Nonenzymic Oxidation of *p*-Hydroxyphenylpyruvic Acid with Singlet Oxygen to Homogentisic Acid. A Model for the Action of *p*-Hydroxyphenylpyruvate Hydroxylase[1]

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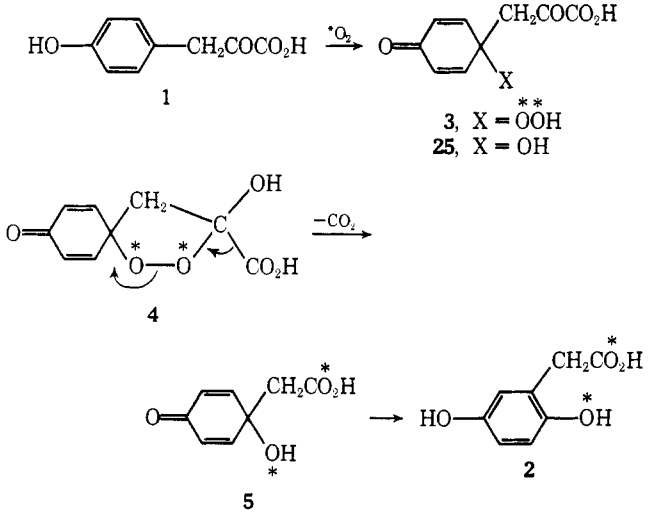
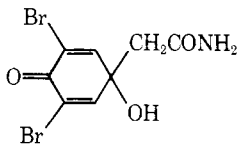
Abstract: The dye-sensitized photooxygenation of *p*-hydroxyphenylpyruvic acid (**1**) and related para-substituted phenols in aqueous media has been investigated. Photooxidation of **1** in its enol form produces *p*-hydroxybenzaldehyde and oxalic acid, whereas **1** in its keto form gives 2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)acetic acid (**5**) which is converted into homogentisic acid at pH 12. The quinol **5** is cyclized reversibly to 1-hydroxy-*cis*-7-oxabicyclo[4.3.0]nona-2-ene-4,8-dione (**13**). The participation of singlet oxygen in the reaction has been shown. Photooxidation of *p*-hydroxyphenylacetic acid and phloretic acid gives the corresponding *p*-quinols, whereas *p*-hydroxyphenethyl alcohol and *N*-acetyltyramine produce the fused bicyclic products **23** and **25**, respectively. The sequence of these reactions is discussed in connection with the mechanism of the action of *p*-hydroxyphenylpyruvate hydroxylase.

The enzyme *p*-hydroxyphenylpyruvate hydroxylase, classified as a monooxygenase,[2] catalyzes the conversion of *p*-hydroxyphenylpyruvate (**1**) into homogentisate (**2**). In this reaction atmospheric oxygen has been shown to be incorporated into both the hydroxy moiety and the carboxyl group of **2**.[3] In order to account for this experimental observation, Lindblad and his coworkers[3] have postulated a mechanism involving a nucleophilic attack of the hydroperoxy group of the intermediate hydroperoxide **3** on the keto group of the side chain. The cyclic peroxide **4** thus formed is converted into the quinol intermediate **5**, which then undergoes migration of the side chain to the ortho position to yield **2** by a mechanism analogous to that of the NIH shift (Scheme I).[4] A similar mechanism was first suggested by Goodwin and Witkop based on the chemical reactions related to the last step.[5] After unsuccessful attempts to synthesize the quinol **5**, they have shown that alkali treatment of 4-carbomethoxymethyl-4-acetoxy-2,5-cyclohexadienone, a derivative of **5**, yielded **2**.[5]

Scheme I



This type of mechanism by which molecular oxygen becomes incorporated via *p*-quinols into aromatic substrates is of particular interest, because it represents an alternate mechanism to one involving an arene oxide intermediate.[4] A quinol intermediate may be potentially involved in the biosynthesis of other natural products.[5-7] Recently, a series of antibiotics including **6**, **7**, and **8** has been isolated from sponges of genus *Verongia* as metabolites of dibromotyrosine,[8,9] and the lactone **7** and hydroquinone **8** are suggested to be derived biogenetically from the quinol **6**.[8c,9]



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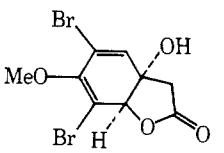

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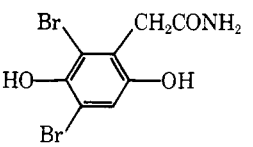

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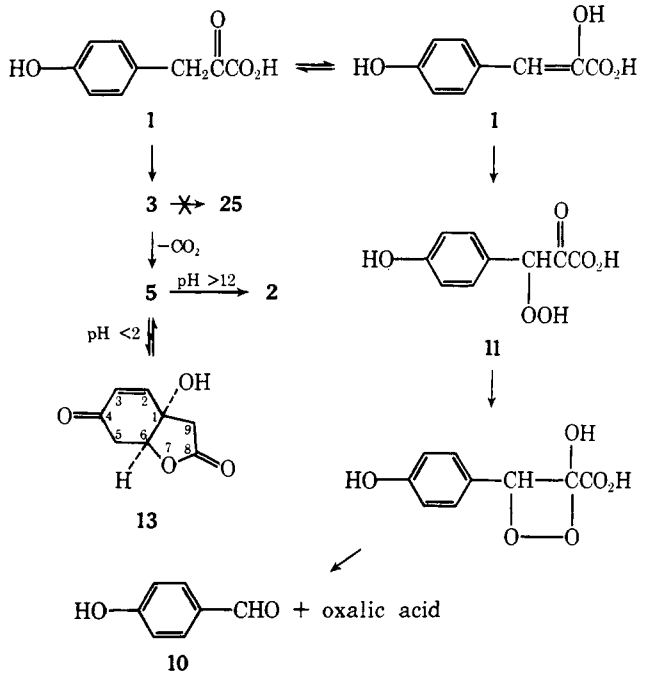
As part of our continuing studies on the reaction of singlet oxygen with aromatic compounds in relation to biological oxygenation,[10] we have investigated the dye-sensitized photooxygenation of **1** and its related phenols, in the hope that an understanding of such model reactions might be of help in the elucidation of the mechanism of the enzymic reaction. The present paper deals with a nonenzymic pathway for the conversion of **1** into **2** via the quinol **5** by oxidation with singlet oxygen. Oxidative cyclizations of some para-substituted phenols via quinols are also described.

