete account of it later.

CONTRIBUTION NO. 193 FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY
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WILLIAM M. POSTMAN
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RECEIVED JUNE 19, 1939

THE FORMATION OF α-ETHYLTHIOGLUCOPYRANOSIDE FROM GLUCOSE ETHYLMERCAPTAL

Sir:

It has been found two years ago [Green and Pacsu, THIS JOURNAL, **59,** 1205 (1937)] that α-ethyl- and α-benzylthioglucosides [Schneider, *et al.*, *Ber.*, **49,** 2054 (1916); **51,** 220 (1918); **61,** 1244 (1928)] are furanosides and not of "normal" (pyranoid) structure as their discoverers believed. This was shown by acid hydrolysis constants, conversion into ethylglucofuranoside, and calculations from Hudson's rules of isorotation. On account of certain irregularities observed by Green and Pacsu during the process of hydrolysis, the behavior of α-ethylthioglucofuranoside in 0.01 *N* hydrochloric acid at 100° was subsequently studied [Pacsu and Wilson, THIS JOURNAL, **61,** 1450 (1939)]. It was found that in this unprecedented hydrolysis about one-half of the α-ethylthioglucofuranoside changed into glucose and mercaptan whereas the other half escaped the hydrolyzing effect of the acid by shifting the furanoid ring into the acid resistant pyranoid ring. The ring shift resulted in the formation of α-ethylthioglucopyranoside, a new thioglucoside, which was isolated and characterized by its tetraacetate. In a recent paper [Brigl, Gronemeier and Schulz, *Ber.*, **72,** 1052 (1939)] which was submitted for publication one month later but appeared one month earlier than the article of Pacsu and Wilson, Brigl and co-workers re-

ported the preparation of the same α-ethylthioglucopyranoside and its tetraacetate. These authors proposed to use glucose ethylmercaptal for disaccharide synthesis and mixed the mercaptal in 22% hydrochloric acid with glucose. Instead of the desired disaccharide Brigl and co-workers obtained this α-ethylthioglucopyranoside in an apparently undetermined yield. As to the mechanism of the reaction the authors stated that the mercaptal lost one mercaptan residue which was partly transferred to the admixed glucose, the whole process being represented by the following two-stage reaction:

(a) $C_6H_{12}O_5(SC_2H_5)_2 = C_6H_{11}O_5(SC_2H_5) + C_2H_5SH$
(b) $C_2H_5SH + C_6H_{12}O_6 = C_6H_{11}O_5(SC_2H_5) + H_2O$

Since we had reasons to believe that this mechanism might not be the correct one, we repeated Brigl's experiment with the modification that we *omitted* glucose. From the reaction mixture we obtained α-ethylthioglucopyranoside in about 20% minimum yield. Also, we obtained the same compound but in somewhat smaller yield (15%) from the reaction of equimolecular quantities of glucose and ethylmercaptan in 22% hydrochloric acid. In our first experiment there was but a mere trace of glucose present in the acetone insoluble residue consisting mainly of barium chloride, whereas in the second experiment, when glucose was used as starting material, the acetone insoluble salt contained a fairly large quantity of unchanged glucose. In both instances the lower rotating component of the reaction mixture represented probably the acid resistant β-ethylthioglucopyranoside, since in neither case could we find glucose ethylmercaptal. These results seem to indicate that the formation in 22% hydrochloric acid of α-ethylthioglucopyranoside from the mercaptal on one hand, and from glucose and mercaptan on the other, are two distinct and independent reactions.

FRICK CHEMICAL LABORATORY EUGENE PACSU
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PRINCETON, NEW JERSEY

RECEIVED JUNE 20, 1939

VITAMIN B_6, A GROWTH PROMOTING FACTOR FOR YEAST

Sir:

The rate of proliferation of *S. cerevisiae* in purified solutions is known to be profoundly affected by a group of substances known as bioses. The multiple nature of bios is firmly established and further evidence of the multiplicity of bios was found in the discovery of the bios action of thiamine [Schultz, Atkin and Frey, THIS JOURNAL, **60,** 490 (1938)].

We have now found that crystalline vitamin B_6 has the properties of a bios factor. This substance acts on the yeast types A and B in an advantageous manner. The work with crystalline B_6 was made possible by the gift of a few milligrams by Merck and Company.

TABLE I

TWENTY-FOUR HOUR GROWTH OF YEASTS A AND B AS INFLUENCED BY VITAMIN B_6, ETC.

Total volume in each case: 30 ml. (seeded with 1 mg. of moist yeast and rocked at 30° for 24 hours). Crop × 4.54 gives mg. of moist yeast. Supplements: inositol (1) 1 mg.; β-alanine (IIA) 0.005 mg.; bios IIB 0.13 mg.; thiamine 0.01 mg.; vitamin B_6 (VI) 0.05 mg.

Ingredients of bios tests: all C. P. sugar, salts, buffer, I and IIA, plus	24-Hour crop Type A	Type B
Nil	Trace	40
II B	15	210
II B plus thiamine	100	120
Vitamin B_6 (VI)	Trace	40
II B plus Vitamin B_6	150	200
II B plus B_6, plus thiamine	170	200

The properties of crystalline B_6 are: (1) stimulation of Type A yeast to produce a 24-hour crop of 100–120; (2) removal of the inhibition imposed on Type B yeast by thiamine; (3) stimulation of Type A yeast to give a high 24-hour crop in the absence of thiamine.

