PHOTOCHEMICAL HYDROXYLATION OF SALICYLIC ACID WITH HYDROGEN PEROXIDE; MECHANISTIC STUDY OF SUBSTRATE SENSITIZED REACTION

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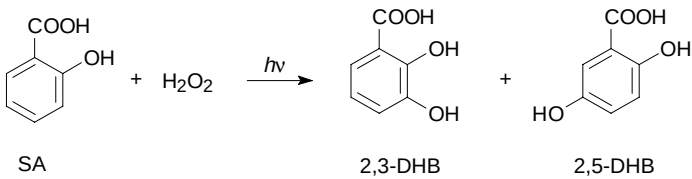
The photochemical hydroxylation of salicylic acid (SA) by hydrogen peroxide proceeds via the same intermediate independently of the excited reactant (i.e. SA or H_2O_2). This conclusion is supported by the similar effect of pH on the quantum yields and on the isomer ratio of formed products: 2,3-dihydroxybenzoic acid (2,3-DHB) and 2,5-dihydroxybenzoic acid (2,5-DHB). The product ratio ([2,3-DHB]/[2,5-DHB]) is not affected by H_2O_2 concentration.

Key words: 2-Hydroxybenzoic acid; Hydrogen peroxide; Photochemical hydroxylation.

In our previous papers we have described very pronounced photocatalytic effects of Fe(III) on photochemical hydroxylation of salicylic acid by hydrogen peroxide[1,2]. The mechanism of the photocatalyzed hydroxylation is evident. It consists in the photoreduction of Fe(III) to Fe(II) and therefore in photogeneration of the Fenton reagent. However, the hydroxylation of salicylic acid (SA) takes place in the absence of added Fe(III) too, although at significantly lower quantum yields. The position of the new hydroxyl group on the aromatic ring is directed mainly by the effect of –OH group on the ring electron density and therefore hydroxylation takes place preferably in *ortho*- and *para*-positions. Thus, hydroxylation of SA by H_2O_2 yields a mixture of 2,3-dihydroxybenzoic acid (2,3-DHB) and 2,5-dihydroxybenzoic acid[3] (2,5-DHB).



(*A*)

The photochemical hydroxylation of aromatic ring by hydrogen peroxide is usually explained in terms of its photodissociation into two hydroxyl radicals with subsequent

attack of the ring[4]. This mechanism is well understood for the irradiation with wavelengths, which are absorbed by the H_2O_2 itself.

$$H_2O_2 \xrightarrow{h\nu} 2\ OH^\bullet \qquad (B)$$

$$Ar + OH^\bullet \longrightarrow ArOH^\bullet \qquad (C)$$

The hydroxylation initiated by excitation of aromatic substrate in the presence of H_2O_2 has been proposed by Ogata[5] for benzoic acid, but the mechanism has not been unambiguously resolved so far. The question remains still open whether hydroxylation occurs at all when only substrate (and not H_2O_2) is excited and what is the mechanism of this "substrate sensitized" hydroxylation. Aim of this work is to contribute to understanding of mechanism of these reactions. SA seems to be suitable substrate for such study as it possesses good chemical stability, solubility in water, and it absorbs at longer wavelengths than H_2O_2 even at relatively low concentrations.

EXPERIMENTAL

Chemicals

Salicylic acid, AR (Reanal, Budapest) was recrystallized from double distilled water. Stabilizer free hydrogen peroxide, AR (Chemical Works, Sokolov), and chromatography standards 2,3-DHB (Fluka) and 2,5-DHB (Merck, Schuchardt) were used as received. Double distilled water was used in all experiments; its quality was checked by comparative experiments with water of high purity[6].

Apparatus and Procedure

A reaction 10 mm cell was placed in thermostatted (20 °C) aluminium block, the reaction solution (2.5 ml) was continuously bubbled with nitrogen to prevent competitive oxidation by dioxygen and to ensure effective stirring. pH was adjusted by addition of 0.5 M $HClO_4$ or 0.1 M K_2CO_3 and checked by a pH meter.