Photooxygenation of *p*-Hydroxyphenylpyruvate. Our earlier finding[11] that the dye-sensitized photooxygenation of 3,5-dihalogenophloretic acid leads to the formation of the dienone spirolactone **9** prompted us to investigate the reaction of **1** with singlet oxygen. In the case of **1**, keto–enol tautomerization must be taken into consideration. In freshly prepared buffered solution or in methanol, **1** exists in its enol form, whereas a solution in which the keto form is predominant is obtained by dissolving the crystals (enol form) in an appropriate buffer and then letting the solution stand for 24 hr (see Experimental Section).[12]

Methylene Blue sensitized photooxygenation of the enol form of **1** (0.01 *M* in methanol) resulted in rapid consumption of an equimolar amount of oxygen with the formation

Scheme II



of *p*-hydroxybenzaldehyde (**10**) in 70% yield along with oxalic acid. This type of cleavage reaction has been frequently observed in the photooxygenation of enols and enol ethers.[13] In the absence of sensitizer, **1** was autoxidized only at a negligibly slow rate under the conditions, in contrast to our previous finding that 4-hydroxy-3,5-diiodophenylpyruvic acid undergoes facile autoxidation only in its enol form to yield a corresponding hydroperoxide analogous to **11**.[14]

When **1** (0.01 *M*) was photooxidized with Rose Bengal as a sensitizer in phosphate buffer at pH 7.0 in which the keto form was preponderant (keto-enol ratio 8:2), three products, **5**, **10**, and *p*-hydroxyphenylacetic acid (**12**), were obtained in 18, 12, and 15% yield, respectively, from the complex mixture of products. The structure of quinol **5** was assigned on the basis of spectral data and following chemical reactions. Sodium borohydride reduction of **5** gave **12** in 72% yield. Treatment of **5** with aqueous alkali (pH 12) at room temperature under nitrogen gave **2** in 80% yield. At below pH 2, quinol **5** readily underwent intramolecular conjugate addition to yield a lactone **13** in 85% yield. The lactone **13** showed the following NMR resonances: δ 6.85 (1 H, d of d, J = 10 and 0.8 Hz, C-2 H), 6.00 (1 H, d, J = 10 Hz, C-3 H), 5.40 (1 H, s, OH), 4.85 (1 H, d of t, X of ABX, $J_{AX} = J_{BX}$ = 5 Hz, J = 0.8 Hz, C-6 H), 2.85 (2 H, d of q, AB of ABX, J_{AB} = 16 Hz, $J_{AX} = J_{BX}$ = 5.0 Hz, $\Delta\nu_{AB}$ = 22.2 Hz, $-CH_2-$ at C-5), and 2.90 (2 H, s, $-CH_2-$ at C-9). The long-range coupling between the olefinic C-2 H and C-6 methine proton indicates a W-type relationship for these two protons, suggesting a quasi-equatorial orientation for the C-6 methine proton. Inspection of Dreiding models suggests that the hydroxy and acyloxy groups must be trans; and accordingly, a quasi-diaxial orientation is expected for the hydroxy and acyloxy groups. Above pH 12, **13** as well as **5** was converted into **2** in 85% yield most likely via quinol **5**. Thus, when the photooxygenated mixture was made alkali without further isolation of the intermediate **5** under nitrogen, **2** was obtained in greater than 25% yield based on the reacted keto form (Scheme II).

There is a possibility that *p*-hydroxyphenylacetic acid (**12**) is a precursor of the quinol **5** in the photooxygenation of **1** since under alkaline conditions the photooxygenation of **12** gave **5** in good yield (see below). However, the following control experiments have shown that **12** cannot be a precursor of **5**. (i) The photooxygenation of **1** at slightly acidic pH (acetate buffer pH 6.0) proceeded smoothly to give **5** in 12% yield, whereas under the same conditions **12** was only sluggishly oxidized. (ii) In the photooxygenation of **1** addition of catalase to the reaction system inhibited the formation of **12** but had no significant effect on the yield of **5**. The result also indicates that the formation of **12** in the photooxygenation of **1** presumably results from the reaction of **1** with hydrogen peroxide formed by the secondary decompositions of the intermediate hydroperoxides. The heterogeneous photooxygenation of **1** using Rose Bengal attached to Amberlite IRA-400[15] as a polymer-based sensitizer[16] under the conditions also gave **5** along with **10** and **12**. The results on the photooxygenation of **1** under various conditions are summarized in Table I.

