The Oxidative Conversion of Hydroquinone Monophosphates to Quinone Ketals*

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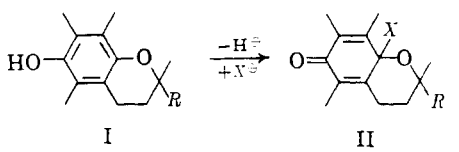
As a result of C—O bond cleavage, the oxidation of hydroquinone monophosphate (or of its dibenzyl ester) with ceric ion in methanol leads to VIII, the dimethyl ketal of *p*-quinone. The yield of *p*-quinone obtained via the alternate pathway, involving P—O bond cleavage and leading to metaphosphate, is less than 5% under these reaction conditions. The 2,6-dibromo derivative of hydroquinone monophosphate is oxidized by *N*-bromosuccinimide in methanol to 2,6-dibromo-*p*-quinone and its dimethyl ketal. In this case, 10–20% of the oxidative reaction proceeds by way of P—O bond cleavage. The effect of ring substituents on the ratio of P—O vs. C—O bond cleavage is examined.

In the preceding paper of this series (Dürckheimer and Cohen, 1964), we reported that the oxidation of a hydroquinone monoether (e.g., α-tocopherol, I) results in the formation of a dienone such as II in the presence of a nucleophile (Martius and Eilingsfeld, 1957; Boyer, 1951). As shown by its high redox potential and instability, such a dienone preserves a significant portion of the free energy expended in the oxidative process. Since one of the goals of the present series of



I II

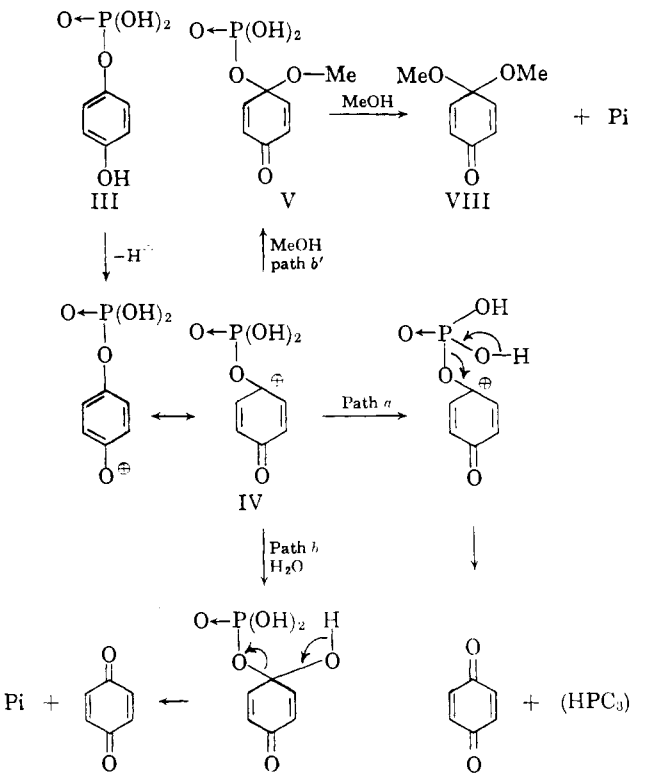
R = $C_{16}H_{33}$; X = OH, $OCOCH_3$, OC_2H_5

Scheme 1

studies is an experimental demonstration of the coupling of oxidation with phosphorylation via a dienone (e.g., II, X = OPO_3H_2), we considered it advisable to first attempt the preparation of a simple analog of II. What was expected to be the most direct synthetic method was the oxidation of hydroquinone monophosphate (III) to the dienone phosphate, V, in the presence of an alcohol.

Although it has been demonstrated (Clark *et al.*, 1961; Wieland and Patterman, 1959; Andrews, 1961) qualitatively that the carbonium ion, IV (as well as some naphthohydroquinone analogs), collapses to *p*-quinone and metaphosphate in aqueous media (path *a*),[1] little or no consideration had been previously given to the alternate sequence (path *b*), from which no metaphosphate is realized. More recently, it has been shown by tracer studies (Lapidot and Samuel, 1962) that *b* is, in fact, the predominant pathway in aqueous media. As is shown in Scheme 3, the nonuseful pathway, *b*, is predominant, and sometimes exclusive, in methanol as well.

