Chemical Studies Concerning the Possible Role of Chromanyl Phosphates and Quinones in Oxidative Phosphorylation

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Plastoquinone (1, 2), vitamin K₁ (3, 4), and possibly coenzyme Q (5-7) are lipophilic quinones believed to be associated with phosphorylation coupled to electron transport (8, 9) (these compounds are designated Ia, II, and Ib, respectively).

In the case of vitamin K₁, anaerobic incubation of the quinone with an electron donor and a \textit{Mycobacterium phlei} preparation yielded a reduced phosphorylated derivative (3) which underwent enzymatic autoxidation to vitamin K with release of inorganic phosphate; this was stated to be a naphthochromanyl phosphate. The 6-chromanyl phosphate (III) of vitamin K₁ (20) has been synthesized (10) and shown to undergo enzymatic oxidation by cytochrome c (with formation of adenosine triphosphate) in an \textit{M. phlei} extract. Monoethyl menadiol 1-phosphate can also serve as substrate for oxidative phosphorylation in the bacterial system (11).

Phosphorylated chromanols of coenzyme Q and plastoquinone have neither been isolated from nor applied to an extract of their respective systems; in fact, they have not been synthesized. However, the chemical relationship of these quinones to vitamin K, especially the 2,3-unsaturation in the isoprenoid side chain which permits reductive cyclization to the chromanol, suggests the possible existence of such an intermediate.

Vilkas and Lederer have proposed (12) a biochemical mechanism of oxidative phosphorylation (Scheme 1); they consider the ring opening of a chromanyl phosphate (IV) to a hydroquinone phosphate (V), which can then be oxidized to a quinone (VI). Inorganic phosphate, according to their hypothesis, would then be reincorporated by addition to the isomeric quinone methine (VII). Wagner et al. (13) have reported chemical evidence in support of this last step, and the nonenzymic oxidation of hydroquinone phosphates has been extensively studied (14-16). It seemed desirable, therefore, to examine the chemistry and properties of chromanyl phosphates, especially in connection with the

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scheme proposed by Vilkas and Lederer, and also to investigate further the isomerization of methyl-substituted quinones to quinone methines.

**EXPERIMENTAL PROCEDURE AND RESULTS**

Infrared, ultraviolet, and nuclear magnetic resonance spectra were recorded on Beckman IR-7, Cary model 14, and Varian A-60 spectrometers, respectively. NMR\(^1\) spectra were run at room temperature, with sample spinning and Si(CH\(_3\))\(_4\) as internal reference in most cases; peak positions are given in \(\tau\) units (14) with the number of protons involved shown in parentheses after each \(\tau\) value.

In the irradiations, the solution was placed in a quartz cuvette at a distance of 8 cm from a 1000-watt, high pressure mercury lamp.

**dl-\(\alpha\)-Tocopheryl Phosphate (VIII; \(R = PO_3H_2, R' = C_{16}H_{33}\))**

The compound is supplied by Calbiochem as the disodium salt. The infrared spectrum (Nujol mull) possessed a band at 3800 cm\(^{-1}\) (OH, water), and the ultraviolet spectrum (in H\(_2\)O) showed maxima at 286 nm (\(\varepsilon = 39.4\)) and 280 nm (shoulder). The infrared spectrum (Nujol mull) had a band at 3300 cm\(^{-1}\) (OH, water), and the ultraviolet spectrum (in H\(_2\)O) showed maxima at 286 nm (\(\varepsilon = 39.4\)) and 280 nm (shoulder). The NMR spectrum (in D\(_2\)O) had broad peaks at \(\tau = 7.8, 8.7, \) and 9.0 (H\(_2\)O as internal reference). The molecular weight was high, (pH 1.1) also showed no spectral change after refluxing for 40 minutes.

