143. *Studies on Phosphorylation. Part XXII.* Phosphorylation accompanying the Oxidation of Quinol Phosphates.*

By V. M. Clark, D. W. Hutchinson, G. W. Kirby, and Sir Alexander Todd.

Oxidation of several quinol phosphates by a variety of oxidising agents has been studied. Evidence is presented for the production of a phosphorylating agent during the oxidation.

The methods used for studying phosphorylation *in vitro* have been closely related to those leading to anhydride formation followed by acylation.[1] As phosphorylating agents, phosphoric anhydrides, *e.g.*, phosphorochloridates [2] and pyrophosphates,[3] have often been used, phosphorylation then proceeding with the expulsion of an anion:

$$\text{Base} + \text{ROH} + (\text{R'O})_2\text{POCl} \longrightarrow \text{RO·PO(OR')}_2 + \text{Base, HCl}$$

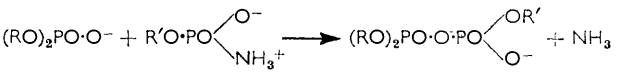
$$(\text{RO})_2\text{PO·O}^- + (\text{R'O})_2\text{POCl} \longrightarrow (\text{RO})_2\text{PO·O·PO(OR')}_2 + \text{Cl}^-$$

$$2(\text{RO})_2\text{PO·O}^- + (\text{R'O})_2\text{PO·O·PO(OR')}_2 \longrightarrow (\text{RO})_2\text{PO·O·PO(OR)}_2 + 2(\text{R'O})_2\text{PO'·O}^-$$

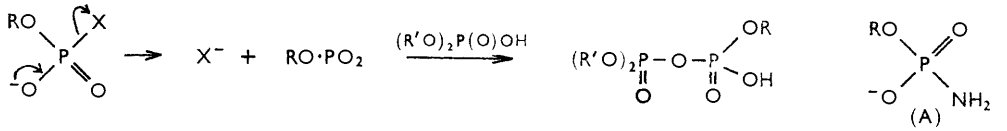
* Part XXI, *J.*, 1960, 4511.

[1] Todd, *Gazzetta*, 1959, **89**, 126; *Proc. Nat. Acad. Sci.*, 1959, **45**, 1389.
[2] Brigl and Müller, *Ber.*, 1939, **72**, 2121; Atherton, Openshaw, and Todd, *J.*, 1945, 382.
[3] Corby, Kenner, and Todd, *J.*, 1952, 1234.

The phosphoramidates require to be in the zwitterionic form to exhibit their acylating function, the expelled group then being a neutral amine molecule:

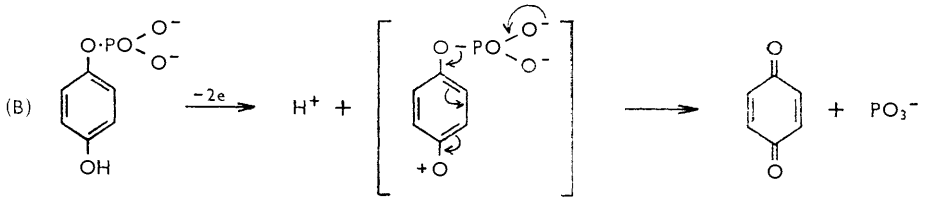


By this method, adenosine diphosphate has been converted into the triphosphate in high yield [4] and coenzyme A has been synthesised.[5] In this type of reaction, the expulsion of the amine molecule may precede rather than accompany acylation; the intermediate would then be a monomeric metaphosphate which would rapidly phosphorylate an appropriate substrate: [6]



In such reactions the heterolysis of the phosphorus–nitrogen bond giving the metaphosphate [7] follows the withdrawal of electrons from the P–N bond as the new N–H bond is being formed during the protonation of the anion (A). The metaphosphate is to be regarded as a hypothetical intermediate, since no monomeric metaphosphate has yet been described, although trimers are well known.[8] The phosphoramidates can be described as metaphosphates which have been converted into complexes or solvates by amines, the esters of phosphoric acid as products from metaphosphates and alcohols, and the pyrophosphates as the products of interaction of metaphosphates and orthophosphates.