The dibenzylphosphate ester of hydroquinone (VI) was prepared from *p*-quinone and dibenzyl phosphite, using triethylamine as base.[2] The possibility of oxidizing VI itself was of interest since it cannot react by path *a*. As a result of the electron-withdrawing nature of the phosphate ester substituent, the oxidation potential of VI is so high that the more common oxidants are ineffective. With tetrachloro-*o*-quinone in methanol or in aqueous acetonitrile, the oxidation of VI is complete only after several days. *N*-Bromosuccinimide, although more effective, may brominate the phenolic ring prior to oxidation. Ceric ion (ceric ammonium nitrate) oxidizes VI instantaneously (cf. Todd, 1962): in aqueous media, the sole products are, as anticipated, quinone and dibenzyl phosphate; in absolute methanol, VI is converted to the quinone ketal, VIII, presumably via the ester, VII.[3] Evidently, the high leaving ability



Scheme 2

* Paper II of a series on Oxidation Mechanisms in Biochemical Processes. For paper I, see Dürckheimer and Cohen (1964).

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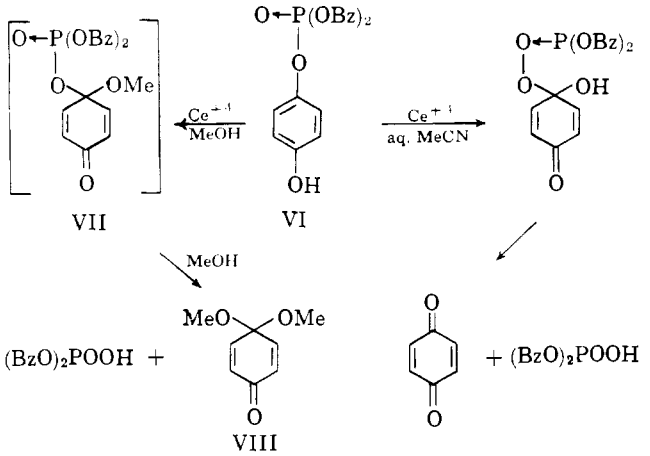
[1] An alternative pathway for phosphorus-oxygen bond cleavage, in polar media, involves the direct attack of the nucleophile on phosphorus without the prior release of metaphosphate. Experimental data do not yet permit a choice of mechanism, nor is any implied herein.

[2] Prepared previously (Clark *et al.*, 1961; Andrews, 1961) with potassium *t*-butoxide as base.

[3] It should be noted that P—O bond cleavage, by nucleophilic attack of methanol on phosphorus, would lead to the formation of *p*-quinone and the triester, methyl dibenzyl phosphate; such a pathway has not been observed in this work.



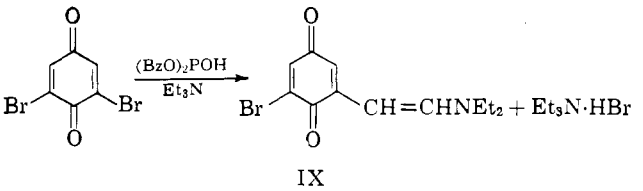
Scheme 3

of the dibenzyl phosphate anion, coupled with the strong resonance stabilization of the remaining carbonium ion, permits a facile solvolysis of VII by methanol. The quinone ketal, VIII (λ_{max}^{EtOH} 213 mμ, ϵ = 9500), was also prepared by oxidation of hydroquinone monomethyl ether with ceric ion in methanol.[4]

