350. *Synthesis of Quinol Monophosphates from Vitamin K_1, Ubiquinone, and Other Quinones, and Experiments on Oxidative Phosphorylation.*

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A number of quinol mono(dibenzyl phosphates) have been synthesised by the action of dibenzyl phosphite on quinones. Hydrogenolysis of these phosphates readily gave the required acid esters. Similarly, from benzyl ethyl phosphite, ethyl hydrogen phosphates were prepared.

Data are presented for the dephosphorylation of quinol monophosphates, in the presence of oxygen, and examples of oxidative phosphorylation are given.

There is evidence that naphthaquinone derivatives [1,2] and ubiquinone [3,4] are involved in oxidative phosphorylation in animal tissues, possibly as the phosphates. It has been suggested that the high-energy phosphate bond might first be formed with the quinols, and some theoretical and experimental evidence favours the formation of a quinol monophosphate or semiquinone phosphate.[5-7] These quinol phosphates have the additional value of being presumably water-soluble. It was therefore of interest to prepare quinol monophosphates, particularly dihydrovitamin K_1 and ubiquinol monophosphates.

One method of preparing quinol monophosphates is the phosphorylation of the monoacyl derivatives followed by deacylation.[8,9] However, the monoesters are often difficult or impossible of access and the deacylation involves concomitant dephosphorylation. It has been shown that dialkyl phosphites react with quinones to give the quinol mono(dialkyl phosphates),[10-12] and the reaction of menaphthone with diethyl phosphite produces the two possible monophosphates.[12] It was therefore thought that, by using suitably substituted phosphites, the substituents could be removed from the phosphate to give the required dihydrogen phosphate. Dibenzyl phosphite, in benzene or acetonitrile with a metal alkoxide as catalyst, reacted readily with a variety of quinones, to give the dibenzyl phosphates. Benzoquinone, for example, reacted vigorously with dibenzyl phosphite, under the standard conditions. The colourless product was a phosphate, and not a

[1] Martius, *Biochem. Z.*, 1956, **327**, 407.
[2] Anderson and Dallam, *J. Biol. Chem.*, 1959, **234**, 409.
[3] Hatefi and Quiros-Perez, *Biochim. Biophys. Acta*, 1959, **31**, 502.
[4] Pumphrey and Redfearn, *Biochem J.*, 1959, **73**, 3P.
[5] Clark, Kirby, and Todd, *Nature*, 1958, **181**, 1650.
[6] Harrison, *Nature*, 1958, **181**, 1131.
[7] Wieland and Pattermann, *Angew. Chem.*, 1958, **70**, 313.
[8] Hirschmann, U.S.P. 2,913,477.
[9] Wieland and Pattermann, *Chem. Ber.*, 1959, **92**, 2917.
[10] Diefenbach, G.P. 937,956.
[11] Ramirez and Dershowitz, *J. Org. Chem.*, 1957, **22**, 1282.
[12] Andrews and Atherton, *J.*, 1960, 4682.

phosphonate, since it did not contain a carbonyl group and gave a monotoluene-*p*-sulphonate the infrared spectrum of which showed no hydroxyl band. Whereas no heating was required with alkylated quinones, reaction with chloranil and 2-chloro-3-methyl-1,4-naphthaquinone was sluggish and heat was necessary. High yields were obtained with the symmetrical alkylated quinones but the unsymmetrical ones gave mixtures and losses were considerable in the fractional crystallisations.

Mono(benzyl ethyl phosphates), prepared from a number of quinones and benzyl ethyl phosphite, were readily converted into the ethyl hydrogen phosphates by hydrogenolysis.

Acylation of the phosphates, either at the dibenzyl or the dihydrogen phosphate stage, gave a series of acyl phosphates.

2-Methyl-1,4-naphthaquinone and dibenzyl phosphite gave a mixture which was separated into two colourless crystalline compounds, m. p. 118—119° and 85—86·5°. These were phosphates, since they both formed non-hydroxylic monobenzoates. From the investigation of the monobenzoates it was concluded that the compound of m. p. 118—119° was 2-methyl-1,4-naphthaquinol 4-(dibenzyl phosphate) and that of m. p. 85—86·5° was the 1-(dibenzyl phosphate).

Hydrogenolysis of 2-methyl-1,4-naphthaquinol 1- and 4-(dibenzyl phosphate) readily gave the dihydrogen phosphates. These were acetylated and converted into the anilinium salts. 2-Methyl-1,4-naphthaquinol 1-acetate 4-(anilinium hydrogen phosphate) was also prepared by phosphorylation of 2-methyl-1,4-naphthaquinol 1-acetate with dibenzyl phosphorochloridate followed by hydrogenation and addition of aniline. 2-Methyl-1,4-naphthaquinol 1-acetate 4-(anilinium hydrogen phosphate) from the phosphite reaction was identical with that from this unambiguous synthesis.

It had been noted [12] that the temperature of the reaction affected the yield of dialkyl phosphates. It has now been found that the solvent has an effect: *e.g.*, in the reaction of dibenzyl phosphite with menaphthone in acetonitrile in presence of potassium t-butoxide at $<40°$, the ratio of 1- to 4-(dibenzyl phosphate) was 4 : 1 whereas, in benzene it was 1·25 : 1.