Table I. Product Distribution in Photosensitized Oxygenation of *p*-Hydroxyphenylpyruvate

Reaction conditions	Sensitizer	Keto–enol ratio[a]	Yields of products, %[b] 5	10	12
Phosphate buffer, pH 7.0	Rose Bengal	8:2	18	12	15
Phosphate buffer, pH 7.0, + catalase	Rose Bengal	8:2	15	8	3
Phosphate buffer, pH 7.0	Rose Bengal–Amberlite IRA-400	8:2	20	14	5
Acetate buffer, pH 6.0	Rose Bengal	9:1	12	5	22
Methanol	Methylene Blue	100% enol		70[c]	

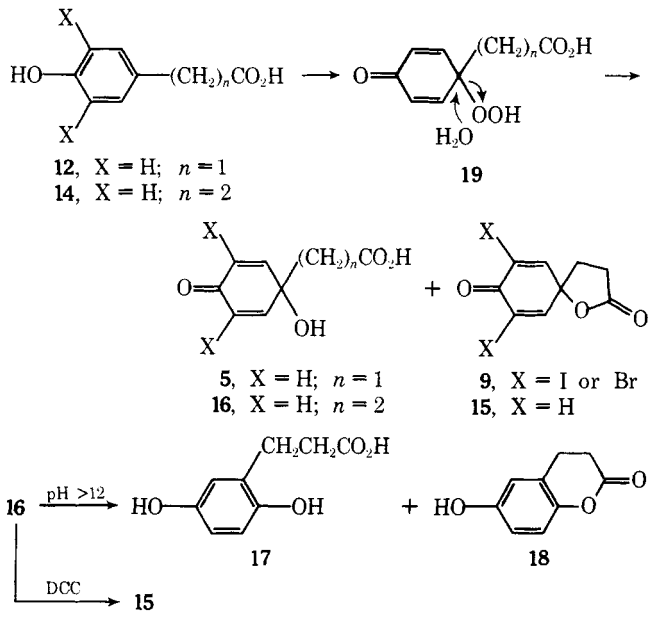
[a] Determined by uv and NMR spectroscopy. [b] Determined by NMR analysis of the ethyl acetate extracts (see Experimental Section). [c] Isolated yield.

Photooxygenation of *p*-Hydroxyphenylacetic Acid and Phloretic Acid. Under more alkaline conditions the dye-sensitized photooxygenation of **12** and phloretic acid (**14**) proceeded at an appreciable rate to give the corresponding *p*-quinols **5** and **16**, respectively. For example, the photooxygenation of **12** (0.05 *M*) in phosphate buffer at pH 8.5 was continued until **12** was completely disappeared. After adjustment to pH 2.5 followed by extraction of the reaction mixture, the crude product was recrystallized from ethyl acetate to yield **5** in 65% yield, thus providing a more convenient method for the preparation of **5**. Under similar conditions, **14** gave the known spirodienone **15**[6,7] and a quinol **16** in 22 and 50% yield, respectively. Treatment of **16** with *N,N'*-dicyclohexylcarbodiimide (DCC) gave **15** in 90% yield. Above pH 12, **16** yielded β-(2,5-dihydroxyphenyl)propionic acid (**17**)[7] and 3,4-dihydro-6-hydroxycoumarin (**18**)[7] in 55 and 25% yield, respectively (Scheme III).

The *p*-quinols **5** and **16** presumably result from the displacement reaction of an initially formed hydroperoxide **19** by water, liberating hydrogen peroxide. The presence of hydrogen peroxide was estimated to be ca. 20% based on reacted **12** by titration of the reaction mixture. The intermediary formation of a hydroperoxide analogous to **19** has been frequently observed in the reaction of phenols with singlet oxygen,[10,17] and the hydroperoxide products are isolable only under mild conditions.[10a,b,17,18]

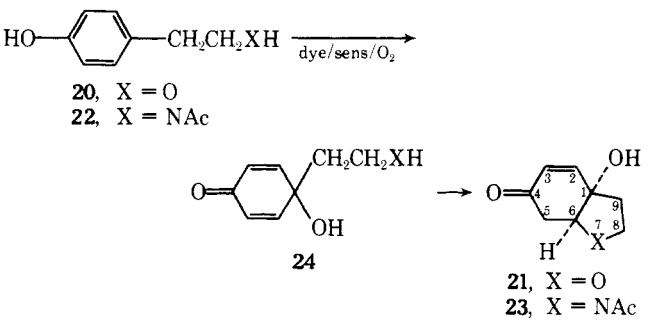
Photooxygenation of *p*-Hydroxyphenethyl Alcohol and *N*-Acetyltyramine. In order to gain insight into the synthetic utility of dye-sensitized photooxygenation in aqueous media for the preparation of *p*-quinols, photooxygenation of other para-substituted phenols was next investigated. Photooxygenation of *p*-hydroxyphenethyl alcohol (**20**) in phosphate buffer at pH 9.0 gave a fused bicyclic product **21** in 51% yield. The 100-MHz NMR spectrum of **21** showed the similar features characteristic to those of **13**, exhibiting a

Scheme III



quartet with additional long-range coupling (1 H, J = 5.6, 5.0, and 1.5 Hz, C-6 H) centered at δ 4.23, a doublet of doublets (1 H, J = 17 and 5.0 Hz, C-5 H) at δ 2.80, and a second doublet of doublets (1 H, J = 17 and 5.6 Hz, C-5 H) at δ 2.57. In addition, a long-range coupling between the olefinic C-2 H and C-6 methine proton was also observed in the NMR spectrum, suggesting a quasi-equatorial orientation for the C-6 methine proton. When the photooxygenation of *N*-acetyltyramine (**22**) in phosphate buffer at pH 8.7 was carried out, a similar bicyclic product **23** was obtained in 47% yield. The products **21** and **23** could result from the intramolecular conjugate addition of the corresponding quinol **24**, respectively (Scheme IV).[19]