A low pressure mercury arc (Spectral lamp Philips 93109 E) served as a source of 254 nm radiation in the hydroxylations initiated by H_2O_2 photolysis. In most of these experiments the initial concentrations of SA and H_2O_2 were 0.5 mmol l^{-1} and 50 mmol l^{-1}, respectively. The major part of incident 254 nm radiation was absorbed by H_2O_2 (75%). Under the used conditions SA absorbs only 18% of incident radiation due to its comparably low absorbance (ε_{254} = 414 mol^{-1} l cm^{-1}).

SA was selectively excited by a high pressure mercury arc HBO 200 W (Osram) equipped with a 100 mm water filter, 300–400 nm band filter UG 1/1.5 g and interference filter UVKSIF 313 nm (both Zeiss, Jena). The initial concentrations $[SA]_0$ = 8 mmol l^{-1} and $[H_2O_2]_0$ = 9.2 mmol l^{-1} were chosen to ensure that practically all incident radiation was absorbed by SA ($A_{313} > 3$).

At both studied wavelengths (i.e. 254 nm or 313 nm) the calculated overall quantum yields Φ were related to the total number of photon absorbed by the reaction mixture at the given wavelength:

$$\Phi = \Delta c\, N_A / [t\, I_v (1 - 10^{-A})] \ , \qquad (1)$$

where Δc is the concentration change (mol l^{-1}) during irradiation time t (s), N_A is the Avogadro's number, I_V is the radiation intensity (quanta s^{-1} l^{-1}), and A is the absorbance of the reaction mixture at the radiation wavelength. The radiation intensities I_v, measured by a ferrioxalate actinometer, were $7.8 . 10^{16}$ photons l^{-1} s^{-1} and $9.1 . 10^{17}$ photons l^{-1} s^{-1} for the low (254 nm) and high (313 nm) pressure mercury arc, respectively.

The concentrations of SA and hydroxylation products (i.e. 2,5-DHB and 2,3-DHB) were determined by HPLC equipped with Separon SGX C18 7 mm reversed phase column (Tessek, Prague), a HPP 4001 pump (Laboratorni pristroje, Prague) and PU 4025 UV detector (Philips Unicam, Cambridge). The eluting solution was: MeOH–H_2O–1 M H_3PO_4 (1 mol l^{-1}) 53 : 46 : 1. Other possible products as benzene, phenol, 1,4-dihydroxybenzene, muconic acid have not been detected.

The UV-VIS absorption spectra were recorded on a PU 8720 spectrophotometer (Philips Unicam, Cambridge). The SA fluorescence quenching experiments were carried out using a Perkin–Elmer LS 50 luminiscence spectrometer. In fluorescence quenching experiments the SA concentration was 1 mmol l^{-1} (pH 3) so that excitation radiation 355 nm was absorbed only by SA. The fluorescence intensities were corrected for a dilution effect after addition of aliquots of H_2O_2.

A laser flash photolysis set-up with an LPX 200 excimer laser (Lambda Physics) was described previously[2].

RESULTS AND DISCUSSION

The Hydroxylation Evoked by Photolysis of H_2O_2

The concentrations of hydroxylation products (i.e. 2,5-DHB and 2,3-DHB) during 254 nm irradiation related to the SA uptake shown in Figs 1 and 2 illustrate the influence of pH on the reaction kinetics. At pH < 3.5 the 2,3-DHB and 2,5-DHB formations proceeded much slower compared with pH ≥ 4.3. The effect of pH on formation of hydroxylation products is more profound for 2,5-DHB then for 2,3-DHB. The detectable concentration of 2,5-DHB at pH 2.3 was reached after 180 min irradiation (Fig. 2, curve *2*) and furthermore its yields are even reduced at pH 3.5 when compared with 2,3-DHB (Figs 1