Vitamin K_1 reacted very similarly to the simple quinones, *i.e.*, the reaction mixture became warm and the orange-yellow colour was practically all discharged. Hydrogenolysis of the oily mono(dibenzyl phosphate) obtained gave the required dihydrovitamin K_1 monophosphate. Benzoylation of the dibenzyl phosphate and, after hydrogenolysis, addition of aniline to the phosphoric acid ester gave a colourless crystalline anilinium salt which was not identical with authentic dihydrovitamin K_1 1-benzoate 4-(anilinium hydrogen phosphate): analytical data and its infrared spectrum showed it to be a phosphate and, therefore, it was concluded that predominantly the 1-phosphate had been formed by the phosphite reaction. Acetylation and propionylation at the dibenzyl phosphate stage enabled the 4-acyl ester 1-phosphates to be prepared. By again using benzyl ethyl phosphite, it was possible to prepare the dihydrovitamin K_1 1-(benzyl ethyl phosphate) and thus the ethyl hydrogen phosphate. Phosphorylation of the benzyl ethyl phosphate with phosphorus oxychloride, followed by hydrogenation, readily gave the dihydrovitamin K_1 1-(ethyl hydrogen phosphate) 4-(dihydrogen phosphate).

Ubiquinone$_{(50)}$ also formed a faintly yellow dibenzyl phosphate in the same way, and hydrogenolysis of this, with Lindlar catalyst in order that the side chain should not be hydrogenated, gave the colourless dihydrogen phosphate.

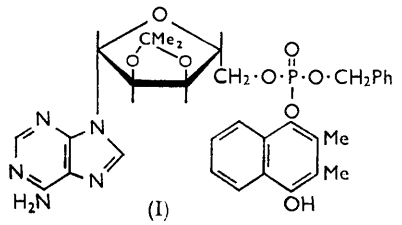
Similarly the monophosphates were obtained from vitamin $K_{2(10)}$ and α-tocopherol quinone. The position of the phosphate group was not proved in these compounds but, by analogy with vitamin K_1, the product was probably mainly the 1-phosphate.

In aqueous solution the monophosphates, particularly the naphthaquinol monophosphates, were relatively unstable in an oxygen atmosphere (see Table 4), in accordance with the oxidative dephosphorylation mechanism described by Clark, Kirby, and Todd.[5] In a Warburg experiment with 2,3-dimethylnaphthaquinol 1-phosphate in buffered solution (pH 6·9) at 37°, there was negligible absorption under oxygen, and at pH 8·3 there

was a slow absorption for 20 min., then a rapid linear absorption for 55 min., by which time the theoretical volume of oxygen for the formation of quinone and orthophosphate had been used. There was also a further slow but small uptake. The extra volume of oxygen required is probably due to the oxidation of the quinone liberated. It was shown that when the theoretical volume of oxygen had been absorbed the liberation of orthophosphate was 80%. At pH 10·5 the uptake was too rapid for satisfactory measurement.

Clark, Kirby, and Todd,[5] and Wieland and Pattermann,[7,9] observed that quinol monophosphates act as phosphorylating agents in the presence of oxidising agents. Similarly, we found that 2,3-dimethylnaphthaquinol monophosphate or dihydrovitamin K_1 monophosphate, when shaken with triethylamine, charcoal, and anhydrous copper sulphate in ethanol under oxygen at 60°, gave the quinone and ethyl phosphate. With dihydrovitamin K_1 monophosphate there was also paper-chromatographic evidence for the formation of a pyrophosphate.

Benzyl phosphites of a variety of complex hydroxy-compounds can be prepared by using the *O*-benzylphosphorous–*OO*-diphenylphosphoric anhydride reagent.[13] Therefore, it was of interest to see if the quinone reaction would readily lead to the phosphate, particularly since the usual procedure (formation of the phosphorochloridate followed by hydrolysis [13,14]) often gives low yields. 2′,3′-Isopropylidineadenosine benzyl phosphite [13] with 2,3-dimethyl-1,4-naphthaquinone readily gave the phosphate (I); anionic fission of the benzyl group, followed by aerial oxidation of an alkaline aqueous solution of the hydrogen phosphate gave the quinone and 2′,3′-isopropylideneadenylic acid. The synthesis of adenylic acid, in low yield, was completed by hydrolysis of the isopropylidene group.



(I)

Experimental

" System A " refers to paper chromatography on Whatman 3MM paper (previously treated with a 5% solution of silicone fluid in light petroleum and dried) with acetic acid–water–propan-2-ol (2·5 : 37·5 : 60 v/v) at room temperature. For " system B " Whatman no. 1 paper and butan-1-ol–acetic acid–water (5 : 2 : 3) were used.

2-*Methyl*-1,4-*naphthaquinol* 1-(*Dibenzyl Phosphate*) *and* 4-(*Dibenzyl Phosphate*).—2-Methyl-1,4-naphthaquinone (17 g.) was stirred with dibenzyl phosphite (31·4 g.) in dry benzene (150 ml.) while N-potassium t-butoxide in t-butyl alcohol–benzene (1 ml.) was added slowly at <40°. After cooling was no longer necessary, the mixture was stirred at room temperature for a further hour, then filtered, washed with water, dried, and evaporated. An oil remained which readily crystallised on addition of carbon tetrachloride followed by cyclohexane. Colourless needles (31 g.; m. p. 109—113°) were obtained that partly dissolved in ether. The insoluble part, recrystallised from ethanol, gave needles (9·7 g.) of the 4-*phosphate*, m. p. 118—119° (Found, in material dried *in vacuo* at 60°: C, 69·5; H, 5·1; P, 7·3. $C_{25}H_{23}O_5P$ requires C, 69·1; H, 5·3; P, 7·1%). Addition of light petroleum (b. p. 40—60°) to the solution of the other part in ether gave the 1-(*dibenzyl phosphate*) (12·2 g.), m. p. 80—81°. Three recrystallisations from ethyl acetate–light petroleum (b. p. 40—60°) raised the m. p. to 85—86·5° (Found, in material dried *in vacuo* at 40°: C, 69·4; H, 5·1; P, 6·9%).