Scheme IV



As was mentioned earlier, dye-sensitized photooxygenation in aqueous media provides an efficient and simple method for the synthesis of *p*-quinols with a potentially biological importance. While various efficient methods have been known for the synthesis of *p*-quinols from 2,4,6-trisubstituted phenols,[8a,b,19,20] only a few synthetic methods have been reported for the selective conversion of 4-substituted phenols into *p*-quinols.[21] Peracetic acid[7] and lead tetraacetate[7] oxidation or anodic oxidation[6,7,22] of phenolic acids such as **14** has been reported to give only the corresponding dienone spirolactone **15** in 5–20% yield.

Mechanism of the Oxidation of *p*-Hydroxyphenylpyruvate. The photooxygenation of **1** (keto form) without sensitizer was negligibly slow and neither **5** nor **12** was detectable in the reaction mixture, indicating that a radical autoxidation is not likely to be involved in the reaction. To determine whether the singlet oxygen process is operating in the photooxygenation, we have tested the inhibitory effect of known singlet oxygen quenchers, 1,4-diazabicyclo[2.2.2]octane (DABCO)[23] or sodium azide,[24] on the rate of photooxygenation of **1**. The rate (k_q) of the photooxygenation of **1** (0.01 *M*) in the presence of the quencher was compared with that of the control experiment (k_0). The ratio (k_q/k_0) was 0.52 for 0.05 *M* DABCO and 0.41 for 0.05 *M* sodium azide. As a further indication for the singlet oxygen participation, we have examined the deuterium solvent effect on the reaction. It has previously been shown that approximately tenfold increase in efficiency of photooxidation is expected for a pure singlet oxygen reaction in going from H_2O to D_2O.[25] The rate of disappearance of the keto form determined by monitoring its absorption maximum (276 nm) increased ca. sixfold in going from H_2O to D_2O. Since the photooxygenation of the keto form gives **5** (maximum yield 25%) and **12** (15%) as the only isolable products except polymeric tars, these results indicate that a large portion of the photooxygenation of the keto form leading to **5** is a singlet oxygen mediated reaction. However, the possibility that a radical process involving sensitizer triplet is operating as a minor competing process cannot be excluded rigorously.[10c] Thus, the reaction of singlet oxygen with **1** gives a hydroperoxide **3** as a primary intermediate. The primary step of some of the phenol photosensitized oxygenations has been suggested to be an electron-transfer process from phenols to singlet oxygen, followed by rapid proton transfer.[10c,17a] The phenoxy radicals thus formed combine with hydroperoxy radicals to give 4-hydroperoxy-2,5-cyclohexadienones.

In contrast to **12** and **14** which give the corresponding *p*-quinols, **1** does not yield *p*-quinol **25** in detectable amounts. It only gives **5** upon photooxygenation within a pH range of 6.0–9.0. This suggests that the hydroperoxy group of **3** reacts much faster with the keto group of the side chain giving a cyclic peroxide **4** than with the solvent. Such reactions of hydroperoxides with the carbonyl group of ketones and keto acids, both intramolecular[26] and intermolecular ones,[27] are well established. The rearrangement of **5** to **2** by aqueous alkali has been known for many years as vinylogous acyloin shifts.[5,28]

It has recently been suggested that a 1,4-*endo*-peroxide[29] and quinoids such as **3**, **4**, and **5** are less likely intermediates because of the lack of exchange of the ^{18}O labeled phenolic function of **1** with water during enzymic transformation.[30] However, if one of the hydroxyls of a hydrated quinoid is removed selectively, or if the quinoid intermediates are stabilized by the possible role of a heavy metal, such quinoid intermediates may still exist. In fact, Lindblad et al.[3] have observed that the new hydroxyl at the 2 position of **2** is exchangeable with $H_2{}^{18}O$ and two deuterium atoms are incorporated into the aromatic ring of **2** from D_2O during the enzymic reaction. These observations have been interpreted in terms of quinoid intermediates.[3]

Speculation on the participation of singlet oxygen in enzymic oxidations is current, such participation having been suggested for the action of the dioxygenases quercetinase,[26a,31] soybeam lipoxygenase,[32] horseradish peroxidase,[33] and for lipid peroxidations.[34] These suggestions are based mainly on the principle of identity of products between the enzymic reaction and the reaction using singlet oxygen, and some are highly speculative. Recent experimental evidence does not infer such participation for lipoxygenase[35] and for some lipid peroxidations.[35,36] On the other hand, it has been demonstrated that singlet oxygen is produced by some biosystems such as the adrenodoxin reductase,[37] xanthine oxidase-xanthine,[38] and rat liver microsomes systems.[39] It has also been demonstrated that superoxide ion ($O_2\cdot^-$), a species suggested to participate in some biological oxidations,[34,40] can produce singlet oxygen

by its dismutation reaction[41] and by electron transfer to a radical cation.[42] There has been no factual basis so far to suggest the singlet oxygen participation in the enzymic conversion of **1** into **2**. A metal–oxygen complex bound to a group on the enzyme is speculated to be involved in the enzymic reaction as the reactive species.[3] However, some metal–oxygen complexes have been shown to react with organic substrates in quite similar manner as that of the singlet oxygen reaction.[33,43,44] Aside from the possibility of the involvement of free singlet oxygen in the enzymic system, our results show that the mode of oxidation of **1** catalyzed by the enzyme is quite analogous to that of the singlet oxygen reaction of **1**. In addition, the sequence of the reaction reported here provides a chemical support for the proposed mechanism of the enzymic reaction.