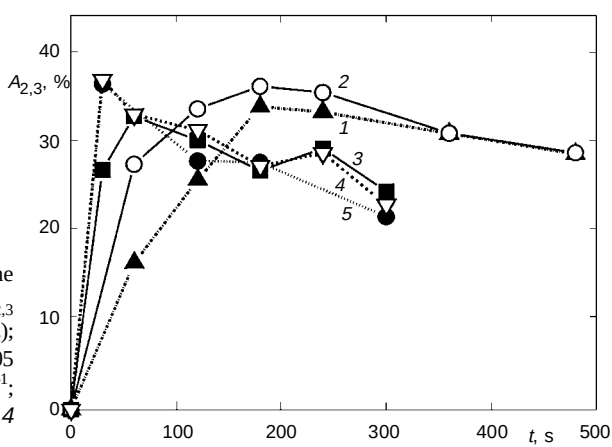


FIG. 1
The yields of 2,3-DHB related to the uptake of SA vs irradiation time. $A_{2,3}$ = $100[2,3\text{-DHB}]_t/([SA]_0 - [SA]_t)$; $[SA]_0$ = 0.5 mmol l^{-1}; $[H_2O_2]_0$ = 0.05 mol l^{-1}; $I_v = 7.8 . 10^{16}$ photons l^{-1} s^{-1}; λ_{irr} 254 nm; pH: *1* 1.4; *2* 2.3; *3* 3.5; *4* 4.3; *5* 9.4

and 2, curves *3*). However, in the pH range 4.3 to 9.4 the yields do not undergo any pH influence (Fig. 1, curves *4* and *5*; Fig. 2, curves *5* and *6*). The maximum quantity of products is reached after 30 min irradiation. The following drop is a result of subsequent photodegradation of both products (namely 2,5-DHB, Fig. 2, curve *6*).

pH affects also the isomer ratio Y = [2,3-DHB]/[2,5-DHB], see Table I. Contrary to it, the initial concentrations of H_2O_2, which were varied (at pH 3.5) from 7.1 mmol l^{-1} (Y = 2.41 ± 0.42) to 1 mol l^{-1} (Y = 2.22 ±0.48), had no effect. Y was not determined at pH < 3.5 because only trace concentration of 2,5-DHB were formed. In the pH range 4.3–8.3 both isomers are produced nearly at equimolar concentrations and Y is constant during 300 min of irradiation (Fig. 3, curve *1*; Table I). In more alkaline medium, pH 9.4,

TABLE I
The effect of pH on the isomer ratio Y = [2,3-DHB]/[2,5-DHB] (error ±10%)

λ_{irr} 254 nm[a]		λ_{irr} 313 nm[b]	
pH	Y	pH	Y
3.5	2.46	3.5	2.47
4.3	1.36	–	–
6.7	1.12	6.5	1.26
8.3	1.25	7.8	1.29
9.4	1.55	–	–

[a] 7.8 . 10^{16} photons l^{-1} s^{-1}, $[SA]_0$ = 0.5 mmol l^{-1}, $[H_2O_2]_0$ = 50 mmol l^{-1}; [b] 9.1 . 10^{17} photons l^{-1} s^{-1}, $[SA]_0$ = 8 mmol l^{-1}, $[H_2O_2]_0$ = 9.2 mmol l^{-1}, irradiation time 30 min

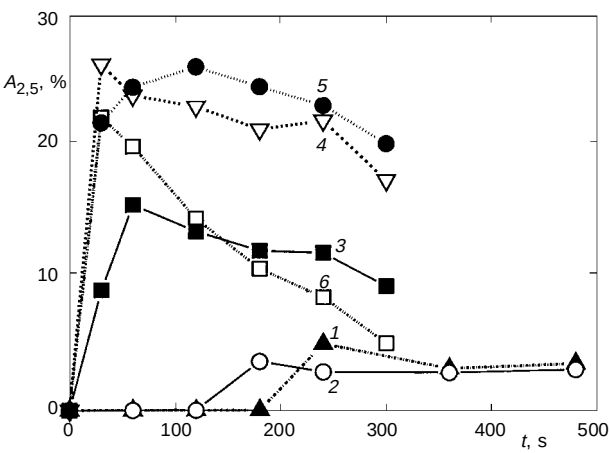


FIG. 2
The yields of 2,5-DHB related to the uptake of SA vs irradiation time. $A_{2,5}$ = 100[2,5-DHB]$_t$/([SA]$_0$ – [SA]$_t$); [SA]$_0$ = 0.5 mmol l^{-1}; $[H_2O_2]_0$ = 0.05 mol l^{-1}; I_v = 7.8 . 10^{16} photons l^{-1} s^{-1}; λ_{irr} 254 nm; pH: *1* 1.4; *2* 2.3; *3* 3.5; *4* 4.3; *5* 8.3; *6* 9.4

the ratio gradually increases from 1.42 to 4.33 after 300 min irradiation as 2,5-DHB is more easily photodegradated compared with 2,3-DHB. This has been proven by experiments, in which 2,3-DHB and 2,5-DHB were used instead of SA.