**Discussion**

dl-\(\alpha\)-Tocopheryl phosphate was not oxidized by dry silver oxide in ether suspension, and was even unchanged after treatment for 20 minutes with 0.1 N auric chloride solution at pH 7. However, when the disodium salt (145 mg) in buffer at pH 7 (30 ml) was treated dropwise with freshly prepared, saturated bromine water (6 ml), the ultraviolet maximum rapidly shifted from 286 nm to 260 nm (approximately 6-fold increase in optical density). After 5 minutes, the solution was acidified and extracted with ether, the ether layer was washed well with water and dried (Na\(_2\)SO\(_4\)), and the solvent was evaporated to give a pale yellow oil (135 mg), which did not contain \(\alpha\)-tocopherylquinone (IX; \(R = C_{16}H_{33}\)) (paper chromatography). The infrared spectrum (smear) had bands at 1765, 1700, and 1650 cm\(^{-1}\). The compound was very unstable, and the initial, well-defined maximum in the ultraviolet spectrum at 259 nm (ether) gave way to an ill-defined spectrum after a few hours. The same oxidation with bromine water took place at pH 5.4 (but not at pH 11.3). However with other solvents (chloroform, ether, or acetic acid) and the free chromanol phosphate, no reaction was observed spectrally upon the gradual addition of a solution of bromine in the organic solvent.

**dl-\(\alpha\)-Tocopheryl phosphate (from 62 mg of disodium salt) was shaken with 95% ethanol (20 ml), 0.1 N aq. sulfuric acid in 0.1 N H\(_2\)SO\(_4\) (12 ml), and ether (peroxide-free, 100 ml) for 3 minutes, according to Kofler (20). The ether layer was washed with water, filtered, and diluted to 1 liter with 95% ethanol, and the \(\alpha\)-tocopherylquinone (IX; \(R = C_{16}H_{33}\)) was estimated by use of the published (21) extinction value at 268 nm (approximately 47% conversion). The \(\alpha\)-tocopherylquinone was identified (after purification on an alumina column) by paper chromatography, ultraviolet spectrum, and infrared spectrum (smear) (maximum at 3500 cm\(^{-1}\), -OH).

**2,3,5,7,8-Pentamethyl-6-chromanol (VIII; \(R' = H, R' = CH_3\))**

Trimethylhydroquinone, prepared by oxidation of 2,3,5-trimethylphenol with Freamy’s salt (22) followed by reduction (sodium dithionite), was treated with isopropyl under acidic conditions according to Smith et al. (23). The crude chromanol was purified either by chromatography on alumina (Merek) in petroleum ether (b.p. 30–60°) and elution with ether-methanol (8:1), or by extraction of the chromanol from a solution in petroleum ether (b.p. 30–60°) with 10% NaOH in methanol-water (1:1). It had a melting point of 95–96.5°; Schudel et al. (24) report 95.5–97°.

A solution of the chromanol (150 mg) in CH\(_3\)OD (1.0 ml) was made 0.02 N with respect to H\(_2\)SO\(_4\) by addition of 1 drop of a solution containing 2 drops of concentrated H\(_2\)SO\(_4\) per ml of CH\(_3\)OD. No change was observed in the peak heights or positions in the NMR spectrum after 3 hours. Repetition of the experiment with ultraviolet irradiation for 1 hour produced no significant change in the NMR spectrum, except for about 15% reduction in the heights of the Ar–CH\(_3\) peaks centered at \(\tau = 7.89\) and Ar–CH\(_2\) peaks centered at \(\tau = 7.42\); the peaks centered at \(\tau = 8.27\) due to the CH\(_2\) protons at position "b" were unchanged. Treatment of the chromanol (50 mg) in CH\(_3\)OD (0.5 ml) with 1 drop of NaOH in D\(_2\)O (making an approximate pD of 9 to 10) under nitrogen again produced no change in the NMR spectrum, even after 1 hour.

**2,3,5,7,8-Pentamethyl-6-chromanol phosphate (VIII; \(R = PO_3H_2, R' = CH_3\))** \(\Delta\) solution of 2,3,5,7,8 pentamethyl-6-
chromanol (1 g) in dry pyridine (5 ml) was cooled and added to a solution of phosphorus oxychloride (1.2 ml) in dry pyridine (5 ml). The mixture was left overnight at room temperature, poured into water and acidified to pH 1, and the precipitate (1 g) was collected; if a gummy solid formed, it was extracted into ether. Recrystallization from ethyl acetate (twice) and then dioxan gave needles, m.p. 150–152° (softening from 130°).