Reaction in acetonitrile gave 36 g. of crude product which was separated into 20·2 g. of m. p. 81—83° and 4 g. of m. p. 115—119°.

2-*Methyl*-1,4-*naphthaquinol* 4-*Benzoate* 1-(*Dibenzyl Phosphate*).—2-Methyl-1,4-naphthaquinol 1-(dibenzyl phosphate) (1·1 g.) was treated in pyridine at room temperature with benzoyl chloride during 2 hr. The *benzoate* (1·06 g.), recrystallised twice by dissolution in hot ether (a little ethyl acetate was added in the second recrystallisation) and addition of light petroleum

[13] Corby, Kenner, and Todd, *J.*, 1952, 3669.
[14] Kenner, Todd, and Weymouth, *J.*, 1952, 3675.

(b. p. 40—60°), gave colourless prisms, m. p. 93—94° (Found, in material dried *in vacuo* at 50°: C, 71·0; H, 5·1; P, 5·7. $C_{32}H_{27}O_6P$ requires C, 71·4; H, 5·1; P, 5·8%).

2-Methyl-1,4-naphthaquinol 1-Benzoate 4-(Dibenzyl Phosphate).—(A) 2-Methyl-1,4-naphthaquinol 4-(dibenzyl phosphate) (1·1 g.) was benzoylated and recrystallised twice as above. The crude product (1·3 g.) had m. p. 80—82° and the recrystallisations gave plates (800 mg.), m. p. 85—86°. There was no m. p. depression on admixture with *2-methyl-1,4-naphthaquinol 4-benzoate 1-(dibenzylphosphate)* prepared as below and the infrared spectra were identical (Found, in material dried *in vacuo* at 50°: C, 71·6; H, 4·8; P, 5·6. $C_{32}H_{27}O_6P$ requires C, 71·4; H, 5·1; P, 5·8%).

(B) 2-Methyl-1,4-naphthaquinol 1-benzoate [15] (0·7 g.) was suspended in a solution of dibenzyl phosphite (0·7 g.) in dry carbon tetrachloride (5 ml.) and triethylamine (0·5 ml.) was added. The mixture was shaken; it became warm, the solid dissolved, and triethylamine hydrochloride was precipitated. After 3 hr., the hydrochloride was filtered off and the filtrate was washed with dilute hydrochloric acid, twice with N-sodium hydroxide, and finally with water, filtered, and evaporated *in vacuo*. The residue (1·12 g.), recrystallised twice from ether–ethyl acetate with light petroleum (b. p. 40—60°), gave plates, m. p. 84—85° (Found, in material dried *in vacuo* at 50°: C, 71·7; H, 5·1; P, 5·7%).

Quinol Dibenzyl (and Benzyl Ethyl) Phosphate.—By the method described for the preparation of 2-methyl-1,4-naphthaquinol 1-(dibenzyl phosphate) a number of analogous compounds were prepared (see Table 1).

TABLE 1. *Quinol phosphates.*

Compound	Reaction in	Recryst. from [d]	M. p.	Yield (%)	Found (%) C	H	P	Formula	Required (%) C	H	P
2,3-Dimethyl-1,4-naphthaquinol 1-(dibenzyl phosphate)	MeCN	EtOAc–Pet	111—112·5°	82	70·0	5·6	7·0	$C_{26}H_{25}O_5P$	69·6	5·6	6·9
,, ,, 1-(benzyl ethyl phosphate)	MeCN	CCl_4	111—112	49	65·2	5·9	8·2	$C_{21}H_{23}O_5P$	65·5	6·0	8·0
Quinol 1-(dibenzyl phosphate)	C_6H_6	EtOAc–Pet	84—86	51	64·9	5·0	8·4	$C_{20}H_{19}O_5P$	64·9	5·2	8·4
,, ,, 1-(benzyl ethyl phosphate)	MeCN		46—51	92	58·8	5·4	9·6	$C_{15}H_{17}O_5P$	58·4	5·6	10·1
Trimethylquinol 1-(dibenzyl phosphate)	MeCN	EtOAc–Pet	100—101	52·9 [b]	67·0	5·7	7·7	$C_{23}H_{25}O_5P$	67·0	6·1	7·5
Duroquinol 1-(dibenzyl phosphate)	MeCN	,,	112—113	81·5	67·6	6·2	7·2	$C_{24}H_{27}O_5P$	67·7	6·4	7·3
Tetrachloroquinol 1-(dibenzyl phosphate)	C_6H_6	,,	143—145	33	47·6	2·6	—	$C_{20}H_{15}Cl_4O_5P$	47·5	3·0	—
,, ,, 1-(benzyl ethyl phosphate)	PhMe	,,	137—139	22	40·3	3·1	7·0	$C_{15}H_{13}Cl_4O_5P$	40·4	2·9	7·0

[a] Purified by extraction of an ethereal solution with N-potassium hydroxide followed by acidification of the alkaline extracts and extraction of the product with chloroform. [b] Total yield of a colourless crystalline mixture of 1- and 4-phosphates (m. p. 75—83°). Fractional crystallisation gave a single pure phosphate, m. p. 100—101°. [c] Under reflux for 3 hr. [d] Pet = light petroleum (b. p. 40—60°).

2,3-Dimethyl-1,4-naphthaquinol 4-Acetate 1-(Dibenzyl Phosphate).—To 2,3-dimethyl-1,4-naphthaquinol 1-(dibenzyl phosphate) in acetic anhydride, a catalytic amount of sulphuric acid (yield

[15] Lindlar, U.S.P. 2,839,570.

86%) or triethylamine (yield 91%) was added. The *product* formed needles, m. p. 105—106° (Found, in material dried *in vacuo* at 60°: C, 68·8; H, 5·1; P, 6·7. $C_{28}H_{27}O_6P$ requires C, 68·7; H, 5·6; P, 6·3%).