The oxidation of hydroquinone monophosphate (III) in aprotic media should proceed exclusively by path *a*, leading to metaphosphate. Indeed, III is oxidized rapidly by ceric ion in anhydrous acetonitrile, presumably with the formation of trimetaphosphate (Clark *et al.*, 1961).[5] Despite the high redox potential of the reagent (E_0 = +1.6 v), no oxidation occurs *ortho* to the phenolic groups, *p*-quinone being the only product detected by ultraviolet spectroscopy and thin-layer chromatography. Under the same reaction conditions, VI is totally inert to attack by ceric ion. With *N*-bromosuccinimide in anhydrous acetonitrile, III is oxidized to *p*-quinone, as well as to 2-bromo- and 2,6-dibromo-*p*-quinone. As in the case of VI, hydroquinone monophosphate is oxidized very slowly by tetrachloro-*o*-quinone in aqueous or alcoholic media. With ceric ion or with *N*-bromosuccinimide in water, III is converted rapidly to *p*-quinone, the ratio of paths *a* and *b* being indeterminable by our techniques. With the latter reagent, oxidation of III apparently proceeds faster than bromination, contrary to the results obtained by oxidation in anhydrous acetonitrile.

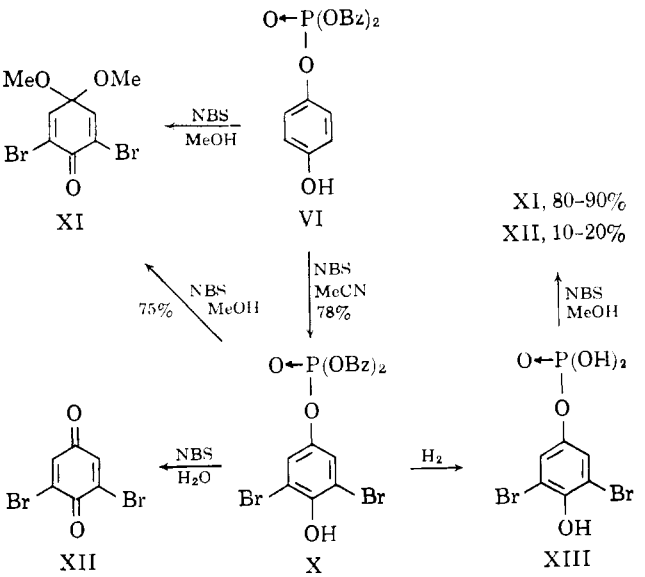
The oxidation of hydroquinone monophosphate in absolute methanol may follow two pathways, *a* and *b'*: path *a* leads to *p*-quinone and metaphosphate; path *b'* leads to the dienone, V, or its solvolysis product, VIII. In this case, the ratio of the pathways may be related to the relative amounts of the two stable and distinct end-products formed, *p*-quinone and the quinone ketal, VIII. In a number of runs with ceric ion as oxidant, the yield of quinone, according to spectroscopic assay, never exceeded 5% while that of VIII was 60–70%. Because the ultraviolet maximum of VIII (at 213 mμ) is often obscured, the dienone content of a sample was checked by the increase in *p*-quinone absorption at 242 mμ after acid hydrolysis. Despite a number of attempts, the dienone, V, could not be detected among the oxidation products (see Experimental). Evidently, the conversion of V to VIII occurs rapidly during the oxidation or the subsequent workup.

Since both the spectroscopic assay and the use of ceric ion were subject to experimental difficulties, we turned to an examination of the dibromo analog of III and VI. The reaction of 2,6-dibromo-*p*-quinone with dibenzyl phosphite led to intensely colored solutions, probably containing substances such as IX (Buckley and Henbest, 1956; Buckley *et al.*, 1957a,b). As noted above, the ring bromination of III with *N*-bromosuccinimide (NBS) could not be achieved without concomitant



Scheme 4

oxidation to the quinone; however, the dibenzyl phosphate, VI, was readily converted into its dibromo