A sample was dried at 100° at 2 mm for analysis.

C₁₉H₂₃O₅P·½C₄H₄N (339.8)

Calculated: C 58.3, H 7.0, P 9.3, N 2.1
Found: C 58.4, H 7.0, P 9.1, N 2.2

The ultraviolet absorption spectrum (in 95% ethanol) showed maxima at 245, 252, 258, and 264 μ (pyridine bands) and at 280 (shoulder) and 286 μ.

An ether solution of the crude chromanyl phosphate was washed with dilute hydrochloric acid and water and then dried, and the ether was removed. Treatment of an ethanolic solution of the residue with the calculated quantity of aqueous NaOH, then cooling, gave the monosodium salt, which crystallized from aqueous ethanol as needles, m.p. 300°. A sample was dried at 100° at 10⁻² mm for analysis.

C₁₉H₂₃O₅PNa (322.3)

Calculated: C 56.2, H 6.3, P 9.6
Found: C 56.1, H 6.2, P 10.0

The ultraviolet absorption spectrum (in H₂O) showed a maximum at 284 μ (ε = 1770), and the infrared spectrum (Nujol) possessed a band at 3400 cm⁻¹ (broad, —OH). The salt (2.9 mg per ml of water) gave a solution with pH 6.3, and no change in the ultraviolet spectrum was observed on heating (100°, 1 hour) in buffers of pH 5.4 or 11.3, or in 5% KOH solution.

Treatment of an ethanolic solution of the crude phosphate with excess ethanolic NaOH solution yielded the sodium salt of 2,2,5,7,8-pentamethyl-6-chromanyl phosphate (VI); R = PO₃H₂, R’ = CH₃ in alkaline D₂O. which lost weight (approximately 30%) on being dried at 100° at 0.6 mm.

C₁₉H₂₃O₅PNa·H₂O (362.3)

Calculated: C 46.4, H 5.8, P 8.6
Found: C 46.2, H 6.2, P 8.7

The molecular weight found by titration with 0.01 N HCl was 366 (a solution of 16.5 mg of the disodium salt in 5 ml of water had pH 8.5). The compound possessed an ultraviolet absorption maximum (in H₂O) at 384 μ (ε = 2300) and had a band in the infrared spectrum (Nujol) at 3000 cm⁻¹ (broad, —OH). 2,2,5,7,8-Pentamethyl-6-chromanol phosphate was obtained by acidification of an aqueous solution of the disodium salt, and after recrystallization from ethyl acetate-petroleum ether (b.p. 30–60°) had a melting point of 200–205°. A sample was dried at 100° at 1 mm for analysis.

C₁₉H₂₃O₅P (300.3)

Calculated: C 56.0, H 7.0, P 10.3
Found: C 55.9, H 7.0, P 10.2

The ultraviolet absorption spectrum (in 95% ethanol) showed a maximum at 255 μ (ε = 2160) and a shoulder at 280 μ (ε = 1880), and the RF value on Whatman No. 1 paper with ethanol-water (2:9) was 0.78.

Oxidation of an aqueous solution of the monosodium or disodium salt of 2,2,5,7,8-pentamethyl-6-chromanol phosphate with freshly prepared bromine water brought about an immediate change in the ultraviolet absorption maximum to 265 μ (8- to 10-fold increase in optical density). Addition of NaBH₄ to this solution gave a maximum at 251 μ (same optical density as original solution), and the change to 265 μ was repeated with the addition of more bromine water. The product contained a phosphate group still, and no free orthophosphate was formed in the reaction (Hanes-Isherwood spray) (25). Use of acetic acid as solvent gave no change in the absorption spectrum other than the small increase in absorption due to bromine.