The heterolysis of the P–N bond in the phosphoramidates can, in theory, be accomplished by removing electrons with an oxidising agent rather than with a proton. The facility of the acid cleavage of the phosphoramidates has, however, precluded their being studied under a wide range of oxidising conditions and attention has been turned to other systems, particularly those in which a phosphorus atom is combined solely with oxygen atoms. In the case of a quinol monophosphate, the scheme (B) can be envisaged:



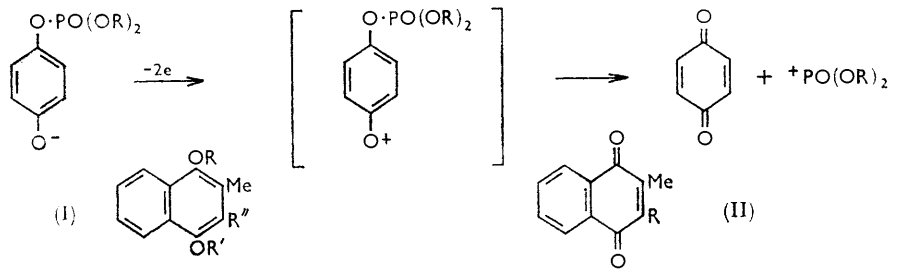
The expected oxidation and concomitant phosphorylation have been substantiated experimentally. Thus, whereas diphenyl 4-hydroxy-2,3-dimethyl-1-naphthyl phosphate is relatively stable to oxidising agents (unchanged by bromine in methanol after 10 minutes at room temperature), the monoesters and the free hydroxynaphthyl dihydrogen phosphate undergo immediate oxidation with bromine to the quinone and various products derived from metaphosphate, *viz.*, trimetaphosphate, pyrophosphate, and orthophosphate. These observations imply that a metaphosphate is easily produced, but not the phosphoryl cation.

4-Hydroxy-3-methyl-1-naphthyl dihydrogen phosphate [I; R = H, R′ = $PO(OH)_2$, R″ = H] in aqueous solution at pH 6·8 in the presence of air is slowly hydrolysed with

[4] Clark, Kirby, and Todd, *J.*, 1957, 1497.
[5] Khorana and Moffatt, *J. Amer. Chem. Soc.*, 1959, **81**, 1265.
[6] Vernon, *Chem. Soc. Spec. Publ.*, 1957, **8**, 23; Bunton, Llewellyn, Oldham, and Vernon, *J.*, 1958, 3574.
[7] Cf. Goehring and Sambeth, *Chem. Ber.*, 1957, **90**, 232.
[8] Van Wazer, " Phosphorus and its Compounds," Interscience Publ. Inc., New York, 1958, Vol. I, Chap. 11.

the liberation of inorganic phosphate (half-life ~4 days); in air-free solution there is no appreciable decomposition for 1 week. In alkaline solution rapid autoxidation greatly complicates further investigation.

The corresponding bisphosphate ("Synkavit") [I; R = R′ = $PO(OH)_2$, R″ = H] is very stable to hydrolysis; after 6 hr. at 80° (pH 5) only about 20% of the phosphorus



appears as inorganic phosphate (personal communication from Dr. K. J. M. Andrews, Roche Products, Ltd.). On treatment in aqueous solution with a variety of oxidising agents, however, rapid decomposition ensues. Ceric sulphate in 3N-sulphuric acid brings about immediate oxidation to the quinone (II; R = H), so does bromine in aqueous solution, one mol. of halogen being used without incorporation into the aromatic nucleus. As the bisphosphate is very resistant to hydrolysis, the dephosphorylation must accompany and not precede the oxidation. Metabolic studies of this bisphosphate, carried out in view of its potent inhibition of mitosis of cultures of chick-heart fibroblasts [9] and its radiosensitising action,[10] have indicated a rapid dephosphorylation *in vivo* [11] and this, too could be oxidative in type. It is noteworthy that the oxidation of dihydrovitamin K_3 (I; R = R′ = R″ = H) by mitochondria is accompanied by phosphorylation,[12] and a rôle associated with phosphorylative reactions has been suggested for vitamin K in studies on animal tissue,[13] bacteria,[14] and chloroplasts.[15]

