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The photolysis ($\lambda = 254$ nm) of tyrosine in aqueous solutions in the absence and presence of oxygen. The reaction of tyrosine with singlet oxygen ☆

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Abstract

In the 254 nm photolysis of deoxygenated aqueous solutions of tyrosine (TYROH, 10^{-3} mol dm^{-3}) TYROH is consumed with a quantum yield of 9.1×10^{-3} while the observed products (quantum yields in parentheses) are 2,2′-bityrosyl (BITYR, 3.1×10^{-3}) and 2-amino-4-ethenyl-hex-4-enic acid (AEHEA, 1.6×10^{-3}). In N_2O-saturated solutions, N_2 (1.8×10^{-2}), in the presence of 2-propanol, H_2 (1×10^{-3}) are formed.

In air-saturated solutions the tyrosine consumption is increased (9.7×10^{-2}); in addition to increased yields of BITYR (1.4×10^{-2}) but unaltered yields of AEHEA (1.6×10^{-3}), 3a-hydroxy-6-oxo-2,3,3a,6,7,7a-hexahydro-1H-indol-2-carboxylic acid (HOHICA, 1.6×10^{-2}), 3,4-dihydroxyphenylalanine (DOPA, 3.8×10^{-2}) and 2,4-dihydroxyphenylalanine (2,4-DHPhe, 2.3×10^{-3}) are observed. The sum of the quantum yields of the O_2-dependent products increases with increasing O_2 and TYROH concentrations, approaching a plateau value of approximately 7×10^{-2} at high reactant concentrations. In D_2O the quantum yields of BITYR, HOHICA and DOPA are increased considerably while those of AEHEA are decreased. Irradiation of Rose Bengal with visible light ($^1O_2$ formation) in the presence of TYROH yields HOHICA (90%) and BITYR (10%).

It is concluded that from the excited singlet state (^{1}TYROH) photoionization and splitting of the phenolic O–H bond occurs giving rise to tyrosine-derived phenoxyl radicals (TYRO$^\bullet$) which are the precursors of BITYR. Also, from ^{1}TYROH a 1,3-H-shift of the phenolic O–H and concomitant ring-opening occurs. Addition of water and loss of carbon dioxide in subsequent thermal reactions yields AEHEA as the final product. Quenching of the TYROH triplet state (^{3}TYROH) by O_2 yields $^1O_2$ which reacts with TYROH by H abstraction. The resulting intermediates TYRO$^\bullet$ and $HO_2^\bullet$ are the precursors of BITYR and HOHICA. In addition, a reaction of O_2 with ^{3}TYROH possibly yields 1,4- and 3,6-endoperoxides which are the precursors of further HOHICA and of DOPA and 2,4-DHPhe, respectively.

Keywords: Photolysis; Tyrosine; Singlet oxygen; Amino acids

1. Introduction

There is a longstanding interest in the photochemistry of proteins and their subunits, the amino acids (for reviews see Refs. [1,2]). The incentive to reexamine the photochemistry of aromatic amino acids in some more detail (see for example Ref. [3]) was induced by a study on the potential by-product formation in the UV disinfection of drinking water [4]. Surface waters contain small concentrations of glycoproteins which stem from the excretion and biodegradation of algae (extracellular matter, EOM). Upon UV disinfection ($\lambda = 254$ nm) of such waters in drinking-water processing this material may undergo photolytic changes. Since the concentration of EOM in surface water is low, it is not expected that potentially toxic–mutagenic material could build up to any significant extent from this source at the low fluences required for disinfection [4–6]. Yet it was of interest to have a closer look at the products and the mechanism of their formation in the case that higher concentrations of glycoproteins irradiated at tenfold higher fluences than required for UV disinfection would reveal a mutagenic potential. During this study, it was shown that no mutagenicity (Ames test) was induced upon UV irradiation of the glycoprotein gum arabic or isolated EOM [7].

The aliphatic amino acids do not absorb the 254 nm radiation to any significant extent, and the absorbance of proteins at this wavelength is mainly owing to the absorption by the aromatic amino acids tyrosine (TYROH, ϵ(254 nm) =

☆ Dedicated to Professor Dr. H.-D. Scharf on the occasion of his 65th birthday.