Although not oxidized by ferric chloride in aqueous solution, 2,2,5,7,8-pentamethyl-6-chromanol phosphate was oxidized by acidic ceric sulfate solution. Under conditions similar to those used for dl-α-tocopherol phosphate, the chromanyl phosphate, as the monosodium salt, the free phosphate, or the form containing 0.5 molecule of pyridine, was rapidly converted into trimethyl(3 methyl 3 hydroxybutyl)-1,4-benzoquinone (IX; R = CH₃) in yields of 39, 69, and 78%, respectively. The product was identified by paper chromatography on alumina-impregnated paper in benzene-chloroform (1:1, 1:0, and 0:1), and by its ultraviolet absorption spectrum (24). The quinone was prepared as described by John, Dietzel, and Emte (26) by oxidation of 2,2,5,7,8-pentamethyl-6-chromanol with ferric chloride, and the light absorption spectrum showed maxima (in 95% ethanol) at 203, 268 (R² = 7.65), and 342 (R² = 11) μ.

The NMR spectrum of 2,2,5,7,8-pentamethyl-6-chromanol phosphate (120 mg in 1.0 ml of CH₂OD) had peaks at τ = 7.25, 7.36, and 7.48 (2); 7.80 and 7.92 (9); 8.10, 8.21, and 8.33 (2); and 8.72 (6). The solution was made 0.02 N with respect to H₂SO₄. The spectrum was unchanged after 3 hours, even with ultraviolet irradiation for 1 hour. The phosphate (105 mg) was dissolved in 0.2 N NaOH in D₂O and again the NMR spectrum (see Fig. 1) was scarcely changed after 20 hours at room temperature followed by irradiation for 1 hour.
Trimethyl(3-methyl-2-butenyl)-1,4-benzoquinone (X; Y = CH₃) — Trimethylhydroquinone (0.9 g), 2-methyl-3-buten-2-ol (1.0 g), fused zinc chloride (0.5 g), acetic acid (0.1 ml), and ether (25 ml) were refluxed for 63 hours, the solution was washed with water and dried, and the ether was removed. The mixture of products was redissolved in dry ether (120 ml) containing sodium sulfate (1 g), and oxidized by shaking with freshly prepared silver oxide (6.8 g) for 1 hour. After filtration and removal of the ether, chromatography on basic alumina (Merck) and elution of the first yellow band with benzene-petroleum ether (b.p. 30–60°) (5:7, 200 ml) gave the quinone (0.60 g, 46%) as a yellow solid. A sample was further purified by chromatography on silica gel.

\[ C_{11}H_{10}O_2 \]  
Calculated: C 77.0, H 8.3  
Found: C 77.0, H 8.2

The ultraviolet absorption spectrum (in 95% ethanol) showed maxima at 259, 266 (ε = 1.72 × 10⁴), and 310 (ε = 954) mU; Martini and Furer (27) report 259 and 268 mU. The NMR spectrum (CD₃OD) had peaks at τ = 2.94, 3.07, and 3.19 (1); 6.80 and 6.90 (2); 8.01 (9); 8.25 (3); and 8.32 and 8.33 (3). The solution was made 0.3 N with respect to NaOH. The spectrum, in particular the height of the —CH₂ peak at τ = 8.01, remained exactly the same after 2 hours. Paper chromatography confirmed that no reaction took place. Another solution (170 mg of quinone in 1 ml of CD₃OD) was made 0.03 N with respect to NaOH by addition of 0.01 ml of NaOH and the NMR spectrum was examined (see Fig. 2). After about 20 minutes the positions and areas of peaks due to —CH and (CH₃)₂C= protons were unchanged, but even after only 10 minutes the quinone —CH₃ and quinone —CH₂ peaks had virtually disappeared and their measured areas (after 20 minutes) were only about 50% (cf. the control experiment) of those prior to addition of alkali. As a control the experiment was repeated with CH₃OH as solvent; after addition of alkali the quinone —CH₃ and quinone —CH₂ peaks showed maxima in the ultraviolet absorption spectrum (in 95% ethanol) at 260 and 266 mU (ε = 954 mU); and the NMR spectrum (CD₃OD) showed peaks at τ = 3.69 (1, triplet, J = 2 c.p.s.), 4.90 (1, triplet, J = 7 c.p.s.), 6.98 (2, doublet, J = 7 c.p.s.), 8.05 (6), 8.24 (3), and 8.35 (3). The solution was made 0.3 N with respect to NaOH in H₂O (0.01 ml) the quinone —CH₂ signal broadened and the measured peak area in the region τ = 8.0 was reduced to about 80% of the original area.