Oxidation of the bisphosphate to the corresponding quinone by bromine can be carried out in aqueous solution over a wide pH range, the metaphosphate intermediate being solvated to orthophosphate. Notwithstanding the water present, this oxidation in the presence of orthophosphate leads to formation of some inorganic pyrophosphate concurrently with precipitation of the quinone; that is, orthophosphate is phosphorylated to pyrophosphate in consequence of an oxidation. Moreover, in the absence of added orthophosphate, 10% of inorganic pyrophosphate was formed; and on oxidation in dry *NN*-dimethylformamide 35% of the phosphorus was transformed into trimetaphosphate and 15% of pyrophosphate was produced. These observations are most readily accommodated by considering the, as yet unknown, monomeric metaphosphate to have been generated in these reactions. Finally, oxidation in glacial acetic acid gave rise to the anhydride, acetyl phosphate, whose formation was detected by the formation of acethydroxamic acid on treatment with hydroxylamine.

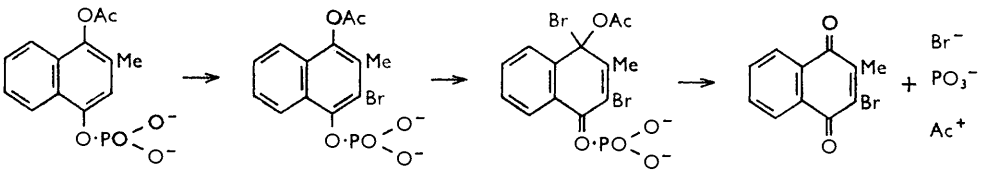
A variety of quinol diphosphates have been oxidised by bromine water to quinones (see Table, p 719). For a variety of oxidising agents, qualitative correlation between their efficacy and the redox potential of the quinol–quinone system was noted. In almost all cases, oxidation by ceric ion or bromine was rapid. Derivatives of catechol required the substitution of chlorine for bromine before they would undergo oxidative dephosphorylation with the production of pyrophosphate. 4-Hydroxy-3-methyl-1-naphthyl

[9] Mitchell and Simon-Reuss, *Nature*, 1947, **160**, 98.
[10] Mitchell, *Brit. J. Cancer*, 1952, **6**, 305.
[11] Neukomm, Peguiron, Lerch, and Richard, *Arch. Int. Pharmacodyn. Therapie*, 1953, **93**, 373.
[12] Colpa-Boonstra and Slater, *Biochim. Biophys. Acta*, 1958, **27**, 122.
[13] Martius, *Biochem. Z.*, 1956, **327**, 407.
[14] Brodie and Ballantine, *J. Biol. Chem.*, 1960, **235**, 226.
[15] Arnon, Allen, and Whatley, *Biochim. Biophys. Acta*, 1956, **20**, 449.

phosphate was only slowly oxidised by acid dichromate, hydrogen peroxide in the presence of tungstate,[16] or dilute nitric acid; neither iodine not potassium ferricyanide produced any apparent change within an hour at room temperature.

Certain other acyl derivatives of dihydrovitamin K_3 (I; R = R′ = R″ = H) undergo oxidation to the quinone. Ceric sulphate rapidly oxidises the disulphate [17] (I; R = R′ = SO_2·OH, R″ = H), presumably to the quinone, whilst bromine in aqueous solution reacts with both the acetate phosphate [I; R = Ac, R′ = $PO(OH)_2$, R″ = H] and the monoacetate (I; R = Ac, R′ = R″ = H) to give 2-bromo-3-methyl-1,4-naphthaquinone (II; R = Br). This nuclear bromination is in contrast to the behaviour of the bisphosphate and 2-methyl-1,4-naphthaquinol (I; R = R′ = R″ = H), both of which give the halogen-free quinone (II; R = H). Since the diacetate (I; R = R′ = Ac, R″ = H) is unaffected by bromine in aqueous dioxan, the phosphorylated phenolic groups appear to be polarisable, and in this sense still phenolic.

Bromination of the acetate phosphate probably takes the following course, oxidation being accompanied by a phosphorylation (by PO_3^-) and an acetylation (by CH_3·CO^+ or its equivalent).



Esters of quinol monophosphates are now readily available from the reaction between a quinone and a diester of phosphorous acid. Ramirez and Dershowitz [18] described the reaction following photochemical activation, but we have found the same products are formed extremely smoothly in the presence of small quantities of base, *e.g.*, potassium t-butoxide. The reaction appears to be general. 2,3-Dimethylnaphthaquinone and dibenzyl phosphite give dibenzyl 4-hydroxy-2,3-dimethyl 1-naphthyl phosphate, and this with lithium chloride [19] in ethyl cellosolve affords the monobenzyl ester, which with bromine gave some P^1P^2-dibenzyl pyrophosphate; the same oxidative phosphorylation has been achieved by hydrogen transfer to another quinone, chloranil.