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350 $dm^3\ mol^{-1}\ cm^{-1}$), phenylalanine ($\epsilon(254\ nm) = 140\ dm^3\ mol^{-1}\ cm^{-1}$) and tryptophan ($\epsilon(254\ nm) = 2900\ dm^3\ mol^{-1}\ cm^{-1}$). The disulphide cystine also absorbs in this wavelength region ($\epsilon(254\ nm) = 270\ dm^3\ mol^{-1}\ cm^{-1}$). TYROH has two absorption maxima in the UV, at 220 nm and at 275 nm. Both absorption bands have been attributed to π-π^* transitions [8,9]. The 254 nm radiation used in this study excites the first one.

The basic photophysical–photochemical reactions to be considered are shown in reactions (1)–(10).

$$\mathrm{TYROH} + h\nu \longrightarrow {}^1\mathrm{TYROH} \tag{1}$$

$${}^1\mathrm{TYROH} \longrightarrow \mathrm{TYROH} + h\nu' \tag{2}$$

$${}^1\mathrm{TYROH} \longrightarrow \mathrm{Products} \tag{3}$$

$${}^1\mathrm{TYROH} \longrightarrow \mathrm{TYROH} \tag{4}$$

$${}^1\mathrm{TYROH} \longrightarrow {}^3\mathrm{TYROH} \tag{5}$$

$${}^3\mathrm{TYROH} \longrightarrow \mathrm{TYROH} + h\nu'' \tag{6}$$

$${}^3\mathrm{TYROH} \longrightarrow \mathrm{TYROH} \tag{7}$$

$${}^3\mathrm{TYROH} \longrightarrow \mathrm{Products} \tag{8}$$

$${}^3\mathrm{TYROH} + O_2 \longrightarrow \mathrm{Products\ (e.g.\ {}^1O_2)} \tag{9}$$

$${}^1O_2 + \mathrm{TYROH} \longrightarrow \mathrm{Products} \tag{10}$$

Excitation leads to the formation of an excited singlet state (reaction (1)) which decays by fluorescence (reaction (2), Φ(fluorescence) = 0.2) [9], product formation (reaction (3)), internal conversion to the ground state (reaction (4)), and intersystem crossing to the triplet state (reaction (5)). Photoionization is one of the processes occurring from the excited singlet state. Laser studies at 193 nm have shown that at this wavelength photoionization is a monophotonic process ($\Phi(e_{aq}^-) = 0.13$) [10]. At 265 nm [11] and 248 nm [10] it is largely biphotonic, but extrapolation of $\Phi(e_{aq}^-)$ to zero light intensity yields an intercept of $\Phi(e_{aq}^-) = 0.03$ [10]; concomitantly the formation of phenoxyl radicals is observed [11,12]. Thus also in the 254 nm photolysis photoionization may contribute significantly to product formation. The triplet state is much longer lived. Besides decaying to the ground state by phosphorescence and intersystem crossing (reactions (6) and (7)) product formation may occur from this excited state as well (reaction (8)). Owing to its long lifetime it can be quenched by oxygen (reaction (9)). A fraction of this reaction leads to the formation of singlet oxygen, ${}^1O_2$. Singlet oxygen formation is common in the photochemistry of phenols (Ref. [13] and references cited therein). TYROH is known to react rapidly with ${}^1O_2$(k(in D_2O) = $2.7 \times 10^8\ dm^3\ mol^{-1}\ s^{-1}$) [14], thus in the presence of oxygen further products may arise from the singlet oxygen reaction (reaction (10)).

Despite the fact that detailed photophysical studies have been carried out, our knowledge about the resulting products and their quantum yields still remained very limited; at 254 nm and in the presence of O_2 Φ(TYROH consumption) = 0.15 has been determined [10], and as products only 2,2′-bityrosyl (BITYR) and 3,4-dihydroxyphenylalanine (DOPA) were detected (no quantum yields given) [15,16].

It will be shown in this study that major products had escaped identification in these earlier studies.