A solution of duroquinone (tetramethyl-1,4-benzoquinone) (20 mg) in CH₃OD (0.5 ml) and CDCl₃ (0.2 ml) was made 0.05 N with respect to H₂SO₄. The height of the —CH₂ peak at τ = 8.0 in the NMR spectrum was unchanged after 1 hour. On addition of 0.01 ml of N NaOH in D₂O (0.01 ml) the quinone —CH₂ signal broadened and the measured peak area in the region τ = 8.0 was reduced to about 60% of its area (and about 90% of its height) in 8 minutes.

2,3-Dimethyl-5-(3-methyl-2-butenyl)-1,4-benzoquinone (X; Y = CH₃) — 2,3-Dimethylhydroquinone (1.5 g), 2-methyl-3-buten-2-ol (0.9 g), fused zinc chloride (1.0 g), acetic acid (0.1 ml), and ether (100 ml) were refluxed for 7 hours, washed with water, and dried, and the ether was removed. The residue was extracted (28) three times with 100-ml portions of warm petroleum ether (b.p. 30–60°), the extracts were filtered, and the solvent was removed. The mixture was oxidized with silver oxide (2 g) in ether (50 ml) containing sodium sulfate (1 g). The product was chromatographed on a column of silica gel (Baker) (30 × 2 cm) with elution by 50 ml each of benzene-petroleum ether

\[ C_{11}H_{10}O_2 \]  
Calculated: C 76.4, H 7.9  
Found: C 76.1, H 7.9

The golden yellow oil (m.p. 22–23°) showed maxima in the ultraviolet absorption spectrum (in 95% ethanol) at 254 mU (ε = 1.67 × 10⁴) and 318 mU (ε = 661); and the NMR spectrum (CDCl₃) showed maxima at τ = 3.09 (1, triplet, J = 2 c.p.s.), 3.90 (1, triplet, J = 6 c.p.s.), 4.98 (2, doublet, J = 7 c.p.s.), 6.98 (2, doublet, J = 7 c.p.s.), 8.0a (9), 8.24 (3), and 8.30 (3).

The first benzene fraction contained a mixture of two compounds, one of which was the quinone described above. After thin layer chromatography on silica gel G (20 × 20 × 0.15 cm) with development by heptane-benzene (2:1) four times, the first benzene eluate (50 ml) contained almost pure 2,3-dimethyl-5-(3-methyl-2-butenyl)-1,4-benzoquinone (X; Y = H) (0.33 g recovered). It was rechromatographed on a short silica gel column (elution with methylene dichloride), and dried at 25° on a 0.1 mm prior to analysis.

\[ C_{11}H_{10}O_2 \]  
Calculated: C 79.4, H 8.9  
Found: C 79.1, H 8.9

The quinone, a yellow oil, showed maxima in the ultraviolet absorption spectrum (in 95% ethanol) at 260 and 266 mU (ε = 1.03 × 10⁴) and 304 mU (ε = 914); and the NMR spectrum (CCl₄) showed peaks at τ = 5.30 (2, triplet), 6.88 (4, doublet), 8.05 (6), 9.10 (6), and 9.12 (6).

2,3-Dimethyl-5-(3-methyl-2-butenyl)-1,4-benzoquinone (X; Y = H) was not suitable for deuterium exchange study, as it rapidly decomposed under the mild alkaline conditions used for

![Fig. 2. NMR spectrum (60 megacycles per second) of trimethyl(3-methyl-2-butanyl)-1,4-benzoquinone (X; Y = CH₃) (17% solution in CD₃OD).](http://www.jbc.org/)