Scheme 5

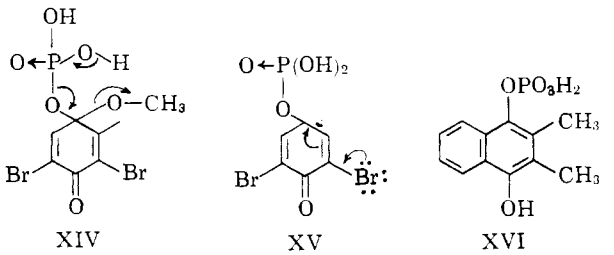
derivative, X, with *N*-bromosuccinimide in acetonitrile. Subsequent oxidation of X with *N*-bromosuccinimide in methanol resulted in the formation of XI, the dibromo analog of VIII. The dibromodienone, XI, is also obtained by the direct reaction of VI with three equivalents of *N*-bromosuccinimide in methanol. In aqueous media, *N*-bromosuccinimide effects the rapid conversion of X into 2,6-dibromo-*p*-quinone (XII). Since the ultraviolet maxima of XI and XII are at 255 mμ and at 288 mμ, respectively, and since both *N*-bromosuccinimide and succinimide have negligible absorption at these wave lengths, direct spectral assay of reaction mixtures was possible.

Selective hydrogenation of the dibenzyl phosphate, X, leads to XIII, the dibromo analog of III. Oxidation of XIII with *N*-bromosuccinimide in acetonitrile or in *t*-butyl alcohol leads to the quinone, XII, and presumably to trimetaphosphate. The hydroquinone monophosphate, XIII, is oxidized by *N*-bromosuccinimide in methanol to a mixture of the quinone ketal (80–90%) and the quinone (10–20%). In methanol containing 10% water, the ketal, XI, is still the major (60%) product. The conversion of XIII to the dibromoquinone, XII, in methanol is accompanied by the formation of metaphosphate, since monomethyl phosphate was formed as shown qualitatively by paper chromatography.[1] An alternative pathway for the formation of the quinone, XII, from XIII may be

[4] Ketals of duroquinone have been prepared (Martius and Eilingsfeld, 1957).

[5] Quantitative aspects of the reaction and its synthetic applicability will be described elsewhere.

visualized by involving release of metaphosphate and methanol from the intermediate, XIV, although the experimental distinction between this route and the direct release of metaphosphate from a carbonium ion intermediate, XV, may be difficult. The higher yield of quinone from XIII than from III *may* be related to the presence of the bromo substituent. If one assumes



XIV XV XVI

Scheme 6

that the extent of formation of quinone, and thus of metaphosphate, is determined by the lifetime of an intermediate carbonium ion (XV), it is evident that substituents which can stabilize a positive charge by resonance, such as bromine, methyl, and methoxyl, should enhance the yield of metaphosphate in methanol, and possibly in water. It is of interest, in this connection, that Lapidot and Samuel (1962) found, by ^{18}O labeling, that XVI is oxidized by the metaphosphate pathway (P-O bond fission) to the extent of 30–35% with bromine water.

Whether the formation of VIII results from a nucleophilic displacement of the phosphate moiety of V or VII by methanol or is preceded by dissociation of the latter species into ion pairs has not yet been determined. We presently favor the ion-pair mechanism, since X could be converted largely and rapidly into XI in the presence of as little as two equivalents of methanol. It is conceivable that dienone phosphates such as V may be more stable in the presence of less reactive alcohols or may be trapped by metal complexing. Further experiments in these directions are in progress.

Experimental[6]

Dibenzyl 4-Hydroxyphenyl Phosphate (VI).—To a solution of 10.8 g (0.1 mole) of *p*-quinone in 250 ml of dry benzene was added 29.0 g (0.11 mole) of dibenzyl phosphite (Aldrich Chemical Co.), followed by 2.5 ml of triethylamine. After one hour at 30–40°, the colorless solution was concentrated to about 80 ml and cyclohexane added until the solution became cloudy. The phosphate crystallized upon cooling, a yield of 33.3 g (90%) being obtained. Following recrystallization from benzene-cyclohexane, 27.0 g (73%) of a product melting at 92.5° was obtained.[7]

Oxidation of VI. A. With tetrachloro-*o*-quinone.—To a solution of 3.7 mg (10 μmoles) of VI in 5 ml of anhydrous acetonitrile (prepared by three distillations from phosphorus pentoxide) was added a solution of 2.5 mg (10 μmoles) of tetrachloro-*o*-quinone in 5 ml of anhydrous acetonitrile. Neither a decrease in absorption at 440 mμ (*o*-quinone band) nor any other change in the ultraviolet or visible spectrum was observed over a period of several weeks. When the same experiment was carried out in methanol as solvent, the peak at 420 mμ decreased slowly; after 3 days, 90% of the absorption had disappeared, the solution having become colorless. In acetonitrile-water (7:3), the oxidant was consumed at approximately the same rate.