Crystalline vitamin B_6 was found to have a certain activity as a fermentation accelerator under the conditions of our fermentation test [Schultz, Atkin and Frey, THIS JOURNAL, **59,** 2457 (1937)]. The stimulation is of about the same type as the nicotinic acid effect [Schultz, Atkin and Frey, *ibid.*, **60,** 1514 (1938)] and may be overcome in the same way, *i. e.*, by adding about 0.05 mg. of B_6 to each test.

There are indications that the growth method may be useful as a method for the determination of vitamin B_6. A growth test in which all factors except B_6 are present in excess will respond to solutions or concentrates in proportion to their B_6 content as indicated by rat curative tests which were made by R. F. Light and L. J. Cracas of our Laboratory.

THE FLEISCHMANN LABORATORIES ALFRED S. SCHULTZ
STANDARD BRANDS INCORPORATED LAWRENCE ATKIN
NEW YORK, N. Y. CHARLES N. FREY

RECEIVED JUNE 10, 1939

VITAMIN B_6 AS A YEAST NUTRILITE

Sir:

In corroboration of the findings of Schultz, Atkin and Frey [THIS JOURNAL **61**, 1931 (1939)], we wish to indicate that we have independently found that vitamin B_6 is effective in yeast growth stimulation. A typical experiment is outlined below.

The basal medium was similar to that used in previous work [Williams and Saunders, *Biochem. J.*, **28**, 1887 (1934)], but contained 0.1 g. of aspartic acid per liter instead of asparagin. It also contained 0.03 mg. of thiamine, 0.3 mg. of β-alanine, and 30 mg. of autolyzed liver extract per liter. The liver extract had been treated with charcoal and with fuller's earth. The yeast seeding was 0.03 mg. of a pure culture isolated from a Fleischmann cake per 12 ml. culture and the growth period was fourteen hours at 30°. The vitamin B_6 used had been generously furnished by Dr. Samuel Lepkovsky.

TABLE I

EFFECT OF VITAMIN B_6 ON YEAST GROWTH

Vitamin B_6 added (γ per culture)	Yeast crop (mg. per 12 ml. culture)
0	4.47
0	4.53
0.0005	4.44
.001	4.95
.005	6.29
.01	6.82
.05	8.01
.1	8.58
.5	7.94
1	8.27

This finding makes more emphatic the close relationship between "B" vitamins and substances effective for the stimulation of the growth of yeasts (as well as other microörganisms).

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ROBERT E. EAKIN
ROGER J. WILLIAMS

RECEIVED JUNE 10, 1939

VITAMIN K ACTIVITY OF SOME QUINONES

Sir:

In view of the recent note of Almquist and Klose [THIS JOURNAL, **61**, 1611 (1939)] and their conclusion "that phthiocol is the simplest member of an homologous series of anti-hemorrhagic substances," we are submitting a report on the potencies of a rather extensive series of quinones. As soon as our investigations on vitamin K indicated a quinone structure [THIS JOURNAL, **61**, 1295 (1939)], we began a survey of the potencies of quinones.

Using the assay procedure previously described [*J. Soc. Exp. Biol. Med.*, **40**, 478 (1939); **41**, 199 (1939)] the following quinones were found to be inactive at a level of 5 mg.: anthraquinone β-sulfonic acid, thymoquinone, tolu-*p*-quinone, dihydro-anthraquinone diacetate, 1,2-naphthoquinone, phenanthraquinone, diamylhydroquinone, *p*-xyloquinone, 2-allyl-1,4-naphthoquinone (tested only at 2.0 mg.) and 1,4-benzoquinone.

With the exception of 2-allyl-1,4-naphthoquinone all of the derivatives of 1,4-naphthoquinone show vitamin K activity. Moreover, the diacetates of two of the dihydro-1,4-naphthoquinones show activity, perhaps due to hydrolysis in the gastro-intestinal tract. 2-Allyl-4-amino-1-naphthol hydrochloride in aqueous solution gives a positive reaction.

Our data are summarized in Table I. They show that the 2-methyl-1,4-naphthoquinone is the most active compound in this group; however, when compared with the natural vitamin K_1 (1000 units per mg.) or K_2 (660 units per mg.), the activity is relatively insignificant. Other more complex derivatives of 1,4-naphthoquinone are being prepared for a study of their physiological activity.

TABLE I

Active compounds	Our standard units per milligram
1,4-Naphthoquinone	1.0
2-Methyl-1,4-naphthoquinone	10.0
2-Ethyl-1,4-naphthoquinone	8.0
Phthiocol, 2-methyl-3-hydroxynaphthoquinone	2.0
2-Bromo-3-methyl-1,4-naphthoquinone	> 0.10
2,3 - Dibromo - 2 - methyl - 1,4 - dioxotetrahydronaphthalene	> 0.10
1,4-Naphthalenediol diacetate	0.50
2-Methyl-1,4-naphthalenediol diacetate	5.00

Our discovery of the activity of 1,4-naphthoquinones and the inactivity of other quinones has been of considerable assistance in developing the structure of vitamin K_1 [THIS JOURNAL, **61**, 1928 (1939)].

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RECEIVED JUNE 19, 1939