(b.p. 30–60°) mixtures (1:20, 1:10, 3:17, 1:5, 1:4, 1:3, and 1:1) and two benzene eluates of 50 and 100 ml. The second benzene fraction (100 ml) contained almost pure 2,3-dimethyl-5-(3-methyl-2-butenyl)-1,4-benzoquinone (X; Y = H) (0.33 g recovered).
trimethyl(3-methyl-2-butenyl)-1,4-benzoquinone \((X; Y = \text{CH}_3)\) and duroquinone. In conjunction with a study of the stability of plastoquinone \((\alpha)\) on chromatographic adsorbents, it was found to behave like plastoquinone and 2,3-dimethyl-5-phytyl-1,4-benzoquinone\(^2\) in that it was rapidly decomposed on columns of basic alumina. A weighed quantity of the pure quinone (2 mg) in cyclohexane was put onto a column (2 \times 0.7 cm) of basic alumina \((\text{Merk})\). After a time varying from 11 to 15 minutes, the material on the column was rapidly eluted and examined by thin layer chromatography on silica gel \(G\) \((5 \times 20 \times 0.25 \text{ cm})\) with development by a benzene-cyclohexane \((2:1)\) solvent mixture. Each of the yellow bands was scraped off the plate and extracted with ether \((2 \text{ to } 10 \text{ ml})\); the mixture of powder and solvent was centrifuged, and the solution was examined by ultraviolet spectroscopy and, after concentration, by paper chromatography on alumina-impregnated paper.

On the thin layer chromatograms, the first band was in all cases unchanged quinone \((\text{plastoquinone had approximate } R_F \text{ of } 0.8, \lambda_{\text{max}} 254 \text{ mg})\). After 14 minutes on the alumina column, plastoquinone was 80\% destroyed, after 5 minutes it was 97.0\% destroyed, and after 8 minutes it was 97.4\% destroyed. The recovery of starting material \(\text{(in the case of } 2,3\text{-dimethyl-5-phytyl-1,4-benzoquinone) was still only } 18\% \text{ after } 8 \text{ minutes on a column of neutral alumina (Weiln).}

Trimethylquinone and trimethyl(3-methyl-2-butenyl)-1,4-benzoquinone were almost completely stable on columns of a basic alumina for these short times, which were not long enough to effect chromanol formation to any great extent.

**DISCUSSION**

With regard to the scheme proposed by Vilkas and Lederer for involving quinones in oxidative phosphorylation \((12)\), attempts were made with the use of model compounds to find chemical evidence in support of some of their proposed steps.

Their scheme \((\text{Scheme } 1)\) is constructed with reference to coenzyme \(Q\) \((\text{ubiquinone})\) or vitamin \(K_\text{a}\), which are possibly associated with oxidative phosphorylation, but it could also apply to plastoquinone, which may be an intermediate in photophosphorylation in chloroplasts. Plastoquinone has no methyl group on the same side of the nucleus as the isoprenoid side chain, but does have two methyl groups on the opposite side of the quinone nucleus; these can equally well partake in quinone methine formation. Analogues or derivatives of \(\alpha\)-tocopherol \((2,2,5,7,8\text{-pentamethyl-6-chromanyl phosphate (VIII); } R = \text{PO}_4\text{H}_3, R' = \text{CH}_3\), dl-\(\alpha\)-tocopheryl phosphate \((\text{VIII}); R = \text{PO}_4\text{H}_3, R' = \text{CH}_3\text{H}_3\)) and trimethyl(3-methyl-2-butenyl)-1,4-benzoquinone \((X; Y = \text{CH}_3)\) were used as model compounds. They were chemically suitable with regard to Scheme 1 (since they possessed nuclear methyl groups and either 2,3-unsaturation in the isoprenoid side chain or a derived chroman ring), even though \(\alpha\)-tocopherol and its derivatives are not believed to play a role in oxidative or photophosphorylation. Coenzyme \(Q\), containing two methoxyl groups, and vitamin \(K\), a 1,4-naphthoquinone, have lower standard oxidation-reduction potentials than a 1,4-benzoquinone substituted by alkyl groups only, which may make the over-all oxidation of \(IV\) to \(VII\) easier in vitro with their respective chromanol phosphates than with 2,2,5,7,8-pentamethyl-6-chromanol phosphate or a chromanyl phosphate derived from plastoquinone. In biological systems, however, oxidation-reduction potentials of the quinones are lower (that of plastoquinone may be lower owing to sulfhydril addition to the free nuclear position), and this distinction is probably not valid. The specific peculiarities of coenzyme \(Q\), vitamin \(K\), and plastoquinone would not be expected to have a marked effect on quinone methine formation or possible ring opening of their chromanophosphates, compared to model compounds with the nuclear substitution pattern of \(\alpha\)-tocopherol \((\text{VIII}; R = \text{H}, R' = \text{CH}_3\text{H}_3)\). Coenzyme \(Q\) or an analogue would, of course, undergo methoxyl displacement in addition to methine formation in alkaline deuteromethanol.

