BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 45, 2866—2871 (1972)

Model Reactions for the Biosynthesis of Thyroxine. XIX. The Formation of a Thyroxine Precursor by Photooxidation of 4-Hydroxy-3,5-diiodophenylpyruvic Acid in Organic Solvents[1-3]

Kanji OMURA, Akira NISHINAGA, and Teruo MATSUURA

Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto

(Received January 20, 1972)

Photooxidation of 4-hydroxy-3,5-diiodophenylpyruvic acid (I) in methanol was found to yield a hydroperoxide (IV). This hydroperoxide reacted anaerobically with 3,5-diiodotyrosine (II) to form thyroxine (III). A photoproduct, presumably IV, apparently accelerated the oxidation of I. Methanol, among several organic solvents, was found to be the best solvent for the photooxidation. Similar oxidations with 4-hydroxy-3-iodo-5-methoxyphenylpyruvic acid (V) and 4-hydroxyphenylpyruvic acid (VI) showed that the rate of oxidation decreased in the order I>V>VI. A product of the photooxidation of I, probably the hydroperoxide IV, also acts as an accelerator in the photooxidation of the desiodo analog VI of I.

A nonenzymic model reaction for the biosynthesis of thyroxine was reported by Meltzer and Stanaback,[4] who showed that autoxidation (pH 7.5) of 4-hydroxy-3,5-diiodophenylpyruvic acid (I) in the presence of 3,5-diiodotyrosine (II) yields thyroxine (III) in moderate yield. Nishinage *et al.*[5] have demonstrated that the reaction takes place in two distinct phases: the enolic form of I is first autoxidized to a hydroperoxide (IV), which then reacts with II to form III in an over-all yield of up to ~40%. Oxygen was found to be required only in the first step, but not in the second.[5]

Structural requirements for the autoxidation of I in aqueous media were found to be the existence of I in its enolic form and the dissociation of the phenolic proton. A solution of I in an organic solvent does not consume oxygen.[6] In organic solvents I exists in its enolic form,[5] but the phenolic hydroxyl is undissociated. This observation prompted us to investigate the photooxidation of I by oxygen in organic solvents. This paper also includes some results obtained in similar photooxidations (sensitized or unsensitized) of two analogs of I, *viz.* 4-hydroxy-3-iodo-5-methoxyphenyl-

1) Part XVIII: A. Nishinaga and H. J. Cahnmann, to be published.

2) Regarded as Part LV in the series of Photoinduced Reactions. Part LIV: T. Nagamachi, A. Nishinaga, and T. Matsuura, *Chem. Lett.*, **1972**, 111.

3) A preliminary communication was published in *Chem. Commun.*, **1969**, 366.

4) R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.*, **26**, 1977 (1961).

5) A Nishinaga, H. J. Cahnmann, H. Kon, and T. Matsuura, *Biochemistry*, **7**, 388 (1968).

6) Unpublished data,

pyruvic acid (V) and 4-hydroxyphenylpyruvic acid (VI).

Results and Discussion

Photooxidation of I. A solution of I in methanol was irradiated at ~5°C with a 100 W high-pressure mercury lamp of Pyrex housing. Oxygen was bubbled through the solution during the irradiation until the absorption of oxygen was nearly quantitative (0.93 mol/mol of I). The solution contained the hydroperoxide IV as shown by the isolation of the diol acid VII after reduction of the photolysate with sodium borohydride. The yield of VII was 57% (based on I). The identity of VII was confirmed by direct comparison with the previously described diol acid VII.[5] Treatment of the photolysate with 3,5-diiodotyrosine (II), described previously,[5] gave thyroxine (III) in 18.6% yield (also based on I). Treatment of a methanolic solution of I with oxygen in the dark, followed by treatment with II yielded no thyroxine (III). It can therefore be concluded that dissociation of the phenolic proton of I, a prerequisite for the autoxidation in aqueous media (pH 7.5),[5] is not required for the photooxidation in methanol.