2. Experimental details

L-Tyrosine (TYROH, Janssen), Rose Bengal (Aldrich), 3,4-dihydroxyphenylalanine (DOPA, Janssen), and D_2O (99.8% Merck) were used as received. 2,2′-Bityrosyl (BTYR) and 3a-hydroxy-6-oxo-2,3,3a,6,7,7a-hexahydro-1H-indol-2-carboxylic acid (HOHICA) had been isolated in mg amounts in a previous study [17]. 2-Amino-4-ethenyl-hex-4-enic acid, (AEHEA) was isolated by preparative high performance liquid chromatography (HPLC) and identified by NMR (Bruker AM 400) and gas chromatography/mass spectrometry (GC-MS, Hewlett-Packard 5971A mass-selective detector, coupled with a HP 5890 Ser. II gas chromatograph). ^{1}H NMR (D_2O): $\delta = 6.64$ (dd; 17.8, 11.4 Hz; 7-H), 5.56 (q; 3×7.0 Hz; 5-H), 5.26 (dd; 17.8, $\leqslant 2$ Hz; 8-Ha), 5.14 (dd; 11.4, $\leqslant 2$ Hz; 8-Hb), 3.74 (dd; 10.3, 4.5 Hz; 2-H), 2.92 (dd; 14.5, 4.5 Hz; 3-Ha), 2.35 (dd; 14.5, 10.3 Hz; 3-Hb), 1.64 (d; 7.0 Hz; 6-H). – ^{13}C NMR (D_2O): $\delta = 177.5$ (C-1), 134.1 (C-7), 133.9 (C-4), 133.4 (C-5), 117.5 (C-8), 56.4 (C-2), 37.8 (C-3), 15.5 (C-6). After trimethylsilylation with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA, Macherey Nagel) the bisTMS-derivative of AEHEA (MW = 299) gave rise to a mass spectrum characterized by the ions m/z (%): 299 (6), 284 (1), 256 (3), 235 (2), 218 (100), 182 (24), 166 (2), 147 (6), 100 (24), 73 (97). Isolated 2,4-dihydroxyphenylalanine (2,4-DHPhe; for the HPLC retention time see below) was characterized as its tri-TMS ether by GC-MS by comparison with the corresponding DOPA derivative. These two isomers have almost identical mass spectra but different GC retention times.

Solutions were made up in Milli-Q-filtered (Millipore) water or in D_2O and irradiated with 254 nm radiation from low-pressure mercury arcs (NK 30/89, Heraeus Noblelight, fluence rate typically 20 $W\ m^{-2}$, and 254 nm irradiator, Gräntzel, Karlsruhe, fluence rate typically 40 $W\ m^{-2}$). Actinometry was performed as described before [18]. For irradiations with visible light in the presence of Rose Bengal a 200 W electrical bulb was used. In the absence of Rose Bengal, TYROH did not yield any photolysis products, even after prolonged irradiation times with this light source.

Separation and quantification of the products was by HPLC on a Nucleosil-5C-18 column (250×4.6 mm, Macherey Nagel) using a Merck/Hitachi 655A-11 Liquid Chromatograph equipped with an L-4000 UV Detector. With water as eluent (flow rate 1 ml min^{-1}) the retention times of TYROH and its products were: HOHICA 4.2 min, DOPA 4.9 min, 2,4-DHPhe 5.5 min, TYROH 6 min, BITYR 6.5 min and AEHEA 9.2 min. The identity of the products was confirmed by GC-MS after trimethylsilylation using authentic material for comparison.

3. Results and discussion

3.1. Photochemistry in the absence of oxygen

It has been shown by flash photolysis that phenoxyl radicals and solvated electrons are formed [10–12]. The yield of phenoxyl radicals exceeds that of solvated electrons, hence it has been concluded that besides photoionization (reaction (11), followed by reaction (12)) there must be another source of phenoxyl radicals, the homolytic splitting of the phenolic O–H bond (reaction (16)).

The solvated electron can be scavenged by N_2O (reaction (14)) and the yield of N_2 measured. In the presence of 2-propanol in sufficient concentration the H-atom is converted into H_2. Reactions (13) and (14) are cage reactions following photoionization (see below). Hence in the value of $\Phi(H_2)$ (in the presence of 2-propanol) the contribution of reaction (15) is included.

$$^1\text{TYROH} \longrightarrow \text{TYROH}^{\bullet +} + e_{aq}^- \qquad (11)$$

$$\text{TYROH}^{\bullet +} \longrightarrow \text{TYRO}^\bullet + H^+ \qquad (12)$$

$$e_{aq}^- + \text{TYRO}^\bullet \longrightarrow \text{TYRO}^- \qquad (13)$$

$$e_{aq}^- + N_2O \longrightarrow N_2 + {}^\bullet OH + OH^- \qquad (14)$$

$$e_{aq}^- + H^+ \longrightarrow H^\bullet \qquad (15)$$

$$^1\text{TYROH} \longrightarrow \text{TYRO}^\bullet + H^\bullet \qquad (16)$$

$$H^\bullet + (CH_3)_2CHOH \longrightarrow H_2 + {}^\bullet C(CH_3)_2OH \qquad (17)$$

$$H^\bullet + \text{TYROH} \longrightarrow {}^\bullet\text{H-Adduct} \qquad (18)$$

$$H^\bullet + O_2 \longrightarrow HO_2^\bullet \qquad (19)$$

When N_2O-saturated aqueous solutions containing 10^{-3} mol dm^{-3} TYROH were photolyzed, $\Phi(N_2)$ was determined at 1.8×10^{-2}, in reasonable agreement with the reported photoionization quantum yield of 3×10^{-2} [10].