B. With *N*-bromosuccinimide.—With two equivalents of *N*-bromosuccinimide in anhydrous acetonitrile, VI was converted selectively to the dibromo derivative, X. The same product was obtained when the reaction was carried out in glacial acetic acid, although bromination proceeded much more slowly. Even in methanol or aqueous acetonitrile, *ortho*-bromination occurred selectively when stoichiometric quantities of oxidant were used. In each case, the course of the reaction was determined by titration of the residual *N*-bromosuccinimide (potassium iodide–thiosulfate), by ultraviolet spectroscopy (persistence of phenolic absorption, absence of dienone or quinone peaks) and by thin-layer chromatography, X being essentially the sole product found. The reaction in acetonitrile was used subsequently as a preparative method for X (see below).

C. With ceric ammonium nitrate.—A solution of 10 μmoles of VI in 5 ml of anhydrous acetonitrile was mixed with a solution of 11.0 mg (20 μmoles) of anhydrous ceric ammonium nitrate[8] in 5 ml of anhydrous acetonitrile. After storage of the mixture for a week at 25°, its ultraviolet spectrum was essentially unchanged; only starting material was detected by thin-layer chromatography. When the same reaction was run in acetonitrile-water (7:3), the yellow color of the ceric ion was discharged immediately, the resulting solution showing a single absorption maximum at 242 mμ (*p*-quinone). *p*-Quinone and dibenzyl phosphate were identified by thin-layer chromatography and were the only products detected.

Oxidation of VI with the ceric salt in methanol also resulted in an immediate discharge of color. After the addition of 0.5 g of sodium bicarbonate (to neutralize the nitric acid liberated as a result of the valence change of cerium), the solution was taken to dryness at 25° and the residue extracted with 10 ml of cyclohexane (spectral grade). The extract showed a peak at 212–215 mμ, with no absorption at longer wave lengths. After dilution of the cyclohexane solution with 4–5 volumes of ethanol and addition of several drops of 2 N sulfuric acid,[9] a new peak gradually appeared at 242 mμ, reaching maximum intensity in 1–2 hours. Thin-layer chromatography revealed the presence of *p*-quinone. The material extracted by cyclohexane was identified as the dimethyl ketal, VIII, by comparison (thin-layer chromatography) with an authentic sample obtained by oxidation of hydroquinone monomethyl ether.

4,4-Dimethoxy-2,5-cyclohexadienone (VIII).—To a stirred solution of 1.24 g (0.01 mole) of hydroquinone monomethyl ether in 25 ml of methanol was added, dropwise, a solution of 11 g (0.02 mole) of ceric ammonium nitrate in 50 ml of methanol. When the reaction was complete, the solution was pale yellow in color. After addition of 5 g of sodium bicarbonate, the solution was taken to dryness at 25° and the residue extracted with 3 × 20 ml of cyclohexane. The cyclohexane solution was concentrated *in vacuo* to a pale yellow oil which was purified by adsorption (from a small quantity of cyclohexane) on 10 g of basic alumina (Woelm, activity 1) and elution with cyclohexane. After removal of the solvent, 1.0 g (65%) of a colorless

[6] Melting points were determined on a Kofler block and are uncorrected. Ultraviolet spectra were determined on a Cary recording spectrophotometer, model 14; infrared spectra were measured on a Perkin-Elmer Infracord; nmr spectra were obtained on a Varian A-60 spectrometer. Microanalyses were performed by Mr. H. G. McCann and his associates of this Institute.