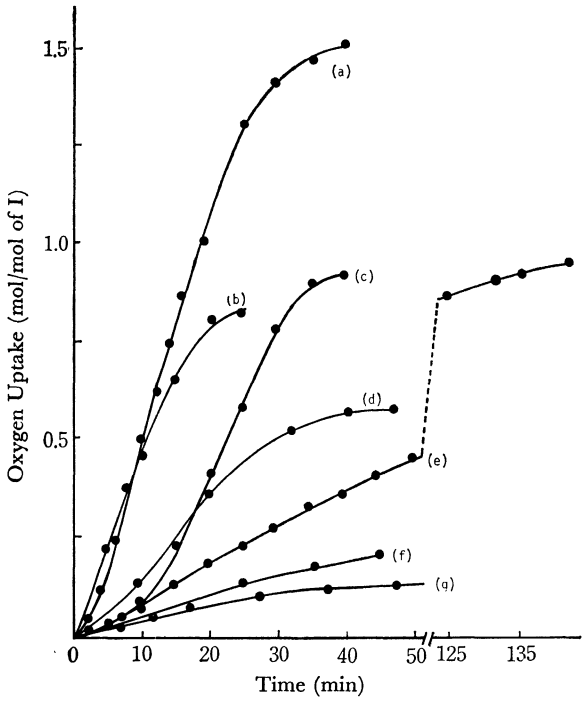


Fig. 1. Uptake of oxygen during photolysis (>2800 Å) of I in various solvents at ~5°C (see footnote a in Table 1). (a) Tetrahydrofuran, (b) acetone, (c) methanol, (d) acetonitrile–methanol (8:1), (e) pyridine, (f) 0.1 M boric acid and 1.1 mM rose bengal, (g) 0.2 M boric acid (pH 4.5).

Table 1. Photooxidation of I and formation of thyroxine (III)[a]

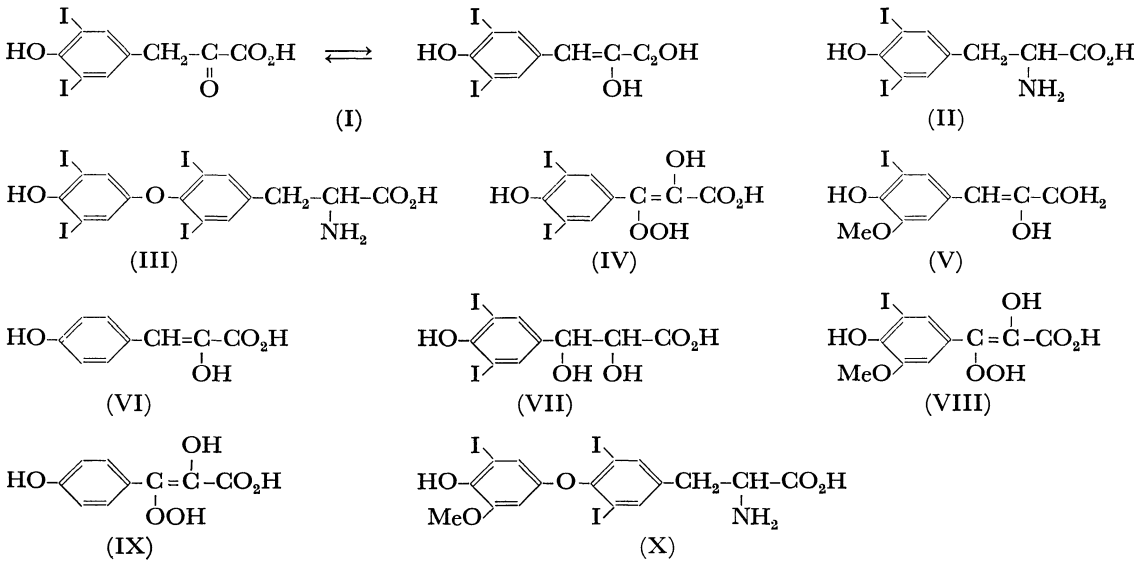
Exp. No.	Solvent	Irradiation time (min)	Oxygen uptake (mol/mol of I)	Yield of III (%)
1	MeOH	40	0.93	18.6
2	MeOH[b]	45	0.88	10.5
3	H_3BO_3 in MeOH (0.2M)	50	1.08	18.6
4	Acetone	25	0.84	11
5	CH_3CN–MeOH (8: 1)	47	0.59	11
6	Tetrahydrofuran	40	1.57	3
7	Pyridine	150	0.98	0
8	0.2M Borate buffer, pH 4.5	60	0.14	—[c]
9	Same+1.1 mM rose bengal	45	0.23	1.1

a) A solution of I (2.3 mmol) in a solvent (100 ml) was irradiated at ~5°C with light above 2800 Å under oxygen-bubbling.
b) Irradiated at 22°C.
c) Not determined.