For assessing the H-atom yield (from reaction (16), with a contribution from reaction (15)) an excess of 2-propanol (0.1 mol dm^{-3}, $k_{17} = 8 \times 10^7$ dm^3 mol^{-1} s^{-1} [19]) over TYROH (10^{-3} mol dm^{-3}, $k_{18} = 4 \times 10^8$ dm^3 mol^{-1} s^{-1} [19]) was used in order to ensure complete H-atom scavenging by 2-propanol. The solutions were deoxygenated with argon prior to irradiation to prevent any scavenging of the H-atoms by O_2 (reaction (19); $k_{19} = 2.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1} [19]). The H_2-yield increased linearly with the absorbed fluence (inset in Fig. 1) and from the slope of a yield vs. dose plot $\Phi(H_2) = 10^{-3}$ was calculated (see for example Table 1).

At this point, no distinction can be made whether the reaction leading to H-atoms is owing to a singlet state or a triplet state reaction (see for example reactions (3) and (5)). If H-atoms arose from the triplet state, the presence of oxygen should reduce the H_2-yield in excess of the H-scavenging capacity of oxygen in the system, since the triplet state can be quenched by oxygen. However, the data presented in Fig. 1 show that the addition of O_2 does not decrease in $\Phi(H_2)$ below the value that can be accounted for by the competition between H-atom scavenging by 2-propanol and O_2 (dotted line in Fig. 1). Hence, it is concluded that H-atom formation is a singlet-state reaction.

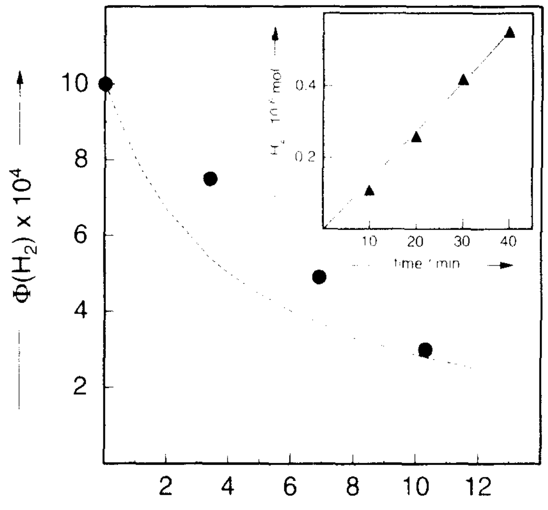
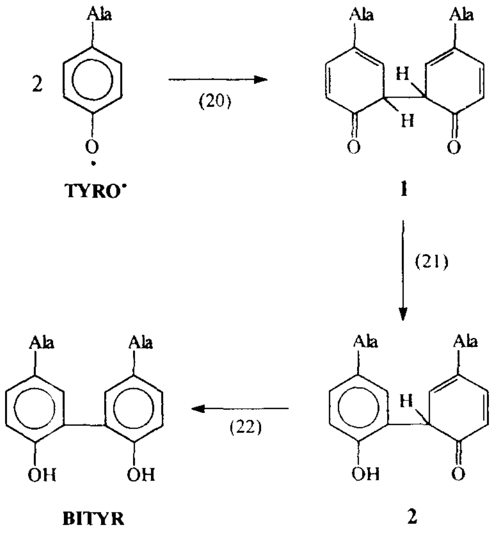


Fig. 1. H_2 quantum yield in the 254 nm photolysis of tyrosine (10^{-3} mol dm^{-3}) in the presence of 2-propanol (10^{-1} mol dm^{-1}) as a function of the O_2 concentration. Inset: H_2 yield as a function of the irradiation time (fluence rate 24 W m^{-2}).

It has been shown recently that 2,2′-bityrosyl, BITYR, is practically the only final product (>90%) when TYRO• radicals interact with one another (reactions (20)–(22)) [17].