As yields of 2,3-DHB and 2,5-DHB are affected markedly by acidity of reaction solution, the respective quantum yields are also governed by pH (Fig. 4) exhibiting the lowest values in acidic solutions pH < 3.5. The reason of decrease of Φ at pH > 8 is not so clear. The explanation might be found in other mechanism involved, namely that in alkaline solution (pH > 8) the degradation of aromatic ring instead of hydroxylation takes place. The Φ dependence smoothes down with prolongation of irradiation time (Fig. 4, curve *2*). The decrease is caused by a subsequent degradation of both products as follows from the kinetics of product formations (Figs 1 and 2), although the ratio *Y* remains constant up to pH 8.3 (Table I). Minor absorption of incident radiation by SA

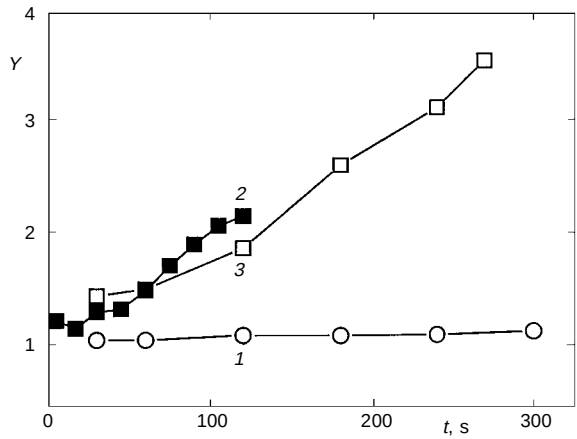


FIG. 3
The isomer ratio Y = [2,3-DHB]/[2,5-DHB] vs irradiation time for various radiation wavelengths. *1* $[SA]_0$ = 0.5 mmol l^{-1}; $[H_2O_2]_0$ = 0.05 mol l^{-1}; pH 6.7; λ_{irr} 254 nm; I_v = 7.8 . 10^{16} photons l^{-1} s^{-1}. *2* $[SA]_0$ = 8 mmol l^{-1}; $[H_2O_2]_0$ = 9.2 mmol l^{-1}; pH 6.5; λ_{irr} 313 nm; I_v = 9.1 . 10^{17} photons l^{-1} s^{-1}. *3* $[SA]_0$ = 0.5 mmol l^{-1}; $[H_2O_2]_0$ = 0.05 mol l^{-1}; pH 6.7; λ_{irr} 300–410 nm; $I_v > 10^{18}$ photons l^{-1} s^{-1}

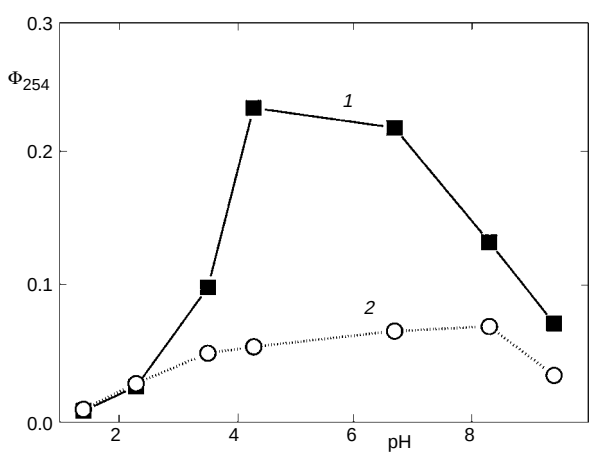


FIG. 4
The effect of pH on the quantum yields of hydroxylation products. $\Phi_{254} = \Phi_{2,3\text{-DHB}} + \Phi_{2,5\text{-DHB}}$; $[SA]_0$ = 0.5 mmol l^{-1}; $[H_2O_2]_0$ = 0.05 mol l^{-1}; λ_{irr} 254 nm; I_v = 7.8 . 10^{16} photons l^{-1} s^{-1}; irradiation time: *1* 30 min; *2* 300 min

does not significantly contribute to the hydroxylation yields since the respective quantum yields are about one order of magnitude lower (vide infra, Table II) in comparison with direct photolysis of H_2O_2 (Fig. 4).