The photochemical conversion of I to IV is an additional example of the resemblance betweeen certain photochemical and biological oxidations.[7] It is similar to the photooxidation of other enolic compounds.[7b,8] Moreover, it provides a new synthetic route to thyroxine.

Solvent Effect on the Photooxidation of I. The effect of solvents on the oxygen consumption of I under irradiation and on the formation of III after treatment of the photolysate with II are shown in Fig. 1 and Table 1. The best yield of III was obtained in methanol at ~5°C (Exp. 1). At room temperature the yield was decreased (Exp. 2). A similar decrease in yield with increased temperature had been observed previously in the autoxidation in an aqueous medium at pH 7.4.[6] It should be noted that the photooxidation in methanol is apparently an auto-catalytic reaction, since the maximam rate was reached only after about 10—15 min (Fig. 1). A similar phenomenon was also observed in the autoxidation of I in an aqueous medium,[5] where a radical chain reaction is believed to occur. In the case of the photooxidation, however, it probably can be attributed to an accelerating effect of the reaction product (IV) (see below). In acetone the rate of the reaction, without any induction period, was higher than with methanol. Acetone, however, seems to promote also the decomposition of the reaction product (IV), as suggested by the lower yield of III (Exp. 4). In tetrahydrofuran induced oxidation of the solvent appears to occur, since the yield of III was low in spite of the absorption of an excess of oxygen (Exp. 6). The slower rate of oxidation in pyridine can be attributed to the absorption of the incident light by the solvent (Exp. 7). It must be assumed that even in this case, the thermally[5] and presumably photochemically unstable hydroperoxide IV is formed, but then destroyed during the long period of irradiation. Consequently, no thyroxine (III) could be isolated in this case after treatment of the reaction mixture with

7) a) T. Matsuura, A. Nishinaga, N. Yoshimura, T. Arai, K. Omura, H. Matsushima, S. Kato, and I. Saito, *Tetrahedron Lett.*, **1969**, 1673; b) T. Matsuura, H. Matsushima, and H. Sakamoto, *J. Amer. Chem. Soc.*, **89**, 6370 (1967); c) T. Matsuura, H. Matsushima, and R. Nakashima, *Tetrahedron*, **26**, 435 (1970); d) T. Matsuura, and H. Matsushima, *ibid.*, **24**, 6615 (1968); e) A. C. Waiss, Jr., R. E. Lundin, A. Lee, and J. Corse, *J. Amer. Chem. Soc.*, **89**, 6213 (1967); f) J. E. Baldwin, H. H. Basson, and H. Krauss, Jr., *Chem. Commun.*, **1968**, 984; and references cited therein.
8) R. H. Young and H. Hart, *ibid.*, **1967**, 827.



diiodotyrosine (II). Oxygen was hardly absorbed in boric acid solution (pH 4.5) on irradiation (Exp. 8), while facile absorption was observed in the autoxidation of I in boric acid–NaOH (pH 7.5).[5] The addition of boric acid to methanol did not alter the extent of oxygen uptake or the yield of thyroxine (III) (Exp. 3).

Accelerating Effect of the Hydroperoxide IV in Methanol. As mentioned above, the conversion of I into IV in methanol is slow at first, but becomes faster with time. A possible explanation for this is a photochemically initiated radical-chain reaction, since similar phenomena can often be found in many autoxidation reactions, including that of I itself,[5] which are believed to proceed *via* a radical-chain mechanism.

An intermittent irradiation experiment seemed, however, to exclude this possibility, since no oxygen uptake was observed during the interruption of irradiation (Fig. 2). Upon resumption of the irradiation the rate of oxygen uptake became the same value which had been reached prior to the interruption of the irradiation. An alternative explanation for the sigmoid shape of the oxygen uptake curve would be an accelerating effect exerted by the photoproduct, presumably the hydroperoxide IV or by one of its degradation products. This possibility was supported by a photooxidation experiment with I in the presence of the hydroperoxide IV which was supplied in the form of a freshly photooxygenated solution of I (Fig. 3). In