The intermediates **1** and **2** formed in reactions (20) and (21) on the way to BITYR are too short-lived (lifetime in the order of milliseconds) to be detected by product analysis [17].

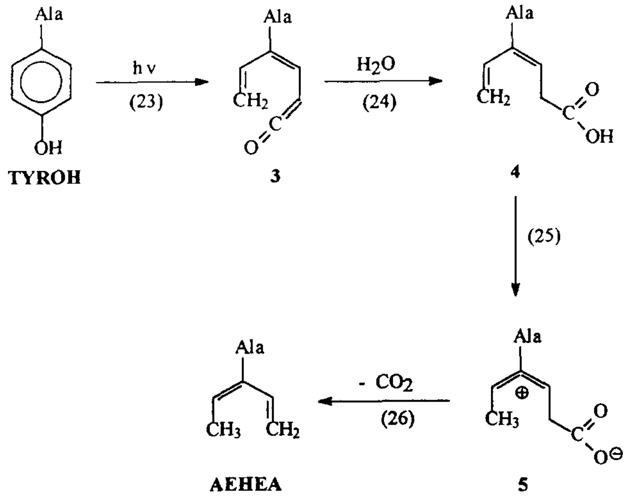
Table 1
Quantum yields (in units of mmol einstein^{-1}) in the 254 nm photolysis of D,L-tyrosine (10^{-3} mol dm^{-3}) in aqueous solution

Product	Ar/H_2O	Air/H_2O	Air/D_2O
TYROH-consumption	9.1	97	160
BITYR	3.1	14	21
AEHEA	1.6	1.6	1.1
HOHICA	absent	16	31
DOPA	absent	38	91
2,4-DHPhe	absent	2.3	n.d.
H_2O_2	absent	33	n.d.
N_2 (N_2O-saturated)	18	n.a.	n.a.
H_2 (2-propanol-added)	1.0	n.a.	n.a.

n.d., not determined; n.a., not applicable.

In addition to BITYR, 2-amino-4-ethenyl-hex-4-enic acid, AEHEA is formed (for their identification see Section 2). Isolated material (>95% pure) was used for calibration in the quantitative determination of the products. From the linear increase of the products with the absorbed dose, quantum yields were calculated. They are compiled in Table 1.

The quantum yield of AEHEA is not reduced in the presence of O_2, and it is thus concluded that it must be formed from the singlet state. It is suggested that in the excited state a 1,3-H-shift concomitant with a fragmentation of the α–C–C bond occurs as the primary process (reaction (23)). In this reaction a dienyl ketene **3** is formed which reacts with water yielding the acid **4** (reaction (24)). Protonation and subsequent decarboxylation (reactions (25) and (26); concertedness of protonation and decarboxylation would be orbital symmetry-forbidden in the electronic ground state) yields AEHEA. The latter reactions are analogous to a sequence observed in another system [20].



Reaction (23) is a novel photochemical reaction. It could be argued that reaction (23) does not proceed in one single step but that in the first step a cyclohexadienone is formed (enol-keto-isomerization) which absorbs a second photon thereby undergoing α-cleavage. Two arguments speak against this two-photon mechanism: (i) 2,4-cyclohexadienones are very short-lived in aqueous solutions (rapid rearrangement to the phenol) [21]. This does not allow the build-up of any significant 2,4-cyclohexadienone steady-state concentrations to absorb the second photon. (ii) The second argument is an experimental one. The yield of AEHEA increases linearly with the absorbed UV-dose even at very low fluence (Fig. 2). If a 2,4-cyclohexadienone were an intermediate an induction period would have been observed at very low fluences. Therefore we conclude that the novel photochemical reaction (23) must be the first step to AEHEA.

It is possible that reaction (23) occurs by a two-step sequence identical to the two-step sequence discussed and refuted above, however with the difference that the entire two-step sequence takes place without intermediate return to the electronic ground state. As for the first step, i.e. enol-keto-isomerization, reversible proton transfers on the excited singlet hyperface are well-known reactions. As for the second step, α-cleavage is the well-documented prominent reaction channel of electronically excited cyclohexa-2,4-diene-1-ones [22].

In D_2O the Φ(AEHEA) is reduced significantly as compared with H_2O as solvent. The exchange of the protons of the phenolic group of TYROH with D_2O is rapid. Hence TYROD is photolyzed under these conditions. The reduction in Φ(AEHEA) is explained by a kinetic isotope effect in the H/D-transfer reaction (23). This reduction in k_{23} is observed experimentally because reaction (23) is in competition with other reactions of the excited singlet state which do not suffer an H/D-isotope effect of the same magnitude.

