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BIOGENETIC TYPE CONVERSION OF p-HYDROXYPHENYLPYRUVIC ACID INTO HOMOGENTISIC ACID[1]

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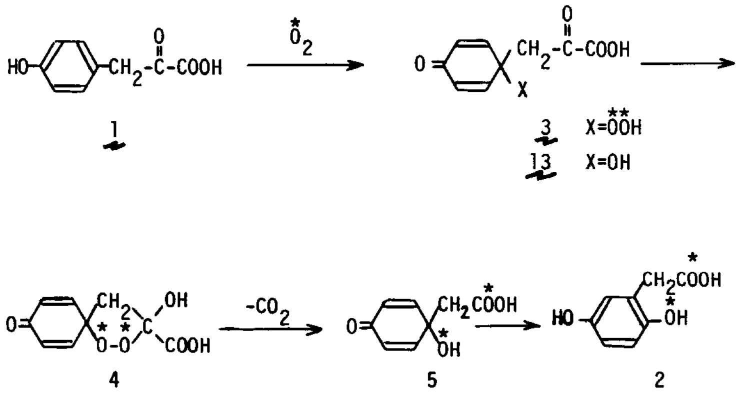
The enzyme p-hydroxyphenylpyruvate hydroxylase classified as a monooxygenase[2] catalyzes the conversion of p-hydroxyphenylpyruvic acid (1) into homogentisic acid (2), in which atomospheric oxygen is incorporated into both the hydroxy and the carboxyl groups of 2[3] In order to account for this experimental observation, Lindblad and his coworkers[3,4] have postulated a mechanism involving nucleophilic attack by the hydroperoxy group of the intermediate hydroperoxide 3 on the α-ketoacid moiety via a cyclic peroxide 4 leading to a 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 NIH shift[7] (Scheme 1). The original idea of this mechanism was suggested by Goodwin and Witkop.[8] After unsuccessful attempts to synthesize the quinol 5, they have shown that alkali treatment of 4-methoxycarbonylmethyl-4-acetoxy-2,5-cyclohexadienone gave 2 which was detected on paper chromatogram. This communication describes a nonenzymic pathway for the conversion of 1 into 2 via the quinol 5