Experimental

Oxidation of 4-Hydroxy-3-methyl-1-naphthyl Phosphate.—4-Acetoxy-3-methyl-1-naphthyl phosphate [20] (100 mg.; dried at 100°/0·1 mm. over P_2O_5) was dissolved in 0·1N-sodium hydroxide solution (5 ml.) with exclusion of air and left at room temperature for 1½ hr. The reaction vessel was opened and the solution quickly adjusted to pH 7 with 3N-hydrochloric acid and examined chromatographically in butan-1-ol–acetic acid–water (5 : 2 : 3) (see below). The chromatogram showed that removal of the acetyl group was complete and that a little inorganic orthophosphate had been produced, presumably by autoxidation, since the solution had become slightly discoloured by the end of the reaction. 0·1N-Iodine solution (5 ml.; containing potassium iodide) was added to this solution of 4-hydroxy-3-methyl-1-naphthyl phosphate; 2-methyl-1,4-naphthaquinone that was immediately precipitated was collected, washed, dried, and recrystallised from light petroleum (b. p. 60—80°) (20 mg.; m. p. and mixed m. p. 103—106°). Titration of the excess of iodine in the filtrate with sodium thiosulphate solution showed that 0·87 mol. of iodine had been consumed. The original acetoxy-phosphate was not affected by iodine under these conditions.

In another experiment an aqueous solution of the naphthaquinol monophosphate at pH 6·8

[16] Cf. Spencer, Todd, and Webb, *J.*, 1958, 2968.
[17] Canbäck and Ehrlen, *Farmacevtisk Revy (Stockholm)*, 1947, **12**, 210.
[18] Ramirez and Dershowitz, *J. Org. Chem.*, 1957, **22**, 1282.
[19] Clark and Todd, *J.*, 1950, 2030.
[20] Baker and Carlson, *J. Amer. Chem. Soc.*, 1942, **64**, 2657; Baker, U.S.P. 2,380,716, *Chem. Abs.*, 1945, **39**, 4727.

(prepared as described above) was divided into two portions, one of which was sealed under a vacuum. The other portion was kept open to the atmosphere at room temperature and examined chromatographically from time to time. After 4 days some 50% of the quinol phosphate had been hydrolysed to orthophosphate, the solution having become deep red. After 7 days the sealed specimen was unchanged.

Oxidation of Various Quinol Phosphates with Bromine.—The quinol diphosphates (supplied by Roche Products, Ltd., and prepared as directed by Andrews *et al.*[21]) and 4-acetoxy-3-methyl-1-naphthyl phosphate were used as their hydrated normal sodium salts. The degree of hydration of these salts was determined by drying at 100°/0·1 mm. over P_2O_5.

Method (*a*). A solution of the sodium salt (500 mg.) in water (5 ml.) was treated at room temperature with a freshly prepared, saturated aqueous solution of bromine. The oxidising agent was added slowly with stirring until a slight excess was present. In all cases, the quinone began to separate almost immediately. Each suspension was cooled to 0° and then filtered, and the quinone washed with water, dried, and recrystallised from light petroleum (b. p. 60—80°).

Method (*b*). The procedure differed from (a) in that sodium acetate (1 g. of the trihydrate) was dissolved in the aqueous quinol phosphate solution before bromine was added. Under these conditions the pH of the solution did not fall below 7 during the reaction.

The results are tabulated. The yields quoted are of purified material; in some cases the yield of crude product was appreciably greater.