Under the condition of "vanishing absorption" (absorbance cm^{-1} < 0.07 [23]) the degradation of TYROH follows a straight line when log([TYROH]/[TYROH]$_0$) is plotted vs. the fluence ([TYROH] = the TYROH concentration at a given fluence, [TYROH]$_0$ without irradiation). Such a plot is shown in Fig. 3.

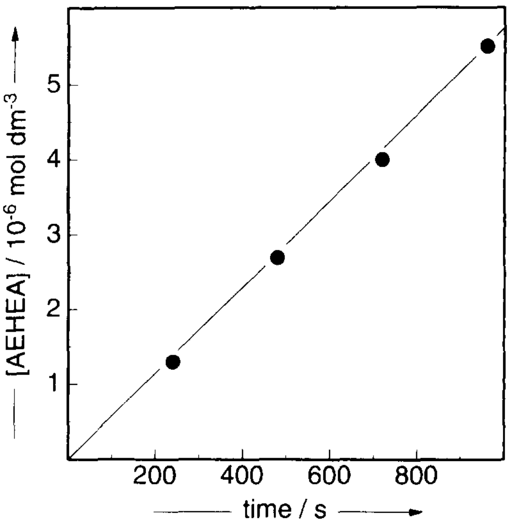


Fig. 2. Photolysis of tyrosine (10^{-3} mol dm^{-3}) at 254 nm in aqueous solution. Formation of AEHEA as a function of the irradiation time. Fluence rate: 20 W m^{-2}.

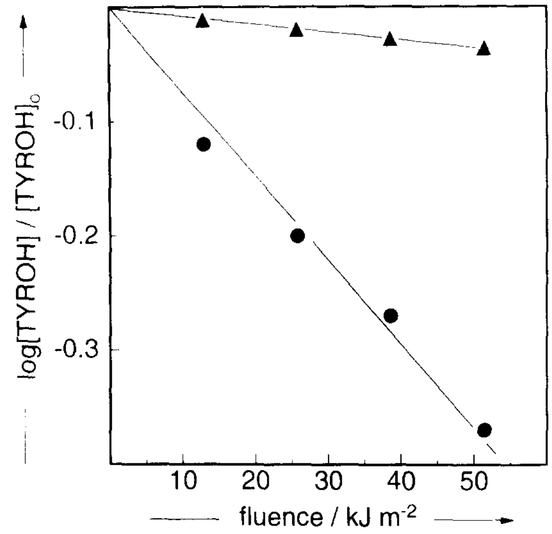


Fig. 3. Photolysis of tyrosine at 254 nm in aqueous solution under the condition of vanishing absorption (10^{-5} mol dm^{-3}). Consumption of tyrosine. (▲) Ar-saturated solutions, (●) air-saturated solutions. log[TYROH]/[TYROH]$_0$ is plotted vs. the irradiation time. Fluence rate: 43 W m^{-2}.

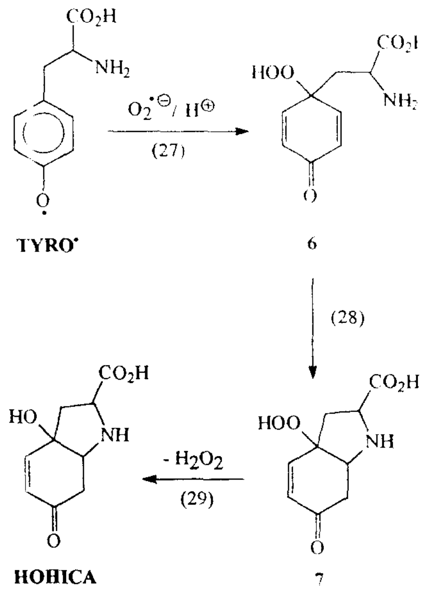
From this figure Φ(TYROH consumption) = 9.1×10^{-3} is calculated. It can be seen from Table 1 that a reasonable material balance has been obtained (note that for one molecule of BITYR formed, two molecules of TYROH are consumed), and that not much material has escaped our attention.

It has been noted above that N_2-formation in N_2O-saturated solutions (2.2×10^{-2} mol dm^{-3}) yielded a quantum yield of $\Phi(N_2) = 1.8 \times 10^{-2}$. This yield is twice as high as the observed TYROH consumption in the absence of N_2O. It is thus concluded that back reactions, e.g. reaction (13), must be of major importance.