The *4-toluene-p-sulphonate*, prepared in 2,6-lutidine, had m. p. 88—89·5° (Found, in material dried *in vacuo* at 40°: C, 61·9; H, 4·7; P, 5·7; S, 6·2. $C_{27}H_{25}O_7PS$ requires C, 61·8; H, 4·8; P, 5·9; S, 6·1%), and had no infrared hydroxyl and carbonyl band.

Quinol Mono(dihydrogen Phosphates) and Mono(ethyl Hydrogen Phosphates).—The quinol dibenzyl phosphates and benzyl ethyl phosphates, described above, were hydrogenolysed in ethanol with 10% palladium–charcoal. The theoretical or nearly theoretical volume of hydrogen was absorbed and after removal of the catalyst the residual acid ester was recrystallised. The yields were virtually theoretical and depended upon the efficiency of the crystallisation. Salts were prepared by the addition of base to the acid in alcohol. Data for the *compounds* prepared are given in Tables 2 and 3.

2-Methyl-3-phytyl-1,4-naphthaquinol 1-(Dibenzyl Phosphate) [*Dihydrovitamin* K_1 *1-(Dibenzyl Phosphate)*].—To a solution of vitamin K_1 (4·5 g.) in benzene, stirred under nitrogen, was added dibenzyl phosphite (3·0 g., 1·15 mole) in benzene (2 ml.) followed by 1·1N-potassium t-butoxide in t-butyl alcohol (1 ml.) containing 10% of benzene. The mixture became warm and was cooled for a few minutes, then stirred at room temperature for 2 hr., diluted with light petroleum (b. p. 40—60°), filtered, and washed with water, dilute hydrochloric acid, water, and finally

TABLE 2. *Dihydrogen phosphates and their monoethyl esters.*

No.	Quinol	M. p.	Found (%) C	H	P	Formula	Required (%) C	H	P	Mol. wt.[f] Found	Req.
1	2,3-Dimethyl-1,4-naphthaquinol	128—129°[a]	53·8	5·35	11·7	$C_{12}H_{13}O_5P$	53·6	4·9	11·6	272	268
2	,, ,, 4-acetate	185—188*[b]	54·5	4·5	9·6	$C_{14}H_{15}O_6P$	54·2	4·9	10·0	314	310
3	,, ,, Et ester	165—166[c]	56·9	5·7	10·6	$C_{14}H_{17}O_5P$	57·0	5·8	10·2	296	296
4	2-Methyl-1,4-naphthaquinol (1-phosphate)	194—195*[b]	51·4	4·9	11·9	$C_{11}H_{11}O_5P$, $0·25H_2O$	51·1	4·5	12·0	262	259
5	,, (4-phosphate)[b]	150*[d]	—	—	11·7	$C_{11}H_{11}O_5P$	—	—	12·2	255	254
6	Quinol	167—168·5[b]	38·4	3·9	16·2	$C_6H_7O_5P$	37·9	3·7	16·3	197	190
7	,, Et ester	Oil	43·7	5·4	14·5	$C_8H_{11}O_5P$	44·0	5·1	14·2		
8	Trimethylquinol	211—212[e]	47·0	5·3	13·3	$C_9H_{13}O_5P$	46·5	5·6	13·3		
9	Duroquinol	237*[c]	48·4	5·8	12·2	$C_{10}H_{15}O_5P$	48·8	6·1	12·6	252	246
10	Tetrachloroquinol	204—206*	21·8	1·35	9·1	$C_6H_3Cl_4O_5P$, $0·5H_2O$	21·4	1·2	9·2		
11	,, Et ester	224—225*[c]	27·9	2·3	8·3	$C_8H_7Cl_4O_5P$	27·0	2·0	8·7		

* With decomp. [a] From EtOAc–cyclohexane. [b] From EtOAc–Pet (Pet = light petroleum of b. p. 40—60°). [c] As *b* but with 5% of MeOH. [d] From Et_2O–Pet. [e] From cyclohexane. [f] By titration.

TABLE 3. *Salts of phosphates.*

Ref. from Table 2	Salt	Recryst. from	M. p.	Found (%) C	H	P	Formula	Required (%) C	H	P
No. 1	Cyclohexylammonium	MeOH–Et_2O	200—203°	60·3	8·1	7·0	$C_{12}H_{13}O_5P$, $1·5C_6H_{13}N$	60·5	7·9	7·4
4-Ac deriv. of No. 4	Anilinium	EtOH–Et_2O	190—191	58·9	5·2	7·8	$C_{19}H_{20}NO_6P$	58·6	5·2	8·0
,,	Triethylammonium	EtOAc–Pet	211—212	57·8	7·4	8·4	$C_{19}H_{28}NO_6P$	57·4	7·2	8·7
1-Ac deriv. of No. 5	Anilinium	EtOH	180—182	57·4	5·1	8·3	$C_{19}H_{20}NO_6P$, $0·5H_2O$	57·3	5·3	7·8
No. 6	Anilinium	EtOH	176—177	51·0	5·0	10·5	$C_{12}H_{14}NO_5P$	50·9	5·0	10·9
No. 7	Cyclohexylammonium	MeOH–Et_2O	175—178	52·6	7·8	9·6	$C_{14}H_{24}NO_5P$	53·0	7·6	9·8
No. 9	Cyclohexylammonium	MeOH–Et_2O–Pet	214—218	55·7	7·9	9·3	$C_{16}H_{28}NO_5P$	55·7	8·2	9·0

twice with 90% methanol (to remove any unreacted dibenzyl phosphite). The petroleum layer was dried (Na_2SO_4) and evaporated *in vacuo*, leaving the *ester* as a pale yellow oil (6·86 g., 97%) (Found: C, 75·6; H, 8·8; P, 5·0. $C_{45}H_{61}O_5P$ requires C, 75·8; H, 8·6; P, 4·4%).