Described phenomena could be accounted for the fact that the nondissociated SA and salicylate (dissociated carboxyl group) differ in the reactivity with $OH^\bullet$. At pH < 3, where nondissociated SA prevails (pK_a = 2.97, ref.[7]), the hydroxylation yields are considerably reduced. Since the reactions of $OH^\bullet$ with SA and salicylate are diffusion-controlled[8], the only difference has to emerge from the stabilization of an intermediate radical adduct.

Hydroxylation Evoked by SA Excitation

The hydroxylation of SA by H_2O_2 initiated by 313 nm radiation (which is absorbed exclusively by SA) occurs, however, the corresponding quantum yields are significantly lower compared with H_2O_2 excitation (Table II). The effect of pH on Φ_{313} resembles to the one observed for Φ_{254}. Furthermore, the isomer ratio Y at 313 nm irradiation matches the one observed for 254 nm radiation (photolysis of H_2O_2), Table I.

It should be also stated that no hydroxylation products were detected in the absence of H_2O_2 at pH 3.5. In neutral solutions, which are more favourable for hydroxylation, only very low quantum yields of 2,3-DHB and 2,5-DHB (in H_2O_2 absence) were observed (e.g. $\Phi_{313} = (1.4 \pm 0.4) \cdot 10^{-3}$ at pH 6.5). From this follows that H_2O_2 acts as a quencher of SA excited states as only in the presence of H_2O_2 hydroxylation occurs at reasonable rate. The low quantum yields of an aromatic ring hydroxylation in the presence of dissolved oxygen has already been described[9]. The trapping of liberated electrons and subsequent formation of $OH^\bullet$ or other reactive oxygen species accounts for this observation.

We have carried out laser flash photolysis and steady-state fluorescence experiments to clarify which excited states of SA initiate the hydroxylation. The former method, suitable for monitoring of excited triplet states, have not shown any transient quenched by H_2O_2 and dissolved O_2. Thus, these states should be excluded. On the other hand,

TABLE II

Quantum yields $\Phi_{313} = \Phi_{2,3DHB} + \Phi_{2,5DHB}$ in case of SA excitation. $[SA]_0$ = 8 mmol l^{-1}; $[H_2O_2]_0$ = 9.2 mmol l^{-1}; λ_{irr} 313 nm, 9.1 . 10^{17} photons l^{-1} s^{-1}, irradiation time 30 min (during first 30 min Φ_{313} remain fairly constant)

pH	3.5	6.5	7.8
Φ_{313}	$(8.2\pm 1.9) \cdot 10^{-3}$	$(1.9 \pm 0.8) \cdot 10^{-2}$	$(2.3 \pm 0.2) \cdot 10^{-2}$

the fluorescence quenching of SA after addition of H_2O_2 can be processed to the Stern–Volmer treatment:

$$F_0/F = 1 + K_{SV}[Q] \ , \qquad (2)$$

where F_0 and F are the fluorescence intensities in the absence and in the presence of a quencher concentration [Q]. As follows from Fig. 5 the experimental data fairly fit the Stern–Volmer equation and the corresponding constant is $K_{SV} = 0.45 \pm 0.01$ mol^{-1} l. Taking the SA fluorescence lifetime 0.4 ns (ref.[10]) into account the quenching rate constant of $1.1 \cdot 10^9$ mol l^{-1} s^{-1} can be evaluated. This constant does not approach the diffusion-controlled rate constant $k_0 = 7.0 \cdot 10^9$ mol l^{-1} s^{-1} given by Smoluchowski relation[11]. The dynamic quenching of tryptophan fluorescence by H_2O_2 has been interpreted by electron transfer from excited indol ring to H_2O_2 (ref.[12]). However, the fluorescence quenching of SA can be attributed to the homolytic splitting of the peroxo bond –O–O– producing OH$^\bullet$. The assumption of the same hydroxylation agent, i.e. OH$^\bullet$, at both irradiation wavelength (254 nm absorbed by H_2O_2 and 313 nm absorbed by SA) is supported further by the similar effect of pH on Φ and on the isomer ratio *Y*. Following Scheme 1 summarizes the mechanisms of 2,3-DHB formation at different excitation wavelengths. (The reaction scheme for 2,5-DHB formation can be drawn by analogy with 2,3-DHB.)