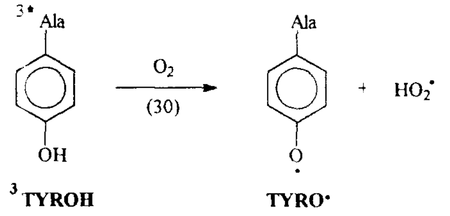
3.2. Photochemistry in the presence of oxygen

From Fig. 3 it can be seen that Φ(TYROH consumption) is considerably higher in air-saturated solutions than in the absence of oxygen. In the presence of oxygen, there are now further prominent products besides BITYR and AEHEA: DOPA (3,4-dihydroxyphenylalanine) and HOHICA [(2S,3aS,7aR)- and (2S,3aR,7aS)-3a-hydroxy-6-oxo-2,3,3a,6,7,7a-hexahydro-1H-indole-2-carboxylic acids]. In addition, 2,4-dihydroxyphenylalanine, 2,4-Phe is also formed in low yields. The formation of DOPA was observed before [15].

We have shown recently that HOHICA is formed in the reaction of $O_2^{\bullet-}$ with the TYROH-derived phenoxyl radical ($k(O_2^{\bullet-} + \text{TYRO}^\bullet) = 1.5 \times 10^9$ dm^3 mol^{-1} s^{-1}) [17]. HOHICA is not a primary product, and peroxidic compounds (e.g. **6** and **7**, $t_{1/2}$ (**7**) = 4.2 h at pH 8 and 20 °C) are its precursor (see for example the abbreviated Scheme, reactions (27)–(29), for details see Ref. [17]).



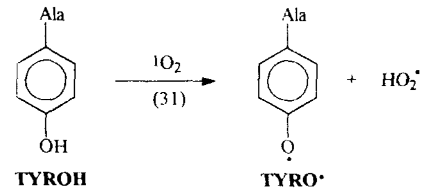
The enhanced yield of BITYR and the formation of HOHICA in the presence of oxygen (Table 1) suggests that in the reaction of ^{3}TYROH with O_2 TYRO$^\bullet$ and $HO_2^\bullet/O_2^{\bullet-}$ radicals are formed (reaction (30)).



Quenching of ^{3}TYROH by O_2 is efficient and leads to the formation of singlet oxygen, $^1O_2$, a reaction well known in the photochemistry of phenols [13]. It turns out that $^1O_2$ reacts relatively efficiently with TYROH.

In a separate experiment, Rose Bengal and visible light have been used to produce $^1O_2$ independent of TYROH excitation. The products resulting from the reaction of $^1O_2$ with TYROH are only HOHICA ($\geq 90\%$) and BITYR ($\leq 10\%$).

From the formation of BITYR it is tentatively concluded that $^1O_2$ may be able to abstract an H-atom from TYROH yielding $HO_2^\bullet(H^+/O_2^{\bullet-})$ plus TYRO$^\bullet$ (reaction (31)), although the reaction is endothermic by about 10 kcal mol^{-1}.



These two radicals are the precursors of HOHICA and BITYR, but it is not excluded that $^1O_2$ yields HOHICA by a route not involving free radicals (e.g. via the endoperoxide **8**, reaction (32)).

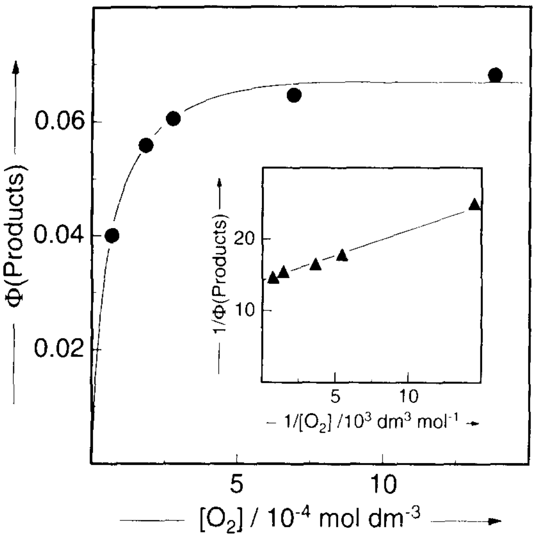
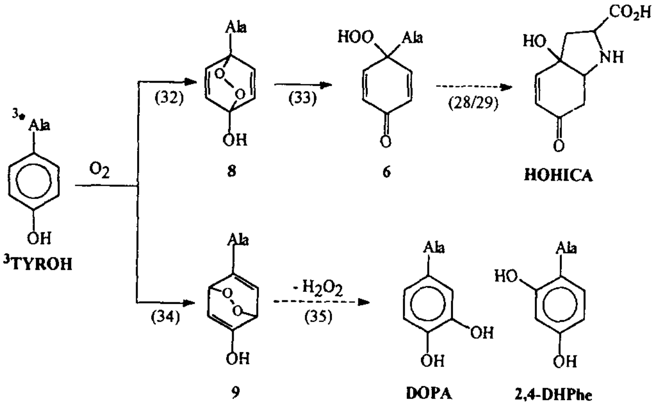