Scheme 1

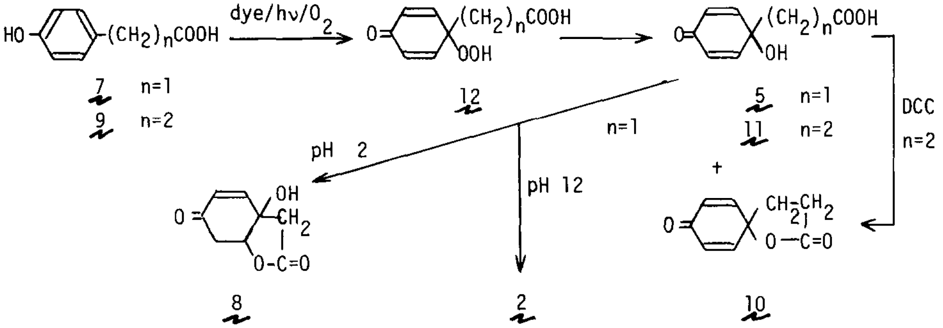


The dye-sensitized photooxygenation[9] of **1** (0.01M) in its enol form[11] resulted in the rapid consumption of an equimolar amount of oxygen with the formation of p-hydroxybenzaldehyde (**6**) in 70% yield along with oxalic acid However, when **1** (0.01M) was photooxidized in phosphate buffer at pH 7.0 in which the keto form was preponderant (keto-enol ratio 8 2),[13] three products, **5**, **6** and p-hydroxyphenylacetic acid (**7**) were isolated in 18, 12 and 15% yield, respectively [14] The quinol **5**[15] was recrystallized from ethyl acetate, mp. 104°C, λ_{max}^{EtOH} 220 nm (log ε 4 18), ν_{max}^{nujol} 3320, 1705, 1670 and 1610 cm^{-1}, NMR (acetone-d_6) δ 7 05 (2H, d, J=10Hz), 6 30(1H, s, OH), 6 07 (2H, d, J=10Hz) and 2 75 (2H, s) Sodium borohydride reduction of **5** gave **7** in 72% yield Treatment of **5** with aqueous alkali (pH 12) at room temperature under nitrogen gave homogentisic acid (**2**) in 80% yield At below pH 2, quinol **5** readily underwent cyclization to yield a lactone **8**[15] in 85% yield, mp 109°C, λ_{max}^{EtOH} 218 nm (log ε 4 12), ν_{max}^{nujol} 3400, 1780, 1670 and 1605 cm^{-1}, NMR (acetone-d_6) δ 6 85 (1h, dd, J=10 and 0 8Hz), 6 00(1H, d, J=10Hz), 5 40 (1H, br s, OH), 4 85 (1H, td, X of ABX, J_{AX}=J_{BX}=5Hz, J=0 8Hz), 3 05 (1H, dd, J_{AB}=16Hz, J_{AX}=5Hz), 2 90 (2H, s) and 2 68 (1H, dd, J_{AB}=16Hz, J_{BX}=5Hz) The photooxygenation of **1** is inhibited by the addition of known singlet oxygen quenchers, 1,4-diazabicyclo[2.2 2]octane (DABCO)[16] and sodium azide,[17] indicating that the reaction may be a singlet oxygen-mediated reaction [18]

The following control experiments have shown that **7** cannot be a precursor of **5** in the photooxygenation of **1** (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 **7** was only sluggishly oxidized (ii) In the photooxygenation of **1** addition of catalase to the reaction system inhibited the formation of **7** but had no effect on the yield of **5** [20] However, under alkaline conditions (phosphate buffer, pH 8.5) the photooxygenation of **7** proceeded at an appreciable rate to give **5** in 65% yield Under similar conditions, phloretic acid (**9**) gave the known lactone **10** [21] (22%) and a quinol **11**[15] (50%), mp 112°C Treatment of **11** with N,N'-dicyclohexylcarbodiimide gave **10** in 90% yield (Scheme 2) The quinols presumably result from the reaction of the initially formed hydroperoxide **12** with water [22]

Unlike **7** and **9** which give **5** and **11** respectively, **1** does not yield the corresponding quinol **13** in detectable amounts It only gives **5** upon photooxygenation within a pH range of 6 0-9 0 which suggests that the hydroperoxy group of **3** reacts much faster with the keto group of the side chain than with the solvent water

In summary, the reaction sequence reported here indicates that **1** in its keto form can react with singlet oxygen to yield **5** most probably via a cyclic peroxide **4**, and that 5 is indeed an intermediate in the nonenzymic conversion of 1 into 2[23] Furthermore, the photosensitized oxygenation in aqueous systems provide a simple alternative method for the synthesis of 4-substituted 4-hydroxy-2,5-cyclohexadienones from phenolic acids[26] Further work on the possible participation of 5 as an intermediate in the enzymic reaction is currently in progress[27]



Scheme 2

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3) B Lindblad, G Lindstedt, and S Lindstedt, J Am Chem Soc , 92, 7446 (1970)
4) Recently Soloway et al[5] have suggested that a 1,4-endo-peroxide[6] and quinoids such as 3, 4 and 5 are less likely intermediates because of the lack of exchange of the O-18 labeled phenolic function of 1 with water during enzymic transformations However, if the subsequent reaction proceeds at a much faster rate than that of oxygen exchange with water, or if one of the two hydroxyls of a hydrated quinoid is removed selectively, such quinoid intermediates may still exist
5) A S Widman, A H Soloway, R L Stern, and M M Bursey, Bioorg Chem , 2, 176 (1973)
6) A H Soloway, J Theoret Biochem , 13, 100 (1966)
7) (a) G Guroff, J W Daly, D M Jerina, J Renson, B Witkop, and S Udenfriend, Science, 1524 (1967), (b) D M Jerina, J W Daly, B Witkop, P Zaltzman-Nirenberg, and S Udenfriend, J Am Chem Soc , 90, 6525 (1968), Biochem , 9, 147 (1970)
8) S Goodwin and B Witkop, J Am Chem Soc , 79, 179 (1957)
9) Methylene blue (in methanol) and rose bengal or rose bengal attached to Amberlite IRA-400[10] (in aqueous solution) were used as sensitizers
10) J R Williams, G Orton, and L R Unger, Tetrahedron Letters, 4603 (1973)
11) In freshly prepared buffered solution or in methanol 1 exists in its enol form, whereas when the buffered solution is allowed to stand for 24 hours the keto form becomes predominant[12]