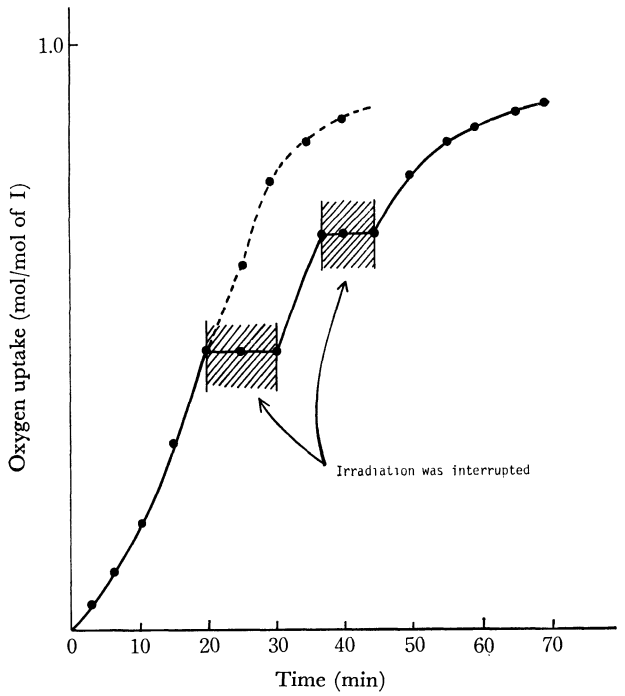


Fig. 2. Intermittent irradiation (>2800 Å) of I (2.3 mmol) in methanol (90 m*l*) at 25°C. ----Intermittent irradiation, ——uninterrupted irradiation.

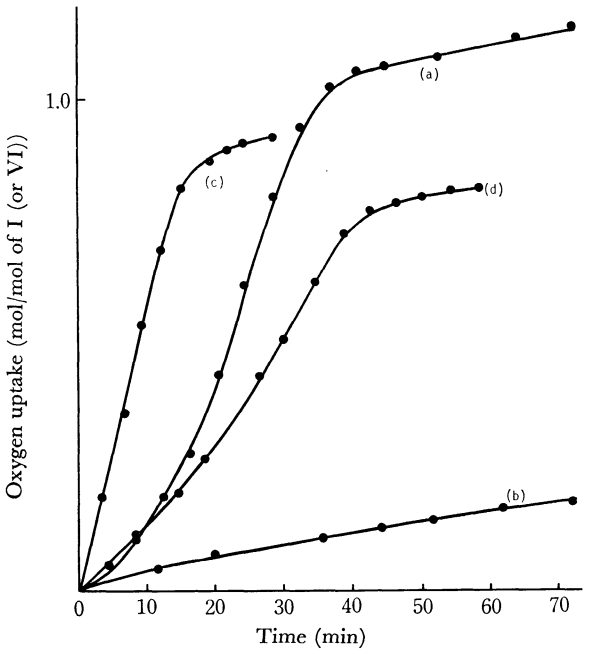


Fig. 3. Effect of the photooxidation product of I on the oxygen uptake of I (1.16 mmol) or VI (1.16 mmol) on irradiation (>2800 Å) in methanol (100 m*l*) at ~2°C. (a) I alone, (b) VI alone, (c) I added to a freshly photooxygenated solution (oxygen uptake 100%) of I (1.16 mmol), (d) VI added to a freshly photooxygenated solution (oxygen uptake 100%) of I (1.16 mmol).

this case the rate of oxygen uptake was maximal even at the initial stage of the reaction. The accelerating action of a photooxygenated solution of I is even more strikingly illustrated by the acceleration of the photooxidation of the desiodo analog of I, 4-hydroxyphenylpyruvic acid (VI), which by itself undergoes only very slow photooxidation (Fig. 3, see also below). The hydroperoxide IV is the main product (~60%) of the oxidation of I, and neither 4-hydroxy-3,5-diiodobenzaldehyde nor 2,6-diiodobenzoquinone, known as degradation products of IV,[5] had an appreciable accelerating effect.

The mechanism of the acceleration remains to be elucidated. The generation of singlet oxygen by the interaction of the excited IV with triplet oxygen[9] seems improbable since the photooxidation of 2,5-dimethylfuran was insufficiently sensitized by the hydroperoxide IV, and addition of a small amount of rose bengal, a typical sensitizer for generating singlet oxygen, had little effect on the photooxidation of I.