Substrate	Method	Product isolated	Yield (%)
2,3-Dimethyl-1,4-naphthaquinol bisphosphate	a	2,3-Dimethyl-1,4-naphthaquinone	62
2-Methyl-1,4-naphthaquinol bisphosphate	a	2-Methyl-1,4-naphthaquinone *	63
1,4-Naphthaquinol bisphosphate †	a	1,4-Naphthaquinone	53
4-Acetoxy-3-methyl-1-naphthyl phosphate	a	2-Bromo-3-methyl-1,4-naphthaquinone	31
Duroquinol bisphosphate	a	Duroquinone	53
	b		54
2,3,5-Trimethylquinol bisphosphate	a	2-Bromo-3,5,6-trimethyl-1,4-benzoquinone	10
	b		70
2-Bromo-3,5,6-trimethylquinol bisphosphate	a	2-Bromo-3,5,6-trimethyl-1,4-benzoquinone	42
	b		51
2,5-Dimethylquinol bisphosphate	a	A mixture of quinones, m. p. 80—100°	10 ‡
	b		15 ‡

* This product melted at 101—103°, even after recrystallisation from light petroleum or ethanol. Authentic 2-methyl-1,4-naphthaquinone melted at 106°, and the infrared spectrum of our product suggested the presence of a small amount of 2-bromo-3-methyl-1,4-naphthaquinone (extra bands at 1280, 1180, 822, and 700 cm.$^{-1}$). † The normal barium salt was used. ‡ Calc. as 3-bromo-2,5-dimethyl-1,4-benzoquinone.

Oxidations of 4-Hydroxy-3-methyl-1-naphthyl Bisphosphate.—(a) *With* Ce^{4+}.[22] To a solution of the tetrasodium salt of this phosphate (500 mg. of the hexahydrate) in N-sulphuric acid (10 ml.) was added 0·1N-ceric sulphate (20 ml.; N with respect to sulphuric acid). The 2-methyl-1,4-naphthaquinone that was precipitated was filtered off, washed with N-sulphuric acid and water, dried, and recrystallised from light petroleum (b. p. 60—80°); it had m. p. 105—106° (100 mg., 62%). The combined filtrate and washings were treated with an excess of potassium iodide, and the liberated iodine was titrated with thiosulphate (Ce consumed, 2·02 equiv.).

(b) *With bromine.* The tetrasodium salt (250 mg.) was oxidised with saturated aqueous bromine (5 ml.) as described above. The precipitated quinone was filtered off and washed, the filtrate and washings were run into an excess of aqueous potassium iodide, and the liberated iodine was titrated with thiosulphate. The bromine content of the aqueous bromine solution was determined separately (Br consumed, 1·03 mol.).

(c) *With other oxidising agents.* An aqueous solution of the tetrasodium salt was stable towards iodine and ferricyanide, but was slowly oxidised by dichromate in 3N-sulphuric acid and by hydrogen peroxide solution containing sodium pertungstate. Oxidation by this last

[21] Andrews, Marrian, and Maxwell, *J.*, 1956, 1844.
[22] Yamagishi, *Ann. Reports Takeda Res. Lab.*, 1954, **13**, 25.

reagent gave a crystalline precipitate of 2-methyl-1,4-naphthaquinone 2,3-oxide,[23] m. p. and mixed m. p. 95—97° (from aqueous ethanol).

Irradiation of an aqueous solution of the tetrasodium salt with ultraviolet light (Hg discharge lamp) in the absence of air brought no hydrolysis.

Formation of Pyrophosphate and Trimetaphosphate in the Oxidation of 4-*Hydroxy*-3-*methyl*-1-*naphthyl Bisphosphate.*—(a) *In aqueous solution.* To a solution of the tetrasodium salt (20 mg. of the hexahydrate) in water (0·25 ml.) one drop of bromine was added. The mixture was left at room temperature for 10 min. and the excess of bromine and precipitated quinone were removed by extraction with cyclohexene (2·5 ml.). The aqueous phase was examined by paper chromatography, with trichloroacetic acid–propan-2-ol–water–ammonia (see below) as solvent. Pyrophosphate was present, together with orthophosphate. The relevant spots were cut out and, together with controls from a developed chromatogram containing no phosphorus, were eluted with water and analysed for phosphorus.[24] The oxidation produced 90% of orthophosphate and 10% of pyrophosphate.

(b) *In non-aqueous solution.* The tetrasodium salt was converted into the free acid by passing its aqueous solution through Dowex-50 (H^+ form) resin. The eluate was concentrated *in vacuo* and the crystalline residue dried (P_2O_5). To a solution of this free acid (35 mg.) in rigorously dried [25] *NN*-dimethylformamide (1 ml.) bromine (0·1 ml.) was added and the mixture left at room temperature for 1 hr. Water (10 ml.) was added, followed by cyclohexene (5 ml.), and the aqueous layer was examined by paper chromatography with the above solvent system. Three phosphorus-containing spots were observed, at R_F 0·66, 0·35, and 0·10, corresponding to ortho-, pyro-, and trimeta-phosphate, respectively. Excision, elution, and estimation, as in the previous experiment, indicated that 50% of orthophosphate, 15% of pyrophosphate, and 35% of trimetaphosphate had been produced.