12) (a) W E Knox and B M Pitt, J Biol Chem , 225, 675 (1957), (b) E C C Lin, B M. Pitt, M Civen, and W E Knox, ibid , 233, 668 (1968)

13) The keto-enol ratio was determined by UV and NMR spectroscopy

14) Products were isolated by acidification (pH 3 5) and extraction of the reaction mixture followed by preparative TLC

15) Satiffactory elemental analyses and mass spectra were obtained for all new compounds

16) C Ouannés and T Wilson, J Am Chem Soc , 90, 6527 (1968)

17) R. Nilsson, P B Merkel, and D R Kearns, Photochem Photobiol , 16, 109 (1972)

18) The rate (k_q) of the photooxidation of 1 (0 01M) in the presence of quencher was compared with that of the control experiment (k_0) The ratio k_q/k_0 was 0 52 for 0 05M DABCO and 0 41 for 0 05M NaN_3 Moreover, the photooxidation rate of 1 ($4x10^{-4}$M) in phosphate buffer (pH 7 0) increased approximately 6-fold in going from H_2O to D_2O [19] However, we cannot exclude the possibility that a free radical process (Type I process) involving sensitizer triplet is operating at least in part as a competing process in the photooxygenation See T Matsuura, N Yoshimura, A Nishinaga, and I Saito, Tetrahedron, 28, 4933 (1972)

19) P B Merkel, R Nilsson, and D R Kearns, J Am Chem Soc , 94, 7244 (1972)

20) The result also indicates that the formation of 7 in the photooxygenation of 1 presumably results from the reaction of 1 with hydrogen peroxide formed by the secondary decompositions of the intermediate hydroperoxides

21) (a) A. I Scott, P A Dodson, F McCapra, and M. B. Meyers, J Am Chem Soc , 85, 3702 (1963), (b) H Iwasaki, L. A Cohen, and B. Witkop, ibid , 85, 3701 (1963), (c) J S Davies, C H Hassall, and J. A. Schofield, J. Chem Soc , 3126 (1964), (d) L Farber and L A Cohen, Biochem , 5, 1027 (1966)

22) The presence of hydrogen peroxide was estimated to be ca 20% (based on reacted 7) by titration of the reaction mixture before and after addition of catalase

23) Although model reactions using singlet oxygen have been reported for certain biological oxygenation systems, the possible participation of singlet oxygen in such biological systems is only suggestive[24] and in some case unlikely [25] It has not yet been shown which tautomer of 1 is involved in the enzymic reaction.[3] However, if the enol tautomer (or mixture of the tautomers) is substrate for the hydroxylase, singlet oxygen is not likely to be involved in the enzymic reaction since none of 7 has been produced in the reaction [3]

24) (a) T Matsuura, H. Matsushima, and R. Nakashima, Tetrahedron , 26, 435 (1970), (b) H. G. Krishnamurty and F J. Simpson, J. Biol. Chem , 245, 1467 (1970), (c) H W.-S Chan, J Am Chem Soc , 93, 2357, 4632 (1971), (d) W L. Smith and W. E. Lands, J. Biol Chem , 247, 1038 (1972), (e) L A Sternson and R A Wiley, Chem.-Biol Interactions, 5, 317 (1972)

25) J I. Teng and L. L Smith, J Am. Chem Soc., 95, 4060 (1973), 96, 2640 (1974).

26) Several oxidation methods including anodic oxidation have been adopted for the synthesis of quinols from phenolic acids.[21] However, in most cases dienone spirolactones are obtained in the yield of 10-45%.

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