This phosphate (3·55 g.) with benzoyl chloride–pyridine at room temperature (2 hr.) gave

an oil (3·76 g.) which was chromatographed on alumina pretreated with methyl formate, elution being with light petroleum (b. p. 80—100°), 1 : 1 light petroleum–benzene, and finally benzene. The petroleum–benzene eluate, on evaporation *in vacuo*, gave 2-*methyl*-3-*phytyl*-1,4-*naphthaquinol* 4-*benzoate* 1-(*dibenzyl phosphate*) as a colourless oil (2·3 g.) (Found: C, 76·2; H, 7·7; P, 4·1. $C_{52}H_{65}O_6P$ requires C, 76·4; H, 8·0; P, 3·8%).

The *propionate* was prepared in pyridine and similarly chromatographed, forming a practically colourless oil (76% yield) (Found: C, 74·3; H, 8·4; P, 4·4. $C_{48}H_{65}O_6P$ requires C, 75·0; H, 8·3; P, 4·0%).

2-*Methyl*-3-*phytyl*-1,4-*naphthaquinol* 1-*Benzoate* 4-(*Dibenzyl Phosphate*).—Dihydrovitamin K_1 1-benzoate [15] (10·0 g.) was dissolved in dry carbon tetrachloride (15 ml.) and dibenzyl phosphite (5·0 g.) was added. The solution was cooled in ice-water and dry triethylamine (2·55 ml.) was added slowly with shaking. The base hydrochloride separated and after ½ hr. the mixture was set aside at room temperature overnight. The hydrochloride was filtered off and the filtrate was evaporated to an oil which was washed in ether with N-sodium hydroxide and then twice with water (sodium sulphate added to break emulsions), dried (Na_2SO_4) and recovered. A 68% yield of the crude 4-(*dibenzyl phosphate*) was obtained which was purified by chromatography on neutral alumina as described for the 1-(dibenzyl phosphate) (Found: C, 77·2; H, 8·1; P, 4·1%).

2-*Methyl*-3-*phytyl*-1,4-*naphthaquinol* 1-(*Dihydrogen Phosphate*).—Dihydrovitamin K_1 1-(dibenzyl phosphate) (2 g.) was hydrogenolysed in absolute ethanol (20 ml.) with 10% palladium–charcoal (200 mg.) (which did not hydrogenate dihydrovitamin K_1 or its 1-benzoate). Uptake (theor.) ceased in 50 min. After removal of the catalyst the alcohol was evaporated off *in vacuo*, to give a hygroscopic oily *acid* which became reddish-brown (Found: C, 69·9; H, 9·8; P, 5·4. $C_{31}H_{49}O_5P$ requires C, 70·0; H, 9·3; P, 5·8%). A paper chromatogram of this product on Whatman no. 1 paper impregnated with silicone fluid (dipped in a 10% solution of silicone fluid in light petroleum and dried) with ethanol–water–acetic acid (2 : 1 : 1) (ascending, 17 hr. at room temperature) showed one major spot (R_F 0·88, visible under ultraviolet light and phosphate-containing), a faint trail (R_F 0—0·1, visible under ultraviolet light, corresponding to vitamin K_1), and a small faint spot (R_F 0·62, inorganic phosphate). The product, in water, had $\lambda_{max.}$ 242, 272, $\lambda_{min.}$ 230, 257 mμ (ε 40,000, 5750 27,750, and 4750, respectively). The infrared spectrum confirmed the presence of hydroxyl and phosphate groups. This acid formed a solid *anilinium salt* of indefinite m. p. (Found, in material dried *in vacuo*: C, 71·4; H, 8·9; N, 2·5; P, 5·2. $C_{37}H_{56}NO_5P$ requires C, 71·0; H, 9·0; N, 2·2; P, 5·0%), $\lambda_{max.}$ 240 mμ (ε 63700) in H_2O.

Dihydrovitamin K_1 4-*Acetate* 1-(*Dihydrogen Phosphate*).—Dihydrovitamin K_1 1-(dibenzyl phosphate) (1·5 g.) was kept for 2 hr. in acetic anhydride (6 ml.) containing a trace of concentrated sulphuric acid. The product, worked up as usual and hydrogenolysed as above, gave the 4-*acetate* 1-(*dihydrogen phosphate* (86%) as a faintly orange oil (Found, in material dried *in vacuo*: C, 66·7; H, 9·0; P, 4·5. $C_{33}H_{51}O_6P,H_2O$ requires C, 66·9; H, 9·0; P, 5·2%), $\lambda_{max.}$ 232 mμ (ε 52,600) in H_2O. The infrared spectrum confirmed the acetate grouping.

Dihydrovitamin K_1 1-(*dihydrogen phosphate*) 4-*propionate*, prepared by hydrogenolysis of its dibenzyl ester, was an amber-coloured oil (99% yield) (Found, in material dried *in vacuo*: C, 68·2; H, 9·4; P, 6·0. $C_{34}H_{53}O_6P,0{\cdot}5H_2O$ requires C, 68·3; H, 9·1; P, 5·2%). Adding methanolic ammonia to its solution in ethanol caused precipitation of the *diammonium salt* which was completed by adding acetonitrile. This had m. p. 160° after softening at 152° (Found, in material dried *in vacuo*: C, 65·4; H, 9·5; N, 4·4; P, 5·0. $C_{34}H_{59}N_2O_6P$ requires C, 65·6; H, 9·6; N, 4·5; P, 4·9%), $\lambda_{max.}$ 237 mμ (ε 62,280) in water.