Sensitized and Unsensitized Photooxidation of V and VI. Photooxidations were also carried out with 4-hydroxy-3-iodo-5-methoxyphenylpyruvic acid (V) and 4-hydroxyphenylpyruvic acid (VI) in methanol in the presence or in the absence of a sensitizer. As shown in Fig. 4, the unsensitized oxygenation of V was slower than that of I. The slowest absorption of oxygen was observed in the case of the desiodo analog VI. The photooxidation of these acids was faster in acetone than in methanol, but the order of the reaction rates was again I>V>VI. Rose bengal was also found to accelerate the photooxidation of V and VI (Figs. 5 and 6). In each case the rate of oxygen consumption increased with increased sensitizer concentration. It

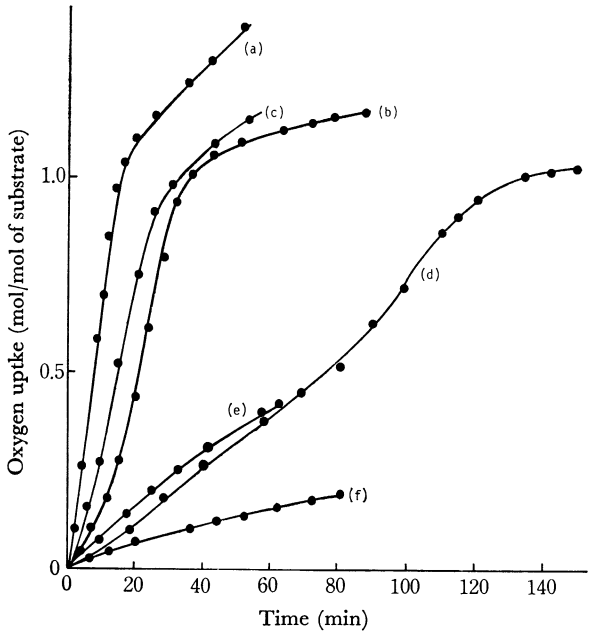


Fig. 4. Oxygen uptake by irradiation (>2800 Å) of I, V and VI (1.16 mmol, respectively) in methanol or acetone (90 m*l*) at ~2°C. (a) I in acetone, (b) I in methanol, (c) V in acetone, (d) V in methanol, (e) VI in acetone, (f) VI in methanol.

9) C. S. Foote, *Accounts Chem. Res.*, **1**, 104 (1968).

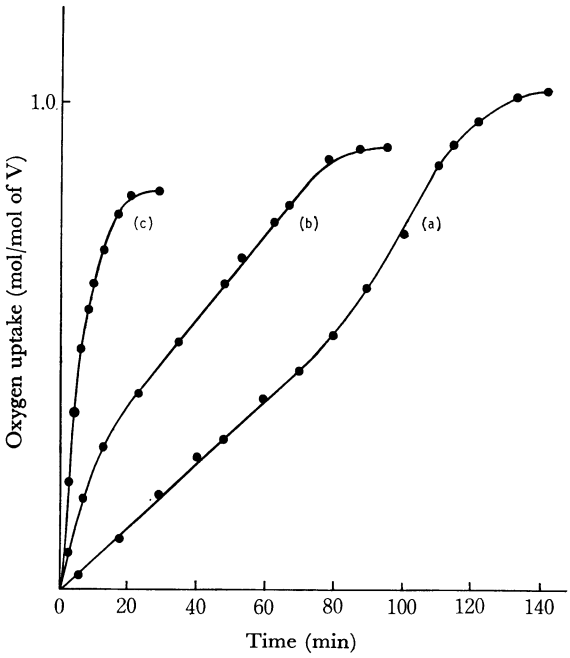


Fig. 5. Oxygen uptake by irradiation (>2800 Å) of V (1.16 mmol) in the presence of various amounts of rose bengal in methanol (90 m*l*) at ~2°C. (a) Without rose bengal, (b) with rose bengal (0.032 mmol), (c) with rose bengal (0.32 mmol).

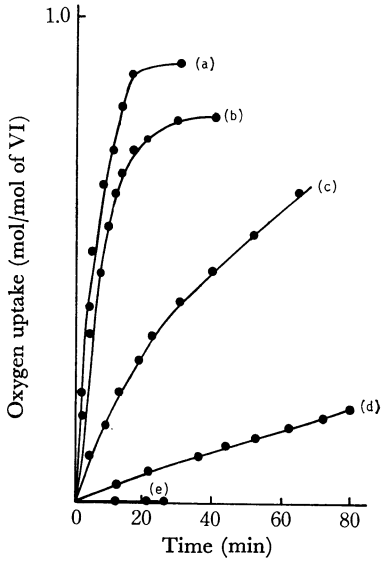


Fig. 6. Oxygen uptake by irradiation (>2800 Å) of VI (1.16 mmol) in the presence of various amounts of rose bengal in methanol (90 m*l*) at ~2°C. (a) Rose bengal (1.1 mmol), (b) rose bengal (0.32 mmol), (c) rose bengal (0.032 mmol), (d) without rose bengal, (e) rose bengal (1.1 mmol) alone (without VI).

should, however, be noted that the amount of rose bengal used was larger than that usually required for a typical photosensitized oxidation. Addition of a small amount of rose bengal, which is sufficient to sensitize the photooxidation of an olefinic compound, had only a weak sensitizing effect.