Oxidation of o-*Hydroxyphenyl Phosphate with Chlorine.*—Chlorine was passed into a solution of cyclohexylammonium *o*-hydroxyphenyl hydrogen phosphate [26] in dry *NN*-dimethylformamide for 15 min. The solution became pale brown and heat was evolved. Paper chromatography with butanol–acetic acid–water (see below) revealed inorganic pyrophosphate. Repetition of the oxidation in the presence of tetra-n-butylammonium phosphate produced a large amount of inorganic pyrophosphate.

Formation of Acetyl Phosphate.—A slight excess of bromine was added to a solution of the tetrasodium 4-hydroxy-3-methyl-1-naphthyl bisphosphate in glacial acetic acid and, after 2 hr., the solution was diluted with water and the precipitated quinone extracted with ether. The presence of acetyl phosphate in the remaining aqueous layer was shown in the following way.[27] A portion of the solution was treated with hydroxylamine hydrochloride followed by aqueous sodium hydroxide and then examined chromatographically with butan-1-ol–acetic acid–water (5 : 2 : 3). A ferric chloride–hydrochloric acid spray revealed a substance (red colour) having the R_F value (0·57) of acethydroxamic acid. Repetition of the experiment using 1,4-naphthaquinol gave none of this product.

Oxidation of 2-*Methyl*-1,4-*naphthaquinol and its* 1-*Acetate with Bromine.*—To a solution of 4-hydroxy-2-methyl-1-naphthyl acetate (300 mg.) in dioxan (10 ml.) containing water (5 ml.) was added saturated aqueous bromine, with stirring, until a slight excess was present. The mixture was diluted with water to 50 ml. and kept at 0° for 30 min.; the precipitated 2-bromo-3-methyl-1,4-naphthaquinone was collected, dried (331 mg.), and recrystallised from light petroleum (b. p. 60—80°) (260 mg., 74%), then having m. p. and mixed m. p. 156—158°. 2-Methyl-1,4-naphthaquinol, when oxidised in analogous fashion, gave 2-methyl-1,4-naphthaquinone (56%), m. p. and mixed m. p. 105—107°.

Dibenzyl 4-*Hydroxy*-2,3-*dimethyl*-1-*naphthyl Phosphate.*—A 1·07N-solution (5 ml.) of potassium t-butoxide in t-butyl alcohol was added to one of 2,3-dimethyl-1,4-naphthaquinone (5 g., 0·03 mole) and dibenzyl phosphite (9·6 g., 0·035 mole) in dry benzene (25 ml.) and the mixture set aside overnight at room temperature in an atmosphere of nitrogen, moisture being excluded. The excess of benzene was then removed *in vacuo*. The residue crystallised on trituration with pentane. Recrystallised from carbon tetrachloride *dibenzyl* 4-*hydroxy*-2,3-*dimethyl*-1-*naphthyl*

[23] Weitz, Schobbert, and Siebert, *Ber.*, 1935, **68**, 1163.
[24] Allen, *Biochem. J.*, 1940, **34**, 858.
[25] Thomas and Rochow, *J. Amer. Chem. Soc.*, 1957, **79**, 1843.
[26] Cherbuliez, Schwarz, and Leber, *Helv. Chim. Acta*, 1951, **34**, 841.
[27] Cf. Stadtman and Barker, *J. Biol. Chem.*, 1950, **184**, 769.

phosphate formed needles (11 g., 82%), m. p. 110° (Found: C, 69·2; H, 5·1. $C_{26}H_{25}O_5P$ requires C, 69·5; H, 5·6%).