If a chromanol phosphate \((IV)\) were able to exist in equilibrium with a hydroquinone phosphate \((V)\), it would be possible to detect this equilibrium by looking for deuterium exchange, with a suitably deuterated solvent, in the 3-position \(\text{(marked “b” in Formulas } IV \text{ and } VIII\text{) of the heterocyclic ring. Such a sensitive method was needed, as no success had been obtained in observing hydroquinone phosphate formation under acidic and alkaline conditions by changes in the ultraviolet spectra of dl-\(\alpha\)-tocopheryl phosphate \((\text{VIII}; R = \text{PO}_4\text{H}_3, R' = \text{CH}_3\text{H}_3)\) and 2,2,5,7,8-pentamethyl-6-chromanol phosphate \((\text{VIII}; R = \text{PO}_4\text{H}_3, R' = \text{CH}_3)\). However, observations of the NMR spectrum of 2,2,5,7,8-pentamethyl-6-chromanol phosphate showed no incorporation of deuterium from alkaline \(\text{D}_2\text{O}\) or acidic \(\text{CH}_3\text{OD}\), even with ultraviolet irradiation, and one must conclude that there is no facile formation of hydroquinone phosphate from chromanol phosphate, with or without equilibrium.

It is known \((29)\) that 2,2,5,7,8-pentamethyl-6-chromanol \((\text{VIII}; R = \text{H}, R' = \text{CH}_3)\) can be obtained in good yield from the hydroquinone of the quinone \(IX\) \((R = \text{CH}_3)\) under mild acidic conditions, but even here the reverse reaction could not be detected; no deuterium exchange at position “b” was observed by NMR spectroscopy with the chromanol in acetic or alkaline \(\text{CH}_3\text{OD}\). These observations would indicate that, if chromanol phosphates are to be implicated as intermediates in oxidative phosphorylation, then conversion into the hydroquinone phosphate before oxidative dephosphorylation is very unlikely (although still a possibility in the presence of enzymes), and a direct oxidation of the chromanol phosphate must be considered.

A step similar to that from the chromanol phosphate \((IV)\) to a quinone was in fact demonstrated; the product was a quinone \((IX)\) with a tertiary hydroxyl group in the side chain instead of a quinone \((VI)\) with the 2,3-side chain unsaturation found in plastoquinone, coenzyme \(Q\), and vitamin \(K\). Of interest is the need for the strong oxidizing agent ceric sulfate for this oxidation of chromanol phosphates; other oxidizing agents, bromine in organic solvents \((\text{including acetic acid}),\) ferric chloride, and auric chloride, do not react with the chromanol phosphates, at least not under conditions in which they oxidize hydroquinone phosphates \((14)\). The nature of the product obtained by the action of bromine water on chromanol phosphates is uncertain.

The chemical properties of chromanol phosphates do not appear to support their participation in oxidative or photophosphorylation which is associated with isoprenoid quinones. For coenzyme \(Q\) and plastoquinone, it is difficult to draw any conclusions as to possible mechanisms involving quinone phosphate moieties from available biological evidence, which is lacking at 3 I thank Dr. O. Wiss, Hoffmann La Roche, for kindly providing samples of plastoquinone and 2,3-dimethyl-5-phytyl-1,4-benzoquinone.
the molecular level. Only in the case of the *M. phlei* system (8) is there evidence for association of a chromanol with oxidative phosphorylation: the naphthochromanol phosphate produced in this system from vitamin K₁ was able to undergo enzymatic oxidation with formation of ATP. However, the question of the site of attachment of the phosphate group to the chromanol being at the nuclear methyl group (a structure favored by Wagner et al. (13)) or at the phenolic oxygen atom does not appear to have been settled. It is stated (8) that the NMR spectrum of the biologically produced compound differs from that of the synthetic chroman of vitamin K₁ in the CH₃ region. Also, the isolated bacterial naphthochromanol phosphate could be oxidatively dephosphorylated by bromine in acetic acid (8); with the reservation concerning the lower oxidation reduction potential in *vivo* of naphthoquinones compared to alkylated benzoquinones (although naphthohydroquinone phosphates and hydroquinone phosphates are equally readily oxidized to the corresponding quinones by bromine (14)), this is surprising for a naphthochromanyl O-phosphate in view of the results obtained with the model benzochromanyl 6-phosphates and oxidizing agents.