In summary, the present chemical model reactions apparently indicate that **1** in its keto form can react with singlet oxygen to form ultimately **2**, and that **5** is indeed an intermediate in the nonenzymic conversion of **1** into **2**. Although no physiological role has been ascribed to the enzyme phenylpyruvate keto–enol isomerase (E.C. 5.3.2.1) present in the rat liver enzyme system,[3] the results reported here also suggest that the keto form of **1** is likely to be involved in the enzymic oxidation of **1**. Furthermore, the dye-sensitized photooxygenation in aqueous systems provides an efficient and simple method for the synthesis of *p*-quinols and their cyclized products which may potentially serve as intermediates in biosynthetic processes.

Experimental Section

General Procedure. Melting points were determined using a hot-stage apparatus and are uncorrected. The following spectrometers were used: NMR, Varian T-60 or HA-100; ir, Jasco IRA-1; uv, Shimazu UV-200; mass, Hitachi RMS-4. Microanalyses were performed by the Microanalytical Center of Kyoto University. Preparative and analytical TLC work was performed on a plate coated with Merck Kieselgel 60 PF. Irradiation was made with a 500-W tungsten bromine lamp (Ushio JPD-C) surrounded by a Pyrex cooling jacket. During irradiation oxygen was bubbled through the solution in a closed circulating system and the consumption of oxygen was followed manometrically. Methylene Blue (in methanol) and Rose Bengal or Rose Bengal attached to Amberlite IRA-400[15] (in aqueous solution) were used as sensitizers.

Photooxygenation of *p*-Hydroxyphenylpyruvic Acid (1) in Phosphate Buffer (Keto Form). *p*-Hydroxyphenylpyruvic acid (**1**, 360 mg, 2 mmol) was dissolved in 200 ml of 0.1 *M* sodium phosphate buffer under nitrogen bubbling and the pH was adjusted to 7.0 with 2 *N* NaOH. In the freshly prepared solution **1** exists exclusively in its enol form: uv 295 nm (log ϵ 3.72); NMR δ 7.95 (2 H, d, J = 8 Hz), 7.20 (2 H, d, J = 8 Hz), 6.64 (1 H, s). The solution was kept standing for 24 hr under nitrogen, in which the keto form became preponderant (keto–enol ratio 8:2). The keto form showed the following spectroscopic properties: uv 276 nm (log ϵ 3.10); NMR δ 7.58 (2 H, d, J = 7 Hz), 7.24 (2 H, d, J = 7 Hz), 4.40 (2 H, s). The keto–enol ratio in the solution was determined by means of uv and NMR spectroscopy as described previously.[14]

(a) Rose Bengal Sensitized Photooxygenation. To a solution of **1** (360 mg, 2 mmol) in 200 ml of 0.1 *M* phosphate buffer prepared as described above was added 10 mg of Rose Bengal. The solution was irradiated under oxygen bubbling until 70 ml (1.5 equiv mol) of oxygen was consumed (2 hr). The reaction mixture was acidified to pH 3.0, saturated with excess NH_4Cl, and extracted with three 300-ml portions of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous Na_2SO_4 and concentrated to a dark oily residue (334 mg). Preparative TLC separation (benzene–EtOH–acetic acid, 50:5:1) of the residue yielded 24 mg (10%) of *p*-hydroxyphenylbenzaldehyde (**10**, R_f 0.85) and 36 mg (12%) of *p*-hydroxyphenylacetic acid (**12**, R_f 0.72). From the band at R_f 0.40 of the TLC, brown solids were isolated, which on recrystallization from ethyl acetate gave 27 mg (8%) of 2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)acetic acid (**5**): mp 103–104°; uv (EtOH) 220 nm (log ϵ 4.18); ir (Nujol) 3320, 1705, 1670, 1610 cm^{-1}; NMR (acetone-d_6) δ 7.05 (2 H, d, J = 10 Hz), 6.30 (1 H, s, OH), 6.07 (2 H, d, J = 10 Hz), 2.75 (2 H, s); mass spectrum *m/e* 168 (M^+), 140, 124, 97. Anal. Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.33; H, 4.60.

NMR analysis (acetone-d_6) of the crude ethyl acetate extracts showed that it contains unreacted **1** (54 mg, 15%), **5** (60 mg, 18%), **10** (27 mg, 12%), **12** (45 mg, 15%), and polymeric tars (ca. 30%), which were determined by the ratio of the NMR absorptions of the following protons: δ 4.10 ($-CH_2-$ of **1**), 7.70 (aromatic 2 H ortho to aldehyde group of **10**), 3.55 ($-CH_2-$ of **12**), and 2.75 ($-CH_2-$ of **5**).

When the photooxygenation of **1** was carried out in the absence of sensitizer under the same conditions for 2 hr, oxygen consumption was negligible and the starting material was recovered quantitatively.