Benzyl 4-Hydroxy-2,3-dimethyl-1-naphthyl Hydrogen Phosphate.—The above dibenzyl ester (10·3 g.) was heated at 80° for 2 hr. in ethyl cellosolve (50 ml.) saturated with anhydrous lithium chloride.[19] 10% Aqueous sodium hydroxide (40 ml.) was added to the cooled solution, and the mixture extracted with ether (4 × 50 ml.). The aqueous layer was acidified and extracted with ether, and the extract washed, dried ($MgSO_4$), and evaporated. The residue recrystallised from chloroform, giving the *monobenzyl ester* as needles (5·96 g., 73%), m. p. 126—127° (Found: C, 63·9; H, 5·8. $C_{19}H_{19}O_5P$ requires C, 63·6; H, 5·3%).

Oxidation. (*a*) With bromine. To a solution of the monobenzyl ester (10 mg.) in dry *NN*-dimethylformamide (5 ml.) dry benzene (5 ml.) was added and then distilled off again *in vacuo* with strict exclusion of moisture. A drop of bromine was added to the solution and after 10 min. cyclohexene (1 ml.). Examination of the mixture by paper chromatography with propan-2-ol–water–ammonia (7 : 2 : 1) showed the presence of monobenzyl dihydrogen phosphate and P^1P^2-dibenzyl dihydrogen pyrophosphate.

(*b*) With chloranil. Chloranil (70 mg.) was added to a similarly prepared solution of the monobenzyl ester (70 mg.) in dry *NN*-dimethylformamide (5 ml.), and the mixture left for 1 hr. at room temperature. Paper chromatography showed the presence of monobenzyl dihydrogen phosphate and P^1P^2-dibenzyl dihydrogen pyrophosphate in the resulting solution.

4-Hydroxy-2,3-dimethyl-1-naphthyl Phosphate.—The above dibenzyl ester (3 g.) in absolute ethanol (30 ml.) was hydrogenated at room temperature and 1 atm. over palladium black (100 mg.). After uptake of hydrogen had ceased the catalyst was removed and the solvent evaporated under reduced pressure in a stream of nitrogen, to give *4-hydroxy-2,3-dimethyl-1-naphthyl dihydrogen phosphate* as hygroscopic needles; recrystallised from carbon tetrachloride, they (1·5 g., 85%) had m. p. 140° (Found: C, 52·4; H, 5·8. $C_{12}H_{13}O_5P,\frac{1}{2}H_2O$ requires C, 52·0; H, 5·1%). The crystals rapidly became pink in air.

Oxidation. To a solution of the free acid (72 mg.) in 0·05M-aqueous potassium carbonate (10 ml.) was added an excess of bromine water, and the mixture was set aside for 15 min. The precipitate was filtered off and dried overnight at 100°/0·1 mm. (42 mg., 96%); it had m. p. 123—124° undepressed in admixture with 2,3-dimethyl-1,4-naphthaquinone.

Oxidation under neutral or acidic conditions gave a virtually quantitative yield of 2,3-dimethyl-1,4-naphthaquinone in each case.

Diphenyl 4-Hydroxy-2,3-dimethyl-1-naphthyl Phosphate.—Prepared by the same method as the above dibenzyl compound, the *diphenyl ester* (yield, 65%) crystallised from ethyl acetate as hygroscopic needles, m. p. 94—95° (Found: C, 65·6; H, 5·4. $C_{24}H_{21}O_5P,H_2O$ requires C, 65·6; H, 5·4%).

The diphenyl ester was recovered unchanged (10 min.) in methanol solution with excess of bromine at room temperature.

Chromatographic Data.—Ascending chromatograms (see Table) were run on Whatman No. 1 paper in solvent systems (A) butan-1-ol–acetic acid–water (5 : 2 : 3) and (B) propan-2-ol (75 ml.)–water (25 ml.)–trichloroacetic acid (5 g.)–ammonia solution (0·25 ml.; *d* 0·88). Phosphorus was detected by the molybdate spray.

	R_F in solvent system A	R_F in solvent system B	Appearance of spot in u.v. light
2-Methyl-1,4-naphthaquinol bisphosphate	0·32	0·76	Blue fluorescence
4-Hydroxy-3-methyl-1-naphthyl phosphate	0·53	—	Blue fluorescence
4-Acetoxy-3-methyl-1-naphthyl phosphate	0·60	—	u.v. absorbing
Inorganic orthophosphate	0·30	0·66	—
Inorganic pyrophosphate	0·15	0·36	—
Inorganic trimetaphosphate	—	0·10	—

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University Chemical Laboratory,
Lensfield Road, Cambridge.

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