Dihydrovitamin K_1 4-*Benzoate* 1-(*Anilinium Hydrogen Phosphate*).—Dihydrovitamin K_1 4-benzoate 1-(dibenzyl phosphate) (0·5 g.) was hydrogenolysed in absolute ethanol (5 ml.) with 10% palladium–charcoal catalyst (50 mg.) (theoretical uptake in 20 min.). After removal of the catalyst, aniline (0·3 ml.) in a little ethanol was added, followed by acetonitrile to turbidity. The precipitated *aniline salt* (200 mg.) had m. p. 146—147° (from ethanol–acetonitrile) (Found, in material dried *in vacuo* at 80°: C, 72·6; H, 8·1; N, 1·8; P, 4·2. $C_{44}H_{60}NO_6P$ requires C, 72·4; H, 8·3; N, 1·9; P, 4·2%). A paper chromatogram (system A; descending, 17 hr.) gave a single spot R_F 0·89, absorbing ultraviolet light and containing phosphate. The mixed m. p. with the isomer prepared as below was 135—149°; the infrared spectra were practically identical, except in the phosphate band region.

Dihydrovitamin K_1 1-*benzoate* 4-(*anilinium hydrogen phosphate*), prepared similarly, had

m. p. 160—161° (from ethanol–acetonitrile) (Found, in material dried *in vacuo* at 80°: C, 72·7; H, 8·2; N, 1·9; P, 4·2%). On paper chromatography it behaved as its isomer but had R_F 0·9.

Dihydrovitamin K_1 1-(Ethyl Hydrogen Phosphate).—Vitamin K_1 (2·5 g.) and benzyl ethyl phosphite (1·2 g.) were treated in benzene (25 ml.) with 0·4N-potassium t-butoxide (1 ml.) in 9 : 1 t-butyl alcohol–benzene. After 2 hr. at room temperature the mixture was washed with sodium sulphate solution, filtered, and dried (Na_2SO_4). Evaporation, finally at 40°/0·1 mm., gave dihydrovitamin K_1 1-(benzyl ethyl phosphate) as a pale yellow oil (3·14 g., 87%). This was hydrogenolysed, as above, to a light brown oil (2·54 g.) which a paper chromatogram indicated as being mainly the required *product* but containing a little vitamin K_1 and another organic phosphate. It was dissolved in ether (30 ml.) and extracted with dilute potassium hydroxide solution (2 × 30 ml.). The alkaline layers were washed with ether, then acidified with dilute hydrochloric acid and extracted with ether. The ether layer was washed with water, dried (Na_2SO_4), and evaporated, finally at 60°/0·1 mm. A light brown oil (1·04 g.) remained (Found: C, 71·4; H, 9·6; P, 5·6. $C_{33}H_{53}O_5P$ requires C, 70·7; H, 9·5; P, 5·5%). A paper chromatogram (system A; ascending, 17 hr.) showed a single spot (R_F 0·92) which absorbed ultraviolet light and contained phosphate.

Dihydrovitamin K_1 1-(Ethyl Hydrogen Phosphate) 4-(Dihydrogen Phosphate).—N-Potassium t-butoxide (*ca.* 0·3 ml.) was added dropwise to a solution of vitamin K_1 (1·0 g.) and benzyl ethyl phosphite (0·5 g.) in dry benzene (2·5 ml.) until no further reaction occurred. After the mixture had been set aside for 1 hr., dry pyridine (10 ml.) was added and the solution was cooled in ice-water. Next was added a cold solution of phosphorus oxychloride (2·2 ml.) in dry pyridine (10 ml.) and the whole was left for ½ hr. at room temperature. The solvent and excess of oxychloride were removed *in vacuo*, dry toluene was added, and the solution was again evaporated *in vacuo*. The residue was partitioned between ether (40 ml.) and water (40 ml.), and the ether layer was washed successively with water (40 ml.), N-potassium hydroxide (2 × 40 ml.), N-hydrochloric acid (40 ml.), and dilute sodium sulphate solution, filtered, dried (Na_2SO_4), and evaporated, finally at 60°/0·1 mm. for 1 hr. Dihydrovitamin K_1 1-(benzyl ethyl phosphate) 4-(dihydrogen phosphate) remained as a pale yellow oil (1·42 g., 88%). This was hydrogenolysed in ethanol (30 ml.) with palladium–charcoal (0·2 g.) for 1 hr. (uptake, 46·7 ml.). After removal of the catalyst evaporation (finally at 60°/0·1 mm. for 2 hr.) gave the *di(phosphate)* as a pale yellow oil (1·04 g.) (Found: C, 62·5; H, 8·4; P, 9·8. $C_{33}H_{54}O_8P_2$ requires C, 61·9; H, 8·5; P, 9·7%). A paper chromatogram (system A; ascending, 17 hr.) showed a single spot, R_F 0·73—0·97, absorbing ultraviolet light and containing phosphorus, that had $\lambda_{max.}$ 234 and 290 (ε 63,000 and 4950), $\lambda_{min.}$ 254 mμ (ε 1250), in EtOH.

In another experiment, with 10 g. of vitamin K_1, methanolic ammonia was added to the final solution, after hydrogenolysis, and the amorphous *diammonium salt* of dihydrovitamin K_1 1-(ethyl hydrogen phosphate) 4-(dihydrogen phosphate) (11·4 g.; 74%) was precipitated with acetonitrile (Found, in material dried *in vacuo*: C, 59·1; H, 8·9; N, 3·8; P, 9·5. $C_{33}H_{60}N_2O_8P_2$ requires C, 58·7; H, 9·0; N, 4·2; P, 9·2%).