Another step in the hypothesis of Vilkas and Lederer—the isomerization of the methylquinone to the corresponding quinone methine—should also be detectable (if there is an equilibrium) by deuterium exchange at the methyl group. Under alkaline conditions, with CD₂OD as solvent, exchange at the nuclear methyl groups was rapid with trimethyl(3-methyl-2-butenyl)-1,4-benzoquinone (X; Y = CH₃), and also with tetramethyl-1,4-benzoquinone (duroquinone) in alkaline CH₃OD, indicating that equilibria are established with quinone methine forms. In the case of the isoprenoid quinone, it would appear that ring closure did not take place to any great extent during quinone methine formation under these conditions, since the NMR signals due to the (CH₃)₃-C(CH) protons and the =CH- proton had virtually disappeared owing to exchange. Hence the quinone methine (XII) analogous to the quinone methine (XIII) from duroquinone may be expected to be in equilibrium with trimethyl(3-methyl-2-butenyl)-1,4-benzoquinone under alkaline conditions, rather than the cyclized form represented by VII in Scheme 1. These observations would not, however, preclude the possibility of phosphate addition across the α,β-unsaturated carbonyl system in the quinone methine prior to a cyclization, if a quinone were involved as phosphate acceptor in a biological system (as may be the case in the *M. phlei* system (8)). The ability to cyclize is still a structural requirement in quinones capable of restoring oxidative phosphorylation in an irradiated *M. phlei* system. 2,3-Dihydro vitamin K₁ with a fully saturated side chain is unable to restore phosphorylation (8), and the 2-methyl group on the nucleus is also necessary; the finding (30) of 2-demethyl vitamin K₁ compounds in *Hemophilus parainfluenzae* is interesting.

The reported (31) dimerization (in 15% yield) of vitamin K₁ (II) under acidic conditions (HClO₄ in CH₂Cl₂) is, from consideration of the proposed structure of the dimer, taken as evidence for the formation of a quinone methine (VII) as intermediate. However, an attempt to incorporate deuterium into the nuclear methyl groups of trimethyl(3-methyl-2-butanyl)-1,4-benzoquinone (X; Y = CH₃) in acidic deuteromethanol was unsuccessful (this quinone and solvent were used for case study by NMR; there was no sign of dimerization in this case, however).

That 2,3-dimethyl-1,4-benzoquinones, with a free position and at least one unsaturated isoprenoid unit, undergo rapid chemical change on columns of basic alumina, with almost complete loss of the quinone after 5 to 8 minutes, could only be rationalized if the nature of the products were known. The products are many, however. The extra methyl group, adjacent to the isoprenoid group on the nucleus of a trimethyl isoprenoid quinone, may sterically stabilize the molecule.

**SUMMARY**

The syntheses and properties of a new chromanyl phosphate and its salts, and of three new isoprenoid benzoquinones, are described. Some of these compounds, together with di-al-tocopheryl phosphate, are used as models to investigate the chemical feasibility of steps in a previously proposed mechanism for oxidative phosphorylation. The chemical evidence for possible participation of chromanyl phosphate is meager; their direct oxidation is difficult, and no evidence was found for an equilibrium with a hydroquinone phosphate. Fully alkylated quinones rapidly exchange hydrogen for deuterium at the carbon atom next to the nucleus under basic conditions; observations indicate that trimethyl(3-methyl-2-butenyl)-1,4-benzoquinone takes part in a base-catalyzed equilibrium with a quinone methine, without concurrent ring closure.

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Chemical Studies Concerning the Possible Role of Chromanyl Phosphates and Quinones in Oxidative Phosphorylation

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