[7] Andrews (1961) reports mp 84–86°.

[8] The commercial anhydrous material was dried *in vacuo* at 25° for 24 hours.

[9] Hydrochloric acid effects partial reduction to a phenolic system.

oil was obtained. The material becomes crystalline in an ice-salt bath but liquefies above 0°; λ_{max}^{EtOH} 213 mμ (ε 9500); $\gamma_{max}^{CCl_4}$ 1695 cm^{-1} (5.90 μ)(s), 1650 cm^{-1} (6.06 μ)(m); nmr (CCl_4): CH_3O, 3.32 ppm, vinyl H quartet, 6.05, 6.23, 6.63, 6.82 ppm.

Anal. Calcd for $C_8H_{10}O_3$: C, 62.32; H, 6.54. Found: C, 62.28; H, 6.47.

Oxidation of III. A. WITH TETRACHLORO-*o*-QUINONE.—As in the case of VI, hydroquinone monophosphate[10] did not react with tetrachloro-*o*-quinone in acetonitrile. In methanol or in aqueous acetonitrile, oxidation is slow, the band at 440 mμ having disappeared after 4 days.

B. WITH *N*-BROMOSUCCINIMIDE.—In anhydrous acetonitrile, oxidation and *ortho*-bromination of III are competitive, since a mixture of *p*-quinone, 2-bromo-*p*-quinone, and 2,6-dibromo-*p*-quinone is obtained, three peaks appearing at 242, 250, and 286 mμ. The reported absorption peak for 2-bromo-*p*-quinone is at 250 mμ (ε 9000) (Steward and Baly, 1906), while 2,6-dibromo-*p*-quinone absorbs at 288 mμ (ε 6850, MeOH). The three quinones are readily separable by thin-layer chromatography; after elution from silica plates, the relative amounts present in a reaction mixture were determined by spectroscopic assay. Using three equivalents of *N*-bromosuccinimide, the reaction mixtures contained approximately 25% *p*-quinone, 25% 2-bromo-*p*-quinone, and 50% 2,6-dibromo-*p*-quinone. Oxidation of III with *N*-bromosuccinimide in aqueous acetonitrile leads only to *p*-quinone, oxidation being faster than bromination in this medium.

C. WITH CERIC AMMONIUM NITRATE.—In aqueous or anhydrous acetonitrile, III rapidly discharges the orange color of ceric ion, *p*-quinone being the only organic product found. The oxidation of hydroquinone monophosphate in methanol was performed as described above for its dibenzyl ester (VI). The methanol solution, after addition of sodium bicarbonate, was evaporated to dryness and extracted with cyclohexane containing 1% ethanol (to ensure complete extraction of *p*-quinone). The cyclohexane extract was diluted, as necessary, with ethanol and examined spectroscopically. On the basis of six runs, the content of *p*-quinone (242 mμ) was found to vary between 0–5%, while that of the quinone ketal (VIII) (213 mμ) was 60–70%. The quinone ketal was converted to *p*-quinone by addition of several drops of 2 N sulfuric acid and storage for several hours at 25°. The increase in absorption at 242 mμ agreed well with that calculated from the dienone content of the sample. The quinone ketal and *p*-quinone were identified by comparison with authentic samples (thin-layer chromatography). The products of acid hydrolysis were negative in molybdate tests for phosphorus. The residue, after the original cyclohexane extraction, was dissolved in N sulfuric acid and examined spectroscopically. Since no *p*-quinone absorption was detected, it was assumed that materials such as the dienone phosphate (V), if originally present, had not survived the working-up procedure.

Dibenzyl 3,5-Dibromo-4-hydroxyphenyl Phosphate (X).—To a solution of 10 g (0.027 mole) of VI in 200 ml of anhydrous acetonitrile was added 11 g (0.062 mole) of *N*-bromosuccinimide and the solution was stored for 2 hours at 25°. After addition of 500 ml of water, an aqueous solution of ascorbic acid was added with shaking until the reaction mixture was almost colorless. The solution was extracted with 3 × 150 ml of benzene and the extract was washed several times with water, dried, and concentrated to 150 ml. Petroleum ether (30–40°) was added until the solution became turbid. The dibromo derivative of VI was obtained as colorless crystals, mp 95–96°, 11.2 g (78%). For analysis, the compound was recrystallized from benzene-petroleum ether without change in melting point.