(b) Conversion of *p*-Hydroxyphenylpyruvic Acid (1) into Homogentisic Acid (2). The photooxygenation of **1** (360 mg, 2 mmol) was carried out under the same conditions as in (a). The reaction mixture was adjusted to pH 12 under nitrogen bubbling and allowed to stand at room temperature for 1 hr. The reaction mixture was acidified to pH 1.0, saturated with excess NH_4Cl, and extracted with three 300-ml portions of ethyl acetate. After drying over anhydrous Na_2SO_4, the extracts were concentrated to a brown viscous residue (270 mg). TLC analysis (benzene–methanol–acetic acid, 50:5:3) of the residue showed a red spot at R_f 0.30 detected by spraying 3-methyl-2-benzothiazolone hydrazone (MBTH)[45] and potassium ferricyanide, which had the same R_f value as that of the authentic homogentisic acid (**2**). Preparative TLC of the residue gave a dark solid (57 mg), which was identical in all respects with an authentic sample of **2**. The yield based on the reacted keto form of **1** was 25%.

(c) Photooxygenation Using Polymer-Based Sensitizer. To a solution of **1** (360 mg, 2 mmol) in 200 ml of 0.1 *M* phosphate buffer at pH 7.0 was added 1.0 g of Rose Bengal–Amberlite IRA-400.[15] The magnetically stirred solution was irradiated under oxygen bubbling until ca. 70 ml (1.5 equiv mol) of oxygen was consumed. Similar work-up of the reaction mixture gave a brown viscous residue (280 mg), which was analyzed by NMR as described in (a). The result is shown in Table I.

(d) Photooxygenation in the Presence of Catalase. To a solution of **1** (360 mg, 2 mmol) in 200 ml of 0.1 *M* phosphate buffer at pH 7.0 was added 10 mg of Rose Bengal and 100 mg of catalase from beef liver (Sigma, Stock No. C-10). The solution was irradiated under the same conditions for 2 hr. Similar work-up of the mixture yielded a brown viscous residue (310 mg), which was analyzed by NMR (Table I).

Photooxygenation of *p*-Hydroxyphenylpyruvic Acid (1) in Acetate Buffer. Under nitrogen bubbling 503 mg (2.8 mmol) of **1** was dissolved in 300 ml of 0.2 *M* sodium acetate buffer. The solution (pH 6.0) was kept standing for 24 hr (keto–enol ratio 9:1). To the solution was added 10 mg of Rose Bengal and the solution was irradiated for 10 hr. During irradiation 57 ml (0.9 equiv mol) of oxygen was consumed. Similar work-up of the reaction mixture gave a viscous solid (314 mg), which was analyzed by NMR (Table I).

Photooxygenation of *p*-Hydroxyphenylpyruvic Acid (1) in Methanol (Enol Form). In methanol **1** exists exclusively in its enol form: uv 287 nm (log ϵ 3.95); NMR (CD_3OD) δ 7.60 (2 H, d, J = 8 Hz), 6.74 (2 H, d, J = 8 Hz), 6.40 (1 H, s). A solution of **1** (360 mg, 2 mmol) in 100 ml of methanol containing 10 mg of Methylene Blue was irradiated under oxygen bubbling for 30 min. After removal of the solvent, preparative TLC separation (benzene–acetic acid–H_2O, 9:1:0.5) of the residue yielded **10** (60 mg, 70% based on the reacted **1**) and unreacted **1** (210 mg). The presence of oxalic acid in the reaction mixture was proven by treatment of the residue with bis(trimethylsilyl)acetamide in acetonitrile followed by GLC analysis (silicone DC 550, 180°).

Photooxygenation of *p*-Hydroxyphenylacetic Acid (12). (a) Preparation of 2-(1-Hydroxyl-4-oxo-2,5-cyclohexadien-1-yl)acetic Acid (5). *p*-Hydroxyphenylacetic acid (**12**, 760 mg, 5 mmol) was dissolved in 200 ml of 0.2 *M* sodium phosphate buffer and the pH was adjusted to 8.5. To the solution 1.0 g of Rose Bengal–Amberlite IRA-400 was added as a sensitizer. The magnetically stirred solution was irradiated under oxygen bubbling until **12** completely disappeared (7 hr). During irradiation 205 ml (1.8 equiv mol) of oxygen was consumed. The reaction mixture was acidified to pH 3.5 and saturated with excess NH_4Cl. The mixture was extracted with

three 300-ml portions of ethyl acetate. The combined extracts were evaporated to give a white solid, which was recrystallized from ethyl acetate yielding 2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)acetic acid (**5**, 260 mg). Further acidification (pH 2) of the reaction mixture followed by repeated extractions with ethyl acetate gave a yellow residue (420 mg), which was dissolved in 100 ml of ethyl acetate. The ethyl acetate solution was extracted with 100 ml of aqueous sodium bicarbonate. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated to a yellow viscous residue, which on recrystallization from ethyl acetate to give 102 mg (12%) of 1-hydroxy-*cis*-7-oxabicyclo[4.3.0]nona-2-ene-4,8-dione (**13**): mp 107–109°; uv (EtOH) 218 nm (log ϵ 4.12); ir (Nujol) 3400, 1780, 1670, 1605 cm^{-1}; mass spectrum *m/e* 168 (M^+), 140, 124, 97. Anal. Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 56.91; H, 4.71. The alkaline extract was acidified to pH 3.0 and extracted with ethyl acetate to give a second crop of **5** (280 mg). The total yield of **5** was 540 mg (65%).