The bond energy of HO–OH is 213 kJ mol^{-1} (ref.[13]). The energy of $^1SA^*$ is at least 272 kJ mol^{-1} (evaluated from the fluorescence peak of SA in water, λ = 440 nm), so the salicylic acid sensitized splitting of HO–OH bond is energetically favourable. Moreover, the value $(1 - F/F_0)$, which expresses the theoretical quantum yield as the fraction of quenching events related to the total number of excited singlet states, provides fur-

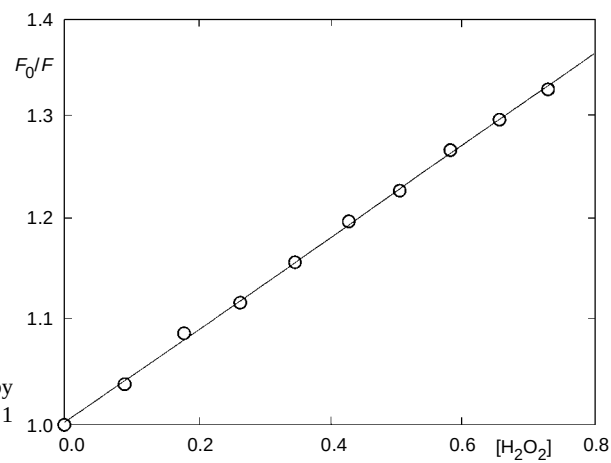
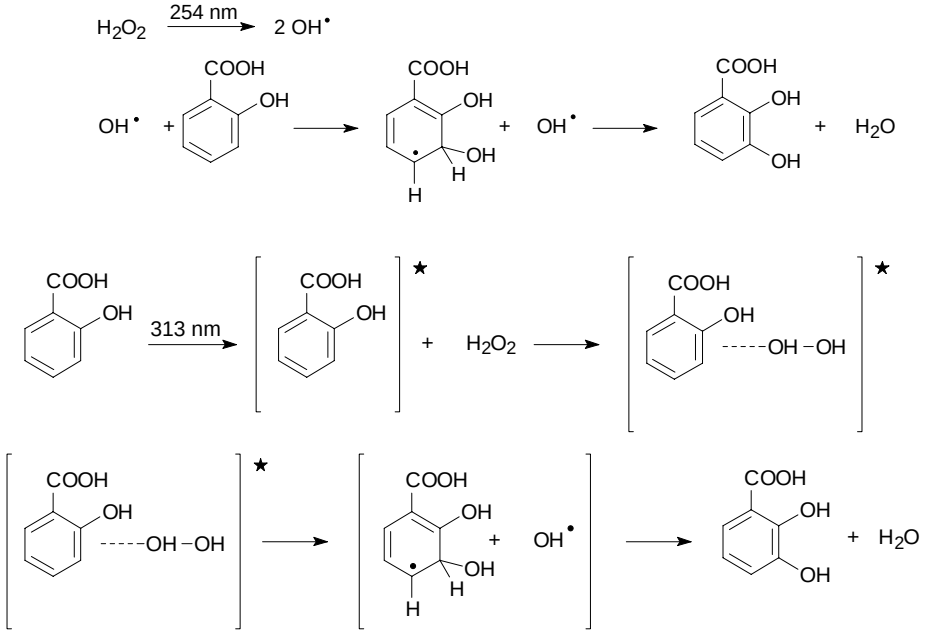


FIG. 5
Quenching of SA fluorescence by H_2O_2; Stern–Volmer plot. $[SA]_0$ = 1 mmol l^{-1};

ther support for the suggested mechanism (Scheme 1). For this estimation the concentration of hydrogen peroxide was considered as constant and equal to the initial one, because its uptake during irradiation was comparable low. Within experimental error the theoretical quantum yield $4.2 \cdot 10^{-3}$ is in accordance with the overall quantum yield $\Phi_{313} = (8.2 \pm 1.9) \cdot 10^{-3}$, which was found experimentally at pH 3.5.



SCHEME 1

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