Anal. Calcd for $C_{20}H_{17}Br_2O_5P$: C, 45.48; H, 3.25; Br, 30.27; P, 5.87. Found: C, 45.47; H, 3.53; Br, 30.26; P, 5.82.

Oxidation of X with N-Bromosuccinimide.—To a solution of 106 mg (0.2 mmole) of X in 4 ml of acetonitrile was added 1 ml of water followed by a solution of 44 mg (0.25 mmole) of *N*-bromosuccinimide in 1 ml of acetonitrile. Oxidation occured quickly and, after 10 minutes, only a trace of starting material could be detected by thin-layer chromatography. The ultraviolet spectrum of the reaction mixture showed a strong peak at 286–287 mμ, due to 2,6-dibromo-*p*-quinone. The mixture was diluted with water and extracted with benzene. The benzene solution was extracted with saturated sodium bicarbonate. Upon acidification of the bicarbonate extract, dibenzyl phosphate separated as a crystalline product, mp 79–80°. The benzene layer contained only 2,6-dibromo-*p*-quinone (thin-layer chromatography).

2,5-Dibromo-4,4-dimethoxy-2,5-cyclohexadienone (XI).—To a solution of 3.18 g (6 mmole) of X in 100 ml of absolute methanol was added 2.16 g (12 mmole) of *N*-bromosuccinimide and the mixture was allowed to stand at 25° for 0.5 hour. After addition of 250 ml of water and 20 ml of saturated sodium bicarbonate solution, the mixture was extracted with 3 × 100 ml of benzene. The benzene extract was dried, concentrated to a small volume, and diluted gradually with petroleum ether (30–40°) until crystallization was complete. The compound was recrystallized from petroleum ether to give 1.34 g (75%) of a colorless product, mp 93–94°; λ_{max}^{MeOH} 255 mμ (ε 7150); $\lambda_{max}^{C_6H_{12}}$ 257 mμ (ε 6780); $\nu_{max}^{CCl_4}$ 1690 cm^{-1} (5.92 μ), 1633 cm^{-1} (6.12 μ). The compound is stable to 2 N sodium hydroxide and may be chromatographed without change on silica gel, but decomposes rapidly to the quinone when treated with 2 N hydrochloric acid.

Anal. Calcd for $C_8H_8O_3Br_2$: C, 30.80; H, 2.58; Br, 51.23. Found: C, 31.43; H, 2.66; Br, 50.64.

The same product was obtained by the reaction of VI with three equivalents of *N*-bromosuccinimide in methanol. When the oxidation of X was carried out in acetonitrile solution containing two equivalents of methanol and an excess of anhydrous sodium bicarbonate, the only products found were the ketal, XI, and a trace of starting material.

3,5-Dibromo-4-hydroxyphenyl Phosphate (XIII).—A solution of 15.2 g of X in 250 ml of absolute methanol, containing 1.5 g of 10% palladium on charcoal, was subjected to hydrogenation. After 14 minutes, hydrogen consumption had reached the value calculated for the removal of two benzyl groups. It is essential to interrupt the hydrogenation at this point since bromine will gradually be removed if the reaction is prolonged. After separation of the catalyst, the solvent was removed *in vacuo*. The residue crystallized on standing and was pressed on a porous plate in order to remove an oily impurity. From time to time, the material was moistened with chloroform, which effected extraction of soluble impurities. The colorless, crystalline material, 6.7 g (67%), melts at 178°. The phosphate is readily soluble in water, alcohol, ethyl acetate, dioxane, and acetone, but not in chloroform or benzene. Recrystallization from a variety of solvent pairs could not be effected. A solution of the crude product in methanol was treated with Norit and evaporated to dryness. The residue was triturated with ben-

[10] Prepared by hydrogenation (Andrews, 1961).

zene, washed several times with the same, and dried *in vacuo*, to give a colorless product, mp 178–180°.