It may be therefore assumed that the mechanism of the rose bengal-sensitization of the photooxidations described in this respect is different from the usual

Table 2. Photooxidation of V and VI in methanol[a)]

Exp No.	Compound	Rose bengal added (mmol)	Irradiation time (min)	Oxygen uptake (mol/mol of substrate)	Peroxide[b)] (%)	Thyroxine analogs[c)] (%)
10	V	—	120	0.90	—[d)]	X (trace)
11	V	0.12	35	0.57	—[d)]	X (1.3)
12	VI	—	60	0.12	—[d)]	—[d)]
13	VI	0.16	35	0.50	12	—[d)]
14	VI	1.1	35	0.80	40	—[d)]

a) A solution of V (2.3 mmol) or VI (4.6 mmol) in methanol (100 m*l* or 220 m*l*, respectively) was irradiated (>2800 Å) at ~2°C under oxygen-bubbling.
b) Estimated by iodometric titration.
c) Isolated after the photolysate was treated with II (see Experimental).
d) Not determined.

photosensitized oxidation, involving singlet oxygen and the triplet sensitizer, in which self-quenching occurs at higher concentrations of the sensitizer resulting in a slower reaction rate. Additional support for this assumption is provided by the finding that the rate of photosensitized oxidation of 2,6-di-*t*-butylphenol, where singlet oxygen is believed to be involved at least in part,[10] decreases with increased sensitizer concentration (Fig. 7).

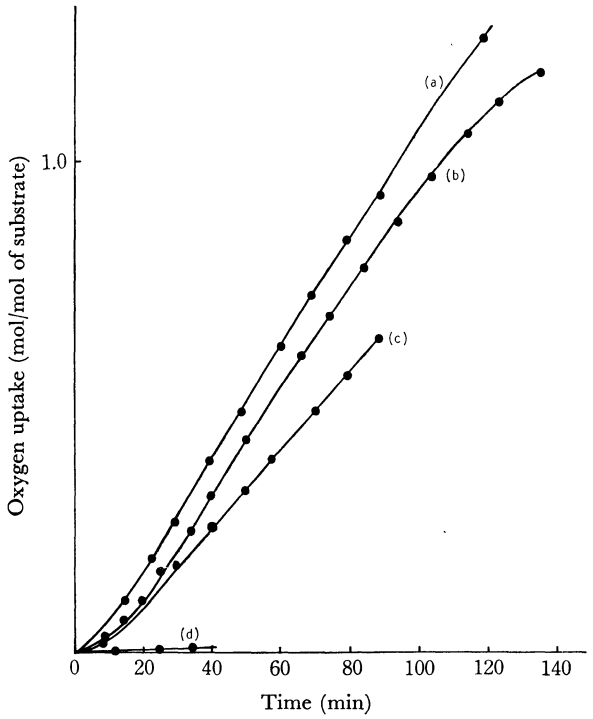


Fig. 7. Oxygen uptake by irradiation (>2800 Å) of 2,6-di-*t*-butylphenol (1.16 mmol) in the presence of various amounts of rose bengal in methanol (90 m*l*) at ~2°C. (a) Rose bengal (0.032 mmol), (b) rose bengal (0.32 mmol), (c) rose bengal (1.1 mmol), (d) without rose bengal.

The structure of the products of the photosensitized oxidation of V and VI has not yet been elucidated. The formation of a hydroperoxide (VIII or IX, analogs of IV) cannot be excluded, since irradiation of VI in the presence of rose bengal yielded a product capable of oxidizing iodide to iodine and the photolysate of V gave a thyroxine analog (X) on reaction with 3,5-diiodotyrosine (II), although in low yield (Table 2).