2-Geranyl-3-methyl-1,4-naphthaquinol (Ammonium Hydrogen Phosphate) (Dihydrovitamin $K_{2(10)}$ Ammonium Hydrogen Phosphate).—Dihydrovitamin $K_{2(10)}$ dibenzyl phosphate, prepared by the method given for dihydrovitamin K_1 1-(dibenzyl phosphate) was hydrogenolysed in ethanol with Lindlar's palladium–barium sulphate catalyst. Absorption was slow and ceased with 82% of the theoretical value. The acid ester obtained was impure but an *ammonium salt* was prepared which on a paper chromatogram (system A; ascending, 17 hr.) had R_F 0·88 and showed only two faint spots for impurity (Found, in material dried *in vacuo*: N, 3·5; P, 7·5. $C_{21}H_{30}NO_5P$ requires N, 3·4; P, 7·6%).

α-Tocopherylquinol Mono(dihydrogen Phosphate).—α-Tocopheryl quinone was converted into the *mono(dibenzyl phosphate)* (2·24 g.) by the method given for dihydrovitamin K_1 (Found: C, 73·3; H, 8·8. $C_{43}H_{65}O_6P$ requires C, 72·9; H, 9·2%). The pale oil (1·88 g.) was hydrogenolysed in the usual way (uptake 127 ml. in 1 hr.). An ethereal solution of the impure acid ester obtained was extracted with dilute potassium hydroxide solution; acidification of the alkaline solution and extraction of the *acid ester* with ether gave a faintly orange-coloured oil (60·5%) (Found, in material dried *in vacuo*: C, 66·2; H, 10·3; P, 5·4. $C_{29}H_{53}O_6P$ requires C, 65·9; H, 10·1; P, 5·9%). A paper chromatogram (system A; ascending, 17 hr.) showed a single absorbent spot under ultraviolet light (R_F 0·93) but there was a trace of phosphate impurity (R_F 0·56). Ultraviolet light absorption in ethanol was min. at 254 mμ (ε 660) and max. at 282 mμ (ε 1760).

Ubiquinol$_{(50)}$ Mono(dibenzyl Phosphate).—Ubiquinone$_{(50)}$ (0·65 g.) was stirred in benzene (1 ml.) under nitrogen while dibenzyl phosphite (0·24 g., 1·2 equivalents) in benzene (0·5 ml.) was added, followed by dilute alkali (a gel formed first). Paper chromatography on Whatman no. 1 paper [treated with a 10% silicone fluid solution in light petroleum; ethanol–water–acetic acid (2 : 1 : 1); ascending, 16 hr.; room temperature] gave a single spot (R_F 0·86) absorbing ultraviolet light and containing phosphate. System A (except that 4 : 1 propan-1-ol–water was used; ascending, for 15 hr.) [16] gave a single, similar spot of R_F 0·87 (ubiquinone$_{(50)}$ has R_F 0·3).

Benzyl Ethyl Phosphite.—A solution of benzyl alcohol (84 ml., 88·3 g.) and diethylaniline (65 ml., 60·8 g.) was added dropwise, during 30 min., to a stirred solution of ethyl phosphorodichloridite (60 g.) in dry benzene (350 ml.) at 5—10° (freezing mixture). The mixture was then stirred for 2 hr. at 0°, water (140 ml.) was added, and the mixture stirred for a further hour. The benzene solution was washed with water (3 × 140 ml.), 3N-ammonia (3 × 250 ml.), and water (3 × 140 ml.), filtered, dried ($MgSO_4$), and evaporated, finally at 100°/0·35 mm. The residue was distilled and the fraction of b. p. 94—107°/0·35 mm. was collected. This *ester*, redistilled through a short column, had b. p. 108—110°/0·35 mm., n_D^{20} 1·5024 (27·3 g., 35%) (Found: C, 53·9; H, 6·9; P, 16·1. $C_9H_{13}O_3P$ requires C, 54·0; H, 6·6; P, 15·5%).

Adenosine 5′-Phosphate.—Crude 2′,3′-isopropylideneadenosine benzyl phosphite [13] (12·3 g.) was treated in dry benzene (40 ml.) with 2,3-dimethyl-1,4-naphthaquinone (4·95 g.) followed by N-potassium t-butoxide in 9 : 1 t-butyl alcohol–benzene (2 ml.) in small portions. After the mixture had been stirred for 1½ hr. it was set aside overnight. Most of the benzene was evaporated off and chloroform was added to the residue. The chloroform solution was washed with water, 0·01N-hydrochloric acid, and water, filtered, evaporated to small bulk, and poured into ether. Crude 2′,3′-*O*-isopropylideneadenosine 5′-(benzyl-4-hydroxy-2,3-dimethylnaphthyl phosphate) (11·0 g.) separated as an oil which solidified on trituration with ether. Part of the material (3·24 g.) was refluxed in dry ethyl methyl ketone (16 ml.) with sodium thiocyanate (0·44 g.) for 2 hr. An oil (1·78 g.) (A) separated which solidified and was collected, washed with acetone, and dried. A second crop (0·88 g.) was obtained from the ethyl methyl ketone mother-liquors. The first crop was dissolved in water and acidified, giving an amorphous precipitate (1·15 g.). Attempted recrystallisation of this precipitate from acetic acid gave an apparently amorphous *substance* (B) of indefinite m. p. (cleared at 160—165°) (Found, in material dried *in vacuo*: C, 50·6; H, 5·1; N, 11·3; P, 4·8. $C_{25}H_{28}N_5O_8P,2H_2O$ requires C, 50·6; H, 5·4; N, 11·8; P, 5·2%). A paper chromatogram (system B) gave a single fluorescent spot (ultraviolet light) (R_F 0·77). Addition of bromine water to a solution of the sodium salt (A) gave an immediate precipitate of 2,3-dimethylnaphthaquinone, m. p. and mixed m. p. 122—123°. The acid (B) (237 mg.) was suspended in water, and 0·098N-sodium hydroxide was added to give a solution of pH 8·4. Oxygen was bubbled through this and the pH was maintained at 8·4—9·6 by further additions. During *ca.* 10 hr. 3·8 ml. of the alkali were required (theor. 4·3 ml.). A paper chromatogram indicated that 2′,3′-isopropylideneadenylic acid was the main product. 0·1N-Hydrochloric acid (9 ml.) was added and the solution was heated on a boiling-water bath for 20 min. A paper chromatogram (Whatman no. 4 paper; 6 : 3 : 1 propan-1-ol–ammonia–water; ascending, 2 hr. at room temperature) showed a trace of isopropylideneadenylic acid, R_F 0·52, adenylic acid, R_F 0·27 (authentic, R_F 0·25), a trace of inorganic phosphate, R_F 0·16, and two small spots (fluorescent in ultraviolet light but not containing phosphate), R_F 0·8 and 0·97 (probably 2,3-dimethylnaphthaquinone and a related substance). The solution was evaporated to small bulk and a little acetone added. A small amount of precipitated brown oil solidified later. Further addition of acetone gave colourless crystals (18·5 mg.), m. p. 169—171° (decomp.). Recrystallisation from water, with the addition of a little acetone, raised the m. p. to 186—188° (decomp.) undepressed on admixture with muscle adenylic acid of m. p. 192° (decomp.).