Anal. Calcd for $C_6H_5O_5Br_2P$: Br, 46.0. Found: Br, 45.2.

Oxidation of XIII with N-Bromosuccinimide.—In anhydrous acetonitrile, XIII is oxidized slowly by *N*-bromosuccinimide to 2,6-dibromo-*p*-quinone, 24 hours being sufficient for completion of the reaction. In a mixture of acetonitrile–*t*-butanol (1:1), the reaction follows the same course, but is complete in 3–4 hours.

When XIII was oxidized with 1.5 equivalents of *N*-bromosuccinimide in methanol (dried over Mg), the reaction mixture, after 1.5 hours, showed a main spectral peak at 255 mμ and a minor peak at 285 mμ. Judging from the spectra of the pure quinone ketal, XI, and 2,6-dibromo-*p*-quinone (XII), the mixture contained 80% of the former and 20% of the latter. A portion of the material was analyzed on a preparative thin-layer plate and, after elution of the individual bands with acetonitrile, the relative amounts of XI and XII were again found spectroscopically to be 80:20. In two additional runs, ratios of 89:11 and 87:13 were obtained. In methanol containing 10% water (by volume), the ratio of XI:XII was found to be 59:41. Using the monotriethylammonium salt of XIII, the ratio in absolute methanol was 79:21. The presence of methyl phosphate was demonstrated qualitatively in several of these runs by paper chromatographic comparison (Ukita *et al.*, 1958), with an authentic sample, prepared by methylation of dibenzyl phosphate with diazomethane and catalytic hydrogenolysis of the product.

Thin-layer Chromatography.—All chromatograms were run on silica gel G. Compounds were located by spraying the plates with 50% sulfuric acid, followed by heating at 100° for 15 minutes. With dimethylformamide as solvent, dibenzyl phosphate showed an R_F value of 0.44 on silica gel G. Identification of products was performed by comparison with authentic samples run on the same plates.

TABLE I
R_F VALUES ON SILICA GEL

Compound	Solvent System[a] A	B	C
VI	0.20		
VIII	0.63		
X	0.33		
XI	0.72	0.60	
XII	0.80	0.69	0.48
2-Br-*p*-quinone			0.34
p-Quinone	0.39		0.18

[a] A = ether, B = ether-cyclohexane (4:1), C = benzene.

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Inhibition of *N*-Acetylneuraminic Acid Aldolase by 3-Fluorosialic Acid*

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3-Fluoropyruvic acid was condensed with *N*-acetylglucosamine or *N*-acetylmannosamine in alkaline aqueous solution to give 3-fluorosialic acid. The compound was isolated in a yield of 1.5 and 3.0%, respectively, by chromatography on Dowex-1 and carbon. The molar absorbancy index of the chromophore in the Ehrlich assay was 8175 at 536 mμ. $[\alpha]_D^{20} = -19.2°$ (H_2O 1.1%). Neutralization equivalent, 330. Infrared spectra were nearly identical with *N*-acetylneuraminic acid except in the C-F absorbing area. The compound reduced ferricyanide. The products from *N*-acetylglucosamine and *N*-acetylmannosamine were identical in several chromatographic systems. Both products were competitive inhibitors and inactivators of *N*-acetylneuraminic acid aldolase. The K_I is 2.5×10^{-3} M for both products compared with a K_m of 1×10^{-3} M. Evidence is presented to show that metals and sulfhydryl groups are not involved in the inhibition.

The sialic acids belong to a family of compounds that have the basic nine-carbon-atom structure of neuraminic acid but differ as to the type and extent of substitution. Neuraminic acid may be regarded as a condensation product of pyruvic acid and *N*-acetyl-D-mannosamine (Comb and Roseman, 1960). The sialic acids occur widely in mammalian tissue, principally in mucoids, mucins, and glycoproteins in association with D-galactose or galactosamine (Castellani

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