Experimental

Materials. 4-Hydroxy-3,5-diiodophenylpyruvic acid was purchased from Osaka Lanoratory of Synthetic Organic Chemicals. 4-Hydroxy-3-iodo-5-methoxyphenylpyruvic acid[11] and 4-hydroxyphenylpyruvic acid[12] were prepared according to the described methods. The solvents were freshly distilled before use.

Oxygen Uptake Experiments. A solution was irradiated using a 100W high-pressure mercury lamp with a Pyrex cooling jacket (ice-water). The reaction vessel was immersed in ice-water. During irradiation oxygen was bubbled through the solution in a closed circulating system equipped with a circulation pump and the consumption of oxygen was determined manometrically. In order to assure a constant irradiation level, the lamp was turned on at least 10 min before the start of the experiment while the solution was shielded from the light. The reaction was started by removal of the shield.

A. Photooxidation of 4-Hydroxy-3,5-diiodophenylpyruvic Acid (I) in Methanol. A solution of I (2.0 g) in methanol (200 m*l*) was irradiated at ~5°C for 70 min. During this period 97 m*l* of oxygen was absorbed. Iodometric titration of an aliquot (20 m*l*) of the light brown photolysate showed that liberated iodine was corresponding to 59% peroxide content. An aliquot (60 m*l*) was treated with sodium borohydride (3.0 g) at 0—36°C under nitrogen-bubbling. The mixture was acidified with dilute HCl and extracted with ether. The ether layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. Vpc analysis of the residue after trimethylsilylation[13] (stationary phase, silicone DC on celite; temperature, 250°C; carrier gas, hydrogen) indicated the presence of 0.36 g of glycol VII (58%). The glycol was isolated as colorless needles by means of preparative tlc on silica gel (toluene–ethyl formate–formic acid, 5:4:1); 110°C with foaming. An authentic sample[5] had the same mp and identical mobility on tlc.

The remaining photolysate (120 m*l*) was deoxygenated by nitrogen-bubbling (10 min, ~2°C), then quickly added to a solution of 3,5-diiodotyrosine (II, 6.0 g) in 0.2M borate buffer (500 m*l*, pH 7.8, ~2°C) with nitrogen-bubbling, during

10) T. Matsuura, N. Yoshimura, A. Nishinaga, and I. Saito, *Tetrahedron Lett.*, **1969**, 1669.

11) T. Shiba, H. J. Cahnmann, T. Matsuura, A. Nishinaga, and H. Sakamoto, *J. Org. Chem.*, **29**, 3016 (1964). We thank Mr. Takeshi Kuri for the preparation of this compound.

12) H. D. Dakin, *J. Biol. Chem.*, **82**, 439 (1929). The corresponding azlactone was converted into the keto acid by treatment with 0.1M HCl under reflux.

13) K. Funakoshi and H. J. Cahnmann, *Anal. Biochem.*, **27**, 150 (1969).

which time the solution was permitted to warm up to room temperature. The reaction mixture was worked up as previously described.[5] A colorless precipitate (0.40 g) of thyroxine (III), identical with authentic sample, was obtained.

Other experiments with I are summarized in Table 1, Figs. 1, 2, 3, and 4.

B. Photooxidation of 4-Hydroxy-3-iodo-5-methoxyphenylpyruvic Acid (V) in Methanol (Table 2-Exp. 11). A solution of V (0.78 g) and rose bengal (0.1 g) in methanol (200 m*l*) was irradiated for 35 min at ~2°C. The oxygen-consumption was 33 m*l*. The photolysate was treated with II (6.0 g) in 0.2M borate buffer (500 m*l*, pH 7.7) under nitrogen and worked up. 3,5,3′-Triiodo-5′-methoxythyronine (X) (21 mg), identical with authentic sample,[11] was obtained.

Other experiments with V are summarized in Table 2, Figs. 4 and 5.

C. Photooxidation of 4-Hydroxyphenylpyruvic Acid (VI) in Methanol (Table 2-Exp. 12—14). A solution of VI (0.84 g) in methanol (200 m*l*) in the presence of various amounts of rose bengal was irradiated under oxygen at ~2°C. The photolysate, when added to a solution of KI in dilute acetic acid, liberated iodine, was iodometrically titrated for peroxide analysis.

Other experiments with VI are summarized in Figs. 3, 4, and 6.

This work was supported by U. S. Public Health Service Research Grant AM 07955 from the National Institute of Arthritis and Metabolic Diseases. The authors wish to thank Dr. H. J. Cahnmann, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md., for his helpful discussion throughout this work.