Phosphorylation of Ethanol.—(*a*) 2,3-Dimethyl-1,4-naphthaquinol 1-(dihydrogen phosphate) (0·4 g.), palladium–charcoal (0·1 g.), anhydrous calcium sulphate (0·2 g.), and triethylamine (0·5 ml.) in dry ethanol (20 ml.) were shaken in oxygen. After the theoretical uptake (18 ml.) the catalyst was filtered off and a drop of the filtrate (now bright yellow owing to 2,3-dimethylnaphthaquinone) was paper chromatographed (system B). Spots of authentic material showed the presence of 2,3-dimethylnaphthaquinone (R_F 0·93), ethyl phosphate (R_F 0·48), and traces of 2,3-dimethylnaphthaquinol monophosphate (R_F 0·69) and inorganic phosphate (R_F 0·36).

[16] Lester and Ramasarma, *J. Biol. Chem.*, 1959, **234**, 672.

The ethyl phosphate was isolated as the monoanilinium salt, m. p. and mixed m. p. 166—168°.

(*b*) Dihydrovitamin K_1 1-phosphate (0·5 g.), palladium–charcoal (0·1 g.), anhydrous copper sulphate (0·2 g.), and dry triethylamine (0·5 ml.) in dry ethanol (20 ml.) were shaken under oxygen at room temperature for 1½ hr. during which time only 5 ml. of oxygen were absorbed.

TABLE 4. *Stability data for 0·2% solutions in aqueous buffer.*

Compound	pH	Temp.	Time (hr.)	Release (%) of phosphate in N_2	Release (%) of phosphate in O_2
2-Methyl-1,4-naphthaquinol 1-phosphate	8·4	37°	2	—	8·2
	8·4	37	4	—	20·6
2-Methyl-1,4-naphthaquinol 4-phosphate	8·4	37	10	—	61·4
	8·4	37	30	4·1	100·5
2-Methyl-1,4-naphthaquinol 4-acetate 1-phosphate	8·4	37	4	<1	<1
2-Methyl-1,4-naphthaquinol 1-acetate 4-phosphate	8·4	37	4	3·7	8·9
2,3-Dimethyl-1,4-naphthaquinol 1-phosphate	3·5	38	4	12·0	21·3
	6·4	38	4	8·3	44·0
	7·5	38	4	9·0	70·0
	9·9	38	4	39·0	94·5
Dihydrovitamin K_1 1-phosphate	7·7	38	4	11·4	45·2
Dihydrovitamin K_1 4-acetate 1-phosphate	7·7	38	4	3·2	5·1
	8·3	38	4	3·5	6·0
Duroquinol 1-phosphate	8·3	37	4	—	2·5
Ubiquinol$_{(50)}$ monophosphate	8·4	37	4	2·2	4·1

The temperature was raised to 60° and in a further 1½ hr. 13 ml. of oxygen were absorbed and then the absorption practically ceased (theor., 21 ml.). The solution was filtered and part of it was partially evaporated and chromatographed (system A; ascending, 17 hr.). The following spots were observed; R_F 0·21, absorbent in ultraviolet light (R_F of vitamin K_1); R_F 0·42, phosphate-containing; R_F 0·66, phosphate-containing (R_F of triethylammonium ethyl phosphate); R_F 0·69, absorbent in ultraviolet light; R_F 0·73—0·94, absorbent in ultraviolet light and phosphate-containing (R_F of dihydrovitamin K_1 monophosphate). A little of the solution was acidified with dilute hydrochloric acid and heated at 100° for 10 min. to decompose any pyrophosphates present. A paper chromatogram, obtained as previously, only then showed 3 spots: R_F 0·14, absorbent in ultraviolet light (R_F of vitamin K_1); R_F 0·78, a phosphate (R_F of ethyl dihydrogen phosphate); and R_F 0·93, ultraviolet light-absorbent and phosphate-containing (R_F of dihydrovitamin K_1 monophosphate). It was concluded that ethyl phosphate had been formed together with at least one and possibly two pyrophosphates.

Stability Data.—The results in Table 4 are self-explanatory.

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