Fig. 4. Product quantum yields (sum of the oxygen-dependent products BITYR, HOHICA, and DOPA) as a function of the O_2 concentration in the 254 nm photolysis of tyrosine (10^{-3} mol dm^{-3}). Inset: 1/Φ(products) vs. 1/[O_2].



For the reaction of ^{3}TYROH with O_2 we have to consider two competitions. The first is the decay of ^{3}TYROH into the ground state (reactions (6) and (7)) and its reaction with O_2 (reaction (9)). Owing to this competition quantum yields of products resulting from reaction (9) and subsequent reactions will depend on the oxygen concentration. The second competition is the decay of $^1O_2$ into its triplet ground state (k (in H_2O) $=5.0\times10^5$ s^{-1}, k (in D_2O) $=3\times10^4$ s^{-1}) vs. its reaction with TYROH (reaction 10; k_{10} (in D_2O) $=2.7\times10^8$ dm^3 mol^{-1} s^{-1} [14]). While at a given O_2 concentration Φ(products) from the direct action of ^{3}TYROH with O_2 do not depend on the TYROH concentration, products resulting from $^1O_2$ do. There is also a strong solvent effect (D_2O vs. H_2O) owing to the prolonged lifetime of $^1O_2$ in D_2O compared with H_2O. An example of the increase of the quantum yield of product formation as a function of the O_2-concentration (first competition) is shown in Fig. 4. The effect of the TYROH concentration on the quantum yield of product formation is shown in Fig. 5.

Interesting reactions are the formation of DOPA and 2,4-DHPhe. These products were not observed when $^1O_2$ was generated by Rose Bengal. We tentatively suggest that they are formed via **9** as precursor (reactions (34) and (35)). Its hydrolysis will yield mainly DOPA and only to a minor extent 2,4-DHPhe (reaction (35)). As it stands, the endoperoxidic products **8** and **9** must remain bona fide intermediates. If formed, **9** must be rather short-lived (lifetime < 15 min) since we were unable to detect by HPLC any precursor of DOPA. The detection of **8** as a precursor of HOHICA was also not possible owing to the formation of another precursor, **7** which is rather long-lived ($t_{1/2}$ (**7**) $=4.2$ h, see above).

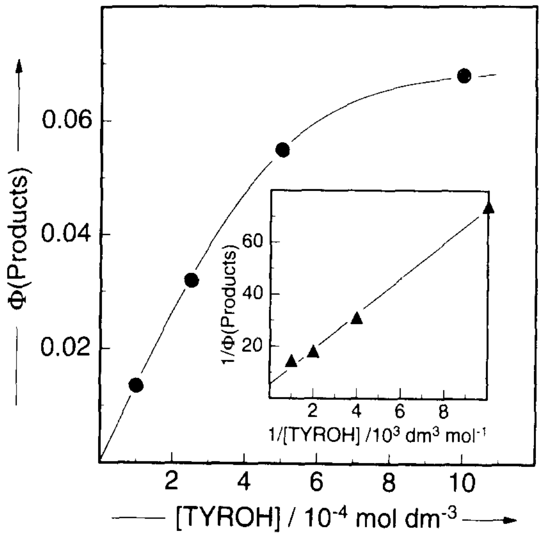


Fig. 5. Product quantum yields (sum of the oxygen-dependent products BITYR, HOHICA, and DOPA) as a function of the tyrosine concentration in the 254 nm photolysis of tyrosine in the presence of 2.5×10^{-4} mol dm^{-3} O_2. Inset: 1/Φ(products) vs. 1/[TYROH].

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