(b) **Rose Bengal Sensitized Photooxygenation.** A solution of **12** (1.0 g, 6.5 mmol) in 200 ml of 0.2 *M* sodium phosphate buffer containing 10 mg of Rose Bengal was adjusted to pH 9.2 with 2 *N* NaOH. The solution was photooxygenated until 105 ml (0.7 equiv mol) of oxygen was consumed (2 hr). Acidification (pH 3.0) followed by similar work-up of the reaction mixture gave a dark yellow residue (850 mg). NMR (acetone-d_6) of the residue showed that it consists of 430 mg (2.8 mmol) of unreacted starting material **12** and 260 mg (1.5 mmol, 41% based on reacted **12**) of **5**. Iodometric titration of the crude reaction mixture indicated that it contains 0.85 mmol (23% based on reacted **12**) of peroxidic products. On similar titration of the crude reaction mixture in the presence of catalase (1 mg, Sigma Stock No. C-10), 0.11 mmol (3%) of peroxidic products was detected. Thus, approximately 20% (based on reacted **12**) of hydrogen peroxide is estimated to exist in the crude reaction mixture.

When the photooxygenation of **12** was carried out in the absence of Rose Bengal under the same conditions, starting material **12** was recovered quantitatively.

(c) **Photooxygenation in Acetate Buffer.** A solution of **12** (1.0 g, 6.5 mmol) in 200 ml of 0.2 *M* sodium acetate buffer at pH 6.0 containing 10 mg of Rose Bengal was photooxygenated for 7 hr. During irradiation 71 ml (0.25 equiv mol) of oxygen was consumed. A similar work-up as described in (a) and subsequent NMR analysis of the residue showed that it contains largely starting material **12** (620 mg) along with polymeric tars (ca. 200 mg) and a small amount of **5** (5 mg).

Reduction of 2-(1-Hydroxy-4-oxo-2,5-cyclohexadien-1-yl)acetic Acid (5). To a solution of **5** (30 mg, 0.18 mmol) in 4 ml of aqueous ethanol was added 25 mg of $NaBH_4$. After stirring for 1 hr, the solution was acidified to pH 2 and saturated with NH_4Cl. Extraction with ethyl acetate gave a white solid, which was recrystallized from methanol to give **12** (20 mg, 72%).

Rearrangement of 2-(1-Hydroxy-4-oxo-2,5-cyclohexadien-1-yl)acetic Acid (5) and 1-Hydroxy-*cis*-7-oxabicyclo[4.3.0]nona-2-ene-4,8-dione (13). (a) With Aqueous Alkali. A solution of **5** (42 mg, 0.25 mmol) in 10 ml of aqueous NaOH (pH 12) was stirred for 1 hr under nitrogen bubbling. The resulting yellow solution was acidified to pH 2, saturated with excess NH_4Cl, and extracted repeatedly with 20-ml portions of ethyl acetate. Evaporation of ethyl acetate yielded a dark brown solid (33 mg, 80%), whose ir spectrum was superimposable with that of the authentic homogentisic acid (**2**).

Similar treatment of **13** (42 mg, 0.25 mmol) with 10 ml of aqueous alkali (pH 12) followed by the same work-up gave 36 mg (85%) of **2**.

(b) **With Acid.** A solution of **5** (42 mg, 0.25 mmol) in 10 ml of water was acidified to pH 1 with 6 *N* HCl. After saturation with NH_4Cl, the solution was extracted with ethyl acetate. Evaporation of ethyl acetate gave a white solid which on recrystallization from ethyl acetate yielded **13** (36 mg, 86%).

Photooxygenation of Phloretic Acid (14). A solution of phloretic acid (**14**, 5.0 g, 30 mmol) in 300 ml of 0.2 *M* sodium phosphate buffer containing 20 mg of Rose Bengal at pH 8.5 was photooxygenated until 692 ml (1.2 equiv mol) of oxygen was consumed. After acidification (pH 2) and saturation with NH_4Cl, the reaction mixture was extracted with ethyl acetate. The extracts were chromatographed on a silica gel column (50 g). Elution with $CHCl_3$–EtOH (98:2) gave a white solid which on recrystallization from ethyl acetate yielded 1-oxaspiro[5.4]deca-6,9-diene-2,8-dione (**15**, 110 mg, 22%), mp 107–108°, which was identical wth the authentic sample prepared by the known method.[7] Further elution with $CHCl_3$–EtOH (95:5) gave a white solid which was recrystallized from ethyl acetate yielding 3-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)propionic acid (**16**): mp 110–112°; uv (EtOH) 226 nm (log ϵ 4.07); ir (Nujol) 3240, 1710, 1665, 1620 cm^{-1}; NMR ($CDCl_3$) δ 6.97 (2 H, d, J = 10 Hz), 6.10 (2 H, d, J = 10 Hz), 5.20 (1 H, br s, OH), 2.30–1.90 (4 H, m); mass spectrum *m/e* 164 ($M^+ - 18$). Anal. Calcd for $C_9H_{10}O_4$: C, 59.33; H, 5.53. Found: C, 59.23; H, 5.52.

Reaction of 3-(1-Hydroxy-4-oxo-2,5-cyclohexadien-1-yl)propionic Acid (16). (a) Dehydration. A solution of **16** (85 mg, 0.48 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC, 186 mg, 0.96 mmol) in 50 ml of dry CCl_4 was refluxed for 5 hr. Insoluble precipitates were removed by filtration and the filtrate was concentrated in vacuo to 30 ml, which on cooling deposited **15** (70 mg, 90%) as white crystals.

(b) **With Aqueous Alkali.** A solution of **16** (150 mg, 0.87 mmol) in 50 ml of 0.2 *N* NaOH was stirred for 2 hr under nitrogen bubbling. The resulting yellow solution was acidified to pH 2, saturated with NH_4Cl, and extracted with ethyl acetate. Evaporation of ethyl acetate gave a viscous oil, which on preparative TLC (ethyl acetate–acetic acid–EtOH, 50:1:1) yielded 3,4-dihydro-6-hydroxycoumarin (**18**, 39 mg, 25%, R_f 0.72) and β-(2,5-dihydroxyphenyl)propionic acid (**17**, 87 mg, 55%, R_f 0.85). These products were identical with the authentic samples prepared according to the known method,[7] respectively.

Photooxygenation of *p*-Hydroxyphenethyl Alcohol (20). A solution of *p*-hydroxyphenethyl alcohol (**20**, 1.02 g, 7.41 mmol) in 260 ml of 0.1 *M* sodium phosphate buffer (pH 9.0) containing 20 mg of Rose Bengal was irradiated under oxygen bubbling until 200 ml (1.2 equiv mol) of oxygen was consumed (13 hr). The reaction mixture was acidified to pH 5.0, saturated with NH_4Cl, and extracted with 500 ml of ethyl acetate. The extracts were purified by preparative TLC (ethyl acetate–EtOH, 10:1) to give a yellow residue. Distillation of the residue gave 580 mg (51%) of 1-hydroxy-*cis*-7-oxabicyclo[4.3.0]nona-2-en-4-one (**21**) as a colorless liquid: bp 105° (1 mmHg); uv (EtOH) 209 nm (log ϵ 4.19); ir (Neat) 3400, 1680, 1070 cm^{-1}; NMR ($CDCl_3$) δ 6.76 (1 H, d of d, J = 11, 1.5 Hz, C-2 H), 5.98 (1 H, d, J = 11 Hz, C-3 H), 4.23 (1 H, q of d, J = 5.6, 5.0, 1.5 Hz, C-6 H), 3.98 (2 H, m, $-CH_2-$ at C-8), 2.80 (1 H, d of d, J = 17, 5.0 Hz, C-5 H), 2.57 (1 H, d of d, J = 17, 5.6 Hz, C-5 H), 2.26 (2 H, m, $-CH_2-$ at C-9); mass spectrum *m/e* 154 (M^+), 136, 110, 82. Anal. Calcd for $C_8H_{10}O_3$: C, 62.32; H, 6.54. Found: C, 62.16; H, 6.45.

Photooxygenation of *N*-Acetyltyramine (22). A solution of *N*-acetyltyramine (**22**, 758 mg, 4.24 mmol) in 260 ml of 0.1 *M* sodium phosphate buffer (pH 8.7) containing 20 mg of Rose Bengal was irradiated under oxygen bubbling until 145 ml (1.5 equiv mol) of oxygen was consumed (6 hr). The reaction mixture was acidified to pH 2.5, saturated with excess NH_4Cl, and extracted with three 300-ml portions of ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4 and concentrated to a viscous residue (660 mg). Preparative TLC (ethylacetate–EtOH, 2:1) of the residue yielded a brown solid which on recrystallization from ethyl acetate–acetone gave 388 mg (47%) of 7-acetyl-1-hydroxy-*cis*-7-azabicyclo[4.3.0]nona-2-en-4-one (**23**): mp 171–172°; ir (Nujol) 3300, 1680, 1595 cm^{-1}; uv (EtOH) 207 nm (log ϵ 4.13), 313 (1.99); NMR (CD_3OD) δ 6.92 (1 H, d of d, J = 11, 1.5 Hz, C-2 H), 5.98 (1 H, d, J = 11 Hz, C-3 H), 4.22 (1 H, q of d, J = 8, 5, 1.5 Hz, C-6 H), 3.80 (2 H, m, $-CH_2-$ at C-8), 2.85 (1 H, d of d, J = 18, 5 Hz, C-5 H), 1.95–2.50 (3 H, m, C-5 and $-CH_2-$ at C-9), 2.05 (3 H, s, $-NCOCH_3$); mass spectrum *m/e* 195 (M^+), 178, 153. Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.28; H, 6.70; N, 7.09.

Experimental Tests for the Singlet Oxygen Participation in the Photooxygenation of *p*-Hydroxyphenylpyruvic Acid (1). (a) Inhibition by Singlet Oxygen Quencher. The rate (k_q) of Rose Bengal sensitized photooxygenation of **1** (2 mmol) in 250 ml of 0.1 *M* sodium phosphate buffer (pH 7.0) in the presence of NaN_3 (10 mmol) or 1,4-diazabicyclo[2.2.0]octane (DABCO) (10 mmol) was compared with that of the control experiment (k_0). Under a fixed set of conditions, the rate of photooxygenation of **1** was followed by measuring the oxygen uptake periodically at 25°. The ratio k_q/k_0 was 0.41 for NaN_3 and 0.52 for DABCO.

(b) **Deuterium Solvent Effects.** Two solutions containing **1** (2.5×10^{-5} *M*), Na_2HPO_4 (4×10^{-5} *M*), and Rose Bengal (3×10^{-6} *M*) were prepared in H_2O (pH 7.0) and D_2O (pD 7.0). The solutions were placed in 10-mm Pyrex test tubes and kept standing for 24 hr under nitrogen atmosphere. A slow stream of pure oxygen was passed through the solutions and the test tubes were sealed with jointed stoppers. The samples were irradiated simultaneously in the "merry-go-round" apparatus immersed in water kept at 25°. The rate of photooxygenation was determined by monitoring the decrease of the absorption maximum of the keto form of **1** (276 nm). The value k_{D_2O}/k_{H_2O} was 6.2.

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